ISSN 2531-1379

HTCT Journal

HEMATOLOGY TRANSFUSION AND CELL THERAPY

VOLUME 48,
ISSUE 3,
JULY/SEPTEMBER, 2026

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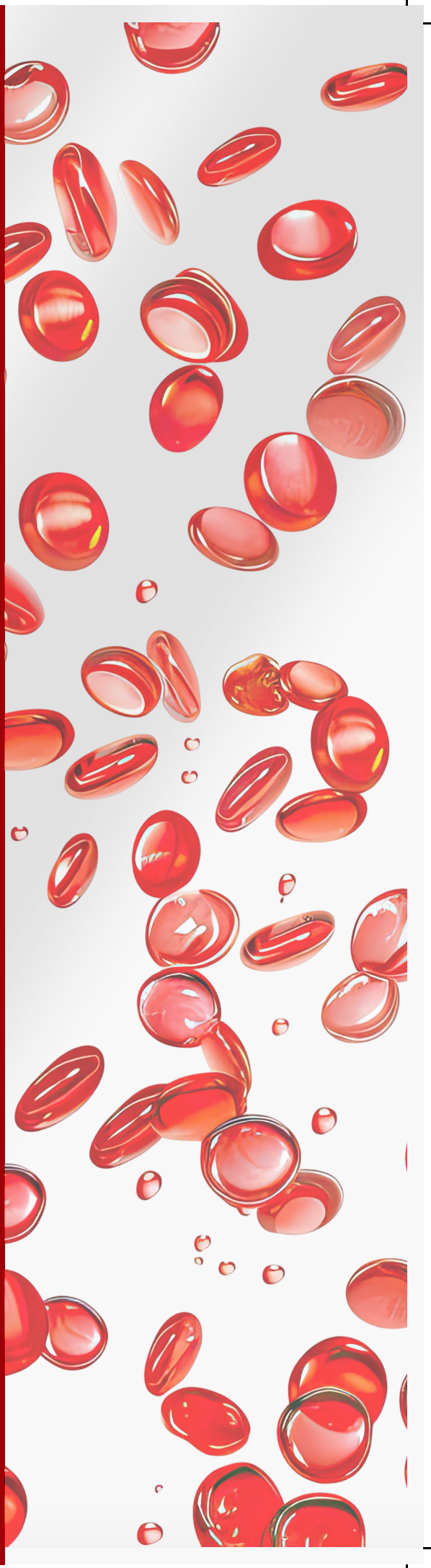

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Original article

Therapeutic plasma exchange in acute liver failure: Unravelling its efficacy and impact

Sonal Sonu *, *Rajesh Kumar, Prince Patel, Gulinder Singh, Amanpreet Kaur, Sonia Gupta, Deepika Aggarwal*

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ARTICLE INFO

Article history:
Received 12 July 2024
Accepted 30 November 2025
Available online 17 April 2026

Keywords:
Acute liver failure
Therapeutic plasma exchange
Model for end-stage liver disease (MELD) scores

ABSTRACT

Background: Acute liver failure presents a significant medical challenge due to its rapid onset, progression, and high mortality rates, often necessitating liver transplantation. Therapeutic plasma exchange has emerged as a potential intervention to modulate the underlying pathophysiological mechanisms and to reduce the need for immediate transplantation.

Methods: This retrospective monocentric study was conducted at a tertiary referral hospital from June 2023 to March 2024 to assess the efficacy of therapeutic plasma exchange in acute liver failure patients. Clinical parameters, including biochemical markers, coagulation status, and Model for End-Stage Liver Disease scores, were analyzed pre- and post-plasma exchange. Additionally, hospitalization duration and adverse events associated with the procedure were documented.

Results: Twenty-five patients with acute liver failure underwent therapeutic plasma exchange resulting in significant improvements in biochemical markers, notably reductions in bilirubin, aspartate transaminase, and alanine transaminase levels (p-value <0.001 for all). Coagulation parameters and Model for End-Stage Liver Disease scores exhibited favorable trends, particularly among discharged patients. Hospitalization duration varied across patient outcomes, indicating the impact of the procedure on clinical course. Adverse reactions were reported but remained manageable, with no severe complications observed.

Conclusion: Therapeutic plasma exchange demonstrates promising efficacy in stabilizing acute liver failure patients, improving biochemical markers, and potentially mitigating the urgency for immediate transplantation. Despite challenges such as donor scarcity and adverse events, the safety profile remains favorable, suggesting the potential of plasma exchange as a valuable adjunct in acute liver failure management. Further research and clinical scrutiny are warranted to fully ascertain the role of therapeutic plasma exchange in optimizing outcomes.

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https://doi.org/10.1016/j.htct.2026.106268

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Introduction

In the complex landscape of acute liver failure (ALF), where every moment is critical, the liver's pivotal role in sustaining life comes sharply into focus. Responsible for synthesizing vital proteins, producing coagulation factors, and detoxifying the bloodstream, dysfunction in ALF precipitates a cascade of challenges, including coagulopathy, toxin accumulation, and profound prognostic uncertainty [1].

ALF is characterized by a rapid clinical deterioration, marked by the emergence of encephalopathy following jaundice and coagulopathy, particularly in patients without preexisting chronic liver disease. The etiology of ALF demonstrates regional variations. Acetaminophen is the dominant etiology of ALF in the developed world; viral and drug-induced etiologies are frequently encountered in developing countries [2]. The immune dysfunction in ALF patients leads to the development of infections, cerebral edema, and multiorgan dysfunction syndrome, necessitating prompt diagnosis and expedited management [3–5].

Despite advances in medical science, the mortality rate of ALF remains dauntingly high, reaching 60–80 % with conservative management alone. Liver transplantation, the gold standard treatment, faces formidable obstacles, chiefly the scarcity of viable donors [6]. In this backdrop, the quest for innovative therapies to bridge the gap between diagnosis and transplantation becomes imperative.

Therapeutic plasma exchange (TPE) is a cornerstone of liver support systems, heralded for its ability to target the underlying pathophysiology of ALF. Endorsed by the American Society for Apheresis (ASFA), TPE offers a multifaceted approach, removing toxins, replenishing coagulation factors, and restoring fluid balance. Its utility is particularly evident in fulminant hepatic failure, where it serves as a vital bridge to transplantation. Current guidelines give a strong grade 1A/I recommendation for high-volume (HV)-TPE but only a weak grade 2B/III recommendation for using any non-HV-TPE in ALF, given the lack of robust data for a low-volume (LV)-TPE approach [7].

This study provides a comprehensive evaluation of the efficacy of TPE in critically ill patients with liver disease. By elucidating the impact of TPE on clinical outcomes, this research aims to refine management strategies for liver failure and optimize therapeutic protocols for this high-acuity population.

Material and methods

Study population

This retrospective, monocentric investigation was conducted at a tertiary referral hospital over a period spanning from June 2023 to March 2024. Patients presenting with acute chronic liver failure (ACLF) and those with secondary ALF arising from concurrent critical conditions such as septic shock or cardiogenic shock were deliberately excluded. This study specifically focused on patients meeting the criteria for ALF, as outlined by the Asian Pacific Association for the Study of the Liver (APASL). ALF is a critical medical emergency characterized by severe hepatic injury resulting in coagulopathy, typically defined by an International Normalized Ratio (INR) ≥1.5, and the presence of hepatic encephalopathy in a patient without pre-existing liver disease. This syndrome is clinically recognized when the duration of illness is <26 weeks; hyperacute and acute presentations typically occur within one to four weeks of symptom onset [8]. The etiologies of ALF in this study included hepatitis A (72 %; *n* = 18), hepatitis E (24 %; *n* = 6), and drug-induced liver injury (4 %; *n* = 1). Yellow fever was not reported, as it is non-endemic in the region. The institute's ethics committee approved the research protocol.

Intervention - therapeutic plasma exchange

Vascular access was established via venous insertion of a 12-French triple-lumen hemodialysis catheter. TPE procedures were performed by a transfusion medicine specialist with a Spectra Optia Apheresis System and MCS$^{+®}$ 9000 Mobile Collection System. Each TPE session involved the exchange of approximately 1–1.5 plasma volume, guided by clinical indications and the treating clinician's requisition. TPE was performed daily following the diagnosis of ALF until clinical recovery or liver transplantation, leading to a variable number of interventions per patient (generally 3–5 sessions). The standardized replacement fluid dosage comprised 10–12 units of fresh frozen plasma (FFP) and saline.

The estimated plasma volume for each patient was calculated using the body weight and hematocrit in the formula to prescribe TPE as follows:

$$\text{Estimated plasma volume} = (0.65 \times \text{body weight}) \times (1 - \text{hematocrit})$$ [9]

Modifications were made in the calculation of the blood volume based on gender and age as follows:

$$\text{Gender – based total blood volume (TBV)} = \text{ideal body weight} \times 75\ \text{mL/kg (male) or 65 mL/kg (female)}$$

$$\text{Age – based modified TBV} = \text{ideal body weight} \times 70\ \text{mL/kg} (< 65\ \text{years old})$$

or 60 mL/kg (≥65 years old) [10]

Each TPE session lasted for 3–4 h. An acid-citrate-dextrose (ACD) anticoagulant solution (1:12 vol ratio anticoagulant to whole blood) was used during the procedure. Rates of citrate flow were meticulously adjusted to keep post-filter ionized calcium concentration in a target range of 0.5–0.6 mmol/L, and caution was taken while supplementing calcium intravenously in the form of calcium gluconate in normal saline (10 mL in 10 % w/v) over 20–30 min with an infusion pump, for every 1000–1200 mL of replacement fluid to avoid citrate toxicity. Calcium concentrations were monitored by venous blood gas (VBG), and calcium gluconate was administered as required. Platelet count, prothrombin time

(PT), INR, and liver function tests (total bilirubin, direct bilirubin, indirect bilirubin, aspartate transaminase [AST], alanine transaminase [ALT] and serum creatinine) were used as pre- and post-procedure laboratory assessments. Outcomes were also assessed through the Model for End-Stage Liver Disease (MELD) score and clinical status.

The laboratory parameters and MELD scores were expressed as mean ± SD with analysis employing the paired *t*-test and one-way ANOVA. GraphPad Prism (Trail Version GraphPad Software) and IBM SPSS Statistics (Version 25.0, IBM Corp., Armonk, New York) were used for data analysis and graph generation. The result was considered statistically significant when the two-tailed p-value was <0.05.

Results

During the study period, 25 patients were diagnosed with ALF and underwent TPE. The cohort had a mean age of 21.48 years, with a marked male predominance (84 %; *n* = 21) compared to females (16 %; *n* = 4). At hospital admission, Grade II and III hepatic encephalopathy (HE) was present in 48 % of cases. Each of the procedures was conducted daily (3–5 procedures; mean of 3.1 procedures). The median treatment time was 240 min (210–260 min). An average of 10–12 units of FFP were used with the average exchanged plasma volume being 3.2 ± 0.8 liters per session. In addition, 56 % of patients required continuous renal replacement therapy (CRRT) due to acute kidney injury at inclusion. Ammonia levels, an important indicator that determines the management of HE, showed a significant decrease from 92.45 μmol/L (± 45.23) pre-procedure to 45.62 μmol/L (± 24.78) post-procedure (p-value = 0.002), which was suggestive of a positive impact of the procedure on hepatic function and metabolic stability. CRRT was temporarily stopped during TPE sessions in patients with acute kidney injury.

In this study, the procedure improved several biochemical parameters. The mean total bilirubin levels dropped significantly from a baseline of 27.48 ± 19.82 mg/dL to 14.64 ± 11.75 mg/dL after the procedure (p-value = 0.001) (Figure 1). Likewise, direct bilirubin levels significantly dropped from 18.85 ± 12.43 mg/dL to 9.88 ± 7.38 mg/dL after the intervention (p-value <0.001) (Figure 2). At the same time, indirect bilirubin levels were also significantly reduced from 8.33 ± 7.74 mg/dL to 4.76 ± 5.14 mg/dL post-procedure (p-value = 0.008) (Figure 3). Reductions in AST and ALT levels were also seen, with post-procedure AST declining from 2243.60 ± 1830.58 IV/L to 199.12 ± 165.49 IV/L (p-value <0.001) and ALT dropping from 2521.00 ± 1739.22 IV/L to 261.08 ± 202.19 IV/L (p-value <0.001) (Figure 4). These results further confirm the potential of this intervention to modulate biochemical markers of liver function. The patients had a mean INR of 2.62 ± 1.55 before TPE, which was significantly decreased to 1.47 ± 0.63 after TPE, indicating a better coagulation profile (Figure 5). Moreover the Prothrombin time reduced from 29.19 ± 17.23 to21.55 ± 23.79 (p-value = 0.32) (Figure 6). The creatinine level dropped from 2.22 ± 184 to 1.36 ± 1.62 mg/dL (p-value = 0.47) (Figure 7) There was a reversible decrease in platelet count during TPE, from 217.04 ± 77.14 to 180.96 ± 78.24 (p-value = 0.063) (Figure 8).

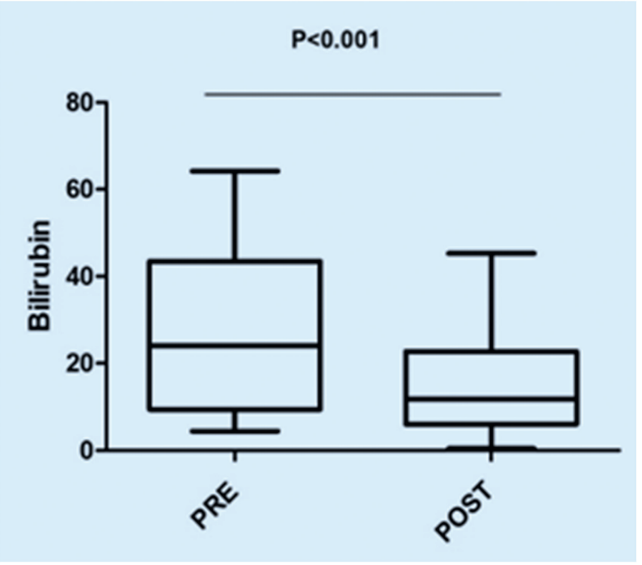

Figure 1 – Impact of therapeutic plasma exchange on bilirubin before and after therapeutic plasma exchange.

Cohen's *d* statistics were calculated to quantify the effect sizes of the differences observed between pre-TPE and post-TPE measurements. The analysis yielded *d* values ranging from 0.32 (95 % confidence interval [95 % CI]: 0.15–0.49) to 1.43 (95 % CI: 1.22–1.63), representing a spectrum from small to large effect sizes across the majority of analyzed parameters (Table 1).

Patient outcomes were compared with hospitalization durations. According to the findings, there is a large difference in the length of stay, showing the shortest was mortality with 8.86 days, significantly diverging from other groups (f = 18.705; p-value <0.001). Discharge against medical advice (DAMA) patients experienced a moderate stay of 18.27 days, while those discharged stayed the longest at 56.00 days (Table 2). The results highlight the impact of patient

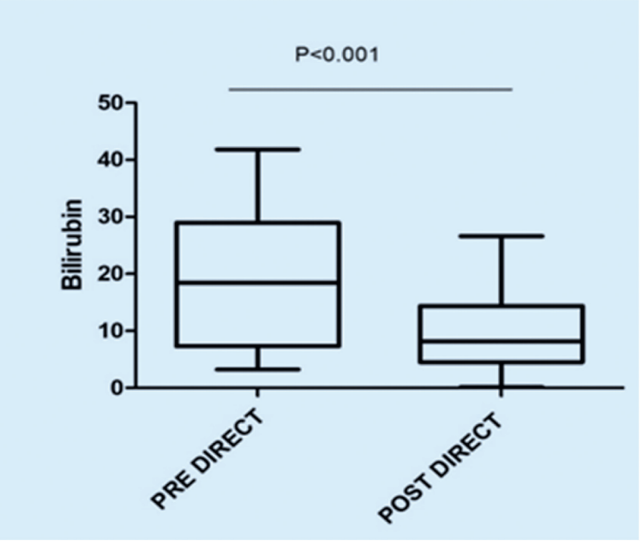

Figure 2 – Direct bilirubin levels before and after therapeutic plasma exchange.

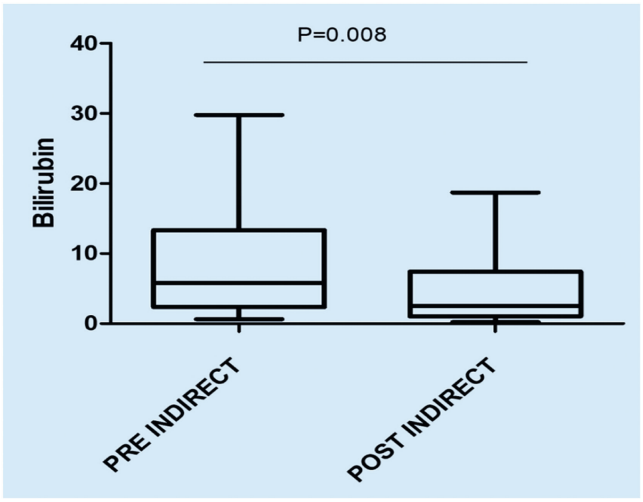

Figure 3 – Indirect bilirubin levels before and after therapeutic plasma exchange.

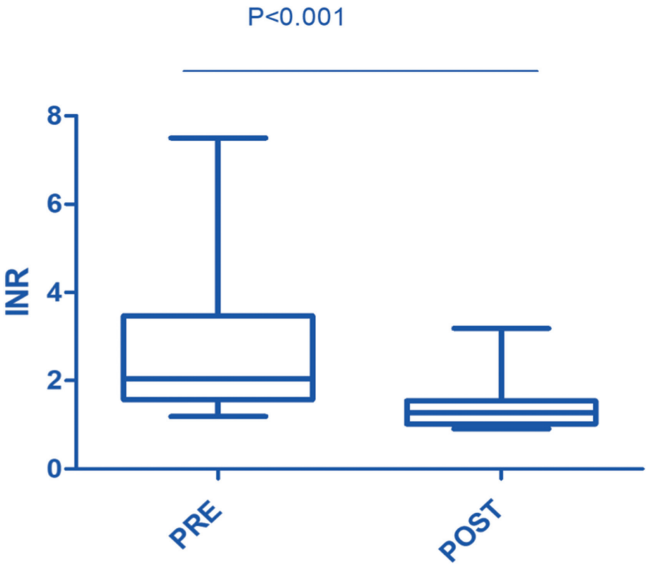

Figure 5 – International Normalized Ratio (INR) levels before and after therapeutic plasma exchange.

outcomes and how substantial savings can be achieved if care pathways are tailor-made to enhance both resource utilization and patient outcomes.

In cases where patients succumbed to their conditions, their MELD score before TPE was 36.683 ± 11.124, decreasing to 28.898 ± 8.766 post-TPE, although there was no significant difference between them (t = 2.104; p-value = 0.080). Conversely, a statistically significant reduction (t = 5.868; p-value = 0.001) in MELD scores was observed among patients who were discharged. In this subgroup, MELD scores decreased substantially from 33.767 ± 10.912 to 11.871 ± 8.016 following TPE demonstrating the clinical utility of TPE in improving the prognosis for surviving patients. An interesting observation was seen in DAMA patients. Despite exhibiting a significant reduction in MELD scores post-TPE, from 27.189 ± 5.998 to 15.822 ± 9.325 (t = 10; p-value = 0.001), these patients faced challenges in obtaining further treatment, likely due to financial constraints (Table 3). This observation highlights a critical barrier to healthcare access, wherein patients who may have been demonstrating improvement were compelled to discontinue treatment prematurely due to financial limitations. Moreover, the statistically significant reduction in MELD scores among patients discharged post-TPE highlights the pronounced positive impact of TPE on disease progression and patient outcomes.

Results show that the use of TPE was viable and well tolerated, facilitating its integration into routine care. However, adverse reactions were described with itching (15 %), urticaria (14 %), and hypocalcemia (20 %) being the most common occurring. However, no major adverse events, such as

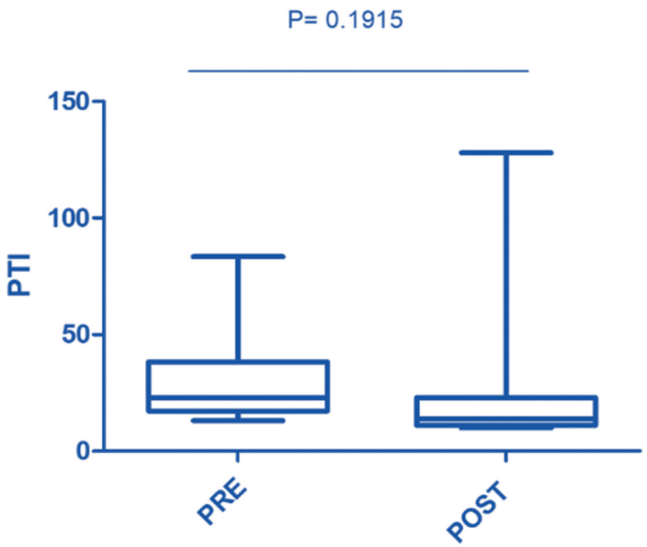

Figure 4 – Prothrombin Time Index (PTI) before and after therapeutic plasma exchange.

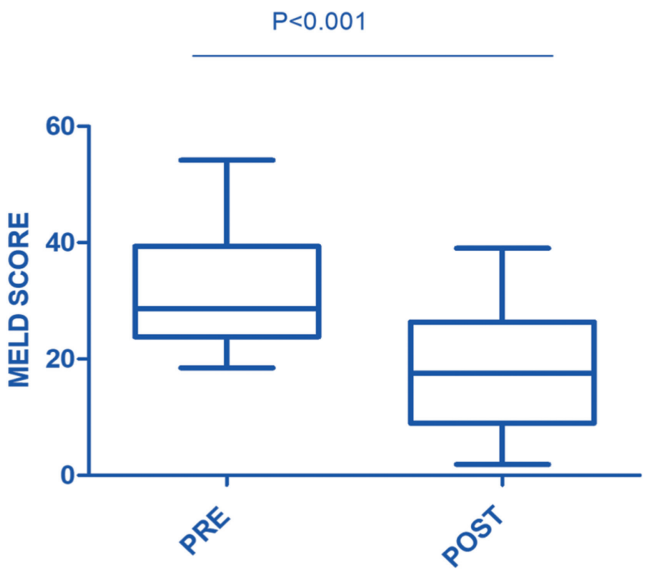

Figure 6 – Model for End-Stage Liver Disease (MELD) score before and after therapeutic plasma exchange.

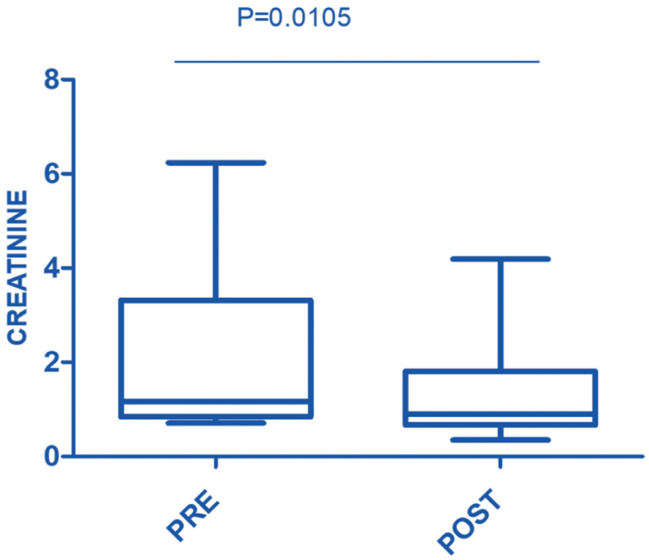

Figure 7 – serum creatinine levels before and after therapeutic plasma exchange.

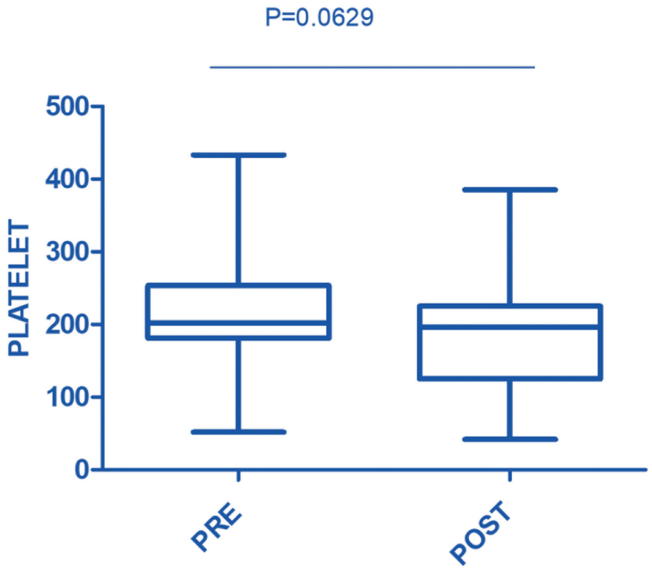

Figure 8 – Platelet count in response to therapeutic plasma exchange.

anaphylactic reactions or transfusion-related acute lung injury (TRALI), were found. Citrate was effectively used as an anticoagulant in all patients, with no significant electrolyte imbalances or acid-base disturbances recorded.

Discussion

The clinical benefit of TPE in patients with ALF was evaluated by analyzing specific biochemical parameters and determining the prognostic utility of the procedure within the current cohort. The prevalence of Grades II and III hepatic encephalopathy (HE) in 48 % of patients upon admission is consistent with the clinical presentations documented by Bernal et al. [11], highlighting the severe neurological impact of ALF. Acute kidney injury necessitating CRRT was present in 56 % of patients at inclusion. In these patients, CRRT was temporarily suspended during TPE sessions. This is in agreement with the management techniques described by Céline Monard et al. [12]. Interruptions of extracorporeal treatments may be needed in the CRRT schedule to avoid complications. One of the significant clinical study findings is a considerable reduction in the serum ammonia level after TPE, suggesting a beneficial effect on hepatic function and metabolic stability, which are critical in the control of HE [13,14]. This highlights the multi-factorial advantages of TPE in ALF, managing the liver and neurological sequelae of ALF. Consistent with the findings of Singer et al. [15], the present study demonstrated significant reductions in bilirubin and improvements in coagulation parameters (INR). Singer et al. emphasized the role of TPE in enhancing liver function by effectively removing hepatic toxins and improving coagulation status. The same is true of the present results, which confirmed that TPE can effectively reach these clinical objectives.

MELD score analysis showed a significant drop in the scores of discharged patients post-TPE (from 33.767–11.871), emphasizing the role of TPE in reducing disease severity. This was comparable to the findings reported by Freeman et al. [16], who observed a 55 % survival rate in post-TPE liver failure patients. The lack of reduction in MELD scores among the deceased (36.683–28.898) indicates that although TPE may improve biochemical parameters, this may not translate to significant survival advantages in critically ill patients. The marked fall in MELDs in DAMA patients (27.189–15.822) is an important impediment to access to health care for financial reasons; these are similar observations to those of Lee et al. [17]. This emphasizes the importance of policy interventions to improve treatment access and adherence. Those who died had spent an average of 8.86 days hospitalized, whereas the average length of stay for those discharged was 56.00 days. The importance of early intervention and persistence of care in managing patient outcomes is an overarching theme highlighted in these studies and research by Shalimar et al. [18].

This study did not directly measure the rate of transplantation. Given the critical role of liver transplantation in ALF management, it is important to acknowledge that at the time of the current study, liver transplant services were not available at the study institution, and eligible patients were referred to other centers for transplantation. However, the findings of this research suggest that TPE may offer a therapeutic window for patient stabilization, potentially reducing mortality while awaiting transplantation. Nevertheless, major improvements in biochemical markers and clinical status raise the chance that TPE could allow delayed transplantation in the very short term by stabilizing patients and allow more time to obtain an appropriate donor. The same result was also seen in the study of Larsen et al., in which TPE was associated with better liver function tests and survival in patients with ALF. Their study showed that early TPE was effective in significantly reducing the severity of liver injury and acted as an important bridge to transplantation when required [19].

Table 1 – Comparative analysis of laboratory parameters before and after therapeutic plasma exchange (TPE).

No.	Parameter	Before TPE mean ± SD	After TPE mean ± SD	t-value	p-value	Cohen's d
1.	Ammonia Level μmol/L)	92.45 ± 45.23	45.62 ± 24.78	1.817	0.002	1.282
2.	Total Bilirubin (mg/dL)	27.48 ± 19.82	14.64 ± 11.75	4.019	0.001	0.59
3.	Direct Bilirubin (mg/dL)	18.85 ± 12.43	9.88 ± 7.38	4.399	<0.001	0.73
4.	Indirect Bilirubin (mg/dL)	8.33 ± 7.74	4.76 ± 5.14	2.867	0.008	0.67
5.	AST (IV/L) (SGOT)	2243.60 ± 1830.58	199.12 ± 165.49	5.552	<0.001	0.91
6.	ALT (IV/L) (SGPT)	2521.00 ± 1739.22	261.08 ± 202.19	6.310	<0.001	1.43
7.	Platelet Count (/μL)	217.04 ± 77.14	180.96 ± 78.24	1.950	0.063	0.44
8.	Prothrombin time (S)	29.19 ± 17.23	21.55 ± 23.79	1.344	0.191	0.32
9.	International Normalized Ratio	2.62 ± 1.55	1.47 ± 0.63	3.843	0.001	0.72
10.	Serum Creatinine (mg/dL)	2.22 ± 1.84	1.36 ± 1.62	2.774	0.110	0.47
11.	MELD score	31.69 ± 9.67	17.82 ± 10.38	6.536	<0.001	0.89

AST: aspartate transaminase; SGOT: Serum glutamic-oxaloacetic transaminase; ALT: alanine transaminase; SGPT: Serum glutamic-pyruvic transaminase; SD: Standard deviation.

Table 2 – Comparison of hospital stay duration by outcome.

Outcome	N	Mean Stay	SD	F-value	P-value
Mortality	7	8.86	6.122	18.705	<0.001
DAMA	11	18.27	10.355		
Discharge	7	56.00	25.794		
Total	**25**	**26.20**	**24.411**		

SD: Standard deviation; DAMA: Discharge against medical advice.

Table 3 – Comparison of Model for End-Stage Liver Disease (MELD) scores before and after therapeutic plasma exchange (TPE) across different outcomes.

Outcome	MELD Score			
	Before TPE mean ± SD	After TPE mean ± SD	t-value	p-value
Mortality	36.683 ± 11.124	28.898 ± 8.766	2.104	0.080
Discharged	33.767 ± 10.912	11.871 ± 8.016	5.868	0.001
DAMA	27.189 ± 5.998	15.822 ± 9.325	10	0.001

SD: Standard deviation; DAMA: Discharge against medical advice.

No severe side effects, like anaphylactic reactions or TRALI, were reported, consistent with the safety profiles [20]. The efficient use of citrate as an anticoagulant without developing major electrolyte abnormalities or acid-base balance appears to align with other observations [21]. Moreover, patients demonstrated significant improvements in patient-reported outcomes regarding quality of life, with the magnitude of benefit exceeding initial clinical expectations. This could indicate that the benefits of TPE extend beyond just biochemical corrections and result in an overall improvement in patient well-being and recovery.

Conclusions

Our data highlight the potential use of TPE as an effective adjunct in ALF. By optimizing biochemical markers and clinical outcomes, TPE facilitates patient stabilization and improves prognosis; this therapeutic strategy serves as a critical bridge to liver transplantation or, ideally, reduces the necessity for an emergency surgical intervention. In the context of ALF management, when the bridge from diagnosis to transplantation is too long, TPE certainly rises as a transitory bridge that may represent potential good patient outcomes and optimal health strategies. Though it has the potential to treat ALF, more studies and clinical judgment support are necessary to exploit the power of TPE in the comprehensive treatment of ALF.

Credit authorship contribution statement

S.S. and R.K. contributed to conceptualization, methodology, analysis, and supervision. They led the writing and editing. P.P. served as the statistician, handling data review and validation. G.S. managed software, validation, and analysis. A.K. focused on visualization and assisted with writing and

editing. S.G. managed resources and administration. D.A. handled data curation and supported writing and editing.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

Editor

Eduardo Rego

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Original article

Bridging the clinical equivalence gap: a nationwide survey on healthcare professionals' perceptions of whole blood-derived versus apheresis platelets in China

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ARTICLE INFO

Article history:
Received 6 April 2025
Accepted 1 March 2026
Available online 17 April 2026

Keywords:
Apheresis platelets
Whole blood-derived platelets
Platelet transfusion
Healthcare professionals
China

ABSTRACT

Introduction: Despite evidence supporting the clinical equivalence of whole blood-derived platelets (WBDP) and apheresis platelets (AP), AP remains the dominant choice for platelet transfusions in many countries. This study aimed to assess Chinese healthcare professionals' preferences, barriers, and conditional acceptance of WBDP as an alternative to AP, especially in the context of platelet shortages and rising clinical demand.

Methods: A nationwide survey targeting physicians, transfusionists, and blood center staff was conducted via the WeChat Mini Program. The survey included 35 questions focusing on attitudes toward AP and WBDP, particularly regarding safety, efficacy, and barriers to WBDP adoption. A total of 781 participants from various healthcare institutions across China completed the survey.

Results: A substantial majority of physicians (78.41%) and transfusionists (93.88%) exhibited a preference for AP compared to WBDP, safety (77.54% and 86.12%) and clinical efficacy (63.77% and 84.9%) as primary factors. Surprisingly, 94.32% of physicians and 89.59% of transfusionists expressed willingness to use WBDP when AP is unavailable, primarily due to blood resource shortages. One of the key findings of this task was that merely 31% of blood center staff and 27% of their hospital customers acknowledged the clinical equivalence between AP and WBDP, with 47% and 53% expressing no opinion. Key barriers to WBDP adoption included concerns over transfusion reactions (92%), efficacy (72%), and safety (68%). To improve the current situation, the following strategies were identified to enhance WBDP adoption: increasing clinical research (60.87%) and physician education (46.96%).

Conclusion: This survey highlights significant reservations among healthcare professionals regarding the clinical equivalence and safety of WBDP. Future efforts should focus on bridging the knowledge gap, optimizing WBDP collection and distribution, and promoting the

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https://doi.org/10.1016/j.htct.2026.106453
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rational use of both platelet products to address shortages and ensure high-quality patient care.

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Introduction

The demand for platelet transfusions has escalated markedly over the past decade, driven by advancements in medical treatments, an aging population, and the increasing prevalence of conditions requiring platelet support such as cancer therapies and major surgeries [1–3]. This escalating clinical demand exacerbates supply-chain challenges for healthcare systems worldwide to ensure a stable and sufficient supply of platelet components [4,5]. Platelets, as critical blood products, play a vital role in managing thrombocytopenia and preventing bleeding in high-risk patients. However, the increasing reliance on platelet transfusions has also highlighted the challenges associated with maintaining an adequate supply, particularly in the face of periodic shortages and logistical constraints.

In recent years, there has been a notable shift in many countries toward the preferential use of apheresis platelets (AP) over whole blood-derived platelets (WBDP) [3,4]. Data from the United States National Blood Collection and Utilization Survey Report showed that AP constituted 95.8 % of platelet transfusions in the United States in 2021 [4]. Meanwhile, the proportion of WBDP distributed saw a 28.2 % reduction compared to 2019 [4]. This trend can be attributed to several factors, including the higher remuneration for AP, its perceived superior quality and consistency, and the efficiency of the apheresis collection process. AP enables the collection of higher platelet yields per donor, thereby minimizing dependency on multiple donations and simplifying the processing workflow [6,7]. These advantages have made AP the preferred choice for many healthcare providers, particularly in settings where high-quality platelet products are essential for complex medical conditions [4,8–10].

Despite the growing dominance of AP, recent studies have demonstrated that WBDP and AP exhibit comparable clinical efficacy and safety profiles [6,10]. Research has shown no significant differences between the two platelet products in terms of bacterial contamination rates, human leukocyte antigen (HLA) alloimmunization, RhD alloimmunization, transfusion-related acute lung injury (TRALI), or febrile nonhemolytic transfusion reactions [6]. The only notable difference is that AP tends to have slightly higher corrected count increments (CCIs), which may be relevant in specific clinical scenarios [10,11]. These findings suggest that WBDP could serve as a viable alternative to AP, particularly in resource-constrained settings or during periods of platelet shortages. However, the widespread adoption of WBDP has been hindered by persistent concerns over its safety, efficacy, and logistical challenges.

In light of these challenges, this study aims to explore the attitudes and perceptions of healthcare professionals in China toward WBDP as a potential alternative to AP. By conducting a nationwide survey, this study seeks to understand the factors influencing the preference for AP, the barriers to WBDP adoption, and the strategies that could enhance its acceptance in clinical practice. The findings of this study will provide valuable insights into the current landscape of platelet transfusion practices in China and inform future efforts to optimize platelet supply chains, mitigate shortages, and ensure high-quality patient care.

Methods

Survey design

The present study was designed to evaluate the attitudes and preferences of healthcare professionals in China toward AP and WBDP. A comprehensive survey questionnaire was developed by the authors, comprising 35 questions divided into sections targeting physicians (15 questions), transfusionists (16 questions), and blood center staff (17 questions). The survey aimed to collect data on participants' basic characteristics, preferences for AP and WBDP, concerns regarding clinical application, and the acceptance of WBDP in the absence of AP. To ensure broad representation, the survey was disseminated through the WeChat Mini Program (Questionnaire Star), a prevalent digital survey platform in China, reaching healthcare institutions nationwide, including hospitals, blood centers, and local blood centers. Several questions included the 'other' option for free-text responses, which were reviewed by two authors and categorized, with discrepancies resolved by a panel of three additional authors. The survey questions are presented in Supplementary Tables S1–4.

Survey participation

A total of 781 participants completed the survey, with 86.04 % from hospitals, 10.37 % from blood centers, and 3.59 % from local blood centers, ensuring a diverse representation of healthcare professionals involved in platelet transfusion practices. The survey was conducted from November 11, 2024, to January 20, 2025, and responses were anonymized to protect participant confidentiality. The questionnaire covered key areas such as the participants' basic demographics, their preferences for AP and WBDP, concerns about the clinical use of WBDP, and their willingness to use WBDP when AP is unavailable. This broad participation ensured a comprehensive understanding of the current attitudes and practices related to platelet transfusion across different regions and types of healthcare institutions in China.

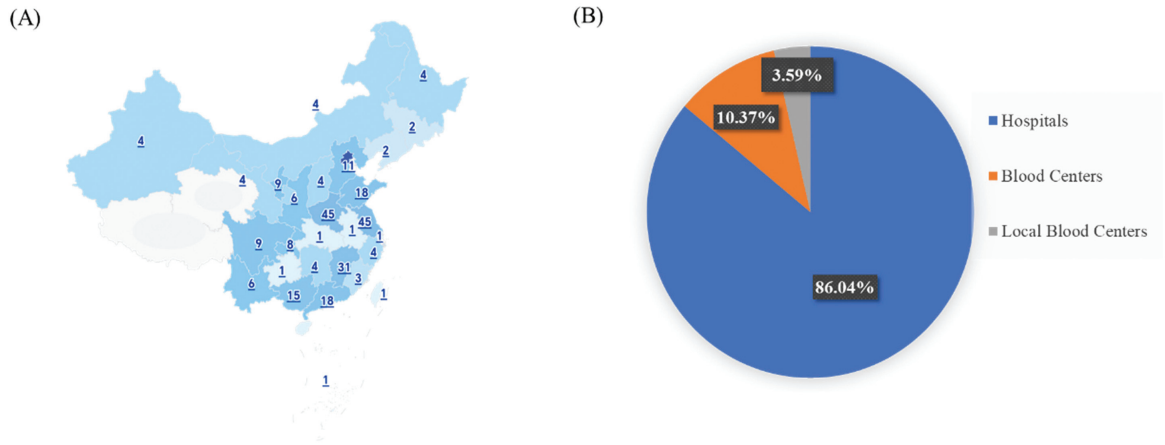

Fig. 1 – Geographical distribution and institutional representation of survey responses across China: a nationwide analysis of 781 participants. (A) Distribution of participating institutions across different regions of China, illustrating nationwide coverage. (B) Proportion of survey participants categorized by institution type, including hospitals, blood centers, and local blood centers.

Statistical analysis

Data were analyzed using descriptive statistics (proportions, means ± SD) with Excel 2010 (Microsoft, Seattle, WA, USA) to summarize respondent characteristics, ensuring a robust and systematic approach to data collection and analysis. The survey responses were aggregated and analyzed to identify trends and patterns in healthcare professionals' attitudes toward AP and WBDP. The analysis focused on key factors influencing preferences, barriers to WBDP adoption, and strategies to enhance its acceptance. The use of descriptive statistics allowed for a clear and concise presentation of the findings, providing valuable insights into the current landscape of platelet transfusion practices in China.

Results

Geographical distribution and institutional representation of survey responses

A total of 781 participants from various institutions across China completed the survey, ensuring broad regional representation. The geographical distribution of respondents spanned all major regions of the country, as illustrated in Figure 1A. This distribution reflects the central role of hospitals in platelet transfusion practices, while also capturing perspectives from blood collection and distribution facilities. The institutional representation, depicted in Figure 1B, underscores the diversity of respondents, providing a comprehensive overview of attitudes and preferences across different healthcare settings.

Baseline characteristics of healthcare professionals

The baseline characteristics of the 781 healthcare professionals surveyed are summarized in Table 1. Of the 176 physicians, 65.91 % were female and the mean age was 35.87 ± 9.03 years. The majority held senior (30.68 %) or intermediate (33.52 %) professional titles, reflecting a well-experienced cohort. Transfusionists (n = 490) were predominantly female (68.16 %), the mean age was 40.21 ± 9.12 years, and 45.1 % held intermediate professional titles. Blood center staff (n = 115) were also mostly female (73.04 %), the mean age was 44.53 ± 9.02 years, and 45.22 % held senior professional titles. These demographics highlight the experienced and diverse

Table 1 – Baseline characteristics of healthcare professionals across different departments.

Category	n	Level of institution (%)	Gender (%)	Age (year) Mean ± SD	Professional title (%)
Physicians	176	Tertiary (76.71) Secondary (22.73) No (0.56)	Female (65.91) Male (34.09)	35.87 ± 9.03	Senior (30.68) Intermediate (33.52) Junior (35.8)
Transfusionists	490	Tertiary (82.65) Secondary (16.33) No (1.02)	Female (68.16) Male (31.84)	40.21 ± 9.12	Senior (34.9) Intermediate (45.1) Junior (20)
Blood Center Staff	115	Tertiary (48.7) Secondary (13.04) No (38.26)	Female (73.04) Male (26.96)	44.53 ± 9.02	Senior (45.22) Intermediate (36.52) Junior (18.26)

Note: The 'No' category indicates that the participant's institution is not classified as a secondary or tertiary hospital (e.g. it is primary healthcare centers or other non-tiered medical facilities).

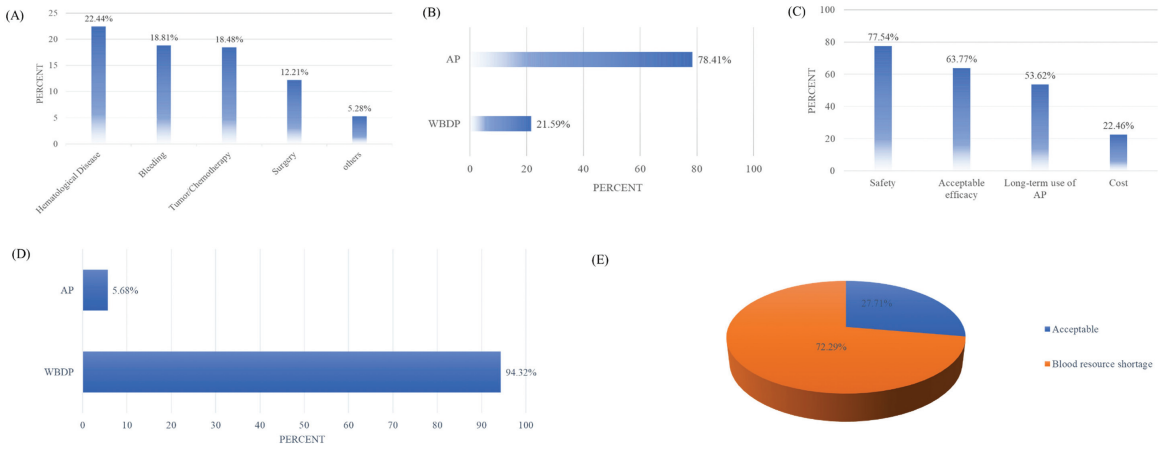

Fig. 2 – Physicians' clinical preferences, barriers, and conditional acceptance of whole blood-derived platelets (WBDP): (A) Platelet transfusion indications among patients. (B) Preference for WBDP versus apheresis platelets (AP) in clinical practice. (C) Key factors influencing physicians' choice between WBDP and AP. (D) Percentage of physicians' willingness (Yes/No) to use WBDP when AP is unavailable. (E) Primary reasons for physicians selecting WBDP as an alternative.

nature of the respondents, ensuring that the survey results reflect informed perspectives from professional healthcare in platelet transfusion practices. Here, professional titles follow the typical hierarchy in China's healthcare system: Junior (e.g., Resident Physician, Technician), Intermediate (e.g., Attending Physician, Supervisor Technician), and Senior (e.g., Associate Chief Physician, Chief Physician, or Senior Technician).

Attitudes of physicians toward apheresis platelets and whole blood-derived platelets

Physicians demonstrated a strong preference for AP over WBDP, with 78.41 % favoring AP for clinical use (Figure 2B). The primary factors influencing this preference were safety (77.54 %) and clinical efficacy (63.77 %), as shown in Figure 2C. These findings underscore the perceived reliability of AP in clinical settings, driven by its established safety profile and consistent efficacy.

When AP was unavailable, 94.32 % of physicians expressed a willingness to use WBDP as an alternative (Figure 2D). However, this acceptance was primarily driven by blood resource shortages, cited by 72.29 % of respondents as the main reason for choosing WBDP (Figure 2E).

Attitudes of transfusionists toward apheresis platelets and whole blood-derived platelets

Similarly, transfusionists exhibited an even stronger preference for AP, with 93.88 % favoring it over WBDP (Figure 3A). Key factors influencing this preference included safety (86.12 %), clinical efficacy (84.9 %), and clinician-patient rapport (68.57 %) (Figure 3B).

Consistent with physicians, a significant proportion of transfusionists would also select WBDP as an alternative only when AP is unavailable in clinical practice (Figure 3C). The primary factors influencing this decision were efficacy (66.67 %) and safety (60 %) (Figure 3D). While this conditional acceptance demonstrates the flexibility of transfusionists in managing platelet shortages, it also highlights the persistent concerns regarding the safety and efficacy of WBDP.

Transfusionists reported a significant increase in workload when using WBDP, with 75 % indicating additional burdens associated with its clinical application (Figure 3E). The primary barriers to WBDP adoption included concerns over transfusion reactions (92 %), safety (73.06 %), and efficacy (58.98 %) (Figure 3F).

Attitudes of blood center staff to the clinical equivalence of whole blood-derived platelets versus apheresis platelets

The next series of questions was designed to understand the attitudes of the blood center staff toward the clinical equivalence of WBDP in comparison with AP. Of the survey respondents, only 31 % agreed or strongly agreed that AP and WBDP were clinically equivalent. Surprisingly, a significant proportion, 47 %, indicated that they did not have an opinion on their equivalency. Meanwhile, 19 % disagreed and 3 % strongly disagreed that the two products were clinically equivalent (Figure 4A).

Blood center staff were also surveyed regarding their perceptions of hospital customers' beliefs about the clinical equivalence of WBDP and AP. Similarly, only 27 % of the respondents believed that their hospital customers would agree or strongly agree that these products are clinically equivalent, while 53 % thought their customers were either unaware or neutral on the issue. About 18 % felt that their customers did not consider the products to be equivalent (Figure 4B). The remaining respondents generally indicated that they had not specifically asked their hospital customers about their opinions, and some noted that hospitals typically relied on the blood center to decide which product to supply.

The primary barriers to the widespread adoption of WBDP, as perceived by blood center staff, included concerns over transfusion reactions (92 %), efficacy (72 %), and safety (68 %) (Figure 4C). To address these barriers, respondents identified increasing clinical research (60.87 %) and physician education

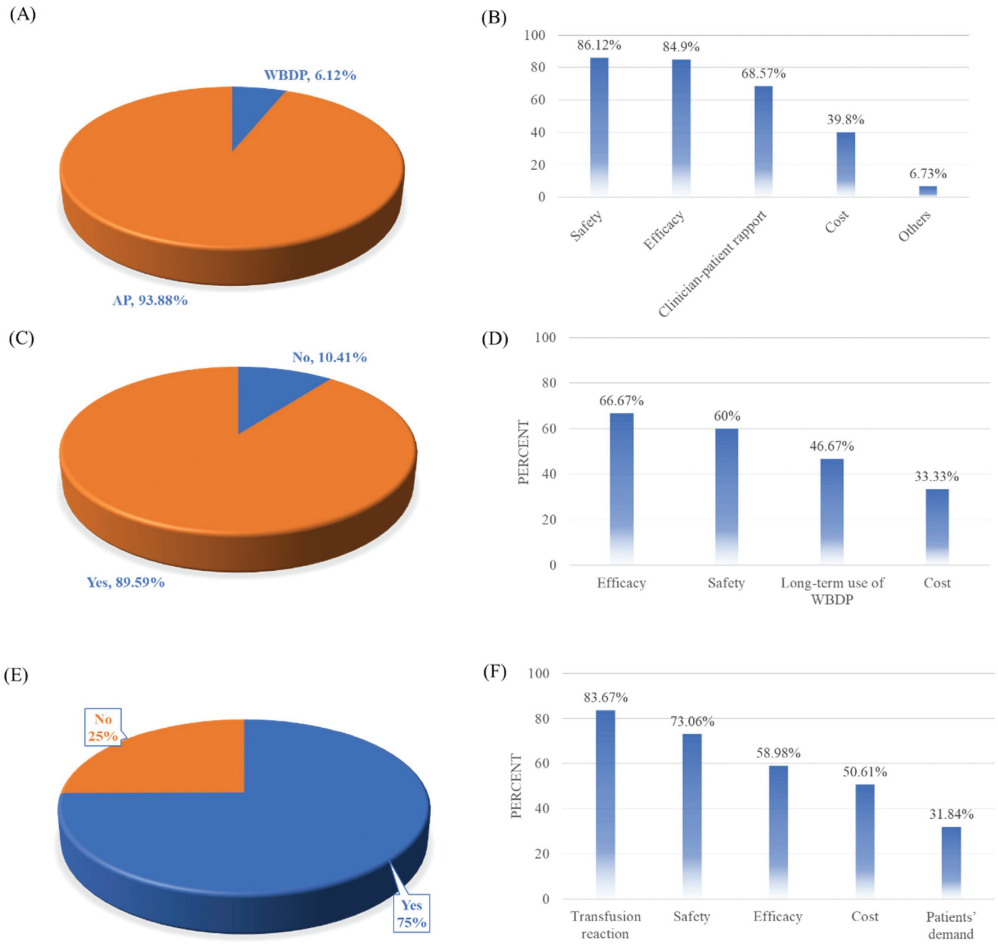

Fig. 3 – Transfusionists' platelet source preferences, workload impact, and barriers to whole blood-derived platelets (WBDP) adoption. (A) Distribution of two types of platelet sources chosen by transfusionists. (B) Key factors influencing transfusionists' decision-making. (C) Transfusionists' acceptance (Yes/No) of WBDP as an alternative when apheresis platelets (AP) is unavailable. (D) Factors influencing transfusionists' selection of WBDP during shortages. (E) Perceived growth in workload for transfusionists due to WBDP use. (F) Primary barriers to clinical application of WBDP identified by transfusionists.

(46.96 %) as key strategies for enhancing WBDP adoption (Figure 4D). Discussion

In China, AP are overwhelmingly preferred over WBDP for clinical transfusions, driven by perceptions of superior safety, efficacy, and long-term clinical experience. The efficiency of AP collection, which generates elevated platelet yields per donation, reinforces its predominance in clinical practice. Conversely, WBDP is often relegated to a secondary option, utilized only during severe blood shortages. Even then, its adoption is constrained by persistent concerns over safety and efficacy, reflecting a persistent skepticism among healthcare professionals. This preference underscores the need to address misconceptions and barriers to WBDP adoption, particularly in light of growing platelet demand and supply challenges.

The data from the present study highlight that the primary limitation to the broader acceptance of WBDP lies in the need to address persistent concerns regarding safety and efficacy. The apheresis process yields higher platelet volumes per donation, reducing donor dependency and streamlining workflows [4,10]. Despite evidence from previous studies demonstrating the clinical equivalence of WBDP and AP in safety and efficacy, this survey reveals that their adoption in China remains conditional, primarily reserved for AP shortages, due to persistent concerns among healthcare professionals. This entrenched preference for AP is sustained by longstanding perceptions of its superiority, reinforced by its standardized collection process. To address this disparity, future initiatives must prioritize evidence-based education to bridge knowledge gaps and optimize the rational use of both platelet products. While AP demonstrates marginally superior clinical CCI outcomes, WBDP remains clinically acceptable, offering cost-effective scalability to address platelet shortages [10,11]. Numerous studies have demonstrated that AP and WBDP exhibit no significant differences in key safety and efficacy metrics, including rates of bacterial contamination, HLA alloimmunization, RhD alloimmunization, TRALI, and febrile non-hemolytic transfusion reactions [6,10]. Despite these robust data, the current survey reveals that a significant knowledge gap persists among physicians, transfusionists, and blood center staff regarding the parity of WBDP with AP. Misconceptions driven by historical preferences for AP,

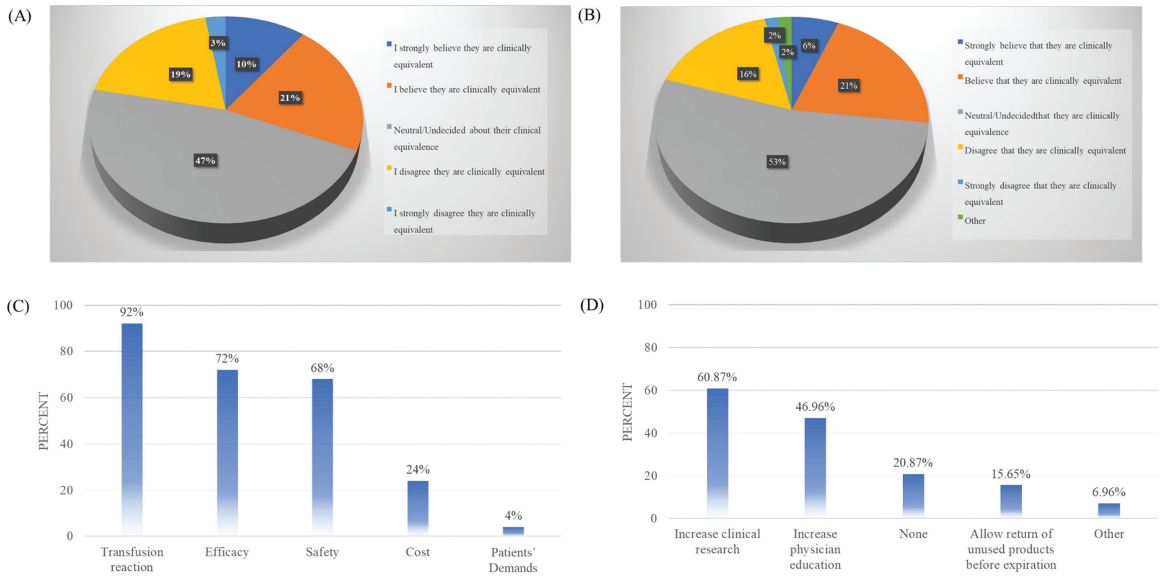

Fig. 4 – Blood center staff's perceptions of clinical equivalence and strategies for whole blood-derived platelets (WBDP) adoption. (A) Attitudes toward clinical equivalence of WBDP and apheresis platelets (AP). (B) Perceived hospital customer beliefs on equivalence. (C) Key concerns hindering WBDP adoption. (D) Proposed strategies to enhance WBDP utilization.

limited dissemination of updated evidence, and fragmented communication between stakeholders hinder the broader adoption of WBDP.

To align practice with evidence, a tripartite collaboration among physicians, transfusionists, and blood center staff is imperative. Firstly, conducting multicenter randomized controlled trials is highly recommended to rigorously assess the clinical equivalence and CCI performance of WBDP. This strategy will provide robust and comprehensive data, ensuring that the outcomes are applicable to a diverse and representative sample of the Chinese population. Leveraging multiple centers will also allow for a broader pool of expertise and resources, thereby enhancing the reliability and credibility of the findings. Secondly, blood centers should optimize centralized WBDP production and distribution to ensure consistent quality and reduce logistical barriers. This requires upgrading infrastructure and adopting standardized protocols for large-scale WBDP preparation. Thirdly, clinical guidelines must explicitly define scenarios for AP versus WBDP use, such as prioritizing WBDP for non-sensitized patients with stable requirements and reserving AP for cases necessitating HLA-matched products or high-frequency transfusions. These guidelines should be codified into national transfusion standards and reinforced through targeted training.

Moreover, educational reforms must address systemic knowledge gaps. Integrating WBDP-focused modules into China's centralized continuing medical education system will standardize training for physicians, transfusionists and blood center staff. Interactive workshops should emphasize practical decision-making algorithms based on patient profiles, platelet availability, and cost-benefit analyses. Concurrently, blood centers must lead quarterly symposia to update hospital teams on WBDP safety data, production advancements, and inventory management strategies. To sustain progress, institutionalized communication channels are critical. Regional transfusion committees should convene multidisciplinary teams to review WBDP utilization metrics, troubleshoot supply chain inefficiencies, and share best practices. Hospitals must establish real-time feedback mechanisms with blood centers to align production with clinical demand, while transfusionists should lead audits to monitor guideline adherence and outcomes. Encouragingly, the evolving attitudes of blood center staff, as evidenced by their active re-evaluation of WBDP's clinical role, signal a paradigm shift. By harmonizing evidence-based education, operational collaboration, and policy reform, China can fully leverage WBDP's potential to achieve platelet supply resilience without compromising patient care.

This study, while providing valuable insights into the perceptions and attitudes of Chinese healthcare professionals regarding platelet transfusion practices, has several limitations that warrant consideration. Firstly, the reliance on self-reported data from participants may introduce response bias, as individuals may not always accurately recall or report their preferences and experiences. Secondly, the demographic and institutional data collected were limited, lacking detailed information such as specific years of experience, exact job roles, hospital size, or blood center capacity, which could influence attitudes toward platelet transfusion practices. Thirdly, the survey was conducted over a specific period, and the findings may not capture changes in attitudes or practices over time or in response to new medical developments or policies. Fourthly, the study primarily used quantitative methods and lacked in-depth qualitative data to explore the reasons behind participants' preferences and concerns. Lastly, the study focuses on healthcare professionals' perceptions and attitudes but does not directly assess patient outcomes related to the use of AP or WBDP. Future research should address these limitations to enhance the robustness and representativeness of the findings, providing a more comprehensive understanding of platelet transfusion practices and their impact on patient care.

Conclusions

This nationwide survey, the first in China to concurrently analyze the attitudes of physicians, transfusionists, and blood center staff, reveals that despite compelling evidence supporting the clinical equivalence of AP and WBDP, AP remains the overwhelmingly preferred choice. This preference is driven by persistent perceptions of superior safety, efficacy, and long-standing clinical experience, which significantly hinder the broader acceptance of WBDP. To address this gap between evidence and practice, targeted educational initiatives, improved interdisciplinary information sharing, and increased investment in local clinical research are essential to build awareness and trust in WBDP. Furthermore, optimizing the collection, storage, and distribution processes for WBDP is crucial to alleviating platelet shortages without compromising patient care. Future efforts must prioritize closing these knowledge gaps, promoting the rational, context-specific use of both platelet products, and fostering a collaborative, multi-stakeholder approach to build a more resilient and sustainable platelet supply system.

When interpreting these findings, several study limitations should be acknowledged. First, the reliance on self-administered questionnaires may introduce response bias, as reported preferences may not fully align with actual clinical behavior. Second, the collected demographic and institutional data were limited; more detailed covariates could offer deeper insights into the drivers of preference. Most importantly, this study assessed professional perceptions but did not evaluate patient-centered outcomes associated with AP versus WBDP use. Future research should incorporate direct measures of clinical efficacy, safety events, and patient recovery to bridge this critical gap between professional perception and patient reality. By addressing these challenges, healthcare systems can move toward a more evidence-based and balanced platelet transfusion strategy, ultimately aiming to improve patient outcomes and mitigate the impact of supply shortages.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

None.

Conflicts of interest

The authors declare no conflict of interests.

Acknowledgments

We sincerely appreciate the participation and insightful responses from healthcare professionals representing a diverse range of institutions throughout China in this survey.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2026.106453.

Editor

Erich Vinicius De Paula

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Original article

Variations in transcranial doppler among pediatric patients with sickle cell disease in the Brazilian Northeast: a cross-sectional study

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ARTICLE INFO

Article history:
Received 13 March 2025
Accepted 3 February 2026
Available online 19 April 2026

Keywords:
Sickle cell disease
Transcranial doppler ultrasound
Stroke
Neurological manifestations

ABSTRACT

Background: Stroke, the most severe neurological complication of sickle cell disease, can result in irreversible sequelae and death. Transcranial Doppler combined with periodic blood transfusions reduces the chances of stroke by 90 %. In Brazil, transcranial Doppler coverage for children is low (around 20 %). This study aimed to analyze the velocity of cerebral blood flow in pediatric patients using transcranial Doppler.

Methods: This cross-sectional study analyzed 42 patients with sickle cell disease aged between 2 and 18 years who underwent transcranial Doppler following the Stroke Prevention Trial in Sickle Cell Anemia protocol. The time-averaged maximum mean velocities of the internal carotid and middle cerebral arteries were analyzed, considering biological, sociodemographic, and clinical factors.

Results: The predominant genotype was hemoglobin SS, and the median age was 10 years (interquartile range: 7–13). Of the total, 42.9 % had never undergone the exam; 7.1 % had a history of stroke with a 66.7 % recurrence rate. Regarding transcranial Doppler, 83 % of patients had normal velocity, 2.4 % abnormal, 2.4 % conditional, 9.5 % low, and 2.4 % had inconclusive results. Higher time-averaged maximum mean velocities were observed in younger patients (r = -0.48; p-value = 0.001), in those with lower levels of hemoglobin (r = -0.37; p-value = 0.024), and with higher leukocyte counts (r = 0.33; p-value = 0.050). Stroke was associated with abnormal transcranial Doppler results (p-value < 0.001).

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https://doi.org/10.1016/j.htct.2026.106458
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Conclusion: Despite recommendations, primary stroke prevention is not yet effectively implemented in the northeast of Brazil, as evidenced by low transcranial Doppler screening rates and the high incidence of stroke in the study population.

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Introduction

Sickle cell disease (SCD) includes a group of autosomal recessive hereditary hemoglobinopathies characterized by a genetic mutation in the β-globin chain of hemoglobin, resulting in sickle hemoglobin (Hb S). The most common form is sickle cell anemia (Hb SS). The Hb SS and Hb Sβ^0 genotypes are associated with the most severe clinical manifestations of the disease [1,2].

According to the National Neonatal Screening Program, the annual incidence of SCD in Brazil was 3.78 per 10,000 live births between 2014 and 2020, representing 1087 new cases. By 2022, the estimated prevalence ranged from 60,000 to 100,000 cases nationwide. This distribution is highly heterogeneous, with the highest incidences recorded in the states of Bahia, Piauí, and the Distrito Federal [3]. Additionally, in 2023, the state of Paraíba reported approximately 300 active cases [4].

The clinical manifestations of SCD are multisystemic, beginning in the early years of life and advancing to a broad range of acute and chronic complications that affect most organs and systems [1,2]. Neurological damage results from vasculopathy, hypercoagulability, thrombosis, hemolysis, and hypoxia [5,6]. Silent cerebral infarction, affecting 27 % of children over six years old, is the most common permanent neurological injury though it can be identified incidentally through nuclear magnetic resonance or screening [7].

Stroke, the worst neurological complication caused by SCD, is associated with motor and cognitive deficiencies, irreversible neurological sequelae, and death. The incidence of stroke in these individuals is 200-fold higher than in the general pediatric population [2,7,8]. Risk factors for ischemic and hemorrhagic stroke include low hemoglobin levels, leukocytosis, hypertension, previous strokes, acute chest syndrome, reticulocytosis, and increased lactate dehydrogenase (LDH). Blood transfusion, corticosteroid therapy, and nonsteroidal anti-inflammatory drugs are also potential risk factors for hemorrhagic stroke [8,9].

Following the Stroke Prevention Trial in Sickle Cell Anemia trial (STOP), a global screening protocol was established recommending annual transcranial Doppler (TCD) beginning at age 2 to monitor cerebral blood flow velocity (CBFV) and prevent stroke [10,11]. In Brazil, the Unified Health System (SUS) has offered this exam for patients with SCD aged from 2 to 16 years since 2012, prioritizing those with the Hb SS and Hb Sß genotypes [3,12]. TCD identifies patients with a higher risk of developing strokes, and actions, such as regular red blood cell transfusions, must be adopted thereby preventing it by 90 % of cases [10–12].

TCD coverage between 2018 and 2021 was 20.6 % in Brazil and 20.8 % in the northeastern rural regions [3,13]. Healthcare inequalities limit access to screening and diagnostic exams, leading to knowledge gaps on the patterns of illnesses in diverse populations, thereby complicating targeted hemotherapy [14,15].

Consequently, this study aimed to assess CBFV using TCD and identify its associated factors in socioeconomically vulnerable pediatric patients.

Material and methods

This cross-sectional analytical study included pediatric patients aged 2 to 18 years diagnosed with SCD via isoelectric focusing or high-performance liquid chromatography. The inclusion criteria were the absence of red blood cell transfusions, fever, or acute clinical events within the four weeks preceding the TCD examination. The patients lived in Campina Grande, Paraíba, Brazil or neighboring municipalities and were under medical follow-up in the Alcides Carneiro University Hospital, a high-complexity public service integrated into the Federal University of Campina Grande. Data were collected from May 2022 to December 2024.

TCD was performed and analyzed by a single licensed radiologist. CBFV values of the internal carotid, middle cerebral, and basilar arteries were obtained. Moreover, biological (sex, age, and ethnic group), sociodemographic (residence, educational level, and family income), clinical (SCD genotype, regularity of outpatient follow-up, vaccination status, hospitalization in the last year, therapies, and history of stroke), and laboratory data (Hb levels, leukocyte count, reticulocyte count, and LDH) were collected via medical records and interviews using a specific questionnaire.

All patients underwent TCD following the Stroke Prevention Trial in Sickle Cell Anemia protocol [11]. A Samsung HS70 ultrasound device was used with a PA1–5A phased array transducer operating in a frequency range of 1.0–5.0 MHz. Assessments were conducted with patients awake, afebrile, and at least four weeks after clinical interventions including red blood cell transfusions.

The assessments focused on the time-averaged maximum velocities (TAMMV) of the internal carotid, middle cerebral, and basilar arteries. The transducer was positioned on the temporal and occipital acoustic windows to obtain images of the studied arteries. A color Doppler identified the points with the highest velocities, and the TAMMV of each artery was recorded at the point of interest. TCD results were classified as normal (TAMMV <170 cm/s), low conditional (TAMMV between 170 and 184 cm/s), high conditional (TAMMV between 185 and 199 cm/s), abnormal (TAMMV ≥200 cm/s), or inconclusive [10–12].

The SPSS software version 3.0 (IBM Corp., Armonk, USA) was used for statistical analysis. Data were presented as absolute and relative frequencies (categorical variables) or measures of central tendency and dispersion (numerical variables). The Chi-square test assessed the associations between categorical variables. Additionally, comparisons between an interval numerical variable and a categorical variable were assessed by Mood's Median Test. Pearson's correlation coefficient (r) was used to measure the intensity and direction of relationships between continuous numerical variables. Correlation values were classified as weak (0.10–0.29), moderate (0.30–0.49), or strong (0.50–1.0). A p-value of <0.05 was considered statistically significant for all tests.

The parents or caregivers of all patients signed an informed consent form, whereas patients over eight years old signed an informed assent form. This study was approved by the human research ethics committee of the Federal University of Campina Grande on August 10, 2022 (CAEE 60,830,422.4.0000.5182).

Results

This study included all 42 patients with SCD aged between 2 and 18 years (median of 10 years: interquartile range: 7–13) receiving clinical follow-up at the referral center during the study period. The Hb SS genotype was found in 29 patients (69 %). Most patients were males (*n* = 27; 64.3 %), of African descent (*n* = 32; 92.9 %), and lived outside the reference area (*n* = 32; 76.2 %). Regarding guardians and caregivers (mostly mothers and fathers), 22 (52.4 %) reported nine years of formal education, and 31 (73.8 %) had a monthly family income of up to one minimum wage (Table 1).

Over half the patients had inconsistent medical follow-up (*n* = 22; 52.4 %) and presented incomplete vaccination records (*n* = 26; 62 %). Also, 33 (78.5 %) were taking hydroxyurea regularly for more than one year with doses ranging from 20 –35 mg/kg/day. Eight patients were not receiving hydroxyurea due to therapeutic decisions (seven had never been prescribed the drug, one had the treatment discontinued by the hematologist), and one patient independently discontinued the treatment. A total of 26 patients had previously received red blood cell transfusions, and two reported chronic transfusion therapy indicated for secondary stroke prevention; these patients received their last red blood cell transfusion more than four weeks prior to the TCD conducted in this study.

Three patients (7.1 %), all with the Hb SS genotype, had experienced a total of five ischemic strokes. Two of these patients had stroke recurrence despite being enrolled in a secondary prevention program. The third patient was not on a transfusion protocol, and the stroke was identified during the study interview, when significant right-sided hemiparesis was noted. From that point on, appropriate clinical and therapeutic measures were taken. The age of the patients at the time of the stroke ranged from 7–11 years old (median age of 7 years and 9 months).

In 2024, 30 patients (71.4 %) were hospitalized, representing a total of 63 admissions. The most common causes of hospitalization were pain crises, infections, and acute chest syndrome. Of the neurological complaints, 25 patients (59.5 %) reported chronic headaches, and two (4.7 %) had epilepsy. No deaths occurred during the study period.

Table 1 – Biological, sociodemographic, and clinical characteristics of pediatric patients with sickle cell disease.

Variables	*n* = 42	%
Genotype		
Hb SS	29	69.0
Hb Sβ^+	7	16.7
Hb Sβ^0	4	9.5
Hb SC	2	4.8
Age (years)		
2 to 5	7	16.7
6 to 10	16	38.0
11 to 18	19	45.3
Ethnic group		
Of African descent	39	92.8
White	3	7.2
Sex		
Male	27	64.3
Female	15	35.7
Educational level of caregivers		
≤ 9 years	22	52.4
10 to12 years	15	35.7
>12 years	5	11.9
Residency		
Campina Grande	10	23.8
Other municipalities	32	76.2
Family income (MW)		
<1 minimum wage	9	21.4
1 minimum wage	22	52.4
>1 minimum wage	11	26.2
Outpatient follow-up		
Regular	20	47.6
Irregular	22	52.4
Vaccination schedule		
Complete	16	38.0
Incomplete	26	62.0
Ischemic Stroke	3	7.1

*Minimum wage = R$ 1302.00 (2022 – approximately $253.00 USD).

TCD results showed increased TAMMV in the middle cerebral and internal carotid arteries in 66.7 % and 26.2 % of the patients, respectively. The TAMMV ranged from 61.6 –221.2 cm/s, with a mean of 119.5 ± 30.8 cm/s (raw data).

Before this study, 18 (42.9 %) patients aged 2–17 years, 13 (72.2 %) of whom were over six years of age, had never undergone a TCD, and only three (7.1 %) performed the exam regularly. Thirty-five (83 %) patients had normal results; most of those with abnormal, conditional or low velocity results had the Hb SS genotype (*n* = 5/6; 83.3 %). Although no statistical significance was observed (p-value = 0.055), higher TAMMV were observed in genotypes associated with the most severe forms of the disease (Hb SS and Hb Sβ^0) (Table 2).

Age progression was associated with a reduction of TAMMV (*r* = −0.48; p-value = 0.001) (Figure 1). No association was found between TAMMV and therapeutic interventions, hospitalizations, or outpatient follow-up. Previous stroke was associated with abnormal TCD results (p-value <0.001) (Table 3).

Regarding laboratory variables, higher TAMMV values were associated with lower Hb levels (*r* = −0.37; p-value = 0.024) and higher leukocyte counts (*r* = 0.33; p-

Table 2 – Transcranial Doppler and TAMMV of cerebral blood flow in pediatric patients with sickle cell disease according to genotype.

Genotype TCD	Hb SS n (%)	Hb Sβ^+ n (%)	Hb Sβ^0 n (%)	Hb SC n (%)	TOTAL n (%)
Normal	23 (79.3)	7 (100.0)	4 (100.0)	1 (50,0)	35 (83.3)
Abnormal	1 (3.4)	0	0	0	1 (2.4)
Conditional	1 (3.4)	0	0	0	1 (2.4)
Low velocity	3 (10.3)	0	0	1 (50.0)	4 (9.5)
Inconclusive	1 (3.4)	0	0	0	1 (2.4)
Total	**29 (100)**	**7 (100)**	**4 (100)**	**2 (100)**	**42 (100)**
TAMMV					**p-value***
Median (cm/s)	119.5	93.7	137.9	110.0	0.055

* Mood's Median Test. Statistically significant differences among groups if p-value <0.05.TCD: Transcranial Doppler; TAMMV: time-averaged median maximum velocity of cerebral blood flow.

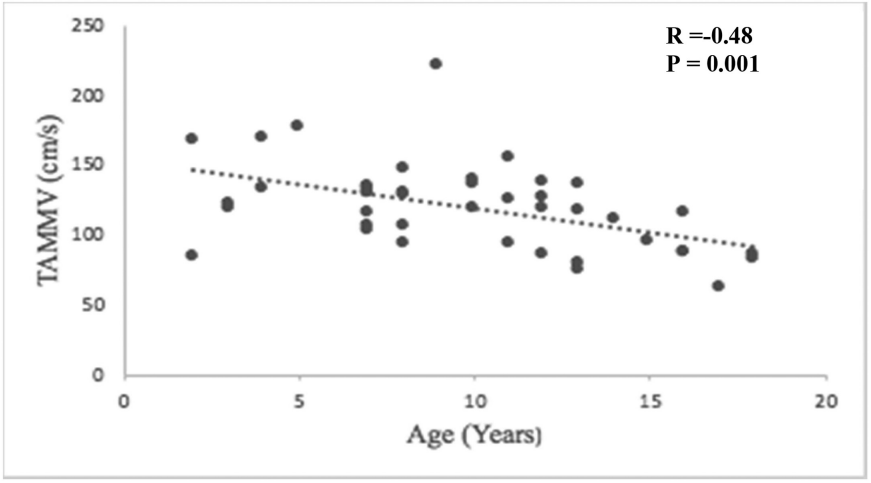

Figure 1 – Pearson correlation between age and TAMMV of cerebral blood flow in pediatric patients with sickle cell disease. TAMMV: time-averaged median maximum velocity

value = 0.050) (Table 4). Further, patients with Hb SS showed the lowest median Hb levels (p-value = 0.038) and highest LDH levels (p-value = 0.032) when compared with the other patients (Table 5).

Discussion

This study analyzed the TCD results of 42 pediatric patients (aged 2–18 years) with SCD. The cohort was predominantly male, of African descent, and of low socioeconomic status, with the Hb SS genotype being the most prevalent. The frequency of stroke (7.1 %) was consistent with literature for patients without appropriate screening, which is 5–17 % during childhood and adolescence [8,13].

Stroke in patients with SCD is associated with inadequate implementation of primary care programs, therapeutic interventions, and inconsistencies in regular TCD screenings. Although most patients in this study were older than six years, nearly half had never undergone a TCD. This finding highlights the limited access to primary prevention during early childhood [14,16].

A Brazilian study showed that almost one-third of children had never performed a TCD, and among them, the prevalence of stroke was 4.4 % [15]. Late and irregular screening for stroke was also observed in a recent national study [17]. In studies conducted in Spain and France, the median age for the first TCD ranged between two and three years, with over 80 % occurring before three years of age [18,19]. These findings indicate the great discrepancy in access to TCD worldwide [3,18–20].

Stroke screening patterns and risk interventions differ between regions. In scenarios where guidelines are not properly implemented, it is unclear how specialists assess patients and apply TCD results. In these scenarios, deviations from guidelines and missed opportunities for stroke prevention have been observed [14,17,21].

Factors hampering access to primary prevention include patient-related barriers (geographic location, poor adherence, low socioeconomic status, caregiver unawareness, and fear of

Table 3 – Clinical characteristics of pediatric patients with sickle cell disease and associations with transcranial Doppler results and TAMMV of cerebral blood flow.

Variable	TAMMV	p-value	TCD		p-value
	Median		Normal N (%)	Other* N (%)	
Use of hydroxyurea					
Yes	115.8	0.133	26 (78.8)	7 (21.2)	0.130
No	130.2		9 (100)	0	
Hospitalization in the previous year					
Yes	121.1	0.733	23 (76.6)	7 (23.4)	0.067
No	109.0		12 (100)	0	
Headache					
Yes	109.2	0.122	18 (90)	2 (10)	0.269
No	129.7		17 (77.2)	5 (22.8)	
ASA					
Yes	110.9	0.694	5 (62.5)	3 (37.5)	0.079
No	121.1		30 (88.2)	4 (11.8)	
Regular blood transfusions					
Yes	117.0	0.751	22 (84.6)	4 (15.4)	0.776
No	127.5		13 (81.2)	3 (18.8)	
Vaccination record					
Updated	126.8	0.340	14 (87.5)	2 (12.5)	0.570
Outdated	117.0		21 (80.7)	5 (19.3)	
Follow-up					
Regular	126.1	0.354	17 (85)	3 (15)	0.782
Irregular	116.6		18 (81.8)	4 (18.2)	
Stroke					
Yes	155.0	0.999	0	3 (100)	**< 0.001***
No	118.8		35 (89.7)	4 (10.3)	

Mood's Median Test: Statistically significant differences between groups if p-value < 0.05.
TAMMV: time-averaged maximum mean velocity; TCD: Transcranial Doppler (Abnormal, conditional, inconclusive); ASA: Acetylsalicylic acid.

Table 4 – Pearson correlation between TAMMV of cerebral blood flow and laboratory variables in pediatric patients with sickle cell disease.

	TAMMV	p-value
Hemoglobin	**−0.37**	**0.024**
Leukocytes	**0.33**	**0.050**
LDH	0.16	0.415
Reticulocytes	−0.01	0.972
Platelets	−0.04	0.815

*LDH: lactate dehydrogenase; TAMMV: time-averaged maximum mean velocity (cm/s).

Table 5 – Associations between medians of laboratory variables and genotypes of pediatric patients with sickle cell disease.

	Hb SS $n = 29$	Hb Sβ^0 $n = 4$	Hb Sβ^+ $n = 7$	Hb SC $n = 2$	p-value
Hemoglobin (g/dL)	7.55	9.15	9.80	8.60	**0.038**
Leukocytes (x 10^9/L)	13.60	10.65	8.20	8.56	0.127
LDH (U/L)	1187.0	639.5	412.5	483.0	**0.032**
Reticulocytes (%)	7.26	6.61	6.75	3.09	0.149
Platelets (x 10^9/L)	390	486	331	276	0.909

Mood's Median Test: Statistically significant differences between groups if p-value < 0.05.
LDH: lactate dehydrogenase.

blood transfusions) and health system limitations (inconsistent TCD availability, a shortage of specialists and equipment, and provider unfamiliarity with prevention guidelines) [15,17,20–22].

In this study, most caregivers attributed the lack of screening to the distance between their residence and the referral center, scheduling difficulties, and a lack of transportation or awareness of primary stroke prevention. Although regular outpatient follow-up and prior TCD examinations correlate with better adherence, the annual rate of missed opportunities for TCD screening remains between 61 % and 88 %. Furthermore, increasing age is associated with a lower likelihood of undergoing the exam [20].

Great variations in occurrence rates among different TCD categories were observed. In this study, the frequency of abnormalities was 2.4 %; however, studies have shown higher (4.8 % to 17 %) [15,18,23,24], lower (1.2 % to 2.1 %), and similar frequencies to our study [17,25,26]. Abnormal TCD results have been documented, including in the Brazilian Northeast, suggesting a lower risk of stroke and the possibility of effective treatments and interventions to prevent cardiovascular diseases [27,28].

Studies have shown divergences in lower TCD flow rates (ranging from 1 % to 8.4 %) [15,18,23,26,28], while a rate of 9.5 % was observed in the current study. This result may be associated with cardiovascular diseases and reflect the complete occlusion of a previous stenotic artery with the development of collateral circulation, thereby, decreasing blood flow in the main artery [28]. Considering conditional TCD, the literature shows a higher prevalence than in this study [15,18,23–26,28].

Several factors may have contributed to these differences: heterogeneity of the genotypes, use of hydroxyurea or other therapeutic interventions, access to screening and diagnostic tools, methodological limitations of different studies, and experience of the operators performing and interpreting the exam [15,20,28]. Environmental variables, such as climate, seasonality, and nutritional deficiencies, may have affected the results too [29].

Associations with previous stroke and abnormal TCD categories were observed. A study involving children with SCD in Jamaica observed an association between abnormal TCD results and previous stroke [29]. This variation is related to progressive vasculopathy, with severe arterial stenosis or Moyamoya syndrome, contributing to further cerebrovascular events. Although patients were enrolled in secondary prevention through chronic transfusion therapy, the recurrence rate observed in this study (66.7 %) was comparable to rates reported in untreated populations (60 % to 92 %) [22].

The TAMMV showed a significant negative correlation with age. Although not statistically significant, higher TAMMV were often observed in patients with Hb SS and Hb Sß^0. These findings corroborate literature showing that increased CBFV is associated with a high risk of stroke, mainly in the most severe forms of the disease and during the first decade of life [8,17,25]. Correlations between TAMMV, genotypes, and age are well-known in pediatric patients with SCD [15,26–29].

The hemodynamics of cerebral blood flow account for the higher TAMMV values observed in younger patients. Newborns show a TAMMV of 24 cm/s in the middle cerebral arteries; this velocity increases gradually until four to six years of age, when it reaches its peak and then a gradual decrease is observed after 10 years of age [30].

Higher TAMMV were associated with lower Hb levels and higher leukocyte counts, which showed a weak positive correlation with LDH levels and no correlation with the reticulocyte count. As expected, the lowest Hb levels and highest LDH levels were observed in patients with Hb SS [29].

Anemia and the effects of severe hemolysis (reduced vascular smooth muscle relaxation, activation of adhesion molecules, increased platelet adhesion and aggregation, increased inflammatory processes, progressive vasculopathy, and compensatory mechanisms for cerebral perfusion) might explain the correlation between reduced Hb levels and increased TAMMV [6,17,28].

The positive correlation between TAMMV and leukocyte counts is linked to chronic inflammation, as leukocytes adhere to other cells and the vascular endothelium, promoting intimal hyperplasia, smooth muscle proliferation, vasculopathy, and subsequent ischemia [6].

Other studies have highlighted the role of reticulocytosis in elevating TAMMV. Reticulocytes released into the circulation express surface adhesion molecules that trigger a cascade leading to cerebral vasculopathy, thereby contributing to impaired cerebral rheology and vascular occlusion [6,29]. Additionally, none of the hemolysis markers are highly specific and may vary depending on clinical conditions (iron deficiency, inflammation, and variations in bone marrow) [5].

The results of this study should be cautiously interpreted since this research was conducted in a single center even though this center is a referral hospital for children with SCD.

Conclusion

This study demonstrated that higher TAMMV were associated with younger age, lower hemoglobin levels, and higher leukocyte counts, while abnormal TCD velocities were linked to a history of stroke. These findings underscore the critical role of TCD in stroke risk stratification for pediatric patients with SCD. Despite current guidelines, primary stroke prevention remains suboptimally implemented in the Brazil Northeast, characterized by high rates of missed evaluations and stroke incidence. Factors such as regularity of follow-up, referral efficiency, and socioeconomic status significantly impact adherence. Targeted interventions are essential to address these barriers and expand TCD access, thereby improving stroke prevention in this vulnerable population.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Taciana Raulino de Oliveira Castro Marques: study conception and design, data acquisition, data analysis, and interpretation, paper composition. Suely Arruda Vidal: study conception and design, results revision, paper revision, approval of the final version. Heráclio Almeida da Costa: exams conduction, results description and analysis, critical review, and approval of the final version. Ariani Impieri Souza: study conception and design, results revision, paper revision, approval of the final version.

Conflicts of interest

none.

Editor

Fernando F. Costa.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Post-operative atrial fibrillation, intraoperative autologous blood salvage and transfusion in cardiac surgery

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ARTICLE INFO

Article history:
Received 9 July 2025
Accepted 18 February 2026
Available online 26 April 2026

Keywords:
Atrial fibrillation
Operative blood salvage
Cardiac surgical procedures
Blood transfusion

ABSTRACT

Background and objectives: Postoperative atrial fibrillation is the most common complication following cardiac surgical procedures. The objective of this study was to assess a possible association of postoperative atrial fibrillation, intraoperative autologous blood salvage, and blood transfusion.

Methods: A total of 357 adult patients undergoing cardiac surgery with the use of intraoperative autologous blood salvage were included. They were divided into two groups: one with 161 patients (45%) who only received their recovered autologous blood, and another with 196 patients (55%) who additionally required the transfusion of homologous blood components. Demographic data, pre- and post-operative laboratory parameters, transfusion profiles, and clinical outcomes (mortality) were compared.

Results: The number of recovered autologous blood units was not associated with an increased risk of postoperative arrhythmia (Odds ratio: 0.75; 95% CI: 0.45–1.11). Intraoperative homologous blood transfusion was associated with a significantly increased risk of death (Hazard ratio: 5.17; 95% CI 1.44–18.56). When autologous salvaged blood volume and homologous components were evaluated together, homologous transfusion was associated with higher risk of postoperative atrial fibrillation (Odds ratio: 2.51, 95% CI 1.18–5.65), whereas number of autologous units was not (OR 0.66, 95% CI 0.39–1.01). Transfusion at any time point (intraoperative, intensive care unit [ICU], or ward) was also associated with an increased risk of death (Hazard ratio: 5.15, 95% CI 1.16–22.94).

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https://doi.org/10.1016/j.htct.2026.106467

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Conclusion: While advanced age was significantly associated with postoperative atrial fibrillation, no association was found with intraoperative autologous blood salvage and blood transfusion.

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Introduction

Postoperative atrial fibrillation (POAF) is the most common complication following cardiac surgical procedures [1]. Its incidence ranges from 20% to 50%, depending on factors such as the type of surgery, patient population, and diagnostic criteria used [2,3]. According to data from the Society of Thoracic Surgeons (STS) Adult Cardiac Surgery Database, POAF occurs in 25% of patients undergoing isolated coronary artery bypass grafting (CABG), 30% of those undergoing isolated valve procedures, and 40% to 50% of those undergoing combined CABG/valve surgeries [4].

In the United Kingdom, approximately 32,000 adult patients across 35 centers undergo cardiac surgery annually, and 30% develop POAF. Even a single episode of POAF extends ICU stay by 12–24 h and hospital stay by 2–5 days [5,6], and nearly triples in-hospital mortality (from 0.5% to 3.3%) and one-year mortality (from 3.7% to 9.9%) [7,8]. POAF increases healthcare costs by approximately £10,000 ($12,500) per patient in the first postoperative year [9]. For these reasons, the prevention of POAF was identified as one of the top ten research priorities by the James Lind Alliance Cardiac Surgery Priority Setting Partnership in 2019, involving patients, caregivers, and healthcare professionals [10].

Multiple studies have confirmed that patients who develop POAF face increased risks of morbidity, mortality [11,12], and complications such as stroke, heart failure, renal dysfunction, and prolonged hospitalization [12,13]. Reducing its incidence may improve clinical outcomes, increasing interest in identifying risk factors that could enable the implementation of preventive strategies.

The pathophysiology of new-onset POAF remains poorly understood. Recent evidence supports an inflammatory mechanism in its development [14,15]. Transfusions of red blood cells (RBCs) or whole blood (WB) modulate the inflammatory response to cardiac surgery by altering various inflammatory mediators and enhancing systemic inflammation [16,17]. Currently, the most effective and safest method to reduce the need for transfusion and its potential complications is intraoperative autologous blood salvage, especially in cardiac surgeries (Class I; Level A evidence) [18]. This technique is a cornerstone of patient blood management [19]. Autologous cell salvage may improve red blood cell viability, preserve their biconcave shape, enhance oxygen delivery, and attenuate the inflammatory response to surgery [20,21].

This retrospective cohort study was designed to assess a potential association between POAF, autologous blood salvage, and transfusion as the primary outcome, and mortality risk as a secondary outcome.

Material and methods

This retrospective observational cohort study collected data from 26 hospitals in four Brazilian states, evaluating 357 adult patients who underwent cardiac surgery with intraoperative autologous blood salvage between January 2021 and December 2023.

The study was conducted in accordance with the Declaration of Helsinki (2008 revision). Due to the retrospective nature of the study and the use of anonymized data, consent was considered unnecessary.

Nearly all types of cardiac surgeries were represented: CABG, valve replacement or repair (VR), aortic aneurysm repair (AAR), the Bentall-de Bono procedure (BB), redirection of blood flow (RBF), myectomy, cardiac tumor resection, and combined procedures (e.g., CABG + VR, CABG + AAR, or VR + AAR). Preoperative and intraoperative variables (volume and number of autologous units recovered) were assessed for potential associations with an increased risk of transfusion.

Cell saver protocol

All cell salvage machines were calibrated and programmed according to a specific protocol based on patient blood volume, with bowl size and flow rates adjusted accordingly for each cycle phase. Blood was collected from skin incision through wound closure using a dual-lumen suction catheter designed for blood collection. Suction pressure was maintained below 100 mmHg to minimize hemolysis. Anticoagulation was achieved using 25,000 IU of heparin diluted in 1000 mL of 0.9% saline solution. Anticoagulant flow was adjusted based on surgical field bleeding rates, and the blood was filtered and stored in a cardiotomy reservoir. Whenever possible, residual blood from the cardiopulmonary bypass circuit was also redirected to the reservoir to maximize autologous blood salvage.

The cell saver system operated in either manual or automatic mode, with automatic mode prioritized to ensure standardized, pre-defined blood processing with minimal operator intervention. Red blood cells were washed and resuspended in 0.9% saline, resulting in a hematocrit of approximately 60%. These washed autologous red blood cells were transferred to a sterile reinfusion bag and transfused intraoperatively.

In the perioperative period, patients requiring additional blood volume beyond their own autologous blood received leukoreduced homologous red blood cell transfusions. In cases of excessive blood loss and cardiovascular instability, transfusions were administered at the discretion of the anesthesiology or intensive care team.

Statistical analysis

Data are presented as mean ((standard deviation), median (interquartile range [IQR]), or frequency (valid %) as appropriate. Comparisons between groups were performed using Student's *t*-test, Mann-Whitney U test, or chi-square test, depending on data distribution and normality assessed via the Shapiro-Wilk test. Logistic regression models and Cox proportional hazards models were used to evaluate associations between variables of interest and postoperative arrhythmia and mortality, respectively. All analyses were conducted using R statistical software (R Foundation for Statistical Computing, 2024).

Results

Patients were divided into two groups: one with 161 patients (45%) who only received their recovered autologous blood, and the other with 196 patients (55%) who also required transfusion of homologous blood components (Table 1). Patients who received homologous blood were more frequently female (p-value <0.001), had lower body weight (p-value = 0.002), and exhibited lower preoperative levels of hemoglobin, hematocrit, and platelets (p-value <0.001).

Regarding autologous blood recovery, the median volume was 505 mL (1.3 units) in the group that required homologous transfusion, compared to 462 mL (1.2 units) in the group that did not require homologous components (p-value <0.001) (Table 2).

The most commonly used blood component in the operating room was packed red blood cells (PRBCs; n = 107), with a median of 2 (IQR = 1.5) units. PRBCs were also the most frequently used component in the ICU.

With respect to surgical type, 163 patients (45.7%) underwent CABG, 129 (36.1%) underwent valve surgery, and 34 (9.5%) underwent aortic aneurysm repair. Other surgical procedures were less frequent.

Table 1 – Patient characteristics (n = 357).

Variable	
Age (years) – mean (SD	58.7 (13.4
Male - n (%)	263 (73.7)
Weight (kg) – mean (SD	78.9 (15.3
State of origin - n (%)	
Bahia	12 (3.4)
Distrito Federal	13 (3.6)
Rio de Janeiro	28 (7.8)
São Paulo	304 (85.2)
Platelets (1000/mm^3) – mean (SD	210.3 (68.4)
Hematocrit (%) – mean (SD	38 (6.6)
Hemoglobin (g/dL) – mean (SD	12.6 (2.3)
Kit (bowl) - n (%)	
55 mL	1 (0.3)
125 mL	35 (9.8)
225 mL	321 (89.9)
pRBC (mL) – mean (SD	556.7 (390.8)
Units salvaged/patient – mean (SD	1.43 (1.0)
Atrial Fibrillation/Arrythmia - n (%)	37 (10.4)
Transfusion - n (%)	196 (54,9)

SD: Standard deviation; pRBC: packed red blood cells.

Table 2 – Patient characteristics per group.

	Homologous blood		P-value
	No (n = 161)	Yes (n = 196)	
Age (years) – median (IQR)	59 (15)	62 (20)	0.243
Female – n (%)	26 (16.1)	68 (34.7)	**<0.001**
Weight (kg) – median (IQR)	80 (18)	77 (22)	**0.005**
Hemoglobin (g/dL) – median (IQR)	13.4 (2.15)	12 (3.2)	**<0.001**
Hematocrit (%) – median (IQR)	40 (6.7)	37 (8.75)	**<0.001**
Platelets (x10^9/L) – median (IQR)	215 (68)	200 (72)	**0.047**
Kit (bowl) – n (%)			0.63
55 mL	0	1 (0.5)	
125 mL	15 (9.3)	20 (10.2)	
225 mL	146 (90.7)	175 (89.3)	
pRBC (mL) – median (IQR)	462 (248)	505 (365.75)	**<0.001**
Autologous units recovered – median (IQR)	1.2 (0.6)	1.3 (1.0)	**<0.001**

IQR: interquartile range; pRBC: packed red blood cells.

Autologous blood transfusion was not associated with POAF (Odds ratio [OR] = 0.75; 95% CI: 0.45–1.11). The only factor independently associated with POAF in the multivariate analysis was older age (OR 1.04; 95% CI: 1.01–1.08) (Table 3).

When autologous salvaged blood volume and homologous components were evaluated together, homologous transfusion was associated with higher risk of POAF (OR 2.51; 95% CI 1.18–5.65), whereas number of autologous units was not (OR 0.66; 95%CI 0.39–1.01). This analysis was adjusted for preoperative hemoglobin (Table 4).

Regarding mortality, intraoperative homologous blood transfusion was associated with a significantly increased risk of death (HR: 5.67; 95% CI: 1.64–19.62). This association remained consistent in the multivariate analysis (HR: 5.17; 95% CI: 1.44–18.56), adjusted for age, sex, preoperative

Table 3 – Blood transfusion and postoperative atrial fibrillation.

	OR	95% CI	P-value
Univariable			
Homologous transfusion	1.13	0.49–2.62	0.77
Autologous units recovered	0.75	0.45–1.11	0.22
Multivariable			
Homologous transfusion	1.02	0.40–2.61	0.96
Age	1.04	1.01–1.08	**0.02**
Male	0.52	0.16–1.73	0.29
Weight	1.01	0.98–1.03	0.63
Preoperative hemoglobin	1.00	0.83–1.20	0.99

Logistic regression analyses were conducted. Covariates included in the multivariable analyses were selected based on biological plausibility (age) and baseline group imbalances (others).
OR: odds ratio; CI: confidence interval.

Table 4 – Simultaneous evaluation of homologous transfusion and autologous transfusion regarding the odds of post-operative atrial fibrillation.

	OR	95% CI	P-value
Homologous transfusion	2.51	1.18–5.65	**0.02**
Autologous units salvaged	0.66	0.39–1.01	0.09
Preoperative hemoglobin	0.99	0.85–1.16	0.92

A multivariable logistic regression analysis was conducted to evaluate the mutual influence of homologous and autologous blood transfusion, adjusted for baseline preoperative hemoglobin.
OR: odds ratio; CI: confidence interval.

hemoglobin, and body weight (Table 5). Transfusion at any time point (intraoperative, ICU, or ward) was also associated with an increased risk of death (HR: 5.15; 95% CI: 1.16–22.94).

Both a higher volume of autologous blood recovered and the use of homologous blood transfusion were associated with an increased risk of death. The number of autologous units recovered was associated with a higher risk of mortality (HR: 1.83; 95% CI: 1.39–2.41), as was older age (HR: 1.04; 95% CI: 1.01–1.08). Conversely, higher preoperative hemoglobin levels were associated with a reduced risk of death (HR: 0.80; 95% CI: 0.67–0.96) (Table 6). Patients who needed homologous blood transfusion had lower survival (log-rank - p-value <0.01, Figure 1).

Discussion

The transfusion-related findings in this study underscore the urgent need to implement Patient Blood Management, as emphasized by the World Health Organization (WHO) in 2021. The WHO highlighted that anemia has a global prevalence,

Table 5 – Homologous blood transfusion and mortality.

	HR	95% CI	P-value
Univariable			
Intraoperative homologous transfusion	5.67	1.64 – 19.62	**<0.01**
Any homologous transfusion	5.89	1.35–25.65	**0.02**
Multivariable			
Intraoperative homologous transfusion	5.17	1.44–18.56	**0.01**
Age	1.03	0.99–1.07	0.11
Male	1.29	0.44–3.74	0.64
Weight	0.99	0.96–1.02	0.71
Baseline hemoglobin	0.90	0.76–1.07	0.25
Any homologous transfusion	5.15	1.16–22.94	**0.03**
Age	1.03	0.99–1.07	0.11
Male	1.14	0.39–3.29	0.81
Weight	0.99	0.96–1.03	0.74
Baseline hemoglobin	0.88	0.74–1.05	0.16

Cox proportional odds regression analyses were conducted. Covariates included in the multivariable analyses were selected based on biological plausibility (age) and baseline group imbalances (others). Analyses were conducted for patients who received homologous blood products in the operating room (intraoperative) and at any time during hospital stay (any), including operating room, intensive care. and ward.
HR: hazard ratio; CI: confidence interval.

Table 6 – Autologous units salvaged and mortality.

	HR	95% CI	P-value
Univariable			
Salvaged units	1.46	1.19–1.80	**<0.01**
Multivariable			
Salvaged units	1.83	1.39–2.41	**<0.01**
Age	1.03	1.00–1.07	**0.04**
Male	1.07	0.36–3.13	0.90
Weight	0.98	0.96–1.02	0.34
Baseline hemoglobin	0.80	0.67–0.96	**0.01**

Cox proportional odds regression analyses were conducted. Covariates included in the multivariable analyses were selected based on biological plausibility (age) and baseline group imbalances (others).
HR: hazard ratio; 95% CI: confidence interval.

affecting billions of individuals worldwide. Preoperative anemia in surgical patients is more prevalent than in the general population and is associated with significantly increased morbidity, mortality, and hospital length of stay across most surgical procedures [22].

When comparing baseline characteristics between groups, preoperative hematocrit, hemoglobin, and platelet levels indicated that the group requiring homologous blood transfusions already presented with anemia at admission (hematocrit median = 37%; IQR: 8.755 and hemoglobin median = 12 g/dL; IQR: 3.2 g/dL; p-value <0.001). In this study, the risk-adjusted impact of preoperative hematocrit on morbidity and mortality is consistent with prior publications in cardiac surgery patients. Multiple studies have demonstrated a significant correlation between preoperative or intraoperative anemia and adverse cardiac surgical outcomes [23–25].

LaPar et al. investigated the relative contribution of preoperative hematocrit and packed red blood cell (PRBC) transfusion to postoperative outcomes following CABG. In this multi-institutional analysis of over 33,000 patient records, the key findings showed that PRBC transfusions had a greater impact than preoperative hematocrit on postoperative mortality, stroke, and renal failure [26]. Therefore, transfusion may be more of a marker of a critically ill patient than the direct cause of death.

In the present cohort, the anemic group also had significantly lower platelet counts (p-value = 0.047), which may explain the higher incidence of reported 'increased intraoperative bleeding' (20 patients – 10.2%), the need for homologous blood components in the operating room (158 patients – 80.6%), and the higher volume of recovered autologous red blood cells (median: 505 mL; IQR: 365.75 mL - p-value <0.001). In contrast, this complication was reported in only two patients (1.2%) in the non-transfused group.

Some authors have suggested that perioperative blood transfusions may induce systemic inflammatory changes, potentially increasing the likelihood of developing POAF in transfused patients during cardiac surgery [14,27,28]. However, the findings of this study are supported by a previous study that showed that intraoperative autologous blood transfusion was not associated with POAF (OR: 1.13; 95% CI: 0.49–2.62), and the number of autologous blood units recovered was also not associated with increased risk of POAF (OR: 0.75; 95% CI: 0.45–1.11) [29]. These results remained

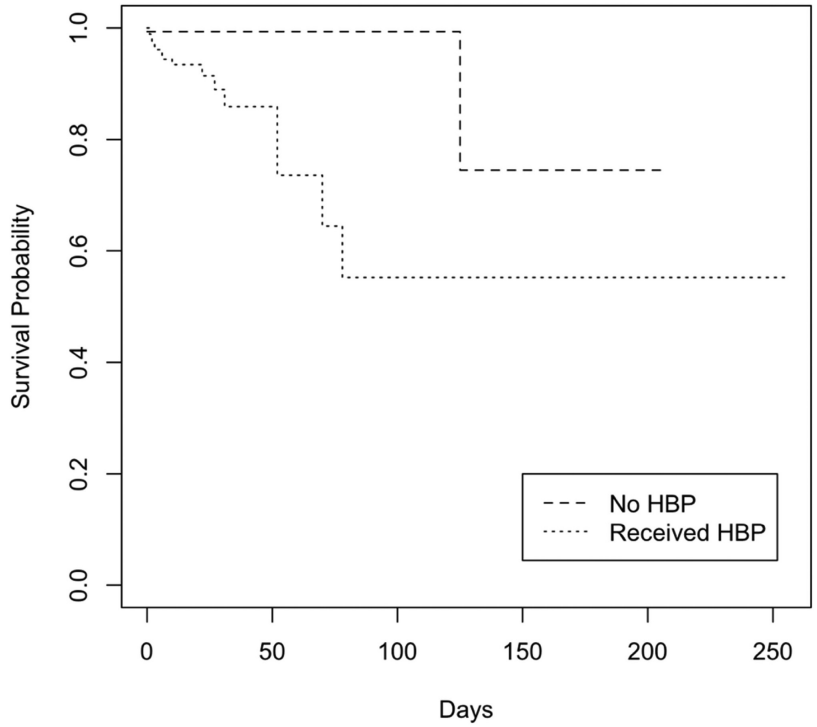

Fig. 1 – Survival analysis stratified according to use of homologous blood products Patients who received homologous blood products (HBP) had lower survival (log-rank - p-value <0.01).

consistent after adjustment for age, weight, sex, and preoperative hemoglobin level.

Limitations

The main limitations of this study are its observational, non-randomized design and the relatively small patient cohort. As a multi-institutional study involving diverse surgical, anesthetic, perfusion, and intensive care teams, the results should be interpreted with caution due to the high degree of inter-team heterogeneity. Furthermore, the clinical significance and long-term implications of POAF in cardiac surgery were not evaluated.

Conclusion

The findings of this multi-institutional retrospective cohort study of cardiac surgical patients indicate no association between POAF, intraoperative autologous blood recovery, and blood transfusion. The only factor associated with POAF was advanced age. Preoperative anemia and blood transfusion independently increased the risk of postoperative morbidity and mortality. Urgent implementation of patient blood management strategies is warranted to improve surgical outcomes.

Ethical considerations

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The author declares no conflicts of interest.

Editor

Eduardo Rego

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Evaluating the treatment of patients with paroxysmal nocturnal hemoglobinuria in Brazil: disparities in access and treatment persistence

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ARTICLE INFO

Article history:
Received 13 February 2025
Accepted 18 March 2026
Available online 27 April 2026

Keywords:
Paroxysmal Nocturnal hemoglobinuria
Eculizumab
Treatment persistence
Geographic access
Quality of life

ABSTRACT

Background: Paroxysmal nocturnal hemoglobinuria is a rare, life-threatening disease that significantly affects quality of life. Despite therapeutic advances, challenges like treatment burden and limited care access persist. This study aimed to evaluate the geographic distribution of paroxysmal nocturnal hemoglobinuria patients in Brazil and explore how travel distance to treatment centers may influence treatment persistence.

Methods: A mixed-methods study was conducted combining quantitative analysis of administrative claims data from the Brazilian Unified Health System and qualitative insights from patient focus groups. The quantitative component included patients with at least one ICD-10 D59.5 code between January 2010 and December 2023. The qualitative component involved a focus group of adult paroxysmal nocturnal hemoglobinuria patients registered with the Brazilian Lymphoma and Leukemia Association (ABRALE). Descriptive statistics were used to summarize demographic, geographic, and treatment-related data.

Results: A total of 769 patients were identified, with 42.4% receiving eculizumab. The mean time to treatment initiation was 172.9 days. Spatial analysis revealed substantial regional variations, with eculizumab-treated patients traveling an average of 87.5 km for care. Persistence analysis showed that 21.2% and 61.0% of patients were non-persistent based using two different methodological approaches. Focus group participants reported limited disease awareness, long travel distances, and extended waiting times, all of which negatively impacted their daily lives and treatment adherence.

Conclusion: This study highlights geographic disparities in access to paroxysmal nocturnal hemoglobinuria treatment in Brazil and suggests that the travel burden may contribute to

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https://doi.org/10.1016/j.htct.2026.106459

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treatment non-persistence. These findings underscore the need for more accessible and patient-centered therapeutic options to promote equitable healthcare delivery.

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Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare disease, with a global prevalence ranging from 1-5 cases per million individuals [1]. PNH is typically diagnosed in adults aged 30 to 40 years. It can occur as a primary condition or concurrently with other bone marrow disorders, most commonly aplastic anemia and myelodysplastic syndromes [2].

Some authors suggest that PNH is underestimated because of low diagnosis rates in individuals with limited symptomatology [3]. The main clinical findings include hemolysis, thrombosis, and bone marrow disorders such as aplastic anemia or myelodysplastic syndromes [4]. Some patients classified as having PNH may have small clones that do not require treatment. However, patients commonly experience chronic fatigue, abdominal pain, and other symptoms that significantly impact their quality of life [4,5].

Currently, several complement inhibitors are approved, each targeting distinct components of the complement cascade. These include terminal pathway inhibitors directed at component 5 (C5) - such as ravulizumab, eculizumab, and crovalimab - as well as proximal or alternative pathway inhibitors targeting C3 (pegcetacoplan), factor B (iptacopan), and factor D (danicopan). These agents also vary in their routes of administration and dosing schedules [6].

Eculizumab was the first approved treatment for patients with PNH [7]. In Brazil, it was approved in 2017 [7], recommended for reimbursement in the Public Healthcare System (SUS) in 2018, and implemented in 2022 [8]. According to PNH Clinical Protocols and Treatment Guidelines (PCDT), the eculizumab regimen includes an initial and maintenance phases. During the initial phase, eculizumab is administered as an intravenous infusion once a week for the first four weeks. This is followed by the maintenance phase with infusions every two weeks [9].

Ravulizumab, a new C5 inhibitor, was approved by the Brazilian National Agency for Sanitary Surveillance in 2019, incorporated into the public system in 2024, and awaits the publication of the new PCDT to make it available for prescription in public services [10].

Brazil faces disparities in access to care [11]. In a country of continental proportions, the distance to care is likely a significant contributor to health inequalities [12]. However, there are no data describing the impact of demographic characteristics on the treatment of PNH patients in the Brazilian public health system.

Objective

This study aimed to assess geographic features and the regional distribution of PNH patients in Brazil. Additionally, it analyzed the distance PNH patients traveled to receive eculizumab treatment in infusion centers and analyzed this travel burden with treatment persistence.

Methods

The study used a mixed-methods approach, incorporating both quantitative and qualitative methodologies. It consisted of two parts: a retrospective database analysis and a focus group study.

Study design and database

The quantitative component was a retrospective analysis of claims databases from the Department of Informatics of the Brazilian Unified Health System (DATASUS) with data extracted from the Hospital Admissions Information System (HAIS) and Outpatient Procedures Information System (OPIS).

The databases of HAIS and OPIS are not linked by a unique identifier; therefore, a probabilistic record linkage was used in this analysis. This linkage used different combinations of patient information from both databases as has been used previously; these included the date of birth, sex, race, city, and postal code [13].

Study population

Patients were identified by the presence of at least one claim with the ICD-10 code D59.5 (PNH) of the International Classification of Diseases, 10th edition (ICD-10) in either the HAIS or OPIS from January 1st, 2010, to December 31st, 2023. The index date was defined as the date of the first ICD code for PNH.

Patients were classified as not treated with eculizumab if they did not have a High Complexity Procedures Authorization record for the drug during the study period (2010–2023), despite having at least one ICD-10 code D59.5. This absence was interpreted as a true lack of treatment within the SUS system, rather than as missing data.

Treatment duration

Time from the index date to treatment initiation was calculated in days. For patients treated with eculizumab, initiation was defined as the date of first eculizumab record. For patients not treated with eculizumab, it was defined as the first PNH-related procedure, that is, any medical procedures associated with PNH management and recorded under the ICD-10 code D59.5, including transfusions and diagnostic procedures. Treatment duration was calculated from the first to

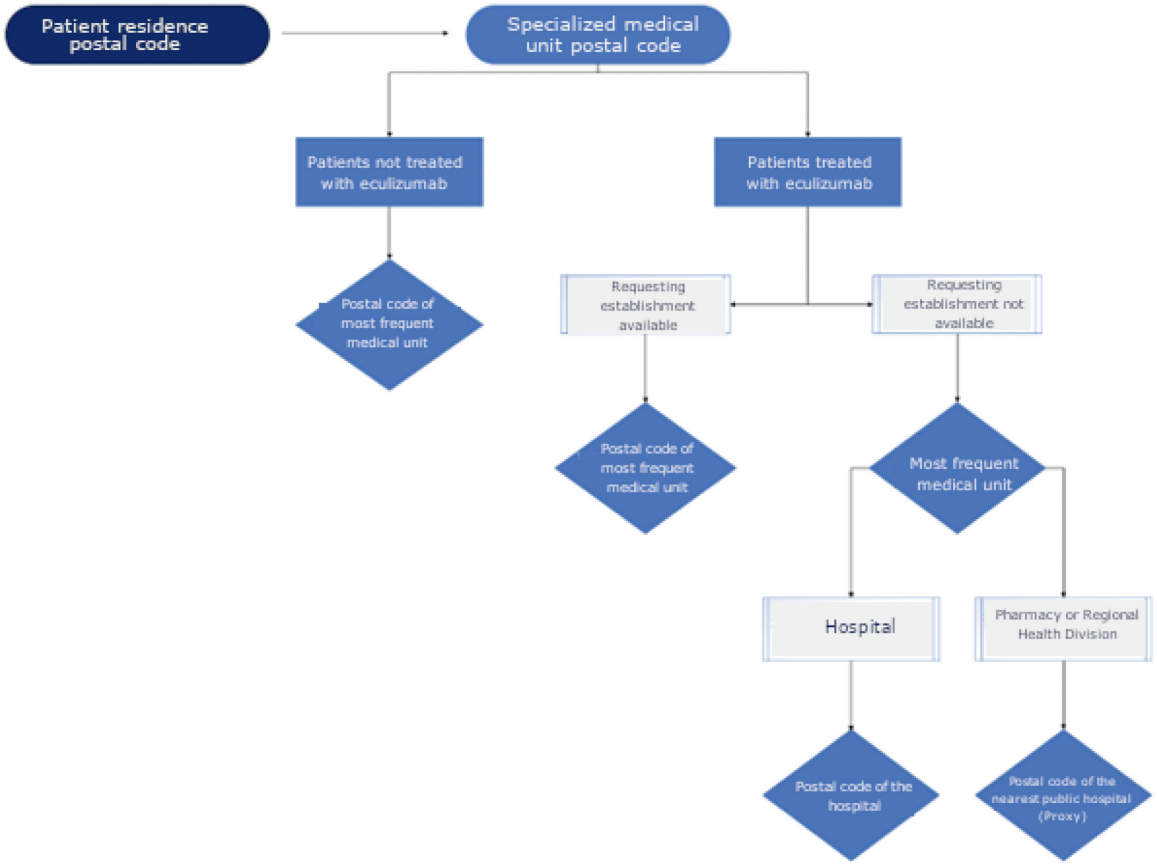

Fig. 1 – Analysis of the distance between the patient's residence and the treatment unit. The flowchart shows the criteria used for measuring the distance according to postal code of the patient's residence and the specialized unit, obtained from databases of the Department of Informatics of the Brazilian Unified Health System (DATASUS).
Note: *If the patient presented discrepant postal code information, the most frequent one was considered.

the last record of eculizumab infusion or PNH-related procedure, respectively.

Distance analysis

The distance between the patient's residence using the postal code and specialized medical units for treatment was calculated by the Google Maps application program interface (Figure 1). Google Maps uses satellite and aerial imagery to calculate the distance between two points. If postal code information was unavailable, the distance was not measured.

For patients not treated with eculizumab, the distance was calculated between the patient's residence and the most frequently specialized medical unit for treatment.

For patients treated with eculizumab, the distance was calculated from the patient's residence to the requesting establishment for eculizumab treatment. If this information was unavailable, the most frequently recorded specialized medical unit was used. When this unit was a hospital, its postal code was used directly. If it was a pharmacy or a regional health division, facilities that do not provide infusion services, the postal code of the nearest public hospital (general or specialized in hemotherapy) was used as a proxy. This approach ensured that distance calculations reflected access to clinically meaningful treatment settings.

Eculizumab persistence analysis

The persistence analysis was measured for patients who received eculizumab treatment based on two approaches:

Approach A: monthly consistency

Each patient was followed from the first to the last eculizumab record or the end of the study period, whichever came first. Patients were classified as 'persistent' if they had at least one eculizumab record in each calendar month during the follow-up. If there was any month without a recorded infusion, the patient was classified as 'non-persistent'. This approach reflects monthly treatment continuity rather than average treatment intervals.

Approach B: infusion interval consistency

This method calculated the average interval between the first and last eculizumab records by dividing the total time span

by the number of infusions. Patients were classified as 'persistent' if the mean interval between infusions was less than or equal to 15 days, consistent with the recommended maintenance dosing schedule. If the average interval exceeded 15 days, they were considered 'non-persistent'.

In both approaches, the date of eculizumab records were extracted from the High Complexity Procedures Authorization records.

Data analysis

All eligible records were analyzed; therefore, no sample size calculation was performed. This study was based on the entire population registered in DATASUS. Continuous variables were described using central tendency and dispersion measures, and categorical variables were expressed as absolute numbers and frequencies. Missing data were reported as such, and no extra data imputation was performed. Statistical analysis was performed using Python version 3.11.0.

Follow-up time was calculated from the index date (first ICD-10 D59.5 claim) to the last available record in the database. Treatment duration was calculated independently, based on the first and last eculizumab infusion records, which may precede or extend beyond the index date.

Focus group

The focus group was part of an independent initiative conducted by ABRALE. The inclusion criteria for participants in the focus group were: 1) being registered in ABRALE's contact system; 2) having a diagnosis of PNH; 3) agreeing to participate by signing the informed consent form; and 4) being over 18 years old. Patients were excluded if: 1) they had passed away, or 2) they had incomplete registration data.

The focus group involved six patients with PNH from different states in Brazil. This meeting was held virtually using the Microsoft Teams platform. Patients were invited via phone contact, with the informed consent form being sent to them. The group was conducted by a researcher from ABRALE following a structured script prepared in Microsoft Word. The questions aimed to explore patients' perceptions and experiences regarding the impact of the disease, the process of accessing treatment, their knowledge about the condition, and the challenges faced in their treatment. The responses were analyzed through discourse analysis and tabulated in Microsoft Excel.

Ethical considerations

The quantitative part of this study was conducted based on the administrative claims databases from DATASUS, which is publicly made available by the Brazilian Ministry of Health. The focus group conducted by ABRALE was submitted and approved by the ethics committee of ABRALE (CAAE: 80532324.0.0000.0071). This study was conducted in compliance with local laws and regulations.

Results

A total of 769 individuals with at least one claim of ICD-10 D59.5 were identified in the database from 2010-2023. The number of PNH cases reported for each year of the study period is summarized in Figure 2.

The year with the most PNH diagnostic records was 2023 with 158 (20.5%) cases.

Of the identified PNH patients (n = 769), the median age at index date was 41.0 (interquartile range [IQR]: 27.0) years old. There was a slight predominance of females (50.3%). Most of the population was White (50.1%) and lived in the southeastern region of Brazil (39.0%). The mean follow-up time for patients was 1.1 (standard deviation [SD]: 2.0) years. Demographic characteristics are summarized in Table 1.

A total of 326 (42.4%) PNH patients underwent treatment with eculizumab. Patients had a median age of 43.0 (IQR: 25.0) years old at the index date. The majority were women (52.5%) and nearly half identified themselves as White (49.8%), with the predominant region of residence being the Northeast (33.7%) of Brazil. The average follow-up time for patients who received eculizumab was 0.7 (SD: 1.4) years (Table 1).

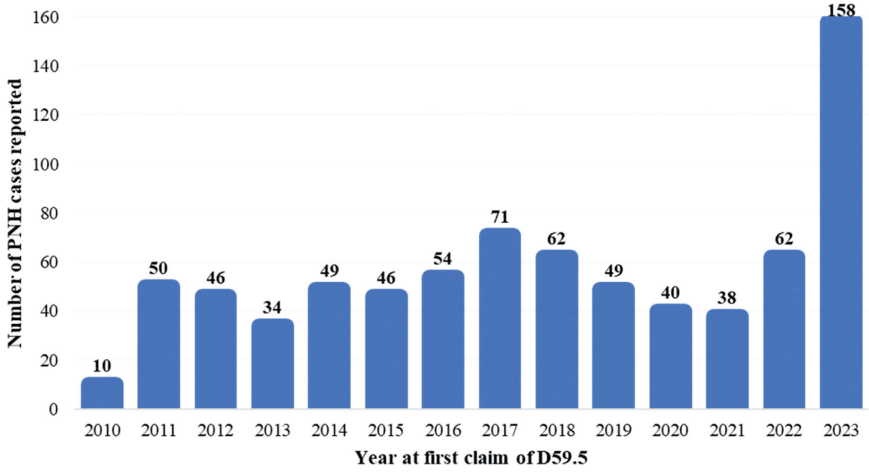

Fig. 2 – Paroxysmal nocturnal hemoglobinuria cases reported for each year in the study period.

Table 1 – Demographic characteristics of paroxysmal nocturnal hemoglobinuria patients.

Characteristic	Total	Treatment	
		Eculizumab (at any time)	Without eculizumab
n (%)	**769 (100)**	**326 (42.4)**	**443 (57.6)**
Age at first claim (index date) - years			
Valid - n (%)	**769 (100)**	**326 (42.4)**	**443 (57.6)**
Median (min - max)	41.0 (1.0 - 92.0)	43.0 (11.0 - 92.0)	38.0 (1.0 - 92.0)
IQR (Q3-Q1)	27.0 (56.0 - 29.0)	25.0 (57.0 - 32.0)	28.0 (55.0 - 27.0)
Gender – n (%)			
valid	**769 (100)**	**326 (42.4)**	**443 (57.6)**
Female	387 (50.3)	171 (52.5)	216 (48.8)
Male	382 (49.7)	155 (47.5)	227 (51.2)
Race - n (%)			
valid	**685 (100)**	**325 (47.4)**	**360 (52.6)**
White	343 (50.1)	162 (49.8)	181 (50.3)
Brown	44 (6.4)	23 (7.1)	21 (5.8)
Black	274 (40.0)	132 (40.6)	142 (39.4)
Yellow	24 (3.5)	8 (2.5)	16 (4.4)
Region of residence - n (%)			
valid	**769 (100)**	**326 (42.4)**	**443 (57.6)**
Southeast	300 (39.0)	93 (28.5)	207 (46.7)
Northeast	203 (26.4)	110 (33.7)	93 (21.0)
South	145 (18.9)	70 (21.5)	75 (16.9)
Midwest	73 (9.5)	34 (10.4)	39 (8.8)
North	48 (6.2)	19 (5.8)	29 (6.5)
Follow-up time*, years			
valid n (%)	**769 (100)**	**326 (42.4)**	**443 (57.6)**
Mean (SD)	1.1 (2.0)	0.7 (1.4)	1.3 (2.2)
Median (min - max)	0.3 (0.0 - 12.3)	0.3 (0.0 - 10.2)	0.2 (0.0 - 12.3)
IQR (Q3 – Q1)	0.8 (0.8 - 0.0)	0.4 (0.5 - 0.1)	1.6 (1.6 - 0.0)

SD: standard deviation; Min: minimum value; Max: maximum value; IQR: interquartile range.

* Time since index date (ICD-10) up to the last date of available patient information in the database.

Patient treatment

The mean time from the index date to the start of PNH treatment was 191.6 (SD: 389.4) days. For patients treated with eculizumab, the mean time was 172.9 (SD: 372.5) days. The mean PNH treatment duration of the entire cohort was 554.0 (SD: 900.7) days. Of the patients treated with eculizumab, the mean treatment duration was 628.8 (SD: 999.5) days (Table 2).

Table 2 – Characteristics of patient's treatment.

	Total	Treatment	
		Eculizumab (at any time)	Without eculizumab
Patients - n (%)	**769 (100)**	**326 (42.4)**	**443 (57.6)**
Time from the index date until PNH treatment (days)			
valid - n	**369**	**236**	**133**
Mean (SD)	191.6 (389.4)	172.9 (372.5)	263.6 (436.7)
Duration of PNH treatment (days)			
valid - n	**369**	**236**	**133**
Mean (SD)	554.0 (900.7)	628.8 (999.5)*	464.9 (504.1)

SD: standard deviation.

* Treatment duration reflects the span between first and last eculizumab infusion, which may not align with follow-up time.

Distance analysis

Of the 769 PNH patients, the distance from the patient's residence to specialized medical units was measured for 677 (88.0%) patients. For patients not receiving eculizumab (n = 363), the distance was calculated based on the specialized medical unit they visited most frequently. For patients treated with eculizumab (n = 314), the location was defined by the requesting establishment for 38 (12.1%) patients and by the most frequent specialized medical unit for the remaining 276 (87.9%).

The spatial analysis revealed significant variability in the distance analysis, with a mean distance of 82.5 (SD: 225.0) km. Patients residing in the northern region had to travel an average of 149.1 (SD: 361.4) km. Those living in the southern, midwestern, northeastern, and southeastern regions traveled 116.4 (357.2) km, 83.0 (113.3) km, 73.2 (180.8) km, and 62.7 (151.2), respectively (Table 3).

Among patients treated with eculizumab (at any time), the average distance traveled was 87.5 (265.2) km. The average distance varied from 52.6 (63.4) km in the southeastern region, 61.7 (82.2) in the Midwest, 64.5 km (216.8 km) in the Northeast, 156.8 km (468.4 km) in the South, to 176.4 (253.8) km in the northern region of the country (Table 3).

Table 3 – Evaluation of the distances from the patient's residence to specialized medical units according to eculizumab treatment.

	Total	Treatment	
		Eculizumab at any time	Without eculizumab
Total cases			
Valid – n (%)	677 (100)	314 (46.4)	363 (53.6)
Distance (km) - Mean (SD)	82.5 (225.0)	87.5 (265.2)	78.2 (183.0)
Region of residence: North			
Valid – n	42	18	24
Distance (km) - Mean (SD)	149.1 (361.4)	176.4 (253.8)	125.8 (438.3)
Region of residence: Northeast			
Valid – n	181	102	79
Distance (km) - Mean (SD)	73.2 (180.8)	64.5 (216.8)	84.4 (119.6)
Region of residence: Midwest			
Valid – n	65	35	30
Distance (km) - Mean (SD)	83.0 (113.3)	61.7 (82.2)	107.8 (138.6)
Region of residence: South			
Valid – n	127	69	58
Distance (km) - Mean (SD)	116.4 (357.2)	156.8 (468.4)	67.4 (117.7)
Region of residence: Southeast			
Valid – n	262	90	172
Distance (km) - Mean (SD)	62.7 (151.2)	52.6 (63.4)	68.0 (180.9)

SD: standard deviation; km: kilometer.

Eculizumab persistence analysis

Among eculizumab users (n = 326), in the patient follow-up approach, 257 (78.8%) PNH patients were classified as 'persistent' and 69 (21.2%) as 'non-persistent.' Considering the interval between the first and last eculizumab records, most patients (n = 199; 61.0%) were classified as 'non-persistent' (Table 4).

Focus group: Results of the paroxysmal nocturnal hemoglobinuria patient experience

Patients in the focus group reported being unaware of PNH before being diagnosed, often receiving a diagnosis after presenting with complications. Although some patients are diagnosed after the first symptoms, some cases can take months between the first symptoms and a correct diagnosis.

> **Patient 2:** "*I was surprised when I found out I had it, and I hadn't sought medical help for PNH. I sought help because I had a liver issue and had a stroke. I had to undergo a liver transplant and was left with this sequel from the stroke. [...] I underwent the transplant, only to then discover the disease.*"

> **Patient 5:** "*I had the first symptoms, but I was diagnosed with bone marrow aplasia. From there, I had relapses, I had a stroke. In 2005, I underwent treatment with thymoglobulin. [. . .] it was then that the hematologist and researcher at the clinic said, 'let's do a test just to clear my doubt.' When he did in 2008, it turned out to be PNH.*"

Table 4 – Eculizumab persistence analysis.

Approach	Eculizumab users (n = 326)	
	Persistent	Non-persistent
Patient follow-up	257 (78.8%)	69 (21.2%)
Interval between first and last eculizumab records	127 (39.0%)	199 (61.0%)

Treatment with eculizumab significantly affects daily activities and professional life. Patients reported losing a significant part of the day to treatment, especially when they had to undergo treatment every two weeks, impacting both employment and quality of life.

> **Patient 4:** "*In my case, it affected me a lot. I am unemployed, but it did impact me because I did the afternoon [treatment] and ended up losing the whole afternoon. And there was also a time when I did the medication pickup, which was on another day.*"

The distance to the treatment center varies among patients. Transportation is one of the difficulties faced, with many patients reporting using public transport, which can be slow and tiring.

> **Patient 1**: "*Generally, I go by bus, but sometimes I also go with my daughter.*"

One of the main difficulties mentioned is the time spent at the treatment center, with long periods of waiting to receive the infusion. Additionally, the need to leave home very early for treatment sessions was highlighted as a constant challenge.

> **Patient 3:** "*The travel time, we know patients who practically leave the night before to get treatment and arrive the next morning, stay all day, then sometimes return the day after. As it's treatment in a public hospital, sometimes we wait a long time there, so imagine a person who works, who needs to be present at work and whose manager won't understand all this every 2 weeks. The infusion time, the travel, and the time we spend at the hospital unit, are the challenges.*"

Patient 5: "*Besides the time you spend. I think today we are very repetitive. The tests, the paperwork to get the medication exemption and then there's a year I have to pay to do it. There's a disease that has no cure, but every six months I have to be there paying and doing new tests to prove that I have it. For me, I think that's harder.*"

The responses of the patients regarding the impact of PNH on their lives ranged from fear to hope. Many expressed hopes for new medications that could simplify treatment, reducing the need for frequent infusions. Others mentioned gratitude for being alive and expecting improvements in available treatments.

Patient 5: "*New medications emerging to stop infusing every 15 days*"

Patient 6: "*I really believe new medications will come, that a lot of genetic therapy projects are being studied. We might be alive to see it or not, but I hope there is a cure waiting for us in the future.*"

Discussion

The findings of this study highlight significant disparities in access to treatment for PNH in SUS. Analysis revealed that patients travel, on average, 82 km to receive eculizumab treatment with notable regional variations. For example, patients in the northern region traveled an average of 149 km, compared to 62 km in the Southeast. These findings reflect geographic inequities in access to specialized care and may contribute to treatment discontinuation, particularly in underserved regions. These results are consistent with previous studies that have shown that geographic barriers can significantly impact access to high-complexity treatments in Brazil [14].

This study identified 769 individuals with at least one claim of ICD-10 D59.5 between 2010 and 2023. Most PNH patients (n = 158; 20.5%) were reported in 2023, which may be attributed to the availability of eculizumab in SUS from September 2022 [8]. This policy change may have led to increased awareness among healthcare providers, improved diagnostic efforts, and more frequent coding of PNH in administrative databases. Additionally, the availability of a high-cost treatment like eculizumab may have encouraged more accurate documentation and reporting of PNH cases to ensure access to therapy.

The spatial analysis demonstrated that patients residing in regions with lower Human Development Index (HDI) scores were required to travel significantly longer distances to access specialized treatment centers. This pattern aligns with prior studies highlighting regional disparities in healthcare accessibility across Brazil [15]. Beyond geographic challenges, patients also reported additional barriers such as inadequate transportation infrastructure, financial hardship, and delays in receiving medical attention. Collectively, these factors may contribute to reduced adherence and the high rates of non-persistence observed among eculizumab-treated patients.

Among eculizumab users, 69 (21.2%) and 199 (61.0%) of PNH patients were classified as 'non-persistent' according to the first and second approaches of persistence analysis, respectively. These findings reflect a substantial burden of treatment discontinuation and are consistent with prior research indicating that the economic burden of treatment, including direct medical costs and productivity loss, can negatively impact adherence [16]. Furthermore, previous studies have identified infusion frequency as a critical factor influencing both treatment preference and long-term persistence [17,18].

The qualitative findings from the focus group provided additional context to the quantitative data. Patients reported that treatment with eculizumab disrupted their daily routines and professional responsibilities due to long travel times, waiting periods, and the frequency of infusions. These insights are consistent with previous literature on the burden of anti-C5 therapies, which require hospital-based administration and are associated with reduced quality of life and work productivity [19,20]. However, it is important to note that the focus group findings are descriptive and based on a small, self-selected sample. Therefore, while they offer valuable perspectives, the results may be subject to selection bias.

The study should be interpreted in the light of several limitations. First, the analysis was restricted to patients who had at least one recorded procedure or hospitalization in the DATASUS databases. As a result, individuals with PNH who did not access care through the public system or who had no recorded procedures were excluded, potentially underestimating the total number of patients with the disease.

Second, the retrospective design and reliance on administrative claims data introduce inherent limitations, including the possibility of incomplete records, misclassification, and information bias. These challenges are common in studies using secondary data sources and have been documented in similar research [21,22].

Another methodological limitation relates to the calculation of travel distances. Due to differences in data availability, the reference point for distance estimation varied between patient groups. For eculizumab-treated patients, the requesting establishment was prioritized; for others, the most frequently visited specialized unit was used. When these units were pharmacies or regional health divisions, which do not provide infusions, the postal code of the nearest public hospital was used as a proxy. Although standardized rules were applied to minimize bias, this approach may not fully capture the actual care location, and caution is warranted when interpreting geographic comparisons.

Additionally, persistence analysis was limited to patients treated with eculizumab, as this group had structured and consistent infusion data. Non-eculizumab therapies lacked standardized documentation, precluding reliable persistence measurement. While this focus ensures methodological rigor, it may limit the generalizability of findings to the broader PNH population.

The static nature of the dataset also presents a limitation. Although DATASUS is regularly updated by the Brazilian Ministry of Health, the dataset used in this study represents a static snapshot extracted at a specific point in time and was not updated thereafter. As such, it does not reflect

subsequent updates. This may result in underreporting of recent procedures, particularly for patients whose treatment occurred near the end of the observation window, potentially leading to misclassification of persistence status.

Furthermore, the lack of clinical data in the DATASUS database precluded assessment of treatment response, adverse events, or reasons for discontinuation. As such, we could not determine whether non-persistence was due to clinical factors or logistical barriers. Future studies incorporating clinical outcomes are needed to better understand these dynamics.

Finally, although ravulizumab was approved by ANVISA in 2019, it was only incorporated into SUS in 2024. As the focus group was conducted prior to or during its early implementation, none of the participants had transitioned to ravulizumab. This limits the study's ability to assess the potential impact of newer therapies on treatment burden and adherence.

Conclusions

The findings of this study provide a comprehensive overview of the profile of PNH patients in SUS, as well as how variability in distance may highlight potential disparities in access to health care. Non-persistence with eculizumab treatment may be associated with a higher patient burden, potentially causing difficulties in achieving better treatment adherence. The availability of alternative therapies for PNH that could facilitate patient access and ensure equitable healthcare delivery for PNH patients may help overcome the differences.

Authors' contributions

RDC and PMC contributed equally to the development of the manuscript and share first authorship. AB designed the study. ABMA and LPL developed the first draft of the manuscript. LVC was responsible for the linkage and quantitative analysis. NM and FF were responsible for the focal groups. All authors contributed equally to the writing of the final version of the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

Alessandro Bigoni, Ana Beatriz Machado de Almeida, Erica Ferreira, Diego Kashiura, and Felipe Thies are Novartis' employees. Lays Paulino Leonel, and Lucas Vieira Cortez are IQVIA Solutions, Brazil employees. Nina Melo and Fabio Fedozzi are employees of the Brazilian Lymphoma and Leukemia Association, Paula de Melo Campos is an employee of the Universidade Estadual de Campinas, and Rodolfo Delfini Cançado is an employee of the Faculdade de Ciências Médicas da Santa Casa de São Paulo.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

The murine Irs1 knockout model, generated by the spontaneous Irs1^{S57X} mutation in homozygosity, exhibits high embryonic lethality, limiting studies of adult hematopoiesis

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ARTICLE INFO

Article history:
Received 9 January 2026
Accepted 23 March 2026
Available online 27 April 2026

Keywords:
Insulin receptor substrate 1
Knockout mice
Fetal lethality
Hematopoiesis

ABSTRACT

Introduction: Insulin Receptor Substrate 1 (IRS1) is differentially expressed in hematological neoplasms suggesting a role in hematopoiesis and neoplastic transformation. Irs1 knockout mice represent a tool to investigate IRS1 function. This study compared hematological parameters of wild-type and heterozygous Irs1^{S57X} mice and assessed fetal lethality in homozygous mice.

Methods: Hematological parameters were analyzed monthly in wild-type and heterozygous mice from 8 to 22 weeks of age. Successive intercrosses failed to yield homozygous knockouts. Fetal lethality was evaluated through timed matings of heterozygous mice, with genotyping performed at various gestational stages (E9.5, E12.5, E15.5 and E18.5).

Results: Heterozygous mice showed no significant differences in body weight or hematological parameters compared with wild-type mice (all p-value >0.05). Homozygous Irs1^{S57X} mice exhibited fetal or postnatal lethality as fetuses developed until gestational stage E18.5 but were either aborted or died shortly after birth.

Conclusion: The Irs1^{S57X} mutation in heterozygosis does not alter phenotype, whereas homozygosity for Irs1^{S57X} is associated with markedly reduced perinatal survival, precluding adult hematopoiesis studies. Future research should focus on fetal hematopoiesis.

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https://doi.org/10.1016/j.htct.2026.106465

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Introduction

The insulin receptor substrate (IRS) family encompasses six cytoplasmic adaptors that regulate numerous cellular processes such as growth, metabolism, survival, and proliferation [1]. Insulin Receptor Substrate 1 (IRS1) is differentially expressed in hematological neoplasms, suggesting its participation in hematopoiesis and neoplastic transformation [2–5]. IRS1 acts as a central adaptor in signal transduction downstream of the insulin and Insulin-like growth factor 1 (IGF1R/IR) receptors, promoting activation of the phosphoinositide 3-kinase (PI3K)-Protein kinase B (AKT)-Mechanistic target of rapamycin (mTOR) signaling cascade [1,6]. Upon ligand binding, IRS1 is phosphorylated on tyrosine residues, creating docking sites for the p85 regulatory subunit of PI3K, leading to PIP3 production and the ensuing recruitment and activation of AKT. Subsequent activation of mTOR, particularly through the mTORC1 complex, stimulates protein synthesis, cell growth, metabolic reprogramming, and inhibition of apoptosis [1,6]. In hematologic malignancies, hyperactivation of this pathway, driven by IRS1 overexpression, autocrine/paracrine IGF1 stimulation, or upstream genetic alterations, contributes to enhanced proliferation, survival, and therapeutic resistance, as well as to metabolic adaptation and interactions with the bone marrow microenvironment [5,7–9]. Thus, IRS1 not only amplifies mitogenic and anti-apoptotic signals but also integrates metabolic and oncogenic cues, positioning it as a potential therapeutic target and biomarker in leukemias and other hematologic cancers. The role of IRS1 in the BCR::ABL1 signaling pathway was first demonstrated by our research group [1,3,10], however the hypothesis of the participation of IRS1 in normal hematopoiesis has not yet been elucidated.

In this scenario, using a murine Irs1 knockout model represents an interesting tool to evaluate the function of Irs1 in normal hematopoiesis. IRS knockout mice have been extensively used for insulin signaling pathway research, particularly in studies related to diabetes, obesity, and development. Irs1 knockout models were generated and first described by two independent research groups in 1994 [11,12].

B6.129S2-Irs1^{smla} mice carry a spontaneous nonsense mutation in serine 57 (Irs1^{S57X}), which in homozygosis produces a knockout animal for Irs1, characterized by low weight and body size. This mutation arose spontaneously in an embryonic stem (ES) cell subclone during the generation of a *SerpinB2*-targeted line. Following the identification of a recombination event, the mutation was segregated from the *SerpinB2* locus, mapped to a 2.78-Mb proximal interval, and backcrossed onto a C57BL/6 background. The original phenotype included reduced growth, decreased survival, and smaller body size at birth, and the colony has since been maintained under controlled breeding conditions to ensure stable transmission of the Irs1^{S57X} allele [13]. The present study aimed to compare the hematological parameters of wild-type and heterozygous mice for the Irs1^{S57X} mutation and to describe the fetal lethality of homozygous mice.

Material and methods

Animals

The protocols for mouse experiments were approved by the Animal Ethics Committee of the Institution. Mice were maintained on a 12:12-h light-dark cycle and housed at the Laboratory for Experimental Animal Studies located at the Regional Blood Center Medical School, Hemotherapy Center of Ribeirão Preto. B6.129S2-Irs1^{smla}/J mice (Jackson Laboratory, stock number: 007240) in a pure C57BL/6 background (≥10 generations) were mated to produce homozygous animals. Genotyping was performed by Sanger sequencing in an ABI 3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Hematological parameters evaluation

For peripheral blood collection, mice were anesthetized with 2 % isoflurane via inhalation. Blood samples were collected into heparinized tubes and analyzed using an automated hematology analyzer. Evaluated parameters included the white blood cell count (WBC), red blood cell count (RBC), hemoglobin level (Hb), hematocrit (Hct), and platelets. Animals were assessed monthly across four age ranges (8–10, 12–14, 16–18, and 20–22 weeks), with body weight recorded immediately prior to blood collection.

Fetal lethality experiments

Mating was performed between heterozygous mice. Females were followed up daily, in the morning, to check for the presence of a vaginal plug. The time that plug is identified is considered as 0.5 days post coitus (dpc) or E0.5, since coitus occurs at night [14]. Once the plug was found, the females were accommodated separately until the date of euthanasia. Euthanasia of the females was performed on gestational days E9.5 (n = 1), E12.5 (n = 1), E15.5 (n = 2), and E18.5 (n = 2). The fetuses were carefully separated from the maternal material to avoid contamination in the genotyping. Throughout the development of this research, we noticed that the successive mating between Irs1^{smla} heterozygous animals did not result in homozygous mice among the offspring.

Offspring genotyping

For early developmental stages (E9.5 and E12.5), DNA was extracted from the entire fetus, whereas for E15.5 and newborns (E18.5), DNA was obtained from the caudal portion. For extraction, 200–400 μL of NaOH (50 μM) was added to each sample, which was then incubated in a dry bath at 98 °C for 60 min (E9.5 and E12.5) for 80 min (E15.5 and E18.5). Subsequently, 50–100 μL of Tris–HCl (1 M, pH 6.8) was added to neutralize the samples. Genotyping was performed via Sanger sequencing.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software Inc.). Differences between groups were analyzed via two-way ANOVA with Tukey's post-test, while Chi-square analysis was used to determine if offspring ratios deviated from Mendelian expectations. All p-values were two-sided, and the significance level was set at 5 %.

Results

The irs1^{s57x} spontaneous mutation, in heterozygous status, did not modulate hematological parameters and body weight

After 28 intercross events, a total of 101 heterozygotes, 46 wild-type mice and 1 homozygous mouse were obtained, with an average of 4.9 (± 1.6) born alive per female. The absence of homozygous knockout mice in the offspring precluded the inclusion of this genotype in the statistical analysis. The single Irs1 knockout mouse that survived the weaning was euthanized at nine weeks of age for analysis. The Irs1-deficient mouse was male and characterized by low body weight (15.64 *g*; WBC: 6.8 × 10^9/L; RBC: 9.79 × 10^{12}/L; Hb: 16.4 g/dL; Hct: 48.5 % and platelets: 311 × 10^9/L). Wild-type (Irs1$^{WT/WT}$) and heterozygous (Irs1$^{WT/S57X}$) mice were monitored for four months starting at eight weeks of age with body weight and peripheral blood parameters being recorded monthly. On comparing the wild type animals (*n* = 7 females and 4 males) and heterozygotes (*n* = 7 females and 12 males), the body weight and peripheral blood hematological parameters did not differ significantly at any of the ages evaluated (all p-value >0.05) (Figure 1).

Since all matings were performed between heterozygous animals, a Mendelian segregation ratio of 1:2:1 was expected. Considering the total number of offspring obtained from intercrosses (*n* = 148), the expected frequencies would be 37 wild-type, 74 heterozygous, and 37 homozygous mice. However, the observed distribution (46 wild-type, 101 heterozygous, and 1 homozygous) markedly deviated from the expected ratio. Chi-square analysis demonstrated a highly significant deviation from Mendelian proportions (χ^2 = 39.25; df = 2; p-value <0.0001), indicating a marked underrepresentation of homozygous animals at birth.

Homozygous fetuses developed until day E18.5; however, with the exception of a single pup, all were identified as aborted or died immediately after birth

An evaluation of the progeny genotype at different gestational stages was performed to elucidate and prove the lethality of homozygous fetuses. A total of eight breeding pairs were established. The average time for plug identification in the evaluated females was 3.17 ± 0.98 days after the formation of mating partners. The presence of the plug indicates coitus, but does not guarantee pregnancy [15]. Euthanasia of the females was performed on gestational days E9.5 (*n* = 1), E12.5 (*n* = 1), E15.5 (*n* = 2), and E18.5 (*n* = 2). All females evaluated showed a positive plug (Figure 2A); however, one female sacrificed on day E9.5 and another on day E12.5 were not pregnant. Representative images of fetuses in different gestational phases can be seen in Figure 2B In each gestational phase, the total number of fetuses, the number of fetuses per uterine horn, the presence of signals of embryo absorption, and the genotype of the offspring were evaluated (Table 1). A female on E15.5 had only one fetus, with a homozygous genotype with signs of absorption being identified in this female (Figure 2C). Both females that would be sacrificed at E18.5 (at term) were maintained together, since the vaginal plug was identified on the same day. On the morning of the euthanasia day (E18.5), both females had given birth. There were no signs

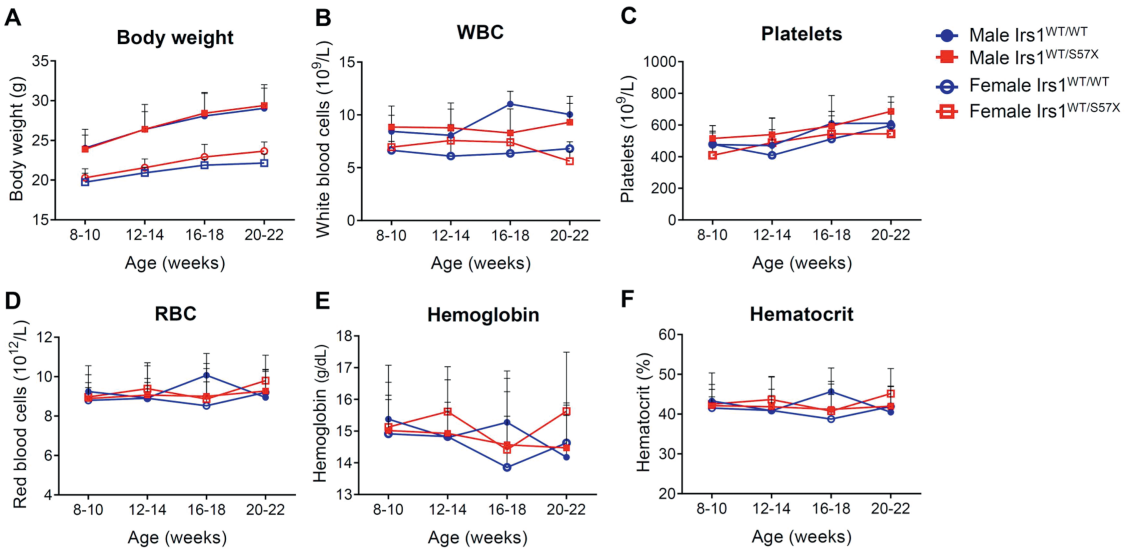

Figure 1 – Body weight and peripheral blood hematological profile in Irs1 mice: serial evaluation. (A) Body weight. (B) White blood cell count (WBC - 10^9/L). (C) Platelets (10^9/L). (D) Red blood cell count (RBC - 10^{12}/L). (E) Hemoglobin level (Hb - g/dL). (F) Hematocrit (Hct - %). Two-way ANOVA test and Tukey post-test; p-values are indicated in the graphs. Irs1$^{WT/WT}$ (wild-type), Irs1$^{WT/S57X}$ (heterozygous).

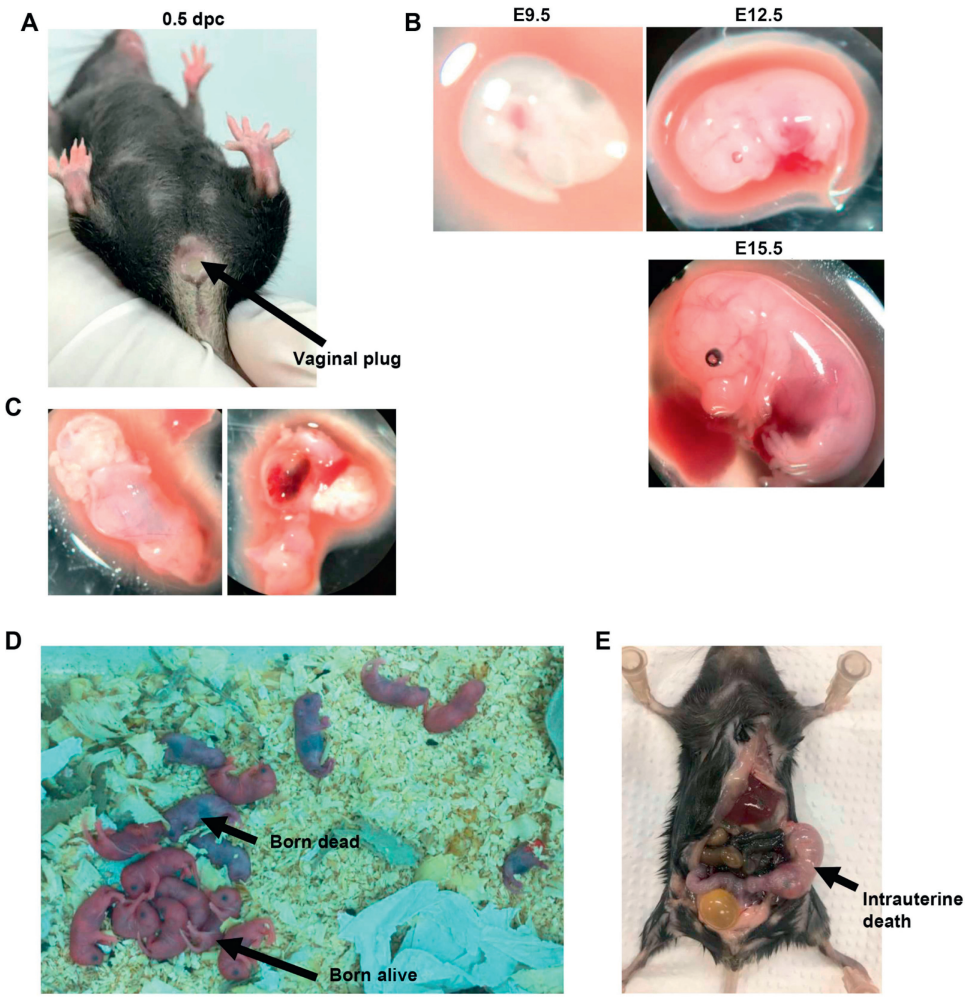

Figure 2 – Evaluation of fetal development in Irs1$^{WT/S57X}$ mice progeny. (A) Appearance of the vaginal plug; the arrow indicates the plug. (B) Visualization of fetuses at different gestational ages (E9.5; E12.5; E15.5); 10X magnification. (C) Evidence of fetal absorption in the uterus of a female at 15.5 days of gestation, 10X magnification. (D) Offspring of two females immediately after birth at E18.5. Both births resulted in one intrauterine death, six dead born and 11 born alive litters. (E) Intrauterine death in a postpartum female; the arrow indicates the fetus in the left uterine horn.

Table 1 – Description of progeny from Irs1$^{WT/S57X}$ crosses at different gestational stages.

Gestational age	E9.5	E12.5	E15.5 #1	E15.5 #2
Fetuses	9	10	7	1
Right horn	2	5	4	1
Left horn	7	5	3	0
Absorption signs	no	no	no	Yes
Collected fetuses	6	10	7	1
Genotype				
Irs1$^{WT/WT}$	3	4	3	0
Irs1$^{WT/S57X}$	3	2	3	0
Irs1$^{S57X/S57X}$	0	4	1	1

D: day
$^{WT/WT}$: Wild-type
$^{WT/S57X}$: heterozygosis.
$^{S57X/S57X}$: homozygous (Irs1 knockout phenotype).

of breastfeeding of litters, indicating that the birth had occurred a few hours previously. Both deliveries resulted in 17 puppies, including 11 born alive and 6 aborted/died after birth (Figure 2D). After euthanasia, the females were dissected for verification, and one of them had a dead fetus in the left uterine horn (Figure 2E). The genotypes of the litters are described in Table 2.

Discussion

Irs1-deficient mice are characterized by a reduction in growth, impaired glucose tolerance, and a decrease in glucose uptake [11,12]. In this study, the spontaneous truncated homozygosis mutation Irs1^{S57X}, on a pure C57BL/6 genetic background, was associated with high fetal or postnatal mortality. In heterozygosis, the S57X mutation did not result in phenotypic changes. In contrast to these findings, Selman et al. described an increased lifespan in Irs1 null females compared to wild-

Table 2 – Characterization of newborn offspring from Irs1$^{WT/S57X}$ crosses.

D18.5 *n* = 2 females	Newborn mice	Irs1$^{WT/WT}$	Genotype Irs1$^{WT/S57X}$	Irs1$^{S57X/S57X}$
Born alive	11	6	4	1
Born dead	6	0	0	6
Intrauterine death	1	0	1	0

D: day.
$^{WT/WT}$: Wild-type.
$^{WT/S57X}$: heterozygosis.
$^{S57X/S57X}$: homozygous (Irs1 knockout phenotype).

type and heterozygotes [16]. Irs1-deficient models generated by targeted disruption do not exhibit significant fetal or postnatal lethality [11,12]. These data suggest that the elevated lethality observed in this study may not be directly related to Irs1 deficiency.

High lethality was described in a knockout model for *SerpinB2*, which spontaneously acquired the Irs1^{S57X} mutation, resulting in the total loss of Irs1 protein expression and the *smla* phenotype [13]. Westrick et al. described a background sensitivity in the Irs1^{S57X} model. Mice backcrossed to C57BL/6 J (at N3 and N4 generations) exhibit less than a 2 % survival rate at two weeks of age, unlike mice backcrossed to 129S1/SvImJ [13]. Genetic background has a relevant impact on phenotype alterations in the insulin receptor pathway [17,18].

In classical Mendelian inheritance, heterozygous intercrosses are expected to generate offspring in a 1:2:1 ratio. In the present study, chi-square analysis revealed a highly significant deviation from this expected distribution at birth, with a profound underrepresentation of homozygous Irs1^{S57X} animals. These findings indicate a markedly reduced perinatal survival associated with homozygosity. Although one homozygous animal survived until nine weeks of age, its extreme underrepresentation among offspring and the significant deviation from Mendelian proportions indicate that survival is rare and likely influenced by modifying genetic or environmental factors. Therefore, while complete lethality cannot be assumed, homozygosity for Irs1^{S57X} markedly compromises postnatal viability. Moreover, although the total number of offspring analyzed at birth was sufficient to detect statistical deviation, the number of pregnancies evaluated at specific embryonic stages was limited. Consequently, the precise developmental window responsible for lethality cannot be definitively established and warrants further investigation in larger cohorts.

An evaluation of the progeny of heterozygous pairs showed no significant implantation failure. Homozygous fetuses developed up to day of birth (E18.5), however, they were identified as aborted or died shortly after birth. Length of gestation is strain-dependent and normally lasts 19 to 21 days [15]. Based on these findings, further hematologic studies on B6.129S2-Irs1^{smla}/J mice should focus on fetal hematopoiesis. This study was first developed to investigate the role of Irs1 in hematopoiesis, however, the absence of Irs1 knockout mice limited the expansion of hematological analysis between genotypes. The number of pregnant female mice at different gestational ages was also insufficient to perform statistical analysis to define an average number of genotypes in each stage. The results of the current study allowed the design of a new project for fetal hematopoiesis evaluation, since the Irs1 knockout fetuses develop until day E18.5.

Conclusion

In summary, the Irs1^{S57X} mutation in heterozygous mice does not affect body weight or hematological parameters. In contrast, homozygosity for Irs1S57X is associated with markedly reduced perinatal survival, preventing studies of adult hematopoiesis. The findings indicate that lethality is not due to implantation failure, as homozygous fetuses develop until E18.5. These results highlight the limitations of this model for postnatal hematopoietic research. Future studies should focus on fetal hematopoiesis to elucidate the role of Irs1 in early hematopoietic development.

Ethics approval

The study was approved by Animal Care and Use Committee of the Institution (CEUA/FMRP - Committee on Ethics in the Use of Animals at Ribeirão Preto Medical School).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Conflicts of interest

The authors declare no competing interests.

CRediT authorship contribution statement

Bruna Alves Fenerich: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jaqueline Cristina Fernandes:** Investigation, Formal analysis, Writing – review & editing. **Cleide Lúcia Araújo Silva:** Investigation, Formal analysis, Writing – review & editing. **João Agostinho Machado-Neto:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fabiola Traina:** Writing – original draft,

Resources, Project administration, Funding acquisition, Data curation, Supervision, Conceptualization.

Acknowledgements

This work was supported by the São Paulo Research Foundation (FAPESP) (grants #2017/25068–8, #2017/19864–6, #2013/08135–2 and #2014/50947–7 [Research, Innovation and Dissemination Centers – Cell Therapy Center]), the National Council for Scientific and Technological Development (CNPq) (465539/2014–9 and 408553/2024–3). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors would like to thank the staff of Laboratory Experimental Animal Studies for the contribution to the maintenance of animals.

Editor

Erich Vinicius De Paula

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Original article

Single-nucleotide variants as potential prognostic biomarkers in newly diagnosed multiple myeloma patients

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ARTICLE INFO

Article history:
Received 14 July 2025
Accepted 18 March 2026
Available online 1 May 2026

Keywords:
Multiple myeloma
SNP array
Single-nucleotide variants
Monoclonal gammopathy of uncertain significance
Prognostic biomarkers

ABSTRACT

Introduction: Microarrays enable high-throughput detection of single-nucleotide variants, making them valuable tools in genetic research. The use of this technology in multiple myeloma, a genetically complex malignancy with highly variable outcomes, may facilitate the identification of novel prognostic biomarkers.

Objective: To identify single-nucleotide variants with prognostic value in newly diagnosed multiple myeloma and to evaluate the ability of microarray technology to distinguish multiple myeloma from monoclonal gammopathy of undetermined significance.

Methods: A total of 56 newly diagnosed multiple myeloma and 14 monoclonal gammopathy of undetermined significance patients were retrospectively analyzed using the Infinium Global Screening Array-24 v3.0. Binary discriminant and principal component analyses were employed to identify single-nucleotide variants associated with post-induction response. Kaplan–Meier curves and log-rank tests were used to evaluate overall survival and progression-free survival.

Results: A total of 692 single-nucleotide variants were associated with post-induction response, of which 42 (t-score >4) were the most discriminant. Variants in the *PTPRD, NOTCH4, SH3RF3, DCC*, and *CSMD1* genes were linked to poorer treatment responses: carriers of alternative alleles showed higher partial remission rates (p-value = 0.005) and early relapse (p-value = 0.021). These patients also showed a reduced 5-year overall survival (p-value = 0.008) and shorter progression-free survival (p-value = 0.017). The current cohort exhibited higher minor allele frequencies for *SH3RF3, PTPRD*, and *CSMD1* relative to broader Latin American datasets. Additionally, 13 single-nucleotide variants were multiple myeloma-specific and eight were specific for monoclonal gammopathy of undetermined significance.

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https://doi.org/10.1016/j.htct.2026.106464
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Conclusion: Single-nucleotide variants of the *PTPRD, NOTCH4, SH3RF3, DCC*, and *CSMD1* genes emerge as promising prognostic biomarkers in newly diagnosed multiple myeloma. Microarray-based single-nucleotide variants profiling shows potential for personalized risk stratification, warranting further validation and functional characterization.

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Introduction

Microarrays have become a cornerstone in genomic research, providing a high-throughput platform to explore genetic variations across the genome. This technology enables researchers to study the impact of rare variants on common diseases, identify mutations in causal genes, and explore the link between environmental factors and phenotypes [1]. At present, hundreds of different microarray chip types are available, some containing over a million genetic markers [2]. Microarray platforms have revolutionized tumor cell analysis, offering a powerful tool to detect genome-wide genetic alterations [3]. Particularly, single-nucleotide polymorphism (SNP) arrays allow for the large-scale evaluation of single-nucleotide variants (SNVs), which could reveal new genetic markers with potential prognostic significance in hematological malignancies [3,4].

Multiple myeloma (MM) is a mature B-cell malignancy characterized by the proliferation of abnormal monoclonal plasma cells in the bone marrow and the overproduction of non-functional immunoglobulins or free immunoglobulin light chains [5]. Patients show a variable clinical course, with some cases progressing rapidly, while others have a more favorable outcome with an overall survival greater than ten years. The disease is associated to multiple genetic events that influence different signaling pathways, modifying the biological features of myeloma cells, and determining proliferative and selective advantages [6].

Despite significant advances in understanding MM genomics, the potential of microarray technology to understand disease biology and identify novel biomarkers remains unexplored. Early studies identified candidate genes involved in the transition from monoclonal gammopathy of uncertain significance (MGUS) to MM [7]. For instance, López-Corral et al. showed an increased incidence of copy number aberrations during disease progression [8]. Similarly, Kim et al. found that increased genomic complexity was associated to poor outcomes in patients receiving bortezomib plus melphalan and prednisone therapy [9]. A recent review highlighted the importance of microarray-based approaches for a better understanding of MM complexity and heterogeneity [10].

The present study conducted a large-scale analysis of SNVs in newly diagnosed MM (NDMM) patients to identify variants with potential prognostic implications. Additionally, as a secondary objective, this study assessed the utility of SNP arrays to differentiate between MGUS and MM.

Materials and methods

Study design and patient characteristics

This retrospective study included 56 adult NDMM patients from Uruguay (n = 35) and Argentina (n = 21). Post-induction response (PIR) rates were defined according to the International Myeloma Working Group (IMWG) recommendations [11]. Early relapse (ER) was defined as relapse within 24 months. Clinical characteristics and treatment information for the patients are described in Table 1. Additionally, 14 patients with MGUS were enrolled (Uruguay, n = 12; Argentina, n = 2), of whom 71.4% were female, with a median age of 65.5 years. MGUS subtypes included IgG (64.3%), IgA (28.6%), and IgM (7.1%).

Table 1 – Clinical characteristics of multiple myeloid patients (n = 56).

Clinical characteristic	
Sex - %	
Male	55.4
Female	44.6
Median age (years) - mean (min-max)	57.4 (27–89)
MM subtypes - %	
IgG	57.1
IgA	25.0
LC	14.3
Other	3.6
Risk staging (ISS) - %	
ISS-1	35.7
ISS-2	33.9
ISS-3	30.4
Bortezomib-based regimens - %	85.2
Post-induction response - %	
Complete response	25.0
VGPR	33.9
PR	26.8
<PR	14.3
Auto-HSCT - %	
No transplantation	32.1
First line	57.1
Second line	10.7
Early relapse (≤24 months) - %	
Yes	48.2
No	51.8

Auto-HSCT: Autologous stem cell transplantation; ISS: international staging system; PR: partial response; VGPR: very good partial response

This study was conducted following the Declaration of Helsinki and received approval from the Ethics Committees of the Institutions involved in the project. All patients provided their written informed consent.

Regarding treatment, in the non-transplant cohort (n = 18), VCD (bortezomib, cyclophosphamide, and dexamethasone) was the most frequent induction regimen (44.4%), followed by VRD (bortezomib, lenalidomide, and dexamethasone; 33.3%). Smaller groups received lenalidomide–dexamethasone, CTD (cyclophosphamide, thalidomide, and dexamethasone), or corticosteroids alone.

For patients undergoing transplant as part of first-line therapy (n = 32), VCD predominated (37.5%), followed by VTD (34.4%), CTD (12.5%), and VRD (9.4%). VAD and VDT-PACE each accounted for approximately 3.1%. In the second-line transplant group (n = 6), VCD was the primary regimen (66.7%), while VTD and CTD each accounted for 16.7%.

Overall, most patients underwent transplant as part of first-line therapy and across all three settings VCD was the most commonly used induction regimen, with a particularly marked predominance in the second-line transplant group. The number of induction cycles was similar across groups, with a median of six cycles in each: non-transplant (interquartile range [IQR] = 4.75), first-line transplant (IQR = 2), and second-line transplant (IQR = 1.25).

Genetic and bioinformatics analysis

Genomic DNA from Uruguayan patients was extracted from peripheral blood for germline variant analysis regardless of the treatment stage. In contrast, Argentinean samples (sourced from bone marrow) were obtained from the DNA archive of the Laboratory of Lymphoid Malignancies (Institute of Experimental Medicine, CONICET-National Academy of Medicine). All MGUS samples were derived from bone marrow; Uruguayan MGUS samples were collected as part of a national prevalence study, while Argentinean MGUS samples were provided by the same CONICET archive. In both cohorts, extraction included the total cellular content of the respective compartments.

The Wizard® Genomic DNA Purification Kit (Promega, Wisconsin, USA) or Quick-DNA Miniprep Kit (Zymo Research, California, USA) were used to extract the DNA from biological samples. DNA concentration and purity were assessed using a NanoDrop Microvolume Spectrophotometer (Thermo Fisher). SNVs were analyzed using the Infinium™ Global Screening Array-24 v 3.0 BeadChip on the Illumina iScan Platform which contains 654,027 markers.

The raw Variant Call Format (VCF) files were annotated using high-performance computing systems for the analysis of large-scale data, referencing the hg19 (GRCh37) genome and utilizing SnpEff/SnpSift 5.0e software along with data from the gwasCat, dbSNP154, ClinVar_20,220,320, gnomAD_r2.1.1, and SnpSift dbNSPF4 databases. Subsequently, the annotated VCF files were read using the vcfR package in RStudio software. Duplicate variants were removed, retaining only those present in 100% of the patients. Variants located on sex chromosomes, pseudogenes, intergenic regions, and those classified as low impact according to American College of Medical Genetics guidelines were excluded [12]. Binary Discriminant Analysis, Principal Components Analysis (PCA), and k-means clustering were employed to identify SNVs associated with the four IMWG treatment response categories.

Statistical analysis

Statistical analyses were conducted using SPSS v.26 and R 4.3.2 software. Descriptive statistics for both quantitative and qualitative variables were calculated using parametric or non-parametric tests as appropriate. Data comparisons between subgroups, along with associations between myeloma characteristics and genotypes, were evaluated through contingency tables and Odds Ratios. The Hardy-Weinberg equilibrium (HWE) was tested using the χ^2 test to identify deviations in genotype frequencies. Genotypic and allele frequencies of variants were analyzed across different populations using χ^2 tests. Allele frequencies in the present cohort were contrasted with European individuals and Latin American Population 2 (LA2), which includes subjects predominantly of European and Native American Ancestry, using information from the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/, last accessed on January 21, 2025) and the Z-score method [13]. Risk assessments were calculated by comparing patient outcomes according to heterozygous and homozygous genotypes.

Overall survival (OS) was defined as the time from diagnosis to patient death, and progression-free survival (PFS) as the time from diagnosis to the first relapse or death from any cause; both were assessed with Kaplan-Meier survival curves and compared using the Log-Rank test. Statistical significance was set as a p-value <0.05 in all tests.

Results

An initial analysis was conducted to identify SNVs associated with the four IMWG treatment response categories. This approach identified 692 statistically significant SNVs distributed across 527 distinct genes, each meeting the criterion of t-score >3. These 692 SNVs effectively classified the dataset according to PIR, although some overlap was observed between the very good partial remission (VGPR) and complete remission (CR) groups (Supplementary Figure S1). The application of a more stringent criterion (t-score >4) identified 42 of the most significant SNVs, located in 39 genes and exhibiting a differential association with cancer (Table 2). Among them, three SNVs were associated with CR, one with VGPR, five SNVs with partial remission (PR), and 33 with <PR.

Subsequently, a search was conducted for SNVs associated with the ER and non-ER categories in patients undergoing front-line autologous hematopoietic stem cell transplantation (auto-HSCT). In this analysis, 151 SNVs distributed across 93 genes were identified as statistically significant based on the criterion of t-score >3 for each group (Supplementary Figure S2). Only 12 overlapping SNVs were found between ER and ≤PR groups, seven of which significantly affect clinical outcomes (Table 3). The examination of allele frequencies for these variants revealed no deviation from HWE. Two patient groups were differentiated based on the presence or absence of alternative alleles at the following loci: *Protein tyrosine*

Table 2 – Genes involved in cancer-associated pathways according to post-induction response.

PIR	SNV (Gene)	Cancer related genes (%)
CR	rs4803750 (*BCL3*), rs10134750 (*BRMS1L*), rs72697384 (*KCNK13*)	100
VGPR	rs1329954 (*OR1J2*)	100
PR	rs7529205 (*HES2*), rs555115 (*IGSF21*), rs6478551 (*TTLL11*), rs6567354 (*SERPINB5*), rs74404771 (*SPON1*)	60
<PR	rs28667972 (*HS3ST4*), rs112754319 (*HS3ST4*), rs2120315 (*SETBP1*), rs78462078 (*OSBP*), rs55902330 (*CCSER1*), rs42582 (*ZNRF2*), rs59350504 (*KCNJ6*), rs144334202 (*SAPCD2*), rs78308355 (*EXOSC7*), rs77262863 (*SHQ1*), rs56264830 (*NOTCH1*), rs75485807 (*PKNOX2*), rs58375792 (*RAB17*), rs2273613 (*VARS1*), rs74624490 (*STX11*), rs114289907 (*RYR2*), rs56312321 (*KLHL29*), rs114442896 (*PRKCE*), rs1550510 (*SLC9A9*), rs13091793 (*SLC9A9*), rs116626750 (*CD38*), rs62364615 (*KIAA0825*), rs75908196 (*CDKAL1*), rs9468254 (*OR2B2*), rs79271049 (*UCN3*), rs62037313 (*CPNE2*), rs55987113 (*CPNE2*), rs111689642 (*TMEM132E*), rs73370119 (*MX2*), rs7716089 (*ANKRD33B*), rs10437360 (*PCDH15*), rs55794691 (*ATP10A*), rs56278472 (*SULT2B1*)	12

SNV: Single-nucleotide variant; PIR: post-induction response; CR: complete response; PR: partial response; VGPR: very good partial response.

phosphatase receptor type D (*PTPRD* rs12343415, rs77411943, rs10978084), *Notch Receptor 4* (*NOTCH4* rs8192588), *SH3 Domain Containing Ring Finger 3* (*SH3RF3* rs76256617), *DCC Netrin 1 Receptor* (*DCC* rs72920200), and *CUB and Sushi Multiple Domains 1* (*CSMD1* rs11781684).

Group A (n = 9) included patients who were either heterozygous or homozygous for the alternative allele, whereas Group B (n = 47) included those homozygous for the reference alleles. Patients in Group A exhibited a significantly higher ≤PR rate compared to those in Group B (88.89% versus 31.91%; p-value = 0.005) (Figure 1 A) and had a higher ER rate than Group B (88.89% versus 40.43%; p-value = 0.021) (Figure 1 B).

An interpopulation comparison of allele frequencies showed that alternative alleles for *SH3RF3*, *PTPRD*, and *CSMD1* gene variants were significantly more frequent in the present cohort than in the LA2 population, with no differences observed compared to Europeans. The minor allele frequencies (MAF) for *SH3RF3* rs76256617 and *PTPRD* rs10978084 observed in the present cohort were 0.018, compared to 0.002 in the LA2 (p-value = 0.047). Additionally, for *CSMD1* rs11781684, a MAF of 0.045 was observed in this study, while in Latin Americans it was 0.040 (p-value <0.001) (Table 4).

Survival analysis revealed that Group A had a 5-year OS rate of 40%, that is, significantly lower than Group B (76.1%). Similarly, the median PFS was markedly shorter in Group A (eight months) compared to Group B (47 months). A multivariate analysis, adjusted for first-line auto-HSCT and International Scoring System (ISS), confirmed that Group A had significantly poorer outcomes for both OS (p-value = 0.008) and PFS (p-value = 0.017) (Figure 2).

Because the majority of Group A cases (8/9) were not transplanted, a sub-analysis was conducted of non-transplanted patients (n = 18: Group A = 8; Group B = 10). ER occurred in 7/8 (87.5%) of Group A versus 8/10 (80.0%) of Group B. Additionally, Group A had a higher proportion of cases with relapse at 12 months; 75% (6/8), compared with Group B 60% (6/10).

The comparison between MM and MGUS patients, following rigorous filtering, resulted in the identification of 103,942 SNVs. Subsequent Binary Discriminant Analysis revealed 251 significant SNVs mapped to 193 genes and successfully clustered MM and MGUS using PCA (Dim1 16.9%, Dim2 4.5%) (Supplementary Figure S3). Differentially represented SNVs between MM (13) and MGUS (8) are shown in Table 5.

Table 3 – Overlapping single-nucleotide variants between early relapse (≤24 months) and ≤ partial response with a significant impact on clinical outcomes.

Gene	ID	Position (GRCh37)	Reference allele	Alternative allele	Genotypic Frequency (%)	Allele Frequency (%) [1]
PTPRD	rs12343415	10,468,666	A	C	AA: 92.9 AC: 7.1	A: 96.4, C: 3.6
	rs77411943	10,266,719	A	G	AA: 96.4 AG: 3.6	A: 98.2, G: 1.8
	rs10978084	9915,288	A	G	AA: 96.4 AG: 3.6	A: 98.2, G: 1.8
NOTCH4	rs8192588	32,188,678	G	A	GG: 96.4 GA: 3.6	G: 98.2, A: 1.8
SH3RF3	rs76256617	110,030,331	A	G	AA: 96.4 AG: 3.6	A: 98.2, G: 1.8
DCC	rs72920200	50,380,636	T	C	TT: 96.4 TC: 3.6	T: 98.2, C: 1.8
CSMD1	rs11781684	3937,801	G	A	GG: 91.1 GA: 8.9	G: 95.5, A: 4.5

[1] All Hardy-Weinberg equilibrium analyses were not significant with p-values greater than 0.05.

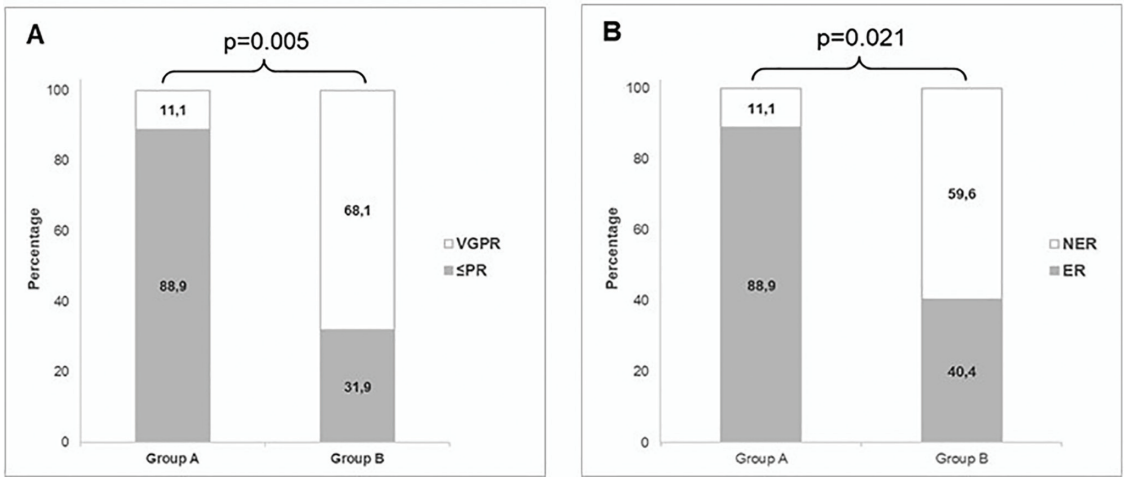

Fig. 1 – Genotype-Based Analysis of partial remission (PR) and early relapse (ER). A) Comparison of ≤PR rates between Groups A and B (Grey ≤PR, White ≥ GPR); B) Comparison of early relapse (ER) rate between Groups A and B (Grey = ER, White = non-ER). Group A included patients carrying homozygous or heterozygous genotypes for alternative alleles, whereas Group B comprised patients homozygous for the reference alleles for variants of the *PTPRD* (rs12343415, rs77411943, rs10978084), *NOTCH4* (rs8192588), *SH3RF3* (rs76256617), *DCC* (rs72920200), and *CSMD1* (rs11781684) genes.

Discussion

This large-scale analysis of SNVs in NDMM patients identified variants associated with IMWG treatment response categories, highlighting the genetic complexity that influences therapy outcomes in this disease. The genetic profile enabled stratification between ER and non-ER in auto-HSCT patients and revealed only 12 overlapping SNVs between the ER and ≤PR groups, seven of which have a significant impact on clinical outcomes, suggesting shared genetic drivers.

The analysis of overlapping SNVs revealed that alternative alleles in the *PTPRD, NOTCH4, SH3RF3, DCC*, and *CSMD* genes were strongly associated with poor outcomes in the present cohort. These genes participate in different cancer-associated signaling pathways. *PTPRD,* located on chromosome 9p23, interacts with pathways such as STAT3, JAK, mTOR, and *β-catenin*; its inactivation has been implicated in various malignancies including colon, gastric, and breast cancers, glioblastoma, melanoma, and with MM pathogenesis [14,15]. Similarly, *NOTCH4* (6p21.32), contributes to oncogenic signaling through interactions with molecules like Sox2, c-MET, and CD44 [16]. *SH3RF3* (*POSH2*)(2q13), defined by its SH3 and ring finger domains, enhances cancer stem cell properties in a JNK-dependent mechanism [17]. The *DCC* gene (18q21), encoding the tumor-suppressor Netrin-1 receptor, is frequently silenced in colorectal cancer [18]. The *CSMD1* gene (8p23) is commonly deleted in cancers such as head and neck squamous cell carcinoma (50%), breast cancer (55%), and lung cancer (46%). In addition, it is associated to tumor mutational burden, mismatch repair deficiency, and PD-L1 expression [19].

The literature offers limited evidence regarding the application of SNP-arrays in MM. Kamada et al. [20]. found a deletion of *PTPRD* (9p23) in MM patients with a non-hyperdiploid karyotype as well as in human MM cell lines. More recently, a

Table 4 – Comparative analysis of alternative allele frequencies between the present cohort and European and Latin American 2 populations.

Gene	SNV ID	Observed MAF	European population [a]		LA2 population [a]	
			MAF	p-value [b]	MAF	p-value [c]
PTPRD	rs12343415	0.036	0.075	0.27	0.0451	0.73
NOTCH4	rs8192588	0.018	0.024	0.91	0.0109	0.56
SH3RF3	rs76256617	0.018	0.007	0.56	0.002	**0.047**
PTPRD	rs10978084	0.018	0.009	0.56	0.002	**0.047**
PTPRD	rs77411943	0.018	0.058	0.18	0.024	0.76
DCC	rs72920200	0.018	0.025	0.73	0.005	0.23
CSMD1	rs11781684	0.045	0.040	0.77	0	**< 0.001**

MAF: minor allele frequency.

[a] Frequencies reported in the dbSNP (https://www.ncbi.nlm.nih.gov/snp/, last accessed on January 21, 2025).
[b] Comparison of the present cohort against European population.
[c] Comparison of the present cohort against Latin American population 2 (LA2) from dbSNP, which includes Latin American individuals with mostly European and Native American Ancestry.

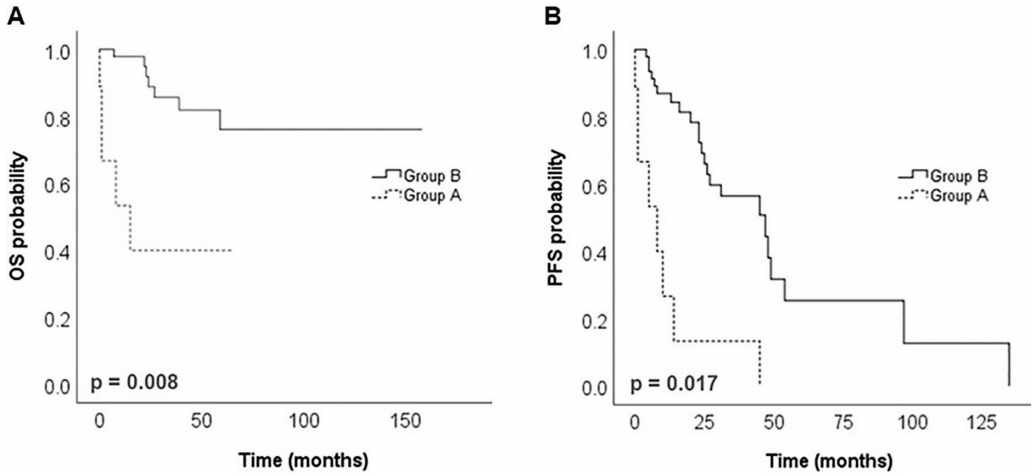

Fig. 2 – Kaplan Meier curves comparing overall survival (A) and progression free survival (B) between Group A (dotted line) and Group B (solid black line). Group A included patients presenting the alternative allele for the *PTPRD* (rs12343415, rs77411943, rs10978084), *NOTCH4* (rs8192588), *SH3RF3* (rs76256617), *DCC* (rs72920200), and *CSMD1* (rs11781684) genes, and Group B patients homozygous for the reference allele.

study by Campo et al. [21]. identified four genetic risk variants associated with the development of bortezomib-induced peripheral neuropathy, including *DCC* rs17748074. These findings, together with the results of the present study, support the use of SNP arrays to identify clinically relevant variants in MM and highlight the need for further studies in diverse MM cohorts.

The population-based comparison of allele frequencies showed that MAFs of *SH3RF3* rs76256617, *PTPRD* rs10978084, and *CSMD1* rs11781684 were higher than expected in the current cohort compared to the LA2 population but not to the European population. These results are likely influenced by the highly heterogeneous genetic structure of Latin American populations. Both Uruguay and Argentina share a strong European ancestry, particularly from Spain and Italy, and have undergone similar demographic processes, including the admixture with Native American and African populations [22–24]. The differences observed between the current cohort and other Latin American populations may reflect distinct genetic backgrounds, likely shaped by regional variations in ancestry components. This is in line with recent genomic studies showing that Latin American populations exhibit marked substructure due to complex admixture histories and demographic processes [25]. Given the underrepresentation of Latin Americans in global genetic studies [26], the findings of this study highlight the importance of further research in genetic characterization of these populations.

Table 5 – Variants differentially represented in multiple myeloma (MM) and monoclonal gammopathy of uncertain significance (MGUS).

MM		MGUS	
Gene	SNV ID	Gene	SNV ID
CFAP74	rs2803337	*OR2W5*	rs10925061
FAF1	rs3827730	*PADI2*	rs11588995
FSTL1	rs1147696	*KCNN2*	rs1487228
SORCS2	rs2285778	*ECE1*	rs213019
ARHGEF37	rs4409073	*PGAP6*	rs763146
UST	rs9390613 rs1045783	*ATP8B4*	rs12915207 rs28706888
NUGGC	rs7840091	*RC3H1*	rs80205789
MMRN2	rs746677		
TRPC6	rs4326755		
ATP10A	rs6576456		
SGF29	rs4788073		
PCP4	rs9983735		

SNV: Single-nucleotide variant.

The present analysis demonstrates that specific SNVs, identified through comprehensive genotyping, indicate that microarray technology can adequately classify distinct groups of PIR and ER patients. Moreover, specific SNVs were associated with poorer induction response, shorter PFS, and reduced OS. Furthermore, the differential representation of several SNVs between MM and MGUS highlights genetic distinctions that may be clinically relevant for both diagnosis and prognosis. These findings underscore the role of SNVs in predicting treatment response and clinical outcomes in MM. Interestingly, a recent meta-analysis using four GWAS datasets found a significant genome-wide association between the G allele of *SKT10* rs28199 and increased MM risk, with no observed heterogeneity among studies [27]. More recently, Zhu et al. [28]. combining microarray analysis and Mendelian randomization, identified the myeloperoxidase (*MPO*) gene as a novel biomarker potentially implicated in MM pathogenesis and progression. Thus, the identification of predictive markers could be used to guide treatment options, as well as to monitor residual disease, providing significant benefits in MM management [10].

Conclusions

In this study, high-resolution SNV genotyping using microarray technology identified several SNVs that can effectively classify patients in different PIR groups. Furthermore, despite

the limited number of patients, the results suggest that the presence of alternative alleles may be associated with inferior induction response, PFS, and OS in NDMM. To our knowledge, this is the first study showing the involvement of specific genes and variants in the context of MM. The findings reported here support the potential predictive role of the identified SNVs in disease progression and prognosis. However, given the small sample size and number of events, further investigation is necessary to elucidate the precise mechanisms underlying these associations and their clinical implications.

Author contributions

All authors contributed to the study's conception and design. DG, FS, and CG were responsible for methodology, data acquisition, analysis, and interpretation of results. DG, SL, and ER were responsible for clinical data collection. ML contributed with bioinformatics analysis. DG, ER, AF, and IS were responsible for the study conceptualization. DG, AF, and IS writing the first draft of the manuscript, and reviewing and editing the final version. All authors read and approved the final version of the manuscript.

Funding sources

This work was supported by grants from IMS and the Laura Rodger Riney Foundation and CONICET (National Research Council), Argentine (PUE: 2018-0042 and PIP 1179).

Ethical approval

The study was approved by the Local Ethics Committees, and it is in accordance with the current version of the Helsinki Declaration. All patients provided their written informed consent.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

None.

Acknowledgements

The authors thanks to the professionals of the Department of Basic Medicine at the Hospital de Clínicas Dr. Manuel Quintela, Montevideo, Uruguay. Particularly to Dr. Daniela Lenz and Dr. Ana Inés Catalán for their invaluable support and to Carolina Ottati for her expertise and dedication.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2026.106464.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Socio-demographic and therapeutic profile of people living with thalassemia in Brazil between 2012 and 2022: A cross-sectional study

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ARTICLE INFO

Article history:
Received 12 November 2025
Accepted 23 March 2026
Available online 2 May 2026

Keyword:
Beta thalassemia
Blood transfusion
Rare disease

ABSTRACT

Objective: To outline the sociodemographic and therapeutic profile of individuals with thalassemia in Brazil between 2012 and 2022, by analyzing data from the Ministry of Health on outpatient care, hospitalization, and mortality records.

Methods: This descriptive, cross-sectional study utilized secondary data from the Outpatient, Hospital, and Mortality Information Systems of the Ministry of Health on patients diagnosed with thalassemia (ICD-10 D56). Analyzed variables included demographics (sex, age, race/ethnicity), geographic location (residence and treatment site), and clinical outcomes (procedures, hospitalizations, and deaths).

Results: In 2022, an estimated 662 patients received outpatient treatment in the Brazilian Unified Health System (SUS), with a predominance of young adults, women, and individuals self-identified as White, who were mostly concentrated in the southeastern and southern regions of the country. Red blood cell transfusion was the most frequently performed outpatient procedure during the period. There were 1278 hospitalizations, mainly of children aged 0–9 years. A total of 153 deaths were recorded, primarily among people aged 50 years or older. The findings characterize the patient profile, identify significant travel challenges to receive treatment, and suggest potential underreporting within reference institutions.

Conclusion: It is essential to improve data recording in public databases to support specific health policies and ensure comprehensive, equitable, and continuous treatment for people living with thalassemia in Brazil.

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https://doi.org/10.1016/j.htct.2026.106461

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Introduction

Thalassemia is a hemoglobinopathy, meaning a genetic condition characterized by the absence or significant reduction in the production of one or more globin chains (alpha or beta) that make up hemoglobin [1,2]. In the most severe form of the disease, the red blood cells (RBCs) produced are more fragile and have a shorter lifespan than normal, resulting in severe anemia [3].

Alpha thalassemia, in which the primary defect is in the production of alpha globin chains, presents as four clinical forms: silent carrier (without clinical manifestations), alpha thalassemia trait (mild anemia), hemoglobin H disease (moderate to severe anemia), and hydrops fetalis (extremely severe and incompatible with life) [4]. Caused by a defect in beta-globin chain synthesis, beta thalassemia presents as three distinct clinical phenotypes: beta thalassemia minor or thalassemic trait (generally asymptomatic), thalassemia intermedia (mild to severe anemia that may require RBC transfusions), and thalassemia major (severe anemia requiring continuous RBC transfusions every 2–4 weeks from the first months of life) [3,4]. The trait forms of thalassemia occur when only one parent is a carrier of the gene for the disease, while the more severe forms occur when both parents are carriers of the alpha or beta thalassemia gene [3,4].

Beta thalassemia is common in the Mediterranean region, the Middle East, and Asia. Alpha thalassemia is more common in Southeast Asia. Migration resulting from globalization has introduced hemoglobinopathies to areas where they were not originally endemic [2]; in Brazil, this occurred mainly through the arrival of Greeks and Italians to the country, with the promise of a better life, in the mid-19th century [4].

Currently, about 5% of the world population carries a gene for some abnormal hemoglobin [2]. In Brazil, it is estimated that 1.1% of the population may have genetic alterations for thalassemia [1]. The Brazilian Thalassemia Association (Abrasta) estimates that about a thousand people in the country are living with severe forms of thalassemia. Among registered individuals with thalassemia, 283 have beta thalassemia major and 222 have beta thalassemia intermedia. These data highlight the importance of proper follow-up and treatment for affected patients [3].

Since early diagnosis is necessary for proper treatment [5], thalassemia is detected via newborn screening at birth and confirmed using hemoglobin electrophoresis. The specific genetic mutation test is rarely used in Brazil due to limited access to this exam [4].

Treatment and follow-up for individuals living with thalassemias are essential to prevent disease-related complications, such as growth retardation, splenomegaly, hepatomegaly, weakened musculature, leg ulcers, extramedullary hematopoiesis, bone deformities of the legs, and craniofacial changes. To prevent these complications, people with beta thalassemia major undergo regular RBC transfusions throughout their lives to maintain adequate hemoglobin levels and minimize symptoms associated with anemia [3,5]. Treatment for intermediate beta or alpha thalassemias may include sporadic or frequent RBC transfusions, depending on each patient's clinical progression [3,5]. Chelation therapy, to manage the iron overload resulting from frequent transfusions, is also an integral part of the treatment plan, in addition to a multidisciplinary approach involving hematologists, palliative care specialists, and other healthcare professionals to provide comprehensive and personalized care [3,5].

Given that thalassemia is a rare disease with limited epidemiological data in Brazil, this study aimed to outline the sociodemographic profile of affected individuals between 2012 and 2022. Additionally, it analyzes outpatient and hospital production, focusing on primary procedures, and assesses mortality rates associated with the condition during the same period.

Methods

A cross-sectional descriptive study was conducted using secondary data from the period from 2012 to 2022 encompassing all types of thalassemia (diagnostic codes D56.0 through D56.9) according to the International Statistical Classification of Diseases and Related Health Problems (ICD-10).

Based on this, categories D56.0 and D56.3 of the ICD-10, which refer to the alpha and beta thalassemia traits, respectively and do not require outpatient treatment, were excluded. Data were retrieved from the Ministry of Health's public databases: the Outpatient Information System (OIS) for ambulatory procedures, the Hospital Information System (HIS) for admissions, and the Mortality Information System (MIS) for mortality data.

The most up-to-date consolidated data in the public databases are related to 2022. Therefore, a retrospective analysis of a decade from 2012 to 2022 was chosen. The datasets were subjected to a cleaning and organization process using Microsoft Excel® to select variables for this study. These variables included sex, age, race/ethnicity, city of residence, city of treatment, state where the procedure/hospitalization took place, cost of procedure/hospitalization, type of procedure performed, number of procedures, year of diagnosis, year the procedure was performed, year of hospitalization, and year of death.

Age was stratified into the following groups: 0–9, 10–19, 20–29, 30–39, 40–49, and ≥ 50 years. The three most prevalent procedures were specifically identified and all other procedures were grouped as 'Others' for the purpose of analysis.

The number of users with thalassemia registered in SUS was estimated by cross-referencing information such as the municipality of residence, sex, ICD-10 category, and the healthcare institution responsible for treatment. To focus on relevant procedures, all types of procedures were selected, ranging from clinical and imaging examinations to blood transfusions and patient follow-up procedures.

It is important to note that the data collected refers exclusively to outpatient and hospital services linked to thalassemia as provided by SUS, and does not include information from the supplementary health network. The public databases were accessed on the data systematization website of the Ministry of Health (available at: https://datasus.saude.gov.br/transferencia-de-arquivos).

In compliance with Brazilian Law No. 12,527/2011 and CNS Resolution 510/2016, this study did not require Research Ethics Committee approval as it used publicly accessible secondary data that contains no personally identifiable information.

Results

Outpatient information system (OIS)

Within the scope of the OIS, an estimated 662 patients underwent outpatient treatment in 2022, with São Paulo having the highest number at 244 estimated patients, followed by Rio Grande do Sul with 113. In contrast, Acre, Roraima, and Rio Grande do Norte did not report any patients in 2022 (Table 1).

Between 2012 and 2022, women accounted for 54.5% of the patients. The main age groups were 20–29 years (29%) and 30–39 years (24.9%), and most patients self-identified as White (86.5%). It is important to note that the majority of patients (64.3%) underwent procedures in cities other than where they resided (Table 2).

A total of 133,893 procedures were performed, with RBC concentrate transfusions being the most frequent. Hospital das Clínicas at Unicamp in Campinas led with a number of nearly 50,000 procedures. In all, 233 facilities were identified as outpatient care locations. The Southeast was the Brazilian region with the highest number of procedures performed for thalassemia (68%), with São Paulo being the State with the largest volume of procedures (86,154). The southern region was ranked second with 24.1% of the outpatient production; Paraná accounted for 29,379 procedures (Table 2).

The year with the highest number of procedures was 2013, with 15,039, while the lowest was in 2020, possibly impacted by the Covid-19 pandemic, when 9713 procedures were recorded. The highest total cost, which occurred in 2022, exceeded two million reals, with an average cost of R$305.31 per procedure.

Table 1 – Estimated number of thalassemia patients in Brazil in 2022.

State	Estimated Patients	
	n	%
São Paulo	244	36.9
Rio Grande do Sul	113	17.1
Minas Gerais	107	16.3
Paraná	23	3.5
Distrito Federal	21	3.2
Mato Grosso	21	3.2
Santa Catarina	20	3.0
Maranhão	18	2.7
Piauí	18	2.7
Goiás	15	2.4
Rio de Janeiro	13	2.0
Espírito Santo	9	1.4
Pará	9	1.4
Bahia	6	0.9
Tocantins	6	0.9
Alagoas	4	0.6
Paraíba	3	0.5
Rondônia	3	0.5
Amapá	2	0.4
Ceará	2	0.4
Mato Grosso do Sul	2	0.4
Amazonas	1	0.2
Pernambuco	1	0.2
Sergipe	1	0.2
Acre	0	0.0
Roraima	0	0.0
Rio Grande do Norte	0	0.0
Total	662	100

Table 2 – Profile of outpatient procedures performed for thalassemia in Brazil between 2012 and 2022.

Variable	Outpatient Procedure n (%)
Race/Ethnicity	
White	115,817 (86.5)
Brown (*Pardo*)	8059 (6.0)
Black	3020 (2.3)
Asian	1450 (1.1)
Indigenous	217 (0.2)
Not declared	5330 (3.9)
Age Group (years)	
0–9	11,662 (8.7)
10–19	27,702 (20.7)
20–29	17,015 (29.2)
30–39	39,190 (24.9)
40–49	9426 (7.1)
≥50	12,612 (9.4)
Gender	
Male	61,020 (45.5)
Female	72,873 (54.5)
Type of procedure	
Red blood cell concentrate transfusion	53,124 (39.7)
Pre-transfusion Tests I*	39,619 (29.6)
Pre-transfusion Tests II**	23,710 (17.7)
Others	17,440 (13.0)
Region of Brazil	
North	2068 (1.5)
Northeast	6852 (5.1)
Central-West	1610 (1.2)
South	32,143 (24.1)
Southeast	91,220 (68.1)
Total	**133,893**

* Determination of ABO blood group and RH system D antigens, as well as the screening for irregular antibodies using the human antiglobulin method accompanied by an auto-control.

** Indirect antiglobulin technique for blood compatibility, also known as crossmatching.

Hospital information system (HIS)

In the HIS, a total of 1278 procedures were recorded during hospitalizations, with 'treatment of hemolytic anemia' being the most common. Women represented the majority of hospitalizations (50.2%), with the 0–9 years age group registering the highest number of cases (431 hospitalizations) (Table 3). About 40% of hospitalized patients lived in cities different to where they were hospitalized, with São Paulo being the State with the highest number of hospitalizations (532). Only 3% of

Table 3 – Profile of hospital admissions for thalassemia in Brazil from 2012 to 2022.

Variable	Hospitalization n (%)
Race/Ethnicity	
White	545 (42.6)
Brown (*Pardo*)	424 (33.2)
Black	47 (3.7)
Asian	10 (0.8)
Indigenous	1 (0.1)
Not declared	251 (19.6)
Age Group (years)	
0–9	431 (33.8)
10–19	233 (18.2)
20–29	170 (13.3)
30–39	132 (10.3)
40–49	89 (7.0)
≥50	223 (17.4)
Gender	
Male	637 (49.8)
Female	641 (50.2)
Region of Brazil	
North	57 (4.7)
Northeast	234 (18.2)
Central-West	83 (6.4)
South	128 (10.0)
Southeast	776 (60.7)
Total	**1278**

Table 4 – Mortality profile of thalassemia in Brazil between 2012 and 2022.

Variable	Death n (%)
Race/Ethnicity	
White	103 (67.3)
Brown (*Pardo*)	34 (22.2)
Black	5 (3.3)
Not declared	11 (7.2)
Age group (years)	
0–9	10 (6.5)
10–19	10 (6.5)
20–29	10 (6.5)
30–39	21 (13.7)
40–49	13 (8.5)
≥50	89 (58.3)
Gender	
Male	65 (42.5)
Female	88 (57.5)
Region of Brazil	
North	6 (3.9)
Northeast	25 (16.4)
Central-West	10 (6.5)
South	22 (14.4)
Southeast	90 (58.8)
Total	**153**

the hospitalizations resulted in admission to an intensive care unit.

The Santiago Children's Hospital in Natal, Rio Grande do Norte, recorded the highest number of procedures (n = 53) during hospitalizations. In Brazil, the year with the most hospitalizations was 2017 with 160 cases, while 2014 and 2015 each had 109 hospitalizations, and 2021 had 105 hospitalizations. The highest total cost occurred in 2014, exceeding 750,000 reals, with an average hospitalization cost of 2382.99 reals. There were 28 hospitalizations for allogeneic hematopoietic stem cell transplantations (HSCT) during the study period, with this procedure alone costing the public treasury more than two million reals. Hospital das Clínicas de Ribeirão Preto accounted for 23 hospitalizations for transplants.

Mortality information system (MIS)

During the study period, Brazil recorded a total of 153 deaths related to thalassemia. Women (57.5%) and individuals aged 50 years or older (37%) accounted for the majority of fatal cases, while 67.3% of the deceased self-identified as White (Table 4). São Paulo had the highest number of deaths, totaling 56 cases. The years 2017 and 2020 had the highest numbers of deaths, with 19 cases each.

Discussion

Data collected between 1970 and 2019 from Italy, a country recognized for its high incidence of thalassemia, reported 7169 patients with thalassemia (5205 of whom had transfusion-dependent thalassemia [TDT] and 1964 with non-transfusion-dependent thalassemia [NTDT]). The majority of the patients were in the 36–50 age group and there were significantly more women than men. The survival cohort included 3777 patients; those with TDT had lower survival rates than NTDT patients (p-value <0.0001). Of the TDT group, the best survival rates were observed among women (71.8 years versus 68.8 years for men). A total of 523 deaths were recorded over the study period. Compared to the general population, patients with TDT had an increased risk of death, while the risk for NTDT was similar to that of the general population [6].

Analyses of outpatient production in the present study identified 662 patients in 2022. The results showed that the profile of people living with thalassemia in Brazil corresponds mostly to young adults aged between 20 and 29, followed by those aged 30–39, with the majority being women and originating from the southern and southeastern regions. São Paulo (Southeast) and Paraná (South) were the predominant states, accounting for 94.4% and 91.4% of all procedures within their respective regions. These factors demonstrate that most people living with thalassemia were born in the 1980s, 1990s and 2000s, when transfusional treatment, typically every two to four weeks, and iron chelation therapy were being established and becoming more widely available both in Brazil and worldwide [7–9]. The treatment protocol enabled a better quality of life and longer survival for people with TDT, preventing death as a consequence of anemia due to lack of treatment, since it is a disease incompatible with life [7–9].

The findings of the current study indicate that while the highest volume of outpatient procedures and estimated patient numbers are concentrated in states with significant Italian heritage, individuals with thalassemia are present across the country due to historical miscegenation and

internal migration [1,10,11]. In several states across the North and Northeast, the majority of procedures were performed on individuals identified as '*Pardo*' (Brown). This contrasts with other Brazilian regions, where the highest percentage of procedures occurred among the white population. The estimated number of patients for 2022 aligns closely with Abrasta's projections. However, as these data only include individuals treated within SUS, they do not account for those receiving care through private health insurance [3].

Packed RBC transfusions were the most prevalent procedures, highlighting the importance of transfusional support in managing thalassemia. However, it is notable that this number was far below what was expected, considering that each patient on a transfusion protocol is estimated to undergo 12–24 transfusions annually, depending on their needs [8]. Some reference healthcare institutions for outpatient thalassemia treatment, such as Hemorio in Rio de Janeiro and Hemope in Pernambuco, do not have their data recorded in the OIS. This underreporting makes it difficult to estimate the number of patients, their treatments, and to fully understand the real situation of thalassemia in the country.

It is interesting to note the drop in the number of procedures in 2020, possibly due to the COVID-19 pandemic, which may have impacted access to health services and outpatient procedures for different conditions, including thalassemia [12].

It is also important to highlight the lack of reporting high-cost procedures involving medications such as iron chelators in the OIS. People with thalassemia use chelators to treat the buildup of iron in the body resulting from transfusions. Each unit of packed RBCs contains between 200 and 250 mg of iron, which is not metabolized by the body and accumulates in organs such as the liver, pancreas, and heart. If the excess iron is not eliminated from the body by iron chelation medications, the patient will develop secondary diseases such as liver cirrhosis, diabetes, and even heart failure [8,13]. Therefore, better articulation and integration of the data is important for unifying information on procedures for ICD-10 [11]. It is known that many patients have difficulty obtaining iron chelators through the public health system, often due to shortages or government bureaucracy [3]. By assessing availability of high-cost medications, it is possible to identify whether patients are truly receiving comprehensive treatment and to plan public policies to ensure the supply of this essential medication for the survival and quality of life of people with thalassemia, thereby preventing legal action.

Most thalassemia patients did not undergo procedures in the city where they reside, indicating a need to travel to major centers to access specialized health services [14]. There is also a notable predominance of women among the patients treated, which suggests a possible difference in access to health services between men and women, since it is known that women tend to be more concerned than men about regularly visiting the doctor and undergoing necessary exams and treatments to maintain their health [15].

Based on the HIS, the mean number of hospitalizations annually was 128 people, with a total of 1278 hospitalizations over the ten years of the study. In 2022, an estimated 19.3% of patients required hospitalization. However, specific triggers or procedures associated with these admissions could not be identified, as the majority were recorded under the generic ICD-10 classification 'treatment of patient with hemolytic anemia,' which lacks clinical detail regarding the circumstances of hospitalization. This demonstrates a lack of coordination among the available databases and raises doubts about the actual number of patients receiving outpatient and inpatient treatment.

Regarding specialized interventions, 28 hospitalizations for allogeneic HSCT were recorded among 26 patients. Allogeneic HSCT may be indicated for transfusion-dependent children and adolescents who do not have severe organ impairment (liver, heart) and have a 100% compatible donor, usually a sibling without the disease or with thalassemia minor [8,13].

Mortality data reveal a substantial disease burden, with 153 deaths recorded during the study period. This figure is notably high relative to the estimated 600 patients currently receiving treatment through the SUS, as identified by both this study and Abrasta data [3]. Unfortunately, the data did not identify the causes of death. It is worth remembering the secondary diseases result from transfusion treatment and iron overload. Death from heart failure secondary to iron accumulation remains a reality worldwide [7,8].

The majority of deaths occurred in individuals over 50 years of age (58%). This trend suggests that with optimized transfusion protocols, effective iron chelation, and multidisciplinary care, extended longevity is increasingly attainable. Consequently, the disease should not be viewed as a death sentence [8,13].

This study has limitations, as it relied on secondary data, leaving the authors dependent on the data entered by the teams responsible for treatment. Therefore, it does not fully represent the true number of people living with thalassemia and receiving transfusions via SUS.

The increased mortality among people with hemoglobinopathies, compared to the general population highlights the need for new therapies and individualized approaches to optimize treatment [6]. Luspatercept, approved in Brazil in 2021, represents an alternative adjunctive therapy for TDT and is intended for use in conjunction with regular transfusions [16,17]. Favorable results have already been published, despite the treatment remaining under clinical study with Brazilian participation [18]. In addition to allogeneic HSCT, curative strategies such as gene therapy are currently under development. These advancements may offer patients transfusion independence and an improved quality of life [19–22], with the expectation of their future integration into the Brazilian healthcare system.

Conclusion

People living with thalassemia in Brazil face invisibility due to the small number of diagnosed cases and the lack of better data in public reporting systems, which hinders a clear understanding of the disease. There is a need for commitment to improve data collection, especially regarding outpatient care, since these are chronic patients who are regularly followed in hematology departments. In the future, improved data might provide a solid foundation for developing health policies

aimed at the prevention, diagnosis, and timely treatment of thalassemia in Brazil.

Financial support

The study was supported institutionally by the Brazilian Association of Thalassemia (Abrasta)

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

Editor

Eduardo Rego

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Potentially malignant oral disorders in patients with fanconi anemia: A cross-sectional study

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ARTICLE INFO

Article history:
Received 15 January 2026
Accepted 23 March 2026
Available online 7 May 2026

Keywords:
Fanconi anemia
Oral leukoplakia
Oral cancer
Hematopoietic stem cell transplantation

ABSTRACT

Introduction: Patients diagnosed with Fanconi Anemia present multiple congenital anomalies, pancytopenia, and defective DNA repair mechanisms, making them more susceptible to malignant neoplasia development, with notably higher incidence of oral squamous cell carcinoma. Hematopoietic stem cell transplantation is the definitive treatment for the hematological manifestations but it is considered a factor that significantly increases the risk of malignant transformation. Oral potentially malignant disorders frequently precede the development of oral carcinoma. Given the specific characteristics of this population, there is a critical need to strengthen surveillance programs and screening strategies for these lesions. The aim of this study was to evaluate the overall prevalence of different oral potentially malignant disorders in patients with Fanconi Anemia, without distinguishing among specific subtypes.

Methods: This was an observational cross-sectional study conducted with individuals who attended the Dental Clinic in the Onco-Hematology department of the Hospital de Clínicas Complex, Federal University of Paraná from March 2022 to January 2024. Demographic and clinical data were collected from patients with no history of oral squamous cell carcinoma.

Results: The study involved 110 patients of both genders (51.91% men and 49.09% women), with a mean age of 19.96 years. Of these, 40.9% presented at least one oral potentially malignant disorder; the presence of these disorders was significantly associated with having undergone hematopoietic stem cell transplantation. Older patients also had a higher risk of developing these lesions.

Conclusions: These disorders should be regarded as distinct entities in this population, with the risk of malignant transformation potentially being higher than in the general population not affected by Fanconi Anemia.

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https://doi.org/10.1016/j.htct.2026.106462

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Introduction

Fanconi Anemia (FA) is a rare autosomal recessive disorder that affects approximately 1 in 360,000 individuals [1,2]. Typically diagnosed in early childhood due to the presence of multiple congenital anomalies and severe pancytopenia, which can progressively result in bone marrow failure [3,4]. Moreover, these individuals exhibit defective DNA repair mechanisms, a critical system responsible for maintaining genome integrity and preventing malignancies [5]. This defect is considered the primary reason for the increased susceptibility to cancer development in individuals with FA [6], particularly acute myeloid leukemia and solid tumors, including oral squamous cell carcinoma (OSCC) [3], which currently stands as the leading cause of mortality in this population.

Hematopoietic stem cell transplantation (HSCT), the definitive treatment for the hematological manifestations of FA, is considered a factor that significantly increases the risk of malignant transformation [7–9], with an earlier onset compared to individuals who have not undergone HSCT [10].

Oral potentially malignant disorders (OPMDs) may precede the development of OSCC. Oral leukoplakia (OL), defined as a "predominantly white patch/plaque that cannot be rubbed off" [11], is among the most common OPMD observed in the oral cavity of FA patients [7]. These lesions, as well as red lesions (oral erythroplakia [OE]) or mixed red-and-white lesions (oral erythroleukoplakia [OLE]), are associated with a risk of malignant transformation [11–15], underscoring the importance of regular follow-up visits and systematic oral screening in FA patients [13].

Among individuals who have undergone HSCT, those who develop chronic graft-versus-host disease (chronic GvHD), a complication arising from alloimmune and autoimmune mechanisms following transplantation, face an even higher risk of malignant transformation [9,16,17]. Clinically, chronic oral GvHD presents with lichenoid features, which may or may not be accompanied by areas of erythema and ulceration and is considered a distinct OPMD entity, separate from OL [11,18]. Histopathologically, the diagnosis of oral chronic GvHD is based on the presence of a lichenoid interface inflammatory infiltrate with lymphocytic exocytosis and variable epithelial apoptosis [18,19], findings that differ from those observed in OL [20,21].

The aim of this study was to identify potential factors associated with the presence of OPMDs in general, without differentiating among OL, OE, OLE, or chronic GvHD, in FA individuals. By identifying patients at increased risk for malignant transformation, this study seeks to support the development of more effective and targeted monitoring and treatment strategies for this population.

Materials and methods

Study design

This was a cross-sectional observational study that was approved by the ethics committee of the Hospital de Clínicas of the Federal University of Paraná (CAAE: 53,235,921.3.0000.0096), and conducted in the dental office of the Onco-Hematology department of the same Hospital between March 2022 and January 2024.

The inclusion criteria for this study comprised patients with a confirmed diagnosis of FA who had attended at least one consultation during the study period. Written informed consent or assent was obtained from all participants prior to their inclusion. Patients were excluded if they had a history of previously diagnosed OSCC or if OSCC was present at the time of the evaluation.

Data collection

Demographic and historic data were obtained through anamnesis and electronic hospital records. Relevant information included age, sex, ethnicity, state of residence, and harmful health habits (use of substances such as alcohol, conventional, or electronic cigarettes), as well as information about HSCT (whether it was performed, the year and type of HSCT). Information regarding donor sex and chemotherapy conditioning for HSCT was not considered in this study.

A thorough physical examination of the oral mucosa was conducted by two dentists with expertise in stomatology.

The following conditions were considered OPMDs: OL, OE, OLE and 'lichen planus-like' features (Figure 1). All of these conditions were analyzed collectively as a single group, referred to as OPMD.

Representative clinical images illustrating the diversity of OPMDs: (A) Oral leukoplakia (OL) presenting as a well-defined white plaque on the palate; (B) Oral erythroleukoplakia (OLE) affecting the gingival mucosa, showing a mixed red and white appearance; (C) 'Lichen planus-like' features on the dorsal and lateral surfaces of the tongue, characterized by reticular and plaque-like white lesions; (D) Oral erythroplakia (OE) on

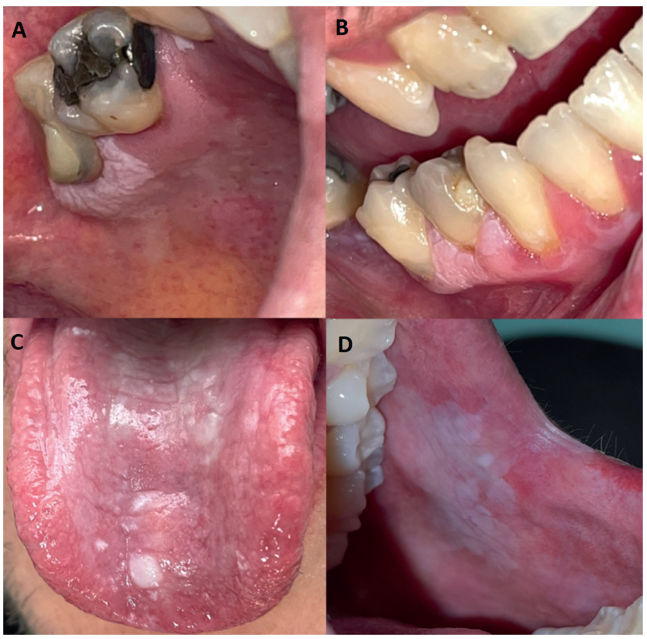

Fig. 1 – Clinical presentation of oral potentially malignant disorders (OPMDs).

the buccal mucosa, presenting as a predominantly erythematous area with subtle white components

Statistical analysis

Statistical analyses were performed using the RStudio software (R4.4). Participants were initially stratified into two groups based on the primary outcome: the presence or absence of OPMDs. Descriptive data analysis was employed to define the general characteristics of the sample. Means and standard deviations were calculated for normally distributed variables, while medians and interquartile ranges were used for variables with non-normal distributions. Categorical variables were described using absolute numbers and percentages.

The potential association between HSCT and the presence of OPMDs was evaluated using univariate and multivariate logistic regression. The multivariate model was adjusted for sex, age, and harmful habits (use of substances such as tobacco, electronic cigarettes, and alcohol). Sensitivity analysis was performed to assess the strength of the association and the influence of covariates. Additionally, among individuals who had undergone HSCT, a separate analysis was performed to investigate the relationship between the time since transplantation and the appearance of OPMDs.

Results

A total of 120 patients diagnosed with FA and no history of OSCC were evaluated. During the initial physical examination, ten patients were found to have lesions compatible with OSCC, all of which were confirmed through histopathological analysis leading to the exclusion of these individuals from the study. Thus, a total of 110 patients, with a mean age of 19.67 years, were included in the statistical analysis. The cohort compromised 56 men (51.91%) and 54 women (49.09%). Among the participants, 45 individuals (40.91%) presented at least one OPMD. When analyzed by age and HSCT status, statistically significant differences (p-value <0.05) were observed between the groups with and without OPMD.

The general characteristics of the sample are summarized in Table 1, while the distribution of OPMDs according to anatomical site within the oral cavity is illustrated in Figure 2.

The association between the presence of OPMDs and HSCT was found to be statistically significant in the univariate regression model, indicating that individuals who had undergone HSCT were up to four times more likely to present OPMDs compared to those who had not undergone the procedure. In the multivariate model, HSCT and age were identified as significant predictive variables for the presence of OPMDs (Table 2).

Of the individuals who had undergone HSCT, the time since transplantation showed an association with the presence of OPMDs in the univariate model (p-value <0.05). However, after adjustment in the multivariate model, this variable lost statistical significance (p-value >0.05), while the individual's age remained a significant factor. This finding suggests that age is a predictive variable for the presence of OPMDs and may act as a confounding factor, explaining the apparent significance of time since HSCT observed in the univariate model (Table 3).

Table 1 – General characteristics of the sample. The values represent the proportion of cases in each category according to the presence or absence of oral potentially malignant disorders.

	OPMD		p-value
	Absent	Present	
n	65	45	
Men – n (%)	28 (43.1)	28 (62.2)	0.075
Women - n (%)	37 (33.6)	17 (15.4)	
Age (years)*	17.17 (7.49)	23.29 (7.82)	**<0.001**
Geographical region - n (%)			0.785
Midwest	5 (7.7)	6 (13.3)	
Northeast	21 (32.3)	17 (37.8)	
North	4 (6.2)	2 (4.4)	
Southeast	13 (20.0)	8 (17.8)	
South	22 (33.8)	12 (26.7)	
Non-White - n (%)	47 (72.3)	28 (62.2)	0.364
White - n (%)	18 (16.4)	17 (15.4)	
HSCT – n (%)	43 (66.2)	40 (88.9)	0.012
Time after HSCT (years)**	7 (2.00–12.00)	9.50 (6.00–18.25)	0.014
Age at HSCT (years)**	9.50 (7.75–12.00)	9.50 (7.75–14.00)	0.605
Type of HSCT - n (%)			
Matched related HSCT	19 (43.2)	17 (41.5)	1.000
Unrelated HSCT	16 (36.4)	10 (24.4)	0.336
Umbilical cord HSCT	1 (2.3)	2 (4.9)	0.950
Haploidentical HSCT	9 (20.5)	11 (26.8)	0.662
Harmful health habits - n (%)	1 (1.5)	4 (8.9)	0.176

HSCT: Hematopoietic Stem-Cell Transplantation.

* The mean and standard deviation were used for variables with a normal distribution.

** The median and interquartile range were applied to continuous variables with non-normal distributions.

After conducting the sensitivity analysis, age and the occurrence of HSCT remained statistically significant predictors across all models (Table 4).

Discussion

The life expectancy of individuals with FA has improved in recent years, largely due to advances in HSCT, which have led to better outcomes in the treatment of bone marrow failure [13]. As a result, another major challenge faced by this population has gained increasing importance: the incidence of malignant neoplasms.

The incidence of OSCC in individuals with FA is up to 700 times higher compared to individuals without the disease matched for age and sex [22]. Moreover, unlike the general population, cancer in FA patients typically manifests in young adults of around 26 years of age [22,23], with a slight predominance in women (2:1) [24,25].

Regarding the tumor site, the lateral border of the tongue is the most commonly affected site, followed by the gingiva, buccal mucosa, and retromolar trigone. Furthermore, FA

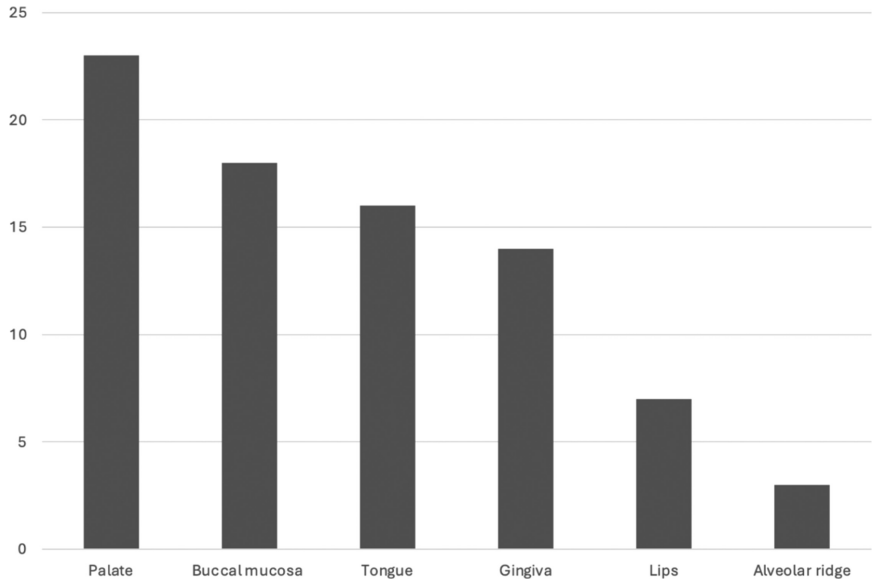

Fig. 2 – Distribution of oral potentially malignant disorders (OPMDs) by anatomical site. Frequency of OPMD occurrence across different regions of the oral cavity.

Table 2 – Association between hematopoietic stem cell transplantation and oral potentially malignant disorders (Logistic Regression).

	OR	95% CI	p-value
Univariate model			
HSCT	4.09	1.41 - 11.84	**0.009**
Multivariate model			
HSCT	6.23	1.55 - 25.04	**0.01**
Age	1.12	1.05 - 1.19	**< 0.001**
Sex	2.46	0.98 - 6.17	0.054
Harmful health habits	7.38	0.55 - 98.98	0.131

OR: Odds Ratio; 95% CI: 95% confidence interval; HSCT: Hematopoietic Stem-Cell Transplantation.

Table 4 – Sensitivity analysis.

	OR	95% CI	p-value
Model 1			
HSCT	4.12	1.3–13	**0.016**
Model 2			
HSCT	4.12	1.3–13	**0.016**
Age	1.11	1.05–1.18	**<0.001**
Model 3			
HSCT	4.19	1.29–13.61	**0.017**
Age	1.12	1.06–1.2	**<0.001**
Sex	2.85	1.16–7.02	0.022

OR: Odds Ratio; 95% CI: 95% confidence interval; HSCT: Hematopoietic Stem-Cell Transplantation.

patients have a higher tendency to develop multiple tumors [25,26]

In general, oral lesions are highly prevalent in the post-transplant period. Many of these lesions are traumatic, frictional, or infectious in nature, and as their potential for malignant transformation is not in question [11], these patients were excluded from this study similar to previous studies [27]. However, some lesions develop independently and may carry a risk of malignant transformation over time. In this study, the classification of OPMDs was based on clinical features after the exclusion of differential diagnoses. Biopsies were recommended only in carefully selected cases, in accordance with standard practice, particularly given that these individuals are already under continuous and intensive medical care. Consequently, the number of patients who underwent histopathological examinations was limited. To be classified as an OPMD, lesions had to be non-removable by scraping and have no identifiable traumatic cause, thereby distinguishing them from frictional keratosis. This approach adhered to the World Health Organization (WHO) criteria for defining leukoplakia and erythroplakia [11,28].

Table 3 – Association between oral potentially malignant disorders and time after hematopoietic stem cell transplantation (Logistic Regression).

	OR	95% CI	p-value
Univariate model			
Time post-HSCT	1.11	1.03 – 1.2	**0.009**
Multivariate model			
Age	1.1	1.02 - 1.19	**0.013**
Sex	1.72	0.82 – 5.8	0.11
Harmful health habits	1.06	0.96 – 1.16	0.23

OR: Odds Ratio; 95% CI: 95% confidence interval; HSCT: Hematopoietic Stem-Cell Transplantation.

It is also important to emphasize that, in the clinical practice of this population, professionals face significant challenges in distinguishing oral manifestations and accurately identifying lesions during OPMD screening. The clinical examination in FA is often complicated by microstomia [29] with this difficulty being further exacerbated in the post-HSCT setting. It is not uncommon for patients to present with

generalized oral mucosal erythema, whitish or brownish striae with a lichen planus-like appearance, and multiple overlapping lesions, making it difficult to determine which lesions should be considered potentially malignant.

These features often differ clinically from the classic OPMDs (OL, OE, OLE) and may closely resemble chronic oral GvHD [19,20,28]. Given the wide spectrum of oral manifestations, the frequent coexistence of overlapping lesions (particularly after HSCT), and the common absence of histopathological confirmation of chronic oral GvHD, this present study focused exclusively on the overall clinical diagnosis of OPMDs, without distinguishing between specific subtypes. Nevertheless, the importance of carefully assessing all oral alterations during the oral examination of this unique population must be emphasized, as a comprehensive and systematic evaluation is crucial for appropriate monitoring and early detection of lesions with malignant potential.

In addition to their heightened susceptibility to malignancies, the prognosis of OSCC in FA patients is particularly concerning. Treatment is typically limited to surgical tumor removal due to the pronounced hypersensitivity these patients exhibit to chemotherapy and radiotherapy, which are generally reserved for more advanced cases with a poorer prognosis [6,26]. Consequently, screening for OPMDs emerges as a key strategy for preventing OSCC in this population. Understanding the potential factors associated with the presence of these oral lesions represents a crucial first step toward enhancing clinical decision-making and optimizing treatment strategies.

A significant percentage of evaluated patients (40.91%) presented with at least one OPMD. The most affected site for OPMDs was the palate (15.97%), corroborating previous findings by Grein-Cavalcanti et al [7]. Interestingly, the buccal mucosa was the second most affected site for OPMDs in this population, aligning with the findings of Mattos et al. who reported a case series of oral cancer at this site [30]. In contrast, studies on the general population without FA indicate that leukoplakia more frequently develops on the lateral/ventral tongue and buccal mucosa, with the palate being an uncommon site [11,12].

The prevalence of OPMDs observed in this study is higher than that reported by other authors who specifically evaluated the prevalence of leukoplakia. For example, Grein-Cavalcanti et al. reported a prevalence of 12%, however, their sample was younger and only included patients who had not undergone HSCT [7]. When considering patients without HSCT in the current study, the prevalence of OPMDs drops to 4.54%, further highlighting the impact of HSCT on the development of lesions. Similarly, Archibald et al. reported a rate of 8.6% for leukoplakia though their study population had a mean age of 11.3 years, which is notably younger than the population in the present study [27]. These differences underscore the importance of age and treatment history in the prevalence of OPMDs among FA patients.

HSCT, along with patient age, was identified as a significant factor associated with the presence of OPMDs. It is already described in the literature that patients undergoing this hematological treatment have a higher prevalence of OSCC [15], and tend to develop the disease at an earlier age compared to those who do not undergo HSCT [10,13,26]. A similar trend seems to occur with OPMDs [31], which may explain the differences in the prevalence of leukoplakia observed in studies evaluating individuals prior to HSCT [7]. One possible explanation for the increased frequency of OPMDs after transplantation is the development of chronic oral GvHD, which may create a microenvironment conducive for epithelial dysplasia and malignant transformation [19,20]. Although chronic GvHD was not analyzed separately in the present study due to the absence of systematic histopathological confirmation, lesions exhibiting lichenoid characteristics were included within the overall OPMD group. Therefore, it is possible that some of the lesions classified as OPMDs in this study may in fact represent manifestations of chronic oral GvHD. This diagnostic overlap reflects the inherent challenges in clinically distinguishing these entities in patients with FA, particularly in the post-HSCT setting.

In the univariate analysis, variables such as sex, ethnicity, and region of origin were not statistically significant and showed no association with OPMD prevalence. However, previous studies suggest that women may have a higher risk of leukoplakia malignancy [32]. Regarding HSCT, the type of transplant did not appear to influence oral lesion development. Future studies focusing on the degree of oral mucositis and chemotherapeutic agents used during HSCT conditioning may provide valuable insights into their long-term effects on oral lesion development.

Tobacco and alcohol consumption were not associated with OPMDs in this study, likely due to the young age of the cohort; OPMDs related to these risk factors typically manifest in older individuals after prolonged exposure. Moreover, it is important to note that in a non-FA population, both alcohol and tobacco consumption do not appear to influence malignant transformation of leukoplakia [11,12].

Some limitations of this study include its cross-sectional design, which precludes the assessment of the progression of OPMDs over time, and the lack of histopathological diagnoses to assess the presence of tissue dysplasia and chronic oral GvHD [20,32]. Longitudinal studies separating OPMDs into their variations with rigorous classification are necessary to understand the risk of malignant transformation of these lesions [33].

Conclusions

This study provides important data on the overall prevalence of OPMDs in individuals with FA, a population known to be at particularly high risk for malignant transformation. The findings of this study show that OPMDs are more prevalent among young adults who have undergone HSCT, regardless of sex or time since transplantation. These results reinforce the need to consider OPMDs in FA as distinct clinical conditions due to the unique characteristics of this population.

However, future studies should clearly distinguish OPMDs according to their specific subtypes, particularly in the context of chronic GvHD. This distinction requires histopathological confirmation, which was limited in the present study and represents an important limitation.

Longitudinal studies are essential to monitor the progression and possible malignant transformation of these lesions

over time in individuals with FA. In this context, the present study should be considered a starting point for future subtype-specific and prospective research aimed at improving surveillance and clinical management strategies in this high-risk population.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

Generative AI and AI-assisted technologies were NOT used in the preparation of this work.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors of the article "Potentially Malignant Oral Disorders in Patients with Fanconi Anemia: A Cross-Sectional Study" declare that there is no conflict of interest.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Hemovigilance in a Brazilian Amazon blood center: A temporal analysis of 5-year consumption patterns

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ARTICLE INFO

Article history:
Received 27 August 2025
Accepted 17 March 2026
Available online 13 May 2026

Keywords:
Blood components
Hemotherapy
Blood treatment
Acute treatment
Brazilian amazon

ABSTRACT

Background: The transfusion of blood components remains a cornerstone in the management of hematological and onco-hematological diseases. Effective blood bank management, combined with the development of predictive models for component utilization, is essential to anticipate local demands, optimize resource allocation, and support the formulation of evidence-based public health strategies.
Aims: To analyze temporal trends in the consumption of blood components at a reference center in the Brazilian Amazon over a five-year period (2019–2023) and to develop predictive models to forecast future demand.
Methods: Transfusion records from the Outpatient and Inpatient Departments were retrospectively collected and categorized by component type. Time-series and regression analyses were applied to assess consumption dynamics. Machine learning models using Auto Regressive Integrated Moving Average and Random Forest algorithms were employed to predict future blood demand.
Results: Overall transfusion demand decreased during the study period; however, irradiated platelet concentrates, cryoprecipitate, and plasma by apheresis showed significant increases, particularly in the Inpatient Unit. These components are predominantly indicated for immunosuppressed patients, such as those undergoing chemotherapy or bone marrow transplantation.
Conclusion: The rising demand for irradiated components reflects the clinical complexity of patients treated in a regional hemotherapy referral center. The findings of this study high-

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https://doi.org/10.1016/j.htct.2026.106460
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light the importance of forecasting tools in supporting transfusion safety, optimizing blood bank management, and guiding public health strategies in the Amazon.

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Introduction

Transfusion therapy has been used since before the 1900s as a palliative treatment for various medical conditions; however, the majority of transfusion demands are associated with hematological malignancies [1–3]. While numerous studies have focused on patient-specific responses to blood components, there remains a significant gap in understanding the transfusion profiles and operational dynamics of blood banks. In Brazil, hemotherapy is based on a voluntary, non-paid model as stated by National Politics of Blood and Products [4]. Data regarding blood consumption varies by Brazilian region, with the highest demand in the southeastern and southern regions [5]. The state of Amazonas is the largest by territory, yet it relies on a single blood bank located in the capital, which is responsible for supplying blood to all 39 healthcare units in the capital city, as well as to 54 municipalities across the state and several neighboring regions.

Data from 2021 indicates that over 23,000 liters of blood were distributed in the state of Amazonas, with an average of 235 transfusions performed daily. Of these, approximately 96% took place in the capital, while only 4% occurred in other municipalities across the state [6]. These Fig. underscore the critical importance of blood donation and the need for a deeper understanding of hospital transfusion profiles and patient demand for blood components.

Several guidelines have been established to provide recommendations for the clinical use of blood, with a particular emphasis on the components. Following donation, whole blood is centrifuged and separated into individual components, allowing a single donation to yield red blood cells (RBCs), platelet concentrate (PC), fresh frozen plasma (FFP), and cryoprecipitate (Cryo). These components can also be obtained through apheresis, a technique that yields higher concentrations of specific components but involves greater operational costs. Each of these blood products has distinct therapeutic purposes and plays a critical role in patient management across a variety of clinical settings [7].

Hemovigilance refers to a set of procedures that encompass the entire blood cycle, from donor recruitment to post-transfusion patient follow-up. It is primarily aimed at monitoring and preventing transfusion-related adverse events. This process is essential for maintaining quality standards, ensuring the safety of therapeutic procedures, identifying adverse reactions, and guiding system improvements [8]. As part of hemovigilance, understanding the patterns of blood component usage over time is crucial for planning future donation campaigns and optimizing resource allocation. Despite its importance, few studies have addressed this approach, even though it is well established that blood donation rates fluctuate throughout the year. Managing large datasets and extracting meaningful insights remain significant challenges for blood bank managers, further emphasizing the need for data-informed strategies in both blood donation and usage [9–12].

This study presents an analysis of hemovigilance data collected over a five-year period (2019–2023) from the regional blood center in the state of Amazonas, Brazil.

Materials and methods

Data availability

This is a descriptive and retrospective study based on data concerning blood component requests and transfusion records from the Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas (HEMOAM), located in Manaus, the capital of the state of Amazonas, Brazil. HEMOAM is the only specialized public hospital in the largest state of Brazil and serves most patients with blood disorders, including primarily onco-hematological conditions (such as leukemia, Hodgkin's and non-Hodgkin lymphomas, and multiple myeloma), as well as sickle cell disease, thalassemia, and coagulation disorders.

Information regarding blood transfusions is recorded daily in the national Hemovigilance software (Hemosys) by authorized hospital staff. This tool manages all transfusion activities within the hospital, and all the data used for analysis was extracted from this software platform. Daily transfusion records from both outpatient (Transfusion) and inpatient (Hospitalization) Departments were collected for the period from January 2019 to December 2023. The Inpatient Department primarily manages patients with acute and chronic leukemia, aggressive lymphoma, severe coagulopathies, and hematopoietic stem cell transplant recipients, all of whom present high transfusion requirements. The Outpatient Department, on the other hand, treats patients with chronic blood disorders, such as sickle cell disease and patients receiving maintenance chemotherapy. Blood components were categorized based on clinical indication and medical specialty, as outlined in Table 1.

Data analysis of crude data

A database was constructed using Microsoft Excel 2010 and analyzed in RStudio. The total number of transfusions was aggregated by day, month, and year over the entire study period. Additionally, the data was stratified by blood component type and by the requesting hospital department. For data visualization, Local Polynomial Regression (LOESS) with

Table 1 – Description of types of blood components used and their management prior to transfusion.

Blood component	Type of component	Description
RBCs	RBCs	Whole blood-derived from blood donations
	Irradiated	Treated with ionizing radiation
	Washed	Washed with isotonic saline
	Filtered	Leukoreduced
	Phenotyped	Phenotyped for ten antigens
PC	PC	Platelets recovered from whole blood donations
	Irradiated	Treated with ionizing radiation
	PCa	Obtained from a single donor via automated apheresis
	Irradiated PCa	Treated with ionizing radiation
FFP		Plasma rich in coagulation factors and plasmatic protein, obtained after double centrifugation and removal of RBCs and PC
Cryo		Precipitated portion of FFP rich in Fibrinogen and Factor VIII

RBC: Red Blood Cell; PC: Platelet Concentrate; PCa: Platelet Concentrates by apheresis; FFP: Fresh Frozen Plasma; Cryo: Cryoprecipitate.
Note: Components requiring multiple interventions (e.g., washed and filtered RBCs) are recorded as separate sub-categories. This classification identifies the specific transfusion requirements of different clinical units and accounts for the cumulative processing steps performed prior to administration.

standard error bands was applied, and variation curves were generated using appropriate R packages.

Poisson regression models

Poisson regression models were employed to analyze the association between transfusion counts and hospital departments and to evaluate daily transfusion rates across different hospital departments over the study period. In the first step, the number of transfusions was treated as the dependent variable that included departments as covariates, estimating the log-relative rate of variation over time. Subsequently, the effect of department-specific dynamics was examined by adjusting for the day of transfusion as a covariate. All analyses were conducted using R software, with a 95% confidence interval. Blood components that showed a positive log-relative rate were selected for subsequent forecasting analyses.

Auto regressive integrated moving average (ARIMA) modeling

To explore time trends and forecast future transfusion demands, ARIMA models were applied to the components identified as increasing in use by Poisson regression. Each ARIMA model was fit separately by component using monthly data from 2019 to 2022, while data from 2023 were used for validation. Optimal model parameters (p, d, q) were selected based on the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and diagnostic plots of the autocorrelation and partial autocorrelation functions. Model performance was assessed using the root mean square error (RMSE) and the Box-Ljung test to evaluate the independence of residuals.

Random forest

To complement the statistical models and evaluate the predictive performance of machine learning techniques, a Random Forest model was applied to predict transfusion volumes for the selected blood components. The dataset was randomly split, with 80% used for training and the remaining 20% for validation. The model was implemented using default parameters, and variable importance was considered to interpret the most influential features in the prediction of transfusion demand.

Results

Blood component use over the years: descriptive dynamics of crude values

To assess the dynamics of each blood component over time, transfusion data were grouped by year and month to identify trends throughout the study period. Although both departments displayed similar overall patterns in blood component usage, irradiated PC emerged as the most frequently requested plasma component, particularly in the Inpatient Department. In contrast, RBC components (specifically washed, filtered, filtered and washed, and irradiated and washed) were more commonly used in the Outpatient Department. The distribution and dynamics of each blood component by department are illustrated in Figure 1.

The transfusion patterns differed significantly between departments over the years. Fisher's Exact Test revealed statistically significant differences in annual transfusion frequencies between departments for multiple components (Figure S1)

The area under the curve (AUC) for each component across departments is presented in Figure S2. Although many components showed similar AUC values between departments, RBC components (except phenotyped & irradiated RBCs), as well as FFP and certain PC subproducts, exhibited higher AUCs in the Outpatient Department.

Monthly distribution patterns varied considerably; while some components showed peaks during specific periods, others followed different trajectories. These differences in demand between departments are likely influenced by the clinical profiles and treatment needs of the patients they serve.

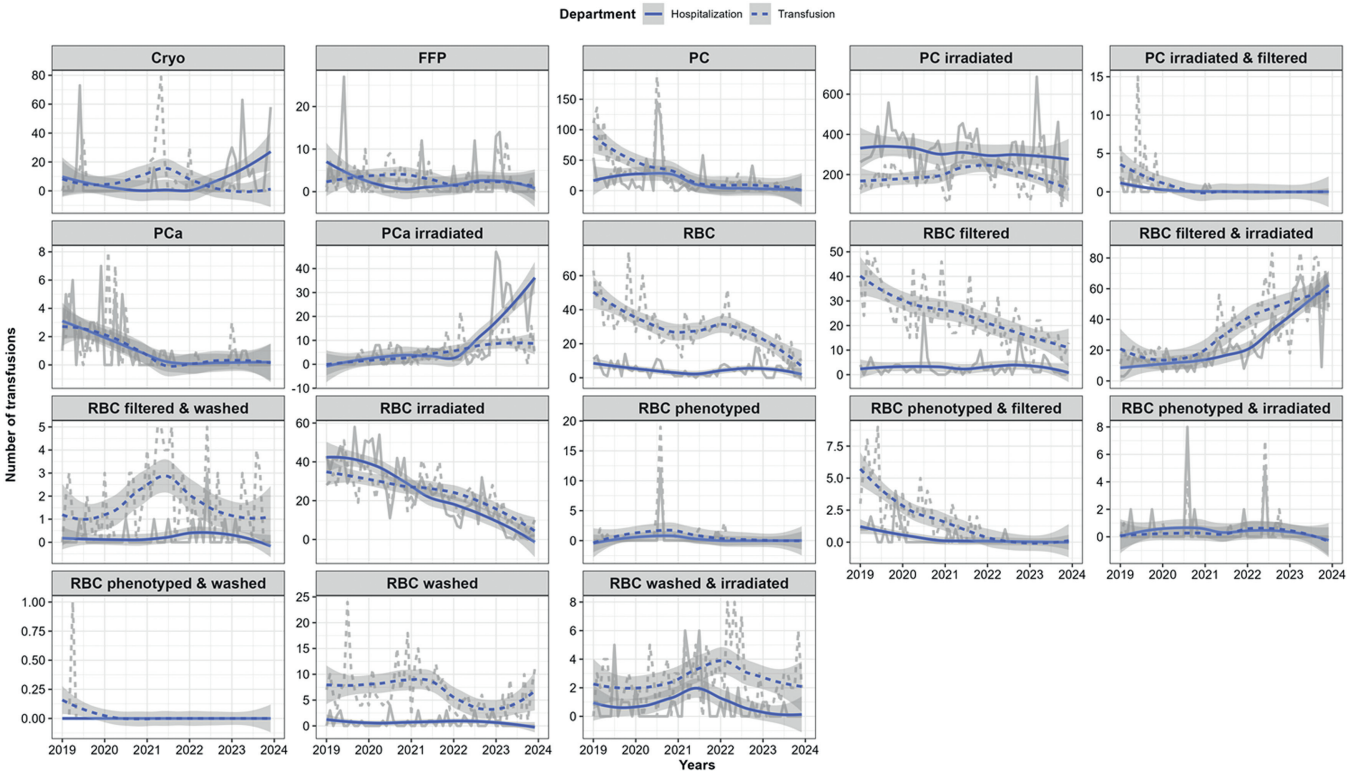

Fig. 1 – Descriptive data regarding blood usage. The dynamics in both hospital departments were reported daily from 2019 –2023. Crude data of each blood component/day are represented by the grey lines, and Locally Estimated Scatterplot Smoothing (LOESS) by blue lines. Departments are categorized by line style as Inpatient (continuous) and Outpatient (dashed). Cryo: Cryoprecipitate; FFP: Fresh Frozen Plasma; PC: Platelet Concentrate; PCa: Platelet Concentrate by apheresis; RBC: Red Blood Cell

Yearly seasonality on blood transfusion requirement per month of the year

When analyzing monthly dynamics, transfusion patterns for components differed significantly between departments when stratified by month (Figure S3). For most of these components, peak transfusion volumes occurred in the middle of the year, suggesting a potential seasonal influence on demand.

Logistic regression analysis of daily trends in blood component usage

Given the apparent seasonal variation in component demand, this study investigated the influence of time (measured in days) on transfusion dynamics across the study period. Although daily transfusion numbers fluctuated, overall usage patterns between departments remained relatively consistent.

This study utilized a Poisson logistic regression model to analyze how the demand for various blood components changed over time (measured in days). While daily numbers fluctuated, the analysis identified clear, statistically significant temporal trends for nearly every component studied.

The majority of blood components, including FFP, PC, and various RBC products, showed a slight but statistically significant decrease in usage per day.

Conversely, three specific components (Cryo, Irradiated PCa, and Filtered & Irradiated RBCs) showed an increasing trend in demand over the study period (Table 2).

The Outpatient Department demonstrated a distinct shift in transfusion requirements compared to inpatient services. There was a significant decrease in the use of irradiated PCa, irradiated PC, and Cryo.

However, there was a substantial and statistically significant increase in the usage of most other products, particularly RBC variations. Notably, washed RBC, filtered RBC, and combinations of those treatments saw increases exceeding 200% (Table 3).

Data-driven forecasting of blood product usage: strategies for hospital resource planning

To estimate the future demand for blood components, the focus was on the components that showed an increasing trend over time based on the day-day analysis, specifically Cryo, irradiated PCa, and filtered & irradiated RBC. A predictive ARIMA model was trained using data from January 2019 to December 2022 to forecast usage patterns for 2023, with model performance validated by comparing predicted values against actual data. An automatic model selection process was used to identify the optimal ARIMA configuration for each component, with selection based on minimal residuals

Table 2 – Statistically significant daily trends in component usage via poisson logistic regression (p-value ≤0.001).

Blood Component	Daily Percentage Change
PCa (irradiated)	+0.19
RBC (filtered & irradiated)	+0.10
Cryo	+0.03
PC (irradiated)	−0.007
FFP	−0.04
RBC (washed)	−0.04
RBC	−0.05
RBC (filtered)	−0.05
RBC (irradiated)	−0.09
RBC (phenotyped)	−0.10
PC	−0.16
PCa	−0.20
RBC (phenotyped & filtered)	−0.26
PC (filtered & irradiated)	−0.50

FFP: Fresh Frozen Plasma; PC: Platelet Concentrate; PCa: Platelet Concentrate by apheresis; RBC: Red Blood Cells; Cryo: Cryoprecipitate.

as well as an evaluation of Autocorrelation Function and Partial Autocorrelation Function diagnostics (Figure S4).

Based on the training data, the ARIMA model predicted an initial increase in 2023 followed by stabilization for both Cryo and irradiated PCa. The usage of filtered and irradiated RBCs was forecasted to remain stable throughout the year. However, actual 2023 data revealed more frequent and pronounced peaks in Cryo usage compared to previous years. The model demonstrated a strong fit (RMSE = 17.81), and the Ljung-Box test confirmed the absence of significant autocorrelation in the residuals (χ^2 = 32.9; p-value = 0.1), indicating a robust model for the underlying trend. Similarly, although irradiated PCa followed an upward historical trend and the model showed a good fit (RMSE = 9; Box-Ljung test χ^2 = 14.2; p-value = 0.94), its variability in 2023 exceeded that observed in the training period. For Filtered and irradiated RBC, while the model predicted a stable trend (RMSE = 21.7; Box-Ljung test χ^2 = 16.2; p-value = 0.87), actual usage in 2023 included a peak higher than any seen during the training period (Figure 3). Overall, the predicted values were consistently lower than the values observed in 2023. Nonetheless, the model captured the temporal structure of the data well, as indicated by the non-significant results of the Box-Ljung tests. This discrepancy between predicted and actual values highlights both the increasing demand for these blood components and the challenges involved in modeling such fluctuations. These findings reinforce the importance of robust forecasting tools to support healthcare planning and resource allocation.

Additionally, a random forest model was applied using the same dataset, this time considering all usage patterns collectively. In several instances, the predicted values from the random forest model were higher than the observed values, suggesting that it may be a more robust tool for capturing complex patterns in component usage. This approach could enhance predictive capacity and support the development of more responsive and efficient blood management and hospital policy strategies (Figure 3).

Table 3 – The following breakdown compares the usage of blood products in the Outpatient Department relative to the Inpatient Department (the reference group), adjusted for date as a potential confounder.

Blood Product	Percentage Change	p-value
RBC (washed)	+227	<0.001
RBC (filtered)	+205	<0.001
RBC (filtered & washed)	+204	<0.001
RBC	+181	<0.001
RBC (phenotyped & filtered)	+164	<0.001
PC (filtered & irradiated)	+122	<0.001
RBC (washed & irradiated)	+115	<0.001
RBC (phenotyped)	+104	<0.001
PC	+68.7	<0.001
Cryo	−18.3	0.01
PC (irradiated)	−46.6	<0.001
PCa (irradiated)	−61.3	<0.001

RBC: Red Blood Cells; PC: Platelet Concentrate; PCa: Platelet Concentrate by apheresis; Cryo: Cryoprecipitate.(Figure 2).

Transfusion reaction profile across departments

Throughout the study period, the number of reported transfusion reactions was monitored. A reduction was observed in both departments from the beginning of the analysis; however, an upward trend began in 2022. While the Outpatient Department showed a slight increase, the Inpatient Department experienced a more pronounced rise in transfusion reactions (Figure S5). Due to data limitations, it was not possible to analyze the reactions by specific blood component, thus, only the overall incidence across departments could be assessed.

Discussion

The current study reflects the transfusion reality of a reference center for hemotherapy procedures located in the northern region of Brazil. The patient profile is predominantly onco-hematological disorders. A temporal analysis of transfusion dynamics of two specialized transfusion therapy departments can contribute to strategies and local patient blood management. Interestingly, this study identified consistent increases in Cryo, irradiated CPa, and filtered & irradiated RBC over a five-year period.

The higher utilization of irradiated components observed in the present study may be attributed to the presence of an active transfusion committee. This committee establishes measures to enhance transfusion safety, minimize the risk of transfusion-associated graft-versus-host disease, and reduce dispensing errors in critically ill onco-hematological patients [13,14].

The feasibility of predicting future blood component usage was demonstrated using a Random Forest modeling approach. The model showed strong agreement with the observed values, highlighting its potential for optimizing quality control, improving the monitoring of transfusion reactions, and guiding resource allocation and strategic planning in hospital management. Additionally, the findings

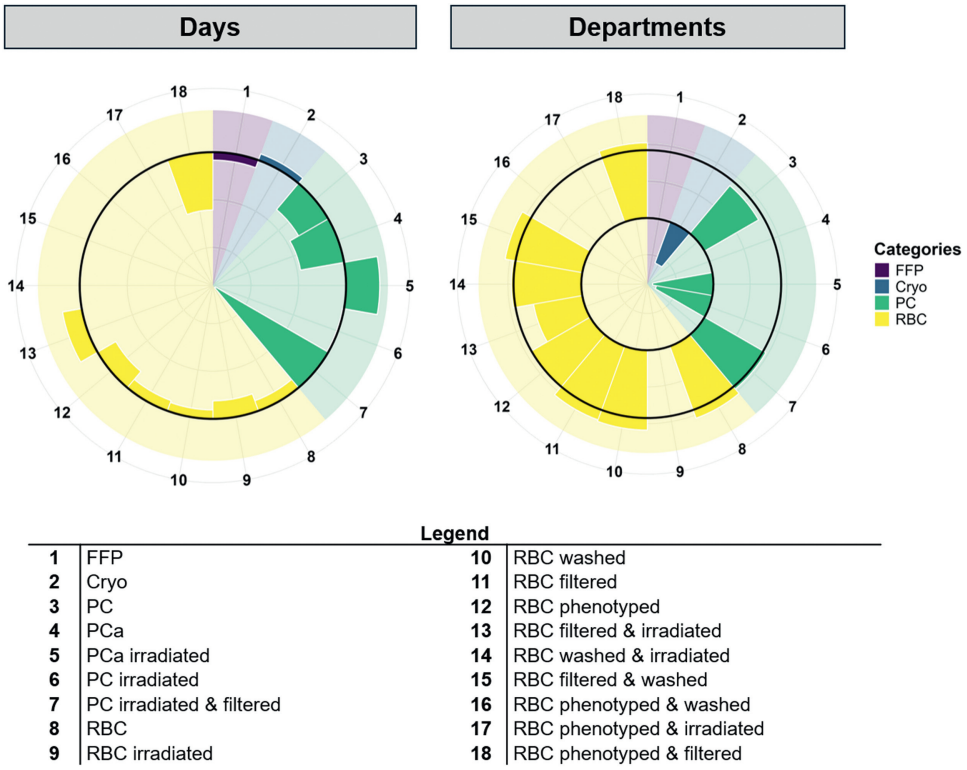

Fig. 2 – Temporal and interdepartmental dynamics of blood component usage. Left (Days): Poisson regression model illustrating daily percentage changes in component demand. The radial axis (log scale: −0.7 to 0.22) depicts the daily dynamic change; the solid black line represents the baseline (zero change). Usage peaked mid-year, followed by a longitudinal decrease in almost all blood components across the study period. Right (Departments): Comparative usage between departments, with the Inpatient Department serving as the reference group. The radial axis (log scale: −4.5 to 7.0) indicates the percentage change for the Outpatient Department. Concentric black lines represent the baseline (inner circle, value of 0) and the 100% increase threshold (outer circle, log value of 100). RBC: Red Blood Cells; PC: Platelet Concentrate; PCa: Platelet Concentrate by apheresis; FFP: Fresh Frozen Plasma; Cryo: Cryoprecipitate

emphasize the importance of seasonality in transfusion patterns, which can assist blood donation campaigns, donor screening, and overall blood surveillance.

It is important to acknowledge that the study period overlapped with the COVID-19 pandemic. While studies have reported significant disruptions in blood donation and transfusion patterns during this period, mainly due to social isolation and decreased donor availability [15,16], the data of this study suggest that the trends observed in 2019 were largely maintained. An overall increase in transfusion rates was described previously [12], however, as an effect of the pandemic, a general reduction in certain blood components was noted, particularly RBC products, possibly influenced by a drop in elective surgical procedures and broader shifts in healthcare utilization during the pandemic [15]. The transfusion profile of patients seemed not to change the tendency that was observed in the previous year [2019]. The reduction seen in components, on the other hand, could have occurred either due to natural reductions or due to COVID-19, especially in respect to RBC products.

The mid-year surge in transfusion demand aligns with seasonal trends observed in other regions, especially during holidays and school vacations, when accidents and emergency procedures tend to rise [17]. Interestingly, this pattern differs from other Brazilian hospitals, such as those in Minas Gerais, where distinct temporal trends have been reported [18]. Notably, seasonal factors also affect donor availability, as holidays and family vacations coincide with a reduction in available donors [19]. In Brazil, summer school holidays occur from late December to February, with another break in July. These periods involve family travel and even affect physician availability, which may contribute to the different healthcare service patterns observed later in the year [17].

Higher demands for Cryo, irradiated PCa, and irradiated PC products were observed in the Inpatient Department, which handles acute and emergency cases. These components are often used in patients experiencing coagulopathies, undergoing oncological treatment or bone marrow transplants, or requiring hemostatic support for surgical procedures [20–23], all of which are the profile of patients attended by this department. The use of irradiated products is particularly relevant for onco-hematological patients to prevent transfusion-associated graft-versus-host disease [24–26]. Given that this hospital is the state reference center for hematological disorders [27], it is expected that such specialized components would be more frequently used in this setting.

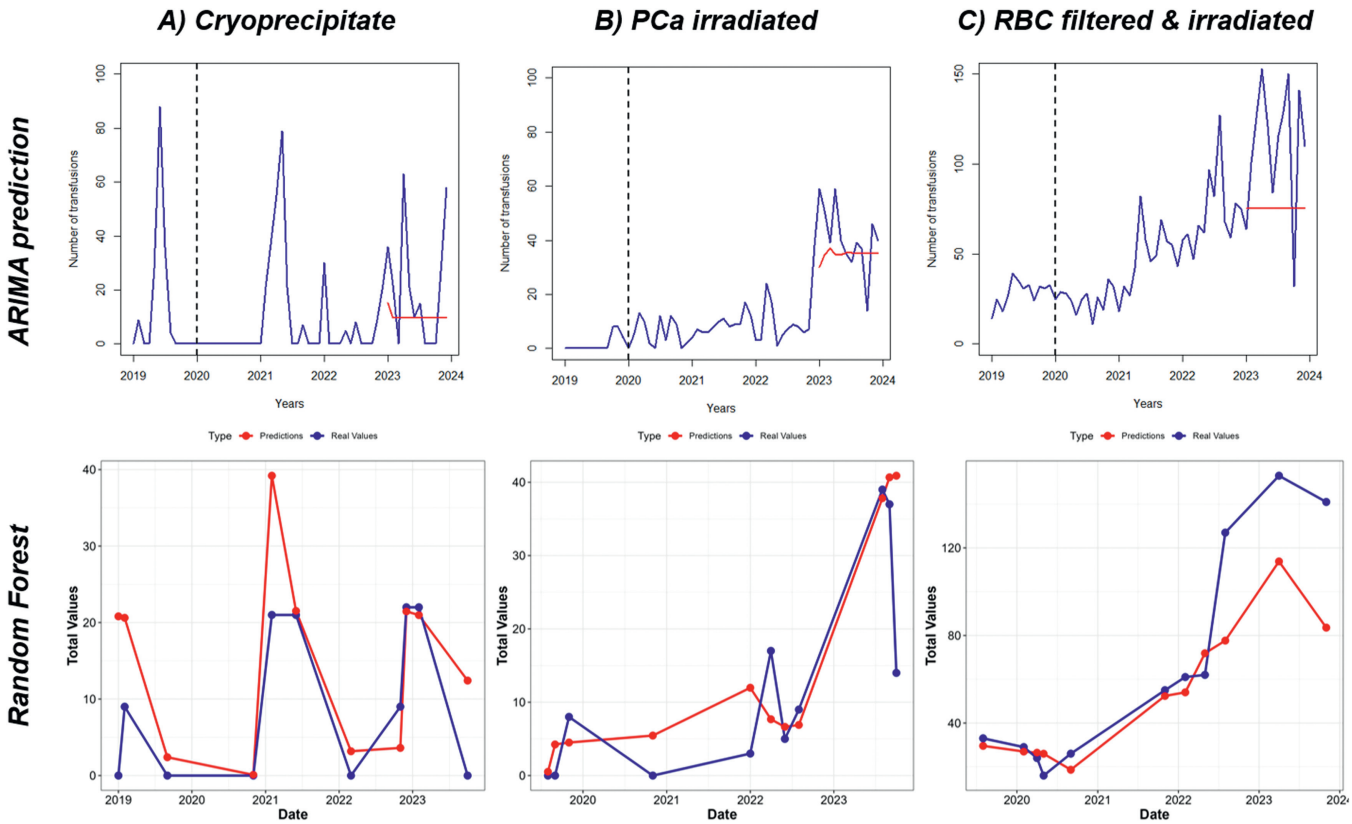

Fig. 3 – Prediction of blood components for 2023 based on auto regressive integrated moving average (ARIMA) model. The prediction of blood components by the ARIMA model and Random Forest analysis regarding the usage of increased blood components: (A) cryoprecipitate, (B) irradiated Platelet Concentrate by apheresis (PCa) and (C) filtered & irradiated Red Blood Cells (RBC). Red lines represent the predicted values in the models, while blue lines are the real values observed. The dashed line represents the beginning of 2020, when the COVID-19 pandemic started.

The profile of patients in the Inpatient Department, who often present with acute bleeding conditions, supports the higher usage of hemostatic components. Despite the limited literature on blood component usage across hospital departments, the findings of this study underscore the need to tailor transfusion strategies to patient profiles and departmental needs [24]. Additionally, the increasing incidence of leukemia in the state of Amazonas [28] may reflect improved diagnostic capabilities and greater access to treatment, contributing to the rising demand for components like Cryo and irradiated PCa, which are recommended to minimize transfusion-related adverse events [29–31]. Given that patients treated at this institution often require frequent transfusions, an observed increase in certain components is expected. However, hospitals dealing with broader patient populations may exhibit different transfusion profiles.

The application of machine learning to healthcare data management, particularly in transfusion medicine, remains limited. While we utilized logistic regression, ARIMA and random forest models, there is potential for broader implementation of predictive analytics. To our knowledge, the study by Mitterecker et al. [32] remains the only work applying machine learning techniques to predict transfusion practices in general patient populations. In contrast, the present study focuses on a longitudinal assessment of a highly transfusion-dependent cohort, emphasizing the need for future research incorporating patient characteristics and follow-up data to refine transfusion management strategies.

An increase in reported transfusion reactions beginning in 2022 likely reflects the impact of a targeted training initiative introduced that year. This program included clinical case discussions, multidisciplinary education sessions, and regular departmental meetings. Consequently, reactions that previously went unrecognized began to be correctly identified and reported. This phenomenon is well-documented in Brazil and in other countries, where underreporting is often due to limited technology and data management infrastructure [33]. Sobral et al. [34] reported that 26% of RBC transfusions in the Federal District had no associated reaction report, emphasizing an urgent need for improved hemotherapy safety and documentation systems [18,35].

Time-series analysis remains underutilized in hemotherapy worldwide. Combined with the lack of technological infrastructure to process health data, especially in remote regions, this highlights a critical need for robust blood surveillance systems [33]. The current study contributes to filling this gap by presenting five years of transfusion data from a major blood center in Brazil. Nonetheless, we acknowledge limitations, including potential underreporting and the

absence of an electronic system for managing transfusion and adverse reaction data.

A limitation of this study is the lack of data regarding the full transfusion supply chain; our analysis focuses on clinical administration and does not account for prior stages such as donation, processing, storage, or distribution. However, this study primarily examined the infusion phase, which represents a critical point in transfusion practice with direct implications on patient outcomes. Our results provide a better comprehension of blood component patterns from a specialized center, to mitigate further storage protocols and implement new public health strategies and professional training.

The findings of this study demonstrate that the patient's profile has a direct influence on transfusion demand but more importantly highlights the importance of local analysis to comprehend blood demand heterogeneity in Brazil. Future studies must integrate data from all the steps of the blood transfusion cycle to provide a broader point of view and enhance a national overview of blood usage in the country.

Conclusions

In recent years, many hospitals have implemented new technologies and tools aimed at identifying innovative administrative strategies with greater efficiency, guiding future public health initiatives with higher impact and improved cost-effectiveness for health systems. The application of machine learning techniques holds considerable promise for enhancing data analysis, interpretation, and decision-making across various healthcare environments, including hospital settings. However, the application of such approaches within the field of transfusion medicine remains limited, due to both intrinsic and extrinsic challenges that hinder the development of effective public health measures in this domain.

The present study identified a consistent increase in the use of irradiated platelet concentrates (irradiated PC and irradiated PCa) and Cryo over the past five years, reflecting a growing demand for these components among patients with hematological and onco-hematological conditions. Furthermore, stratification by hospital department revealed that the Inpatient Department, responsible for urgent care, exhibited a higher demand for blood components associated with hemostatic support, consistent with the underlying disease burden in this particular patient population.

The data presented in this study contribute not only to local hospital planning but also to a broader understanding of transfusion practices. Analyzing temporal trends and integrating data through advanced technological tools should be considered a priority. These processes are vital for optimizing clinical decisions and guiding management strategies in transfusion medicine.

Author contributions

Conceptualization: T.A.L., J.S.C., A.L.S.-J. and E.C.C.; Data curation: C.H.A.C., T.R.A., A.L.S.-J. and E.C.C.; Formal analysis: J.A.C.C. and A.L.S.-J.; Funding acquisition: A.L.S.-J. and E.C.C.; Investigation: C.H.A.C., T.R.A. and J.A.C.C.; Methodology: C.H.A.C., T.R.A. and J.A.C.C.; Project administration: T.A.L., J.S.C., A.L.S.-J. and E.C.C.; Supervision: J.S.C., A.L.S.-J. and E.C.C.; Visualization: J.A.C.C. and A.L.S.-J.; Writing – original draft: C.H.A.C., T.R.A., and J.A.C.C.; Writing – review & editing: T.A.L., J.S.C., A.L.S.-J. and E.C.C. All authors reviewed and approved the final draft of the manuscript.

Ethics approval statement

Prior to data collection, this study was approved by the ethics committee of the Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas (HEMOAM) under protocol number #6.575.757.

Funding information

C.H.A.C. and T.R.A. holds an undergrad scholarship from Fundação de Amparo à Pesquisa do Amazonas (FAPEAM) via Programa de Apoio à Iniciação Científica from HEMOAM (PAIC—HEMOAM) for this work. J.A.C.C. and E.C.C. hold a MSc and PhD scholarship, respectively, from Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). A.L.S.-J. holds a post-doctoral fellowship from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Any of the funders had any decision on conceptualization, design, data collection, analysis, decision to publish nor preparation of this manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors would like to thank to the hospital staff for providing the information and materials to collect the data, especially Nursing Management, and the Research department for all their support. The authors declare that they have no competing interests.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2026.106460.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Demographic and prognostic factors affecting survival in acute myelomonocytic leukemia with abnormal eosinophils

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ARTICLE INFO

Article history:
Received 17 April 2025
Accepted 30 November 2025
Available online 13 May 2026

Keywords:
National cancer database
Prognostic factors
Insurance status
Socioeconomic factors
Overall survival
Age
Race
Comorbidities
Acute myeloid leukemia

ABSTRACT

Introduction: Acute myelomonocytic leukemia with abnormal eosinophils is an extremely rare, malignant acute myeloid leukemia subtype. Using the National Cancer Database, this study evaluated the demographic and prognostic factors affecting the overall survival and mortality rates of patients. To the extent of our knowledge, this is the most comprehensive study to date on the prognostic and socioeconomic factors of this leukemia subgroup.

Methods: Patients with acute myelomonocytic leukemia with abnormal eosinophils were identified in the National Cancer Database between 2004 and 2020 using the ICD-O-3 histology code 9871. Time-to-event outcomes were analyzed by the Kaplan-Meier method, log-rank tests, and multivariable Cox hazard regression models to understand the association of demographic and prognostic factors on survival.

Results: A total of 2138 patients were identified. Patients with a Charlson-Deyo score of 2 or 3 had a 91% (Hazard Ratio = 1.91; 95% Confidence Interval: 1.52–2.39; p-value ≤0.001) and 79% (Hazard Ratio = 1.79; 95% Confidence Interval: 1.34–2.40; p-value ≤0.001) increased mortality risk, respectively, compared to those with a score of 0. Compared to patients with private insurance/managed care, those with other government insurance, Medicaid, and Medicare had a 46% (Hazard Ratio = 1.46; 95% Confidence Interval: 0.87–2.45; p-value ≤0.156), 57% (Hazard Ratio = 1.57; 95% Confidence Interval: 1.26–1.97; p-value ≤0.001), and 32% (Hazard Ratio = 1.32; 95% Confidence Interval: 1.11–1.58; p-value ≤0.001) greater mortality risk, respectively.

Conclusion: Generally, older patients with comorbidities who did not have private insurance had poorer overall survival. No significant difference in overall survival was associated with median household income quartiles.

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https://doi.org/10.1016/j.htct.2026.106463

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Introduction

Acute myelomonocytic leukemia with abnormal eosinophils (AMMLEo) is an extremely rare, malignant acute myeloid leukemia (AML) subtype. Acute myelomonocytic leukemia (AMML) is a type of AML, a group of blood cancers marked by a rapid increase in the white blood cell count [1]. The most common cytogenetic changes in this form of leukemia include inv(16)(p13q22) or the variant t(16;16)(p13;q22). AML with inv(16)(p13q22), is commonly seen in young patients and typically exhibits both granulocytic and monocytic differentiation with abnormal eosinophils [2].

AML itself makes up about 15–20 % of all acute leukemia cases and according to Surveillance, Epidemiology, and End Results (SEER) program data, AMMLEo accounts for 5–8 % of all AML cases. AMMoL originates within the bone marrow and peripheral blood; while the disease manifests across a broad age spectrum, it is most frequently diagnosed in pediatric and young adult populations [3].

Chemotherapy is the standard treatment for AMMLEo [1,4]. AMMLEo presents with a documented five-year survival rate of around 51.7 %, regardless of grade. Survival analysis revealed that individuals with eosinophilia had an overall survival rate of 64 % at 36 months, with a median survival time of 23.3 months. Conversely, patients without eosinophilia exhibited a lower overall survival rate of 38 %, with a median survival time of 15.8 months [5]. Understanding the variables affecting prognosis holds significance for patients and healthcare providers. However, due to its rarity, there is a lack of comprehensive studies on factors influencing overall survival. Prior investigations have indicated that factors such as older age, complex cytogenetic abnormalities, and specific genetic mutations correlate with reduced overall survival, with the presence of certain chromosomal rearrangements associated with poor prognosis and more aggressive disease [6,7].

This study aims to comprehensively expand on factors influencing the prognosis of AMMLEo. An emphasis is placed on demographics including age, sex, race, income, and education as well as clinical aspects such as tumor size, analytical stage, neoadjuvant chemotherapy, neoadjuvant radiation, distance traveled for health care, and Charlson-Deyo (CD) comorbidity score. The goal is to offer a thorough understanding of how these factors collectively affect overall survival in individuals with this type of leukemia.

Materials and methods

This is a retrospective cohort study of patients diagnosed with AMMLEo from 2004 to 2020 and registered in the National Cancer Database (NCDB). The NCDB is a clinical oncology database derived from hospital registry data of >1500 Commission on Cancer-accredited facilities with information on patient characteristics, tumor staging, tumor histology, type of first treatment, disease recurrence, and survival. This patient data was made accessible to the authors through the Participant User Data Files program.

Patients with AMMLEo were identified from NCDB data using the ICD-O-3 histology code 9871, which classifies tumors based on their morphological and histological features. Patients were then selected if they had behavior code 3 (invasive). Patients were excluded from the cohort if they had any missing clinical or demographic data or if they had concurrent tumors.

Covariates

Patients were analyzed by age, sex, race, education, income, insurance status, tumor size, analytical stage, CD comorbidity score, primary anatomic site, primary radiation, primary and adjuvant chemotherapy, and distance traveled for health care.

Race was categorized into three groups: White, African-American, and Other. The race group categorized as other includes American Indian, Aleutian or Eskimo, Chinese, Japanese, Filipino, Hawaiian, Korean, Vietnamese, Kampuchean, Asian Indian or Pakistani not otherwise specified (NOS), Asian Indian, Micronesian NOS, Other Asian, Asian NOS, Oriental NOS, and Pacific Islander NOS.

Income was measured by median household income from 2016 to 2020 for the ZIP code where the patient resided at the time of diagnosis. Education was measured in 2020 by the percentage of residents within the patient's ZIP code of residence who did not graduate from high school.

The staging was measured by the NCDB analytical stage. Clinical staging was used when the variable NCDB analytical stage was not available. Insurance status was categorized into five groups: uninsured, private, Medicare, Medicaid, and other government insurance.

Distance traveled for health care was measured by the miles between the patient's residence and the hospital that reported the case. The CD score, which measures comorbidities, was used to classify patients into groups with scores of 0, 1, 2, and ≥3 [8].

The primary outcome of interest was overall survival, defined from the date of diagnosis until the date of death, censored at the date of last contact in the database. Independent prognostic factors were identified using a multivariable Cox hazard regression model. Kaplan-Meier curves were plotted and the overall survival of the variables of interest was measured at two, five, and ten years using survival tables. Variables included in the multivariable Cox model were age, sex, race, insurance status, education, income, CD comorbidity score, analytic stage, primary anatomic site, primary radiation, and primary and adjuvant chemotherapy, all of which were a priori variables of interest. Patients within the same facility were accounted for using a robust sandwich covariance matrix. LOESS methods were employed to examine the functional form of continuous variables, while log-log survival curves and the inclusion of time-dependent covariates were utilized to evaluate the proportional hazards assumption for each variable.

Statistical considerations

The descriptive statistics, unadjusted survival analysis, and multivariable analysis for this study were conducted using the IBM Statistical Package for the Social Sciences (SPSS) version 27 (IBM Corp., Armonk, NY). Patients with any missing clinical or demographic data were excluded from the cohort. Bonferroni correction was used to adjust the p-value threshold for multiple comparisons, ensuring the family-wise error

rate remained controlled at $\alpha = 0.05$. The University of Arizona Biomedical Institutional Review Board (IRB) reviewed this study (IRB Submission ID: STUDY00003534, Approval No 2001,750–01) and determined that it does not involve human subjects research as defined by the Department of Health and Human Services (DHHS) and Food and Drug Administration (FDA) regulations. Consequently, IRB approval and ongoing review were not required.

Results

After applying inclusion and exclusion criteria, a total of 2138 patients were included in this analysis after excluding 531 patients due to missing data. Table 1 lists the demographics and clinical factors of the cohort.

This cohort was slightly male-dominant (55.5 %) with an overwhelming majority of patients being White (86.4 %). The primary payor of diagnosis for patients was private insurance/managed care (50.7 %) followed by Medicare (30.1 %), which was followed by Medicaid (13.4 %). Patients were mostly spread among income brackets with the slight majority of patients earning more than $74,063 (35.0 %).

The majority of patients (51.1 %) were located in metro area counties with a population exceeding 1 million people. Furthermore, the average age at diagnosis was 51.4 years (standard deviation = 19.2; range: 0–90 years). The overwhelming majority of patients (73.8 %) had a CD score of 0, while 362 patients (16.9 %) had a CD score of 1. Figs. 1–6 show the Kaplan-Meier survival curves of patient cohorts separated by various demographic and clinical groups.

Table 1 – Demographics of the subjects.

Variable	n = 2138 (100 %)
Sex – n (%)	
Male	1185 (55.5)
Female	952 (44.4)
Race – n (%)	
White	1847 (86.4)
Black	164 (7.7)
Other	127 (5.9)
Age (years)	
Mean ± Standard Deviation	51.38 ± 0.415
Median (Interquartile Range	53.00 (38.5–67.5)
ZIP Code-Level Median Household Income (2016–2020, $) – n (%)	
<$46,277	380 (17.8)
$46,277-$57,856	496 (23.2)
$57,857-$74,062	513 (24)
≥$74,063	749 (35)
ZIP Code-Level Education (high-school degree, 2020) – n (%)	
≥15.3 %	475 (22.2)
9.1 %–15.2 %	598 (28)
5 %–9 %	597 (27.9)
<5 %	468 (21.9)
Insurance Status – n (%)	
Uninsured	91 (4.3)
Private	1085 (50.7)
Medicaid	286 (13.4)
Medicare	644 (30.1)
Other Government	32 (1.5)
Distance Traveled for healthcare (Miles)	
Mean ± Standard Deviation	37.474 ± 1.678
Median (Interquartile Range)	17.2 (0–35.25 Miles)
Charlson-Deyo comorbidity score – n (%)	
0	1578 (73.8)
1	362 (16.9)
2	120 (5.6)
>3	78 (3.6)

In this cohort, the most common primary site was the bone marrow in 2130 cases (99.6 %). Regarding management, none of the patients underwent primary site surgery. The majority of the cohort received chemotherapy as their primary treatment modality (90.6 %), while 5.7 % received immunotherapy and 3.6 % underwent radiation therapy.

In the present analysis, older age was associated with decreased survival since the oldest age group of 76–100 years had the lowest survival rates at 12 % for two-year survival and 5.5 % for five-year survival. 'Other races' exhibited the highest two-year (77.1 %), five-year (64.6 %), and ten-year survival rates (57.9 %) and showed a 34 % decrease in mortality compared to Whites (Hazard Ratio [HR] = 0.66; 95 % Confidence interval [95 % CI]: 0.48–0.90; p-value = 0.009). Univariate analysis revealed that African-Americans had the lowest survival rates compared to Whites and other races (p-value <0.001). However, after multivariate adjustment, this difference was not statistically significant (HR = 0.82; 95 % CI: 0.64 –1.06; p-value = 0.135). Females demonstrated better survival in univariate analysis (p-value = 0.003), but this advantage was not significant after multivariate adjustment, showing a paradoxical increase in mortality compared to males (HR = 1.03; 95 % CI: 0.91–1.17; p-value = 0.642).

In analyzing CD scores, patients with a score of 2 or 3 had a 91 % (HR = 1.91; 95 % CI: 1.52–2.39; p-value <0.001) and 79 % (HR = 1.79; 95 % CI: 1.34–2.40; p-value <0.001) increased mortality when compared to those with a score of 0. Regarding insurance status, patients with private insurance/managed care had the greatest two-year, five-year and ten-year survival rates compared to those with Medicare, who had the lowest. Furthermore, compared to patients with private insurance/managed care, those with Medicaid had a 57 % greater mortality risk (HR = 1.57; 95 % CI: 1.26–1.97; p-value <0.001), followed by patients with Medicare who had a 32 % greater mortality risk (HR = 1.32; 95 % CI: 1.11–1.58; p-value <0.001). On comparing levels of income, there was a 26 % decrease in mortality for median household incomes ≥$74,063 compared to median household incomes of $46,227-$57,856 (HR = 0.74; 95 % CI: 0.61–0.90; p-value = 0.002).

Regarding the primary anatomic site, after adjusting for all else, a primary site of bone marrow had increased mortality when compared to all other sites (HR = 0.38; 95 % CI: 0.16- 0.86; p-value = 0.021). In an analysis of treatment modalities, primary chemotherapy demonstrated a 70 % reduction in mortality (HR = 0.30; 95 % CI: 0.25–0.37; p-value < 0.001), while primary immunological treatment showed no statistically significant effect on mortality (HR = 0.83; 95 % CI: 0.58–1.18; p-value = 0.303).

No significant difference in overall survival was associated with education level. Moreover, the quartile with fewer than 5 % of individuals lacking a high school diploma demonstrated the highest two-year, five-year, and ten-year survival rates. In the NCDB data, there were no significant mortality differences associated between patients with CD scores of 2 and ≥3. AJCC staging was not applicable in this cohort Table 2.

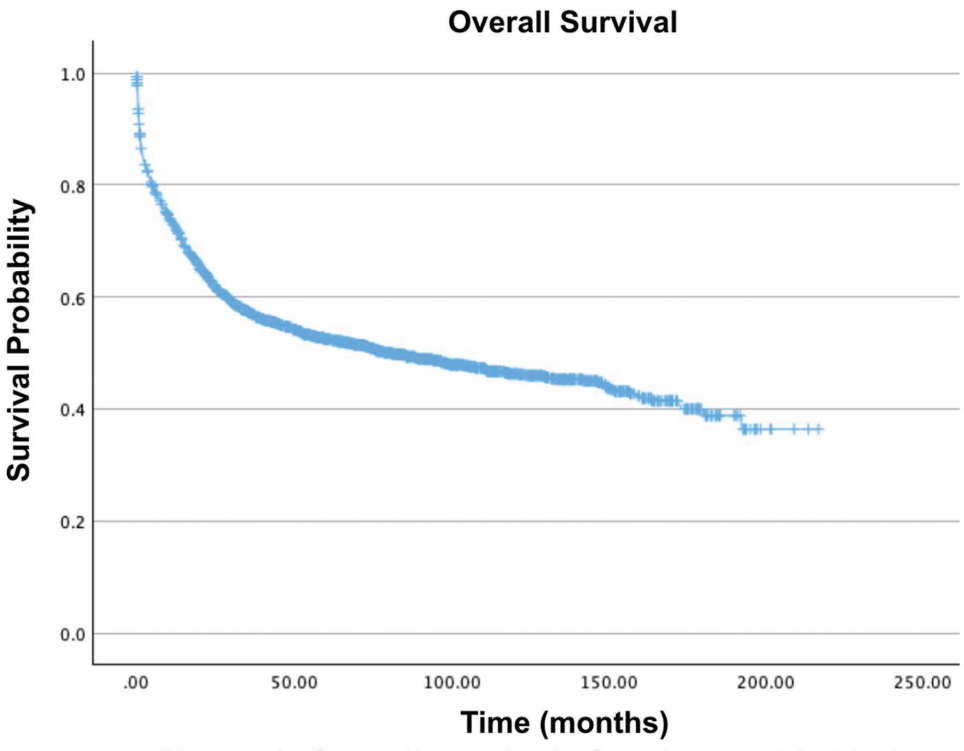

Fig. 1 – Overall survival of patients with acute myelomonocytic leukemia with abnormal eosinophils.

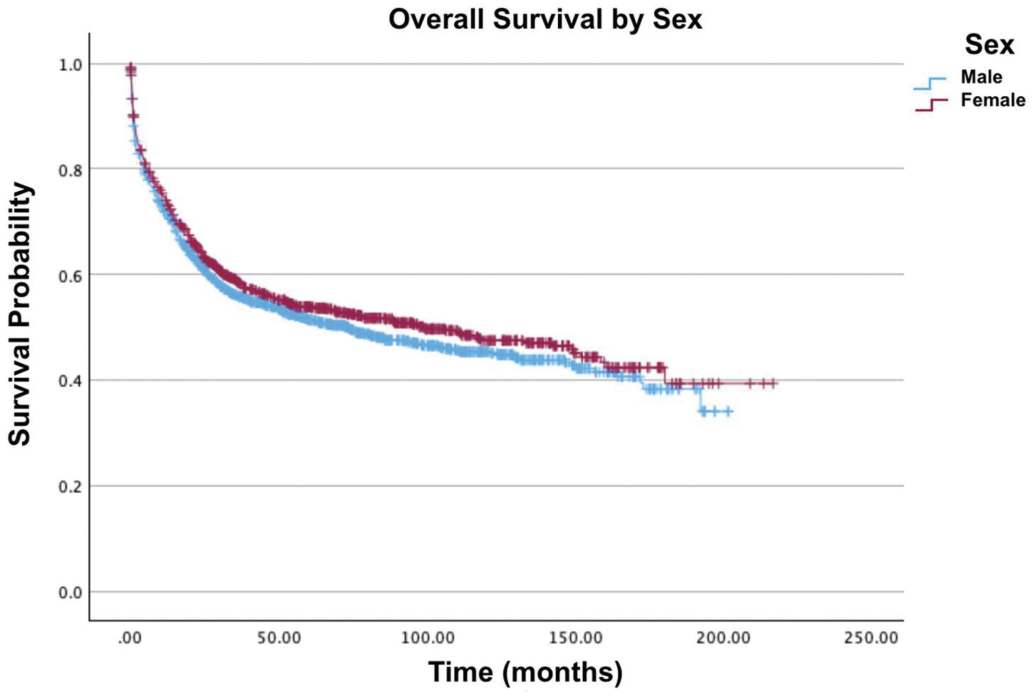

Fig. 2 – Overall survival by sex of patients with acute myelomonocytic leukemia with abnormal eosinophils.

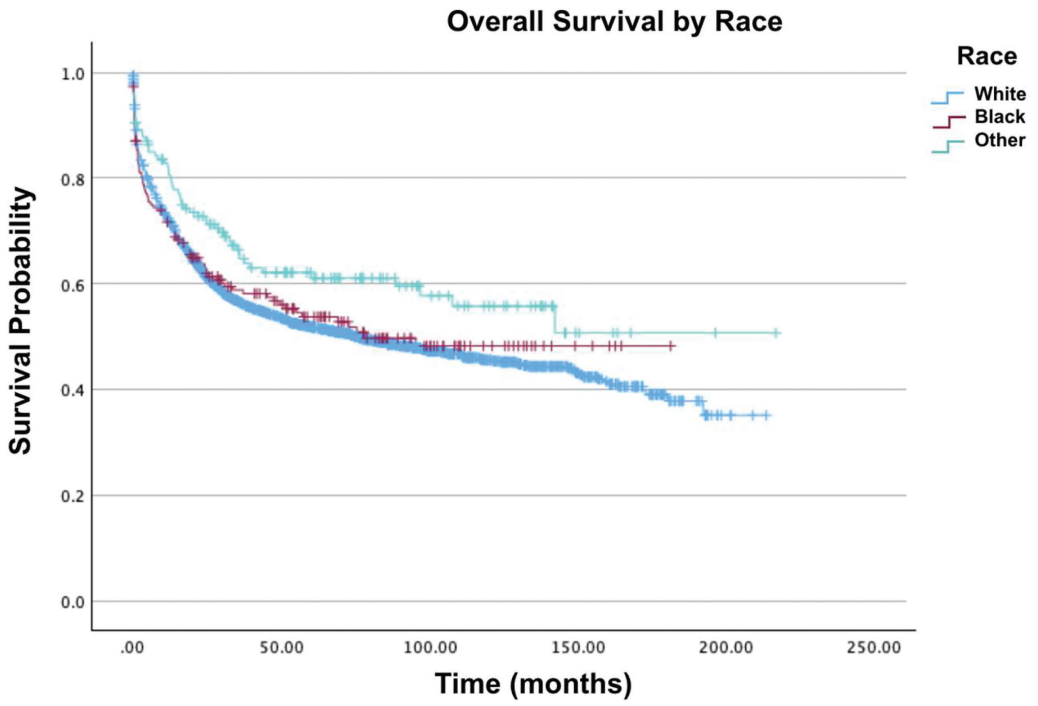

Fig. 3 – Overall survival by race of patients with acute myelomonocytic leukemia with abnormal eosinophils.

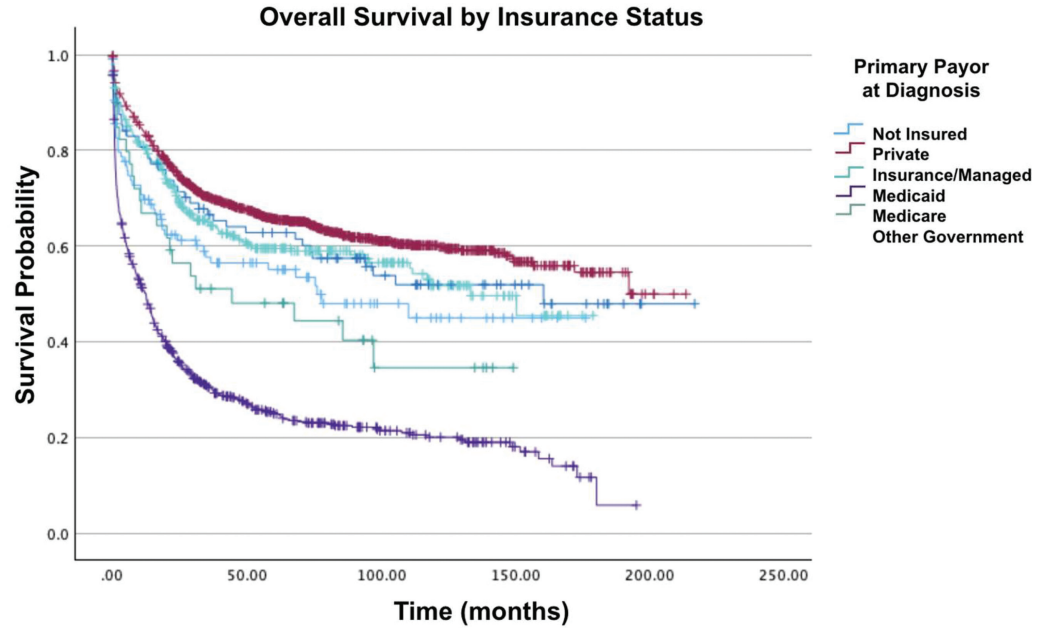

Fig. 4 – Overall survival by insurance status of patients with acute myelomonocytic leukemia with abnormal eosinophils.

Discussion

Research on AMMLEo has been limited due to its rarity. However, this study represents one of the largest to examine prognostic factors for AML and is notable for highlighting the significance of the primary anatomic site. The descriptive statistics of this cohort align with the previously reported characteristics of AML patients with abnormal marrow eosinophils. Notably, males have a slightly higher likelihood of developing this form of AML compared to females, consistent with other studies indicating a mild male predominance

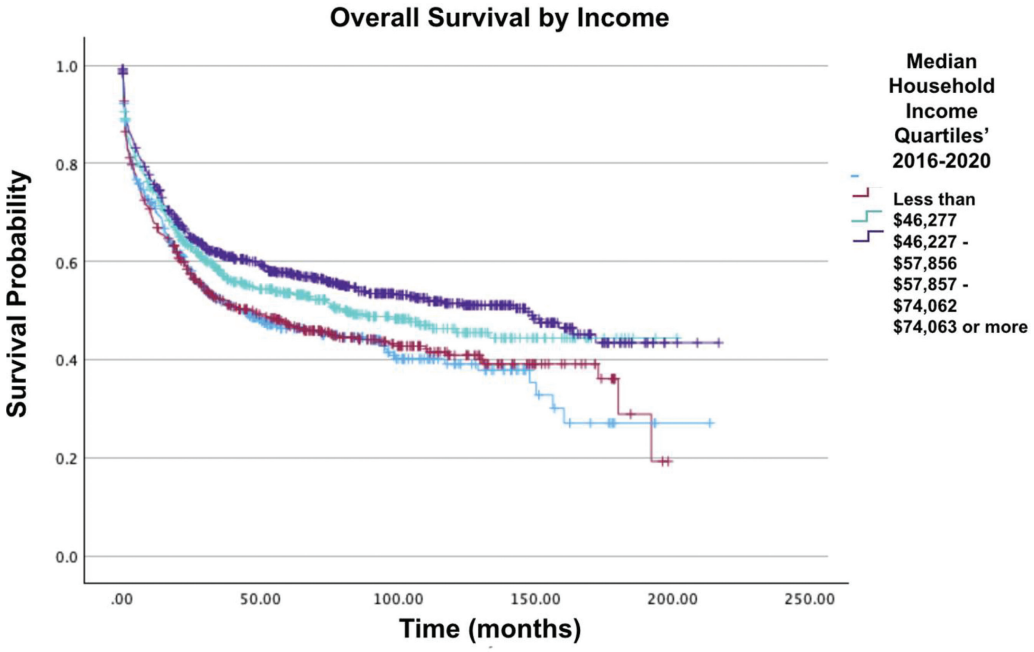

Fig. 5 – Overall Survival by income of patients with acute myelomonocytic leukemia with abnormal eosinophils.

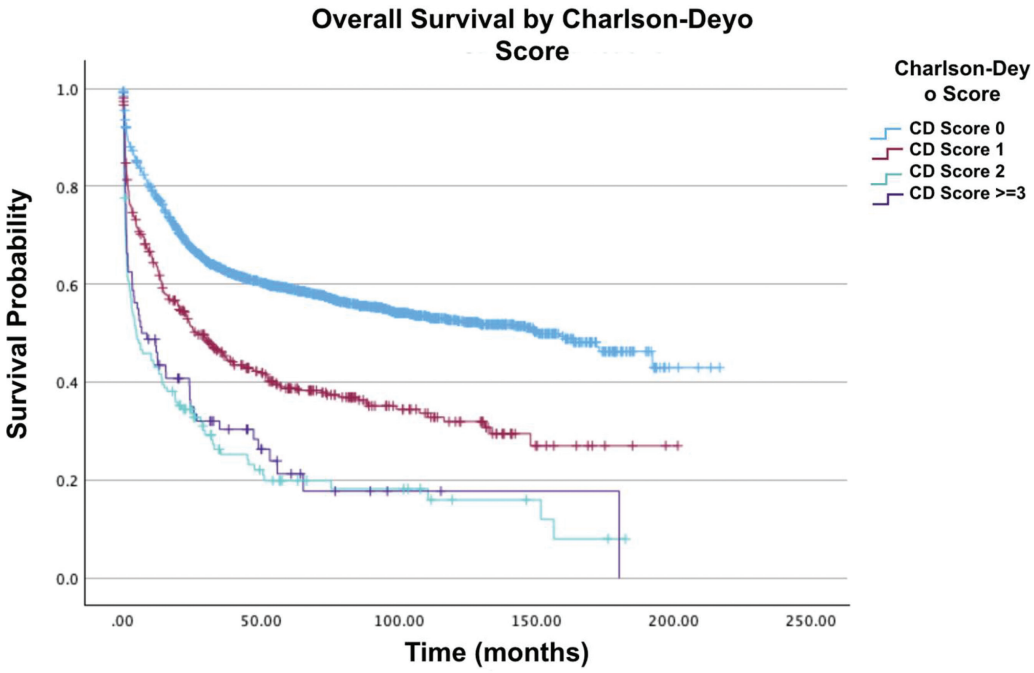

Fig. 6 – Overall survival by Charlson-Deyo of patients with acute myelomonocytic leukemia with abnormal eosinophils.

[7,9,10]. Nonetheless, some reports suggest an equal incidence between the sexes, implying that any actual difference in incidence is likely minimal [11].

The NCDB gathers and analyzes this information to better understand patient prognosis. Although our analysis does not distinguish between AMMLEo-specific mortality and overall mortality, the strong correlation between cancer stage and mortality suggests that patients likely succumbed to cancer-related causes. This underscores the importance of early detection and diagnosis of AMMLEo, as patients diagnosed at an earlier stage and with smaller tumors generally have better survival rates.

AMMLEo is a rare subtype of this hematologic cancer, characterized by the presence of atypical eosinophils in

Table 2 – Confidence intervals of various demographics.

Variable	HR (95 % CI)	p-values
Age (5 years)	1.18 (1.15–1.22)	<0.001
Males versus Females	1.03 (0.91–1.17)	0.642
Race and Ethnicity		
White versus Black	0.82 (0.64–1.06)	0.135
White versus Other	0.66 (0.48–0.90)	0.009
Black versus Other	0.80 (0.54–1.19)	0.27
Charlson-Deyo Score		
0 versus 1	1.29 (1.10–1.51)	0.002
0 versus 2	1.91 (1.52–2.39)	<0.001
0 versus ≥ 3	1.79 (1.34–2.40)	<0.001
1 versus 2	1.48 (1.15–1.91)	0.002
1 versus ≥ 3	1.40 (1.02–1.90)	0.036
2 versus ≥ 3	0.94 (0.66–1.33)	0.728
ZIP code-level median household income (2020 US Dollars		
<$46,277 versus $46,227-$57,856	1.09 (0.90–1.33)	0.376
<$46,277 versus $57,867-$74,062	0.91 (0.73–1.13)	0.381
<$46,277 versus ≥$74,063	0.81 (0.65–1.02)	0.072
$46,227-$57,856 versus $57,857-$74,062	0.83 (0.69–1.00)	0.047
$46,227-$57,856 versus ≥$74,063	0.76 (0.61–0.90)	0.002
$57,857-$74,062 versus ≥$74,063	0.89 (0.74–1.07)	0.229
Insurance		
None versus Private	0.66 (0.47–0.92)	0.014
None versus Medicaid	1.03 (0.71–1.50)	0.867
None versus Medicare	0.87 (0.61–1.24)	0.44
None versus Other Government	0.95 (0.52–1.75)	0.881
Private versus Medicaid	1.57 (1.26–1.97)	<0.001
Private versus Medicare	1.32 (1.11–1.58)	0.001
Private versus Other Government	1.46 (0.87–2.45)	0.156
Medicaid versus Medicare	0.84 (0.65–1.09)	0.196
Medicaid versus Other Government	0.93 (0.54–1.60)	0.779
Medicare versus Other Government	1.10 (0.65–1.86)	0.727
No chemotherapy versus yes Chemotherapy	0.36 (0.32–0.40)	0.01

HR: Hazard Ratio; 95 % CI: 95 % Confidence interval.

the peripheral blood or bone marrow. Chemotherapy remains the primary treatment, with specific regimens tailored to individual patient factors such as age, overall health, and genetic mutations of leukemic cells. Standard chemotherapy induction regimens for AML typically comprise cytarabine in combination with an anthracycline, such as daunorubicin or idarubicin [7,12]. For patients with actionable mutations, personalized approaches include targeted therapies such as FLT3 inhibitors (e.g., midostaurin) for FLT3-ITD variants, and IDH1/IDH2 inhibitors (e.g., ivosidenib or enasidenib) for IDH1- or IDH2-mutated disease [12,13]. Additionally, stem cell transplantation, either autologous or allogeneic, is considered for patients with high-risk disease or those achieving remission after initial chemotherapy [14].

In cases of AMMLEo, patients with Medicaid or no insurance have a 99 % higher mortality risk compared to those with private insurance [7]. High-income patients show a significant survival benefit over lower-income patients. Additionally, more patients with Medicaid or no insurance are treated at community cancer centers (9.3 % and 6.3 %, respectively) compared to those with Medicare or private insurance (4.2 % and 3.2 %, respectively). Facility type correlates with insurance status, with community cancer centers generally having poorer overall survival rates compared to academic/research programs, comprehensive community cancer programs, or integrated network cancer programs. Facility volume influences outcomes, with higher volume centers being potentially associated with reduced mortality risk [15,16]. Community cancer programs usually encounter anywhere from 100 to 500 newly diagnosed cancer cases annually. In contrast, comprehensive community cancer programs and academic institutions deal with over 500 cases per year. Additional investigations are necessary to grasp the connections between insurance coverage, facility types, facility workload, and overall survival rates. This deeper understanding could enhance patient referrals and accessibility to specific facilities.

One study found that for patients under 60 years old with inv(16) or t(16;16) chromosomal abnormalities had complete remission rates of 92 % and ten-year survival rates of 55 %, whereas patients with a normal karyotype had slightly lower rates of 90 % and 38 %, respectively. Additionally, patients with t(8;21) experienced a complete response rate of 97 % and a ten-year overall survival rate of 61 %. The presence of eosinophilia may lead to even better rates of remission and survival [17].

In cases of AMMLEo, individuals with Medicaid encounter a 57 % higher risk of mortality compared to those with private insurance. Medicare patients face a 32 % elevated risk, while other government insurance holders experience a 46 % increased risk compared to privately insured patients. Moreover, patients in the highest income category demonstrated a notable survival advantage over those in lower-income brackets. These findings align with existing literature, including a Danish study showing low socioeconomic status negatively impacted AML survival (HR = 1.49; 95 % CI 1.25–1.76) [18], and prior research revealing 2.4 times higher mortality in pediatric AML patients from poorer neighborhoods [19].

The five-year survival rate of 51.7 % for patients with AMMLEo reported in this analysis is notable. However, the ten-year survival rate of 45.5 % falls short of the 61 % reported in a study of 5876 young adult patients treated in the United Kingdom Medical Research Council Trials. One potential factor contributing to this difference could be the selection criteria, which included all candidates receiving chemotherapy, immunological treatment, and radiation as their primary form of therapy [20].

The present study is not without limitations. As a retrospective cohort study, it is subject to the inherent limitations of this design, including the lack of granularity that could be achieved with a prospective study. While retrospective analyses are valuable for exploring large datasets, they are constrained by pre-existing data collection practices. For instance, we were unable to capture more nuanced clinical details that may influence outcomes, such as detailed treatment responses or additional comorbidities not registered in the NCDB. Moreover, relying solely on variables obtained from the NCDB and contending with missing data within our cohort limits the breadth of the analysis. Although efforts were made to adjust for potential confounders, we cannot rule out the possibility of other covariates that may impact survival outcomes.

Another limitation arises from the exclusive recording of overall survival rather than cancer-specific survival of the NCDB. This data gap makes it difficult to differentiate between deaths specifically caused by AMMLEo and those resulting from other causes. However, overall survival remains a valid and meaningful endpoint, particularly in large-scale studies such as ours, where cancer-specific mortality data may not always be available. Given that AMMLEo is a rare morphologic subtype, it is reasonable to consider overall survival as a key metric for evaluating long-term patient outcomes.

The strength of this study lies in its large sample size, despite the rarity of AMMLEo. Using the NCDB, covering over 70 % of U.S. cancer diagnoses, we note potential inconsistencies as it excludes non-Commission on Cancer-accredited facilities. Despite its retrospective nature and limited data, the findings of this study align with existing literature, supporting the conclusions.

Conclusion

Several factors associated with worse overall survival in AMMLEo patients were identified. Generally, older African-American patients with comorbidities who did not have private insurance had decreased overall survival. Also, no survival benefit was found for the primary sites (bone marrow and peripheral blood), median household income, and patient sex. These findings are clinically relevant and contribute to AMMLEo comprehension, especially as this rare cancer subtype becomes more accurately defined.

Disclosure statement

The study protocol was reviewed by the Creighton University Institutional Review Board (IRB# 2001,750–01). The IRB determined that this project does not constitute human subjects research as defined by 45 CFR 46.102(f); consequently, formal informed consent was not required

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Financial relationships

No financial relationships currently or within the prior three years with any organizations that may have interest in the submitted work.

Authors contribution

All authors contributed to all aspects of the research, including research design, data analysis, and manuscript preparation. Furthermore, all authors reviewed, edited, and approved the final version of the paper.

Conflicts of interest

The authors declare they have no competing interests.

Editor

Eduardo Rego

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Impact of the changes in chronic myeloid leukemia classification proposed by the 2022 World Health Organization: a single-center Brazilian study

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ARTICLE INFO

Article history:
Received 14 November 2025
Accepted 15 April 2026
Available online 16 May 2026

Keywords:
Chronic myeloid leukemia
Imatinib
Survival
Accelerated phase
2022 WHO classification

ABSTRACT

Introduction: The latest edition of the 2022 World Health Organization (WHO) classification for chronic myeloid leukemia omitted the definition of accelerated phase.
Methods: This study evaluated the impact of using the updated classification in chronic myeloid leukemia patients treated upfront with imatinib by analyzing the outcomes of 139 newly diagnosed patients, previously classified according to the 2017 WHO criteria.
Results: Using the updated WHO Classification, eight (6%) cases previously characterized as accelerated phase were classified as chronic phase.
Overall survival (OS) was comparable under both the 2017 and 2022 WHO classifications. For patients in the chronic phase, OS was 86% at 60 months and 70% at 96 months (compared to 85% and 69% respectively under 2022 criteria). Conversely, outcomes were significantly poorer for advanced stages, with 0% survival for the accelerated phase and 25% for blast crisis (p-value <0.0001). Progression-free survival was 85% and 69% for the chronic phase and 25% for blast crisis (p-value <0.0001) by the 2017 WHO classification and 83% and 68% for chronic phase and 25% for blast crisis (p-value <0.0001) according to the 2022 WHO classification. Independent predictors of overall survival included basophil count, 2022 WHO classification, and age at tyrosine kinase inhibitor therapy initiation. For progression-free survival, the 2022 WHO classification (specifically blast crisis) and age at diagnosis were identified as significant factors.
Conclusions: Age, elevated basophil count, and advanced disease at diagnosis remain associated with worse outcomes. The omission of the accelerated phase did not significantly affect the prognosis in this cohort. However, it may affect the treatment of individual patients in Brazil, since higher doses of tyrosine kinase inhibitors are indicated for those who meet the criteria for the previous accelerated phase definition.

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https://doi.org/10.1016/j.htct.2026.106470
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Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm that accounts for approximately 15–20% of adult leukemias [1–5]. CML is characterized by the reciprocal translocation t(9;22)(q34;q11), resulting in the fusion gene *BCR::ABL1*, which encodes a tyrosine kinase protein, the primary therapeutic target for tyrosine kinase inhibitors (TKIs) [2–6]. Classically, CML has been described as a triphasic disease: chronic phase (CP), accelerated phase (AP) and blast crisis (BC) [7]. CP is the initial stage, in which the percentage of blasts in the peripheral blood (PB) or bone marrow (BM) is <10%. Patients who do not respond to treatment with TKIs progress to the advanced stages of the disease (AP and BC), which morphologically resemble acute leukemia [2,3,5,7]. Currently, the 10-year overall survival (OS) rate for CML is estimated to be 80%–90% [8].

In the 2017 World Health Organization (WHO) Classification, AP was defined as persistent neutrophilia, splenomegaly, and/or thrombocytosis; persistent thrombocytopenia unrelated to therapy; 20% or more basophils in PB; 10–19% blasts in PB and/or BM; additional clonal cytogenetic abnormalities (ACAs) of Philadelphia positive (Ph+) cells at diagnosis in the major route (according to Ph+ cells, trisomy of chromosome 8, isochromosome 17q, trisomy 19); complex karyotype and 3q26.2 abnormalities; or any new clonal abnormality that occurs during therapy. Progression to BC is defined by ≥20% blasts in the BM or PB, or the infiltrative proliferation of extramedullary blasts. However, the detection of any lymphoblasts in the PB or BM, even at levels below 10%, indicates lymphoid blast crisis (Table 1) [7].

However, the new WHO classification of 2022 omitted the AP definition, emphasizing mainly the factors associated with high risk that mark the transition from CP to BC, such as the accumulation of mutations, ACAs, and resistance to TKIs. The exclusion of AP was also justified by the decrease in the number of patients who progress to advanced phases after TKI therapy, which has increased the overall survival (OS) rate thereby improving the prognosis of AP patients [13].

On the other hand, the 2022 International Consensus Classification (ICC) of Myeloid Neoplasms and Acute Leukemias maintained the triphasic disease definition. The identification of ACAs marks the transition from CP to AP, characterized mainly by 10–19% blasts in the bone marrow or peripheral blood, in addition to 20% or more basophils in peripheral blood [9].

The differences between the definitions of the two classifications may influence treatment decisions and prognostic analyses, as most studies that approved the current TKIs had used the definition of AP to include patients in clinical trials [14].

This study aimed to evaluate the impact of the 2022 WHO classification on the categorization and prognosis of CML patients treated with first-line imatinib.

Methods

This was a single-center, observational, retrospective study. This study analyzed electronic medical records of newly diagnosed CML patients at treated at Centro de Hematologia e Hemoterapia, Universidade Estadual de Campinas, a Brazilian university institution (from January 2015 to December 2023). Participants received first-line imatinib (400–600 mg/day) and underwent regular clinical follow-ups. Clinical outcomes were compared across the 2017 WHO, 2022-ICC, and 2022 WHO classifications. Cases with insufficient clinical and laboratory data to calculate the disease stage before starting treatment were excluded. This study was approved by the local Institutional Review Board.

OS was calculated from the start of imatinib administration until the date of death or last follow-up. Progression-free survival (PFS) was calculated from the start of imatinib therapy until transformation to AP or BC or death. Survival was analyzed using the Kaplan-Meier method, and differences between curves were assessed using the log-rank test. The cut-off date for this analysis was May 2024. Patients were stratified using the European Treatment and Outcome Study (EUTOS) long-term survival score (ELTS) [15] and the Sokal score [16]. Responses were analyzed according to the definitions of the European LeukemiaNet (ELN) recommendations 2006–2020 [10–12].

Results

A total of 256 electronic medical records of patients followed at the center were analyzed (Figure 1), of which 117 (46%) were excluded for the following reasons: diagnosis outside the study period and lack of clinical information before starting imatinib therapy (patients referred from other centers). The clinical and laboratory characteristics of the 139 (54%) patients evaluated in this study are described in Table 2.

Additional chromosomal abnormalities (ACA) were found in 25/139 (18%) patients: seven (28%) were major route alterations, specifically of trisomy of chromosomes 8 and 19 and isochromosome [i(17q)], four (16%) other high-risk alterations (trisomy 21, 3q26.2, monosomy 7/7q−, 11q23, complex karyotypes) and 14 (56%) low-risk alterations (any other).

BCR::ABL1 transcripts were identified in 133 (95.5%) of the 139 cases: b3a2 in 84 (63.2%) patients, b2a2 in 46 (34%), both in two (1.4%), e1a2 in one (0.7%), and p230 in one (0.7%) case. Two patients died before starting imatinib treatment and 137 started imatinib in a median time of ten days.

According to the 2017 WHO and 2022-ICC classifications, 125 (90%) were in the CP, eight (6%) in the AP, and six (4%) in the BC phase; and according to 2022 WHO, 133 (96%) were in the CP and six (4%) in the BC phase (Figure 1). There was a significant change in the 2022 WHO classification compared to

Table 1 – 2017-WHO, 2022-ICC, 2020-ELN and 2022-WHO classification for Chronic Myeloid Leukemia (CML).

	2017-WHO [7]	2022-ICC [9]	2020-ELN [10–12]	2022-WHO [13]
Accelerated phase	PB or BM blasts 10%–19%		PB or BM blasts 15%–29% PB blasts + promyelocytes ≥30%, with blasts <30%	Not recognized
	PB basophils ≥20%			
		Not included	Persistent thrombocytopenia ($\leq 100 \times 10^9$/L) unrelated to therapy	
	Persistent or increasing splenomegaly (unresponsive to therapy)	Not included	Not included	
	ACA in pH^+ cells at diagnosis, including major route, complex karyotype, or 3q26.2 abnormalities, at diagnosis	ACA in Ph+ cells at diagnosis or the acquisition of major route ACAs on treatment	Clonal chromosome abnormalities in Ph+ cells (CCA/Ph+), major route, on treatment	
	Any new clonal chromosomal abnormality in pH+ cells that occurs during therapy			
	Provisional: - Failure to achieve CHR to first TKI - Any indication of resistance to two sequential TKIs - Occurrence of >2 mutations of BCR::ABL1 during TKI treatment	Not included	Raises concern: - ACAs in Ph+ cells - Resistance to two TKIs - Detection of a *BCR::ABL1* kinase domain mutation	
Blast crisis	PB or BM blasts ≥20%		PB or BM blasts ≥20%	PB or BM blasts ≥20%
	Extramedullary blast proliferation, apart from spleen	Myeloid sarcoma	Extramedullary blast proliferation, apart from spleen	Myeloid sarcoma
		Presence of morphologically apparent lymphoblasts (>5%) warrants consideration of lymphoid BP-CML	Not included	Presence of increased lymphoblasts in PB or BM

WHO: World Health Organization; ICC: International Consensus Classification; ELN: European LeukemiaNet; ACA: additional clonal cytogenetic abnormalities; BM: bone marrow; CHR: complete hematologic remission; PB: peripheral blood; WBC: white blood cells; TKI: Tyrosine kinase inhibitor; Ph+: Philadelphia positive.

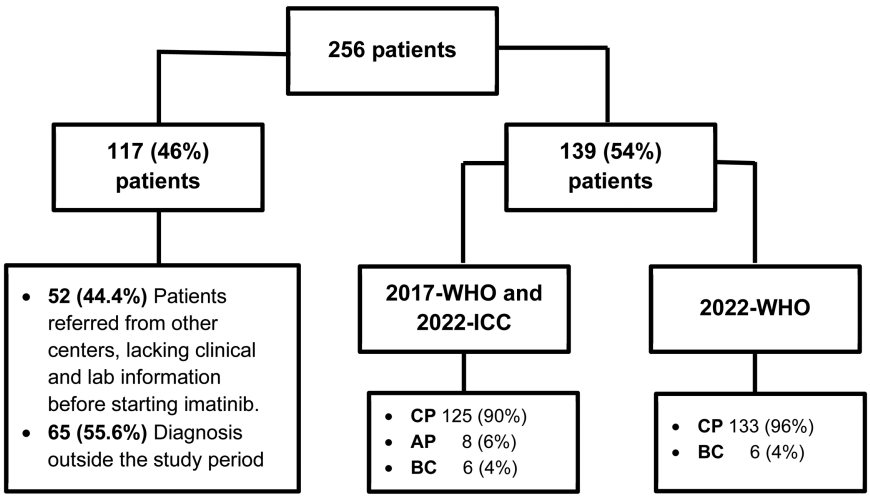

Figure 1 – Flowchart of patients with chronic myeloid leukemia treated with Imatinib (n = 256).

Table 2 – Clinical and laboratorial data on chronic myeloid leukemia patients at diagnosis (n = 139).

Variable	
Age (years) - median (range)	53 (17–83)
Sex, female – n (%)	71 (51)
Spleen size (cm from BCM) - median (range)	0 (0–30)
Laboratory data - median (range)	
White blood cell count (x 10^9/L)	117.2 (5.4–588)
Basophils (%)	3.2 (0–18)
Blasts in peripheral blood (%)	2 (0–60)
Platelets (x 10^9/L)	387 (71–3862
Hemoglobin g/dL	11.4 (3.5–16.6)
Eosinophils (%)	2 (0–6)
Bone marrow blasts (%)	1 (0–63)
Bone marrow basophils (%)	1.5 (0–15)
Sokal score – n (%)	
Low	37 (26.6)
Intermediate	59 (42.4)
High	39 (28.1)
Not available	4 (2.9)
ELTS – n (%)	
Low	55 (39.6)
Intermediate	47 (33.8)
High	33 (23.7)
Not available	4 (2.9)

BCM: Below costal margin; ELTS: European long-term survival score.

the 2017 version (p-value <0.0001%). Eight of 139 (6%) patients were reclassified from AP to CP.

Treatment response

In the entire group, the response to first-line imatinib treatment was as follows: complete hematologic response (CHR) was achieved by 135 (97%) patients; complete cytogenetic response (CCyR) by 83 (60%) and major molecular response (MMR) by 65 (46.5%). Fourteen (10%) patients lost CHR, seven (8.5%) lost CCyR and 15 (23%) lost MMR. Overall, 11 (8%) patients progressed to the advanced phases, four to AP and seven to BC. Eleven (8%) required hematopoietic stem cell transplantation (HSCT). There were 22 (16%) deaths, 15 (68%) of which were CML related.

Of the eight patients in AP who were reclassified as CP, five switched to a second-generation TKI because of imatinib failure, and two of them required allogeneic HSCT; one of whom died from the procedure. One patient progressed to BC, one patient was lost to follow-up, and four are currently under treatment (Table 3).

Based on Sokal risk scores, OS was 100%, 87%, and 56% for low, intermediate, and high-risk groups, respectively, while corresponding PFS rates were 100%, 85%, and 56% (both p-value <0.0001; Figure 2). Survival outcomes remained consistent across classifications: under 2017 WHO/2022 ICC criteria, CP OS was 86% at 60 months and 70% at 96 months, compared to 0% for AP and 25% for BC (p-value <0.0001; Figure 3). Under 2022 WHO criteria, CP OS was 85% and 69% at 60 and 96 months, respectively and 25% for BC (p-value <0.0001; Figure 3). Similarly, 2017 WHO/2022 ICC PFS was 85% (60 months) and 69% (96 months) for CP, 0% for AP, and 25% for BC (p-value <0.0001; Figure 4), while 2022 WHO PFS was 83% (60 months) and 68% (96 months) for CP and 25% for BC (p-value <0.0001; Figure 4). Multivariate analysis identified basophil count (p-value = 0.013), 2022 WHO phase, and age at TKI initiation (p-value = 0.019) as independent predictors for OS. For PFS, 2022 WHO specifically BC (p-value <0.0001) and age at diagnosis were the sole independent factors (p-value = 0.010)

Discussion

The omission of the AP classification did not significantly affect OS or PFS rates in CML patients treated with first-line imatinib in the present cohort. Age, higher basophil count, and advanced disease at diagnosis were independent factors of worse outcomes. This finding corroborates the literature, which argues that it is essential to develop predictive tools to quickly identify patients at higher risk of CML-induced progression and death, warranting closer molecular monitoring and treatment intensification, such as frontline second

Table 3 – Summary of clinical characteristics of chronic myeloid leukemia (CML) patients who were reclassified from accelerated phase (AP) (2017-WHO/ 2022-ICC) to chronic phase (CP) by 2022-WHO.

Patient #	Sex	Age at diagnosis, (years)	Blasts in PB at diagnosis (%)	BM blasts at diagnosis (%)	BM basophils at diagnosis (%)	Progression to advanced phases	Response to first-line treatment with imatinib	Second-generation TKI during follow-up	Disease status in the last follow-up	Treatment in the last Follow-up (dose mg)	Situation in the last Follow-up
1	F	50	12	10.5	4.5	No	CHR	Yes	CHR	Post HSCT	Deceased
2	M	36	13	2.5	1.5	No	CHR (lost to follow-up)	Yes	MMR	Nilotinib (800)	Alive - Transferred to other hospital
3	F	69	11	8.2	0.8	Yes	CHR	Yes	BC	Dasatinib (100)	Deceased
4	F	57	13	0	5	No	CHR CCyR MMR	No	MMR	Imatinib (600)	Alive - on treatment
5	F	58	11	1.5	1	No	CHR	Yes	DMR	Dasatinib (100)	Alive - on treatment
6	M	30	12	12	5	No	CHR	Yes	PMR	Post HSCT	Alive - on treatment
7	F	69	19	15.8	1.8	No	CHR CCyR MMR	No	DMR	Imatinib (600)	Alive - Lost follow-up
8	M	58	4	12	3.5	No	CHR CCyR MMR	No	MMR	Imatinib (600)	Alive - on treatment

PB: Peripheral blood; BM: Bone marrow; TKI: tyrosine kinase inhibitor; F: female; M: male; allo-HSCT: allogeneic hematopoietic stem cell transplantation; CHR: Complete hematologic response; CCyR: Complete cytogenetic response; MMR: Major molecular response; DMR: Deep molecular response.

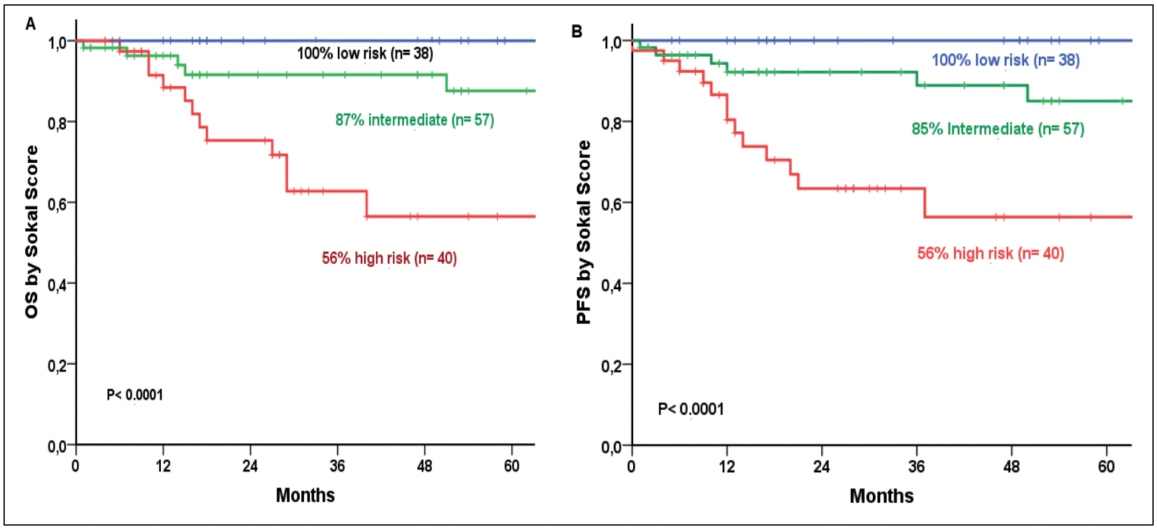

Figure 2 – Overall Survival (2A) and Progression-Free Survival (2B) by Sokal score.

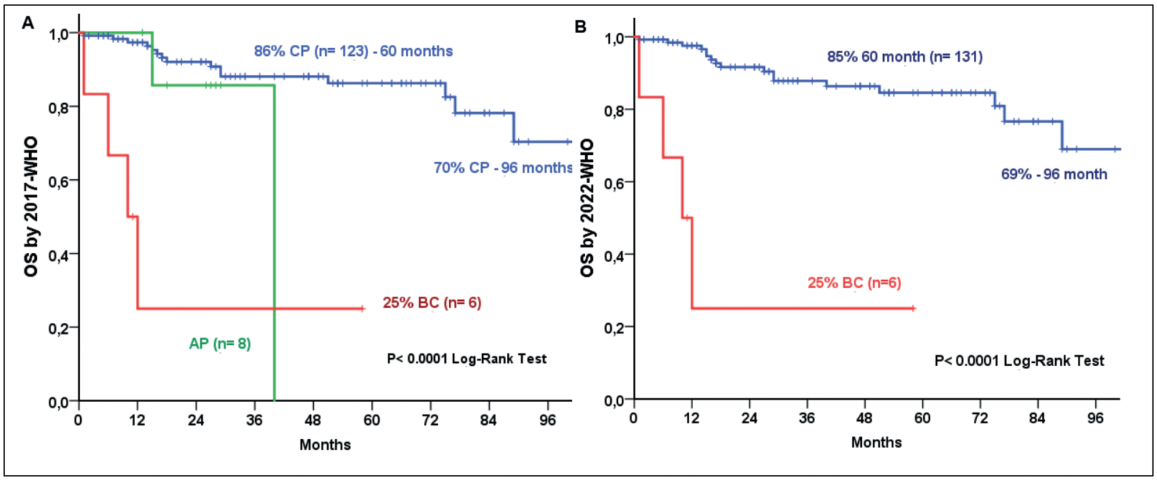

Figure 3 – Overall Survival by 2017 WHO (A) and 2022 WHO (B) classifications.

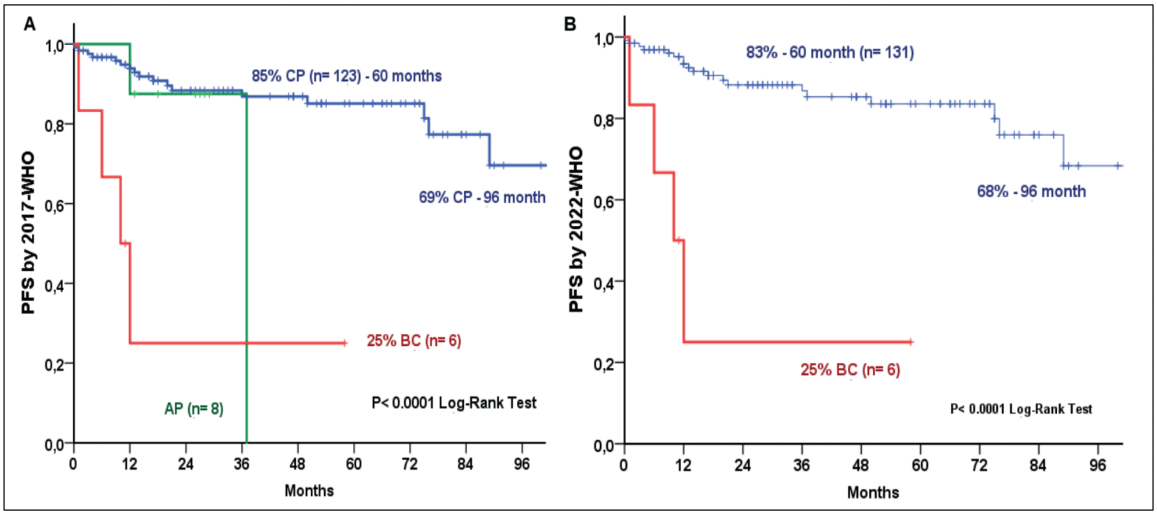

Figure 4 – Progression-Free Survival by 2017 WHO (A) and 2022 WHO (B) classifications.

generation TKIs, early switch to a more potent TKI, eligibility for allogeneic HSCT, or new agents [17].

The rationale for the change to the 2022 WHO classification was the improvement of CML survival in the last decades after TKI therapy introduction. AP was omitted in the updated classification, but high-risk features associated with CP progression and resistance to TKI were stressed [13].

A possible alternative to complete exclusion could be to simplify the definition of AP, as defined by the 2022-ICC. Variables that were considered less-specific definers of AP (platelet count and persistent splenomegaly unresponsive to therapy) were omitted due to more robust evidence of the association of other parameters with a higher risk of progression to worse outcomes [14]. There is a need for further discussion in this respect, as it remains a heterogeneous disease with marked variability in individual prognosis [18].

In a real-world multicenter study [19] using the Czech 'INFINITY' registry, patients diagnosed with AP (by 2020-ELN, 2022-ICC, and 2017 WHO criteria) had a significantly worse prognosis than those in the CP group, even those classified as high-risk by the ELTS. These findings support the recognition of AP as a distinct clinical entity at diagnosis. In the current cohort of AP patients, only three of eight are still on imatinib, and five have switched to a second-generation TKI due to resistance.

On the other hand, Yang et al. [20] analyzed 2122 consecutive CML patients diagnosed between 2006 and 2023. Using the 2022 ICC, they found that response milestones, OS, and PFS, were consistent with results obtained using the 2020-ELN criteria. AP patients with increased basophil counts had similar transformation-free survival (TFS) and OS compared with CP subjects classified as intermediate-risk by the ELTS, whereas AP patients with increased blasts had worse TFS but similar OS compared with those with CP and high-risk ELTS. Similar findings were observed using the 2022-ICC. The authors supported omitting AP but suggested updating the ELTS risk classification to include a very high-risk cohort with increased blasts and/or decreased platelets.

Kantarjian et al. reviewed the outcomes of both *de novo* CML-AP and 'transformed' CML-AP (evolving from CML-CP) treated in the era before TKI therapy (before 2000) and in the TKI therapy era (after 2000). There was an improvement in survival from 30%–66% in *de novo* CML patients with AP, but less progress in transformed AP. The authors noted that many clinical trials used the AP definition for patient stratification, and omitting AP may impair patient recruitment and coverage of treatment costs [18].

The omission of AP presents not only theoretical but also practical implications. It may also influence ongoing scientific research with TKI-based regimens or new therapies, as well as community practice in CML, including considering HSCT, since pathologists who follow the 2022 WHO criteria will not report progression to AP. These patients cannot be included in such protocols [18].

The potential challenges that the omission of AP for individual patients in Brazil should not be underestimated, as, in the public healthcare system in Brazil, only imatinib is available as the first-line treatment for CML and higher doses of imatinib are only reimbursed using the previous definition of AP and not for high-risk CP patients according to ELTS.

Conclusions

Age, higher basophil counts, and advanced disease at diagnosis remain relevant factors associated with worse treatment outcomes. Omitting the AP from the 2022 WHO classification did not significantly impact OS or PFS in this cohort of CML patients treated with first-line imatinib. However, its exclusion may influence individual clinical management, which has historically relied on the AP definition for treatment decisions.

Funding

This study was supported by the National Council for Scientific and Technological Development (CNPq) through the Institutional Scientific Initiation Scholarship Program (PIBIC) Quota 2023/2024.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

None

Acknowledgement

This study was supported by the National Council for Scientific and Technological Development (CNPq) through the Institutional Scientific Initiation Scholarship Program (PIBIC).

Editor

Erich Vinicius De Paula

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Original article

Derived hematological indices during peripheral blood stem cell mobilization in the plerixafor era: insightful dynamics but limited predictive value

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ARTICLE INFO

Article history:
Received 9 August 2025
Accepted 23 March 2026
Available online 2 June 2026

Keywords:
Peripheral blood stem cells
Plerixafor
Granulocyte colony-stimulating factor
Derived hematological indexes
CD34$^+$ cell count

ABSTRACT

Introduction: Derived hematological indices reflect immune activation, but their dynamics during the plerixafor era and their predictive value for stem cell yield remain unclear.

Objectives: To assess changes in derived hematological inflammatory markers during peripheral blood stem cell mobilization in autologous patients and allogeneic donors, and to evaluate their correlation with CD34$^+$ stem cell yield. These markers include ratios (neutrophil-to-lymphocyte, platelet-to-lymphocyte and lymphocyte-to-monocyte), and composite indices (systemic immune-inflammation and systemic inflammation response indices).

Methods: A retrospective analysis was conducted of 127 autologous and 53 allogeneic mobilization events between January 2019 and March 2024. Blood counts and derived indices were analyzed before and after granulocyte colony-stimulating factor administration with and without plerixafor. CD34$^+$ cell enumeration was performed after mobilization. Dynamic changes in inflammatory indices and their correlation with CD34$^+$ counts were assessed.

Results: After mobilization, there was a significant increase in the neutrophil-to-lymphocyte ratio, systemic immune-inflammation index, and systemic inflammation response index, and a decrease in the platelet-to-lymphocyte and lymphocyte-to-monocyte ratios in both groups. While allogeneic donors had higher CD34$^+$ counts (median 110 cells/μL versus 48 cells/μL in autologous), no consistent correlation was found between dynamic inflammatory marker changes and CD34$^+$ yield. Plerixafor use in autologous patients significantly

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https://doi.org/10.1016/j.htct.2026.106474

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influenced the platelet-to-lymphocyte ratio and systemic immune-inflammation index dynamics, highlighting its role in modulating the acute inflammatory response in the plerixafor era.

Conclusions: Inflammatory markers change significantly during mobilization with granulocyte colony-stimulating factor, reflecting acute immune activation. However, the studied markers do not predict $CD34^+$ yield and should not replace direct enumeration. Their role may be better suited to complement biological understanding rather than clinical decision-making.

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Introduction

Peripheral blood stem cell (PBSC) mobilization induces significant hematological changes, including elevated white blood cell (WBC) counts, increased $CD34^+$ cell counts, and occasional thrombocytopenia [1–3]. These shifts reflect the physiological response to mobilization and the systemic demand for hematopoietic progenitor cells. The introduction of agents such as plerixafor, which antagonizes the CXCR4-SDF1α axis, has further improved mobilization efficiency, particularly in patients who respond poorly to granulocyte colony-stimulating factor (G-CSF) alone [4].

Pre-apheresis peripheral blood $CD34^+$ cell enumeration remains the gold standard for predicting stem cell yield and guiding collection timing [1]. However, this technique requires flow cytometry, which can be time-consuming and resource-intensive. Conversely, simple, cost-effective markers derived from routine complete blood counts, such as the Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Lymphocyte-to-Monocyte Ratio (LMR), and composite indices like the Systemic Immune-Inflammation Index (SII) and Systemic Inflammation Response Index (SIRI), have gained attention as surrogate markers of inflammation and immune activation [5–7]. These indices have been used in cancer settings to assess prognosis, treatment response, and systemic inflammation however, their potential predictive value for stem cell yield remains unclear [8–10].

G-CSF–mediated mobilization is known to provoke a transient inflammatory response, which may be reflected in these derived markers [11]. While historical data on hematological indices exists for G-CSF mobilization, there is a scarcity of data regarding their behavior in the current plerixafor era. Plerixafor's unique mechanism, antagonizing the CXCR4-SDF1α axis, may alter the systemic inflammatory milieu differently to G-CSF alone. Understanding the dynamics could enhance our ability to monitor and optimize mobilization strategies without relying solely on $CD34^+$ enumeration. This study addresses this gap by including a significant proportion of patients requiring plerixafor.

This study evaluates changes in derived hematological inflammatory indices during PBSC mobilization in both autologous patients and allogeneic donors. It also investigates whether these markers correlate with $CD34^+$ cell counts and could serve as alternative indicators of mobilization adequacy.

Methods

This study was a retrospective observational analysis conducted at a tertiary care center, assessing autologous patients and allogeneic donors who underwent PBSC mobilization followed by apheresis for hematopoietic stem cell transplantation (HSCT). The data collection period spanned from January 2019 to April 2024. The study was approved by the Institutional Review Board.

The study population included autologous patients undergoing mobilization as part of treatment for hematologic malignancies (e.g., lymphoma, multiple myeloma) and allogeneic donors, typically related healthy individuals, undergoing mobilization for matched or haploidentical recipient transplantation.

The inclusion criteria were autologous patients or allogeneic donors who received G-CSF-based mobilization, with or without plerixafor. Those with incomplete pre- and post-mobilization hematological and $CD34^+$ cell count data were excluded.

Data collection

Clinical and laboratory data were extracted from hospital medical records, including patient files and donor evaluation charts. Apheresis unit records, which included mobilization regimen details and stem cell product characteristics, were collected.

The following variables were collected and entered into a standardized proforma:

- Demographic details: Age, sex.
- Mobilization regimen: Number of G-CSF doses, use and number of doses of plerixafor.
- Laboratory parameters:
- Hemoglobin, hematocrit, total white blood cell (WBC) count, percentage of neutrophils, lymphocytes, monocyte and platelet count, recorded before and after mobilization.
- Peripheral blood $CD34^+$ counts (pre-apheresis) measured by flow cytometry.

Calculation of derived inflammatory indices *[6,8,12,13]*

Derived hematological inflammatory markers were calculated from complete blood count (CBC) values as follows:

- Neutrophil-to-Lymphocyte Ratio (NLR) = Absolute neutrophil count / Absolute lymphocyte count
- Platelet-to-Lymphocyte Ratio (PLR) = Platelet count / Absolute lymphocyte count
- Lymphocyte-to-Monocyte Ratio (LMR) = Absolute lymphocyte count / Monocyte count
- Systemic Immune-Inflammation Index (SII) = (Neutrophil count × Platelet count) / Lymphocyte count
- Systemic Inflammation Response Index (SIRI) = (Neutrophil count × Monocyte count) / Lymphocyte count

For each index, values were calculated both before and after mobilization, and dynamic changes (Δ values) were computed as post-mobilization minus pre-mobilization values. Also to analyze the magnitude of change, mobilization signatures of these indexes were estimated by post-value / pre-value between autologous patients and allogeneic donors who received plerixafor or not.

Statistical analysis

Data were analyzed using statistical software. Continuous variables were expressed as median with range. Comparisons of pre- and post-mobilization values were performed using paired statistical tests (e.g., Wilcoxon signed-rank test or paired *t*-test, depending on distribution). Differences between autologous and allogeneic groups were assessed using the Mann–Whitney U test or independent *t*-tests. Correlations between derived inflammatory indices and $CD34^+$ counts were assessed using Spearman's rank correlation coefficient. Receiver Operating Characteristic (ROC) curves were plotted to evaluate the predictive ability of dynamic indices for achieving $CD34^+$ counts >50 cells/μL. A p-value <0.05 was considered statistically significant.

Results

Reports of autologous patients and allogeneic donors who underwent stem cell mobilization and harvesting for transplantation were consecutively analyzed during the period from January 2019 to March 2024. The number of events taken for analysis in the autologous setting was 127 while it was 53 events for allogenic donors.

The median ages in the autologous and allogenic settings were 47 years and 39 years, respectively. Patients received G-CSF mobilization alone in 69.3 % of the events and plerixafor along with G-CSF in the remaining 30.7 %. While donors received G-CSF mobilization in 98.1 % and only 1 (1.9 %) donor required plerixafor along with G-CSF for mobilization. The number of days that G-CSF was administered is shown in Table 1.

Table 2 shows the baseline blood counts of both autologous patients and allogenic donors before and after the administration of G-CSF. There was a significant increase in the WBC count after mobilization in both groups. Additionally, lymphocyte percentages dropped, while neutrophil percentages rose sharply, indicating a shift in the immune cell profile. After mobilization the median $CD34^+$ counts in autologous patients was 48 cells/μL whereas it was 110 cells/μL in allogenic donors.

Table 1 – Demographics and mobilization regimen used in autologous patients and allogenic Donors.

	Autologous (n = 127) Frequency	Allogenic (n = 53) Frequency
Age (Years) - Median (Range)	47 (4–65)	39 (2–69)
Gender – n (%)		
Male	80 (63)	29 (54.7)
Female	47 (37)	24 (45.3)
G-CSF (days) – n (%)		
5	84 (66.2)	49 (92.5)
6	36 (28.3)	3 (5.7)
7	6 (4.7)	0 (0)
8	1 (0.8)	1 (1.9)
Plerixafor (dose) – n (%)		
None	88 (69.3)	52 (98.1)
1	35 (27.6)	1 (1.9)
2	4 (3.1)	-

G-CSF: granulocyte colony-stimulating factor.

Table 3 shows the CD34 enumeration details by flow cytometry. Regarding mobilization efficacy, 11.3 % of autologous patients failed to reach the minimum threshold (<20 cells/μL), whereas all allogeneic donors exceeded this level. Furthermore, suboptimal mobilization (<50 cells/μL) was observed in 52.4 % of autologous patients, while the vast majority of allogeneic donors (92.5 %) achieved optimal counts (>50 cells/μL).

In autologous donors, the NLR increased significantly from a normal baseline of 2.1 to 11.7 post-mobilization. Conversely, LMR, which was low at baseline (3.2), further declined to 1.3. While PLR was high before mobilization (148.8), it dropped sharply to 57.9. The SII increased from 0.565 to 1.925, and the SIRI rose from 1201.7 to 30,102.5, reflecting a robust systemic inflammatory response during the mobilization process.

Similar trends were observed in autologous patients, characterized by a sharp increase in NLR (1.4 versus 8.5) and SIRI (564.0 versus 21,309.6), alongside a decrease in PLR (103.1 versus 58.1) and LMR (6.5 versus 1.6). Figure 1 provides a visual representation of the shifts in all derived inflammatory markers following mobilization.

Table 4 summarizes the dynamic changes in derived inflammatory markers following mobilization in both autologous patients and allogeneic donors. While dynamic changes in NLR and SII were observed in both groups, the differences were not statistically significant. However, significant differences between autologous and allogeneic cohorts were identified for the LMR (p-value = 0.003), MLR (p-value = 0.027), PLR (p-value = 0.006), and SIRI (p-value = 0.030), indicating that these specific markers may reflect differential inflammatory or hematologic responses to mobilization in the two populations.

Table 5 summarizes the impact of plerixafor use on inflammatory indices in autologous patients undergoing PBSC mobilization. At baseline (prior to mobilization), there were no statistically significant differences in any of the inflammatory markers between patients who received G-CSF alone and those who received G-CSF with plerixafor. However, post-mobilization, patients who received plerixafor showed significantly lower PLR (p-value < 0.01) and SII (p-

Table 2 – Blood Picture of autologous patients and allogeneic donors before and after mobilization with granulocyte colony-stimulating factor (G-CSF) (Median with range).

	Autologous Patients		Allogenic Donors	
	Before	After		After
Hemoglobin (g/dL)	11.9 (7.7–15.6)	11.7 (7.8–14.9)	13.1 (8.5–16.2)	13.3 (8.3–16.1)
Hematocrit (%)	36.7 (23.4–46.9)	35.9 (24.9–44.5)	40.7 (26.6–47.9)	40.5 (27–48.5)
WBC Count (x $10^3/\mu$L)	6.60 (1.41–4.60)	42.10 (13.70–96.20)	7.69 (4.90–10.50)	43.20 (18.60–82.86)
Platelet Count (x$10^3/\mu$L)	256 (71–769)	178 (48–511)	264 (155–442)	218 (125–405)
Neutrophils (%)	60 (6–90)	84.2 (5.3–95)	52.1 (24.5–76)	83.5 (8.6–87.9)
Lymphocytes (%)	28 (7–64)	7 (3–35)	37.3 (19.9–59)	9.2 (5.2–18)
Monocytes (%)	8 (2.5–83.5)	5.25 (1–84)	6 (2–12.4)	6 (2– 0.8)
CD34$^+$ cells (cells/μL)	Not done	48 (2–316)	Not done	110 (34–486)

WBC: White blood cell.

Table 3 – Flow cytometric enumeration of peripheral blood CD34$^+$ counts in autologous patients and allogenic donors.

		Autologous		Allogenic	
		Frequency	Percent	Frequency	Percent
CD34$^+$ Count	**<20** cells/μL	14	11.3	0	0
	>20 cells/μL	110	88.7	51	100
CD34$^+$ Count	**<50** cells/μL	65	52.4	2	7.5
	>50 cells/μL	59	47.6	49	92.5

value = 0.005) compared to those mobilized with G-CSF alone, suggesting a modulation of the inflammatory response by plerixafor. Further analysis of dynamic changes (Δ values) in these indices revealed that PLR (p-value < 0.01) and SIRI (p-value = 0.03) were significantly different between the two groups. No significant differences were observed in the dynamic changes of NLR, LMR, MLR, or SII. These findings suggest that while plerixafor does not influence baseline inflammatory status, it may attenuate specific inflammatory responses after mobilization, particularly for platelet- and monocyte-associated indices.

No significant correlation was found between peripheral blood CD34$^+$ cell counts and the dynamic changes in derived variables. Consequently, ROC analysis (Figure 2) demonstrated low predictive power, with AUC values ranging between 0.5 and 0.6.

Table 6 captures the dynamic shift in inflammatory markers, highlighting how plerixafor significantly blunts the velocity of change of specific indices. Subdividing the autologous cohort based on plerixafor administration demonstrated that the drug significantly alters PLR kinetics (p-value < 0.01).

Discussion

This study aimed to evaluate the dynamics of derived hematological inflammatory markers during PBSC mobilization in both autologous patients and allogeneic donors. In addition, it explored the correlation of these markers with CD34$^+$ cell enumeration, which remains the gold standard for assessing mobilization efficacy and guiding apheresis timing.

G-CSF mobilization led to significant hematological shifts in both autologous patients and allogenic donors, including elevated total WBC and neutrophil counts, and relative lymphopenia. Consequently, derived inflammatory indices such as NLR, SII, and SIRI increased markedly, indicating a robust acute inflammatory response. In contrast, PLR and LMR decreased significantly after mobilization. These changes reflect the characteristic pro-inflammatory state induced by G-CSF and are consistent with earlier studies linking such indices to systemic inflammation and cancer prognosis [8,14–16].

The results revealed that while inflammatory markers change consistently in both autologous and allogeneic settings, some parameters, specifically LMR, MLR, PLR, and SIRI, differed significantly between the groups. These differences likely stem from the disease burden and exposure to prior treatment in autologous recipients, conditions that are absent in healthy allogeneic donors.

Notably, mobilization efficiency, as measured by CD34$^+$ counts, was significantly higher in allogeneic donors (median = 110 cells/μL) compared to autologous patients (median = 48 cells/μL). Only one donor required plerixafor, while 27.6 % of autologous patients did. This highlights the greater mobilization challenges in the latter group.

In the present cohort, the use of plerixafor introduced a distinct inflammatory signature compared to mobilization with G-CSF alone. While baseline values were similar, the plerixafor subgroup exhibited significantly lower post-mobilization PLR (p-value < 0.01) and SII (p-value = 0.005). More importantly, the dynamic shifts for PLR (p-value < 0.01) and SIRI (p-value = 0.03) were significantly altered in these patients. These findings suggest that the addition of a CXCR4 antagonist does more than just enhance CD34$^+$ egress; it may exert a unique modulatory effect on the systemic inflammatory milieu. By potentially attenuating G-CSF-induced responses in platelet and monocyte-associated indices, plerixafor appears to create a different kinetic profile, emphasizing the need to interpret these biomarkers differently in the modern plerixafor era of mobilization.

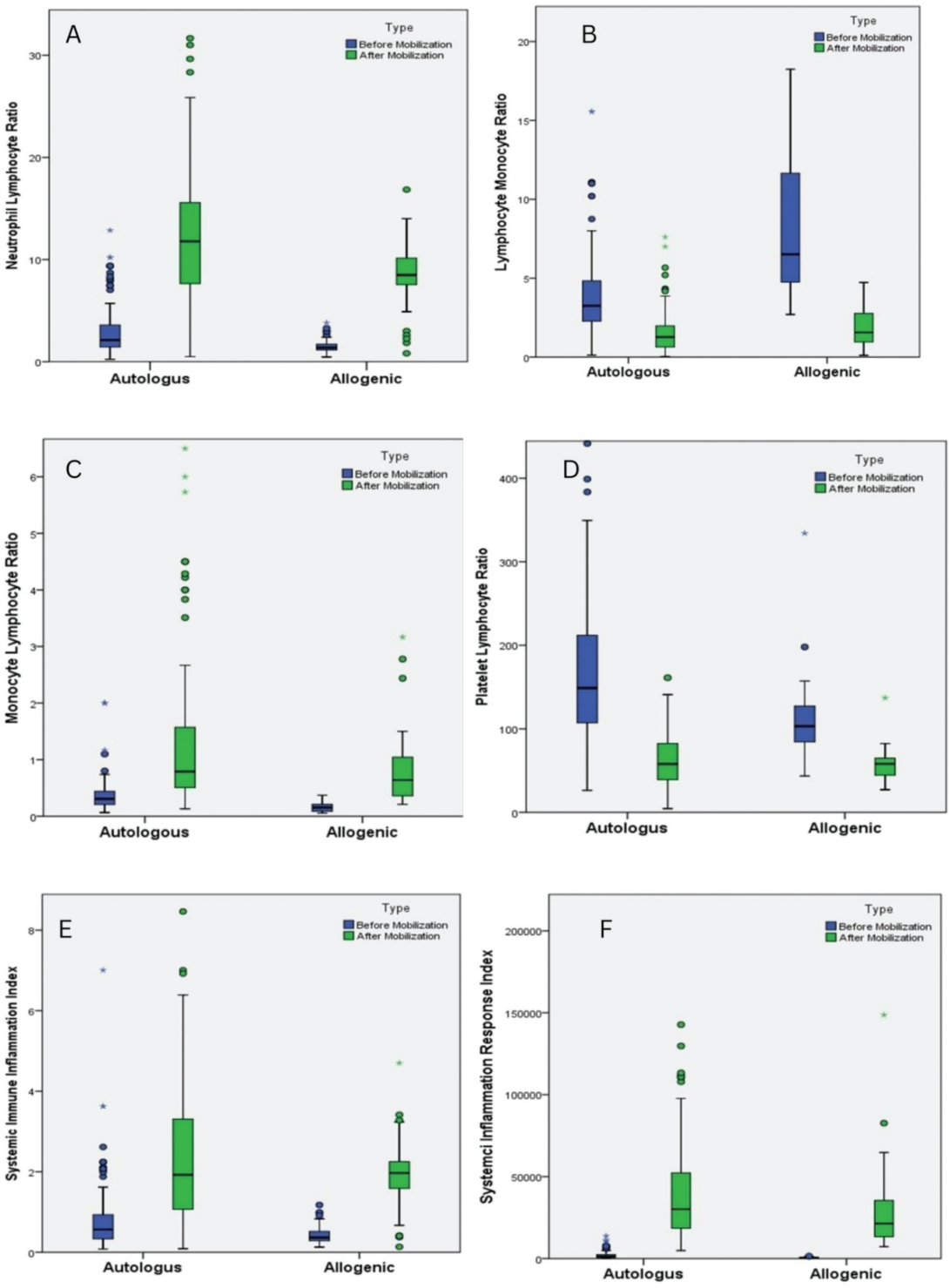

Figure 1 – Changes in Derived Blood Indices before and after mobilization in Autologous patients and allogenic donors.

Interestingly, SII and SIRI showed the highest fold-increases after mobilization: 2-fold in autologous patients and between 5- and 37-fold in allogeneic donors. This disparity highlights a profound, albeit varying, systemic response to G-CSF across the two cohorts. While the magnitude of these shifts coincided with the superior mobilization observed in the allogeneic cohort (median 110 $CD34^+$ cells/μL versus 48 cells/μL in autologous), the data of this study did not show a direct correlation between individual index increases and stem cell yield within the groups. Therefore, rather than serving as a direct measure of mobilization efficacy, these indices likely reflect the intensity of the acute inflammatory stimulus caused by the mobilization regimen. These findings contrast with their traditional prognostic role in oncology, where elevated baseline values typically correlate with poor clinical outcomes [14,15]. Future dose-effect analyses would be

Table 4 – Dynamic Changes in Inflammatory markers with mobilization in autologous patients and allogenic donors.

	Autologous		Allogenic		p-value
	Median	Range	Median	Range	
Dynamic NLR	4.144	−1.0 to 30.8	4.845	−1.0 to 14.1	0.786
Dynamic LMR	−0.639	−1.0 to 12.4	−0.787	−1.0 to 0.5	**0.003**
Dynamic PLR	−0.612	−1.0 to 1.8	−0.480	−1.0 to 0.4	**0.006**
Dynamic SII	2.363	−1.0 to 22.1	3.619	−1.0 to 10.8	0.285
Dynamic SIRI	26.163	0 to 530.5	40.531	0 to 118.3	**0.030**

NLR: Neutrophil-to-Lymphocyte Ratio; LMR: Lymphocyte-to-Monocyte Ratio; PLR: Platelet-to-Lymphocyte Ratio; SII: Systemic Immune-Inflammation Index; SIRI: Systemic Inflammation Response Index.

Table 5 – Changes in blood indexes with plerixafor in autologous patients [median (range)].

Inflammatory Variable	Before Mobilization			After Mobilization		
	No Plerixafor	With Plerixafor	p-value	No Plerixafor	With Plerixafor	p-value
NLR	2.14 (0.2–12.9)	2.107 (0.6–10.2)	0.496	12.31 (0.5–31.7)	9.42 (0.9– 29.6)	0.241
LMR	3.03 (0.5–11.1)	3.83 (0.1–15.6)	0.181	1.31 (0.1–7.6)	1.17 (0.5–5.7)	0.52
PLR	148.85 (26.3–685.2)	148.85 (26.3–685.2)	0.987	62.09 (12.9–140.9)	40.20 (4.5–161.1)	**<0.01**
SII index (x 10^9/L)	0.57 (0.1–7.0)	0.56 (0.1–3.6)	0.368	2.33 (0.1–8.5)	1.59 (0.2–5.0)	**0.005**
SIRI index (x 10^9/L)	1294.22 (159.4–13,802)	1116.78 (135–10,354)	0.143	27,398.25 (4862–267,904)	34,633.86 (6524–470,854)	0.461

NLR: Neutrophil-to-Lymphocyte Ratio; LMR: Lymphocyte-to-Monocyte Ratio; PLR: Platelet-to-Lymphocyte Ratio; SII: Systemic Immune-Inflammation Index; SIRI: Systemic Inflammation Response Index.

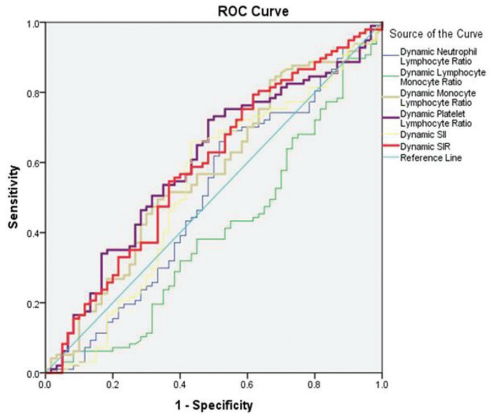

Figure 2 – Receiver Operating Characteristics curve demonstrating dynamic changes in blood indices comparing before and after mobilization in autologous patients and allogenic donors with peripheral blood CD34⁺ Count >50 cells/μL.

Table 6 – Mobilization signature ratios (post-mobilization/pre-mobilization velocity) in autologous patients mobilized with and without plerixafor.

Inflammatory Variable	Plerixafor		p-value
	No (n = 92)	Yes (n = 35)	
NLR	5.32 (0.34–31.81)	5.38 (0.35–18.39)	0.840
LMR	0.39 (0.03–2.00)	0.29 (0.02–13.42)	0.158
PLR	0.46 (0.09–2.76)	0.25 (0.04–1.31)	**<0.01**
SII	3.91 (0.22–14.16)	2.34 (0.19–23.08)	0.070
SIRI	25.80 (1.98–531.45)	33.75 (1.83–289.51)	0.158

NLR: Neutrophil-to-Lymphocyte Ratio; LMR: Lymphocyte-to-Monocyte Ratio; PLR: Platelet-to-Lymphocyte Ratio; SII: Systemic Immune-Inflammation Index; SIRI: Systemic Inflammation Response Index.

required to determine if the degree of index elevation can quantitatively predict yield.

LMR, previously associated with better survival in malignancy, decreased significantly (by 50–70 %) in both groups, indicating mobilization-associated lymphopenia and monocyte predominance [16]. This shift aligns with effective mobilization, although it diverges from survival data, again highlighting the distinct immune environment during stem cell collection.

Despite these significant changes, no dynamic marker showed a consistent correlation with CD34⁺ counts. ROC curve analyses yielded only marginally elevated area under curve (AUC) values for PLR, SIRI, and MLR and failed to establish any of these markers as reliable predictors for peripheral blood CD34⁺ cell counts above 50 cells/μL. This underscores the limited utility of inflammatory markers as standalone biomarkers in pre-apheresis decision-making. Enumeration of CD34⁺ cells by flow cytometry remains the most accurate, rapid, and actionable method for assessing mobilization success.

The mobilization signature ratio (the ratio of post- to pre-mobilization values) was analyzed to further explore the plerixafor era dynamics. The most striking finding was in the PLR, where the velocity of change was significantly lower in the plerixafor group (0.25 versus 0.46 in the G-CSF alone group; p-value < 0.01). This indicates a more pronounced suppression of PLR when plerixafor is added to the regimen.

Although prior studies have explored WBC counts, total nucleated cells, and even biochemical markers like CRP and albumin as surrogate predictors of stem cell yield, their utility remains suboptimal. [17].

A major strength of this analysis is its alignment with contemporary mobilization strategies. By analyzing a real-world cohort of autologous patients where plerixafor was utilized for suboptimal responders, the study provided a more comprehensive view of how modern pharmacological interventions influence these indices.

The findings of the present study align with this, suggesting that while derived hematological indices provide insight into the physiological milieu of mobilization, they lack specificity and predictive strength for clinical utility. Future research should explore composite scoring systems, approaches integrating inflammatory indices with clinical and laboratory parameters to improve predictive accuracy for mobilization success.

Conclusion

PBSC mobilization with G-CSF, with or without plerixafor, is associated with pronounced shifts in derived hematological inflammatory markers, including significant increases in NLR, SII, and SIRI, and reductions in LMR and PLR. These changes reflect an acute systemic inflammatory response, characteristic of effective stem cell mobilization. Although the inflammatory indices vary between autologous and allogeneic settings and are influenced by mobilization regimens, particularly plerixafor use, they do not reliably predict $CD34^+$ stem cell yield. The absence of correlation and low predictive accuracy of ROC analysis limits their application as standalone biomarkers. Flow cytometric enumeration of $CD34^+$ cells remains the most robust and clinically meaningful measure for guiding stem cell collection.

Financial disclosure

There was no funding utilized for this study.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

There was no conflict of interest.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Psychometric properties of the hematopoietic cell transplantation frailty scale: A prospective observational study

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ARTICLE INFO

Article history:
Received 9 April 2025
Accepted 23 March 2026
Available online 25 May 2026

Keywords:
Frailty
Hematopoietic stem cell transplantation
Psychometrics
Frailty scale
Hematologic neoplasms

ABSTRACT

Introduction: Frailty is common in patients undergoing hematopoietic stem cell transplantation, thereby increasing toxicity risk and reducing post-transplant survival. This study evaluated the psychometric properties of the Hematopoietic Cell Transplantation Frailty Scale to assess transplant candidates. Additionally, it characterized the participants` sociodemographic and clinical profiles.

Methods: One hundred and two patients with hematologic malignancies participated in the study. Reliability was evaluated through internal consistency (Cronbach's alpha), factorial analysis with varimax rotation, and construct validity via hypothesis testing. Scale items were grouped in three dimensions: (1) objective, (2) subjective, and (3) laboratory parameter assessments.

Results: Participants had a mean age of 40.5 ± 14.5 years; 15 (14.7%) were classified as 'frail,' 48 (47.1%) as 'pre-frail,' and 39 (38.2%) as 'fit.' Multiple myeloma was the most common diagnosis (32.4%). Internal consistency was acceptable (α = 0.63). The first dimension explained 55.3% of the variance, while the second contributed 31.5%, totaling 86.8% of the explained variance. Construct validity confirmed only one hypothesis: patients with a performance status below 90 had significantly higher frailty scores than those with a performance status of 90–100 (p-value <0.01). Other hypotheses comparing frailty classification (frail, pre-frail, and fit) across Disease Risk Index categories (low-moderate, high-very high, not evaluable), Hematopoietic Cell Transplantation Comorbidity Index (<3, ≥3), transplant type (autologous, allogeneic), and age groups (18–59, ≥ 60 years) were rejected (p-value >0.05).

Conclusion: The Chilean version of the Hematopoietic Cell Transplantation Frailty Scale demonstrates adequate reliability and validity, supported by a strong factorial structure to assess transplant candidates. Frailty was associated with a lower performance

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https://doi.org/10.1016/j.htct.2026.106471
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status, but not with the Disease Risk Index, the Comorbidity Index, transplant type or age.

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Introduction

Hematopoietic stem cell transplantation (HSCT) is used with curative intentions for hematologic neoplasms and other potentially lethal diseases [1]. Improvements in clinical practice, along with the increase in the population life expectancy, have expanded the indications of both autologous and allogeneic HSCT [2].

Frailty is a syndrome characterized by reduced physiological reserve in patients, which in HSCT patients increases the risk of toxicities and reduces post-transplant survival [3–6]. Additionally, frailty has been associated with poor post-HSCT outcomes in both young and old individuals [7].

Thus, identifying and treating frailty in HSCT candidates can improve final transplant outcomes. Different tools have been developed to diagnose frailty, most of which are adapted from validated geriatric frailty scales [6,8]. Nevertheless, a significant number of HSCT candidates are not geriatric patients, limiting the applicability of these scales for younger individuals [6,8].

While frailty scales are being developed for implementation in the HSCT setting, there is still no consensus on the best assessment methodology, target population, cost-effectiveness, and predictive accuracy [9].

Professionals at Princess Margaret's Hospital developed the Hematopoietic Cell Transplantation Frailty Scale, a tool to assess frailty and functionality in HSCT candidates regardless of age. It is a multidimensional assessment composed of eight items specifically selected to be administered in the daily clinical practice, with no additional waiting time for patients and using existing human resources [9,10].

The HCT Frailty Scale corresponds to a quick and easy-to-implement tool that combines functional variables related to frailty along with laboratory biomarkers that categorize adult HSCT candidates into three categories: 'fit,' 'pre-frail' and 'frail' [10].

The HCT Frailty Scale still lacks psychometric studies in the Chilean population. As the validation process is dynamic and continuous, frailty scales acquire greater relevance as they advance and deepen with psychometric studies that allow the strengthening of their use in the daily clinical practice [11]. Therefore, the use of validated instruments tailored to the specific population is recommended for clinical applications. Thus, the objective of this study is to evaluate the psychometric properties such as reliability and validity of the HCT Frailty Scale in a sample of Chilean HSCT candidates, and, subsequently, characterize their sociodemographic and clinical profiles.

Methods

This observational study with a cross-sectional design followed the Consensus-based Standards for the selection of health Measurement Instruments (COSMIN) guidelines [12].

Participants

This psychometric study included 102 patients (aged ≥18 years) diagnosed with onco-hematological diseases who were candidates for autologous or allogeneic HSCT at a public hospital in Santiago, Chile.

The exclusion criteria were people with disabilities and cognitive deficits that limited their ability to understand instructions and sign an informed consent. Additionally, patients with insufficient comprehension of Spanish were excluded. The Mini-Mental Abbreviated test was used to evaluate the cognitive dimension [13].

Procedure

Patients were recruited during their first consultation with a hematologist in the HSCT program. Those who met the eligibility criteria were invited to participate in this study. The study objectives and the details of their participation were explained to those who voluntarily agreed to participate and signed an informed consent form.

The evaluations, conducted by two physical therapists with clinical experience of HSCT patients, were performed in a physical medicine and rehabilitation polyclinic between December 2023 and January 2025.

A convenience sampling method was used. The sample size was estimated based on the main objective of this study, which was to evaluate reliability in psychometric studies. It was calculated that a minimum of 100 participants was required to obtain reliable estimates of Cronbach's alpha [14].

This study adhered to ethical standards of the Declaration of Helsinki and was approved by the scientific ethics committee of the Eastern Metropolitan Health Service (December 5, 2023). The HCT Frailty Scale was administered approximately 45 days before transplantation as part of the routine pre-transplant evaluation. Patients identified as 'pre-frail' or 'frail' were referred to a structured prehabilitation program, in line with institutional clinical care protocols.

Hematopoietic cell transplantation frailty scale

This scale was created by professionals at the Princess Margaret Cancer Center, Toronto, Canada, and is composed of eight items: Clinical Frailty Score (CFS), Instrumental Activities of Daily Living (IADL) test, Hand Grip Score (GS), Timed Up and Walk Test (TUGT), Self-Reported Health Question (SRH-Q), Single Falls Question, Self-Reported Health Levels (SRH-Q), and the measurement of the serum albumin and C-reactive protein (CRP) levels [9,10].

A Jamar® hydraulic dynamometer (J A Preston Corporation, New York, USA) was used for the handgrip strength test.

The HCT Frailty Scale allows patients who are candidates for HSCT to be categorized as 'fit', 'pre-frail' and 'frail' according to the score resulting from the sum of the weighted values of the eight items of the scale.

Karnofsky functional status scale (KPS)

A numerical scale was used to quantify the functional status of patients concerning their degree of independence in daily activities and self-care needs. Patients are assessed using an 11-category scale ranging from 0 to 100, where a higher score indicates greater ability to perform daily activities [15]. In this study, patients were grouped according to the following ranges: <90 and 90–100.

Disease risk index (DRI)

The Disease Risk Index (DRI) is a validated tool to categorize HSCT patients with hematologic malignancies. Its purpose is to stratify patients into broad categories of disease risk. The following categories were used in this study: low-moderate, high-very high, and not assessable [16].

Hematopoietic cell transplantation-comorbidity index (HSCT-CI)

Hematopoietic Cell Transplantation Comorbidity Index (HSCT-CI), also known as the Sorror Comorbidity Index, is a score that estimates the risk of transplant mortality based on patient comorbidities and the type of conditioning indicated. Based on the score obtained, patients receive scores of 0 to 4. The following ranges were used in this study: <3 and ≥3 [17].

Sociodemographic and clinical background

Information was compiled on variables (age, sex, schooling, marital status, employment status, occupation) and clinical variables (treatments received, smoking, alcohol consumption, weight, height, body mass index [BMI], diagnosis, transplant type, treatments received).

Statistical analysis

The data were tabulated in an Excel spreadsheet and analyzed with the Statistical Package for Social Sciences (SPSS) version 30 software. Descriptive statistics were reported as frequency, mean, median, and interquartile range.

Regarding psychometric properties, reliability was assessed through internal consistency and construct validity using hypothesis testing.

Cronbach's alpha analysis was used to evaluate the internal consistency and dimensionality of the scale employing the values described by Loewenthal [18]. These authors suggested that an internal consistency of 0.60 was acceptable for scales with less than ten items such as the HCT Frailty Scale (eight items). In addition, a factor analysis was conducted to identify the factor loading and instrument dimensions, considering Barlett's test assumptions (p-value <0.05), and using the Kaiser-Meyer-Olkin test.

For these analyses, the eight items of the scale were grouped into three dimensions: (1) subjective dimension, grouping items 2, 5, and 6, (2) objective dimension, grouping items 1, 3, and 4, and (3) laboratory parameters grouping items 7 and 8.

Hypothesis testing using the Mann-Whitney U test was used to evaluate the construct validity of the HCT Frailty Scale. Five hypotheses were tested employing this test:

(1) Patients with a Karnofsky Performance Status (KPS) <90 have higher frailty scores compared to those with a performance status of 90 to 100.
(2) Patients with high to very high DRI have higher frailty scores compared to those with a low to moderate DRI.
(3) Patients with HSCT-CI ≥3 have a higher frailty score compared to those with HSCT-CI <3.
(4) Patients who are candidates for allogeneic HSCT have higher frailty scores compared to those who are candidates for autologous HSCT.
(5) Older adult patients (≥60 years) have higher frailty scores compared to younger adult patients (18–59 years).

Results

Sociodemographic and clinical characterization of the participants

A total of 102 patients participated, with an average age of 40.5 years. Fifty-two (51.0%) were women and fifty (49.0%) were men. The most prevalent diagnosis was multiple myeloma in 33 patients (32.4%), followed by acute lymphoblastic leukemia in 22 patients (21.6%). Of the total sample, fifty-nine patients (57.8%) were candidates for autologous HSCT, 34 (33.3%) for allogeneic haploidentical HSCT, and nine (8.8%) for allogeneic HSCT with an identical family donor (IFD). Regarding frailty, 15 (14.7%) individuals were categorized as 'frail', 48 (47.1%) as 'pre-frail' and 39 (38.2%) as 'fit'. Table 1 presents all the sociodemographic and clinical characteristics of the participants (Table 1).

Psychometric properties

Internal consistency and dimensionality

A Cronbach's α of 0.63 was obtained for the total scale, indicating acceptable internal consistency. For the objective dimension which grouped items 1, 3 and 4, Cronbach's α was 0.61. For the subjective dimension which grouped items 2, 5, and 6, Cronbach's α was 0.40 and for laboratory parameters that grouped items 7 and 8, the Cronbach's α was 0.10 (Table 2).

Factor analysis and varimax rotation

Regarding the analysis of the scale components with varimax rotation, the Kaiser-Meyer-Olkin test of sample adequacy

Table 1 – Characterization of study participants (n = 102).

Variable	
Sex – n (%)	
Female	52 (51.0)
Male	50 (49.0)
Age - years – median ± SD (range)	40.5 ± 14.5 (37.6–43.4)
18–59 years – n (%)	98 (96.1)
≥ 60 years – n (%)	4 (3.9)
Anthropometric Measurements – median ± SD (range)	
Height - m	1.64 ± 0.10 (1.62–1.66)
Weight - kg	74.8 ± 17.5 (71.4–78.3)
Body Mass Index - kg/cm^2	27.4 ± 5.2 (26.4–28.4)
Diagnosis – n (%)	
Plasma cell disorders: (Multiple Myeloma; Amyloidosis)	34 (33.3)
Acute Leukemias: (Acute Lymphoblastic Leukemia; Acute Myeloid Leukemia; Acute Leukemia of Ambiguous Lineage)	34 (33.3)
Mature Lymphoproliferative disorders (Hodgkin Lymphoma; Non-Hodgkin Lymphoma)	25 (24.5)
Myeloproliferative neoplasms (Chronic Myeloid Leukemia)	2 (2)
Myelodysplastic Neoplasms/ Aplastic anemia (Aplastic anemia, MDS, Hypoplastic MDS)	7 (6.9)
Type of Treatment – n (%)	
Chemotherapy/Radiotherapy/ Immunotherapy	2 (2.0)
Chemotherapy/Immunotherapy	7 (6.9)
Chemotherapy/Radiotherapy	8 (7.8)
Chemotherapy	78 (76.5)
Immunotherapy	5 (4.9)
Not Declared	1 (1.0)
Type of HSCT – n (%)	
Autologous	59 (57.8)
Allogeneic - Identical Family Donor	9 (8.8)
Allogeneic - Haploidentical	34 (33.4)
DRI – n (%)	
Low-Moderate	78 (76.5)
High-Very High	19 (18.5)
Not Evaluable	5 (4.9)
Karnofsky Score – n (%)	
90–100	52 (51.0)
<90	50 (49.0)
HSCT-CI – n (%)	
< 3	91 (89.2)
> = 3	11(10.8)
Patient Categorization According to HCT Frailty Scale – n (%)	
Frail	15 (14.7)
Pre-frail	48 (47.1)
Fit	39 (38.2)

Mean ± Standard Deviation [Lower Limit; Upper Limit, 95% Confidence Interval].
DRI: Disease Risk Index; HSCT-CI: Hematopoietic Cell Transplantation–Specific Comorbidity Index.

produced a score of 0.74, indicating acceptable adequacy. Bartlett's test of sphericity was significant (p-value <0.01), supporting the relevance of performing the factor analysis. The first dimension (objective evaluation) accounted for 55.3% of the variance, making it the strongest factor in the scale.

Table 2 – Reliability of the HCT Frailty Scale (n = 102).

HCT Frailty Scale Dimensions	Item	Cronbach's Alpha
Objective Assessment	Item 1: Clinical Frailty Scale Item 3: Timed Up and Go Test (TUGT) Item 4: Handgrip Strength	0.61
Subjective Assessment	Item 2: Instrumental Activities of Daily Living (IADL) Item 5: Self-reported Health Question Item 6: Falls in the Last Six Months	0.40
Laboratory Parameter Assessment	Item 7: Serum Albumin Level Item 8: C-Reactive Protein	0.10

Total Cronbach's Alpha of the HCT Frailty Scale: 0.63.

The second dimension (subjective evaluation) accounted for 31.5%, bringing the cumulative variance explained by both dimensions to 86.7%. Finally, the third dimension had a low eigenvalue (0.39) and explained only 13.2%, suggesting that it is a weak factor, that is, its component items contribute less to the overall scale (Table 3). Figure 1 represents the sedimentation graph, which shows that the slope decreases from factor 2 of the scale, specifically in factor 3, which presented a low eigenvalue (Figure 1 & Table 3).

Construct validity

Construct validity confirmed only one hypothesis: patients with a Karnofsky performance status <90 had higher frailty scores compared to those patients with performance status of 90 to 100 (u = 2048.0; p-value <0.01).

The remaining four hypotheses were rejected (p-value >0.05). There was no statistically significant difference for the subgroups: DRI (K = 3.87; p-value = 0.14), HSCT-CI (U = 349.000; p-value = 0.07), type of HSCT (U = 1.252; p-value = 0.90), and age (K = 5.373; p-value = 0.06) (Table 4).

Complementary to the hypothesis tests, Figure 2 represents the relationship map between frailty categories and other variables (DRI, type of HSCT, Karnofsky score, and HSCT-CI). In general, the connections established in the map reflect the strength of the association between the variables; the thickness of the lines indicates the magnitude of these associations. Although the aforementioned hypotheses were rejected, autologous HSCT candidates tend to be pre-frail compared to allogeneic candidates (Figure 2A). Low DRI is associated with all three categories of frailty concerning the other indices in this category (Figure 2B), as is a HSCT-CI index between 1–2 (Figure 2C). Additionally, the lower performance status score is more strongly associated with pre-frail and fit individuals (Figure 2D). Regarding patient frailty categorization, Supplemental Table 1 presents the classification of each domain of the HCT Frailty Scale as normal or abnormal, according to established cutoffs. These individual scores were summed to obtain a total frailty score (range: 0 –10.5), which allows categorization of patients as 'fit', 'pre-frail', or 'frail'.

Table 3 – Variance explained by extracted factors.

HCT Frailty Scale Dimensions	Eigenvalue	% of Variance	Cumulative %
Objective Assessment Item 1: Clinical Frailty Scale Item 3: Timed Up and Go Test (TUGT) Item 4: Handgrip Strength	1.65	55.26	55.26
Subjective Assessment Item 2: Instrumental Activities of Daily Living (IADL) Item 5: Self-reported Health Question Item 6: Falls in the Last Six Months	0.94	31.51	86.77
Laboratory Parameter Assessment Item 7: Serum Albumin Level Item 8: C-Reactive Protein	0.39	13.22	100.00

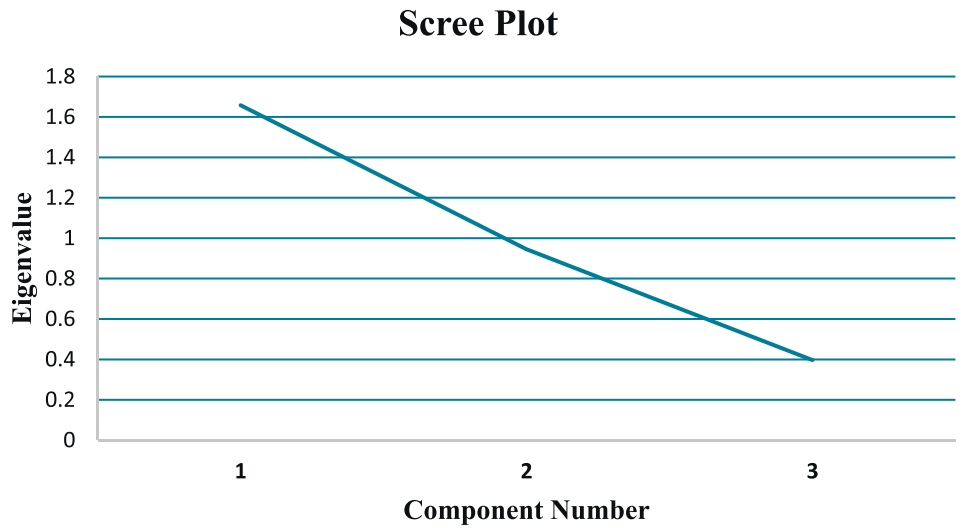

Figure 1 – Scree plot of the scale dimensions.1. Objective assessment: Item 1: Clinical Frailty Scale; Item 3: Timed Up and Go Test (TUGT); Item 4: Handgrip strength. 2. Subjective assessment: Item 2: Instrumental Activities of Daily Living (IADL); Item 5: Self-reported health question; Item 6: Falls in the last six months. 3. Laboratory parameter assessment: Item 7: Serum albumin level; Item 8: C-reactive protein.

Table 4 – Prevalence of the Hematopoietic Cell Transplantation Frailty Scale by Disease Risk Index, performance status, Hematopoietic Cell Transplantation Comorbidity Index, and transplantation type.

	Frail	Pre-frail	Fit	Test value; P-value
Hematopoietic Cell Transplantation (HSCT)	Autologous: 6 (40) Allogeneic: 9 (60)	Autologous: 32 (66,.7) Allogeneic: 16 (33.3)	Autologous: 21 (53.8) Allogeneic: 18 (46.2)	U = 1.252; p-value = 0.90
Performance status (Karnofsky Score)	90–100: 14 (13.7) <90: 1 (1.0)	90–100: 28 (27.5) <90: 20 (19.6)	90–100: 10 (9.8) <90: 29 (28.4)	U = 2048.0; p-value <0.01
Disease Risk Index (DRI)	Low-Moderate: 12 (11.8) High-Very high: 3 (2.9) Not Evaluable: 0 (0)	Low-Moderate: 37 (36.3) High-Very high: 10 (9.8) Not Evaluable: 1 (1.0)	Low-Moderate: 29 (28.4) High-Very high: 6 (5.9) Not Evaluable: 4 (3.9)	K = 3.87; p-value = 0.14
Hematopoietic Cell Transplantation–Specific Comorbidity Index (HSCT-CI)	<3 13 (86.7) > = 3 2 (13.3)	<3 40 (83.3) > = 3 8 (16.7)	<3 38 (97.4) > = 3 1 (2.6)	U = 349.000; p-value = 0.07
Age	18–59 years ≥ 60 years	18–59 years ≥ 60 years	18–59 years ≥ 60 years	K = 5.373; p-value = 0.06

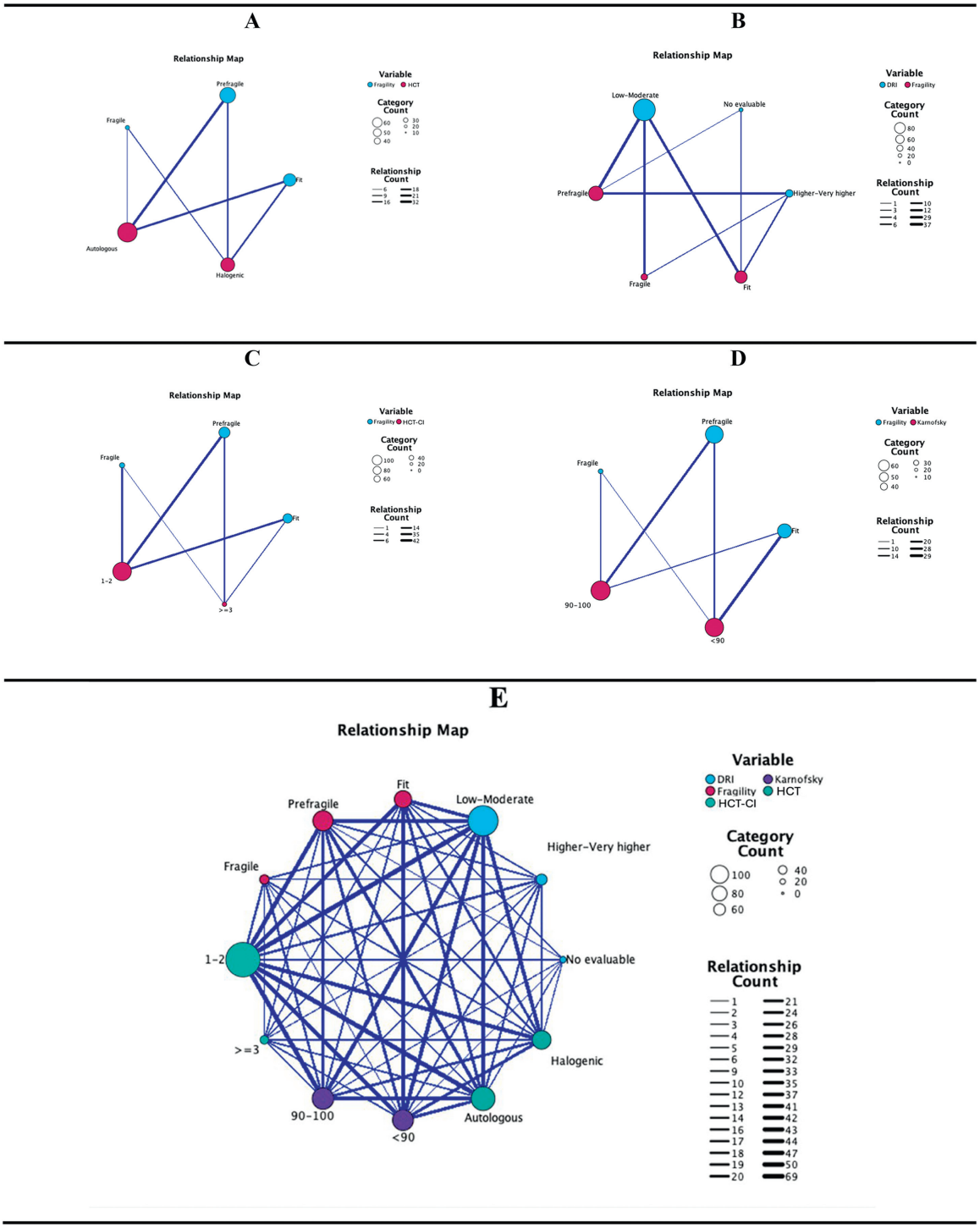

Figure 2 – Relationship map between frailty categories according to the HCT Frailty Scale and DRI, type of HSCT, Karnofsky score, HSCT-CI, and all variables combined. A: Relationship between frailty and HSCT. B: Relationship between frailty and DRI. C: Relationship between frailty and HSCT-CI. D: Relationship between frailty and performance status. E: Relationship between frailty and HSCT, DRI, HSCT-CI, and performance status

Discussion

This study demonstrates that the Chilean Spanish version of the HCT Frailty Scale is adequate in terms of internal consistency and construct validity in the assessment of frailty in HSCT.

The sociodemographic background of this study identified a young population with a mean age of 40.5 ± 14.5 years, exhibiting some degree of frailty. This finding is consistent with previous studies that indicated that frailty is not exclusive to the geriatric population [3,5,10]. Additionally, patients with hematological

malignancies who are candidates for HSCT may also exhibit this syndrome [5,9,10].

In terms of frailty, the majority of participants were categorized as 'prefrail' (n = 48; 47.1%) followed by 'fit' (n = 39; 38.2%) and a minority as 'frail' (n = 15; 14.7%). Similar results were reported in studies conducted by the original authors of the HCT Frailty Scale. In their initial consultation assessment, 53.4% were classified as 'pre-frail', 28.8% as 'fit' and 18.2% as 'frail' [5,10,19].

The criteria used to assign patients to these categories are summarized in Supplementary Table 1, which details the classification of each HCT Frailty Scale domain (normal versus abnormal) based on established cutoffs, and how these scores determine the overall frailty status.

Regarding the psychometric properties, the scale demonstrated acceptable internal consistency, with Cronbach's alpha values of 0.63 for the total scale. Internal consistency is a measure of the correlation between items in a measurement instrument. Generally, values are acceptable between ≥0.70 and ≤0.95. However, when interpreting the value of the coefficient of internal consistency, it is convenient to take into account the length of the scale. It is generally recommended that scales with ten homogeneous items should achieve an adequate reliability [20]. Nevertheless, many modern scales comprise fewer than ten items. In these cases, internal consistency can still be acceptable with values below 0.70. This was the case in this study, where the HCT Frailty Scale (with eight items) achieved a Cronbach's alpha of 0.63, which is considered acceptable [18].

In this study, construct validity confirmed only one hypothesis related to performance status with results similar to those reported in the study by Salas et al., who found that patients with a KPS <90 were more likely to be frail than those with a higher KPS [9].

Contrary to expectations, the results of this study indicate that frailty is a common syndrome in both patients with HSCT-CI ≥ 3 and those with a HSCT-CI <3. This finding is consistent with Salas et al., who reported that frailty was independent of the initial comorbidity index [19]. This was also the case for patients with both high-very high and low-moderate DRI.

Regarding age, the results of this study show that comparable levels of frailty can be found in both younger (under 60 years) and older adults (≥60 years). Rejecting the initial hypothesis, it was found that young adult candidates for HSCT may also be frail due to multiple factors, such as underlying disease, prior treatments, and existing complications [10,21,22].

Finally, the type of HSCT (autologous or allogeneic) did not influence the frailty score. Similar results were obtained in the study by Singh et al., where similar distributions of frailty domains were observed for autologous and allogeneic HSCT [23]. Considering that most studies on frailty in HSCT have focused exclusively on allogeneic HSCT [10,24] the findings of the present study are noteworthy as a significant percentage of autologous HSCT candidates (57.8%), who may also present pretransplant frailty, was included.

One of the limitations of this study was the absence of published psychometric studies of the HCT Frailty Scale, which restricted the possibility of comparing these findings with other studies. However, a nationally representative sample was involved, evaluating HSCT candidates from all regions of the country.

In addition, it is important to note that although frailty assessments are currently recommended prior to HSCT [4,24,25], they have not yet been systematically incorporated into pre-HSCT evaluations.

Likewise, the implementation of pre-HSCT frailty assessments remains an area of emerging interest and active research [1]. Therefore, this study represents a breakthrough in the area, demonstrating how frailty interacts with key factors in HSCT candidates, and identifying clinically relevant patterns for decision-making in this population.

Finally, as frailty status is dynamic in nature, i.e., the transition from 'fit' to 'pre-frail' and 'frail' is not always linear, it is potentially reversible and can be modified through targeted interventions [26]. Therefore, routine frailty assessment and monitoring is gaining importance in HSCT patients [1,18] and could be a preliminary step in designing specific, multidisciplinary interventions, including prehabilitation and post-HSCT rehabilitation programs. Therefore, the management of this syndrome should focus on its identification, treatment, and prevention of its potential consequences [27] aiming to reduce the negative impact of HSCT on the patient's quality of life, morbidity, and mortality.

Conclusions

The Chilean version of the HCT Frailty Scale has demonstrated adequate reliability and validity, with a factorial structure that supports its use in assessing frailty in HSCT candidates. Frailty was associated with lower performance status, but not with DRI, HSCT-CI, transplant type, or age.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

None.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors are very grateful to the hematologists from the HSCT program at the Hospital del Salvador and to all patients who participated in this study.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2026.106471.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Developing infographics to implement patient blood management in the surgical pathway: From concept to design

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ARTICLE INFO

Article history:
Received 5 August 2025
Accepted 3 February 2026
Available online 30 May 2026

Keywords:
Health communication
Health education
Blood transfusion
Blood conservation
Patient care management

ABSTRACT

Introduction: Effective communication plays a crucial role in health education by facilitating the dissemination and assimilation of complex clinical concepts. Infographics are promising visual tools to promote evidence-based practices, especially for multifaceted strategies like patient blood management.

Objective: The aim of this study was to develop and evaluate infographics to communicate Patient Blood Management principles along the surgical pathway.

Methods: Using a user-centered, iterative Design Thinking approach, a multidisciplinary team of healthcare professionals, a graphic designer, and patient blood management specialists developed infographics across five stages: narrative construction, sketching, digital design, expert validation, and assessment of real-world applicability.

Results: Infographics representing patient blood management pillars were created incorporating icons, color gradients, symbolic pathways, and QR codes linking to supplementary content. The materials were accessible, visually engaging, and technically accurate, facilitating knowledge retention and practical application of patient blood management protocols.

Conclusion: Infographics can overcome educational barriers and enhance the engagement of healthcare professionals in patient blood management implementation. By combining scientific evidence with visual communication, infographics support cultural shifts towards rational blood use, aligning with global surgical safety and public health objectives.

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https://doi.org/10.1016/j.htct.2026.106473

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Introduction

Communication, whether visual, written, or verbal, is a fundamental pillar for the effective dissemination of knowledge. The way information is conveyed, through images, texts, or speech, directly shapes individuals' perception, assimilation, and engagement with the presented content [1]. In this context, infographics represent a powerful fusion of visual and written communication, structuring complex information into clear and accessible representations [2,3].

The human brain processes images much faster than isolated texts, and the simultaneous combination of visual and textual stimuli activates multiple brain areas related to memory and learning [4,5]. This integration facilitates understanding and retention of knowledge, translating complex information into more easily assimilated concepts and promoting greater engagement and practical application [6].

In healthcare, where the volume of technical information is high and time for assimilation can be limited, innovation in communication plays an even more strategic role. Infographics emerge as innovative tools to translate scientific data and clinical protocols into visually appealing and educational formats, aiding continuous education and adherence to evidence-based practices [7].

To validate this context, the concept of Patient Blood Management (PBM) was chosen as the subject for applying infographics. PBM, recognized by the World Health Organization (WHO) as a patient-centered, systematic, evidence-based approach to improve patient outcomes by managing and preserving a patient's own blood, while promoting patient safety and empowerment, encompasses three main pillars: management of preoperative anemia, minimization of intraoperative blood loss, and optimization of postoperative care [8–11].

Given the importance of PBM for patient safety and healthcare sustainability, infographics are a powerful tool to overcome cultural barriers, raising awareness and engaging healthcare professionals. Thus, this study describes the development of these visual materials, highlighting their creation, design strategies, and potential impact on public health education, underscoring the transformative role of visual communication in modernizing and enhancing the dissemination of clinical knowledge.

Methods

General graphic design framework

The development of the infographics followed an iterative creation approach focused on the end-user's information absorption. It is based on the principles of Design Thinking [12] for the development of graphic elements. The adopted approach placed the content-consuming professional as the main focus in the development process, emphasizing continuous iterations, collective feedback, and careful consideration of the context in which the innovation was implemented.

Development team

The infographic development process was conducted by a multidisciplinary team composed of professionals including a nurse, a biomedical scientist, biologists, a graphic designer, and an engineer. Some members of this team are PBM specialists (certified by the Sociedad Iberoamericana de PBM [SIAPBM] or the Society for the Advancement of Patient Blood Management [SABM]).

User-centered development stages

For the creation of the infographics, five structured development stages were carried out to explore specific aspects, using techniques such as storytelling [13], brainstorming for graphic elements, and feedback iterations of the surgical patient journey (Figure 1).

The first stage involved the multidisciplinary team sharing the initial project ideas. During this session, the team shared their practical experiences related to PBM and the challenges associated with understanding its principles. These narratives helped shape the initial infographic content, directly connecting it to practical, real-world clinical scenarios. Additionally, brainstorming was employed in this stage to generate creative ideas and explore innovative approaches to infographic construction. This exchange aimed to stimulate concrete reflections on clinical practice problems and explore possible educational solutions that could be translated into visual elements.

In the second stage, the first infographic prototypes were presented, developed as hand-drawn sketches by the design

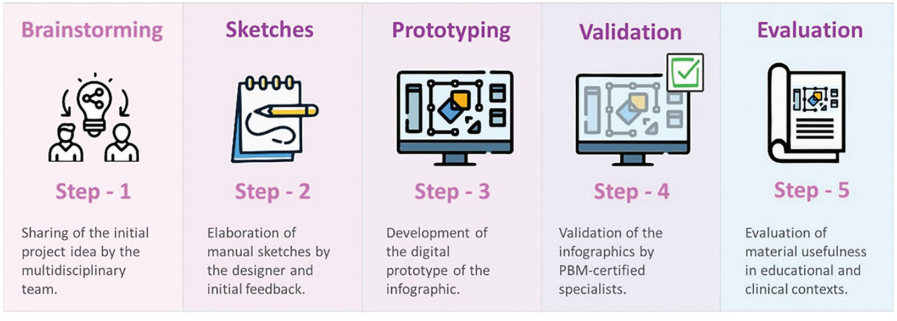

Fig. 1 – Illustration of the infographic development stages.

professional. These sketches served as basic, schematic representations of the infographics, allowing the team to explore content organization, visual hierarchy, and initial layout before moving on to detailed designs. The initial feedback on the sketches from the team was crucial to identify needs such as simplifying technical language, increasing the use of icons, and structural adjustments to facilitate the understanding of different audiences.

Based on the adjusted sketches, the third stage focused on developing digital prototypes. During this phase, graphic elements such as colors, diagrams, and figures were refined, prioritizing message clarity and visual impact. This iterative approach between sketches and prototypes ensured that changes reflected end-user needs and maintained consistency with established educational and design principles.

The fourth stage consisted of an analysis of the infographics by the PBM-certified experts. In this phase, the content was thoroughly reviewed to ensure technical accuracy and practical relevance.

Finally, the usefulness of the materials was validated in potential real educational and clinical contexts, allowing a final analysis of their applicability and effectiveness as educational tools.

The Design Thinking methodology [12] was present throughout all development stages, acting as the central axis for the conception, refinement, and validation of the graphic representations. This creative and iterative approach model enabled a structured construction of the materials, ensuring that the infographics were visually appealing, scientifically grounded, and didactically effective in disseminating PBM concepts. Moreover, the iterative and collaborative development process guaranteed that the infographics met the target audience's expectations and contributed to the dissemination of PBM principles.

Digital version of the infographic

The infographic development utilized CorelDRAW version 23 and Adobe Photoshop version 25.12.0. CorelDRAW was used to create illustrations, vector elements, and structured layouts, while Adobe Photoshop was employed for image editing and manipulation, ensuring visual refinement and the final quality.

Results

Infographic 1: the patient blood management of anemia in the 'Surgery verse'

Conceptual description

This infographic simplifies a complex medical protocol using a gamified visual learning experience. It leverages a metaphor of a journey or a 'verse' to guide viewers through the PBM process for anemia before surgery.

The innovative approach is evident in how it visualizes the decision-making pathways. Patients are represented as figures on a path, creating a clear narrative flow. The initial 'Check Control for Anemia' acts as a critical gatekeeper. Instead of just listing criteria, the infographic shows the diverging paths: those who are cleared proceed directly to the goal ('Surgery'), while those who are not are rerouted for intervention.

The detour is where the educational power truly shines. It graphically represents the diagnostic and treatment phases, a 'blood analysis' followed by 'treatment' with key supplements (iron, vitamin B12, Folic Acid, erythropoietin). The color-coded bar for treatment is a simple yet effective way to represent a spectrum of care. The concept of a 'Detour Return' highlights the iterative nature of PBM: it is not a one-and-done process. Patients are re-evaluated in a second 'check for anemia' before they can be cleared for surgery. This loop reinforces a core principle: patient optimization is paramount and may require multiple steps.

By personifying the patient's journey and using visual cues like green checkmarks for success and red 'X's for setbacks, the infographic transforms a dry medical guideline into a compelling, easy-to-understand narrative.

Visual elements

The dark background creates contrast and highlights the vibrant colors of technical figurative elements, making the visual flow clearer and more appealing. For the figurative elements, shades of blue, purple, and cyan are used to represent stages such as hematological analysis and medical guidance.

The use of minimalist stylized human figures facilitates understanding of the patient's trajectory. Iconography such as a microscope, doctor, and blood are stylized in a simple and clear manner. The patient's path is represented by a continuous curved line, providing a sense of movement and continuity. Arrows indicate directions and actions. Text is minimal and direct, complementing the visuals. The use of symbols predominates to convey messages quickly (Figure 2).

Figure 2 Fe; Iron; VIT B12: Vitamin B12; EPO: Erythropoietin; TSat: Transferrin saturation; Hgb: Hemoglobin; MCV: Mean corpuscular volume; IV: Intravenous; ESA: Erythropoiesis stimulating agents.

QR codes and informative supplementation

As part of the additional content of Infographic 1, a QR code was incorporated, directing users to supplementary materials on preoperative anemia management. This includes a detailed flowchart guiding decision-making according to the type of anemia, covering everything from initial laboratory screening to defining the appropriate therapeutic approach. The image presents a structured scheme for differential diagnosis of anemia, considering parameters such as hemoglobin, ferritin, transferrin saturation, vitamin B12, and reticulocytes. Based on these data, the flowchart guides the healthcare professional in choosing treatment, including oral or intravenous iron supplementation, vitamin B12 or folate replacement, erythropoietin use, and other strategies according to the etiology of the anemia (Figure 2).

Infographic 2: the patient blood management 'Winner's lap' in the 'Surgery verse'

Conceptual description

This infographic cleverly transforms the medical interventions into a structured, metaphorical 'winner's lap', a concept familiar from racing, to underscore the goal of a successful, safe surgery.

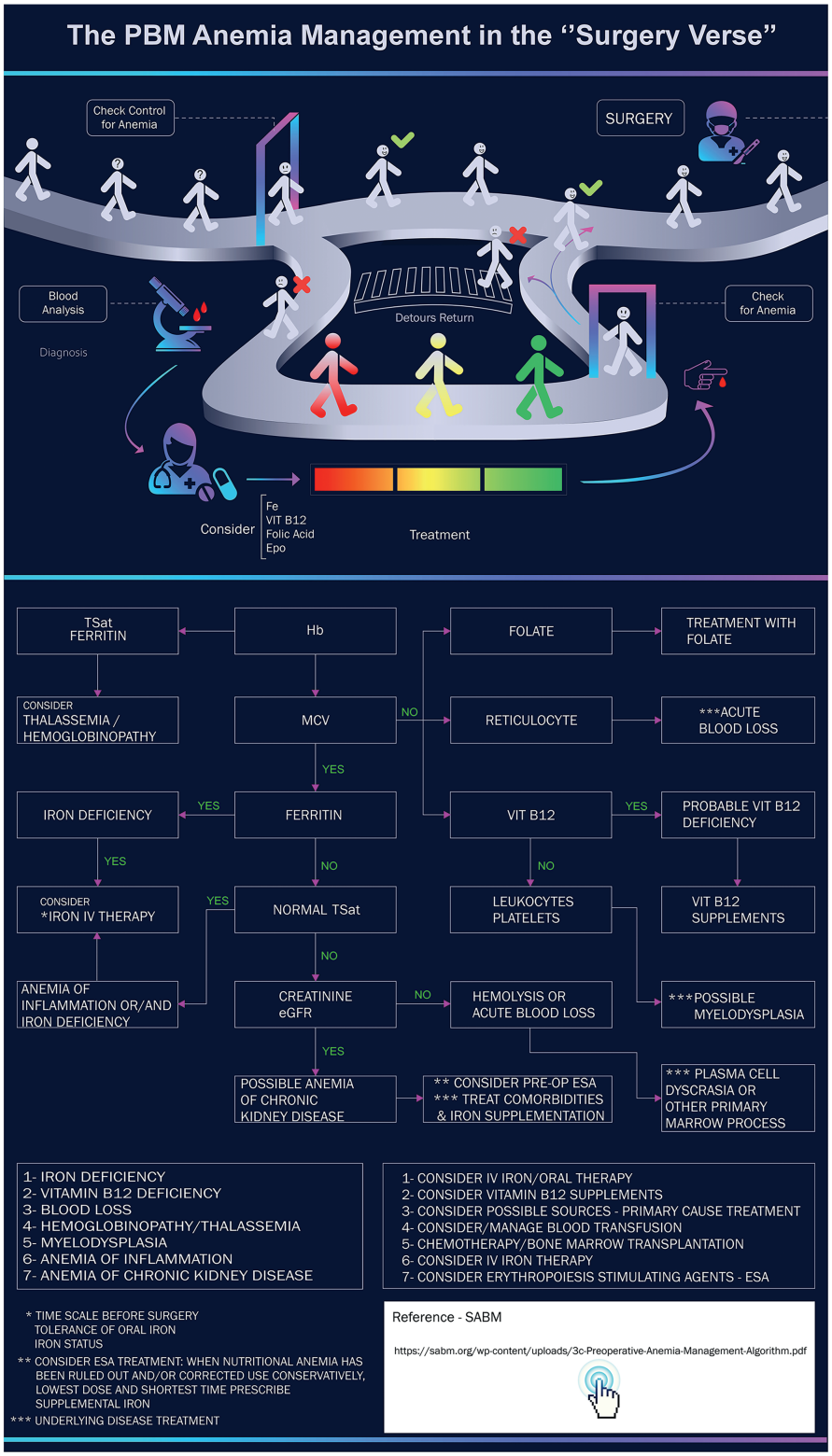

Fig. 2 – Patient blood management of anemia in the 'Surgery Verse'. This gamified visual narrative guides viewers through preoperative anemia screening, diagnostic tests, and sequential treatments (iron, vitamin B12, folic acid, erythropoietin). The iterative re-assessment step ensures patient optimization before surgery. Stylized patient Fig.s, color-coded paths, and minimal text create an engaging and intuitive clinical decision flow. Accessible via QR code, this resource provides an in-depth diagnostic algorithm considering hemoglobin, ferritin, transferrin saturation, vitamin B12, and reticulocytes. It guides personalized treatment choices including oral/IV iron, vitamin supplementation, and erythropoietin use, supporting evidence-based clinical decisions. ESA: Erythropoiesis-stimulating agent.

The innovation lies in its use of a circular, continuous path to represent the perioperative process. It eschews a linear flowchart for a dynamic loop, emphasizing that PBM is not just a pre-surgical checklist but a continuous cycle of care. Patients, again represented as figures on a path, first encounter a 'Pre-surgery check-point' that includes obtaining informed consent and creating a PBM surgical plan. This step is presented as a crucial start line. The central part of the infographic is a visual tour of the operating room, where various PBM strategies are presented as numbered stations around a central operating table. This format effectively breaks down the intraoperative phase into a series of distinct, yet interconnected, interventions. This 'pit stop' style of presentation makes the various tools and techniques of PBM easy to digest and remember.

After the central 'winner's lap' of interventions, the patient figure moves towards a 'Postoperative check-point' and then onto the 'Recovery Road'. This sequential layout reinforces the full spectrum of care, from pre-op planning to post-op recovery (Figure 3).

Visual elements

As described in the Infographic 1, the background, the figurative elements and, the use of minimalist stylized human figures facilitates understanding of the patient's trajectory.

Iconography and the patient's path were chosen to represented a sense of movement and continuity. The use of symbols predominates to convey messages quickly (Figure 3).

QR codes and informative supplementation

The inclusion of a QR code for a 'Quick Reference' further enhances its utility as a modern, accessible learning resource. This infographic describes all processes numbered in the surgical phase and the guidelines for each one (Figure 3).

Infographic 3: the patient blood management 'Energy wheel' in the 'Surgical verse'

Conceptual description
This infographic presents an innovative model for understanding PBM by reframing the process as a dynamic system. Instead of a linear path or a static checklist, it uses the metaphor of an 'Energy Wheel' to illustrate how various interventions work together to drive a patient towards a successful outcome.

The central spinning turbine visually represents the core engine of PBM, with each blade symbolizing a critical component. This design shows that all these elements are interdependent and must be activated in a coordinated manner for the system to function effectively. The wheel is surrounded by eight key PBM strategies, each with a corresponding icon presented as the fuel or power that turns the wheel.

The patient's journey is shown as a path leading into and out of this central system. Patients are depicted entering the process, engaging with the 'Energy Wheel,' and then proceeding on the 'Recovery Path' toward 'Patient Discharge' and the 'FINISH' line. This graphical representation transforms a set of guidelines into an active, process-oriented model, highlighting that PBM is not a static state but a continuous, dynamic effort to power a patient's recovery (Figure 4).

Visual elements
As described in the Infographic 1, the background, the figurative elements and, the use of minimalist stylized human figures facilitates understanding of the patient's trajectory.

Concise and direct texts associated with each icon ensure the message is quickly assimilated, while the overall composition reinforces clarity and organization of the information (Figure 4).

QR codes and informative supplementation
This interactive link provides access to detailed strategies for managing hemostasis and anticoagulation, addressing ways to prevent both bleeding and thrombotic complications, as well as offering recommendations for rigorous monitoring of postoperative bleeding. In addition, the QR code directs users to therapeutic approaches for different types of anemia, with content on strategies to optimize oxygen delivery and consumption, highlighting interventions that ensure adequate tissue perfusion and minimize metabolic demand in anemic patients (Figure 4).

Discussion

This study demonstrates the potential of infographics as effective tools to communicate and disseminate the principles of PBM across surgical care. The visual materials developed present clinical pathways and protocols in a structured, intuitive format that enhances understanding and engagement, particularly within multidisciplinary healthcare teams.

The choice of PBM as the central theme is justified by its growing relevance in the global health landscape, particularly following recommendations from the WHO, which recognizes this approach as essential for patient safety and the sustainability of healthcare systems [8,9]. Despite these recognized benefits, PBM implementation still faces significant challenges, especially cultural resistance within healthcare institutions where liberal transfusion practices remain entrenched. Traditional didactic strategies may fall short in conveying the complexity of PBM or in promoting long-term behavioral change. In this context, infographics offer a compelling alternative by simplifying technical content and supporting knowledge retention [13].

The visual narratives developed in this study, such as the 'Surgery Verse' journey and the 'Energy Wheel', reflect a novel application of design thinking in health education. They position PBM not as a collection of isolated interventions, but as an integrated, patient-centered process across the perioperative continuum. By visually aligning patient pathways with the three pillars of PBM, these tools reinforce the concept of coordinated, continuous care.

Incorporating QR codes for supplementary content further enhances interactivity and accessibility. This hybrid model, combining static visualizations with dynamic digital resources, addresses diverse learning styles and supports the ongoing education of healthcare professionals in time-constrained environments.

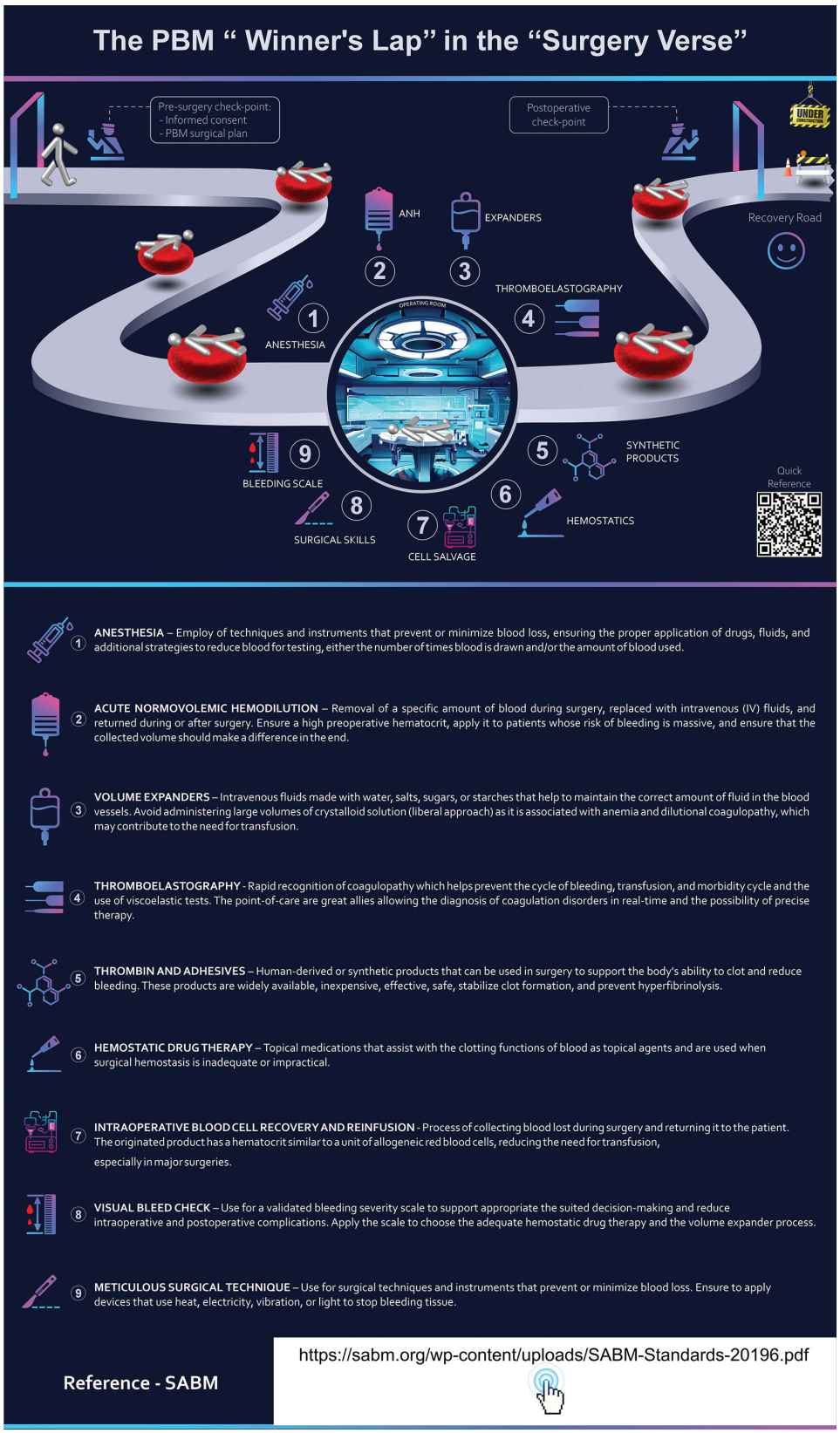

Fig. 3 – The patient blood management (PBM) 'Winner's Circle' in the 'Surgery Verse'. This infographic depicts PBM as a continuous, circular pathway encompassing preoperative planning, intraoperative interventions, and postoperative recovery. Patient Fig.s progress through numbered 'pit stops' around a central operating table, visually breaking down complex surgical blood management strategies into digestible steps. Linked via QR code, this guide summarizes each intraoperative PBM strategy with clear indications and usage guidelines, enhancing accessibility and supporting clinicians in applying best practices during surgery.

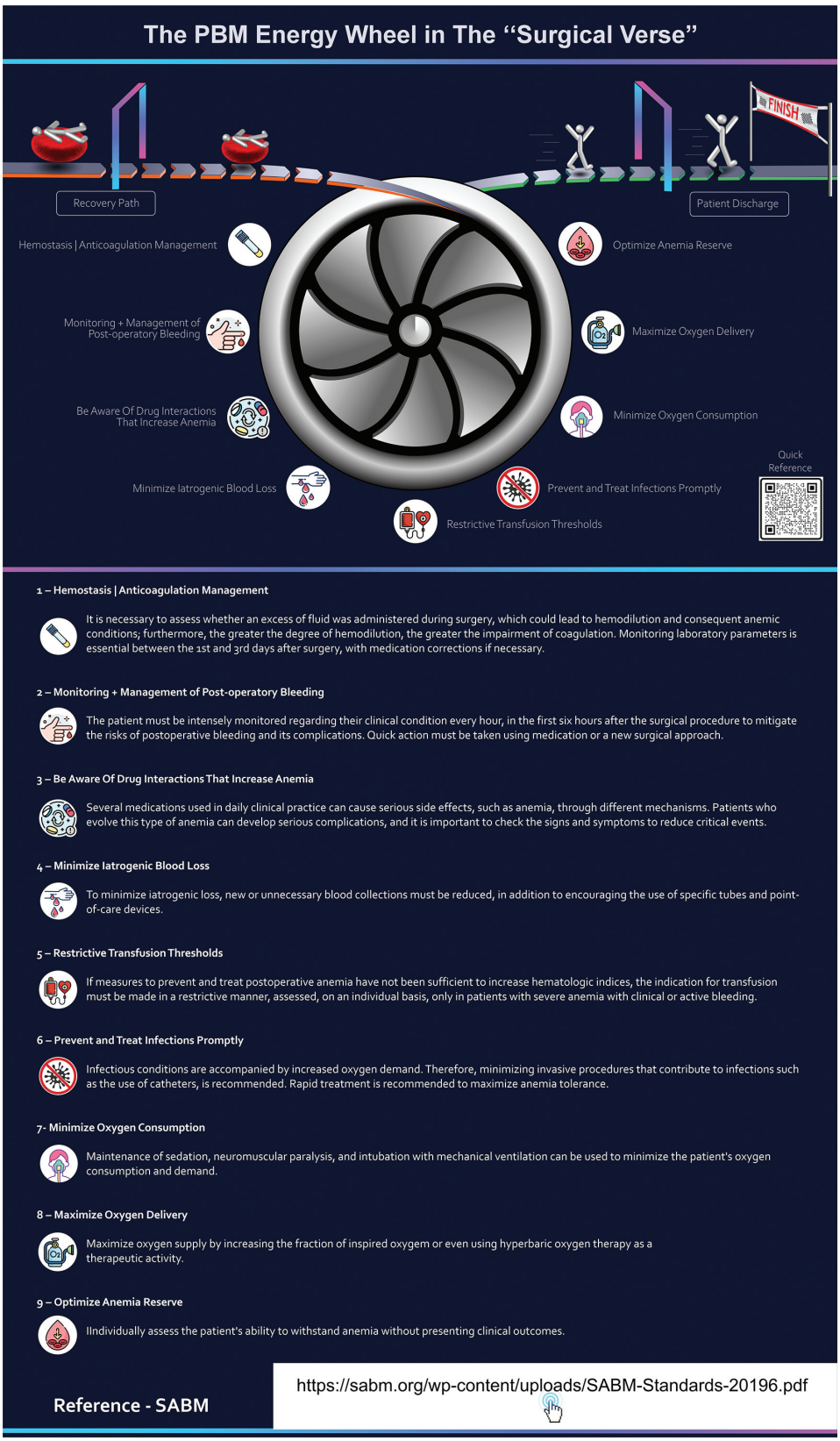

Fig. 4 – The patient blood management (PBM) 'Energy Wheel' in the 'Surgery Verse'. This innovative model uses a spinning turbine metaphor to emphasize the interdependent PBM strategies powering the patient's journey from intervention to recovery. Each blade represents a critical component, reinforcing the need for coordinated, continuous care to achieve optimal outcomes. Accessible through QR code, this content provides detailed recommendations on bleeding and thrombosis prevention, postoperative monitoring, and therapies to optimize oxygen delivery and consumption, reinforcing safe and effective blood management.

Framing PBM within the concept of the surgical patient journey is especially relevant. This journey comprises interconnected stages, from preoperative evaluation to postoperative rehabilitation, each requiring coordinated interventions to optimize outcomes. PBM, when integrated into this pathway, enables resource optimization and enhances clinical effectiveness through strategies such as anemia screening and correction, blood conservation during surgery, and structured recovery planning [10]. A fragmented approach may undermine these efforts, while an integrated perspective fosters adherence to evidence-based practice.

Nevertheless, this study has limitations. While the infographics were developed using a robust design methodology and expert input, their effectiveness was not assessed through formal educational interventions or clinical trials. Therefore, their impact on knowledge acquisition, behavioral change, and clinical outcomes remains to be determined. Furthermore, although the development included feedback from PBM-certified professionals, broader usability testing with frontline clinicians and patients would strengthen the generalizability and applicability of the tools.

Future studies should explore the impact of such infographics using both quantitative and qualitative methods, including pre- and post-intervention assessments, focus groups, and implementation science frameworks. Their adaptability across various healthcare settings, cultural contexts, and languages should also be explored to assess scalability.

From a public health perspective, increasing awareness and adherence to PBM is urgent [8,9]. Global blood shortages, exacerbated by the COVID-19 pandemic, underscore the need for sustainable, rational strategies in transfusion medicine. By translating complex evidence into engaging, accessible formats, infographics may function not only as educational tools but also as catalysts for institutional change and policy development.

The methodology described in this study—anchored in user-centered design, iterative development, and multidisciplinary collaboration—offers a replicable framework for other health education topics beyond PBM. Clinical protocols involving complex decision-making, such as perioperative safety or chronic disease management, could benefit from similar infographic-based approaches to improve knowledge transfer and adherence. By emphasizing co-creation with content experts and end-users, and by integrating visual narratives and interactive elements like QR codes, this model can be adapted to diverse educational settings and learner profiles. Its flexibility supports implementation across clinical disciplines and institutional contexts, reinforcing its value as a scalable strategy for translating evidence-based practices into effective, engaging, and actionable learning tools.

Conclusion

Well-designed infographics can facilitate the translation of complex clinical guidelines, such as those related to PBM, into practical, actionable strategies. They support professional education, enhance multidisciplinary communication, and align with global efforts to improve patient safety and the sustainability of healthcare systems. While further evaluation is warranted, this study offers a replicable model for integrating visual communication into evidence-based practice, particularly in contexts where cognitive overload, time constraints, or educational gaps may hinder effective clinical decision-making.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

I declare no conflicts of interest.

CRediT authorship contribution statement

Matheus Santos Moitinho: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Bianca Meneghini:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Rosangela Monteiro:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **Anderson Manucci:** Methodology, Investigation, Writing – original draft. **Maria Carolina Guido:** Conceptualization, Writing – original draft, Writing – review & editing. **Guilherme Rabello:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **Fabio Biscegli Jatene:** Conceptualization, Supervision, Project administration.

Editor

Eduardo Rego

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Prevalence of parvovirus B19 infection in patients with hematological diseases in the Western Brazilian Amazon

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ARTICLE INFO

Article history:
Received 22 October 2025
Accepted 25 April 2026
Available online 30 May 2026

Keywords:
Hematological diseases
Parvovirus B19V
Epidemiology
Prevalence
Blood center
Western Amazon

ABSTRACT

Introduction: Parvovirus B19 (B19V) infects erythroid precursor cells, compromising red blood cell production. This can trigger severe anemia and other hematological diseases in susceptible patients. However, data on B19V epidemiology in patients with hematological diseases are scarce, especially in the northern region of Brazil. The aim of this study was to estimate the prevalence of IgG and IgM antibodies against B19V, and to detect B19 DNA, in patients with hematological diseases undergoing

Methods: Plasma samples from 421 patients were subjected to enzyme immunoassay for the detection of anti-B19V IgG and IgM antibodies. Subsequently, all samples underwent real-time polymerase reaction for viral DNA detection.

Results: More than half (55.11%) of the study population was seropositive for IgG anti-B19 antibodies. Notably, women (prevalence ratio: 1.62; 95% CI: 1.10–2.41; p-value = 0.016) and individuals aged 60 and above (prevalence ratio: 7.72; 95% CI: 3.77–15.80; p-value = 0.016) showed the highest seroprevalence rates. Patients with thrombophilia, lymphoma, and anemia also exhibited elevated seroprevalence, with thrombophilia having the highest rate (prevalence ratio: 6.52; 95% CI: 1.78–23.88). Twelve patients were positive for IgM anti-B19, and the presence of B19V DNA was confirmed in two patients (0.46%).

Conclusions: Patients with hematological diseases, particularly thrombophilia, exhibit a high seroprevalence of B19V, indicating frequent prior exposure. However, recent infections and viremia are uncommon. The elevated seroprevalence among women and adults aged 60 and older suggests that these groups may be at greater risk. This underscores the need for enhanced epidemiological surveillance and targeted B19V testing to improve patient management.

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https://doi.org/10.1016/j.htct.2026.106477

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Introduction

Parvovirus B19 (B19V), discovered in 1975, belongs to the *Parvoviridae* family and the genus *Erythroparvovirus* [1]. Its genome is composed of single-stranded DNA (ssDNA) of approximately 5 kb that encodes three main proteins, VP1, VP2, and NS1, alongside several smaller accessory proteins that contribute to viral function [2]. It is classified into three genotypes (1, 2 and 3) and has an affinity for proerythroblasts in the bone marrow, binding to the P (Globoside) antigen on their membranes [3,4]. The NS1 protein has been identified as the main mediator of apoptosis in B19V-infected cells, playing a crucial role in the development of clinical complications [5]. This virus is commonly related as the causative agent of erythema infectiosum, popularly known as the "fifth disease", due to its distinctive skin rash [6].

B19V is ubiquitous, infecting most people at some point in their lives [7]. Its transmission occurs mainly through respiratory droplets, viremic blood transfusions, and the transplacental route [8]. Although B19V infections often manifest asymptomatically or with mild symptoms, it is notable that the virus has the ability to temporarily interfere with erythropoiesis, culminating in hematologic disorders [9]. Notable clinical manifestations include idiopathic thrombocytopenic purpura (ITP) and severe anemia in immunocompromised patients, alongside other disorders characterized by the suppression of erythropoiesis (red blood cell production) [10].

Some rare hematological manifestations have been associated with B19V infection since the identification of this virus and its tropism by proerythroblasts, including bicytopenias, pancytopenia, myelodysplastic syndrome and leukoerythroblastopenia [11]. Studies indicate a case frequency of 15%-30% of B19V infection in patients with acute lymphoblastic leukemia (ALL) [12,13]. Furthermore, studies conducted in Tunisia reported a prevalence of 65% for B19V infection among blood donors and 56.5% among patients with sickle cell disease [14,15].

A study conducted in Saudi Arabia investigated B19V infection in patients with sickle cell disease, revealing a prevalence of 37.6% in this population [16]. In the northern region of India, a prevalence of 53.4% of B19V infection was observed in children with hemato-oncological disorders [17]. In Brazil, the first reports of B19V infection date back to 1985, when three pregnant women were referred for the detection of rubella antibodies in Rio de Janeiro [18]. In addition, case studies point to B19V infection as a strong complicating factor in the clinical course of hematological diseases [18]. Despite its global prevalence in hematological disorders, comprehensive epidemiological studies on B19V, especially in Brazil's northern region, are lacking. Case studies underscore the significant impact of B19V on the clinical course of hematological diseases.

Individuals with hematologic disease infected with B19V have a decrease in the production of viable erythrocytes, exacerbating the disruption of hematologic homeostasis [19]. This decrease is particularly evident in patients with anemia and leukemia [20]. In patients with a compromised hematopoietic system, such as in cases of leukemia or bone marrow transplant recipients, B19V infection can lead to serious blood disorders, including pancytopenia, bone marrow necrosis syndrome, and fat embolism syndrome [21]. In some rare cases, B19V infection can also complicate the course of acute leukemia [13]. In patients with chronic hemolytic anemia, B19V infection can trigger an aplastic crisis, temporarily stopping the production of blood cells in the bone marrow and aggravating the anemia [22]. B19V-associated ITP is a condition that results in thrombocytopenia, characterized by a low platelet count in the blood. In children, ITP can develop as a chronic condition after an acute viral infection [23].

The intricate relationship between B19V infection and hematologic diseases underscores the pivotal role of epidemiological studies in shaping clinical strategies and public health interventions. By thoroughly estimating B19V prevalence across diverse hematological conditions, this knowledge not only enhances patient care but also guides preventive measures. Epidemiological studies serve as the initial step in developing tailored patient management approaches, facilitating a deeper understanding of the intricate link between viral infections and hematologic disorders. This study is a cross-sectional investigation of parvovirus B19V infection in patients with hematological diseases in the Western Brazilian Amazon.

Materials and methods

Ethical approval

This study was approved by Human Research Ethics Committee (CAAE 63474122.1.0000.0009) of the Foundation of Hematology and Hemotherapy of the Amazon state (HEMOAM). All individuals signed an informed consent form prior to their participation in the study. Consent for individuals under 18 years of age was obtained from one of the parents or legal guardians. Confidentiality and the right to withdraw from the study at any time were assured to all participants.

Study design and population

This study was conducted from November 2022 to March 2023 at the HEMOAM Foundation (FHEMOAM) outpatient clinic and included patients with a range of hematological disorders. The study involved 421 patients with diverse hematological conditions residing in different cities and towns within the western Brazilian Amazon region. Both male and female patients with ages ranging from 1-96 years old were eligible for inclusion. Detailed demographic information was gathered from the electronic medical record system IDOCTOR at FHEMOAM. Employing a cross-sectional observational

approach, the study adopted consecutive sampling as its selection method, with the sample size determined based on a 95% confidence interval.

Serological analysis

Serum samples were tested for the presence of anti-B19V IgG and IgM antibodies using an enzyme immunoassay (Anti-Parvovirus B19 ELISA, Euroimmun, Lubeck, Germany), following the manufacturer's recommendations. The optical density (OD) resulting from the test was measured in a spectrophotometer (Molecular Devices, San Jose, CA, USA) employing a 450 nm filter. Positivity was estimated by calculating the ratio of the sample OD to the calibrator OD, considering the manufacturer's indicated cut-off values. Serum levels of anti-B19V IgG were estimated in IU/mL by interpolating the standard curve provided by the manufacturer. The assay demonstrates 100% sensitivity and specificity for anti-B19V IgG detection. For anti-B19V IgM, the sensitivity and specificity are 100% and 97.9%, respectively. The detection limit of the tests was defined as a value of three times the standard deviation of an analyte-free sample, with the final value being the lowest detectable antibody concentration. Seroprevalence was defined a priori by anti-parvovirus B19 IgG positivity, since IgG is the standard epidemiological marker used to assess previous infection and cumulative exposure within a population. Anti-B19V IgM positivity was analyzed separately as an indicator of recent infection and was not included in the main seroprevalence estimate.

Nucleic acid extraction and B19V DNA detection

All serum samples underwent DNA extraction using the Blood Lysate protocol of the PureLink Genomic DNA Mini Kit (Invitrogen, Waltham, MA, USA), following the manufacturer's recommendations. Detection of B19V DNA was achieved by real-time polymerase reaction (PCR) on the QuantStudio 5 equipment (Applied Biosystems, Waltham, MA, USA). The amplification reaction utilized 200 ng of DNA, forward and reverse primers (5 μM; 5′-CAC CCC CAT GCC TTA TCA TC-3′ and 5′-TTG TAC GCT AAC TTG CCC AG-3′), and a FAM-labeled probe (10 μM; 5′-FAM-CAG TCA TGC AGA ACC TAG AGG A-3′). Procedures were conducted using the TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems, Waltham, MA, USA) in a total volume of 10 μL. The reaction consisted in an initial denaturation at 95°C for 20 seconds, succeeded by 45 cycles consisting of 3 seconds at 95°C and 30 seconds at 60°C.

Statistical analysis

Multinomial logistic regression and prevalence ratio (PR) were used to investigate the association between the likelihood of B19 infection and demographic variables or different types of hematological diseases. PR was determined by dividing the prevalence among individuals exposed to specific demographic characteristics or hematological diseases by the prevalence among those not exposed. Linear regression was used to test the relationships between plasma concentrations of anti-B19V IgG and continuous quantitative variables. The student's t-test and one-way ANOVA were used to test the difference between means of two or more variables, respectively. The results were considered statistically significant when the p-value was less than 0.05 for a 95% confidence interval (95% CI). R version 4.3.0 software was used for statistical analysis.

Results

Prevalence of B19V infection according to demographic factors and type hematological diseases

Because IgM primarily reflects recent infection, whereas IgG reflects previous exposure and may persist long term, IgM-positive results were not incorporated into the main estimate of seroprevalence, which was designed to represent cumulative prior exposure in the study population. The seroprevalence of B19V infection in the study population was 55.11% (95% CI: 0.50–0.60). Seroprevalence rates were higher in women than men (seroprevalence: 59.92% versus 40.14%; PR: 1.62; 95% CI: 1.10–2.41; p-value = 0.016). The seroprevalence increased with age, with the highest seroprevalence in patients over 60 years old (72.22%) and the lowest in young people aged 1–18 years (27.01%) (Table 1). Increasing age was positively correlated with a rise in seroprevalence (PR = 5.41

Table 1 – Parvovirus B19 prevalence in the study population according to sex and age.

Category	n (%)	IgG positive n (%)	PR (95% CI)	p–value
Sex				
Men	169 (40.14)	81 (47.93)	Ref.	
Women	252 (59.86)	151 (59.92)	1.62 (1.10–2.41)	0.016
Age (years)				
01–18	137 (32.54)	37 (27.01)	Ref.	
19–30	66 (15.68)	44 (66.67)	5.41 (2.86–10.21)	<0.0001
31–40	48 (11.40)	33 (68.75)	5.95 (2.90–12.18)	<0.0001
41–50	63 (14.96)	44 (69.84)	5.81 (3.03–11.14)	<0.0001
51–60	53 (12.59)	35 (66.04)	5.26 (2.66–10.40)	<0.0001
60+	54 (12.83)	39 (72.22)	7.72 (3.77–15.80)	<0.0001

PR: Prevalence Ratio; 95% CI: 95% Confidence Interval; Ref.: Reference
The values of PR, 95% CI, and p-value were obtained from multinomial regression analysis

Table 2 – Parvovirus B19 seroprevalence according to hematologic disorders.

	Age range	Sex		Total (%)	IgG positive n (%)	PR (95% CI)	p−value
		F	M				
Leukemia	22 ± 30	55	51	106 (25.18)	46 (43.40)	Ref.	
ALL	11 ± 17	24	32	56 (52.83)	17 (30.36)	Ref.	
CLL	43 ± 81	3	2	5 (4.72)	2 (40.00)	1.53 (0.23−10.00)	0.657
AML	22 ± 39	10	8	18 (16.98)	8 (44.44)	1.84 (0.62−5.46)	0.275
CML	39 ± 52	15	8	23 (21.70)	16 (69.57)	5.24 (1.83−15.06)	0.002
Undetermined	01 ± 13	3	1	4 (3.77)	3 (75.00)	–	–
Anemia	34 ± 41	113	43	156 (37.05)	90 (57.69)	1.83 (1.11−3.01)	0.018
Iron-deficiency	37 ± 50	25	7	32 (20.51)	24 (75.00)	Ref.	
Aplastic	22 ± 49	8	7	15 (9.62)	13 (86.67)	2.17 (0.40−11.74)	0.37
Fanconi	26	1	–	1 (0.64)	–	–	–
Sickle Cell	15 ± 26	21	15	36 (23.08)	15 (41.67)	0.24 (0.08−0.67)	0.007
Hemolytic	26 ± 46	11	4	15 (9.62)	9 (60.00)	0.50 (0.14−1.85)	0.298
Megaloblastic	52 ± 72	14	2	16 (10.25)	8 (50.00)	0.43 (0.12−1.53)	0.191
Microcytic	13 ± 36	2	1	3 (1.92)	2 (66.67)	0.67 (0.05−8.36)	0.753
Pernicious	48	–	1	1 (0.64)	–	–	–
Undetermined	31 ± 46	31	6	37 (23.72)	19 (51.35)	–	–
Lymphoma	34 ± 41	25	32	57 (13.54)	35 (61.40)	2.08 (1.08−4.00)	0.029
Hodgkin's	20 ± 30	15	12	27 (47.37)	17 (62.96)	Ref.	
Non-Hodgkin's	42 ± 56	9	19	28 (49.12)	17 (60.71)	0.91 (0.31−2.70)	0.864
Undetermined	06 ± 28	1	1	2 (3.51)	1 (100.00)	–	–
Hemophilia	13 ± 25	2	19	21 (4.99)	13 (61.90)	2.12 (0.81−5.54)	0.125
Leukopenia	51	1	–	1 (0.24)	1 (100.00)	–	–
Myeloma	39 ± 65	4	4	8 (1.90)	3 (37.50)	0.78 (0.18−3.44)	0.746
Thrombocytopenia	32 ± 51	15	8	23 (5.46)	14 (60.87)	2.03 (0.81−5.10)	0.132
Polycythemia	51 ± 72	2	–	2 (0.48)	1 (50.00)	1.30 (0.08−21.42)	0.852
ITP	19 ± 36	16	7	23 (5.46)	8 (34.78)	0.70 (0.27−1.78)	0.449
Thrombophilia	47 ± 61	14	4	18 (4.27)	16 (88.89)	6.52 (1.78−23.88)	0.005
Von Willebrand	15 ± 56	5	1	6 (1.43)	5 (83.33)	6.52 (0.74−57.76)	0.092
Total		252	169	421	232(55.11)		

PR: Prevalence Ratio; 95% CI: 95% Confidence Interval; Ref.: Reference; ITP: Idiopathic Thrombocytopenic Purpura; ALL: Acute Lymphoblastic Leukemia; CLL: Chronic Lymphocytic Leukemia; AML: Acute Myeloid Leukemia; CML: Chronic Myeloid Leukemia; F: Female; M: male
The values of PR, 95% CI, and p-value were obtained from Multinomial Regression Analysis. Numbers in bold indicate statistically significant values

for the age group of 19−30 years compared to the reference group and PR = 7.72 for patients over 60 years old; p-value <0.0001 for both comparisons).

B19V seroprevalence varied depending on the type of hematological disease, with higher rates observed in patients with thrombophilia (88.89%), followed by lymphoma (61.40%), anemia (57.69%), and leukemia (43.40%). Seroprevalence rates were higher among the following patient groups: anemia (PR: 1.83; CI: 1.11−3.01; p-value = 0.018), lymphoma (PR: 2.08; 95% CI: 1.08−4.00; p-value = 0.029), chronic myeloid leukemia (PR: 5.24; 95% CI: 1.83−15.06; p-value = 0.002), and thrombophilia (PR: 6.52; 95% CI: 1.78−23.88; p-value = 0.005). Notably, patients with thrombophilia were 6.5 times more likely to have been exposed to B19V than those with other hematological diseases, such as leukemia. Conversely, patients with sickle cell anemia demonstrated a significantly lower likelihood of infection. (PR: 0.24; 95% CI: 0.08−0.67; p-value = 0.007) (Table 2).

The prevalence of anti-B19V IgM was 2.85% (12/421), indicating recent serological evidence of infection. Most IgM-positive individuals were women (n = 9) and were aged 30 years or older (n = 8) (Table 3). Of the IgM-positive patients, seven (58.33%) were also positive for IgG anti-B19V. B19V DNA was detected in two patients (0.46%) and was analyzed separately as molecular evidence of detectable viremia. Notably, both patients were negative for anti-B19V IgG and IgM. One of the patients positive for B19V DNA had acute lymphoblastic leukemia, and the other had chronic myeloid leukemia. The

Table 3 – Positive cases for the presence of IgG and IgM anti-B19V in patients with hematological diseases.

Hematological diseases	Sex	Age	IgM	IgG
Leukemia (Undetermined)	F	1	+	–
ITP	F	10	+	–
Thrombocytopenia	M	10	+	–
Aplastic Anemia	F	12	+	+
Hodgkin's Lymphoma	F	30	+	+
Non-hodgkin's lymphoma	M	30	+	+
Thrombocytopenia	F	39	+	+
AML	M	50	+	+
Megaloblastic Anemia	F	69	+	–
Non-hodgkin's lymphoma	F	70	+	–
Thrombophilia	F	72	+	+
Anemia (Undetermined)	F	75	+	+

ITP: Idiopathic Thrombocytopenic Purpura; AML: Acute Myeloid Leukemia; F: Female; M: Male; +: Positive; –: Negative.

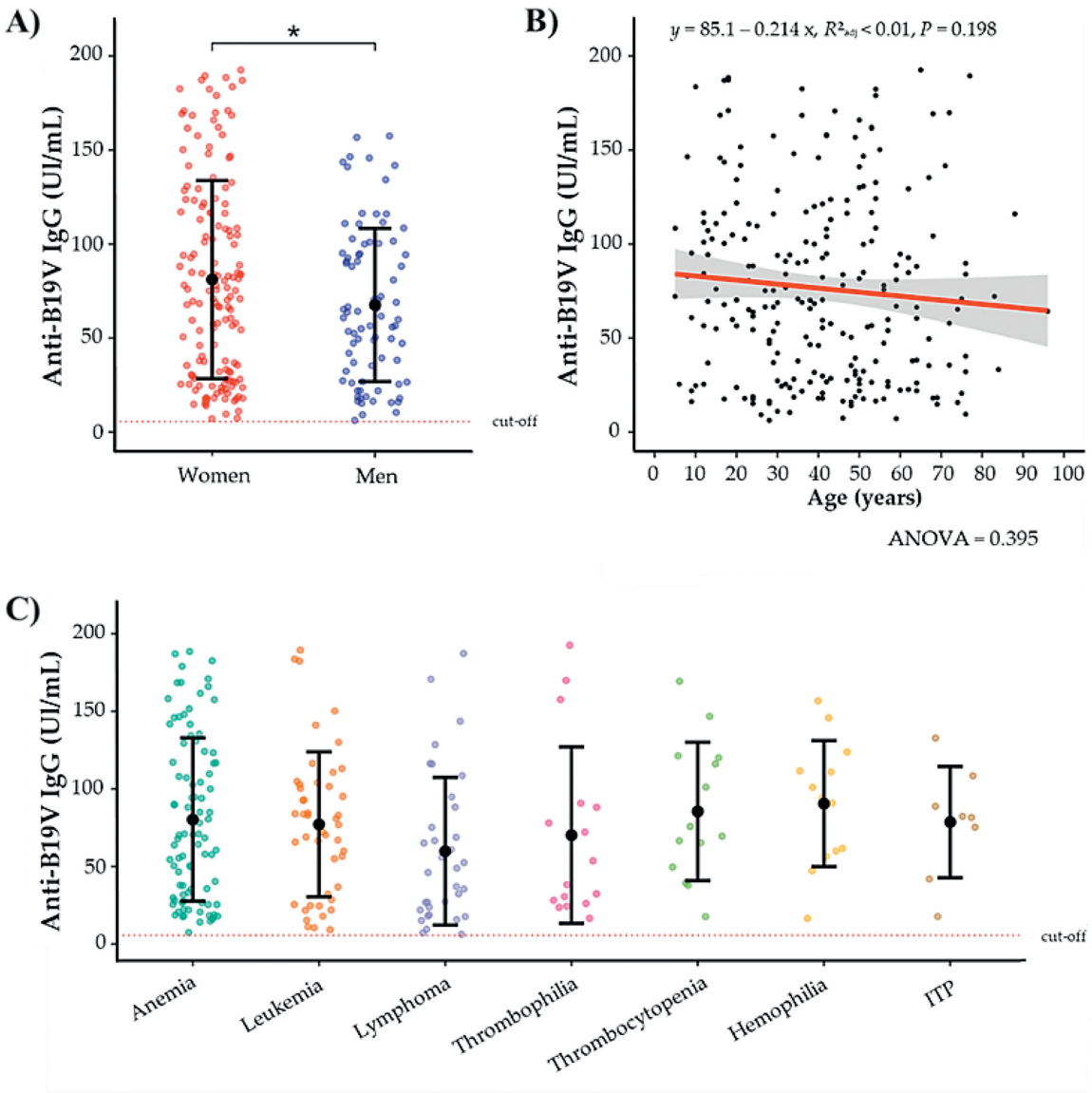

Figure 1 – Serum anti-B19V IgG antibody levels in patients with hematological diseases. Serological analysis of serum anti-B19V IgG levels according to sex (A), age (B) and type of hematological disease (C). The difference between means of serum anti-B19V IgG antibody levels by sex was assessed using a student's t-test. The correlation between serum anti-B19V IgG antibody levels and age was evaluated by linear regression. Data in A and B are represented by mean and standard deviation. *p-value = 0.046.

overall positivity, defined as the presence of one or more laboratory markers (anti-B19V IgG, IgM, or DNA), was 56.77% (239/421). However, this composite measure was not used as the primary endpoint because these markers reflect different stages or manifestations of B19V infection.

Analysis of serum anti-B19V IgG levels

Serum anti-B19V IgG levels were higher in women than in men (81.11 ± 52.67 IU/mL; p-value = 0.046) (Figure 1A). There was no correlation between anti-B19V IgG serum levels and age (Figure 1B). While patients with hemophilia (90.48 ± 40.63 IU/mL), thrombocytopenia (85.42 ± 44.59 IU/mL), and anemia (80.21 ± 52.62 IU/mL) exhibited numerically higher serum anti-B19V IgG levels, these differences were not statistically significant compared to other hematological conditions (Figure 1C).

Discussion

The prevalence of B19V infection has been assessed in many countries, including China, the United States, Saudi Arabia, Turkey, and Croatia, with prevalence ranging from 20%-60% [16,24–27]. However, few studies have addressed the circulation of B19V among patients with hematological diseases worldwide. In the context of Brazil, particularly in the western Brazilian Amazon region, there is limited epidemiological information available on B19V infection.

This study found a high seroprevalence of B19V infection (55.11%) in individuals with hematological diseases in the western Brazilian Amazon, which was slightly higher than that found in other regions of Brazil (30%-53%) and India (53.40%), but much higher than that found in Tanzania (29%), Saudi Arabia (37.6%), and the United States (35%) [16,17,27–31]. These findings underscore the importance of understanding regional variations in B19V prevalence and its potential impact on individuals with severe hematologic conditions.

In this study, women exhibited higher seroprevalence rates and notably elevated serum IgG anti-B19 levels compared to men. Freitas et al. found a higher seroprevalence of B19V infection among women (59.70%) than men (35.40%) in the northern Brazilian city of Belém do Pará [32]. Similar findings emerged from a study in England and Wales, which reported a 28.5% higher seroprevalence of anti-B19V IgG among women compared to men [33]. Additionally, certain studies have reported a higher seroprevalence of B19V infection in pregnant women, often linked with fetal mortality [34–36]. The reasons behind the higher B19 infection rate in women are still elusive. Moreover, this study did not investigate the possible connection between pregnancy and B19V.

The findings of the present research demonstrated a positive correlation between age and B19V seroprevalence. Patients older than 60 years showed the highest seroprevalence, while patients aged 1 to 18 years showed the lowest. However, serum anti-B19V IgG levels did not correlate significantly with age. This pattern aligns with a study from São Paulo State, where sickle cell anemia patients ≥40 years had the highest B19V seroprevalence (93.3%) and those aged 1–9 years the lowest (20.7%), consistent with cumulative, age-related exposure. [31]. Another study conducted in Australia with healthy individuals also linked higher seroprevalence to B19V infection with increasing age, in which individuals aged 65 to 80 years showed the highest seroprevalence of B19V infection [7].

In Germany, the seroprevalence of B19V is around 20% in children aged 1 to 3 years and 67% in adolescents [37,38]. Adolescents may contribute to the spread of B19V infection in school settings, given the high rates of contact and transmission [37,38]. However, the seroprevalence of B19V infection in children and adolescents in this study was very low compared to older individuals. One plausible explanation for the distinct epidemiological pattern identified in Brazil might be that children and adolescents with hematological diseases, due to their fragile health, frequently miss school, where B19V transmission commonly occurs. However, this possibility was not specifically investigated.

In this study, patients with thrombophilia, lymphoma, anemia, and chronic myeloid leukemia had a high prevalence of B19V infection, suggesting an increased likelihood of past B19V exposure. However, no correlation was observed between serum anti-B19V IgG levels and the type of hematological disease. Susceptibility to B19V infection has been correlated with different hematological diseases, especially leukemia, lymphoma, and anemia [39]. Prior studies have demonstrated an increased susceptibility to B19 infection among patients with anemia, as evidenced by a significantly higher prevalence of B19V infection (87%) compared to those with other hematological malignancies, such as chronic myeloid leukemia (18.70%) and lymphomas (52%) [40,41]. Anemia is the main consequence of B19V infection due to the destruction of erythrocyte percussor cells during the viral cycle [42].

Strikingly, the findings of the present study revealed that patients with thrombophilia exhibited the greatest seroprevalence rate for B19V infection, which was up to six times higher than that of patients with other hematological diseases. To date, there is no known direct association between thrombophilia and B19V infection, but viral infections are known to be linked to blood clotting disorders [43]. For example, B19V can infect endothelial cells, which are key regulators of coagulation, thereby activating them and initiating the coagulation cascade [43,44]. Further studies with larger sample sizes are needed to confirm the association between thrombophilia and increased exposure to B19V infection, as well as the impact of this infection on the development and prognosis of this hematological disease.

Conversely, sickle cell anemia showed a lower likelihood of contracting B19V infection in this study. This contradicts previous findings that reported a high seroprevalence of B19V among individuals with sickle cell disease [45,46]. These contrasting outcomes warrant further investigation into potential factors influencing the interplay between sickle cell anemia and B19V infection, such as variations in genetic predisposition and environmental factors. Understanding these discrepancies may provide deeper insights into the complex relationship, shedding light on novel mechanisms influencing viral interactions within specific genetic conditions.

This study found that 12 patients were seropositive for IgM and seven were seropositive for both IgG and IgM anti-B19V antibodies. This finding is consistent with the results of a similar study in India, which reported that 53.40% of patients with hematological diseases were positive for IgG anti-B19V and 6.70% were positive for IgM antibodies [17]. Interestingly, only two patients in this study were positive for B19V DNA and neither of them had detectable IgM or IgG anti-B19V antibodies. This suggests that B19V infection in these cases may have been in its early stages, as B19V viremia is often short-lived and can be difficult to detect, especially if blood samples are not collected during this period [5].

Given the pivotal role of transfusions, the lack of mandatory B19V screening in Brazil and most international jurisdictions represents a significant transfusion-safety gap [47,48]. This gap in screening could facilitate the transmission of B19V through blood or plasma, posing serious health risks to patients with hematological conditions. Complications such as anemia, prolonged red blood cell aplasia, fever, erythema, and even pancytopenia may arise [49,50]. While lacking longitudinal data on post-transfusion outcomes, this study still offers crucial information for blood safety. Uncovering the prevalence of this under-researched virus is key to future decisions about mandatory screening.

This study had limitations, including restricted access to some medical records, which precluded a detailed assessment of specific diagnoses. In many instances, this information was incomplete, compromising further analyses. Another problem was related to the parents' refusal to allow children to participate in the study at the time of data collection. However, we understand the situation due to the

sensitivity and severity of some patients. This limitation may have affected the representativeness of the sample in the case of some hematological diseases, such as myeloma and polycythemia.

Despite the limitations mentioned above, this study provides an important contribution to B19V surveillance in the Brazilian Amazon region, particularly among patients with hematological diseases. It presents valuable pioneering epidemiological information on the dynamics of B19V infection in this specific population, raising questions about its clinical implications.

Conclusions

Parvovirus B19 is highly prevalent among patients with hematological diseases undergoing treatment at a renowned blood center in Manaus, Amazonas, Brazil. The data suggest marked cumulative B19V exposure, disproportionately affecting women and older individuals. Prior B19V exposure was more frequent in thrombophilia, lymphoma, anemia, and chronic myeloid leukemia, with thrombophilia showing the greatest prevalence. These data indicate substantial cumulative exposure but low active viremia, supporting targeted B19V testing in compatible clinical scenarios and motivating larger, longitudinal studies in the region. Given the central role of transfusions and the absence of mandatory B19V screening in Brazil, these findings also highlight a potential transfusion-safety gap warranting periodic surveillance.

Authors' Contributions

Conceptualization, Pontes GS and Souza PHR; methodology, Souza PHR, Barbosa AN, Alves CEdC, Oliveira LC, Mineiro LS, Mejía MdCC and Naveca FG; research, Souza PHR, Barbosa AN, Alves CEdC, Oliveira LC, Abreu RSA and Mineiro LS; formal analysis, Souza PHR, Barbosa AN, Alves CEdC, Mejía MdCC, Naveca FG, Oliveira LC and Mineiro LS; elaboration - preparation of original draft, Pontes GS, Souza PHR and Barbosa AN; Writing - review & editing, Pontes GS, Souza PHR, Barbosa AN, Alves CEdC; supervision, Pontes GS; project management, Pontes GS; resources/acquisition of financing, Pontes GS. All authors have read and agreed to the published version of the manuscript.

Humans Ethics and Consent to participate declarations

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval was granted by Human Research Ethics Committee (CAAE 63474122.1.0000.0009) of the Foundation of Hematology and Hemotherapy of the Amazon state (FHEMOAM). All individuals signed the informed consent form prior to their participation in the study.

Funding statement

This research was funded by the Coordination for the Improvement of Higher Education Personnel - CAPES (Finance code PROCAD AMAZÔNIA 88881.200581/201801).

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials.

Conflicts of interest

The authors have no conflicts of interest to declare for this study.

Acknowledgments

We thank the University of the State of Amazonas (UEA), the Foundation of Hematology and Hemotherapy of the State of Amazonas (FHEMOAM), Research Support Foundation of the State of Amazonas (FAPEAM) and the Laboratory of Virology and Immunology of the National Institute of Amazonian Research (INPA) for the support provided throughout this study.

Editor

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Predictive factors for poor mobilization in autologous stem cell transplant: a multivariate model

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ARTICLE INFO

Article history:
Received 2 October 2025
Accepted 23 March 2026
Available online 30 May 2026

Keywords:
Autologous stem cell transplantation
CD34+ cells
Poor mobilization
Predictive model

ABSTRACT

Introduction: Inadequate stem cell mobilization remains a major challenge in autologous stem cell transplantation. Although multiple risk factors for poor mobilization have been suggested, their clinical utility is limited. This study aimed to identify predictors of poor mobilization and develop a clinically applicable predictive model.

Material and Methods: A retrospective analysis of patients who underwent autologous stem cell transplantation was conducted. Poor mobilization was defined as a total $CD34^+$ cell collection of less than 2×10^6 cells/kg or the requirement for extended apheresis with multiple collection cycles.

Results: A total of 430 patients with Hodgkin's lymphoma (n = 134), non-Hodgkin's lymphoma (n = 113), and multiple myeloma (n = 183) were included in this study. Poor mobilization was observed in 28.6% (123/430) of patients. A predictive model was developed using multivariate analysis, achieving an area under the receiver operating characteristic curve of 0.97 (p-value <0.001) when including the $CD34^+$ cell count and 0.98 (p-value <0.001) when excluding it. Independent risk factors for poor mobilization included low platelet count, bone marrow infiltration, advanced disease status, and exposure to ≥2 alkylating agents. The final model demonstrated a specificity of 98.7% (95% CI: 96.7–99.6%) and a sensitivity of 91.1% (95% CI: 84.6–95.4%).

Conclusion: This predictive model can be applied before the initiation of the mobilization process. With sensitivity, specificity, positive predictive value, and positive likelihood ratio all exceeding 90% in both versions, the model demonstrates high effectiveness and clinical utility.

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https://doi.org/10.1016/j.htct.2026.106475
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Introduction

Autologous hematopoietic stem cell transplantation (aHSCT) is a therapeutic intervention for patients with lymphoma and multiple myeloma (MM) [1,2]. The procedure is typically performed using peripheral blood stem cells (PBSCs). Because hematopoietic stem cells (HSCs) normally circulate in the peripheral blood in very low numbers, they must first be mobilized into the circulation before collection by apheresis [3].

Poor mobilization can result in insufficient PBSC yield for aHSCT [4,5]. This represents a significant challenge, as a minimum of 2×10^6 $CD34^+$ cells/kg is required to ensure successful transplantation. This threshold is necessary to achieve rapid and sustained hematopoietic recovery, while also reducing the risks of prolonged hospitalization, blood transfusion requirements, and infection [6,7]. The optimal HSC dose is approximately 5×10^6 $CD34^+$ cells/kg [8]. Failure to achieve adequate mobilization may necessitate re-mobilization or, in some cases, prevent transplantation altogether.

Several factors have been associated with an increased risk of mobilization failure, including bone marrow infiltration, older age, and myelodysplastic changes [9–11]. However, the predictive accuracy of these parameters for identifying suboptimal mobilization remains uncertain. Although predictive models have been proposed to address this limitation [12], their applicability across different patient populations has not been fully validated. Early identification of patients at high risk for poor mobilization is essential, as it could minimize resource use, facilitate timely consideration of alternative strategies, and ultimately improve patient outcomes.

This study aimed to identify predictive factors and develop a new risk model for poor mobilization in aHSCT candidates. Additionally, the study validated an existing predictive model [12], developed a novel scoring system based on specific criteria, and compared their performance to optimize clinical decision-making.

Material and methods

This retrospective study was conducted at the Hematology, Oncology, and Stem Cell Transplantation Research Center, Shariati Hospital in Tehran, Iran. Patients eligible for aHSCT with a diagnosis of MM, non-Hodgkin lymphoma (NHL), or Hodgkin's disease (HD) between January 2013 and December 2021 were included. The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences, and all data were collected and analyzed anonymously. Diagnoses of lymphoma were confirmed by pathology review, and diagnoses of MM were established jointly by hematologists and pathologists.

The inclusion criterion was age ≥18 years. Patients with acute or chronic leukemia or those previously mobilized with plerixafor were excluded, as the latter group typically represents poor mobilizers and could bias the predictive analysis. Clinical data, including disease characteristics, prior therapies, mobilization regimen, and apheresis outcomes, were recorded.

Mobilization failure was defined as a total $CD34^+$ cell yield of $<2 \times 10^6$ cells/kg or the requirement for large-volume apheresis involving multiple collection cycles. Stem cell collections were performed using a continuous-flow cell separator following the institutional protocol.

The primary dependent variable was poor mobilization. Independent variables were defined as follows:

- Extensive bone marrow (BM) infiltration was defined based on the most recent BM assessment performed prior to stem cell mobilization, and not at the time of initial diagnosis. Extensive BM infiltration was considered as >60% plasma cells in patients with MM or >30% lymphomatous infiltration in patients with lymphoma.
- Advanced-phase disease: prior treatment with at least two lines of cytotoxic chemotherapy.
- Prior marrow-toxic exposure: previous therapy with lenalidomide, melphalan, or fludarabine.
- Extensive radiotherapy: prior radiation involving marrow-bearing sites.

Statistical analysis

The association between individual variables and poor mobilization was first assessed using univariate logistic regression, with results expressed as odds ratios (ORs) and 95% confidence intervals (95% CIs). Subsequently, multivariate logistic regression with forward stepwise selection was performed to identify independent predictors of mobilization outcomes.

The GITMO (Gruppo Italiano Trapianto Midollo Osseo) consensus score and the Predicted Poor Mobilizer (pPM) [12] were applied to this dataset generating scores for each patient according to the respective criteria in order to assess the reliability of previously published models. Given the central importance of the pre-apheresis $CD34^+$ cell count, this study developed two distinct predictive models: one including the pre-mobilization $CD34^+$ cell count and one excluding it. The primary objective was to design a model capable of estimating the probability of poor mobilization at the earliest possible stage, prior to the initiation of apheresis.

Each model classified patients into two categories based on their final score: predicted poor mobilizers and predicted successful mobilizers. Final scores were calculated using different cut-offs, with the cut-off selection ultimately dependent on clinical preference. The objective was to develop a scoring system capable of reliably identifying patients at high risk of mobilization failure; therefore, the threshold that maximized the positive likelihood ratio (LR) was selected. Model performance was assessed by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative LRs, each reported with 95% CIs. LRs and their 95% CIs were expressed as ratios of proportions.

Internal validity of the final model was evaluated through cross-validation. Calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test and by plotting calibration curves comparing observed versus predicted probabilities. Discriminative ability was evaluated using receiver operating characteristic (ROC) curve analysis with the area under the ROC curve (AUC) being calculated according to previously

Table 1 – Qualitative characteristics of patients based on disease type.

		Diagnosis							
		HL (n = 134)		**NHL** (n = 113)		**MM** (n = 183)		**Total** (n = 430)	
		Failed mobilization		**Failed mobilization**		**Failed mobilization**		**Failed mobilization**	
		Yes n (%)	**No** n (%)	**Yes** n (%)	**No** n (%)	**Yes** n (%)	**No** n (%)	**Yes** n (%)	**No** n (%)
Diagnosis		44	90	37	76	42	141	123	307
Gender	Female	19 (36)	33 (64)	15 (37)	25 (63)	17 (23)	57 (77)	51 (31)	115 (69)
	Male	25 (31)	57 (69)	22 (30)	51 (70)	25 (23)	84 (77)	72 (27)	192 (73)
Extensive BM involvement	No	41 (35)	76 (65)	31 (33)	64 (67)	0 (0)	4 (100)	72 (33)	144 (67)
	Yes	3 (18)	14 (82)	6 (33)	12 (67)	42 (24)	137 (76)	51 (24)	163 (76)
Refractory disease	No	30 (31)	66 (69)	31 (34)	59 (66)	39 (22)	139 (78)	100 (28)	264 (72)
	Yes	14 (37)	24 (63)	6 (26)	17 (74)	3 (60)	2 (40)	23 (35)	43 (65)
Advanced phase disease	No	21 (23)	70 (77)	13 (18)	59 (82)	31 (20)	125 (80)	65 (20)	254 (80)
	Yes	23 (54)	20 (46)	24 (59)	17 (41)	11 (41)	16 (59)	58 (52)	53 (47)
Relapse	No	10 (30)	23 (70)	11 (23)	37 (77)	40 (24)	128 (76)	61 (25)	188 (75)
	Yes	34 (34)	67 (66)	26 (40)	39 (60)	2 (13)	13 (87)	62 (34)	119 (66)
Previous extensive radiotherapy	No	28 (26)	78 (74)	23 (25)	69 (75)	36 (22)	128 (78)	87 (24)	275 (76)
	Yes	16 (57)	12 (43)	14 (67)	7 (33)	6 (32)	13 (68)	36 (53)	32 (47)
Disease status at mobilization	CR	24 (26)	70 (74)	26 (30)	62 (70)	35 (20)	138 (80)	85 (24)	270 (76)
	PR	20 (50)	20 (50)	11 (44)	14 (56)	7 (70)	3 (30)	38 (51)	37 (49)
Large volume apheresis	No	17 (16)	90 (84)	9 (11)	76 (89)	12 (8)	141 (92)	38 (11)	307 (89)
	Yes	27 (100)	0 (0)	28 (100)	0 (0)	30 (100)	0 (0)	85 (100)	0 (0)
Toxic marrow exposure	No	43 (32)	90 (68)	37 (33)	75 (67)	8 (7)	110 (93)	88 (24)	275 (76)
	Yes	1 (100)	0 (0)	0 (0)	1 (100)	34 (52)	31 (48)	35 (52)	32 (48)
Maintenance IMiDs	No	44 (33)	90 (67)	37 (33)	76 (67)	8 (7)	110 (93)	89 (24)	276 (76)
	Yes	0 (0)	0 (0)	0 (0)	0 (0)	34 (52)	31 (48)	34 (52)	31 (48)
≥ 2 Alkylating agents	No	4 (10)	37 (90)	10 (20)	41 (80)	41 (23)	141 (77)	55 (20)	219 (80)
	Yes	40 (43)	53 (57)	27 (44)	35 (56)	1 (100)	0 (0)	68 (44)	88 (56)
Previous alkylating chemotherapy	No	0 (0)	0 (0)	1 (100)	0 (0)	33 (25)	97 (75)	34 (26)	97 (74)
	Yes	44 (33)	90 (67)	36 (32)	76 (68)	9 (17)	44 (83)	89 (30)	210 (70)
Use of IMiDs	No	44 (33)	90 (67)	37 (33)	75 (67)	7 (17)	34 (83)	88 (31)	199 (69)
	Yes	0 (0)	0 (0)	0 (0)	1 (100)	35 (25)	107 (75)	35 (25)	108 (75)

*: percentage in parentheses refer to rows in each category.
BM: bone marrow; HL: Hodgkin disease; NHL: non-Hodgkin lymphoma; MM: multiple myeloma; IMiDs: Lenalidomide or Thalidomide.

described methods [13]. AUCs were compared using the estimated covariance matrix.

The required sample size was determined based on the primary outcome of poor mobilization. Assuming a 20% poor mobilization rate, a power of 80%, and $\alpha = 0.05$, detection of an AUC ≥ 0.6 required a total of 405 mobilization procedures (including 81 failures). Statistical analyses were performed using SPSS version 24 (IBM Corp., Armonk, NY) and MedCalc version 20 (MedCalc Software, Ostend, Belgium). A p-value <0.05 was considered statistically significant.

Results

A total of 430 patients, including Hodgkin's lymphoma (n = 134), non-Hodgkin's lymphoma (n = 113), and MM (n = 183), were enrolled in this study. The median age was 47 years. The median number of chemotherapy courses prior to mobilization was nine. Overall, 15.5% of patients had received prior marrow-toxic exposure, including melphalan or fludarabine in lymphoma patients and maintenance lenalidomide in MM patients. At the time of mobilization, 17.5% of patients were in partial remission, while the remainder had achieved complete remission. BM biopsy was performed in all patients prior to mobilization. Median BM infiltration was 35% in patients with mobilization failure compared with 45% in patients with successful mobilization. Baseline demographic and clinical characteristics by disease group are summarized in Tables 1 and 2.

Overall, 123 patients (28.6%) were classified as poor mobilizers, while 307 patients (71.4%) achieved adequate stem cell collection for transplantation. The causes of suboptimal mobilization were an unsatisfactory peak $CD34^+$ cell count (<20 cells/μL) in 19 (15.4%) patients, insufficient total $CD34^+$ yield ($\leq 2 \times 10^6$ cells/kg) in ten (8.1%) patients, and both criteria in 94 (76.4%).

Patients with failed mobilization demonstrated significantly greater BM infiltration and lower pre-mobilization $CD34^+$ cell counts compared with those who mobilized successfully. Quantitative parameters associated with mobilization outcome are detailed in Table 3.

Poor mobilization risk factors

Univariate regression analysis was used to evaluate the association between different variables and poor mobilization. Significant predictors (p-value <0.001) included intact BM

Table 2 – Quantitative characteristics of patients based on disease type.

	Diagnosis											
	HL			NHL			MM			Total		
	~x	Min	Max	~x	Min	Max	~x	Min	Max	~x	Min	Max
Age (years)	34.21	17.90	67.55	43.88	17.70	64.88	55.32	28.87	77.03	46.96	17.70	77.03
BM infiltration (%)	40	20	80	40	25	70	40	20	85	40	20	85
Harvested volume (mL)	540	180	2100	559	180	1950	418	164	2685	470	164	2685
Harvested nucleated cell ($x10^7$ /kg)	13.55	3.60	68.80	13.36	3.90	44.65	13.01	1.74	31.24	13.25	1.74	68.80
pre-mobilization $CD34^+$ count ($x10^6$ /kg)	15.10	1.00	70.00	15.00	2.50	75.00	18.00	1.00	40.00	17.00	1.00	75.00
Harvested $CD34^+$ ($x10^6$ /kg)	3.00	.50	10.20	2.80	.52	12.00	3.40	.46	16.04	3.12	.46	16.04
WBC (/mm^3)	5.05	2.00	12.40	4.70	1.50	11.30	5.70	1.70	22.40	5.10	1.50	22.40
Hemoglobin (mg/dL)	12.10	7.70	17.60	12.30	7.80	17.50	12.80	9.40	18.70	12.50	7.70	18.70
Platelet (/mm^3)	160	22	414	162	34	419	193	31	387	175	22	419
Number of chemotherapy courses	12	6	26	11	6	16	6	4	16	9	6	26

BM: bone marrow; HL: Hodgkin disease; NHL: non-Hodgkin lymphoma; MM: multiple myeloma; ~x :median.

Table 3 – Quantitative parameters based on failed mobilization.

	Failed mobilization					
	No			Yes		
	~x	Min	Max	~x	Min	Max
Age (years)	46.90	18.28	69.35	46.96	17.70	77.03
Bone marrow infiltration (%)	45	25	85	35	20	55
Harvested volume (mL)	439	164	2685	600	250	2100
Harvested nucleated cell ($x10^7$ /kg)	13.05	1.74	68.80	13.63	3.60	52.07
Pre-mobilization $CD34^+$ ($x10^6$ /kg)	20.00	3.00	75.00	8.00	1.00	25.00
Harvested $CD34^+$ ($x10^6$ /kg)	3.90	2.00	16.04	1.70	.46	8.80
WBC count (/mm^3)	5.50	1.60	22.40	4.20	1.50	12.70
Hemoglobin (mg/dL)	13.00	8.10	18.70	11.60	7.70	15.40
Platelet count (/mm^3)	200	31	419	110	22	322
Number of chemotherapy courses	9	6	18	10	8	26

reserve, history of relapse, prior extensive radiotherapy, advanced disease status at mobilization, BM infiltration, pre-mobilization $CD34^+$ cell count, white blood cell count, hemoglobin level, platelet count, number of prior chemotherapy courses, toxic marrow exposure (fludarabine, melphalan, or lenalidomide), and treatment with more than two alkylating agents. In contrast, gender, age, refractoriness, and underlying hematologic diagnosis were not significantly associated with mobilization failure (Table 4).

Verifying previous models

This dataset was first applied to the previously proposed pPM and GITMO scoring systems [20]. In the pPM model, scores ranged from 8.7 to 24.7 with a median of 13.5. Using the published cut-off of 7.86, all patients in the cohort were classified as poor mobilizers, indicating limited discriminative value in this setting.

For the GITMO score, values ranged from 0–7 with a median of 1. Based on the consensus, the cut-off was set at ≥2. At this threshold, sensitivity for predicting mobilization failure was 70.7% (95% CI: 61.8–78.6%) and specificity was 69.1% (95% CI: 63.6–74.2%). The positive likelihood ratio was 2.3 (95% CI: 1.9–2.8). With a poor mobilization prevalence of 28.6% in this cohort, the positive predictive value (PPV) was 47.8% (95% CI: 42.8–52.8%) and the AUC for predicting poor mobilization was 0.70 (95% CI: 0.66–0.75).

Modeling

Given the modest performance of the pPM and GITMO scores in the current dataset, further analyses were conducted to develop a more robust predictive model. Adjusting cut-off values within these models resulted in reduced sensitivity and specificity with positive predictive values (PPVs) ranging only from 40.3% to 56.7%.

The predictive role of the $CD34^+$ cell count in the mobilization outcome is well established [4–8]. In accordance with the primary objective of this study to develop a model capable of identifying poor mobilizers prior to apheresis, two distinct models were constructed: one including the pre-mobilization $CD34^+$ cell count and one excluding this variable.

Table 4 – Mobilization failure predictors according to univariate logistic regression.

Candidate factor	β	p-value	OR	95% CI	
				Lower	Upper
Gender (Female)	0.173	0.427	1.189	0.776	1.821
Age	−0.003	0.744	0.997	0.982	1.013
Diagnosis		0.089			
HL	0.495	0.051	1.641	0.997	2.702
NHL	0.478	0.073	1.613	0.957	2.719
Intact bone marrow	−0.462	0.032	0.630	0.413	.962
Refractory disease	0.349	0.219	1.417	0.813	2.472
Advanced phase disease	1.457	<0.0001	4.293	2.706	6.810
Relapse	0.479	0.026	1.614	1.059	2.460
Previous extensive radiotherapy	1.272	<0.0001	3.569	2.093	6.086
Disease status at mobilization (PR)	1.186	<0.0001	3.274	1.958	5.475
BM infiltration	−0.175	<0.0001	0.840	0.809	0.871
Pre CD34$^+$	−0.572	<0.0001	0.564	0.496	0.642
WBC count	−0.290	<0.0001	0.748	0.660	0.848
Hemoglobin level	−0.621	<0.0001	0.537	0.455	0.635
Platelet count	−0.053	<0.0001	0.949	0.938	0.959
Number of chemotherapy courses	0.150	<0.0001	1.162	1.091	1.237
Toxic marrow exposure	1.233	<0.0001	3.430	2.007	5.864
>2 Alkylating agents	1.128	<0.0001	3.091	2.005	4.766

HL: Hodgkin lymphoma; NHL: Non-Hodgkin lymphoma; PR: partial remission; OR: odds ratio; 95% OI: 95% confidence interval.

Multivariate logistic regression was performed using the variables found significant in the univariate analysis. In the model excluding the CD34$^+$ cell count, the final regression model was formulated after five steps using forward stepwise selection. The improvement employing the chi-square test was 344.64 (p-value <0.001), indicating strong statistical significance. The Cox-Snell and Nagelkerke R^2 values demonstrated that the model explained between 55% and 79% of the variance in poor mobilization. This model achieved a sensitivity of 89.4%, specificity of 94.1%, PPV of 89.4%, NPV of 94.1%, and an overall accuracy of 92.8%.

When the CD34$^+$ cell count was incorporated into the multivariate regression, the final model was achieved after four steps. The improvement employing the chi-square test was 415.28 (p-value <0.001), with Cox–Snell and Nagelkerke R^2 values showing that 62% to 89% of the variance in outcome could be explained.

Independent predictors identified in the two models were as follows:

- Model excluding the pre-mobilization CD34$^+$ cell count: platelet count, BM infiltration, advanced disease status, full-course chemotherapy, and the use of more than two alkylating agents.
- Model including the pre-mobilization CD34$^+$ cell count: platelet count, BM infiltration, extensive radiotherapy, and the CD34$^+$ cell count.

Cut-off optimization yielded excellent discriminative performance. In the CD34$^+$ model, a threshold >0.219 produced an AUC of 0.97, with sensitivity 97.6% and specificity 88.3%. In the model excluding the CD34$^+$ count, a cut-off >0.502 resulted in an AUC of 0.98, with sensitivity 94.3% and specificity 97.7% (Figure 1; Tables 5 and 6).

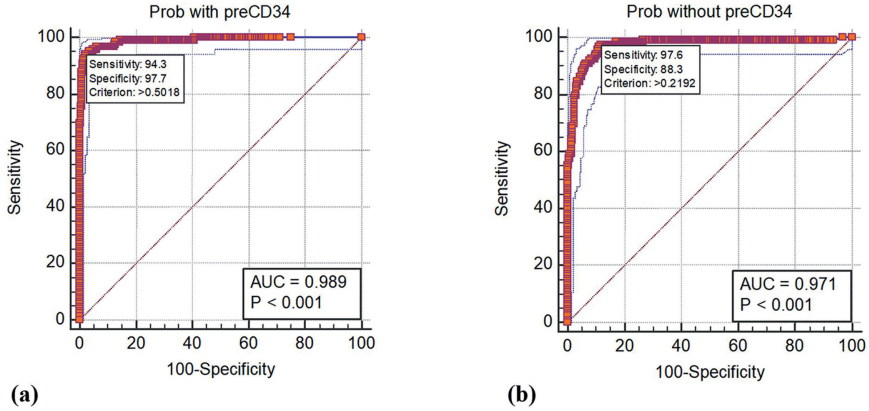

Figure 1 – Area under the receiver operating characteristic curves for poor mobilization using (a) the model including the pre-mobilization CD34$^+$ count and (b) the model excluding the pre-mobilization CD34$^+$ count.

Table 5 – Statistical measures of predicting models for poor mobilization.

Measure	Value	95% CI
Model excluding the pre-mobilization CD34⁺ cell count		
Cut-off	0.71	
Sensitivity	0.911	0.846 - 0.954
Specificity	0.987	0.967 - 0.996
Positive Likelihood Ratio	69.89	26.36 −185.31
Negative Likelihood Ratio	0.09	0.05 - 0.16
Disease prevalence*	28.60%	24.38% - 33.13%
Positive Predictive Value*	0.965	0.913 - 0.987
Negative Predictive Value*	0.965	0.940 - 0.979
Accuracy*	0.965	0.943 - 0.980
Model including the pre-mobilization CD34⁺ cell count		
Cut-off	0.43	
Sensitivity	0.911	0.846 - 0.954
Specificity	0.935	0.901 - 0.959
Positive Likelihood Ratio	13.98	9.12 - 21.43
Negative Likelihood Ratio	0.10	0.05 - 0.17
Disease prevalence*	28.60%	24.38% - 33.13%
Positive Predictive Value*	0.848	0.785 - 0.896
Negative Predictive Value*	0.963	0.937 - 0.978
Accuracy*	0.928	0.899 - 0.950

95% CI: 95% confidence interval.

* These values are dependent on the disease prevalence.

The probability of mobilization failure according to the model score without the CD34⁺ cell count, can be calculated as below:

$$\text{Poor Mobilization Probability} = \frac{e^{(model\ score - 10.082)}}{e^{(model\ score - 10.082)} + 1}$$

The AUCs of the GITMO score, pPM score and the scores of the models with and without CD34⁺ inclusion were compared. The AUC of previous models were significantly inferior to both the models constructed in this study (p-value <0.0001). Figure 2 shows the result.

Discussion

This study evaluated mobilization outcomes in patients undergoing aHSCT, with the primary aim of identifying prognostic risk factors and assessing the applicability of previously published predictive scores (GITMO and pPM). A secondary objective was to develop a practical predictive model capable of identifying patients at high risk of mobilization failure, ideally independent of the well-established predictor: pre-apheresis CD34⁺ cell count. To this end, four models were examined: the GITMO score, the pPM score, and two newly developed models (one including and one excluding the pre-mobilization CD34⁺ cell count).

The pPM score demonstrated poor performance in the current dataset, essentially failing to predict poor mobilization. The GITMO score also showed limited accuracy, with modest sensitivity (70%), specificity (69%), and an AUC of 0.70. In contrast, the new predictive models performed substantially better, each achieving an AUC greater than 0.9, with excellent sensitivity, specificity, and PPVs. Using optimized cut-offs of 0.71 (model excluding the pre-mobilization CD34⁺ cell count) and 0.43 (model including the pre-mobilization CD34⁺ cell count), both models demonstrated strong clinical utility in predicting mobilization failure.

Several factors may explain the discrepancy between the present findings and those of prior studies. The pPM score developed by Olivieri et al. [14], was based on variables markedly different from those of this patient population. For

Table 6 – Independent predictive factors for mobilization failure identified by forward stepwise method with multiple logistic regression.

Model excluding the pre-mobilization CD34⁺ cell count					
Variables in the Equation	β	p-value	Odds ratio	95% CI	
				Lower	Upper
Advanced phase disease	1.086	0.010	2.961	1.292	6.788
Bone marrow infiltration	−0.156	<0.0001	0.855	0.810	0.904
Platelet count	−0.043	<0.0001	0.958	0.946	0.970
Full courses of previous therapy	2.443	0.001	11.512	3.660	36.204
More than two Alkylating	1.615	0.001	5.027	2.000	12.637
Model including the pre-mobilization CD34⁺ cell count					
Variables in the Equation	β	p-value	Odds ratio	95% CI	
				Lower	Upper
Previous extensive radiotherapy	1.933	0.012	6.912	1.525	31.333
Bone marrow infiltration	−0.118	0.001	0.889	0.830	0.952
Pre-mobilization CD34⁺ cell count	−0.518	<0.0001	0.596	0.502	0.708
Platelet count	−0.033	<0.0001	0.967	0.954	0.981

95% CI: 95% confidence interval.

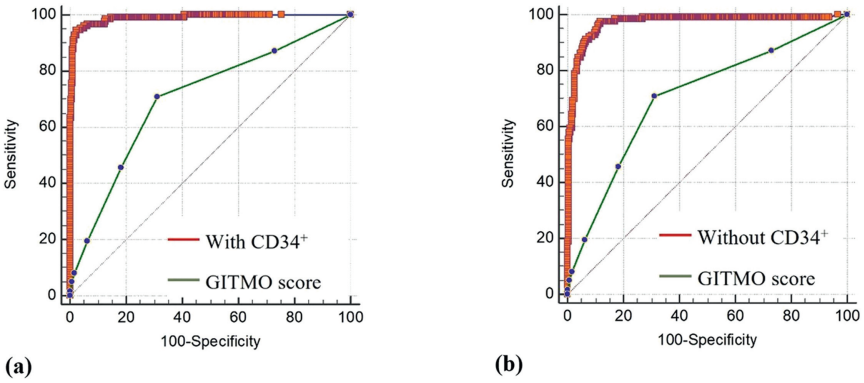

Figure 2 – Comparing the area under the receiver operating characteristic curve of the GITMO (Gruppo Italiano Trapianto Midollo Osseo) score with (a) the model including the pre-mobilization CD34⁺ count and (b) the model excluding the pre-mobilization CD34⁺ count.

example, in this study the median number of prior chemotherapy courses was nine (range: 2–26), compared with only 1–6 in the Italian study. Likewise, conditioning regimens differed substantially: Olivieri et al. identified granulocyte colony-stimulating factor (G-CSF) use without chemotherapy as a strong negative predictor ($\beta = 2.25$), whereas in this study all patients received G-CSF alone. Age distribution also varied, with the present median patient age being 46 years, while 26% of patients in the Italian cohort were younger than 45 years. Moreover, Italian patients tended to undergo aHSCT earlier in their disease course, resulting in lower cumulative exposure to marrow-toxic therapy compared to this study.

These contextual differences highlight why previously published models may lack external validity across diverse patient populations. Despite recent advances in peripheral blood stem cell collection including apheresis instrumentation, mobilization failure remains a challenge, with reported rates ranging from 5% to 40% [15–17]. The transfused stem cell dose, directly linked to the number of CD34⁺ cells collected, is a key determinant of engraftment success [14,18]. While circulating CD34⁺ cell counts remain the most reliable predictor of mobilization success, their utility is limited by the fact that they are only available immediately prior to apheresis. This delay prevents early identification and stratification of patients who might benefit from alternative mobilization strategies.

The new models address this limitation by incorporating additional predictive variables that are assessable before apheresis. In the model excluding the CD34⁺ cell count, independent predictors of mobilization failure included platelet count, BM infiltration, advanced disease status, history of full-course chemotherapy, and prior exposure to more than two alkylating agents. When the CD34⁺ cell count was included, the independent predictors were platelet count, BM infiltration, pre-mobilization CD34⁺ cell count, and prior extensive radiotherapy. These findings suggest that predictive models can be tailored to different clinical contexts, either allowing earlier patient stratification without pre-mobilization CD34⁺ cell data or achieving maximum predictive accuracy including the pre-mobilization CD34⁺ count.

A 28.6% rate of mobilization failure was observed in older patients in this study, whereas other investigators such as Rossi et al. [19] and Wuchter et al. [20] reported rates as high as 46%. This discrepancy is likely attributable to differences in mobilization techniques between studies, but it warrants further investigation. Demiroğlu et al. reported that higher pre-mobilization platelet counts, and the use of chemotherapy-based mobilization regimens were associated with greater mobilization success. While the platelet count was also identified as a significant predictor in the present study, the mobilization regimen could not be examined since our protocol was chemotherapy-free [21].

Although no single measure of accuracy can fully capture the clinical value of a predictive tool, the AUC is widely considered a reliable indicator of discriminative performance, as it is independent of cut-off values or disease prevalence [22]. In this respect, the new models demonstrated excellent predictive capability, each achieving an AUC greater than 0.95.

One important consideration in evaluating predictive scores across studies is the discrepancy between the minimal threshold and the optimal target dose of CD34⁺ cells. Defining poor mobilization solely by the minimum cut-off of 2×10^6 CD34⁺ cells/kg may underestimate clinical requirements, as higher doses are often needed depending on the patient's condition. This variability may affect the accuracy and applicability of predictive models.

The primary goal of this study was to establish a practical tool for decision-making before mobilization. These new models incorporate easily obtainable variables available prior to apheresis and classify patients as 'poor mobilizers' or 'successful mobilizers.' Such information can guide clinical planning, including reconsideration of mobilization targets, early use of CXCR4 antagonists, cytokine therapy, or large-volume apheresis. The strong positive likelihood ratio (>10), combined with sensitivity and specificity above 85%, supports the reliability and potential generalizability of these models across different settings.

In the present cohort, plerixafor was used only on-demand, limiting the ability to evaluate or recommend its upfront use. Nevertheless, based on this model, it would be reasonable to consider early administration of plerixafor in patients classified as poor mobilizers, in line with recent reports [36]. Similarly, because G-CSF–based mobilization was employed exclusively, conclusions regarding chemotherapy-based mobilization strategies cannot be provided.

In addition to cytokine-based approaches, chemotherapy-based mobilization strategies, particularly vinorelbine combined with G-CSF, have been reported as effective alternatives for poor mobilizers, leading to higher pre-apheresis $CD34^+$ cell counts, predictable timing of collection (typically on days 7–8), and acceptable toxicity with a relatively low risk of infectious complications, while remaining substantially more cost-effective than plerixafor [4,6,10].

As a future direction, prospective studies could apply this predictive framework prior to mobilization, without reliance on circulating $CD34^+$ counts, to identify poor mobilizers and randomize them between G-CSF–based mobilization with early or on-demand plerixafor versus chemotherapy-based mobilization with vinorelbine. In addition to apheresis success rates, such studies should incorporate pharmacoeconomic analyses to assess the cost-effectiveness of these competing strategies.

Finally, although the findings of this study suggest that adapting mobilization approaches according to the predictive model may be clinically beneficial, external validation in an independent cohort is required. Furthermore, prospective clinical trials are essential to confirm the effectiveness and safety of implementing this model in routine practice.

Conclusion

This study developed a predictive model for inadequate mobilization that excludes the pre-mobilization $CD34^+$ cell count, making it applicable prior to the initiation of the mobilization process. Both versions of the model demonstrated sensitivity, specificity, positive predictive value, and positive likelihood ratio exceeding 90%. Early identification of poor mobilizers enables individualized monitoring, supports optimized decision-making, and ultimately enhances patient outcomes.

Funding

This research received no specific grant from funding agencies in the public, commercial, or non-profit sectors.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank the Research Institute for Oncology, Hematology, and Cell Therapy, Tehran University of Medical Sciences, Tehran, Iran, for their support.

Editor

Erich Vinicius De Paula

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Original article

Early diagnostic accuracy of immature platelet fraction for sepsis: a systematic review and meta-analysis

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ARTICLE INFO

Article history:
Received 22 November 2025
Accepted 18 March 2026
Available online 30 May 2026

Keywords:
Early detection
Immature platelet fraction
Sepsis

ABSTRACT

Background: The immature platelet fraction, a rapid and accessible indicator of bone marrow thrombopoietic activity, is considered a possible biomarker for the early diagnosis of sepsis. This study aims to evaluate the diagnostic precision of immature platelet fraction testing for the early identification of sepsis. This is, to our knowledge, the first systematic review and meta-analysis addressing this problem.

Methods: A literature search was carried out on the PubMed and Scopus databases from August 1 to August 8, 2025. Studies that met the inclusion criteria were analyzed to calculate sensitivity, specificity, diagnostic odds ratio, Deeks' funnel plot, and summary receiver operating characteristic. Sub-group analysis was carried out to evaluate differences in immature platelet fraction diagnostic performance in adults excluding pediatrics data.

Results: Three studies published between 2013 and 2021 satisfied the selection criteria and were incorporated into the analysis. Meta-analysis indicates that the immature platelet fraction possesses moderate diagnostic accuracy for identifying sepsis. The pooled sensitivity was 74% (95% CI: 53%–87%), and the pooled specificity was 70% (95% CI: 48%–85%). The combined diagnostic odds ratio was 6.54 (95% CI: 2.90–14.76). Moderate heterogeneity was observed between the included studies ($I^2 = 59.57\%$; $\tau^2 = 0.30$).

Conclusions: Immature platelet fraction may represent a complementary biomarker for sepsis, but limited evidence and methodological heterogeneity necessitate cautious interpretation as these pooled estimates were derived from a limited number of studies. Further standardized prospective studies are required to establish its clinical utility.

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https://doi.org/10.1016/j.htct.2026.106476

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Introduction

Sepsis is a serious worldwide health concern, especially in developing countries. Sepsis, by definition, is severe and fatal organ failure caused by the body's reaction to an infection that it cannot control [1]. Globally, sepsis accounts for an estimated 48.9 million cases and 11 million deaths annually, representing 19.7% of all fatalities worldwide. Furthermore, data analysis across 109 million individual death records underscores the substantial global burden of the condition [2]. Purba et al. reported a case fatality rate among patients receiving treatment in intensive care units of 69% [3]. The mortality rate for severe sepsis and septic shock can range from 25% to 70%, making early identification imperative [4]. The elevated death rate underscores the importance of early identification to promptly and correctly commence therapy. Given the central role of platelet activation and consumption in sepsis-induced coagulopathy, biomarkers reflecting real-time thrombopoietic activity may provide additional diagnostic value. The immature platelet fraction (IPF), which reflects increased bone marrow platelet release in response to peripheral consumption, represents a readily available hematological parameter that might complement existing sepsis biomarkers [5]. However, the diagnostic performance of the IPF for early sepsis identification has not been systematically evaluated.

Timely identification of sepsis necessitates an integration of clinical assessment and laboratory indicators. Frequently employed measures encompass procalcitonin (PCT), C-reactive protein (CRP), lactate, and a complete blood count [6,7]. PCT serves as a measure for systemic bacterial infection, CRP signifies an acute inflammatory response, and lactate reflects tissue hypoperfusion. Nevertheless, the use of these parameters has constraints: PCT and CRP lack specificity for coagulation activity. The platelet count alone indicates the quantity of platelets and does not represent the activity of bone marrow production. All these indicators remain of low efficacy in preventing multiorgan dysfunction syndrome resulting from sepsis. This syndrome is initiated by persistent microcirculatory abnormalities, which ultimately lead to death [8–10].

Among the numerous mechanisms contributing to microcirculatory disorders, dysfunctions in the coagulation system are pivotal in sepsis-induced organ damage [11]. Despite the contentious nature of microthrombi in sepsis, microvascular alterations may transpire even in the absence of evident thrombotic occurrences [12]. The severity of a patient's condition may deteriorate when systemic inflammation triggers coagulation factors and platelets [13]. This highlights the necessity of including coagulation indicators within the laboratory assessment for sepsis diagnosis [5].

Sepsis triggers Tumor Necrosis Factor-alpha (TNF-α), interleukin(IL)-1β, and IL-6 as inflammatory mediators, which activate the vascular endothelium [14]. The activation of endothelial cells leads to the release of tissue factors that trigger the coagulation cascade, resulting in the formation of microthrombi and the consumption of significant quantities of platelets, frequently culminating in disseminated intravascular coagulation. The reduction in platelet count prompts the bone marrow to enhance thrombopoiesis and release an IPF into the bloodstream, resulting in an elevated intravascular platelet count [14,15]. A high IPF value in sepsis signifies that thrombocytopenia results from platelet consumption or destruction, with bone marrow function remaining intact. In contrast, a low IPF shows a failure in bone marrow production.

The IPF is the percentage of reticulated platelets that still contain residual RNA from megakaryocyte formation and is a direct indicator of thrombopoietic activity in the bone marrow [16,17]. IPF testing may be conducted automatically with fluorescence flow cytometry alongside a complete blood count. The benefit of the IPF compared to other metrics is in its capacity to identify alterations in platelet production at an early stage, prior to a substantial decline in total platelet count, hence serving as a biomarker for the early detection of thrombocytopenia in sepsis [18].

Numerous studies indicate that the IPF has great diagnostic accuracy for the early identification of sepsis [19–27]. The results for diagnostic testing are still pending for some individuals; therefore, this study seeks to ascertain the definitive outcomes. However, existing evidence includes both adult and pediatric populations, which may influence diagnostic performance due to age-related hematological differences. This study sought to evaluate the diagnostic precision of IPF testing for the early identification of sepsis. This is, to our knowledge, the first systematic review and meta-analysis addressing this problem.

Methods

Search strategy

This study followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for reporting on diagnostic test accuracy of studies [28]. PubMed and Scopus databases were searched for relevant articles using keyword searches tailored to the topic of the IPF in sepsis. PubMed and Scopus were selected due to their broad coverage of biomedical, laboratory medicine, and diagnostic accuracy literature. These databases are widely used in systematic reviews of diagnostic test accuracy studies and were considered sufficient to capture relevant evidence on IPF in sepsis. The search period, from August 1 to August 8, 2025, used keywords such as Reticulated Platelet OR Immature Platelets OR Immature Platelet Fraction OR IPF AND sepsis. No restrictions were applied regarding publication year or article type. All references found were entered into the Mendeley reference manager software, with duplicate records being removed.

Study selection

The research selection procedure was separately executed by two authors (S.W., I.P.Y.P.) employing a blinded review methodology. Disputes were settled through discussion until a consensus was achieved, augmented by the perspective of a third author (A.A.W.L.). The initial round of selection comprised the examination of titles and abstracts, followed by a comprehensive assessment of papers that fulfilled the preliminary criteria. This manuscript was prospectively

registered in the PROSPERO database under the registration ID number CRD420251123676.

Included studies were original investigations documenting IPF examination results in patients who exhibited no sepsis criteria at arrival but developed sepsis during the research period of the original article. The exclusion criteria encompassed: review articles, letters to the editor, commentaries, conference abstracts, case reports, small case series (fewer than 50 cases), articles in languages other than English, *in vitro* or animal studies, studies pertaining to sites/sample types unrelated to sepsis, studies with overlapping data from larger publications, and studies focused on non-sepsis diseases. Several potentially relevant studies could not be included due to inaccessible full texts and insufficient diagnostic data, which may have limited the completeness of available evidence (Figure 1).

Data extraction

Data extraction was performed independently by two authors (S.W., I.P.Y.P.), with discrepancies resolved through consensus of three authors (S.W., I.P.Y.P., A.A.W.L.). Extracted data included: first author's name and year of publication, country, study period, study design, patient age, total number of sepsis cases and controls, sensitivity, specificity, the IPF cut-off, and the number of positive and negative cases in both the sepsis and control groups.

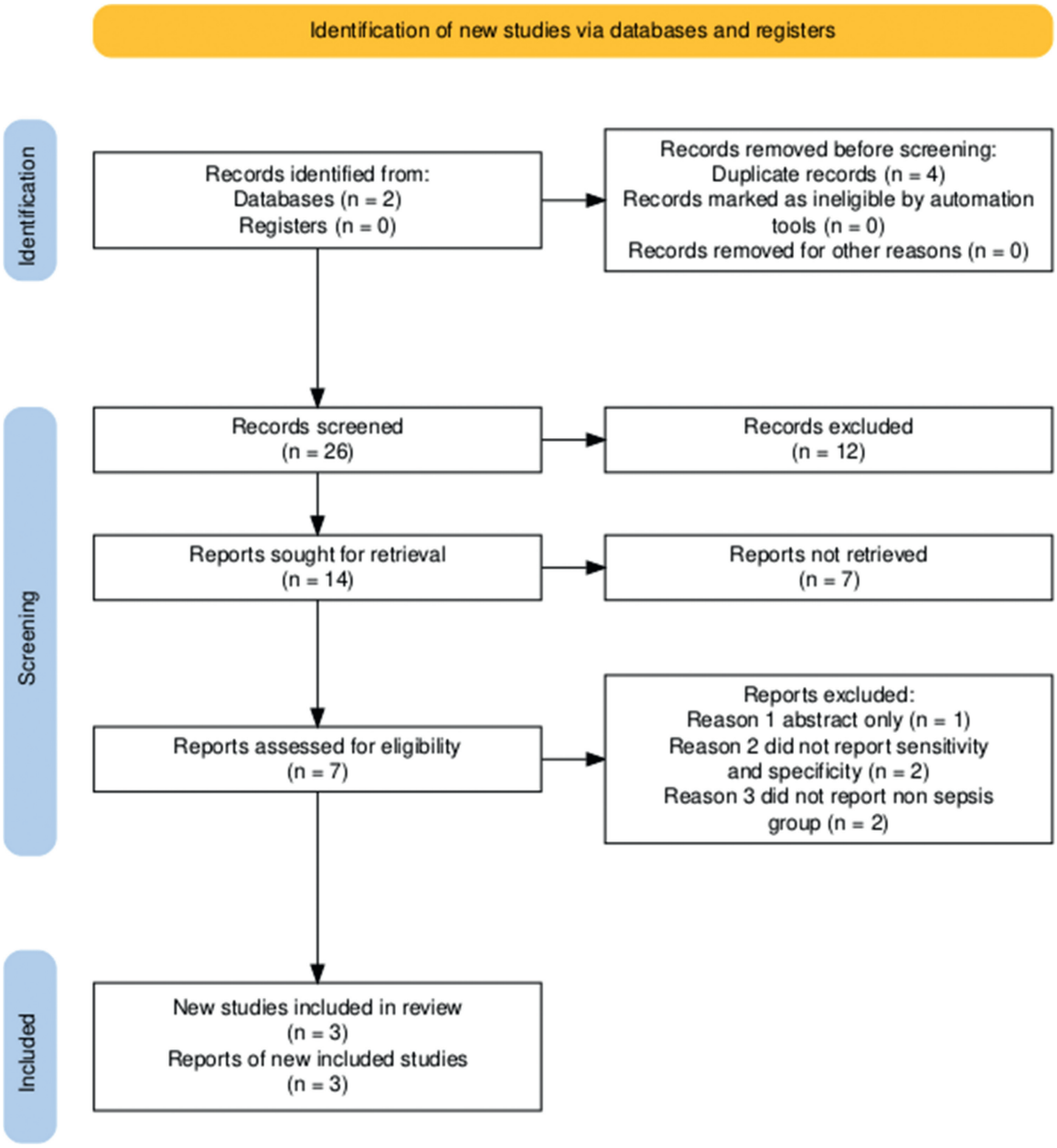

Figure 1 – Flow diagram of the literature search and selection process.

If one of the data points was unavailable, the sensitivity and specificity calculations were carried out using the diagnostic test calculator in Review Manager version 5.4.

Study quality assessment

Study quality was evaluated using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies 2) instrument in four domains: study selection, index test, reference test, and sampling flow and timing.

Statistical analysis

Statistical analyses were performed using Stata software version 17. For each study, sensitivity and specificity were calculated univariately using random effects. The combined diagnostic odds ratio (DOR) was calculated using a random effects model of the IPF test in diagnosing sepsis. A summary receiver–operating characteristic (SROC) curve was generated by plotting individual and pooled sensitivity and specificity points to assess overall diagnostic accuracy. Subgroup analyses were performed based on the adult sepsis population. Finally, potential publication bias was evaluated using a Deeks' funnel plot, recommended for systematic reviews of diagnostic test accuracy studies.

Results

Literature search: study characteristics

The study identification and selection process for this systematic review began with a literature search of two databases (n = 2) without additional reference to register sources. Of the 30 records found, four were removed due to duplication. No records were removed due to automatic ineligibility or other reasons. The remaining 26 records were then screened based on the title and abstract, yielding 14 potentially relevant reports. Of these, seven reports were not accessible for full-text review. A total of seven reports were fully assessed for eligibility. A further four were eliminated for the following reasons: one report was available only in abstract form, two reports did not report sensitivity and specificity values, and two reports did not include a non-sepsis comparison group.

Finally, three studies from Italy, South Korea, and Turkey, published between 2013 and 2021, met the inclusion criteria for this systematic review and meta-analysis [19–21]. The IPF cutoff points varied across the studies. The total sample size included in this review was 451 patients. Additional methodological details regarding the characteristics of each study, reference standard for sepsis diagnosis, and the timing of the IPF measurement are summarized in Table 1. All included studies measured the IPF at or near hospital admission and all analyzed studies used IPF testing to detect sepsis in a population of patients with suspected infection or critical illness.

Table 1 – Study characteristics of the included analysis.

Study	Country	Study design	Sepsis Definition	Timing IPF	ROC compare content	Cutoff values of IPF%	No. of patients		Age (mean ± SD/median)	
							Case	Control	Case	Control
De Blasi et al. 2013[19]	Italy	Prospective	Sepsis-2	1 hour after ICU admission	Sepsis versus non-sepsis	4.7	33	31	59.6 (19.3)	55.4 (15.9)
Park et al. 2016[20]	South Korea	Cross–sectional	Sepsis-2	At the time of admission	Sepsis versus non-sepsis	3.1	215	47	66 (20–92)	52 (20–84)
Turkmen et al. 2021[21]	Turkey	Prospective	Sepsis-2	At the time of admission	Sepsis versus non-sepsis	2.7	78	47	21 (2–86)	23 (2–120)

IPF: Immature platelet fraction; ROC; Receiver operating characteristic; SD: Standard deviation; ICU: Intensive care unit.

Quality assessment and bias analysis

Based on the QUADAS-2 assessment [29], two studies were assessed as having an 'unclear risk of bias' in the Index Test and Reference Standard domains due to insufficient information regarding blinding procedures and details of the reference test implementation. Overall, the methodological quality of all three studies was considered of low concern (Figure 2).

Diagnostic accuracy

Data from eligible studies were analyzed to calculate the diagnostic accuracy of the IPF test for sepsis. A forest plot of sensitivity and specificity is shown in Figure 3. The pooled sensitivity of the IPF test for diagnosing sepsis was 74% (95% Confidence interval [95% CI]: 53%–87%), and the pooled specificity was 70% (95% CI: 48%–85%).

A meta-analysis of the three studies assessing the diagnostic accuracy of the IPF for detecting sepsis yielded a pooled DOR of 6.54 (95% CI: 2.90–14.76), indicating that patients with sepsis were approximately 6.5 times more likely to have a positive IPF result than patients without sepsis (Figure 4). The combined effect significance test (Test of $\theta = 0$) showed statistically significant results (z = 4.52; p-value ≤0.001), confirming that the IPF has significant diagnostic ability in differentiating patients with and without sepsis. Heterogeneity analysis (Test of $\theta i = \theta j$) yielded a Q statistic of 5.01 (degrees of freedom = 2) with p-value = 0.08 and an I^2 value of 59.57%. This shows that the variation in results between studies can largely be explained by differences in methodology or population characteristics, but is not large enough to obscure the combined effect results. The SROC curve shows summary points for sensitivity and specificity that fall within a reasonably broad area of the curve, supporting the ability of the IPF as a diagnostic parameter for sepsis (Figure 5). The publication bias evaluation using Deeks' funnel plot showed a p-value = 0.730 showing there was no evidence of publication bias in the included studies (Figure 6).

Subgroup analysis was performed to evaluate the diagnostic accuracy of the IPF specifically in the adult patient population. This was done as one of the included studies used a pediatric population, which has physiologically different hematologic characteristics than the adult population, potentially affecting the pooled estimates. [21]. Results from subgroup analyses of two studies involving adult patients showed a pooled sensitivity of 85% (95% CI: 80%–89%) and a pooled specificity of 68% (95% CI: 57%–77%) (Figure 7) [19,20]. For this evaluation, the DOR, SROC, and Deeks' funnel plot analysis were not included due to the limitations of the study. Although the sensitivity values were higher compared to the overall analysis, the specificity values were relatively similar, indicating that the IPF still exhibits moderate discriminatory ability in the adult population.

Discussion

This meta-analysis demonstrates that the IPF holds considerable promise as a complementary biomarker for sepsis

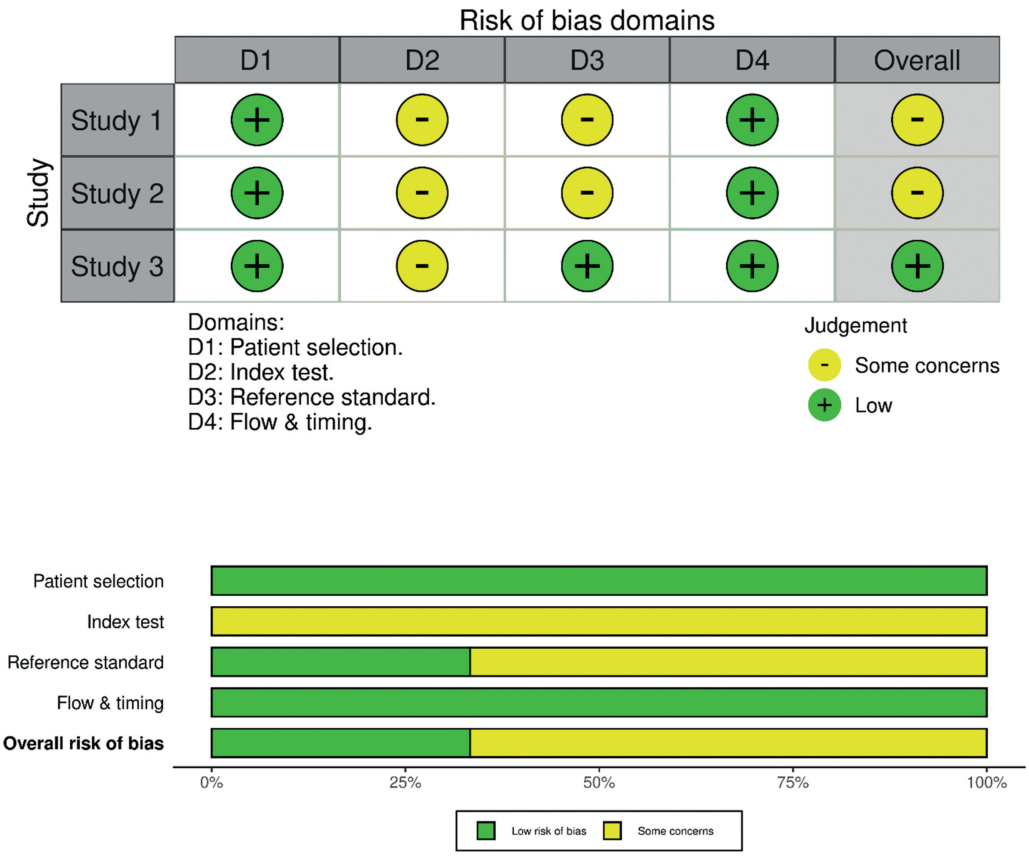

Figure 2 – Study quality assessment using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool.

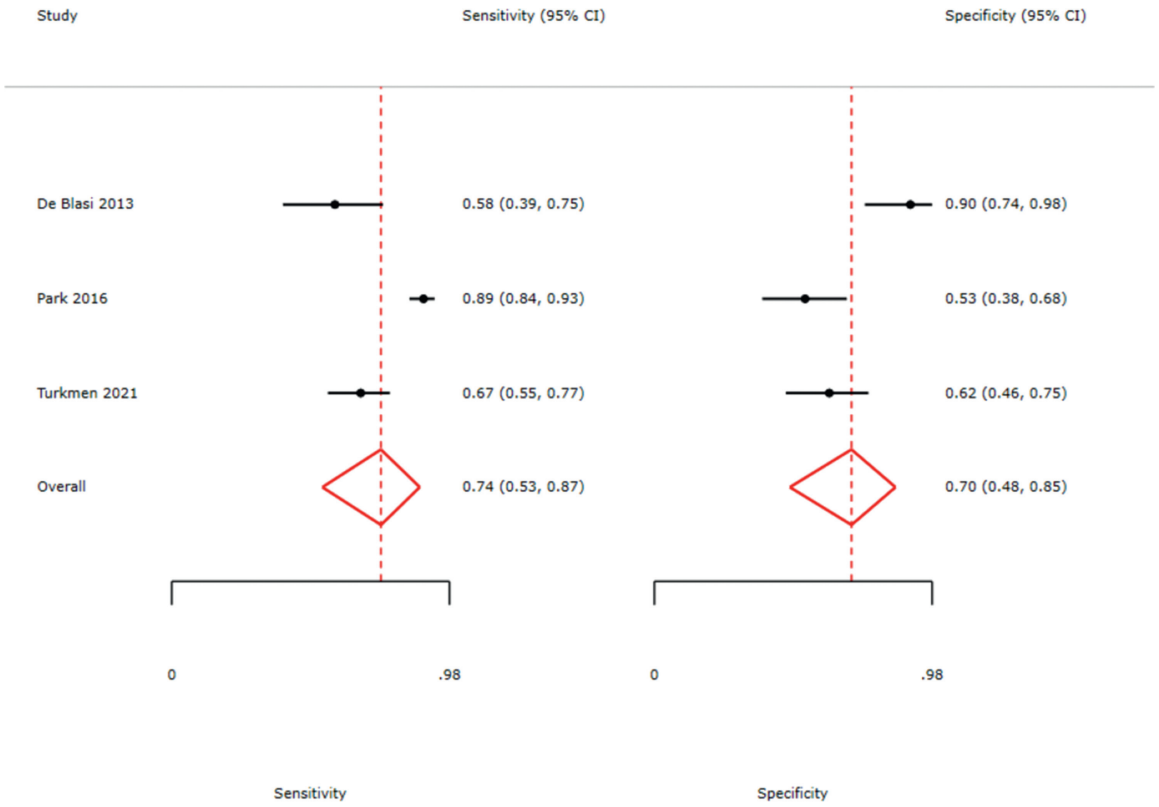

Figure 3 – Forest plots of pooled sensitivity and specificity for sepsis diagnosis. The analysis illustrates the diagnostic performance of the immature platelet fraction across three included studies. Point estimates (black circles) and 95% confidence intervals (horizontal lines) are provided for each study. The red diamonds represent the pooled summary estimates, yielding an overall sensitivity of 0.74 (95% CI: 0.53–0.87) and a specificity of 0.70 (95% CI: 0.48–0.85).

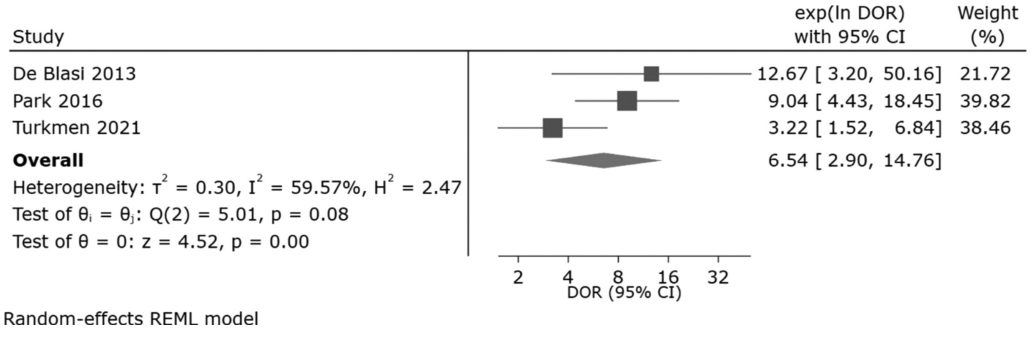

Figure 4 – Forest plot of the diagnostic odds ratio (DOR) for immature platelet fraction (IPF) in Sepsis Diagnosis. Analysis, utilizing a random-effects REML model, yielded a pooled DOR of 6.54 (95% CI: 2.90–14.76). This indicates that the odds of a positive IPF result are 6.54 times higher in patients with sepsis compared to those without the condition. Moderate heterogeneity was observed (I^2 = 59.57%; Q = 5.01; p-value = 0.08). The test of overall effect was statistically significant (z = 4.52; p-value < 0.001), supporting the diagnostic utility of IPF for sepsis identification.

detection in mixed adult and pediatric populations. The IPF exhibited a sensitivity of 74% and a specificity of 70%. These results indicate a moderate diagnostic capacity for both identifying the condition and ruling out health in suspected cases. Clinically, this indicates that the IPF is dependable as an initial screening instrument, although not strong enough to serve as the sole test for diagnostic confirmation. These findings offer the first indication that the IPF might serve as a pertinent supplementary test, particularly when utilized with other known metrics such as PCT and CRP levels.

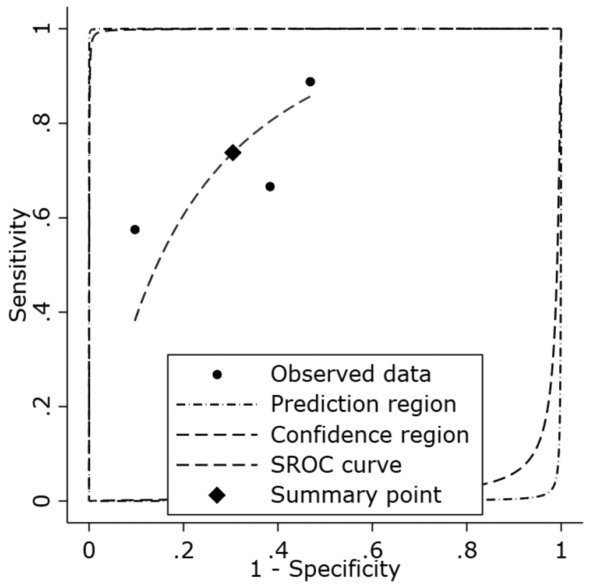

Figure 5 – Summary receiver operating characteristic (SROC) curve of included studies. The SROC analysis integrates three studies evaluating the diagnostic accuracy of the immature platelet fraction for sepsis detection. Individual circles represent the observed sensitivity and specificity for each included study. The solid diamond indicates the pooled summary point, reflecting a combined sensitivity estimate of approximately 0.74 and a specificity of 0.70.

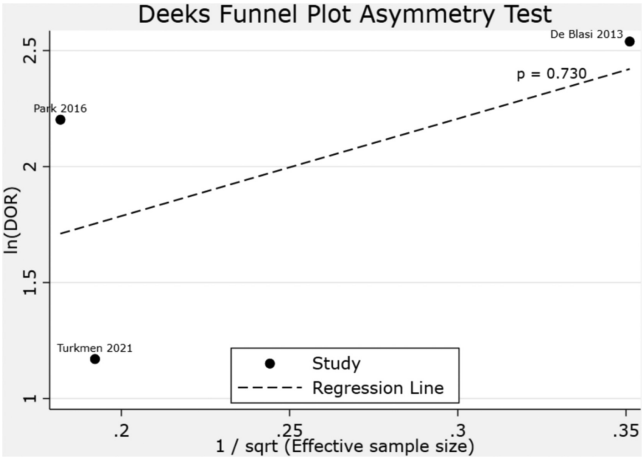

Figure 6 – Deeks' funnel plot assessing the potential publication bias of the three studies. No evidence for publication bias was found (p = 0.73).

These findings align with literature reporting that the IPF is increased in sepsis patients due to stimulation of megakaryocytes in the bone marrow in response to platelet destruction and consumption during systemic inflammation [16]. The activation of the inflammatory response, mainly via the production of pro-inflammatory cytokines like IL-6 and TNF-α, stimulates expedited platelet maturation, leading to a notable rise in the quantity of immature platelets discharged into the bloodstream [14,15]. This situation indicates the body's compensatory efforts to maintain hemostasis while simultaneously signaling an ongoing severe inflammatory process.

In the adult population subgroup analysis, without the research on pediatric patients, sensitivity rose to 85% and specificity to 68%. This heightened sensitivity indicates that the IPF may be more effective in identifying sepsis in adult patients than in the pediatric group. The pathophysiological explanation lies in the stability of the adult hematopoietic system, which regularly reveals an increase in immature platelet formation in response to sepsis [30]. In pediatrics, physiological variability in platelet production, the development of the immune system, and varying hematopoietic responses might affect the IPF levels even without infection, hence diminishing diagnostic accuracy [30].

This finding aligns with previous reports that other inflammatory biomarkers, such as PCT and CRP, also show differences in diagnostic performance across age groups [31]. Physiological PCT levels exhibit variations across age groups, with healthy pediatric individuals presenting higher levels than healthy adults, although healthy newborns have the lowest levels. The variations in baseline levels may affect the interpretation of PCT values in sepsis diagnosis, indicating that the appropriate cut-off may differ between age groups [31]. Likewise, CRP levels may be increased owing to non-infectious reasons in young patients, including vaccinations or minor inflammatory conditions. CRP levels may rise substantially post-vaccination, with up to 85% of preterm newborns exhibiting an increase, and adults experiencing an average elevation of around 30% following influenza vaccination. This indicates that an elevation in CRP does not invariably signify infection, particularly during the post-immunization phase [32,33]. This meta-analysis aligns with many studies indicating that the IPF was considerably elevated in septic patients exhibiting neutrophilia compared to non-septic controls [34,35]. The IPF could distinguish thrombocytopenia resulting from peripheral damage (e.g., in sepsis) from thrombocytopenia caused by bone marrow hypoproduction [36–39].

Sepsis triggers massive immune system activation accompanied by activation of the coagulation system [40]. A crucial mechanism is the production of microthrombi resulting from disseminated intravascular coagulation or subclinical coagulation events. This disease markedly elevates platelet consumption. Consequently, the bone marrow enhances thrombopoiesis, characterized by the discharge of immature platelets (platelet reticulocytes) into the bloodstream [40,41]. The IPF, measured by fluorescent flow cytometry on modern hematology analyzers, reflects the proportion of immature platelets in the blood and is a direct indicator of thrombopoietic activity [42].

From a clinical standpoint, the IPF offers numerous advantages: it is readily accessible and can be concurrently measured with a complete blood count on most contemporary hematology instruments; it is expeditious, yielding results within minutes, thereby facilitating prompt clinical decision-making in emergency departments or intensive care units; and it is non-invasive, negating the necessity for additional

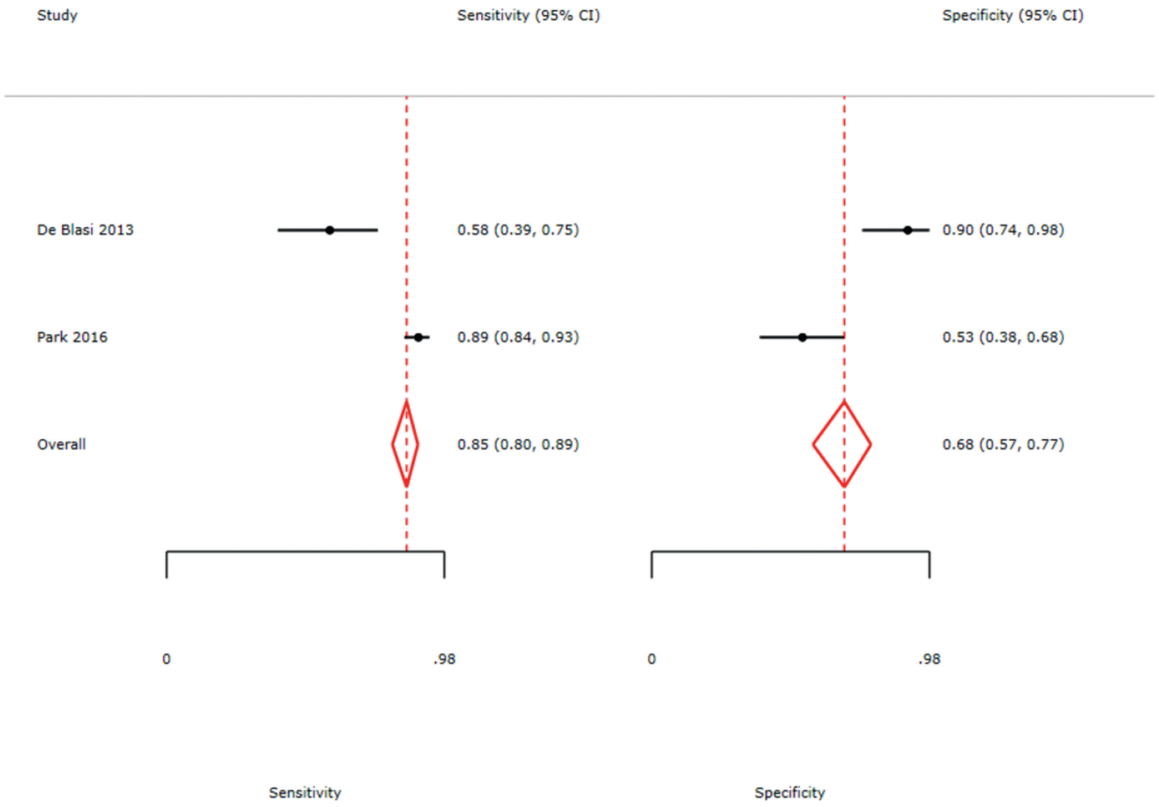

Figure 7 – Subgroup analysis of adult sepsis patients only.

blood sampling [37]. Consequently, the data of the present study suggest that the IPF should be included in a sepsis diagnostic panel rather than utilized as an independent test. Integrating the IPF with PCT, CRP, or a clinical score like the quick Sequential Organ Failure Assessment (qSOFA) could enhance diagnostic precision and assist in patient risk stratification [23,43]. The interpretation of the IPF necessitates the evaluation of confounding circumstances, including acute bleeding, postoperative states, significant trauma, autoimmune diseases, and hematologic illnesses such as idiopathic thrombocytopenic purpura (ITP), all of which may elevate the IPF in the absence of sepsis [44]. Conversely, decreased bone marrow production resulting from chemotherapy, certain viral infections, or dietary deficits might lower the IPF count. In pediatrics, characteristics such as hematopoietic maturation, comorbidities, and nutritional conditions may further influence this value [45,46].

Several limitations must be acknowledged. The number of papers fulfilling the inclusion criteria was small ($n = 3$), resulting in a low strength of evidence. Secondly, discrepancies in research design, sepsis definitions, IPF measurement techniques, and applied cutoffs may affect the heterogeneity of the findings. Third, two of the three studies were observational, and subgroup analyses were constrained since just one study investigated a pediatric population, rendering adult-pediatric comparisons exploratory.

This meta-analysis should be interpreted with caution as the small number of included studies may reduce the precision of pooled estimates and limit generalizability. Additionally, heterogeneity may arise from differences in sepsis definitions, patient age groups, hematology analyzers, IPF measurement techniques, and applied cut-off values. Consequently, the data of this study suggest that the IPF should be included in a sepsis diagnostic panel rather than utilized as an independent test. This complementary role is consistent with the intended use of the IPF as an adjunct to established biomarkers and clinical assessment rather than a replacement. Further research should highlight the need for future standardization of IPF measurement, including harmonization of analytical platforms, calibration procedures, and clinically validated cut-off values. Establishing standardized protocols is essential to facilitate broader clinical implementation and improve the reliability of the IPF as a diagnostic adjunct in sepsis. Research should encompass a fair representation of pediatric and adult populations to elucidate variations in diagnostic performance attributable to age. Assessing the interplay of the IPF with additional biomarkers in multivariate predictive models may enhance its application in comprehensive diagnostic algorithms.

Conclusions

The IPF may serve as a complementary diagnostic biomarker for sepsis due to its rapid availability and integration into routine hematological testing. However, the limited number of studies and methodological heterogeneity warrant cautious interpretation. Future large-scale prospective studies using standardized sepsis definitions, IPF measurement techniques, and validated cut-off values are needed to confirm its clinical utility.

Author contributions

Conceptualization: SW, IPYP; Data curation: IBAPM, IGPS; Formal analysis: SW, IPYP; Investigation: IBAPM, IGPS; Methodology: IBAPM, IGPS, AAWL; Resources: SW, IPYP; Software: IBAPM, IGPS; Supervision: AAWL; Validation: IBAPM, IGPS, AAWL; Visualization: IBAPM, IGPS, AAWL; Writing–original draft: SW, IPYP; Writing–review & editing: all authors. All authors read and approved the final manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest and sources of funding

The authors state that there are no conflicts of interest to disclose.

Acknowledgements

The authors would like to express their sincere gratitude to the members of the RISEarch Research Group for their valuable collaboration, insightful discussions, and continuous support throughout the course of this study.

Editor

Erich Vinicius De Paula

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Original article

Association of *Toxoplasma gondii* infection with hematological parameters and CD4⁺ cell counts among pregnant women attending antenatal care in a public hospital of Northwest Ethiopia

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ARTICLE INFO

Article history:
Received 21 March 2025
Accepted 15 April 2026
Available online 2 June 2026

Keywords:
Toxoplasma gondii
Hematological parameters
CD4⁺ cell count
Pregnant women
Ethiopia

ABSTRACT

Background: *Toxoplasma gondii* affects one-third-of the global population and may alter hematological parameters and CD4⁺ T cell counts, especially during pregnancy. This study evaluated the association of *T. gondii* with alterations in hematological values in pregnant women attending a public antenatal care hospital in Northwest Ethiopia.

Methods: An analytic cross-sectional study of 554 pregnant women (301 seropositive, 253 seronegative) attending antenatal care at a public hospital from 2022 to 2023 assessed T. gondii exposure using ELISA IgG/IgM kits (Human Diagnostics, Germany). Blood samples collected in EDTA tubes were analyzed for hematological profiles using a Coulter Hematology analyzer, and the CD4⁺ cell count with a BD FACSPresto™. Data were analyzed with SPSS 21.0. Descriptive statistics and independent sample t tests were performed: normality was confirmed using the Kolmogorov-Smirnov test

Results: Significant differences were observed in hematological values (white blood cell count, hemoglobin, hematocrit, red blood cell count, lymphocytes, neutrophils, and mean corpuscular volume) between seropositive and seronegative women (p-value < 0.001). Platelet counts showed no significant variation (p-value = 0.811). However, CD4⁺ cell counts were significantly lower in toxoplasmosis-infected women (p-value < 0.001).

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https://doi.org/10.1016/j.htct.2026.106480
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Conclusion: Toxoplasma gondii infection is associated with alterations in hematological parameters and immunological profiles in pregnant women. Routine screening during antenatal care and preventive education are recommended.

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Introduction

The parasite *Toxoplasma gondii* is a single-celled, eukaryotic, coccidian protozoan that must live inside cells [1]. It is in the group of phylum Apicomplexa and relies on the actomyosin system, an active process that invades host cells. Several protozoan parasites utilize the actomyosin system, causing serious illnesses in a range of hosts, including humans and other warm-blooded domestic and wild animals [2]. *T. gondii* is a prevalent zoonoses, affecting one-third-of the global population [3,4]. It causes toxoplasmosis, a potentially fatal disease in immunocompromised patients, and can lead to congenital defects in the fetuses of women infected during pregnancy [5,6]. Unlike other coccidian, it can infect any nucleated cell, including immature red blood cells (RBC) in warm-blooded animals [7].

T. gondii has a complex life cycle, infecting most warm-blooded species. Similar to *Plasmodium*, it infects cells by invading the host cell membrane or disrupting the cytoskeleton, often resulting in cell rupture [8,9]. This process, known as cell lysis, occurs during the parasite's exit [10]; The exact mechanism of the exit is still unclear, but it is a facultative heterogeneous process, involving sexual and asexual phases in different hosts [11,12].

T. gondii infection can occur in two stages: acute toxoplasmosis, which is often asymptomatic in healthy adults, and chronic persistence of cysts in immune-competent individuals [13]. Chronic infection and cell-mediated immunodeficiency can cause toxoplasmosis symptoms to reoccur and lead to a decrease in the $CD4^+$ cell count [14]. Immunocompromised individuals may experience headaches, confusion, poor coordination, seizures, lung problems, and blurred vision due to retinal inflammation [15]. Latent toxoplasmosis typically presents with mild symptoms but can cause lesions in the heart, skeletal muscle, and central nervous system [16,17]. In humans, the initial *T. gondii* infection typically does not show any noticeable symptoms. However, some individuals may experience ocular toxoplasmosis or cervical lymphadenopathy [18].

During pregnancy, the parasite can cross the placenta and infect the fetus, posing significant risks to both the fetus and the mother. Vertical transmission from an infected mother can lead to miscarriage, congenital infection, severe fetal damage, retinochoroiditis, and even death or infertility in women [4,12,19].

$CD4^+$ T cells play a crucial role in controlling both acute and chronic *Toxoplasma* infections. While $CD8^+$ T cells are essential for managing chronic infection, their maintenance depends on $CD4^+$ T cells. $CD8^+$ T cell exhaustion can lead to reactivation of the infection [20,21]. Non-exhausted antigen-specific $CD4^+$ T cells can restore function and prevent infection reactivation [22].

In humans, infection with *T. gondii* induces both cellular and humoral immune responses in immunocompetent individuals. The immune system significantly reduces the number of parasites, although the encysted form survives. Acquired immunity is long-lasting and protects against reinfection. Cellular immunity is a critical component of the host's immune response to *Toxoplasma* infection [23].

Global infection rates of *T. gondii* in pregnant women aged from 18 to 49 range from 2% to 90% [11]. Risk factors and transmission routes significantly impact the health of pregnant women. Specifically, infection rates correlate with environmental exposure to cat populations and are further influenced by hygiene practices, such as hand washing, sanitation, and the consumption of contaminated food or water [5,12,24]. Accurate detection and the use of sensitive diagnostic methods are crucial for effective control and treatment of infections. This study aims to determine the effect of *T. gondii* infections on hematological parameters and on the $CD4^+$ cell count among pregnant women attending antenatal care at a public hospital in Northwest Ethiopia. The findings provide baseline information for planning and implementing control and prevention strategies, as well as for advancing the understanding of the epidemiology and management of toxoplasmosis.

Materials and methods

Study design and setting

An analytic cross-sectional study was conducted to assess the effect of T. gondii infection on hematological parameters and on the $CD4^+$ cell count among pregnant women receiving antenatal care at a public hospital in Northwest Ethiopia. Serological testing employing an enzyme-linked immunosorbent assay (ELISA) was performed between 2022 and 2023. Participants were categorized into two groups: those who tested positive for toxoplasmosis (seropositive) and those who tested negative (seronegative - control group).

Study participants

The study population included pregnant women aged 18 to 49 who attended antenatal care during the study period and provided consent to participate. However, pregnant women unable to communicate due to medical conditions and those

who were human immunodeficiency virus (HIV)-positive were excluded from the study.

Data collection tools and procedures

Seropositive participants were identified by positive results for parasite-specific IgG and IgM antibodies using ELISA kits (Human Diagnostics, Germany) following the manufacturer's instructions. Seronegative participants had negative test results.

Differences in mean values of hematological parameters and $CD4^+$ cell count were analyzed between seropositive and seronegative groups to understand how *T. gondii* infections affected these variables.

Qualified phlebotomists collected 3 ml of venous blood from each participant using sterile needles and syringes. The samples were immediately transferred into labeled tubes containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. These samples were then used for hematological analysis and $CD4^+$ T cell counts within one hour of collection. A Coulter Automated Hematology Analyzer was employed for the comprehensive blood counts.

This analyzer provided quantitative data regarding the total number of RBC, white blood cells (WBC), lymphocytes, neutrophils, and platelets. Additionally, the analyzer measured hematocrit (Hct), hemoglobin (Hb), and mean corpuscular volume (MCV).

This study utilized FACSPresto™ (BD Biosciences), a single-platform automated analyzer designed for point-of-care testing. This device provided absolute $CD4^+$ T cell counts, $CD4^+$ percentages, and Hb levels.

Data quality control

Laboratory technologists received a one-day training session that focused on the study's purpose and significance, the registration of laboratory test results, and the importance of maintaining the confidentiality of these results. Standard operating procedures (SOP) and manufacturer instructions were strictly followed throughout the procedures, and all reagents were prepared according to the manufacturer's instructions. The lead investigator maintained the quality of the test result by strictly adhering to the laboratory SOP, from the pre-analytic phase of blood collection to the post-analytical phase of reporting results. The investigator also frequently checked and ensured adherence to these procedures. Training was provided to laboratory technologists and supervisors to reduce variations in data collection (information bias). This training included standardized instructions on using and interpreting each test.

Data processing and analysis

Data were entered into EpiInfo, version 7.1.5.2, and then exported to the Statistical Package for Social Science (SPSS) version 21. Normality of distribution was evaluated using the Kolmogorov-Smirnov test. Data cleaning was performed to ensure accuracy, consistency, and the handling of missing values. Descriptive statistics, such as mean and percentages, were used to describe the data, which were then presented using text and tables. An independent t-test was conducted to determine the mean difference between the seropositive and seronegative groups. A p-value <0.05 and a 95% confidence interval (CI) indicated statistically significance.

Results

Participants characteristics

A total of 554 pregnant women receiving antenatal care were enrolled, 301 (54.3%) of whom were seropositive for *T. gondii*. The majority were aged 26–30 (42%) and resided in towns (78.9%) (Table 1).

Table 1 – Characteristics of pregnant women (n = 554) attending antenatal care in a public hospital of Northwest Ethiopia.

Variable	n (%)	Seropositive n (%)	Seronegative n (%)	p-value
Age				
18–20	37 (6.7)	25 (67.6)	12 (32.4)	0.021
21–25	125 (22.6)	64 (51.2)	61 (48.8)	
26–30	233 (42.1)	125 (53.6)	108 (46.4)	
31–35	102 (18.4)	53 (52.0)	49 (48.0)	
36–43	57 (10.3)	34 (59.6)	23 (40.4)	
Residence				
Urban	437 (78.9)	218 (49.9)	219 (50.1)	0.003
Rural	117 (21.1)	83 (70.9)	34 (29.1)	
Occupation				
Student	40 (7.2)	25 (62.5)	15 (37.5)	0.045
Government	177 (31.9)	89 (50.3)	88 (49.7)	
Daily labor	34 (6.1)	19 (55.9)	15 (44.1)	
Farmer	68 (12.3)	49 (72.1)	19 (27.9)	
Merchant	39 (7.0)	20 (51.3)	19 (48.7)	
Other	196 (35.6)	99 (50.5)	97 (49.5)	
Education level				
Illiterate	120 (21.7)	80 (66.7)	40 (33.3)	0.008
Elementary	66 (11.9)	39 (59.1)	27 (40.6)	
Secondary	143 (25.8)	66 (46.2)	77 (53.8)	
Higher education	225 (40.6)	116 (51.6)	109 (48.4)	

Association of T. gondii *infection with hematological parameters*

T. gondii has the ability to infect almost every type of nucleated cell, including immature RBC (erythroblasts). The effect of T. gondii infection on different hematological parameters, including WBC, lymphocytes, neutrophils, RBC, Hb, Hct, MCV, and platelets, was examined. Significant differences were found in the mean values of most of these parameters between seropositive and seronegative participants. However, the platelet counts did not show any significant difference (p-value = 0.811) (Table 2).

Association of T. gondii *infection with the CD4⁺ cell count*

On comparing seropositive and seronegative pregnant women, there was a significant difference in the mean $CD4^+$ cell counts with the seronegative group having a significantly higher count (Table 3).

Discussion

This study highlights a significant association between *T. gondii* infection and hematological alterations in pregnant women. Specifically, women who tested seropositive for *T. gondii* exhibited significantly lower levels of WBC, lymphocytes, neutrophils, RBC, Hb, Hct, and $CD4^+$ T cell counts compared to their seronegative counterparts. These hematological disturbances may reflect the parasite's ability to impair hematopoiesis through direct invasion of progenitor cells or immune-mediated mechanisms [25].

The findings of this study align with earlier reports from Tikrit city [25], Saudi Arabia [26], and Iraq [27] that also documented hematological suppression among infected pregnant women. WBCs play an essential role in controlling toxoplasmosis [28], and the parasite's ability to invade nucleated cells including immature RBC suggests a plausible pathophysiologic mechanism. The clinical implications are concerning, as such alterations could compromise maternal health and fetal outcomes [28]. Neutrophilia was notably higher in seropositive women, indicating an acute inflammatory response potentially induced by the rapid multiplication of tachyzoites [10,26,29]. Neutrophils, guided by chemokine gradients toward the sites of tachyzoite invasion, are among the first responders during infection [26,29].

Interestingly, while some studies from Thailand [30] and Libya [26] report contrasting findings regarding WBC levels, the present results revealed significant leukopenia in infected cases. This suggests regional variability or potential influence from other confounding factors such as nutrition or co-infections. *Toxoplasma* infection can lead to a significant decline in WBC counts in pregnant women. Because these cells are essential for orchestrating both innate and adaptive immune responses, their depletion may compromise the host's ability to control the parasite [25]. Crucially, *T. gondii* infection was also associated with a marked reduction in $CD4^+$ T cell counts. $CD4^+$ T lymphocytes are pivotal in orchestrating adaptive immunity and ensuring long-term immunological memory [31]. The findings of the current study support existing evidence that lower $CD4^+$ counts in seropositive pregnant women may result from suppressed interleukin (IL)-12 and interferon gamma (IFN-γ) production and impaired cytotoxic T cell activity. This immune suppression renders these women more susceptible to opportunistic infections and may contribute to reactivation of latent toxoplasmosis or other latent pathogens. Although most participants were in the age range of 18–49 years, significant immune alterations were consistent across the spectrum. The observed decline in $CD4^+$ T cell levels corroborates previous work [32] and underscores the pathogen's capacity for immune modulation. Notably, some discrepancies were observed when compared to published studies [33–35]. These inconsistencies may be attributed to differences in study design, population demographics, or diagnostic methodologies.

Despite its strengths, this study is constrained by its cross-sectional nature, limiting causal inferences. Moreover, potential confounders like nutritional deficiencies or unrecognized infections were not exhaustively assessed. Nonetheless, the consistent pattern of hematological and immunological disruptions in *T. gondii*-positive pregnant women suggests a need for routine screening and timely intervention, particularly in endemic settings. By characterizing the hematological impact and immunologic suppression associated with toxoplasmosis, this research reinforces the importance of early diagnosis and immune monitoring in pregnant women.

Table 2 – Association of *T. gondii* infection with hematological parameters of pregnant women attending antenatal care in a public hospital of Northwest Ethiopia.

Parameter	Seropositive mean (95% CI)	Seronegative mean (95% CI)	P-value
White blood cells ($\times 10^3/\mu L$)	6.2 (6.0–6.4)	7.1 (6.9–7.3)	<0.001
Lymphocyte ($\times 10^3/\mu L$)	2.03 (2.00–2.05)	2.00 (1.98–2.02)	0.059
Neutrophils ($\times 10^3/\mu L$)	2.01 (2.00–2.02)	2.02 (2.00–2.03)	0.038
Red blood cells ($\times 10^6/\mu L$)	3.9 (3.8–4.0)	4.4 (4.3–4.5)	<0.001
Hemoglobin (g/dL)	11.2 (11.0–11.4)	12.1 (11.9–12.3)	0.004
Hematocrit (%)	34.1 (33.8–34.4)	36.8 (36.5–37.1)	<0.001
Mean corpuscular volume (fL)	85.2 (83.9–86.5)	87.1 (85.8–88.4)	0.051
Platelet ($\times 10^3/\mu L$)	218 (210–226)	221 (213–229)	0.811

95% CI: 95% confidence interval.

Table 3 – Association of *T. gondii* infection on the $CD4^+$ count of pregnant women Public Hospital Northwest Ethiopia.

Parameter	Seropositive Mean (95% CI)	Seronegative Mean (95% CI)	P-value
$CD4^+$ cells/μL	1550 (1500–1613)	1996 (1987–2000)	<0.001

95% CI: 95% confidence interval.

Proactive management can not only mitigate maternal complications but also reduce the risk of vertical transmission to the fetus.

Conclusion and recommendation

The study demonstrates that *T. gondii* infections during pregnancy are associated with significant alterations in immune and hematological profiles. We recommend routine screening during antenatal care visits and conducting further research into the implications in humans. Additionally, it is advisable to provide education on prevention and risk factors.

Funding

This research did not receive any grants from any funding agencies.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics, approval, and consent to participate

Ethical clearance was obtained from the Research and Ethical Review Committee of the University of Gondar with a reference number of VP/RTT/05/280/2022. Written consent was secured from all participants. All methods were carried out in accordance with relevant guidelines and regulations.

Authors' contributions

Conceptualization: Eden Woldegerima, Mastewal Birhan, Mequant Melese,Tadelo Wondmagegn, Nega Berhane; Data analysis: Eden Woldegerima, Destaw Fetene Tesshome, Mequanente Dagnaw; Investigation: Eden Woldegerima, Tadelo Wondmagegn, Nega Berhane; Methodology: Eden Woldegerima, Destaw Fetene Tesshome, Mequanente Dagnaw; Project administration: Eden Woldegerima, Nega Berhane; Supervision: Eden Woldegerima, Nega Berhane; Writing - original draft: Eden Woldegerima, Mastewal Birhan, Mequant Melese,Tadelo Wondmagegn, Nega Berhane; Writing - review & editing: Eden Woldegerima, Destaw Fetene Tesshome, Mequanente Dagnaw, Nega Berhane.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgements

The authors express their gratitude to the University of Gondar for providing the opportunity for this study. In addition, the authors thank study participants for providing invaluable information and data collectors and supervisors for their dedicated effort and approved the manuscript.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Dose-dependent mortality risk of plasma transfusion in critically ill patients

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ARTICLE INFO

Article history:
Received 12 September 2025
Accepted 20 May 2026
Available online 23 June 2026

Keywords:
Plasma transfusion
Frozen plasma
In-hospital mortality
Critically ill patients
Dose-response relationship
Restrictive transfusion strategy

ABSTRACT

Objectives: Plasma transfusion is commonly used in intensive care units, often for non-bleeding indications despite limited evidence. This study aimed to evaluate the association between plasma transfusions and in-hospital mortality among critically ill patients.

Methods: A retrospective cohort study was conducted using data from 282 adult intensive care unit patients who received transfusion therapy between January and December 2020. Patients were grouped by their exposure to plasma transfusion and further by the transfusion volume. First, multivariable Cox regression coupled with propensity score matching was used to control for baseline confounders and quantify the independent association of plasma transfusion with in-hospital mortality. Subsequently, Cox models and Kaplan-Meier survival curves were employed to elucidate the dose-response relationship between transfusion volume and mortality risk, with additional subgroup analyses.

Results: In the unadjusted analysis, plasma transfusion was significantly associated with increased in-hospital mortality (Hazard ratio = 4.199; 95% confidence interval: 2.53−6.97; p-value <0.001). This association persisted after propensity score matching adjusted for key confounders (Hazard ratio = 3.271; 95% confidence interval: 1.30−8.21; p-value = 0.012). Kaplan-Meier survival analysis demonstrated significantly lower survival probabilities in the transfusion group (log-rank p-value <0.05). Furthermore, a dose-dependent relationship was revealed: mortality risk increased at higher volumes, reaching statistical significance at >800 mL (Hazard ratio = 4.09; 95% confidence interval: 1.19−14.04; p-value = 0.025). Subgroup analysis indicated that the elevated risk was particularly pronounced among patients who concurrently received red blood cell transfusions.

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https://doi.org/10.1016/j.htct.2026.106491

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Conclusion: Plasma transfusion is independently and dose-dependently associated with increased mortality in critically ill patients, particularly when used without clear indications. These findings support a restrictive, evidence-based approach, emphasizing correction of underlying coagulopathy over prophylactic transfusion. Strict adherence to guidelines and an individualized risk-benefit assessment are essential to improve patient safety.

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Introduction

In the field of critical care medicine, transfusion therapy is frequently employed as a supportive treatment to address anemia, replenish blood volume, and correct coagulopathy [1]. The use of frozen plasma (FP) in intensive care units (ICUs) remains prevalent. Epidemiologic data from a recent large international multicenter study indicate that roughly 10% of critically ill patients receive plasma transfusions during their hospital stay, of which up to 37% are given for non-hemorrhagic indications [2]. This aligns with earlier observations from studies conducted in the UK, Australia, and other countries [3–5].

Several international guidelines [6–10] outline limited indications for FP use, such as active bleeding with coagulation factor deficiencies, plasma exchange, specific coagulopathies requiring replacement therapy, and massive transfusion protocols. However, the evidence supporting prophylactic FP use in critically ill patients remains limited and controversial [11]. Studies have shown that early high-ratio plasma transfusion (plasma-to-red-cell ratio ≥1:2) in patients undergoing massive transfusion can reduce mortality rates [12–14]. Conversely, research conducted in Hiroshima indicated that the lack of improvement in coagulation following FFP transfusion was independently and significantly associated with death within 28 days [15]. Additionally, Qin et al. found that early FFP transfusion in sepsis patients was significantly correlated with an increased risk of mortality [16].

Despite this, plasma transfusion remains prevalent in current clinical practice, with its potential risks often underestimated. A retrospective audit at a tertiary hospital revealed that up to 51.8% of plasma transfusions were administered outside of guideline recommendations, including unnecessary prophylactic use [17]. Such inappropriate transfusions waste limited blood resources and expose patients to a range of transfusion-related adverse effects [18,19].

Employing a retrospective cohort analysis, this study investigates the relationship between plasma transfusion dosage and mortality risk among critically ill patients. It aims to evaluate clinical outcomes to establish evidence-based risk thresholds and provide decision support for optimizing transfusion practices.

Material and methods

Study design

This single-center retrospective cohort study was conducted at Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University), a tertiary Grade A general hospital directly under the Hunan Provincial Health Commission. As a regional medical center, it holds major responsibilities in clinical care, teaching, research, and the management of critically ill patients. Its comprehensive ICU is equipped with standard monitoring and life-support devices, serving a diverse population of critically ill medical and surgical patients. This setting provided an adequate case source for the study. The admitted cases were patients hospitalized in the ICU who received blood transfusion therapy from January 2020 to December 2020. This study was approved by the hospital's Ethics Committee.

Inclusion and exclusion criteria

The inclusion criteria were age ≥18 years and ICU stay ≥24 h. Required core data items were available and complete in all medical records. These data comprised: (1) baseline demographic characteristics (sex, age) and disease severity (Acute Physiology and Chronic Health Evaluation II [APACHE II] score); (2) key pre-transfusion laboratory parameters (hemoglobin [Hb], prothrombin time [PT], activated partial thromboplastin time [APTT], fibrinogen, among others); (3) detailed records of plasma and other blood product transfusions; and (4) in-hospital survival outcomes.

The exclusion criteria were patients aged <18 years, ICU stay <24 h (to minimize the inclusion of patients dying rapidly from their primary disease), and patients receiving plasma exchange. For patients with multiple ICU admissions, data collection was restricted to the hospitalization during which they received their first plasma transfusion.

After applying these criteria, 282 critically ill patients were included in the final cohort.

Data collection

Plasma transfusion involves the administration of various plasma preparations, including fresh frozen plasma (FFP), frozen plasma (FP), cryoprecipitate-reduced plasma, and cold supernatant. Indications for plasma transfusion are categorized as prophylactic or therapeutic. Prophylactic transfusions correct coagulation abnormalities in non-bleeding patients or reduce perioperative bleeding risks in those undergoing invasive procedures or surgery. Therapeutic transfusions are primarily used for hemostatic support in actively bleeding patients, antithrombin III (AT3) supplementation, and plasma exchange. The indications for plasma transfusion applied in this study were based on

contemporary international guidelines [6,9], which align with current clinical practice in China.

Plasma transfusion is indicated in a range of critical clinical scenarios, including severe bleeding due to single or multiple coagulation factor deficiencies; dilutional coagulopathy following massive blood loss or transfusion; cases requiring emergent reversal of warfarin anticoagulation; heparin resistance associated with AT3 deficiency; disseminated intravascular coagulation (DIC); thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS); as well as in the setting of major trauma, extensive burns, and large-volume autologous blood salvage (>1000 mL in adults). Plasma transfusion is also utilized during therapeutic plasma exchange procedures.

Patients were divided into two groups, plasma transfusion group and no plasma transfusion group, according to whether they received plasma transfusion during hospitalization in the ICU. The following data were collected: demographic data, ICU hospitalization time, APACHE II score, blood transfusion data, and pre-transfusion laboratory tests, including Hb, C-reactive protein, procalcitonin, PT, APTT, fibrinogen, AT3 activity, total bilirubin, alanine aminotransferase (ALT), creatinine and others.

Statistical analysis

All analyses were conducted in Statistical Package for Social Sciences (SPSS) 25.0. For continuous variables, normally distributed data are summarized as means ± standard deviation and compared using Student's *t*-test. Non-normally distributed data are reported as medians and interquartile range and compared using the Wilcoxon test. For categorical variables, data are presented as numbers (percentages) and compared using the Chi-square (χ^2) test.

First, multivariable Cox proportional hazards regression was performed on the overall cohort to identify risk factors for mortality and to estimate the unadjusted association between plasma transfusion and patient outcomes, reporting results as hazard ratios (HR) with 95% confidence intervals (95% CI). To better isolate the effect of plasma transfusion by minimizing baseline confounding and enhancing intergroup comparability, 1:1 propensity score matching (PSM) was subsequently performed. In the matched cohort, a Cox proportional hazards model (PSM model) was applied to re-evaluate the association between plasma transfusion and mortality risk. This model was further adjusted for key covariates (age, patient type, PT) to assess robustness. Survival probabilities were visualized using Kaplan-Meier curves, and the log-rank

Table 1 – Baseline characteristics after propensity score matching.

Variable	All (n = 110)	Plasma (n = 55)	Non-plasma (n = 55)	Z/χ2	p-value
Sex				0.00[3]	1.000
Male	68(61.8%)	34(61.8%)	34(61.8%)		
Female	42(38.2%)	21(38.2%)	21(38.2%)		
Age (years)	58.8 ± 16.1	57.4 ± 15.9	60.1 ± 16.3	0.89[1]	0.373
Length of ICU stay (days)	12.3 ± 13.7	12.1 ± 12.7	12.5 ± 14.7	0.49[2]	0.625
APACHE Ⅱ score	23.6 ± 7.7	23.4 ± 7.1	23.8 ± 8.3	0.32[1]	0.750
Patient category				0.93[3]	0.335
Surgical care	63(57.3%)	34(61.8%)	29(52.7%)		
Medical care	47(42.7%)	21(38.2%)	26(47.3%)		
Sepsis				0.69[3]	0.405
Yes	15(13.6%)	9(16.4%)	6(10.9%)		
No	95(86.4%)	46(83.6%)	49(89.1%)		
Hemoglobin (g/L)	77.2 ± 26.2	78.5 ± 27.5	75.8 ± 25.0	0.14[2]	0.891
C-reactive protein (g/L)	113.0 ± 93.9	116.8 ± 91.4	109.1 ± 97.0	0.65[2]	0.515
Procalcitonin (g/L)	17.9 ± 29.6	19.7 ± 30.9	16.0 ± 28.5	0.67[2]	0.505
Prothrombin time (s)	14.5 ± 4.3	14.7 ± 5.0	14.2 ± 3.6	0.66[2]	0.511
APTT (s)	36.5 ± 16.9	37.0 ± 13.2	36.0 ± 20.1	1.81[2]	0.071
Fibrinogen (g/L)	3.9 ± 2.1	4.0 ± 2.1	3.8 ± 2.1	0.61[2]	0.544
AT3 (%)	65.8 ± 20.7	66.3 ± 20.9	65.3 ± 20.7	0.14[2]	0.888
Total bilirubin (μmol/L)	41.2 ± 54.6	39.7 ± 48.3	42.6 ± 60.6	0.04[2]	0.971
ALT (U/L)	220.4 ± 763.9	177.2 ± 491.3	263.6 ± 965.8	0.93[2]	0.351
Creatinine (μmol/L)	159.6 ± 162.5	171.0 ± 173.1	148.1 ± 152.0	0.13[2]	0.893
RBC transfusion				1.23[3]	0.268
Yes	83(75.5%)	39(70.9%)	44(80.0%)		
No	27(24.5%)	16(29.1%)	11(20.0%)		
Platelet transfusion				0.05[3]	0.829
Yes	29(26.4%)	14(25.5%)	15(27.3%)		
No	81(73.6%)	41(74.5%)	40(72.7%)		
Cryo transfusion				0.79[3]	0.376
Yes	13(11.8%)	5(9.1%)	8(14.5%)		
No	97(88.2%)	50(90.9%)	47(85.5%)		

ICU: Intensive care unit; APTT: activated partial thromboplastin time; ALT: Alanine aminotransferase; AT3: antithrombin III; Cryo: Cryoprecipitate.
Note:[1] = student t-test; [2] = Wilcoxon test; [3]= Chi-square (χ^2) test.

test was used to compare survival between transfusion groups. A two-sided p-value <0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 282 critically ill patients met the inclusion criteria, of which 192 survived, accounting for 68.09% of the total number. Baseline characteristics, including gender, age, duration of ICU stay, APACHE II score, and patient category, were similar between the two groups. However, patients in the plasma transfusion group exhibited worse coagulation function (prolonged PT and APTT; lower fibrinogen and AT3 levels), poorer liver function (elevated total bilirubin), and received more platelets and cryoprecipitate. They also had milder anemia (higher Hb levels), received fewer red blood cell (RBC) transfusions, and had a higher in-hospital mortality rate. PSM (1:1) yielded a final cohort of 110 patients for analysis. Comparison of the matched groups demonstrated no statistically significant differences in any baseline characteristics (all p-value >0.05) (Table 1).

Risk factors for mortality

The multivariable Cox regression results are presented in Table 2. Significant independent predictors of mortality were age (HR = 1.04 per year increase; p-value = 0.029), plasma transfusion (HR = 3.34; p-value = 0.017), and RBC transfusion (HR = 10.74; p-value = 0.034). Notably, a longer ICU stay was associated with a lower mortality risk (HR = 0.94 per additional day; p-value = 0.043), a result potentially confounded by survivor bias. Furthermore, surgical patient status was a strong protective factor compared to medical patient status (HR = 0.13; p-value = 0.002).

Table 2 – Predictors of mortality by Cox regression analysis.

Variable	Hazard Ratio	95% CI	p-value
Sex	1.03	0.68–1.56	0.948
Age (years)	1.04	1.03–1.06	0.029*
Patient category	0.13	0.07–0.23	0.002*
Length of ICU stay (days/)	0.94	0.92–0.97	0.043*
APACHE Ⅱ score	1.06	1.03–1.09	0.096
Sepsis	2.52	1.32–4.79	0.231
Hemoglobin (g/L)	1.00	0.98–1.01	0.883
C-reactive protein (g/L)	1.00	1.00–1.00	0.797
Procalcitonin (g/L)	1.01	1.00–1.02	0.408
Prothrombin time (s)	1.10	1.04–1.16	0.130
APTT (s)	1.01	1.00–1.02	0.364
Fibrinogen (g/L)	0.99	0.85–1.15	0.944
AT3 (%)	1.02	1.01–1.03	0.166
Total bilirubin (μmol/L)	1.00	1.00–1.01	0.480
ALT (U/L)	1.00	1.00–1.00	0.464
Creatinine (μmol/L)	1.00	1.00–1.00	0.323
Plasma transfusion	3.34	2.19–5.09	0.017*
RBC transfusion	10.74	4.21–27.39	0.034*
Platelet transfusion	2.16	1.29–3.63	0.214
Cryo transfusion	0.47	0.21–1.05	0.433

95%: CI: 95% Confidence interval; ICU: Intensive care unit; APTT: activated partial thromboplastin time; ALT: Alanine aminotransferase; AT3: antithrombin III; Cryo: Cryoprecipitate.
Cox proportional hazards regression was performed on the propensity score-matched cohort, adjusting for all available covariates.

Table 3 – Association of plasma transfusion with in-hospital mortality.

Model	Hazard Ratio	95% Confidence interval	p-value
Model 1	4.199	2.53–6.97	<0.001*
PSM	3.271	1.30–8.21	0.012*

95%: CI: 95% Confidence interval; PSM: Propensity score-matched. Model 1 is the univariate Cox regression. The PSM Model is derived from a propensity score-matched analysis, incorporating adjustments for the following covariates: age, patient category, red blood cell transfusion status, and intensive care unit length of stay.

Association between plasma transfusion and mortality

As shown in Table 3, plasma transfusion was significantly associated with increased in-hospital mortality. In the unadjusted model (Model 1), the HR was 4.199 (95% CI: 2.53–6.97; p-value <0.001). After adjusting for key covariates (age, patient category, RBC transfusion, and ICU length of stay) via PSM, the association remained significant, albeit attenuated, with an HR of 3.271 (95% CI: 1.30–8.21; p-value = 0.012).

Subgroup analysis

Subgroup analyses were performed using the PSM cohort to assess the association between plasma transfusion and mortality risk across strata of age, patient category, RBC transfusion status, and ICU length of stay (Figure 1). After covariate adjustment, a statistically significant association was observed only for the subgroup of patients who had received RBC transfusions (HR = 2.92; 95% CI: 1.22–7.00; p-value = 0.016). For the subgroups stratified by age (<60 or ≥60 years), patient category (surgical or medical), and ICU stay (≤14 or >14 days) the association between plasma transfusion and mortality risk was not statistically significant (all p-value >0.05).

Dose-response relationship

Following PSM adjustment, a dose-response relationship was observed between plasma transfusion volume and in-hospital mortality (Table 4). Mortality risk increased progressively with higher volumes, reaching statistical significance only at the highest dose. Compared to no transfusion, the risk was elevated but not significant with ≤400 mL (HR = 2.27; 95% CI: 0.86–6.01; p-value = 0.100) and 401–800 mL (HR = 2.52; 95% CI: 0.78–8.10; p-value = 0.121). A significantly higher risk was observed with volumes >800 mL, representing a 4.09-fold increase in mortality (HR = 4.09; 95% CI: 1.19–14.04; p-value = 0.025).

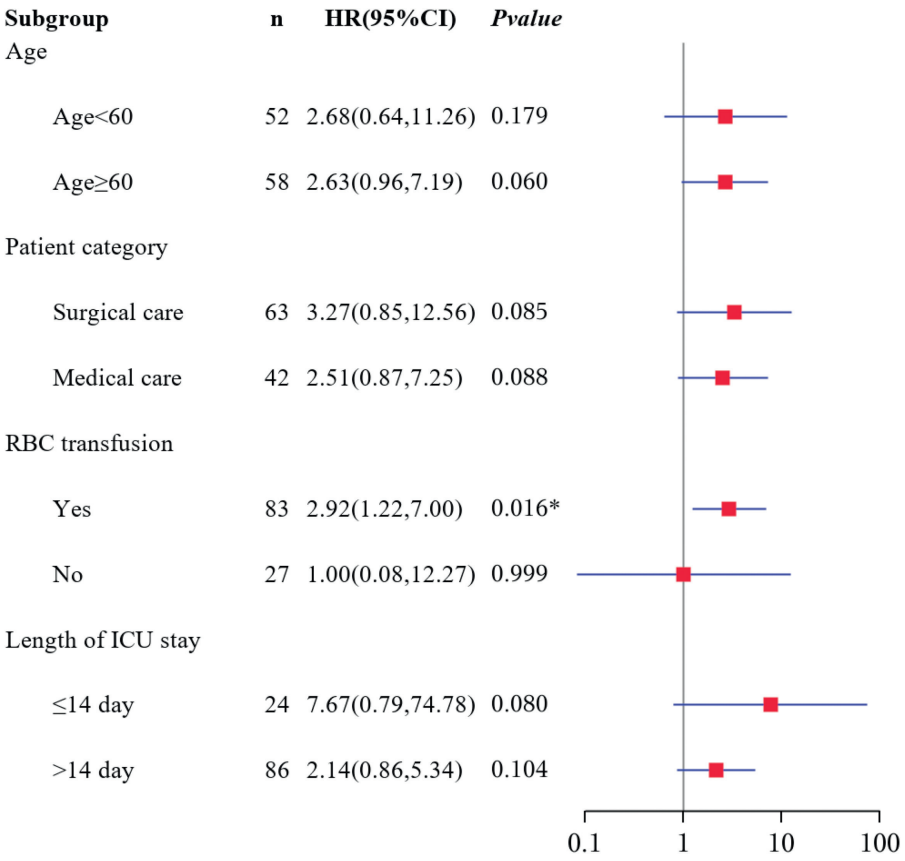

Fig. 1 – Subgroup analysis of the association between plasma transfusion and in-hospital mortality. The model was fit on the propensity score-matched (PSM) cohort, with adjustment for age, patient category, red blood cell (RBC) transfusion status, and intensive care unit (ICU) length of stay.

Survival analysis

Survival analysis demonstrated that patients receiving plasma transfusion had significantly lower survival probabilities than non-transfused patients. In the unadjusted analysis, the transfusion group exhibited a 4.17-fold higher mortality risk (Figure 2a). In the PSM cohort, plasma transfusion remained significantly associated with increased mortality, with an adjusted hazard ratio of 2.60 (Figure 2b). The differences in survival curves were statistically significant in both analyses (log-rank p-value <0.05).

Table 4 – Association between plasma transfusion volume and in-hospital mortality.

Variable	Hazard Ratio	95% CI	p-value
<400	2.27	0.86–6.01	0.100
401–800	2.52	0.78–8.10	0.121
≥801	4.09	1.19–14.04	0.025*

95%: CI: 95% Confidence interval.
The model was fit on the propensity score-matched cohort, adjusted for age, patient category, red blood cell transfusion status, and intensive care unit length of stay. Patients who received no plasma transfusion (0 mL) served as the reference group.

Discussion

This retrospective study of 282 critically ill ICU patients aimed to investigate the impact of plasma transfusion and its dosage on mortality, clarifying the risks and benefits associated with plasma use in critical care. After adjusting for baseline confounders employing PSM, the mortality risk in the plasma transfusion group remained significantly elevated, with a hazard ratio of 3.271 (95% CI: 1.30–8.21; p-value = 0.012). Moreover, a dose-dependent relationship was evident, as the risk increased to 4.09-fold (95% CI: 1.19–14.04; p-value = 0.025) when the transfusion volume exceeded 800 mL.

Patients receiving plasma transfusions presented with poorer baseline clinical characteristics, including more severe coagulopathies, a higher incidence of sepsis, and worse liver function. This suggests that transfusion decisions largely followed clinical indications, targeting sicker patients. However, after balancing 19 baseline variables using PSM (Table 1), plasma transfusion remained independently associated with increased mortality. This indicates that, beyond reflecting illness severity, plasma transfusion itself may confer additional risk.

The increased mortality risk associated with plasma transfusion may be linked to several factors. First, immune-

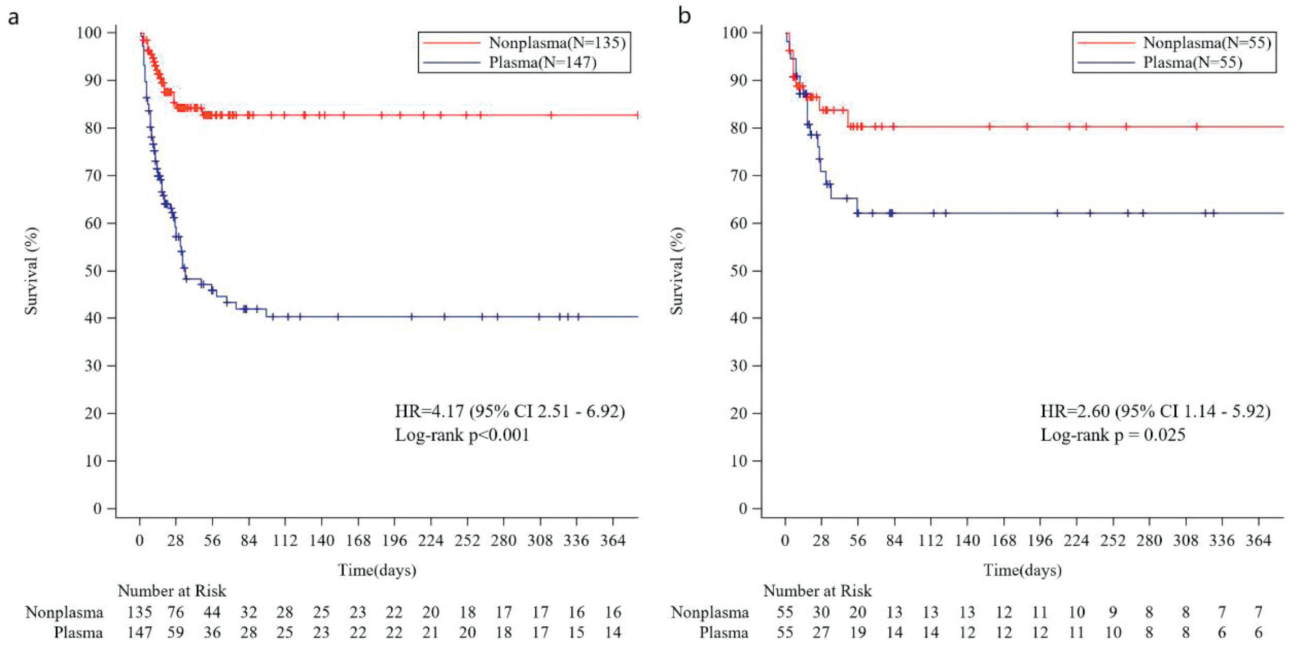

Fig. 2 – Kaplan-Meier survival estimates. (a) Estimates derived from the original (unmatched) cohort. (b) Estimates derived from the propensity score-matched (PSM) cohort.

inflammatory storms [20,21], where plasma contains various bioactive substances (complement, cytokines) that can activate systemic inflammatory responses, leading to febrile non-hemolytic transfusion reactions, allergic reactions, transfusion-related acute lung injury (TRALI) [22], and even hemolysis [23], potentially causing direct patient mortality or complicating their condition. TRALI and transfusion-associated circulatory overload (TACO) are leading causes of transfusion-related deaths, identified as independent risk factors for ICU mortality [24,25]. For instance, the UK SHOT report attributed 52.54% of transfusion-related deaths to TACO among a total of 59 cases [26]. Second, although volume overload is effective for volume replacement and coagulation correction [27], critically ill patients often have impaired physiological functions and limited compensatory capacities, making them less tolerant to large-volume plasma transfusions, thus increasing the risk of acute left heart failure, pulmonary edema [28], citrate toxicity-induced hypocalcemia and myocardial depression. Third, plasma administration can exacerbate coagulation imbalance [29]. Because plasma is rich in procoagulant factors such as thrombin and fibrinogen, it may alter blood flow dynamics and disrupt the homeostatic balance, thereby increasing the risk of thrombosis. This can trigger secondary inflammation, tissue edema, or necrosis, ultimately worsening clinical outcomes [30]. A study on FFP transfusion efficacy in ICU patients with decompensated cirrhosis and coagulopathy found no benefit in terms of mortality but increased hospital stays and multi-organ failure risk [31].

Subgroup analysis provided further insights, revealing a statistically significant increase in mortality specifically among patients who received RBC transfusions (HR = 2.92; 95% CI: 1.22–7.00; p-value = 0.016). In contrast, this association was not significant in subgroups stratified by age, patient category, or ICU length of stay. This suggests that patients requiring concomitant RBC and plasma transfusion represent a particularly vulnerable population. In these critically ill patients that are often facing anemia, active bleeding, or hemodynamic instability, plasma may compound risks through volume overload, inflammatory activation, or coagulopathy. Thus, in multi-component transfusion settings, the risks of plasma transfusion appear amplified, necessitating cautious reassessment even when coagulation parameters seem to justify its use. Clinical practice should adhere strictly to evidence-based guidelines, prioritize treating underlying conditions, and exercise heightened vigilance when considering plasma alongside other blood products.

This study has several limitations. First, as a retrospective observational analysis, it may be subject to residual confounding despite adjustment for known factors via PSM. Second, the exact time interval between plasma transfusion and death was not consistently recorded in the medical records, limiting our ability to establish a clear temporal relationship or infer causality. To partially address this, we excluded patients with an ICU stay of <24 h, thereby reducing the likelihood of including deaths unrelated to transfusion or driven solely by the underlying disease. Nevertheless, future studies should aim to collect time-to-event data more systematically to enable time-varying exposure analyses. Third, we were unable to directly quantify specific adverse reactions such as TRALI or TACO, limiting our ability to confirm the proposed mechanisms. Fourth, the sample size was relatively modest, particularly in subgroup and dose-response analyses, which may have reduced statistical power and limited the generalizability of our findings. Therefore, these results should be

considered exploratory and warrant validation in larger, multicenter prospective studies.

Conclusions

This study demonstrates that plasma transfusion is independently associated with significantly increased in-hospital mortality in critically ill patients, with a strong dose-dependent effect. The association remained significant after adjusting for confounding factors in PSM. Plasma use, particularly in the absence of active bleeding or clear indications, may cause more harm than benefit, likely due to complications like TRALI, TACO, and thrombosis.

Current clinical practices frequently diverge from established guidelines, potentially exposing patients to avoidable risks. Consequently, a more restrictive and evidence-based strategy for plasma transfusion is strongly warranted. Clinicians are encouraged to avoid prophylactic use, particularly in hemodynamically stable patients, and should instead focus on addressing the underlying causes of coagulopathies. These findings underscore the need for stricter adherence to transfusion guidelines and highlight the importance of future prospective studies to enhance clinical decision-making in critical care settings.

Plasma transfusion should be regarded as a high-intervention therapy that carries substantial risks and requires meticulous patient-specific evaluation before administration. Future research efforts should aim to refine existing guidelines through well-designed prospective studies and large-scale randomized controlled trials, with the goal of establishing precise, individualized dosing strategies. Ultimately, the objective is to improve patient outcomes by maximizing the therapeutic benefits of plasma transfusion while minimizing unnecessary exposure to risk.

Funding

This study was supported by the Hunan Provincial Health Commission General Project (No. 202204133544).

Author contributions

All listed authors have contributed to the manuscript substantially and have agreed to the final submitted version. MZ contributed to the concept of the article, wrote the draft, and modified it. CSL and FY supported in data treatment and analysis and reviewed the article. ZH, SF and FFC helped in data collection and modified the article. XSH reviewed and modified the article. HJG did the data analysis, modified thoroughly the whole article, including the tables and the figures. PL helped in funding and reviewed the article.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgement

The authors would like to thank Ping Weng for his valuable help in statistical analysis guide.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Survival of trauma patients requiring massive transfusion protocol activation: A retrospective cohort study at a tertiary hospital in Malaysia

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ARTICLE INFO

Article history:
Received 22 May 2025
Accepted 16 April 2026
Available online 27 June 2026

Keywords:
Trauma injury
Blood transfusion
Massive transfusion protocol

ABSTRACT

Background: Trauma is one of the main causes of morbidity and mortality worldwide. Understanding the factors affecting the survivability of trauma patients is crucial, especially those requiring the activation of the massive transfusion protocol. This study aimed to determine survival outcomes and identify factors associated with mortality among trauma patients requiring massive transfusion activation.

Materials and methods: This was a retrospective cohort study of 71 trauma patients who required activation of the massive transfusion protocol. The trauma patients were divided into Survivor (n = 41) and Non-survivor groups (n = 30). The activation criteria for massive transfusions were an assessment of blood consumption score ≥2.

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https://doi.org/10.1016/j.htct.2026.106489

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Glasgow coma scale
Haemoglobin

Results: The hospital mortality rate for trauma patients who required activation of the massive transfusion protocol was 42.3%. Most of the patients were male (90.1%), of Malay ethnicity (49.3%), and had blood group O (40.8%). The commonest cause of trauma was road traffic accidents (84.5%). Patients with severe Glasgow Coma Scores had approximately 4.2 times higher odds of mortality compared with those with non-severe Glasgow Coma Scores (p-value = 0.014). Haemoglobin level was independently associated with outcome, with lower haemoglobin levels observed among non-survivors (adjusted odds ratio: 1.38; 95% CI: 1.03–1.85; p-value = 0.030).

Conclusions: The survivability of trauma patients who need massive transfusion protocol activation was associated with Glasgow Coma Score and haemoglobin level on arrival. It is crucial to determine factors that affect the survivability of trauma patients for the early identification of those at risk and to initiate prompt management and resuscitation in the future.

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Introduction

Trauma is one of the leading causes of morbidity and mortality worldwide. In 2021, injuries in Southeast Asia affected an estimated 35.5 million people and caused approximately 317,000 deaths, highlighting a substantial regional mortality burden [1]. Haemorrhage is the most common preventable cause of death for trauma patients [2,3]. The risk of hospital mortality is increased by uncontrolled haemorrhage, which leads to a lethal triad vicious cycle of coagulopathy, hypothermia, and acidosis [4]. Effective coordination between the trauma team is pivotal to ensure the timely management of massively haemorrhage trauma patients.

Implementing the massive transfusion protocol (MTP) is crucial in replacing blood loss and maintaining haemodynamics until definitive care is provided. It is a proactive, standardised protocol designed to facilitate communication between different services and avoid delays in management, including blood product transfusion, for patients requiring a massive transfusion [5]. Early delivery of blood products through predefined MTP in resuscitation was associated with decreased length of hospital stay and mortality rate [6,7]. Trauma patients presenting with low systolic blood pressure, high heart rate, low haemoglobin (Hb), elevated international normalised ratio (INR), decreased base excess, and high lactate have a significantly higher risk of requiring massive transfusion [8].

Mortality risk factors of trauma patients found in previous studies were age, comorbidity, mechanism of injury, Glasgow Coma Scale (GCS), pulse rate, respiratory rate, blood pressure, cardiopulmonary resuscitation performance, blood transfusion, and occurrence of complications [9,10]. Currently, there is limited local and regional data evaluating survival outcomes and mortality-associated factors among trauma patients requiring MTP activation in Southeast Asia. Our hospital functions as a referral trauma centre; however, outcome performance and determinants of mortality have never been evaluated. Therefore, this study was conducted to determine survival outcomes and identify factors associated with mortality among trauma patients requiring MTP activation. This study provides context-specific evidence and actionable predictors to enhance protocol implementation in middle-income country trauma systems.

Materials and methods

Research setting and ethics

This was a retrospective cohort study that included all trauma patients who required activation of the MTP (n = 71) at Hospital Sultan Ismail Johor Bahru (HSIJB) from 1st June 2022 to 31st May 2024. The activation criteria for MTP were an Assessment of Blood Consumption (ABC) score ≥2. As the protocol was newly implemented, a simple trigger was selected to ensure timely activation and workflow compliance that will be evaluated as local outcome data accumulate. HSIJB is a specialist tertiary hospital with 704 beds located in Johor Bahru, Johor, Malaysia. It is a leading East Johor hospital cluster and receives referrals from the district hospitals. Ethical approval for this study was obtained from the Medical Research and Ethics Committee, Ministry of Health Malaysia (research number NMRR ID-24-02690-RL4).

Massive transfusion protocol for trauma patients

An institutional MTP for trauma patients was implemented on 1st June 2022. All providers and staff were systematically educated about the protocol before implementation. It is a standardised protocol designed to facilitate communication between trauma team members. Trauma team members were representatives from the Emergency and Trauma Department (ETD), Surgical Department, Anaesthesiology Department, Orthopaedic Department, Transfusion Medicine Unit (TMU), and Radiology Department.

The emergency physician, surgeon, and anaesthesiologist were the authorised personnel to activate the MTP. If the patient was at the ETD, the Surgical Department or ETD medical officer is nominated as the MTP coordinator. The Anaesthesiology Department medical officer was nominated as the MTP coordinator if the patient was in the operation theatre or the Intensive Care Unit (ICU). The coordinator was responsible for notifying the activation or termination of MTP, calling

the TMU for box preparation, ensuring cold chain maintenance of blood products, and ensuring compliance with the transfusion procedure, including complete documentation. Upon activation, the coordinator conveys only essential information: the patient's name, diagnosis, location, ABC score, MTP activator, and the coordinator's name and contact number.

To initiate the protocol, the first MTP box was requested by sending a completed transfusion request form and 6 mL of the patient's whole blood to the TMU. The sample was collected in a 5.4 mg K2EDTA BD Vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). For the subsequent MTP box, a second blood transfusion request form and an additional 3 mL whole blood sample were required.

The standard MTP Box 1 contained four units of group-crossmatched (GXM) packed red blood cells (PRBCs), four units of fresh frozen plasma (FFP), and four units of platelet concentrate. To maintain a 1:1 PRBC-to-FFP ratio, the number of GXM PRBC units was reduced by the number of "Safe O" units (Group O Rh(D) positive, direct Coombs test negative) already supplied during the emergency phase. The standard MTP box 2 contained four fully crossmatched PRBCs and four units of FFP. In addition, six units of cryoprecipitate were added to box 2 upon request by the attending clinician based on clinical assessment and fibrinogen level (if available). Subsequent MTP boxes were alternated between box 1 and box 2 if MTP needed to be continued (Figure 1). For transportation, an insulated blood box with an ice pack was used for PRBC or FFP, and without ice for platelets or cryoprecipitate. Screening for transfusion-transmitted infections in all blood and blood components was negative. The laboratory turnaround time for each box was 30 minutes.

Whenever possible, patients or their authorised next-of-kin signed written informed consent forms for blood transfusions. However, two fully registered clinicians decided on urgent blood transfusions if no family member was available. The patients were transfused according to ABO-specific blood components. A haematology analyser XN-450, Sysmex Corporation, Japan, was used for point-of-care testing of the complete blood count (CBC). A viscoelastic haemostatic assay test was not available at the hospital.

If the patient was in the ETD, the attending emergency physician or surgeon was responsible for MTP deactivation and the attending anaesthesiologist or surgeon was responsible for MTP deactivation if the patient was in the operating theatre or ICU. The MTP deactivation criteria included both anatomic (control of bleeding) and physiological (haemodynamically stable) statuses.

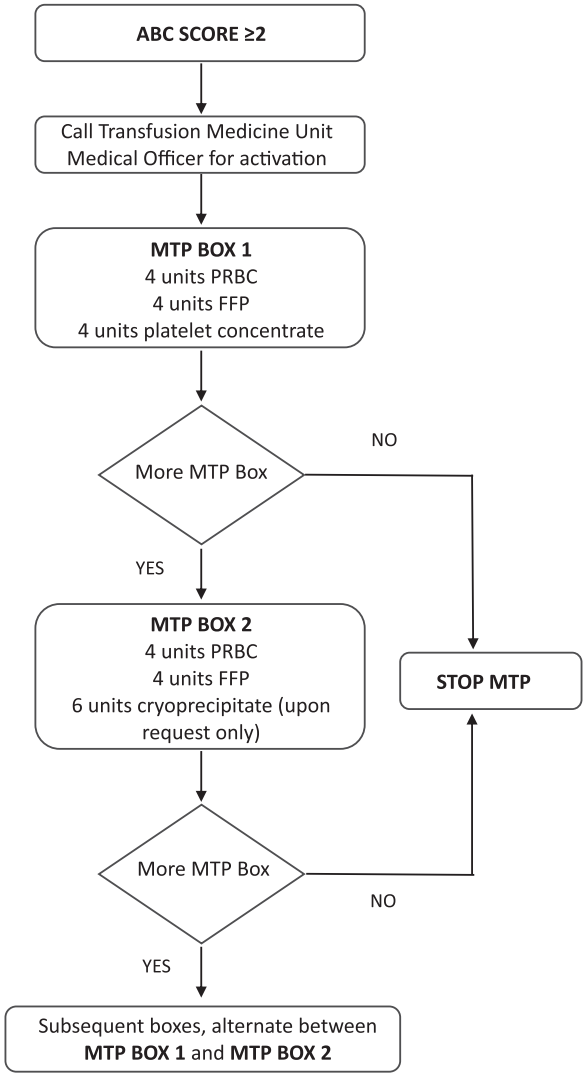

Fig. 1 – Massive transfusion protocol for trauma.

Variables and research tools

Patient data were collected regarding demographics (age, gender, ethnicity, blood group, referral status, comorbidities, and mechanism of trauma) and clinical characteristics (Glasgow Coma Scale [GCS] score, pulse rate, systolic and diastolic blood pressure, and total MTP boxes delivered). Additionally, laboratory parameters (Hb, white blood cell count, and platelet count) and clinical outcomes (adverse transfusion reactions, length of ICU and hospital stay, and mortality) were recorded. All the data was retrieved retrospectively using the Total Hospital Information System (THIS), an institutional electronic medical record platform used for comprehensive clinical documentation, including patient management records and blood bank transfusion data. Survivability was defined as the ability to remain alive and be discharged from the hospital after being admitted due to a traumatic injury.

Statistical analysis

All the data were tabulated and analysed using the Statistical Package for the Social Science (SPSS) Statistics Version 29 (IBM, New York, USA). Numerical variables were expressed as median and interquartile range (IQR) and categorical variables were expressed as count and percentage. Simple logistic regression was performed to examine associations between clinical and laboratory variables and survival, followed by multiple logistic regression to identify factors independently associated with survival. Results are presented as adjusted odds ratios (OR) with 95% confidence intervals (95% CIs). The level of significance was set at a p-value <0.05.

Result

Demographics of the patients

The hospital mortality rate for trauma patients who required activation of the MTP was 42.3%. The cohort was predominantly male, reflecting the typical distribution of severe trauma in our setting. Road traffic accidents accounted for the majority of cases, indicating that high-energy blunt trauma was the principal mechanism leading to MTP activation (Table 1). Other mechanisms, including falls, industrial injuries, and assault, were relatively uncommon. The ABO blood group was unknown for two patients due to early mortality after MTP was activated.

Clinical characteristics and laboratory parameters of the patients

In most cases, the TMU delivered one (45.1% of patients) or two (36.6% of patients) MTP boxes. The highest number of MTP boxes delivered to patients was five boxes indicated for three patients (4.2%). Lower initial GCS scores and haemoglobin (Hb) levels were observed in the non-survivor group, indicating that these patients presented with greater physiological compromise than survivors (Table 2).

Outcome of the patients

The median length of ICU stay was five days, while the median total hospital stay was 12 days (Table 3) reflecting the substantial resource utilisation of trauma patients requiring MTP activation. Transfusion-related adverse events were uncommon with only two cases of mild allergic reactions and no severe transfusion reactions being reported. Both cases were limited to urticaria and resolved with standard treatment.

Independent factors associated with non-survival

Patients with severe GCS had approximately 4.2 times higher odds of mortality compared with those with non-severe GCS (p-value = 0.014). Additionally, Hb level was independently associated with outcome, with lower Hb levels observed among non-survivors (adjusted OR: 1.38; 95% CI: 1.03–1.85; p-value = 0.030) (Table 4).

Table 1 – Demographics of the patients.

Variable	All patients (n = 71)	Non-survivor (n = 30)	Survivor (n = 41)
Age (years) - median (IQR)	33.00 (29)	34.00 (31)	32.00 (28)
Gender – n (%)			
Male	64 (90.1)	28 (93.3)	36 (87.8)
Female	7 (9.9)	2 (6.7)	5 (12.2)
Ethnicity – n (%)			
Malay	35 (49.3)	15 (50.0)	20 (48.8)
Chinese	16 (22.5)	7 (23.3)	9 (22.0)
Indian	7 (9.9)	3 (10.0)	4 (9.8)
Other ethnicities	13 (18.3)	5 (16.7)	8 (19.5)
Blood group – n (%)			
O	29 (40.8)	14 (46.7)	15 (36.6)
A	15 (21.1)	7 (23.3)	8 (19.5)
B	23 (32.4)	6 (20.0)	17 (41.5)
AB	2 (2.8)	1 (3.3)	1 (2.4)
Unknown	2 (2.8)	2 (6.7)	0 (0.0)
Referral case – n (%)			
No	60 (84.5)	27 (90.0)	33 (80.5)
Yes	11 (15.5)	3 (10.0)	8 (19.5)
Comorbidity – n (%)			
No	56 (78.9)	25 (83.3)	31 (75.6)
Yes	15 (21.1)	5 (16.7)	10 (24.4)
Type of comorbidity – n (%)			
Diabetes mellitus	6 (8.5)	2 (6.7)	4 (9.8)
Hypertension	6 (8.5)	2 (6.7)	4 (9.8)
Dyslipidaemia	2 (2.8)	0 (0.0)	2 (4.9)
Cardiovascular disease	4 (5.6)	3 (10.0)	1 (2.4)
Cerebrovascular disease	1 (1.4)	0 (0.0)	1 (2.4)
Respiratory disease	3 (4.2)	2 (6.7)	1 (2.4)
Renal disease	2 (2.8)	2 (6.7)	0 (0.0)
Cause of trauma – n (%)			
Road traffic accident	60 (84.5)	26 (86.7)	34 (82.9)
Industrial injury	2 (2.8)	1 (3.3)	1 (2.4)
Fall	8 (11.3)	3 (10.0)	5 (12.2)
Assault	1 (1.4)	0 (0.0)	1 (2.4)

IQR: Interquartile range

Table 2 – Clinical characteristics and laboratory parameters of the patients.

Variable	All patients (n = 71)	Non-survivor (n = 30)	Survivor (n = 41)
GCS on arrival – n (%)			
Severe (score ≤ 8)	24 (33.8)	16 (53.3)	8 (19.5)
Non-severe (score > 8)	47 (66.2)	14 (46.7)	33 (80.5)
Pulse rate on arrival (beats/minute) – median (IQR)	120.00 (33)	120.00 (24)	122.00 (38)
Systolic blood pressure on arrival (mmHg) – median (IQR)	92.00 (44)	90.00 (53)	93.00 (41)
Diastolic blood pressure on arrival (mmHg) – median (IQR)	61.50 (31)	62.00 (39)	61.00 (29)
Haemoglobin on arrival (g/dl) – median (IQR)	13.00 (2.4)	12.40 (4.5)	13.10 (1.4)
White cell count on arrival ($x10^3/\mu L$) – median (IQR)	19.00 (10.3)	18.90 (8.7)	19.10 (11.6)
Platelet count on arrival ($x10^3/\mu L$) – median (IQR)	244.00 (73)	234.00 (61)	251.00 (81)

GCS: Glasgow Coma Scale; IQR: Interquartile range

Discussion

The mortality rate of trauma patients who needed MTP activation ranged from 16.9% to 67.0% [11–15]. Based on the results of this study, the majority of trauma patients who needed MTP activation were male with road traffic accidents being the most common mechanism of injury. These findings were consistent with the results of other studies [13,16,17]. Males were more frequently involved in road traffic accidents, which may be attributed to a higher prevalence of high-risk behaviors, such as speeding, driving under the influence (DUI), and aggressive driving [18]. The National Trauma Database of eight public hospitals in Malaysia showed males were 6.5 times more likely to be involved in major trauma than females. The majority of cases resulted from blunt trauma (96.3%) and were unintentional in nature (91.2%), with road traffic accidents (76.8%) being the most frequent cause of injury [19].

Delays in MTP activation and delivery of blood components were associated with prolonged duration to achieve haemostasis and reduced chances of survival [20]. Massive blood transfusion has been defined in various articles as the replacement of an entire blood volume within 24 hours, transfusion of more than ten units of PRBC in 24 hours, transfusion of more than four units of PRBC within one hour with expectation for more transfusion support, or replacement of 50% of total blood volume within three hours [21–23]. The temporal-based definition is not useful during the acute management of severe haemorrhage trauma patients. Therefore, a simpler and reliable predictive score is needed that helps clinicians activate MTP faster. The ABC score had a sensitivity between 60% and 89% and a specificity between 84% and 86% [24–26]. The advantage of the ABC scoring system lies in its use of non-laboratory variables, those derived from history, physical examination, and Focused Assessment with Sonography in Trauma (FAST) scans. These parameters are easily applied in ETD settings and facilitate early resource mobilisation.

The observed association between low GCS scores and mortality is clinically plausible, as a markedly reduced neurological status may reflect either primary traumatic brain injury or cerebral hypoperfusion secondary to haemorrhagic shock. In patients requiring MTP activation, the GCS likely represents a surrogate marker of overall injury burden and physiological compromise at presentation. The trauma patients who required massive transfusions had a lower GCS score, higher pulse rate, lower systolic blood pressure, lower Hb level, higher Injury Severity Score (ISS), higher INR, and longer hospital stay [27]. The GCS is widely used to assess the level of consciousness of trauma patients at the site of injury and in the hospital. Previous studies found that the GCS score was a predictive factor for mortality after trauma [28,29]. A retrospective study involving 513 trauma patients found that a unit increment of the GCS score resulted in a 40% decrease in the risk of death (OR: 0.63; 95% CI: 0.59–0.67; p-value <0.05) [16].

Table 3 – Outcome of the patients.

Variable	All patients (n = 71)	Non-survivor (n = 30)	Survivor (n = 41)
Adverse transfusion reaction – n (%)			
No	69 (97.2)	30 (100.0)	39 (95.1)
Yes	2 (2.8)	0 (0.0)	2 (4.9)
Length of ICU stay (days) – median (IQR)	5.00 (11)	1.00 (4)	8.00 (7)
Length of hospital stay (days) – median (IQR)	12.00 (22)	2.00 (7)	17.00 (21)
Time of death – n (%)			
≤ 6 hours	NA	7 (23.3)	NA
6 < x ≤ 24 hours		7 (23.3)	
> 24 hours		16 (53.3)	

ICU: Intensive Care Unit; IQR: Interquartile range; NA: Not Applicable

Table 4 – Variable logistic regression modelling

Variable	Simple logistic regression		Multiple logistic regression	
	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
GCS on arrival				
Severe (score ≤8)	0.21 (0.07-0.61)	0.004	0.24 (0.08-0.75)	0.014
Non-severe (score >8)	1	NA	1	NA
Pulse rate on arrival	1.00 (0.98-1.02)	0.878	NA	NA
Systolic blood pressure on arrival	1.00 (0.98-1.02)	0.914	NA	NA
Diastolic blood pressure on arrival	1.00 (0.98-1.02)	0.893	NA	NA
Haemoglobin on arrival	1.37 (1.05-1.79)	0.021	1.38 (1.03-1.85)	0.030
White blood cell count on arrival	0.99 (0.94-1.05)	0.815	NA	NA
Platelet level on arrival	1.00 (1.00-1.01)	0.372	NA	NA

GCS: Glasgow Coma Scale; OR: Odds ratio; 95% CI: 95% Confidence interval; NA: Not applicable
P-value <0.05 is considered significant

Identifying trauma patients with severe bleeding on hospital arrival is crucial to initiate appropriate intervention and life-saving procedures such as massive transfusion and damage control resuscitation. In the initial part of acute bleeding, the Hb and haematocrit levels may appear normal because red blood cells and plasma are lost concomitantly [30]. Lower Hb level in trauma patients often reflects the severity of haemorrhage and ongoing blood loss, which can lead to reduced oxygen delivery to vital organs and subsequent organ dysfunction. The decreases in the Hb level on hospital arrival are associated with trauma severity and the requirement for haemostasis intervention [31]. Bruns et al. reported that a Hb ≤10 g/dL was associated with significant haemorrhage in 87% of trauma patients and a three-fold increase in the need for interventions to stop the haemorrhage (OR: 3.14; 95% CI: 1.18-8.35; p-value <0.05) [32]. In addition, severe haemorrhage may lead to trauma-induced coagulopathy and tissue hypoperfusion, which further worsens patient outcomes. In this setting, early activation of MTP is essential to facilitate rapid and balanced blood component replacement, thereby restoring circulating volume, improving oxygen delivery, and correcting coagulopathy. Calcium and fibrinogen levels are currently not routinely monitored within our institution's massive transfusion protocol, highlighting an opportunity to enhance practice following evolving clinical guidelines.

The length of hospital stay in this study (median: 12 days) was consistent with the average length of hospital stays for major trauma patients in other hospitals in Malaysia (ranging from 3-18 days) [19]. Among trauma patients, factors affecting the length of hospital stay are age, gender, mechanism of injury, infection, type of injury, and Injury Severity Score (ISS) [33]. This study found that 46.6% of trauma patients who needed MTP activation succumbed within 24 hours of admission. A retrospective analysis of 1,470 major trauma patients found that 68% of deaths occurred within the first 48 hours and were mainly due to haemorrhagic shock and traumatic brain injury [34].

This study provides institution-specific data on outcomes among trauma patients requiring MTP activation. The implementation of MTP facilitates coordinated communication between the trauma team members. However, challenges in implementation remain, including the timeliness of activation and blood product delivery, as well as adherence to the protocol in emergency settings. Higher levels of protocol compliance have been associated with lower mortality, highlighting the importance of timely activation, appropriate laboratory monitoring, and protocol-based administration of blood products [35]. Although this study did not directly evaluate protocol adherence or transfusion timing, the findings provide preliminary institutional data that may support ongoing evaluation and optimisation of the MTP workflow. Measures to improve compliance may include periodic education of healthcare providers, discussion of MTP cases during trauma team meetings, regular MTP simulation drills, and placement of clear protocol flowcharts in key areas such as the trauma bay and transfusion medicine unit.

This study has several limitations, including its retrospective design, single-centre setting, and relatively small sample size. In addition, adjustment for potential confounders was limited due to incomplete data. Important variables such as injury severity scoring, transfusion volume, timing, and biochemical markers, including lactate and international normalised ratio (INR), were not consistently available in the dataset.

Conclusion

In conclusion, the survivability of trauma patients who need MTP activation was associated with the GCS score and Hb level on arrival at the hospital. It is crucial to determine factors that affect the survivability of trauma patients for early identification of those at risk and to initiate prompt management and resuscitation in the future.

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors would like to thank the Director-General of Health Malaysia for the permission to publish this paper. We acknowledge the Director of Hospital Sultan Ismail Johor Bahru for their support throughout the research.

Editor

Eduardo Rego

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Predictors of survival among children with Burkitt lymphoma treated at a single center in Tanzania: a retrospective cohort study

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ARTICLE INFO

Article history:
Received 17 August 2025
Accepted 25 April 2026
Available online 6 July 2026

Keywords:
Burkitt lymphoma
Mortality
Predictors
Children

ABSTRACT

Background: Despite improvements in the management of patients with Burkitt lymphoma, the survival rate in low- and middle-income countries remains poor. This study aimed to determine the predictors of 18-month overall survival among children with Burkitt lymphoma.
Methods: A retrospective cohort study was conducted at the Pediatric Oncology unit of Muhimbili National Hospital in Dar-es-salaam, Tanzania. The study included a cohort of 123 children who were diagnosed with Burkitt lymphoma from January 2012 to December 2017. Predictors of mortality were assessed using Cox regression analysis. A two-tailed p-value <0.05 was considered statistically significant.
Results: The median age of the patients was seven years and the majority 61.8% (76/123) were males. Abdominal presentation was the most common tumor site in 39% (48/123). The 18-month overall survival rate was 54.0%. Key predictors of mortality included human immunodeficiency virus (HIV) infection (adjusted Hazard ratio [aHR] = 5.12; 95% CI: 1.39–19.0; $p < 0.01$) and elevated lactate dehydrogenase levels (>500 U/L; aHR = 2.50; 95% CI: 1.14–14.71; $p = 0.03$).
Conclusion: Most patients presented with abdominal Burkitt lymphoma. Despite the tumor's high chemosensitivity, treatment response in this cohort was marginal, characterized by moderate overall survival and low event-free survival. HIV infection and elevated lactate dehydrogenase levels were significant predictors of mortality. Improving outcomes will require optimized early diagnosis, HIV screening, and rigorous patient follow-up.
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https://doi.org/10.1016/j.htct.2026.106496
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Introduction

Burkitt lymphoma (BL) is a highly aggressive non-Hodgkin lymphoma (NHL) which was first described in 1958 by Denis P Burkitt in Kampala, Uganda [1]. His findings followed observations of an increased frequency of children with masses involving the face or abdomen [1,2]. BL is characterized by clonal proliferation of mature B-lymphocytes due to a chromosomal translocation t(8,14) that results in dysregulation of the c-MYC oncogene [3–5]. The World Health Organization (WHO) has classified BL into three distinct clinical variants: endemic, sporadic, and immunodeficiency-associated, the latter of which is predominantly observed in human immunodeficiency virus (HIV)-positive patients [6]. BL is the most common childhood cancer in equatorial Africa, accounting for 30%–50% of all childhood malignancies in children <15 years of age [2] and 90% of all childhood lymphoma [7]. The incidence of endemic BL in Tanzania, like in many sub-Sahara African countries, is difficult to ascertain due to the lack of a centralized population-based registry on childhood cancer [8].

Despite its aggressive nature, BL is highly responsive to chemotherapy. Some patients achieve complete remission with single-agent regimens, and relapse is rare beyond one year after treatment [9]. While cure rates for BL in resource-rich settings exceed 90%, most cancer treatment centers in sub-Sahara African report survival rates ranging between 25% and 62% [10]. Studies on reduced-intensity BL chemotherapy protocols have been adapted to suit the available resources and the level of supportive care in resource-limited settings [11–13]. Other studies have shown consistent and reproducible findings, leading to universally accepted protocols for use in these environments [14,15]. Factors hindering the development of resource-appropriate protocols in these settings include a lack of well-designed multicenter clinical trials for endemic BL, diverse sociocultural challenges, and comorbidities, such as HIV and severe malnutrition, that negatively impact treatment outcomes [11]. In addition to clinical variables, several socioeconomic factors unique to resource-limited settings significantly influence BL outcomes. Chief among these are diagnostic delays resulting from limited healthcare access, and the financial burden of care associated with low socioeconomic status. These systemic barriers frequently culminate in high rates of treatment abandonment, further compromising survival [10,16].

This study aims to review the clinical profile, survival, and predictors of mortality of children with BL treated in the Pediatric Oncology Unit (POU) at Muhimbili National Hospital (MNH) from January 2012 to December 2017.

Methods

Study design and setting

The POU was established in April 2011 after relocating from the Ocean Road Cancer Institute (ORCI), the country's primary cancer center. MNH is a national hospital and a teaching facility for the Muhimbili University of Health and Allied Sciences (MUHAS) in Dar es Salaam. The pediatric unit consists of two wards and a hostel providing both outpatient and inpatient services. With a 96-bed capacity, the unit averages 400 pediatric cancer admissions annually. Critically ill patients receiving chemotherapy or radiotherapy are admitted to the Upendo ward, while stable patients are cared for in the Tumaini ward. The hostel provides accommodation for pediatric patients from upcountry until their treatment is complete.

Patients' characteristics

All children aged less than 18 years who were diagnosed histologically with BL over a period of six years from January 2012 to December 2017 were included in this study if they had complete clinical information as well as follow-up information. Moreover, all patients who were included in the analysis were screened for HIV status during normal clinic attendance. Patients who died or abandoned care before the initiation of treatment, patients with a diagnosis of BL based on clinical suspicion, and those with missing information on treatment were all excluded from the analysis.

Sample size and sampling procedure

The sample size was calculated using Kelsey method for cohort studies via OpenEpi [17] based on the following assumptions for comparing survival rates between early and advanced tumor stages: an 18-month survival rate of 51% for Stages I/II and 21% for Stages III/IV, based on previous research [10]. Additional parameters included a 95% confidence level ($\alpha = 0.05$), a two-sided significance test, 90% power ($\beta = 0.10$), a 1:1 ratio between groups, and a 10% adjustment for loss to follow-up. A minimum sample size of 123 was deemed adequate to detect a 30% difference in survival rate. Sampling of the study subjects was based on the convenience sampling method, in which all participants meeting the inclusion criteria were consecutively enrolled until the sample size was obtained.

Data collection

Data were extracted from patient files and electronic databases and recorded using a custom-designed data collection sheet. The extracted data included demographic (age and sex), clinical characteristics (tumor site, nutritional status, HIV status, chemotherapy treatment administered and response to treatment at discharge), and follow-up data (date of last visit and date of death).

Institutional chemotherapy regimens for Burkitt lymphoma

Since 2011, children diagnosed with BL at MNH have been treated with the International Network for Cancer Treatment and Research (INCTR-03–06) protocol. The first-line regimen consists of a combination of cyclophosphamide, vincristine, and methotrexate (COM), supplemented by intrathecal methotrexate and cytarabine for central nervous system (CNS) prophylaxis or treatment [18]. Additionally, with this protocol, patients with refractory disease or early relapse were treated using a combination of etoposide, mesna, ifosfamide and

cytarabine (EMIC) as a salvage regimen. Previous published data on the study using this BL treatment protocol reported overall survival (OS) rates of 67% and 62% at one and two years, respectively but there have been no follow-up review on the performance of this protocol of children treated using routine clinical patient care at MNH [19].

Tumor staging

Patients in this study were staged using the St. Jude/Murphy staging system. The staging process included a clinical examination, a complete blood count, and an evaluation of peripheral blood films. Additionally, bilateral bone marrow aspirates and biopsies, cerebrospinal fluid (CSF) analysis, and imaging by computed tomography (CT) or magnetic resonance imaging (MRI), were performed for patients whose parents or guardians could afford them. Bone marrow involvement was defined as the presence of malignant lymphoma cells in a bone marrow smear [20]. Confirmation of CNS involvement was based on the presence of at least one of the following: lymphoma cells in the CSF cytology, intracranial or parameningeal lesions on imaging, cranial nerve palsy, or clinical spinal cord compression.

Risk group classification

In this study, risk group classification was based on the INCTR-03–06 protocol in which patients were stratified as low-risk or high-risk similar to a previous study [19]. The low-risk group consisted of patients with a single extra-abdominal tumor the widest diameter of which was <10 cm. Furthermore, patients were classified as high-risk if they presented with features not seen in the low-risk group, such as advanced tumor stages (Stage III/IV), elevated lactate dehydrogenase (LDH) levels, intra-abdominal disease, a tumor diameter ≥10 cm, or involvement of the CNS or bone marrow.

Tailoring of therapeutic regimen according to the patient risk group

Low-risk patients received three courses of cyclophosphamide, vincristine or oncovin, and methotrexate (COM) at three-week intervals. In contrast, high-risk patients received six courses of COM combined with intrathecal methotrexate and cytarabine at two-week intervals. With the exception of low-risk patients, treatment began with a pre-phase consisting of prednisone, followed by low-dose cyclophosphamide, vincristine, and prednisone (COP). Therapy was subsequently intensified for high-risk patients who failed to respond to COM, as well as for any patient with residual viable cells following the consolidation phase.

Treatment response criteria

Treatment responses were categorized as follows: complete response (CR), defined as no evidence of disease post-treatment; partial response (PR), a reduction in tumor size of 50% or greater; no response (NR), characterized by persistent disease; and response not assessed (NRA) for patients who died or abandoned care prior to assessment [19]. Treatment response was assessed using clinical factors, including the Eastern Cooperative Oncology Group (ECOG) performance status, imaging (CT or MRI), and laboratory studies such as LDH levels. Tumor size reduction of ≥50% was evaluated after two weeks of chemotherapy. Evaluation methods included the monitoring of LDH levels, the clearance of lymphoma cells from the bone marrow, and radiographic assessment via CT or MRI.

Measurement of variables

Wherever possible, the parents or guardians of patients who were diagnosed and treated from January 2012 to December 2017 were contacted by phone from January to December 2019. Caregivers who answered the phone were asked about the progress of the patients, whether they had died after treatment or relapsed. Additionally, patients were monitored at the last follow-up appointment visit in the facility. Patients who were unreachable by phone or who did not respond to call attempts were categorized as lost to follow-up. OS was defined as the time from the date of diagnosis or starting treatment to the date of death from any cause [21].

Statistical analysis

Data were anonymized and recorded in the Epidata database version 2.0 and then exported to IBM SPSS version 20 for statistical analysis. Categorical variables are summarized as frequencies and percentages whereas continuous variables are reported as medians and interquartile ranges (IQR). The log-rank test was used to estimate the differences in survival groups. Cox regression analysis was performed to examine if any factors were associated with mortality. Factors associated with mortality in the univariate analysis with a p-value <0.2 were entered in the multivariate analysis to determine predictors of mortality. Kaplan-Meier curves were used to estimate the OS at 18 months since the start of treatment. A two-tailed p-value <0.05 was considered statistically significant.

Results

A total of 161 children clinically suspected of having BL were identified from the admissions register, 27 (16.8%) of whom were excluded after reviewing their case notes as they had other malignancies. Of the 83.2% (134/161) children that remained as cases of BL, 8.2% (11/134) were excluded due to death or abandonment of care before treatment initiation. A total of 91.8% (123/134) children were finally eligible to be included in the final analysis (Figure 1).

Demographic and clinical characteristics of the patients

The median age at diagnosis was 7 (range: 4 –10) years, with a male to female ratio of 1.6:1. The majority (n = 102; 82.9%) presented with symptoms of less than three months duration. Abdominal involvement accounted for over one-third (n = 48; 39.0%) followed by jaw involvement found in 31 (25.2%) of the cases. Most of the patients (n = 69; 56.1%) had endemic BL. A CSF evaluation was performed in 97 (78.9%) children, among

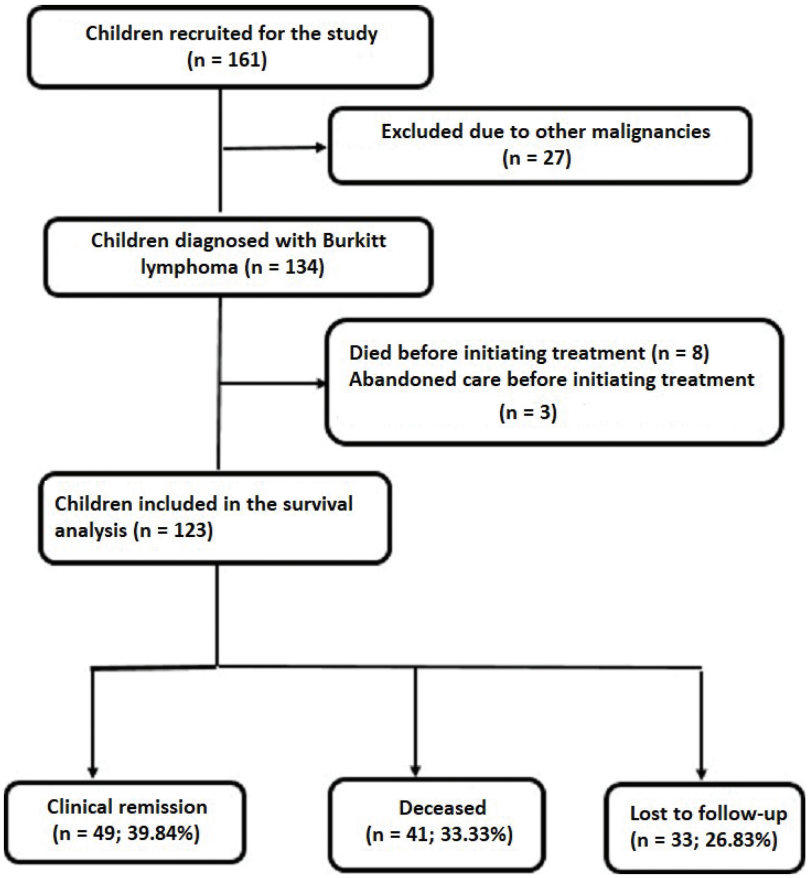

Figure 1 – Flow chart showing enrolled study children and their outcome.

whom 39 (40.2%) had CNS involvement. A bone marrow evaluation was performed in 98 (79.7%) patients, of whom 12.2% (n = 12) had bone marrow involvement. Regarding risk group classification, the vast majority 69.9% (n = 86) of the patients were in the high-risk group. Among the 98 patients (79.7%) who underwent clinical staging, Stage IV was the most prevalent, accounting for 38.8% (n = 38/98) of the cohort, followed by Stage III in 28.6% (n = 28/98) of cases (Table 1).

Treatment regimens of the patients and related complications

Most patients were treated with chemotherapy only (n = 51; 41.5%) followed by chemotherapy and surgery (n = 28; 22.8%). Over one-third (n = 48; 39.0%) of those who were treated with chemotherapy were prescribed the COM regimen. Most patients (n = 63; 51.2%) underwent 1–15 cycles of chemotherapy. Additionally, 21 patients (17.1%) had treatment-related complications (Table 2**).**

Treatment responses of children with Burkitt lymphoma to first- and second-line regimens using the INCTR-03-06 protocol

Of the 90.1% (111/123) of children who received first-line chemotherapy, 55.9% (62/111) and 23.4% (26/111) achieved a CR and PR, respectively, while 2.7% (3/111) had NR. Twenty patients (18.0%) could not be assessed for response following first-line treatment; of these, 9.9% (11/111) died and 8.1% (9/111) were lost to follow-up (Figure 2). Subsequently, all patients with PR or NR, along with three patients referred from an external cancer center, were switched to second-line chemotherapy. In total, 32 (28.8%) of the patients transitioned to second-line regimens, 28.5% (8/32) and 59.4% (19/32) of whom achieved CR and PR, respectively and were discharged home for palliation. Additionally, of the nine children with bulky abdominal disease who were kept on a hybrid regimen of first and second lines, six (66.7%) children achieved CR.

Predictors of mortality among children with Burkitt lymphoma

In univariate analysis, the risk of death was three times higher in HIV infected children compared to HIV negative children (unadjusted Hazard Ratio [uHR] = 3.07; 95% CI: 1.19–7.59; p = 0.02). Additionally, children in advanced disease risk groups (C and D) had a 2.3-fold increased risk of mortality compared to those in risk group B (uHR = 2.34; 95% CI: 1.09–5.05; p = 0.03). Moreover, it was observed that children with increased LDH levels (>500 U/L) had increased risk of death compared to their counterparts (uHR = 1.58; 95% CI: 1.28–3.20; p = 0.04). Multivariate Cox regression analysis was performed, adjusting for CSF involvement, risk stratification, HIV status, and serum LDH levels. HIV-positive status and elevated LDH (>500 U/L) remained independently associated with an increased risk of mortality. Specifically, HIV-positive patients had a fivefold higher risk of death compared to HIV-negative patients (aHR = 5.1; 95% CI: 1.38–19.0; p = 0.02). Furthermore, patients with LDH levels >500 U/L faced a significantly higher risk of mortality than those with LDH levels ≤500 U/L (aHR = 2.50; 95% CI: 1.14–14.71; p = 0.03). Although children in

Table 1 – Demographic and clinical characteristics of children with Burkitt lymphoma (n = 123).

Variable	n	%
Sex		
Male	76	61.8
Female	47	38.2
Age at diagnosis (years)		
<10	41	33.3
≥10	82	66.7
Duration of illness (months)		
<3	102	82.9
≥3	21	17.1
Tumor sites		
Abdomen	48	39.0
Jaw	31	25.2
Abdomen and jaw	25	20.3
Others *	19	15.5
CSF testing		
Positive	39	31.7
Negative	58	47.2
Missing data	26	21.1
Bone marrow evaluation		
Positive	12	12.2
Negative	86	69.9
Missing data	25	20.3
Tumor staging		
Stage I	10	8.1
Stage II	22	17.9
Stage III	28	22.8
Stage IV	38	30.9
Missing data	25	20.3
Risk groups		
Low-risk	12	9.8
High-risk	86	69.9
Missing data	25	20.3
Subtypes of Burkitt lymphoma		
Endemic	69	56.1
Sporadic	46	37.4
HIV-associated	8	6.5
HIV status		
Positive	8	6.5
Negative	115	93.5
LDH (U/L)		
<250	3	4.1
250–500	16	21.9
>500	54	74.0
Hemoglobin (g/L)		
<70	17	13.8
70–99	27	22.0
100–110	57	46.3
>110	22	17.9

CSF: cerebrospinal fluid; LDH: lactate dehydrogenase.

* Other tumor sites included orbit, ovary, neck, chest, femur, and spinal column.

Table 2 – Treatment regimens for the patients and related complications (n = 123).

Variable	N	%
Treatment modalities		
Chemotherapy	51	41.5
Chemotherapy + surgery	28	22.8
Chemotherapy + surgery + radiotherapy	19	15.4
Chemotherapy + radiotherapy	15	12.2
Chemotherapy + surgery + physiotherapy	10	8.1
Types of chemotherapy regimens given		
COM	48	39.0
R-COM	11	8.9
CHOP	23	18.7
ITmCH	9	7.3
EMIC	32	26.0
Number of cycles		
1–15	63	51.2
16–31	34	27.6
>31	26	21.2
Treatment related complications		
Nausea	9	7.3
Vomiting	4	3.3
Anaemia	5	4.1
Others	3	2.4
None	102	82.9

COM: cyclophosphamide + vincristine + methotrexate; R-COM: Rituximab + cyclophosphamide + vincristine + methotrexate; CHOP: cyclophosphamide + doxorubicin + vincristine + prednisolone; ITmCH: intrathecal methotrexate + cytarabine + hydrocortisone; EMIC: etoposide + mesna + ifosfamide + cytarabine.

risk groups C and D exhibited higher mortality rates compared to those in risk group B, this difference did not reach statistical significance (aHR = 1.83; 95% CI: 0.42–8.08; p = 0.42) (Table 3).

Survival rates of children with Burkitt lymphoma

At the end of the study period, 49 (39.8%) of the patients were in clinical remission. However, 33 patients (26.8%) were lost to follow-up. Among these, 13 (39.4%) abandoned treatment before response assessment, while the remaining 20 were unreachable by telephone: 11 (33.3%) had inactive numbers and nine (27.3%) did not respond to repeated call attempts. A total of 82 patients (66.7%) were censored in the survival analysis. Of these, 33 (40.2%) were censored due to loss to follow-up, while 49 (59.8%) were censored because they had not experienced the event of interest (death) by the end of the study period. Death was confirmed for 41 (33.3%) of the children analyzed in the study, with 11 (26.8%) deaths attributed to disease and treatment-related complications during treatment and the other 30 (73.2%) occurring at home.

The mean survival time from the initiation of treatment to 18 months was 13 months (95% CI: 11.7–14.3), with an estimated 18-month OS rate of 54.0% (Figure 4). The estimated OS rate among children with elevated LDH (>500 U/L) was shorter than that of children with LDH levels of ≤500 U/L (44.9% versus 72.4% - Log rank: p < 0.001) (Figure 5). Also, children who were HIV positive had reduced OS compared to children who were HIV negative (29.2% versus 61.7% - Log rank: p = 0.01) (Figure 6). Although the OS rate for children with jaw disease was higher compared to children with abdominal disease (70.5% versus 56.0%), the difference was not statistically significant (Log-rank: p = 0.43) (Figure 7).

Discussion

This study sought to assess the clinical profile and predictors of mortality of children with BL who were treated at a

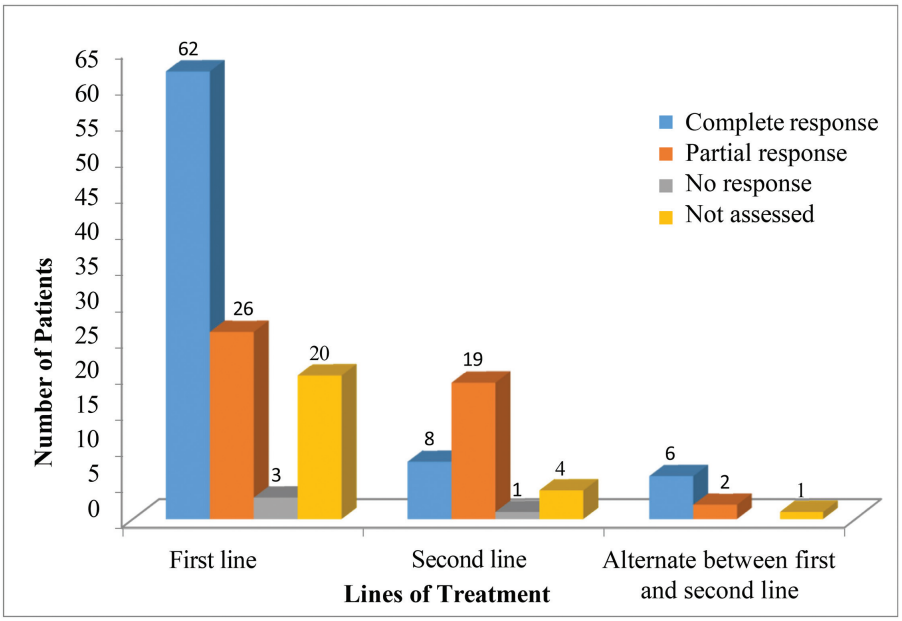

Figure 2 – Treatment responses of first and second line regimens of the INCTR-03-06 protocol.

Table 3 – Cox proportional hazards analysis for predictors of mortality of children with Burkitt lymphoma (n = 123).

	Patient status		Univariate analysis		Multivariate analysis	
Variable	Dead	Alive	uHR (95% CI	p-value	aHR (95% CI)	p-value
Sex						
Female	15 (31.9)	32 (68.1)	1.0			
Male	26 (34.2)	50 (65.8)	0.93 (0.60- 1.76)	0.83		
Age (years)						
<10	26 (63.4)	15 (36.6)	1.0			
≥10	15 (18.3)	67 (81.3)	0.56 (0.18–1.71)	0.31		
Tumor sites						
Jaw	9 (29.0)	22 (71.0)	1.0			
Abdomen	17 (35.4)	31 (64.6)	1.29 (0.58–2.88)	0.53		
Others	6 (31.6)	13 (68.4)	1.13 (0.74–5.11)	0.62		
CSF test						
Negative	12 (20.7)	46 (79.3)	1.0		1.0	
Positive	14 (35.9)	25(64.1)	1.83 (0.86–3.89)	0.12	0.64 (0.15- 2.85)	0.56
Bone marrow test						
Negative	14 (25.5)	41 (74.5)	1.0			
Positive	4 (33.3)	8 (66.7)	1.29 (0.43- 0.39)	0.65		
Tumor staging						
Stage I and II	10 (31.2)	22 (68.8)	1.0		1.0	
Stage III and IV	31 (47.0)	35 (53.0)	2.34 (1.09–5.05)	0.03	1.83 (0.42- 8.08)	0.42
Risk group						
Low-risk	3 (25.0)	9 (75.0)	1.0			
High-risk	37 (43.0)	49 (57.0)	1.27 (1.35–8.13)	0.49		
HIV status						
HIV-	36 (31.3)	79 (68.7)	1.0		1.0	
HIV+	5 (62.5)	3 (37.5)	3.07 (1.19–7.95)	0.02	5.12 (1.38–19.0)	0.02
LDH (U/L)						
≤500	10 (25.0)	30 (75.0)	1.0		1.0	
>500	31 (37.3)	52 (62.7)	1.58 (1.28–3.20)	0.04	2.50 (1.14- 14.71)	0.03
Hb (g//L)						
≥115	6 (27.3)	16 (72.7)	1.0			
<115	35 (34.7)	66 (65.3)	1.32 (0.71–2.47)	0.3		

uHR: unadjusted Hazards ratio; aHR: adjusted Hazard ratio; CI: confidence interval; CSF: cerebrospinal fluid; HIV: human immunodeficiency virus; LDH: lactate dehydrogenase; Hb: haemoglobin.

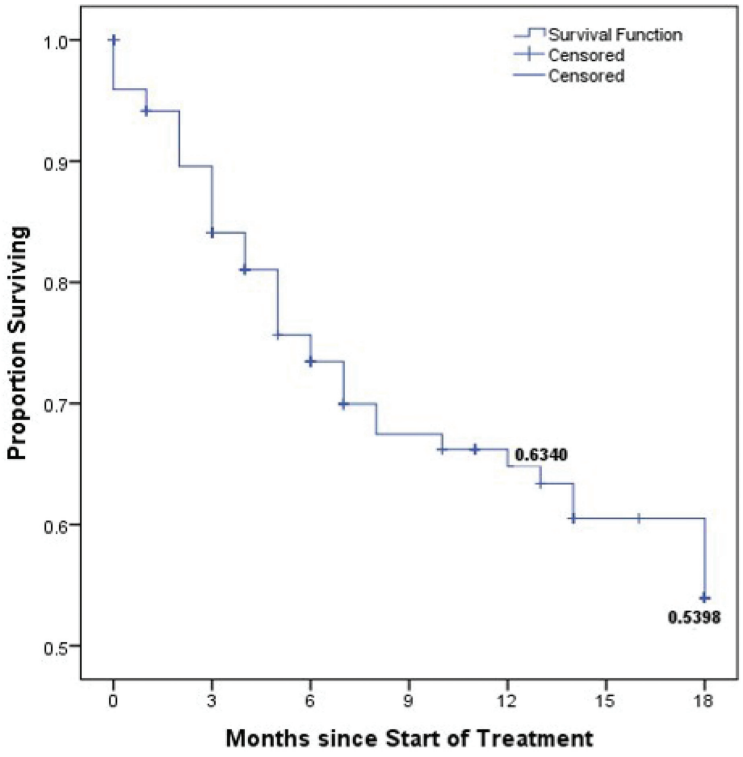

Figure 3 – Kaplan-Meier curve of the overall survival of patients treated for Burkitt lymphoma.

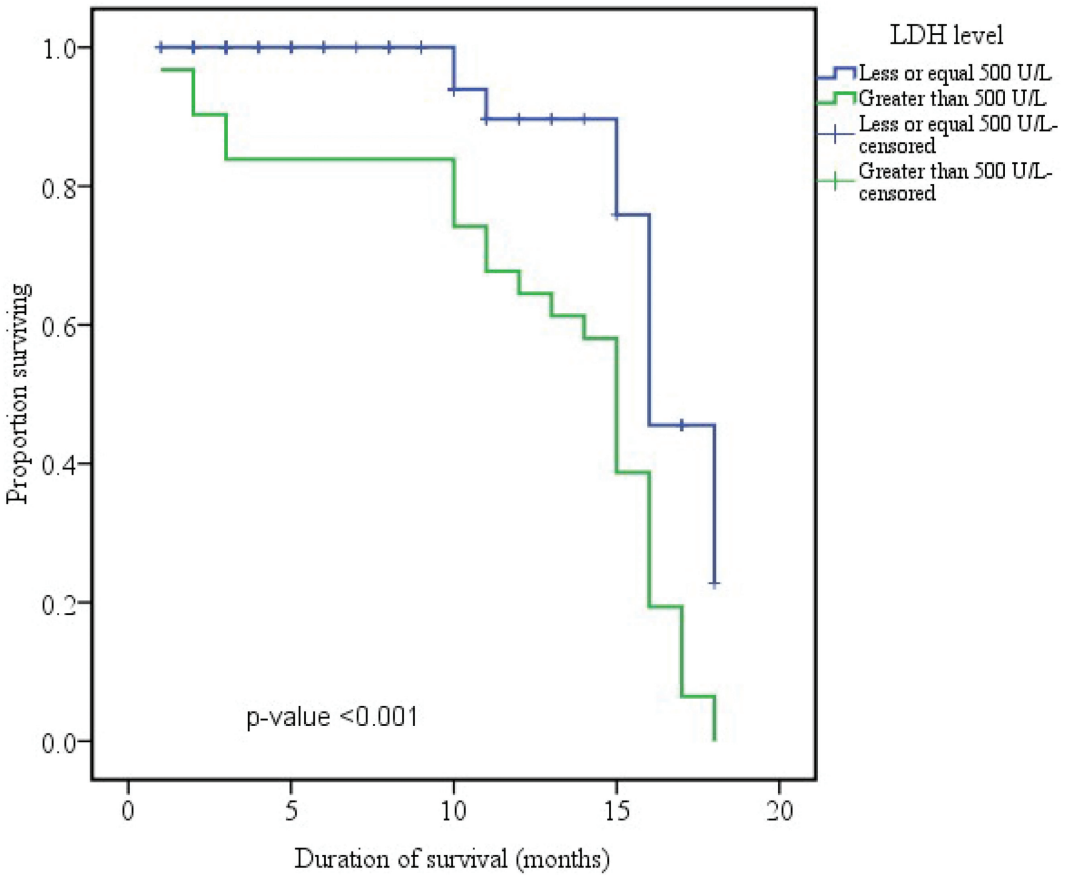

Figure 4 – Kaplan-Meier survival analysis of patients stratified by serum lactate dehydrogenase (LDH) levels.

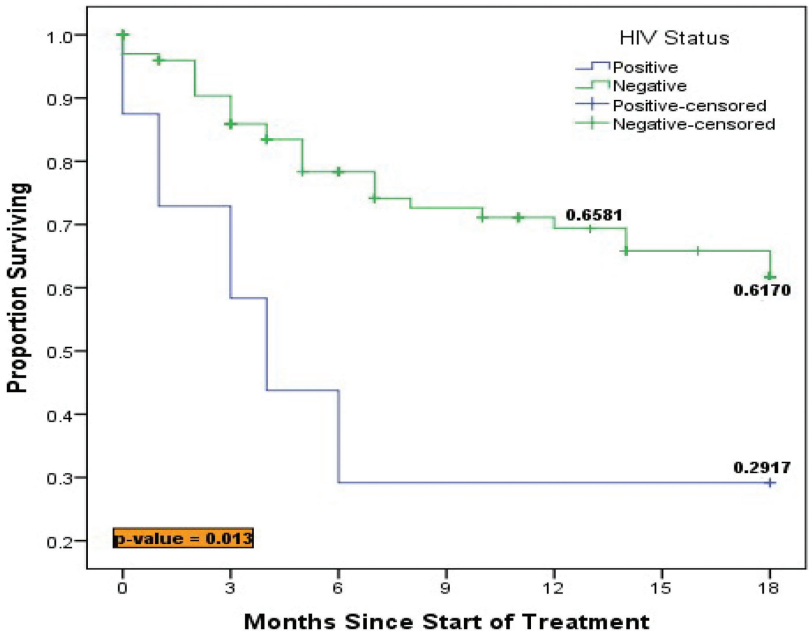

Figure 5 – Kaplan-Meier curves comparing overall survival of patients stratified by HIV status.

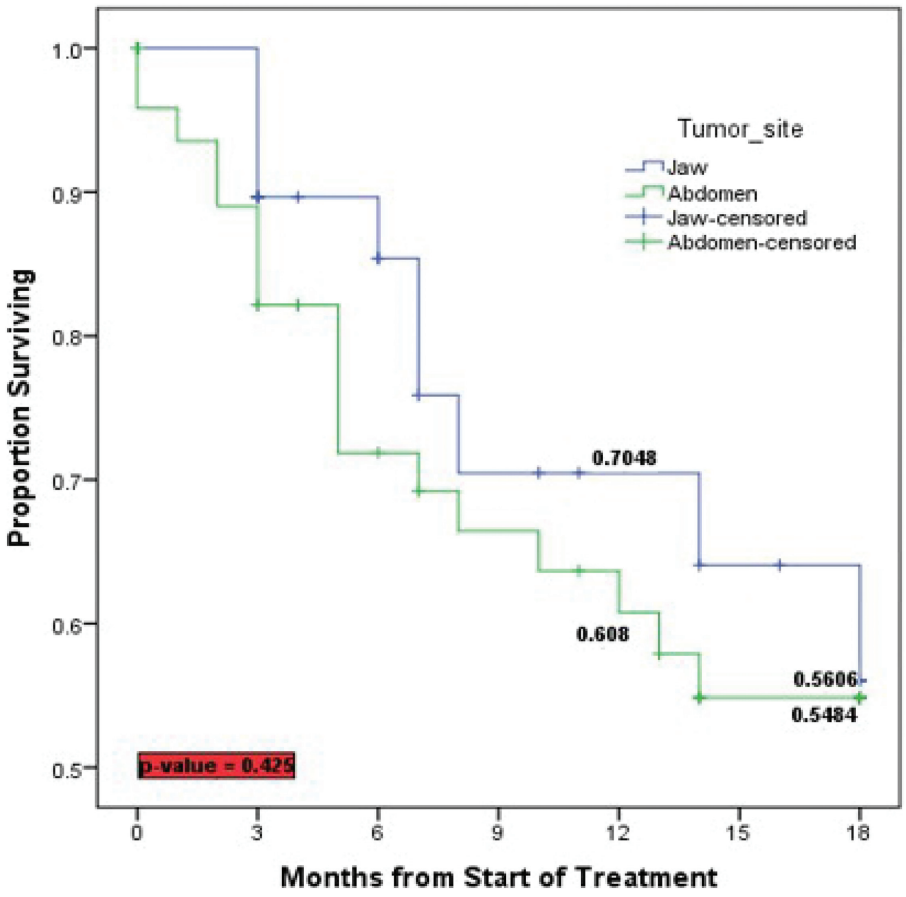

Figure 6 – Kaplan-Meier curves comparing overall survival of patients stratified by tumor site.

national hospital in a resource-constrained setting in the southern region of sub-Sahara African. The key findings of this study demonstrate a moderate OS rate. Furthermore, HIV-positive status and elevated LDH levels were identified as significant independent predictors of mortality in this patient population.

In the present study, abdominal manifestations were the most common clinical presentation of BL, followed by jaw involvement. This aligns with findings from studies conducted in Mozambique and Uganda [7,22]. Similarly, research from Malawi, Northern Uganda, Cameroon, and Tanzania has reported a clear abdominal predilection in the clinical presentation of BL [10,23–25]. The rising proportion of patients presenting with abdominal disease may be partially attributed to advancements in diagnostic modalities. The widespread availability of abdominal ultrasound, CT, and MRI in equatorial Africa has enhanced the detection of intra-abdominal involvement, which is in contrast to historical trends where jaw involvement was the predominant clinical feature [26].

Regarding treatment responses in this study, over half of the patients achieved CR on first-line chemotherapy of the INCTR protocol. The CR rate in this study (55.9%) was lower than those reported in other regional cohorts; for instance, Ngoma et al. [19], Park et al. [27], and Stanley et al. [10] observed CR rates of 75.8%, 76.3%, and 88%, respectively. However, a slightly lower proportion (44.5%) was reported in Uganda [2]. Discrepancies in CR rates across these studies may be attributed to variations in chemotherapy protocols, as well as differences in clinical staging, risk stratification, and the performance status of the patients at presentation. Despite marked variations in CR rates following first-line chemotherapy across these studies, the early mortality rate in this cohort did not differ significantly from rates reported in previous research. Furthermore, the proportion of patients achieving CR after transitioning to second-line regimens was low, a finding consistent with outcomes reported in other studies [10,19,27].

In this study, the 18-month OS was 54.0%; this is slightly lower than the 1-year and 2-year OS rates of 67.0% and 62.0%, respectively that were reported by Ngoma et al. in Tanzania in 2012 [19]. In a similar cohort from Malawi, the 18-month OS rate was reported to be 54% [14], which is consistent with the findings observed in the present study. In Uganda, McGoldrick et al. reported a 4-year OS rate of 44%, with 88% of their cohort treated using the COM regimen [28]. Furthermore, in the study of Fahad et al. [29] in the United States, the 5-year OS was 90.4%, that is 54.0% higher than the 18-month OS rate found in the present study. Amani et al. also reported a 5-year OS rate of 56% among children treated with BL in Saudi Arabia, 2% higher than the 18-month OS rate in the present study.

Several factors may explain the discrepancies in OS observed across these studies, most notably the wide variation in chemotherapy intensity across sub-Saharan Africa. For instance, the high-intensity LMB protocol used in South Africa yielded a superior OS rate of 64.7%. In contrast, the JOOTRH protocol utilized in Kenya resulted in a 1-year OS of 45%, while the lower-intensity CHOP regimen, previously used in Malawi for non-Hodgkin lymphoma including BL, demonstrated a lower 1-year OS rate of 29.0% [10,30,31]. Since the implementation of the INCTR-03-06 protocol, a standardized, non-trial treatment strategy primarily based on the COM regimen, there has been a significant improvement in the OS of patients with BL [19,32]. The difference in the risk groups as well as tumor stages of patients in the studies may help to explain the discrepancies in the OS rates. Studies have shown that, patients who are classified into high risk groups (C and D), generally have a poorer clinical outcome compared to patients who are in low risk groups (A and B) [33,34].

Studies from the sub-Sahara African region have shown several factors that can influence the clinical outcome of the patients. In the present study, the clinical stage of the disease at the time of diagnosis, was strongly associated with prolonged survival. Children with advanced disease (risk groups C and D) were at increased risk of dying compared to children who were in risk group B. This finding agrees with the observation in the studies carried out in the United States, South Africa, and Ghana [29,31,35]. Children with advanced stage BL present in fragile states with a multitude of complications and huge tumor cell burden, which increases their risk of early mortality [36]. Further, most children with cancer in low- and middle-income countries present late to cancer treatment centers, this contributes significantly to advancing the disease before initiation of primary treatment [37]. HIV infection was another predictor of mortality in the current study. HIV-positive patients had shorter survival compared to HIV-negative children. This observation aligns with the findings of Orem et al. in Uganda, who reported that HIV-positive status was significantly associated with inferior OS [38]. Another similar finding was reported in the study of Stefan et al. in South Africa, which found that the mortality rate was significantly higher in the HIV-positive group compared to the HIV-negative group (73% versus 23.0%) [31]. HIV infection remains a significant negative prognostic factor, despite the concurrent administration of antiretroviral therapy (ART) and cytostatic treatment [31].

LDH, an enzyme present in most body cells, is released into the bloodstream when cells are damaged or destroyed [39]. LDH is a known biomarker of tumor burden as well as of cell turnover [40–42], and therefore high levels of LDH in cancer patients suggest a larger amount of cancerous tissue and rapid cell death [43]. In this study, patients who had an elevated level of LDH (≥500 U/L) had a higher mortality rate. This finding is in agreement with the findings of the studies by Cairo et al. [44], Buckle et al. [10], and Galleze et al. [41].

In the present cohort, children with jaw involvement exhibited superior OS compared to those with abdominal BL, although the difference did not reach statistical significance. This trend aligns with previous studies that have consistently demonstrated more favorable survival outcomes in patients with jaw presentations relative to those with intrathoracic or abdominal involvement. For example, Nkurumah et al. reported poorer prognosis of children with BL involving the abdomen than those having jaw involvement [45]. Two review articles, one by Linch and the other by Crombie & LaCasce, explained that jaw involvement carries a better prognosis than intra-thoracic or intra-abdominal involvement [46,47]. Several factors may explain this prognostic advantage. First, jaw involvement is more characteristic of pediatric BL than adult cases, and children generally achieve

superior clinical outcomes compared to their adult counterparts [48–50]. Additionally, jaw-associated BL often carries a more favorable prognosis due to its typically localized nature at presentation, which contrasts with the more disseminated disease often seen in other primary sites. Furthermore, jaw lesions are more accessible for biopsy or primary surgical resection, which, when combined with chemotherapy, may improve patient outcomes compared to those with deep-seated visceral disease [51,52].

Other factors such as age, sex, and hemoglobin level did not affect the OS of the patients in this study. This is contrary to the observations by Stanley et al. which showed that mortality was associated with being aged >9 years, and being female [10].

Limitations of this study

The retrospective nature of the study contributed to recall bias in one way or another as not all caretakers could recall the exact time of event (death or relapse). The sample size, which is relatively small, affected the power of the study. Because data were collected from a single institution, the findings may not be generalizable to other settings across the region. Additionally, potential biases in predictor selection, and lack of external validation were other factors which affected the findings of this study.

Conclusion

In this study, abdominal involvement was the most frequent clinical presentation, and approximately half of the patients achieved CR following first-line chemotherapy. The 18-month OS rate remained low, with HIV infection and elevated LDH levels identified as key predictors of early mortality. Consequently, thorough pretreatment evaluation of pediatric BL should mandate HIV screening alongside bone marrow and CSF investigations, as these factors are critical determinants of patient survival.

Ethical clearance and consent to participate

Ethical clearance was obtained from the Research and Publication Committee of MUHAS. Permission to conduct the study in the pediatric department was obtained from the Research Ethics Committee of the MNH. All patients' information was anonymized using unique codes for the purpose of confidentiality. Verbal consent was obtained from patents or caregivers during the interview and they were informed of their rights to participate including withdrawing at any point from the interview.

Funding

No funding was received for the research, authorship, or publication of this article.

Patient consent statement

The caregivers of the study subjects gave their informed consent.

Authors' contribution

AAS, AIN, NSM, SJ: study designing, conceptualization, supervision, and data curation. AAS, JJY, AIN: methodology, statistical analysis, and interpretation, writing the first draft of the manuscript. All authors reviewed the final version of the manuscript critically and agree to take responsibility for the intellectual part of it.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

None of the authors have competing interests to disclose regarding this work.

Acknowledgements

We are grateful to the team of medical doctors at the POU as well as caretakers for their support. Also, we acknowledge that this work was presented at a scientific conference in (USCA, 2020) and later it was published as an abstract (DOI:10.1200/JCO.2020.38.15_suppl.e22507).

Editor

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Platelet counts, but not neutrophil counts, are associated with non-bleeding-related mortality in patients with hematological malignancies and post-chemotherapy febrile neutropenia

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ARTICLE INFO

Article history:
Received 17 November 2025
Accepted 1 June 2026
Available online 10 July 2026

Keywords:
Febrile neutropenia
Sepsis
Neutrophils
Platelets

ABSTRACT

Introduction: Neutrophils and platelets are key elements of the host immune response to sepsis, participating in both pathogen clearance and in sepsis-related tissue damage. Although it is widely recognized that patients with hematological malignancies and chemotherapy-induced febrile neutropenia are at increased risk of severe sepsis complications, the prognostic impact of neutrophil and platelet counts on sepsis-related outcomes remains controversial, with conflicting results regarding the magnitude of neutropenia. Moreover, to our knowledge, no studies have addressed the association of thrombocytopenia with outcomes in febrile neutropenia in patients with hematological malignancies. This study investigated the association of neutropenia and thrombocytopenia in a consecutive cohort of 113 patients with underlying hematological malignancies who were admitted to an academic tertiary hospital with chemotherapy-induced neutropenia.

Methods: Neutrophil and platelet counts were obtained at fever onset and 24, 48 and 72 hours thereafter.

Results: Neutrophil counts at the time of febrile neutropenia were not associated with sepsis-related mortality or other negative outcomes. However, a lower platelet count at fever onset was associated with non-bleeding sepsis-related mortality and the need of organ support. Moreover, the trajectories of both neutrophil and platelet counts in the first 72 hours after the onset of febrile neutropenia were also associated with negative outcomes.

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https://doi.org/10.1016/j.htct.2026.106498

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Conclusion: These results highlight the heterogeneous role of platelets in the immune response and pave the way for studies exploring to what extent severe thrombocytopenia modulates the molecular and cellular mechanisms of post-febrile neutropenia sepsis in these patients.

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Introduction

Sepsis is a severe clinical condition that can be described as an unregulated systemic inflammatory response developed by the host in response to a pathogen [1]. In 2017 approximately 48.9 million incident cases of sepsis and 11 million sepsis-related deaths were recorded worldwide according to the Global Burden of Disease (GBD) study [2].

Sepsis manifests as an exaggerated inflammatory response in which tissue injury may be mediated by pathogens or by the host immune response. It involves several elements such as the complement system, coagulation cascade, endothelium, and circulating cells, including neutrophils and platelets [3]. Neutrophils and platelets seem to play a dual role contributing to both pathogen clearance and secondary tissue injury [4].

In patients with cancer, sepsis is frequently associated with chemotherapy-induced neutropenia and thrombocytopenia. Several studies have demonstrated that post-chemotherapy neutropenia is a risk factor for sepsis complications when compared to non-neutropenic sepsis [5,6]. However, the association of neutrophil counts with sepsis outcomes among patients with cancer and post-chemotherapy neutropenia is less evident [7,8]. Regarding platelet counts, to our knowledge, no studies have addressed the association of the magnitude of thrombocytopenia with the risk of febrile neutropenia (FN) in patients with hematological malignancies who typically present more severe thrombocytopenia than patients with other cancers. Moreover, most studies that explored the impact of neutrophil and platelet counts in FN considered only a single time-point analysis.

Neutrophils and platelets play critical roles in the host response to pathogens and act as putative mediators of secondary tissue injury in sepsis. Therefore, this study explored the association between the trajectories of these cellular counts within the first days of fever onset and clinical outcomes in a consecutive cohort of patients with hematological malignancies and post-chemotherapy neutropenia.

Methods

Study population

This was a retrospective study conducted at an academic tertiary hospital in Campinas, SP, Brazil. The study population included all patients with chemotherapy-induced neutropenia admitted between 2018 and 2021, identified through the laboratory database of complete blood counts (CBC). The filtering strategy involved: (i) identification of all results with an absolute neutrophil count (ANC) $<1.0\times10^9$/L; (ii) selection of the first CBC result that reached this level; (iii) selection of patients with a diagnosis of any hematological malignancies from the electronic medical records with a history of chemotherapy; and (iv) selection of patients with severe neutropenia (ANC $<0.5\times10^9$/L). Each individual medical record was then evaluated and patients without a history of fever during their admission were excluded. The study was approved by the local institutional review board, which waived the requirement for obtaining written informed consent from participants (#59359022.7.0000.5404), and it was performed according to the Declaration of Helsinki.

Chemotherapy regimens and supportive treatment protocol

Patients were submitted to intensive chemotherapy regimens which included: induction or consolidation chemotherapy for acute leukemias, or salvage chemotherapy regimens for lymphoma and plasma cell neoplasms. Hematopoietic stem cell transplantation conditioning regimens were used as per protocol based on diagnosis. All admitted patients with FN received filgrastim until neutrophil recovery. An empirical antimicrobial regimen was initiated immediately after fever onset and modified upon pathogen identification or specific clinical criteria, in accordance with institutional and international protocols. Platelet and red blood cell transfusions were used as normally indicated in the context of post-chemotherapy cytopenias.

Clinical and laboratory data, and study outcomes

Clinical and laboratory data were manually retrieved from the electronic medical records. FN was defined as the presence of fever (>37.8 °C) in patients with an ANC $<0.5\times10^9$/L. Sepsis was defined as the presence of life-threatening organ dysfunction in patients with clinical or laboratory evidence of infection [9]. The presence of these criteria was strictly adjudicated from patient records by one of the investigators (EVDP). The following outcomes were evaluated: sepsis-related death within 30 days from FN onset, septic shock, venous thromboembolism, need for mechanical ventilation, vasopressor agents or renal replacement therapy, and a composite outcome termed any organ support, defined as the need for any of the aforementioned supports. In order to facilitate interpretation, the neutrophil-to-platelet ratio (NPR) was calculated by multiplying the ratio of neutrophil to platelet counts (both in x 10^9/L) by 100.

Statistical analysis

Quantitative data were expressed as medians (with percentiles), minimum, maximum, mean, and standard deviation. Data distribution was assessed using appropriate statistical tests. Group comparisons were performed using t-tests or Mann-Whitney tests (2 groups), and ANOVA or Kruskal-Wallis tests (≥3 groups), depending on data normality. Correlations were evaluated using Pearson's or Spearman's tests. Diagnostic accuracy of neutrophil and platelet counts, and the neutrophil-to-platelet ratio were assessed by receiver operating characteristic (ROC) curve analysis and univariate/multivariate associations with primary and secondary outcomes. Statistical significance was set for p-values <0.05. Analyses were performed using Statistical Package for Social Sciences (SPSS) v. 25.0 and GraphPad Prism v8.0.

Results

In total, 5,759 CBC results with neutropenia were identified during the study period, of which 113 were from patients with severe neutropenia, hematological malignancies and fever, as illustrated in the study flowchart (Supplementary Figure 1). Patient characteristics at the time of FN onset are shown in Table 1.

In the first 30 days following FN onset, sepsis-related mortality was 16.8% (n = 19). Of note, none of these nineteen patients died due to bleeding complications. The characteristics of patients stratified by two of the most relevant outcomes (sepsis-related mortality and need of any organ support) are shown in Supplementary Table 1.

Neutrophil counts were similar between patients irrespective of sepsis-related mortality. In contrast, platelet counts were significantly lower in deceased patients (Figure 1 a-b), while no difference was observed in the NPR (Figure 1c).

Furthermore, the time-course of neutrophil and platelet counts were evaluated from the onset of FN up to 72 hours in patients stratified by relevant clinical outcomes. As shown in Figure 1d the trajectory of platelet counts was significantly different, with a downward trend in deceased patients and a more stable trajectory in patients who survived. A similar, albeit not significant, trend was observed in neutrophil counts (Figure 1e) when patients were stratified by survival status. In fact, when patients were stratified by the need of any organ support, the upward trend of neutrophil counts in the first 72 hours after FN onset in patients who survived was more evident and statistically significant.

Since platelet, but not neutrophil counts, were significantly associated with 30-day sepsis-related mortality at the time of FN onset, the prognostic accuracy of platelet counts for the prediction of sepsis-related mortality and the need for any organ support in this population was explored. As shown in Figure 2, ROC curves demonstrate that the platelet count at the time of FN onset could significantly predict both outcomes (Figure 2a-b). Similar results were observed with the platelet count 72 hours after FN onset (data not shown). Using a cutoff of 13.0×10^9/L identified by the ROC curve analysis, a significant difference is observed in the survival curve of the study population (Supplementary Figure 2).

Table 1 – Clinical and laboratory characteristics at the time of febrile neutropenia onset and outcomes (n = 113).

Characteristic	
Sex - n (male: female)	54:59
Age - median (IQR)	54.0 (37.5-60.0)
Diagnosis - n (%)	
Acute leukemia	53 (46.9)
Lymphomas	26 (23.0)
Plasma cell neoplasms	18 (15.9)
Other	16 (14.2)
Comorbidity index - median (IQR)	2 (2-3)
Treatment modality - n (%)	
Intensive chemotherapy	78 (69.0)
HSCT	24 (21.2)
Other	4 (3.5)
qSOFA score - median (IQR)	0 (0-1)
ECOG performance status - median (IQR)	3 (3-3)
Neutrophil count (x 10^9/L) - median (IQR)	0.04 (0.01-0.25)
Platelet count (x 10^9/L) - median (IQR)	21 (10.0-55.5)
Neutrophil:Platelet ratio - median (IQR)	0.13 (0.03-0.84)
Outcomes	
Bloodstream infection (yes) - n (%)	38 (33.6)
Mechanical ventilation (yes) - n (%)	26 (23.0)
Renal replacement therapy (yes) - n (%)	10 (8.8)
Vasoactive drugs (yes) - n (%)	29 (25.7)
Septic shock (yes) - n (%)	25 (22.1)
Venous thromboembolism (yes) - n (%)	8 (7.0)
Any organ support (yes) - n (%)	38 (33.6)
30-day sepsis-related mortality - n (%)	19 (16.8)

HSCT: hematopoietic stem cell transplantation; qSOFA: quick sequential organ failure assessment; ECOG: (Eastern Cooperative Oncology Group Performance Status).

Finally, to further investigate whether the association of platelet count at FN onset with mortality was dependent on disease severity, the association of platelet count with disease severity markers, namely quick sequential organ failure assessment (qSOFA), performance status and presence of bloodstream infections was analyzed. As shown in Figure 3, while this association was confirmed after 72 hours, suggesting that platelet counts are at least partly driven by disease severity, no such difference was present at fever onset (Figure 3).

Discussion

Whether neutropenia influences outcomes in patients with hematological malignancies is still controversial. Moreover, the influence of thrombocytopenia, on sepsis-related outcomes among patients with hematological malignancies presenting chemotherapy-induced neutropenia has not been systematically explored. The main contribution of this study was to demonstrate that platelet counts at FN onset are associated with 30-day sepsis-related, non-bleeding mortality.

Despite the common association in clinical practice between neutropenia and sepsis severity, several studies concur with these results, corroborating the concept that neutropenia is not a reliable predictor of sepsis-related mortality in patients with hematological malignancies [7,8,10–14]. Accordingly, it has been emphasized that the sole presence of

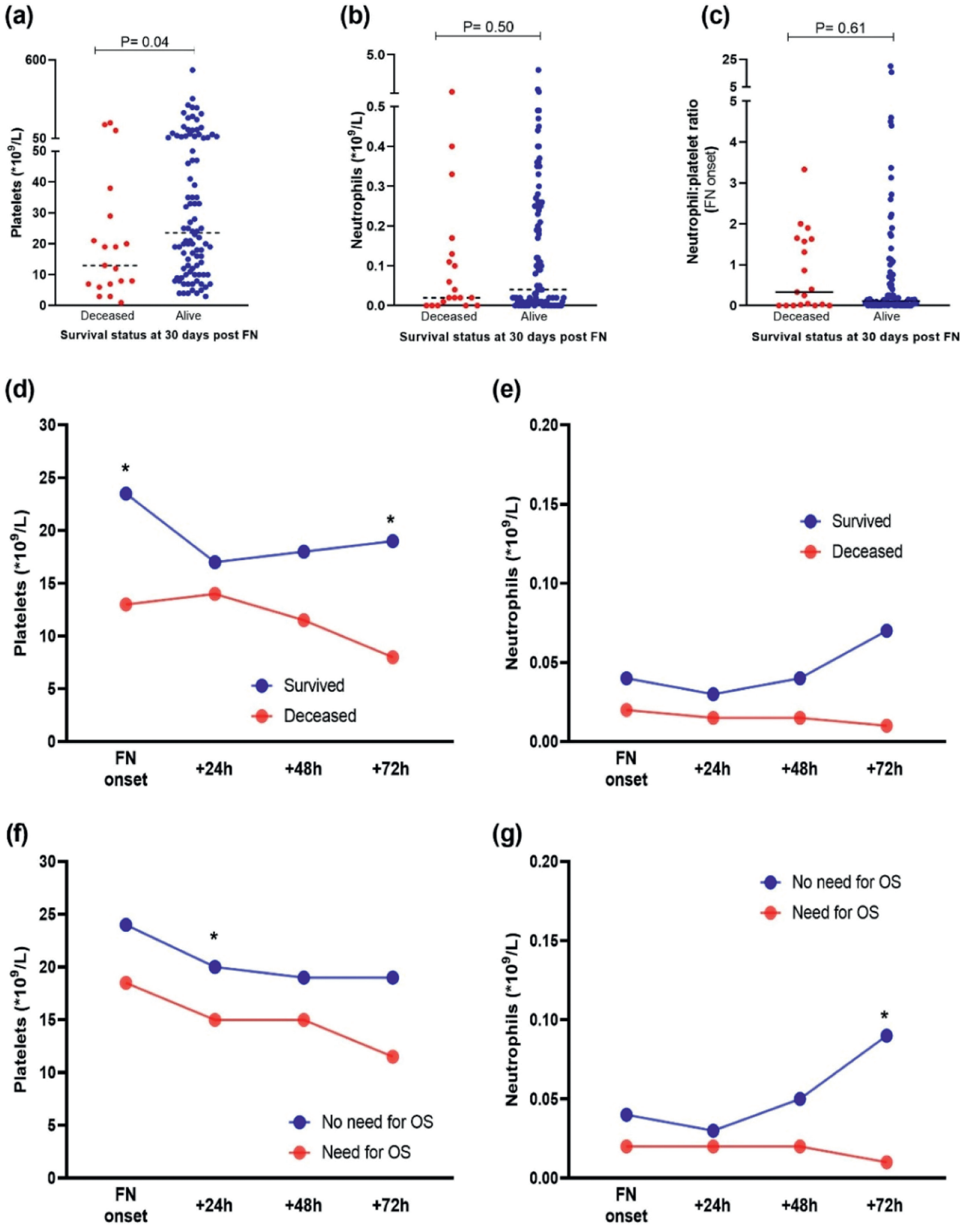

Figure 1 – Dot blot and line graphs showing platelet count (a), neutrophil count (b), and neutrophil-to-platelet ratio (c) at the onset of febrile neutropenia (FN), stratified by 30-day survival status (Mann-Whitney test). Panels (d) and (e) depict the time course of the platelet and neutrophil counts, respectively, from FN onset up to 72 hours, according to survival status. Panels (f) and (g) show the time course of the platelet and neutrophil counts from FN onset up to 72 hours, according to the need for organ support (OS).

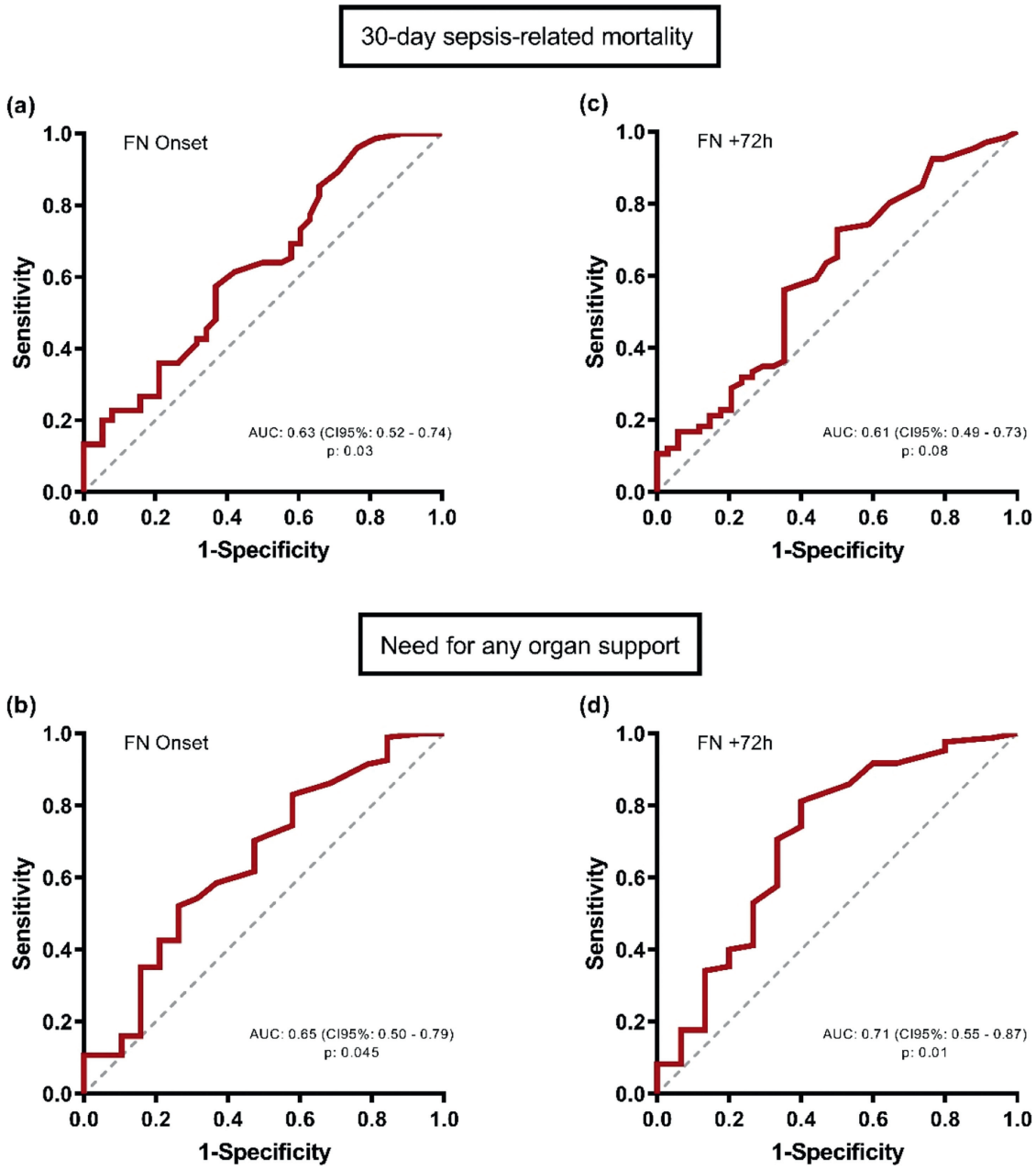

Figure 2 – Diagnostic accuracy of platelet counts at fever onset and at 72 hours to predict clinical outcomes. Receiver operating characteristic curves of platelet count at fever onset (a-b) and after 72 hours (c-d) to predict 30-day sepsis-related mortality (Panels a and c) and need of any organ support (Panels b and d). FN: febrile neutropenia.

neutropenia should not be a criterion to prioritize the admission of patients to intensive care [15]. In addition, this study explores whether the time-course of neutropenia during the first 72 hours after fever onset provides additional prognostic information. This study demonstrated that while the neutrophil count at fever onset was not associated with sepsis severity, non-surviving patients exhibited a distinctly different kinetic trajectory of neutrophil recovery. Whereas surviving patients initiated neutrophil recovery within 48 hours of fever onset, deceased patients had persistently reduced counts, yielding a statistically significant difference in neutrophil levels by 72 hours post-FN onset. Whether a more protracted pattern of neutrophil recovery represents a consequence of a complicated clinical course that hampers the increment of neutrophils after FN onset, or a cause of the negative outcomes of these patients cannot be determined by our data, although the fact that the difference was observed only after 72 hours supports the former hypothesis.

In addition, the data of this study demonstrate an association between platelet counts and adverse sepsis-related outcomes. This relationship is well known, with the platelet count serving as one of the established biomarkers of organ failure in sepsis [16]. However, among patients who develop thrombocytopenia as a consequence of chemotherapy this

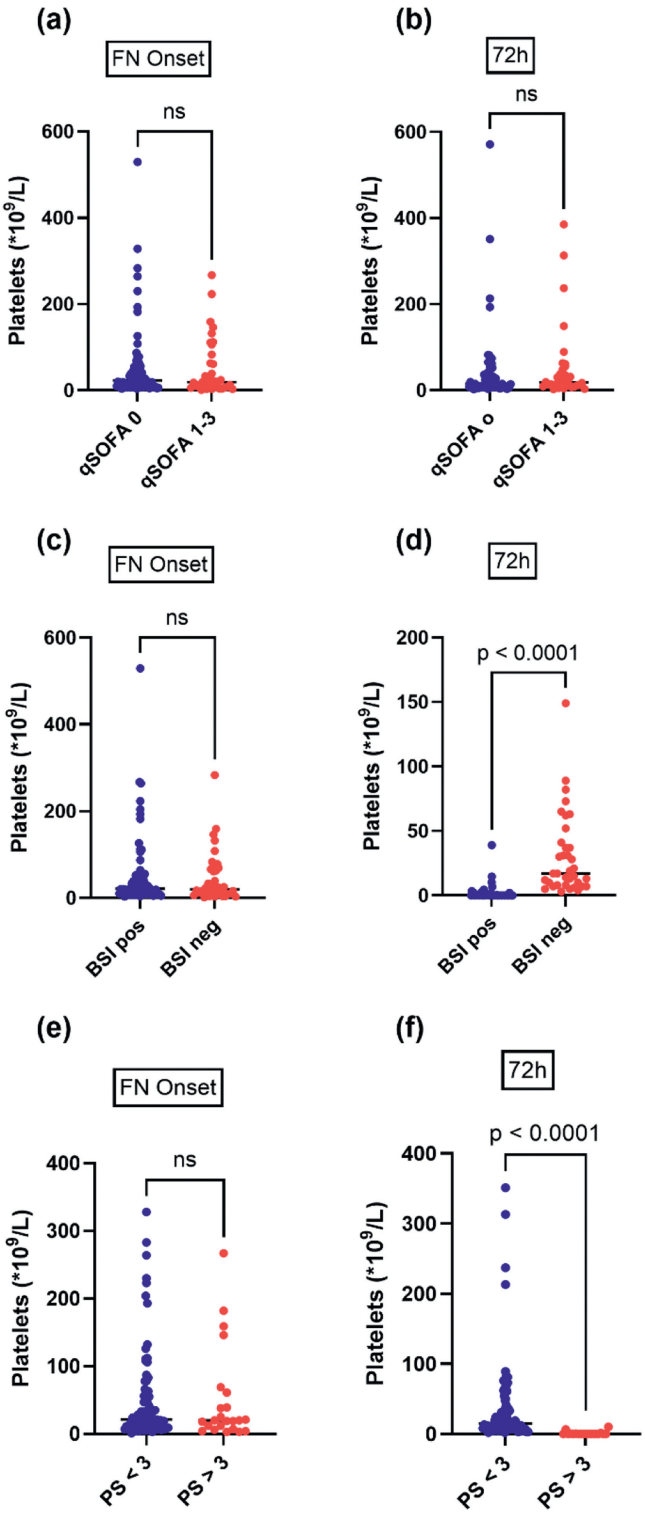

Figure 3 – Dot blots of platelet counts at FN onset (left panels) and 72 hours after FN onset (right panels) stratified by quick sequential organ failure assessment (a-b), presence of bloodstream infection (BSI) (c-d) and performance status (PS) (e-f) (Mann–Whitney test). ns: non-significant.

association is less clear. In a comprehensive review that included 65 studies that explored predictors of outcomes in FN [17], only one study identified platelet count as a predictor variable; however, the study included all types of cancer, with thrombocytopenia (platelet count $<50\times10^9$/L) being considered a categorical variable [18]. Two previous retrospective cohort studies also reported an association between thrombocytopenia and worse outcomes after FN but both included all types of cancer patients and considered thrombocytopenia (platelet count $<50\times10^9$/L) as a categorical variable [19,20]. Hematological malignancies, which require chemotherapeutic regimens that more frequently induce severe thrombocytopenia, are a well-known predictor of FN severity when associated with fungal infections [21]; consequently, it cannot be excluded that the association observed in these studies could be driven by cancer type. Notably, none of the 19 FN-related deaths included in the present analysis were attributed to bleeding, suggesting that thrombocytopenia could negatively impact other mechanisms of the innate immune response. This raises the possibility that severe thrombocytopenia could affect platelet-mediated vascular protection in inflammatory settings, as shown in *in vivo* studies [22,23]. However, a probable hypothesis is that thrombocytopenia results, at least in part, from a more complicated clinical course in these patients. Regarding the trajectory of platelet counts, a similar pattern to the one observed for neutrophil counts was noted, with patients who eventually died from sepsis complications presenting a more protracted recovery of platelet counts. Again, whether this represents a cause or consequence of their clinical course remains to be determined.

Another goal of this study was to explore whether the NPR could provide more precise prognostic information, based on the importance of the interaction between these cells in the physiopathology of host response to sepsis. This hypothesis was not confirmed.

Limitations of this study include those inherent to retrospective designs, such as missing data and recall bias. To minimize these, medical records were reviewed using a standardized form on the Research Electronic Data Capture (RedCap) platform. Additionally, the primary outcomes (mortality and need for organ support) are less prone to such biases. The relatively small sample size limited the feasibility of classical multivariate analysis. To address this, a stratified analysis of platelet counts by severity (qSOFA: 0 vs. 1–3; ECOG: <3 vs. ≥3, and BSI presence vs. absence) was performed. A statistically significant difference in platelet counts was observed at 72 hours, which likely reflects the progression of infection and sepsis, leading to thrombocytopenia as described in the literature. In contrast, at FN onset no significant differences in platelet counts were observed across any of the severity stratifications. This suggests that the platelet count at FN onset may display some degree of independence from these severity indicators. Although this independence may not be absolute, the absence of differences at FN onset followed by their emergence at 72 hours supports the interpretation that thrombocytopenia becomes progressively influenced by the worsening infectious and septic process over time. Nonetheless, one strength of this study was the analysis of a consecutive cohort of neutropenic and severely thrombocytopenic patients with hematological malignancies and FN, whose baseline and clinical characteristics aligned with the literature. Uniquely, this study evaluated the trajectory of

neutrophil and platelet counts, enhancing the robustness of the findings compared to previous studies based solely on a single value at fever onset.

Another limitation is that the precise timeframe between chemotherapy and FN was not recorded, although based on the expected kinetics of neutrophil depletion and recovery after the chemotherapy regimens considered in the study, the nadir of neutrophil count occurs 7-14 days after chemotherapy. Moreover, platelet transfusions were not included in the analysis, which may be a confounding factor. However, since transfusions followed the contemporary standard protocols, we believe that platelet counts used in the analyses illustrate the dynamics of platelet count variations in patients submitted to intensive chemotherapy. Furthermore, the precise time between fever onset and antimicrobial administration was not recorded, which is a well-characterized prognostic factor in FN.

In conclusion, a low platelet count at the time of fever onset was associated with sepsis-related mortality and other negative outcomes in a population of patients with FN and hematological malignancies, independent of bleeding. Neutrophil counts at the time of FN were not associated with sepsis-related mortality or other negative outcomes, although trajectories of both platelet and neutrophil counts within the first 72 hours after FN onset were associated with sepsis mortality. These results highlight the heterogeneous role of platelets in the immune response [24], and pave the way for studies exploring to what extent severe thrombocytopenia modulates the molecular and cellular mechanisms of post-NF sepsis in these patients.

Funding sources

This study was funded by the Sao Paulo Research Foundation (FAPESP), grants 2023/04541-8, 2022/13216-0, 2021/12945-6, 2023/03765; and Capes Finance code 01.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

GRS and LQSJ collected and analyzed clinical data and drafted the manuscript; CRPM collected and revised all laboratory data; ERMK analyzed outcome data and drafted the manuscript; BKLD was responsible for the clinical cohort; EVDP designed the study, oversaw and provided resources contributed to data analysis, and drafted the manuscript; all authors revised and approved the manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2026.106498.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Seroprevalence of transfusion-transmitted infections: A five-year multicentric study of 87,878 blood donors in the Mumbai region, India

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ARTICLE INFO

Article history:
Received 9 April 2025
Accepted 15 April 2026
Available online 10 July 2026

Keywords:
Transfusion-transmitted infections
Seroprevalence
Blood donors
Blood safety
Hemovigilance

ABSTRACT

Introduction: Blood transfusion is a critical component of modern medicine, yet the risk of transfusion-transmitted infections remains a significant challenge. Despite stringent screening protocols, infections such as hepatitis B, hepatitis C, human immunodeficiency virus, and syphilis continue to pose risks. Understanding the seroprevalence of transfusion-transmitted infections in blood donors is essential for improving transfusion safety and shaping public health strategies. The objective of this study is to evaluate transfusion-transmitted infection seroprevalence, analyze trends, and assess seropositivity by age, donation type, and co-infections over five years.

Materials and methods: A five-year cross-sectional study (2020–2024) was conducted at three blood centers: Jagjivan Ram Hospital Blood Bank (Mumbai), Bloodline Charitable Blood Bank (Thane), and Sadguru Charitable Blood Centre (Navi Mumbai). The study assessed the prevalence of transfusion-transmitted infections by screening 87,878 donor samples for human immunodeficiency virus, hepatitis B, hepatitis C, syphilis, and malaria using standardized diagnostic methods. Donor demographics, infection patterns, and regional variations were analyzed statistically.

Results: The overall seroprevalence of transfusion-transmitted infections was 0.78%, with Hepatitis B being the most common infection (0.48%), followed by syphilis (0.144%), human immunodeficiency virus (0.078%), and hepatitis C (0.075%). No malaria cases were detected. Seropositivity was highest among younger donors (18–30 years) and predominant among males (97%). Mumbai recorded the highest seroprevalence, followed by Navi Mumbai and Thane. Co-infections were rare, with human immunodeficiency virus with syphilis being the most frequently observed combination.

Conclusion: This study identifies Hepatitis B as the most common transfusion-transmitted infection, followed by syphilis, human immunodeficiency virus, and hepatitis C. This study

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https://doi.org/10.1016/j.htct.2026.106492
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emphasizes the need for better detection strategies, specifically the use of enzyme-linked immunosorbent assay, electrochemiluminescence Immunoassay, and individual donor nucleic acid testing to improve blood safety. Transfusion-transmitted infection screening plays a vital role as a quality indicator for blood banks, driving method evaluation and improvements. Mumbai's structured referral system ensures seropositive donors receive counseling and treatment, reinforcing blood safety protocols.

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Introduction

Approximately 118.54 million units of blood are collected globally each year, with 40% of the supply coming from high-income countries, which make up 16% of the world's population [1]. Blood is often regarded as the essence of life, but when transfused, it carries both the potential to heal and the hidden risk of harm. Despite rigorous screening measures, Transfusion Transmitted Infections (TTIs), including Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), and syphilis, continue to pose significant challenges to transfusion safety worldwide [2].

The seroprevalence of TTIs varies by region, shaped by donor demographics, socio-economic factors, and advancements in screening. Recognizing these patterns is key to improving blood safety and public health strategies.

Infected blood transfusions pose significant health risks, affecting not only recipients but also their communities. Ensuring transfusion safety involves improving donor selection, screening, and pathogen inactivation to minimize infections [3]. Optimal use of blood minimizes unnecessary transfusions, reduces infection risks, and conserves resources. Evidence-based practices and patient blood management enhance safety and ensure a sustainable blood supply.

Although more sensitive methods are available to detect TTI markers, false negatives still occur due to factors like asymptomatic carriers, donations during the window period, highly variable viral strains, and technical errors. As a result, preventing TTIs remains a significant challenge in transfusion medicine [4].

Hepatitis B remains the most common TTI, affecting 66% of the population in high-prevalence areas, with two billion infections and 400 million chronic carriers, causing 60% to 80% of primary liver cancers and 620,000 deaths annually [5,6]. Hepatitis C affects 3% of the global population, placing 170 million at risk, with India having 10–24 million active cases and a seroprevalence of 0.09% to 2.02% [7]. HIV, though declining, still had 1.7 million new cases in 2019, with India's adult prevalence at 0.2% to 0.3% [8].

The objective of this study is to evaluate TTI seroprevalence, analyze trends, and assess seropositivity by age, donation type, and co-infections over five years. This study is novel in its comparative analysis of TTI prevalence across multiple blood centers using different serological methods, offering insights into detection effectiveness and regional variations.

Materials and methods

A Multicentric cross-sectional study was conducted from January 1, 2020 to December 31, 2024, across three blood centers: the Blood bank, Jagjivan Ram Hospital (Western Railways, Mumbai), Bloodline Charitable Blood Bank (Thane), and Sadguru Charitable Blood Centre (Navi Mumbai). This study was conducted in Mumbai due to its dense population, cosmopolitan nature, and significance as a major healthcare hub, making it an ideal location to assess TTI prevalence in a diverse donor population. The western region of Mumbai includes Thane and Navi Mumbai, with one center selected from each zone.

Ethical approval was granted by the Institutional Review Board (IEC No EC/20/00156).

The inclusion criteria were blood donors aged 18–65 years, weighing at least 45 kg, and with a hemoglobin level of at least 12.5 g/dL. The upper age limit for first-time donors was 60 years, while repeat donors had an upper age limit of 65 years. These criteria were based on the Standard Operating Procedures of the respective blood centers, following the guidelines for blood donor selection and referral [9].

The exclusion criteria were individuals with high-risk behavior, asthmatics on steroids, individuals taking anticoagulants, and others as specified in the Standard Operating Procedures for blood donor selection and referral [9].

Healthy blood donors were evaluated by trained blood transfusion officers through a comprehensive medical history assessment and physical examination, adhering to the Blood Centre's standard operating procedures. Donors filled out a questionnaire in English, Marathi, or Hindi, providing personal information (name, age, gender, etc.) along with health details (general well-being, medical history, high-risk behaviors, etc.). After receiving counseling from a trained professional, eligible donors gave their consent and were assigned a unique identification number. Blood collection involved drawing 2 mL of venous blood for serological testing and 2 mL of blood in ethylenediaminetetraacetic acid (EDTA) for malaria testing with both procedures being performed under aseptic precautions.

Serological analyses

At Jagjivan Ram Hospital Blood Center (Western Railways, Mumbai), transfusion-transmitted infections (TTIs) were

screened using serological methods. Separate serum samples were tested for HIV, HBV, HCV, syphilis, and malaria. HIV detection was performed using 4th generation Qualisa™ HIV Ag and Ab ELISA kits (Tulip Diagnostics Pvt. Ltd.), while HBsAg and HCV were detected using 3rd generation Qualisa™ ELISA kits (Tulip Diagnostics Pvt. Ltd.). Syphilis was diagnosed using the Carbogen Kit (Tulip Diagnostics Pvt. Ltd.), which is based on the Rapid Plasma Reagin (RPR) method and therefore represents a non-treponemal test. Malaria screening involved a peripheral smear examination with conventional microscopy and Rapid Diagnostic Tests (RDTs) using Bioline malaria antigen P.f HRP-II for *Plasmodium falciparum* and p-LDH for *Plasmodium vivax*.

At the Bloodline Charitable Blood Bank (Thane), serological screening was first performed using ELISA for HIV, HBV, and HCV, and the Carbogen kit (RPR method, Tulip Diagnostics Pvt. Ltd.) for syphilis. For samples that tested reactive by ELISA, additional confirmatory testing was performed using individual donor Nucleic Acid Testing (ID-NAT) for HIV, HBV, and HCV, in accordance with the national guidelines, the Drugs and Cosmetics Act, and under the regulatory framework of the Drugs Controller General of India (DCGI).

At Sadguru Charitable Blood Centre (Navi Mumbai), electrochemiluminescence immunoassay (ECLIA) was used instead of ELISA for detecting HIV, HBV, and HCV, while syphilis screening was performed using the Carbogen kit (RPR method, Tulip Diagnostics Pvt. Ltd.), in alignment with their testing protocol.

All three participating blood centers (Mumbai, Thane, and Navi Mumbai) used the Carbogen kit (Tulip Diagnostics), which is based on the Rapid Plasma Reagin (RPR) method, a non-treponemal test for syphilis screening. No treponemal assays (such as TPHA or FTA-ABS) were used in this study.

NAT in India is regulated under the Drugs and Cosmetics Act, 1940 & Rules 1945, and its adoption has been encouraged by the National Blood Transfusion Council (NBTC) and the National AIDS Control Organisation (NACO) guidelines of 2017. However, NAT is not mandatory in India; it remains optional, subject to availability of infrastructure, resources, and licensing. In the present study, NAT was performed only at the Bloodline Charitable Blood Bank, Thane, because this center had the required infrastructure and regulatory approval to implement individual donor NAT in addition to conventional serological screening. At the Jagjivan Ram Hospital (Mumbai) and Sadguru Charitable Blood Centre (Navi Mumbai), NAT was not adopted due to resource constraints and reliance on ELISA or ECLIA-based serological screening methods, which are still considered standard and permissible under Indian regulations.

Seropositive samples were first repeated in duplicate using the same screening assay (ELISA/ECLIA/Carbogen-RPR). A sample was considered reactive only if at least two out of three results were positive. At the Bloodline Charitable Blood Bank (Thane), samples that were initially reactive for HIV, HBV, or HCV on ELISA were further confirmed by individual donor NAT. For syphilis, only the Carbogen kit was used; donors with repeatedly reactive results were referred to government-approved reference laboratories for confirmatory treponemal testing. In case of discrepant results, donors were deferred and not included in the final seropositive count. All confirmed reactive donors were counseled confidentially, and their blood bags were discarded in accordance with biomedical waste management regulations.

Data collected were entered into Microsoft Excel Spreadsheet for Analysis.

Results

Over a five-year period, a total of 87,878 blood donors were screened, of which 689 were found to be seropositive, yielding an overall seroprevalence rate of 0.78%. The annual seroprevalence rates for the years 2020, 2021, 2022, 2023, and 2024 were 0.93%, 0.59%, 0.67%, 0.89%, and 0.84%, respectively. The breakdown of seropositive cases for each infection type revealed that 69 (0.078%) donors were positive for HIV, 427 (0.48%) for HBV, 66 (0.075%) for HCV, and 127 (0.144%) for syphilis, with no cases of malaria being detected during the study period. Over time, there was a noticeable decline in the seroprevalence of HIV, HBV, and HCV, while the number of syphilis cases initially increased before gradually decreasing. Hepatitis B remained the most common infection among donors over the years, followed by syphilis, HCV, and HIV (Table 1).

Table 1 – Total seroprevalence in five years.

Year	No. of donors	No. of seropositive donors (n)						Sero (+) (%)
		HIV	HBV	HCV	Syphilis	Malaria	Total	
2020	12,699	9 (0.07%)	89 (0.7%)	5 (0.03%)	16 (0.13%)	0	119	0.93%
2021	17,294	14 (0.08%)	65 (0.3%)	8 (0.04%)	16 (0.17%)	0	103	0.59%
2022	18,844	16 (0.08%)	82 (0.4%)	12 (0.06%)	17 (0.13%)	0	127	0.67%
2023	19,648	18 (0.19%)	93 (0.4%)	21 (0.1%)	44 (0.2%)	0	176	0.89%
2024	19,393	12 (0.14%)	98 (0.5%)	20 (0.1%)	34 (0.1%)	0	164	0.84%
Total	87,878	69 (0.078%)	427 (0.48%)	66 (0.075%)	127 (0.144%)	0	689	0.78%

Table 2 – Prevalence of different transfusion-transmitted infections among males and females.

Sex	No. of seropositive donors (n)					
	HIV	HBV	HCV	Syphilis	Malaria	Total
Male	69	415	63	122	0	669 (97%)
Female	0	12	3	5	0	20 (3%)
Total	69	427	66	127	0	689

The majority of seropositive donors were male (97%), with only a small fraction (3%) of the total seropositive cases found among females. This trend is consistent across all infections, with males showing a significantly higher seroprevalence for HIV, HBV, HCV, and syphilis (Table 2).

The highest number of seropositive cases was observed in the 18–30 age group, with 279 cases (40.49%), while the lowest percentage was seen in those over 60 years, with just two cases (0.29%) (Table 3).

Based on the regional distribution of TTIs, Mumbai exhibited the highest seroprevalence (0.97%), followed by Navi Mumbai (0.91%) and Thane (0.63%). Although Thane had the largest absolute number of seropositive donors, its adjusted prevalence was the lowest, these figures indicate regional variations, with Thane showing the most significant number of seropositive cases, especially for HBV (Table 4).

Co-infection of HIV with other TTIs was observed in a few cases. Notably, there were five instances of HIV and syphilis co-infection, with four occurring in 2024. Additionally, one donor in 2024 was found to be co-infected with HIV, HBV, and HCV. All co-infected individuals were male, with the majority of cases being reported in the blood bank of Jagjivan Ram Hospital. These co-infections underscore the complexity of TTIs in blood donors, especially in relation to HIV (Table 5).

Discussion

Each blood transfusion carries a 1% risk of transfusion-related reactions and the potential for TTIs [10]. Developed countries have significantly reduced TTIs in the past two decades through effective prevention efforts, while developing nations still struggle with early-stage, hospital-based blood transfusion policies [11]. Blood transfusions should follow national guidelines, considering the needs of the patient, minimizing costs and waste, ensuring safety, and maintaining effectiveness [12]. Given the long-term consequences of TTIs on recipients, healthcare organizations are responsible for ensuring safe blood transfusion services [13]. Continuous improvements in donor screening, selection, and testing methods are essential to reduce the risk of acquiring TTIs.

The total seroprevalence of TTIs in this study was 0.78%, which is low compared to other studies, such as Mendhe VV et al. (1.01%) [14], Lakshmikumar MT et al. (1.07%) [15], Patel SK et al. (1.19%) [16], and Bagde S et al. (1.46%) [17]; however, it is higher than the findings of Sharma RL et al. (0.36%) [18]] (Table 6). This lower prevalence may be attributed to stringent donor selection criteria, increased awareness among donors, effective pre-donation screening to exclude high-risk individuals, and a higher proportion of voluntary non-remunerated blood donors. Additionally, factors such as a predominantly young donor population, better socioeconomic conditions, higher literacy rates, and improved access to medical facilities contribute to reducing the overall burden of transfusion-transmitted infections.

In the present study, the vast majority of donors (97%) were male, which is concordant with findings from studies by Rao and Annapurna, [25] Rose et al. [26] Arora D et al. [27] and Singh B et al. [28] all of whom reported male donors making up >90% of the donor population. The disparity in TTI seroprevalence between male and female donors may be influenced by male-specific risk factors, such as greater social exposure and polygamous relationships. Female donor underrepresentation is due not only to lower participation but also higher deferral rates, with many women being deferred for reasons like low hemoglobin levels and health-related factors. Additionally, limited awareness, lack of motivation, and insufficient education contribute to the lower female donor participation.

Table 3 – Age wise distribution of transfusion-transmitted infections.

Age Group (Years)	No. of seropositive donors (n)					
	HIV	HBV	HCV	Syphilis	Malaria	Total
18–30	29	154	32	64	0	279 (40.49%)
31–40	24	147	19	33	0	223 (32.36%)
41–50	13	107	9	27	0	156 (22.64%)
51–60	3	16	6	3	0	28 (4.06%)
Above 60	0	2	0	0	0	2 (0.29%)

Table 4 – Region wise distribution of transfusion-transmitted infections.

Region	Total Donations	No. of seropositive donors (n)						
		HIV	HBV	HCV	Syphilis	Malaria	Total TTI positive cases	Prevalence (%)
Mumbai	27,520	36	144	21	66	0	267	0.97%
Thane	44,782	23	199	15	44	0	281	0.63%
Navi Mumbai	15,576	10	84	30	17	0	141	0.91%

Table 5 – Co-infection of seropositive donors.

No	Co-Infection	Age	Sex	Year	Region
1.	HIV And Syphilis	22	Male	2022	Mumbai
2.	HIV And Syphilis	47	Male	2024	Mumbai
3.	HIV And Syphilis	24	Male	2024	Mumbai
4.	HIV, HBV and HCV	47	Male	2024	Mumbai
5.	HIV And Syphilis	42	Male	2024	Mumbai

In this study, the highest prevalence of TTIs was observed in the 18–30 age group (40.49%), while the lowest was found in the over 60 age group (0.29%). This trend is concordant with studies by Mandal et al. [29] who reported peak prevalence in the 26–35 age group, and Mendhe et al. [14] who found the highest seropositivity in the 21–30 age group, with the lowest rates in those aged 50 and above. The higher TTI prevalence in the 18–29 age group may be due to greater social exposure, risk behavior, and first-time donations where infections are detected. In contrast, lower rates in donors above 60 years likely reflect reduced high-risk activities, cumulative screening, and safer practices among long-term voluntary donors.

All blood donors in this study were voluntary and unpaid. The predominance of voluntary donations underscores the significance of encouraging voluntary blood donation as a strategy to minimize the risk of transfusion-transmitted infections (TTIs), aligning with the objectives of India's National Blood Policy [30]. Additionally, the majority of repeat donors were voluntary and underwent regular testing, which likely contributed to the lower TTI rates observed in this group.

Hepatitis B emerged as the most prevalent transfusion-transmitted infection (TTI) in the current study, with a seroprevalence of 0.48%. This finding is concordant with similar studies, such as Cheema S et al. (HBV = 0.49%) [24], Mendhe VV et al. (HBV = 0.7%) [14], and Bhawani Y et al. (HBV = 1.41%) [31] that reported comparable rates of HBV prevalence among blood donors. The consistent occurrence of hepatitis B across multiple studies highlights the continued relevance of this infection in the context of transfusion safety. While blood banks implement screening procedures, the persistent prevalence of HBV underscores the need for enhanced donor education, vaccination programs, and the continued advancement of screening technologies. Additionally, it is important to consider the region-specific variations in prevalence, as they may reflect local epidemiological patterns and stress the importance of targeted interventions based on local prevalence data.

It is crucial to detect Hepatitis B during the window period; the implementation of NAT could potentially reduce the risk. However, the high cost of NAT limits its widespread use in many centers [32].

In this study, syphilis was identified as the second most prevalent transfusion-transmitted infection (TTI), with a seroprevalence of 0.144%, followed by HIV at 0.078% and HCV at 0.075%. These results are concordant with those observed in similar studies conducted by Patel SK et al. [16] and Sharma RI et al. [18] which reported comparable rates of syphilis, HIV, and HCV among blood donors.

No cases of malaria were detected among the donors in this study, which is concordant with the findings of other studies, including those by Kaur G et al. [33] Makroo RN et al. [21] and Sethi B et al. [34] where none of the donors tested positive for malarial parasites.

Co-infection was observed in 5 (0.005%) of the seroreactive donors, which is lower than that reported by Kaur R et al. (co-infection = 1.2%) [35] and Kaur H et al. (co-infection = 4.04%) [36]. The discordance in co-infection rates may stem from differences in study populations, geographic regions, testing

Table 6 – Regional variations in the seroprevalence of transfusion-transmitted infections across India.

Place	Publication Year	Total no of donors	Total seroprevalence %	HIV	HBV	HCV	Syphilis	Malaria
Karnataka Bommanahalli BP et al. [19]	2014	19,413	2.22%	-	2.12	0.1	-	-
Gurjat Dhruva GA et al. [20]	2014	10,788	0.93%	0.074	0.68	0.074	0.065	0.037
New Delhi Makroo RN et al. [21]	2015	180,477	11.9%	0.24	1.18	0.43	0.23	0
Western Himalayas Raina S et al. [2]	2015	27,995	0.83%	0.10	0.49	0.21	0.03	-
Gujrat Assessment of NACO supported blood banks- [22]	2016	606,683	1.11%	0.11	0.64	0.15	0.18	0.039
Gujrat Sharma RI et al. [18]	2018	13,724	0.36%	0.03	0.29	0	0.04	0
Andhra Pradesh Naik VSS et al. [23]	2020	54,937	2.41%	0.23	1.82	0.31	0.04	0.01
Haryana Cheema S et al. [24]	2022	10,797	1.07%	0.03	0.49	0.50	0.05	0.009
Maharashtra Mendhe VV et al. [14]	2023	12,193	1.01%	0.09	0.7	0.106	0.04	0
Gujrat Patel SK et al. [16]	2024	20,392	1.19%	0.09	0.76	0.14	0.20	0
Present Study	**2025**	**87,878**	**0.78%**	**0.078**	**0.48**	**0.075**	**0.144**	**0**

protocols, and time periods, as well as variations in sample size and representativeness. These factors can influence the detection and prevalence of co-infections across studies. Co-infections are significant because they affect the course of disease and the quality of life, and share common risk factors and transmission routes.

In the present study, regional analysis showed that Mumbai had the highest seroprevalence (0.97%), followed by Navi Mumbai (0.91%) and Thane (0.63%). Although Thane contributed the largest absolute number of seropositive donors, the adjusted prevalence was lowest when calculated against the total donor pool. Importantly, seroprevalence values in this study were derived from serological assays alone. NAT primarily adds value by identifying window-period infections that are seronegative but NAT-positive, thereby reducing residual transfusion risk. Since no NAT-only cases were detected during the study period, prevalence values remained unaffected. The observed regional variations are therefore more likely explained by differences in donor demographics, distribution of first-time versus repeat donors, and local epidemiological factors rather than by assay type alone.

These findings emphasize the need for targeted interventions and region-specific strategies to enhance blood safety, particularly in areas with higher TTI prevalence.

Donors identified with TTIs are directed to Integrated Counseling and Testing Centers for HIV and government-approved facilities for HBV, syphilis, and malaria, ensuring they receive proper medical evaluation and necessary treatment [37].

Strengths of this study

The strength of this study lies in its large sample size, multicentric design, and diverse serological methods, enhancing the reliability and generalizability of findings. By incorporating data from multiple regions and using ELISA, ECLIA, and individual donor NAT, it provides a comprehensive analysis of TTI prevalence while enabling regional comparisons and assessing the effectiveness of different detection techniques.

Limitations

The study is limited by its focus on the Mumbai-Thane-Navi Mumbai region, which may not fully represent broader or rural populations with different socio-economic factors. This regional scope may affect the generalizability of TTI prevalence across diverse settings.

Conclusion

TTI screening serves as a crucial quality indicator for blood banks, facilitating method evaluation and enhancement. This study highlights Hepatitis B as the most common TTI, followed by syphilis, HIV and HCV. The evaluation of ELISA, ECLIA, and individual donor NAT emphasizes the importance of refining detection strategies to improve blood safety. Additionally, Mumbai's structured referral system ensures seropositive donors receive necessary counseling and treatment thereby strengthening the effectiveness of blood safety protocols.

Research involving human participants

This study was been conducted in accordance with ethical guidelines. (IEC No EC/20/00156).

Informed consent

Informed consent was obtained from all participants included in the study.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

No conflicts of interest to declare.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Real-world use of intravenous ferric derisomaltose in Brazilian patients with iron deficiency anemia

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ARTICLE INFO

Article history:
Received 20 December 2025
Accepted 31 May 2026
Available online 20 July 2026

Keywords:
Iron deficiency anemia
Intravenous iron
Ferric derisomaltose
Hemoglobin
Hypophosphatemia
Oral iron intolerance

ABSTRACT

Background: Iron deficiency anemia remains a major global health concern, especially among women, children, and people with chronic illnesses. Oral iron therapy is the standard treatment, but it is often limited by gastrointestinal side effects or poor absorption. Intravenous ferric derisomaltose is a newer formulation that allows high-dose, rapid iron replenishment with a favorable safety profile. This study aimed to assess the effectiveness and safety of intravenous ferric derisomaltose in patients with iron deficiency anemia who could not tolerate or did not respond to oral iron.

Methods: This was a prospective, open-label, single-center trial conducted in São Paulo, Brazil. Adult patients (≥18 years) with iron deficiency anemia received 1 or 2 infusions of intravenous ferric derisomaltose (up to 1000 mg per infusion). Hematologic and iron parameters were measured at baseline, Week 4, and Week 8. Safety outcomes included adverse events and serum phosphate levels.

Results: Thirty patients were treated with 25 requiring a second infusion. Significant increases in hemoglobin, serum ferritin, transferrin saturation, and reticulocyte hemoglobin content were seen at both time points. By Week 8, 81% achieved a hemoglobin increase ≥2 *g*/dL, and 54% achieved an increase of ≥3 *g*/dL. Four (8%) mild adverse events occurred and three patients (10%) experienced transient, asymptomatic hypophosphatemia, which resolved without intervention.

Conclusion: This study contributes to the growing evidence supporting the use of intravenous ferric derisomaltose as a safe and effective alternative for distinct populations with iron deficiency anemia. In this real-world clinical setting, the treatment is effective for treating patients with iron deficiency, providing rapid hematologic recovery with a favorable safety profile.

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https://doi.org/10.1016/j.htct.2026.106501

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Introduction

As the most prevalent hematologic disorder globally, anemia affects approximately 1.8 billion individuals; among these cases, iron deficiency anemia (IDA) accounts for nearly 50% of the total burden [1]. IDA can be caused by inadequate dietary intake, poor absorption, ongoing blood loss, or high iron requirements. It has a tremendous public health significance affecting children, adolescents and women of reproductive age worldwide [1–4].

Historically, oral iron supplementation has been recommended as the first-line therapy for IDA, especially in outpatient and resource-limited settings [1–4]. Gastrointestinal side effects, including nausea, constipation, abdominal discomfort, and diarrhea, occur frequently and contribute to poor adherence in up to 40% of treated patients, thereby limiting its effectiveness in real-world practice [5]. Additionally, inflammation, gastrointestinal disorders, previous bariatric surgery, or ongoing blood loss can hinder oral iron absorption and reduce treatment efficacy [2,3,5]. In this context, intravenous (IV) iron replenishes iron stores rapidly, thereby quickly raising hemoglobin (Hb) levels, and thus has gained recognition as an effective and well-established treatment for patients who are intolerant of, unresponsive to, or unsuitable candidates for oral iron supplementation [1–4]. IV Ferric Derisomaltose (IFD) is a newer drug capable of delivering high doses of iron in a single or a few infusions [6]. This approach benefits patient convenience and helps ensure compliance, ultimately improving treatment outcomes [6,7]. The immediate and long-term effects of IFD on Hb and iron stores have been documented in both clinical trials and real-world studies, showing notable safety advantages and few hypersensitivity reactions [6–8]. Furthermore, randomized comparative trials suggest that the risk of clinically significant hypophosphatemia with IFD is lower than with other IV iron formulations [8]. Pharmacoeconomic analyses demonstrate a balance among efficacy, safety, and cost, especially in healthcare systems aiming to maximize efficiency while maintaining high-quality care [8–10]. These findings supported the inclusion of IV iron therapies, including IFD, in international guidelines for iron deficiency and IDA across various clinical conditions [9–11].

Given the global prevalence of IDA and the limitations of oral therapy, and as the evidence supporting IV agents continues to grow, this study aims to assess the effectiveness and safety of IFD in adults with IDA.

Methods

Study design and ethical approval

This trial was a prospective, open-label, single-center clinical study of IFD treatment in patients with IDA who were intolerant of, or unresponsive to oral iron therapy. Unsatisfactory response to oral iron was defined as a failure to achieve an increase of 1 g/dL in Hb concentration from baseline after at least four weeks of treatment (100–200 mg/day of oral iron) in the previous 12 months. Oral iron intolerance was defined as the inability to complete treatment due to gastrointestinal symptoms, including nausea, abdominal pain, constipation, or diarrhea [9]. The etiology of IDA was determined based on the patient's clinical history (e.g., heavy menstrual bleeding) and investigations into underlying causes, including upper and lower gastrointestinal endoscopy and H. pylori assessment [9]. The study was conducted at Hospital Samaritano de São Paulo, Brazil, from May 2023 to December 2023, in accordance with the Declaration of Helsinki and good clinical practice guidelines. Ethical approval was obtained from relevant ethics committees before starting study, and all participants provided written informed consent.

Inclusion and exclusion criteria

Participants were adults (≥18 years) diagnosed with IDA, defined as Hb levels below 12 g/dL for females and below 13 g/dL for males, and serum ferritin <30 ng/mL or <100 ng/mL if transferrin saturation (TSAT) <20%. Patients with active infections, chronic liver disorders, known hypersensitivity to IV iron, a history of blood transfusion, or severe conditions requiring urgent hospital treatment were excluded. Diagnostic criteria were strictly adhered to in accordance with internationally recognized guidelines [9,12].

Intervention and dosage

Treatment involved administering a single IV injection of IFD (Monofer®; Pharmacosmos) based on body weight and baseline Hb levels, following the dosing matrix used in recent clinical trials [12–15]. Specifically aligning with Food and Drug Administration (FDA) recommendations, the maximum infusion dose was limited to 1000 mg over 15–30 min in outpatient settings [7,12,13]. When necessary, a second dose of 500 or 1000 mg was administered seven days after the first. Baseline blood tests, including Hb, mean corpuscular volume (MCV), serum ferritin (SF), TSAT, reticulocyte Hb content (Ret-He), and serum phosphate level, were performed before the first infusion, with follow-up tests at Weeks 4 and 8. Participants were observed during infusion and for 30 min afterward for signs of hypersensitivity reactions., [13,14].

Statistical analysis

Continuous variables are presented as medians with interquartile ranges (IQRs). Clinical groups were compared using the Kruskal-Wallis test. Statistical significance was defined as p-values <0.05. All analyses and figures were generated using GraphPad Prism.

Primary and secondary outcomes

The primary outcome was the change in Hb level from baseline to Weeks 4 and 8. Secondary outcomes included the percentage of patients achieving an improvement in Hb ≥2 g/dL and ≥3 g/dL; as well as the incidence and types of adverse events (AEs), including hypophosphatemia (serum phosphate <2mg/dL).

Results

Patient characteristics

A total of 30 participants were enrolled in the study; 28 (93.3%) were female. The mean age was 48.2 years (range: 23–83), with a median of 46 years. Regarding the reason for treatment, 23 (76.6%) had an unsatisfactory response to oral iron, and seven (23.3%) had intolerance to oral iron. Among the 28 women, 15 (53.3%) experienced abnormal uterine bleeding associated with uterine myoma. Six (20%) of the 30 patients had gastrointestinal bleeding, and eight (26.6%) had a history of bariatric surgery. Among the two male participants in this cohort, the main reason for iron deficiency was gastrointestinal bleeding.

The 30 participants received an initial infusion of 1000 mg of IFD. Of the twenty-five who required a second infusion, fifteen received an additional 1000 mg, and ten received 500 mg, based on iron-deficiency estimates.

Efficacy

Treatment with IFD led to significant increases in Hb and iron status markers at Weeks 4 and 8 (Table 1). The primary goal was achieved with statistical significance. Hb levels gradually increased to ≥12 g/dL over time (Figure 1 and 2). By Week 4, 69% of participants had Hb levels >2 g/dL, increasing to 81% by Week 8 (Figure 3). Similarly, 54% reached ≥3 g/dL at the latter timepoint, indicating excellent overall hematologic recovery in this cohort (Figure 4). The majority of patients achieved Hb gains within eight weeks (Figure 5). Of the 55 infusions administered, only four adverse events occurred: dizziness (n = 1), diarrhea (n = 2), and anxiety (n = 1). All were mild and self-limiting. No hypersensitivity reactions were observed at any time point; similarly, median serum phosphate levels remained stable throughout the study period (Figure 1). Mild, asymptomatic hypophosphatemia (serum phosphate <2 mg/dL) occurred in three out of 30 subjects (10%) at Week 4 (Table 2). In all three cases, serum phosphate levels returned to normal by Week 8 without intervention. There were no cases of severely reduced serum phosphate (<1 mg/dL).

Discussion

Anemia is a significant public health concern, affecting roughly a quarter of the world's population [1,2]. It primarily impacts women of reproductive age, children, and individuals with chronic conditions such as inflammatory bowel disease and kidney problems [1,3]. Although oral iron supplements are the first-line treatment, their effectiveness is often limited by gastrointestinal side effects and low adherence [5].

The FERWON-IDA trial evaluated the safety and efficacy of high-dose IFD in 1512 adult patients with IDA and intolerance or lack of response to oral iron, regardless of etiology, compared with IV iron sucrose (IS). The co-primary non-inferiority endpoint for Hb change was met. The Hb increased from baseline to Week 4 by 2.14 g/dL and to Week 8 by 2.49 g/dL, confirming non-inferiority. The authors concluded that IFD was a more convenient method of IV iron administration than IS, without sacrificing efficacy or safety [12].

Two similar randomized, open-label trials compared the safety and efficacy of IFD and IS in non–dialysis-dependent chronic kidney disease, randomizing 3050 patients to a single 1000-mg infusion of IFD or multiple infusions of IS [13,14]. The coprimary endpoints, safety, and change in Hb were assessed at Week 8 and were similar.

This Brazilian cohort represents a real-world population from a tertiary referral center. Hb levels increased significantly from baseline to Week 8, with 81% of patients achieving an increase of ≥2 g/dL and 54% achieving ≥3 g/dL. These findings are highly consistent with results from large international trials evaluating IFD [12–15], even in a smaller, more heterogeneous population (including abnormal uterine bleeding, gastrointestinal bleeding, and post-bariatric surgery).

A recent randomized clinical trial evaluated whether single-dose IV iron for the primary treatment of maternal IDA in the second trimester was superior to twice-daily oral iron. The study demonstrated that IV iron (IFD and ferric carboxymaltose) was safe and effective, reducing the incidence of low-birth-weight infants and increasing the incidence of attaining a maternal nonanemic state without the use of additional iron or blood transfusions [15]. The authors emphasize that clinical guidelines should address the potential benefit of single-dose IV iron as the primary treatment for moderate iron-deficiency anemia in pregnancy [15].

Table 1 – Baseline characteristics and laboratory evolution of the study population (n = 30).

Variable	Baseline	Week 4	Week 8	p-value
Hemoglobin (g/dL)	9.3 (8.5–10.3)	11.6 (10.8–12.5)	12.6 (11.8–13.3)	<0.01
MCV (fL)	75.9 (67.8–82.6)	82.5 (77.9–86.8)	84.2 (80.2–88.4)	<0.01
Reticulocytes (%)	1.4 (1.1–2.3)	1.4 (1.1–2.2)	1.4 (1.0–1.8)	0.90
RET-He (%)	22.9 (19.4–28.7)	30.4 (27.6–32.8)	32.9 (28.7–33.6)	<0.01
Serum Iron (μ/dL)	21 (15.5–34)	53.5 (40–88)	60 (45–85)	<0.01
Transferrin saturation (%)	6 (4–10)	20.5 (14–33)	23 (18–29)	<0.01
Serum Ferritin (μ/L)	9.5 (5.5–18)	163 (50–314)	134 (85–257)	<0.01
Serum Phosphate (mg/dL)	3.4 (2.8–3.8)	3.1 (2.6–3.7)	3.2 (2.6–3.5)	0.30

Data are presented as median with interquartile range (25th–75th percentile).
Clinical groups were compared using the Kruskal–Wallis test.
MCV: Mean Corpuscular Volume; RET-He: Reticulocyte Hemoglobin Equivalent.

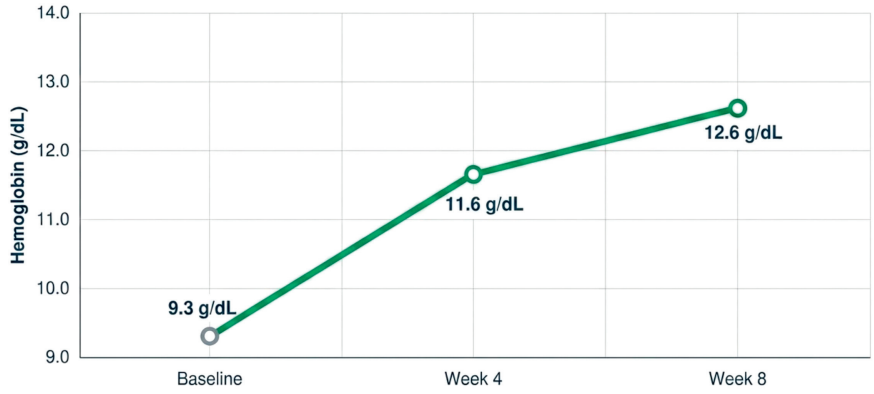

Fig. 1 – Hemoglobin trajectory illustrating rapid and complete normalization by Week 8.

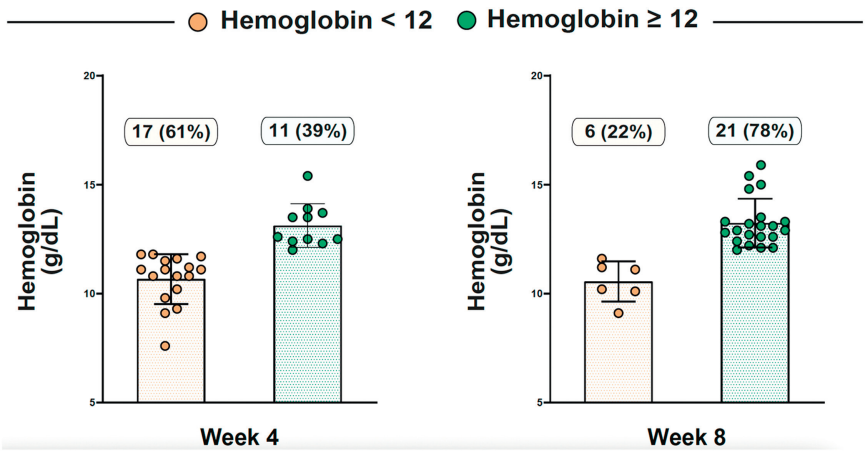

Fig. 2 – Distribution of patients achieving Hb ≥12 g/dL vs. <12 g/dL from baseline to Week 8.

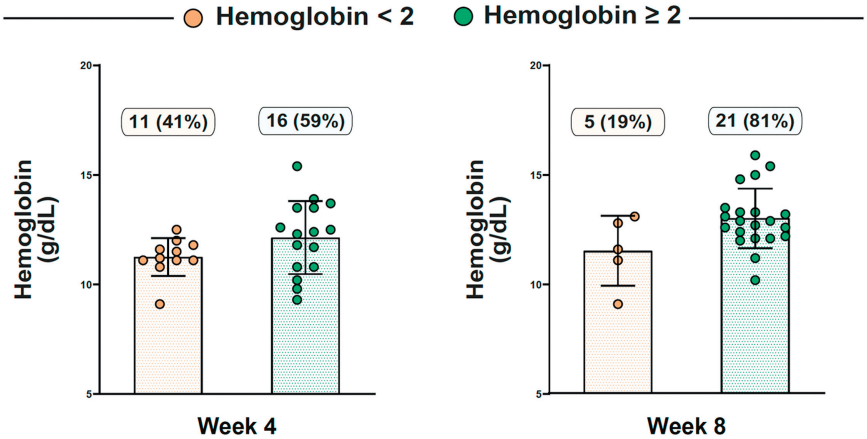

Fig. 3 – Distribution of patients achieving increases of ≥2 g/dL vs. <2 g/dL of hemoglobin from baseline to Weeks 4 and 8.

Results from the IFD trial in the United States involving over 2000 patients showed that the incidence of severe hypersensitivity reactions ranged from 0.3% to 0.8% [12,13,16]. In the present study, the treatment was widely accepted, with mild AEs occurring rarely (8%) and no moderate or severe hypersensitivity reactions [6–8,11]. These results are consistent with the contemporary understanding that modern IV iron formulations carry a low risk of severe hypersensitivity reactions.

Awareness of hypophosphatemia, a relatively common AE, is essential because of its potential consequences (e.g., bone loss, osteomalacia), especially after repeated high-dose

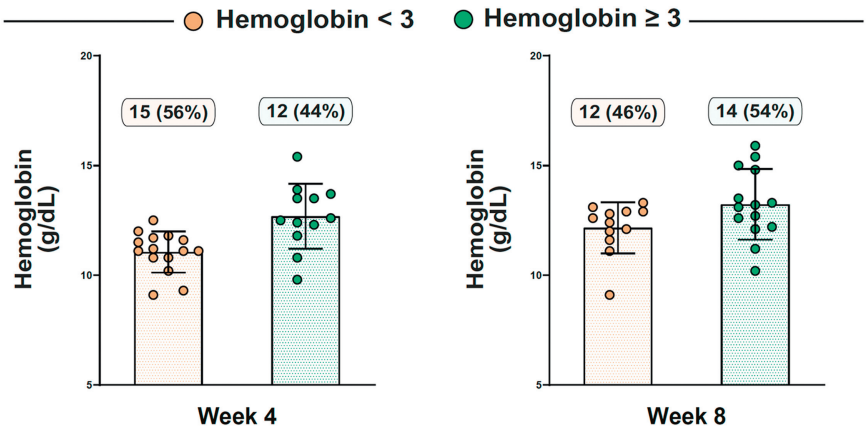

Fig. 4 – Distribution of patients achieving an increase ≥3 g/dL vs. <3 g/dL of hemoglobin from baseline to Weeks 4 and 8.

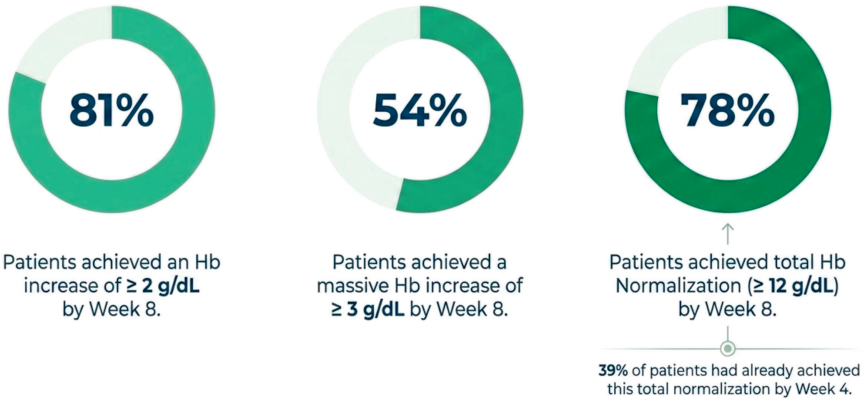

Fig. 5 – The majority of patients achieved hemoglobin gains within eight weeks. Hb: Hemoglobin.

treatment [6]. In the FERWON-IDA trial, hypophosphatemia (3.9% with IFD and 2.3% with IS) was temporary, with no cases of severe hypophosphatemia (serum phosphate <1.0 mg/dL) associated with either treatment [12]. Similarly, in the FERWONNEPHRO trial, few patients treated with IFD (3.2%) and IS (0.8%) developed hypophosphatemia, and none experienced severe hypophosphatemia [13].

In the current study, hypophosphatemia occurred in 10% of participants, was mild and asymptomatic, and self-limited (tended to resolve spontaneously within eight weeks of observation). No severe hypophosphatemia was observed. These results are consistent with published evidence showing that IFD has a significantly lower risk of clinically significant hypophosphatemia than other IV iron compounds, such as ferric carboxymaltose [12–14,16].

Table 2 – Serum phosphate levels in patients with transient hypophosphatemia from baseline to Week 8.

Patient	Serum phosphate level (mg/dL)		
	Basal	Week 4	Week 8
#1	2.8	1.7	2.4
#2	4.9	1.4	3.8
#3	3.4	1.4	3.7

The results of this study demonstrate that IFD is effective for treating patients with IDA who are intolerant of or unresponsive to oral iron. While existing literature suggests benefits regarding adherence and resource utilization, the findings of this study specifically confirm the clinical effectiveness and safety of this formulation within a Brazilian real-world cohort [6–10]. Expanding access to modern IV iron formulations in low- and middle-income countries could help reduce ongoing disparities in anemia treatment. They may decrease the need for transfusions, hospital visits, and longer treatment durations. Such efforts are essential for developing better treatment options that benefit individuals and communities worldwide through collective actions [1–3,7,9].

Despite some limitations of this study such as a relatively small number of patients that limit statistical power and generalizability; a single-center design that may restrict diversity in patient populations and healthcare settings; a short follow-up period (eight weeks) that only captures short-term efficacy and safety rather than long-term outcomes; and the absence of a comparator arm (such as IS or ferric carboxymaltose), it still makes a meaningful contribution to the literature on IDA treatment. It provides a foundation for larger, more comprehensive research. Additionally, it adds value to the Latin American literature, which remains underrepresented in international datasets on anemia management.

Conclusion

This study contributes to the growing evidence supporting the use of IFD as a safe and effective alternative for specific IDA populations. In this real-world clinical setting, the treatment is effective for treating patients with iron deficiency, providing rapid hematologic recovery with a favorable safety profile.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare institutional support (ferric derisomaltose and all laboratory tests) from Pfizer.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

False-positive test results: prevalence, time trends, and factors associated with false-positive serological markers in blood donation screening

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ARTICLE INFO

Article history:
Received 22 November 2025
Accepted 1 June 2026
Available online 18 July 2026

Keywords:
Blood donors
Donor selection
Serologic tests
False-positive reactions
HIV

ABSTRACT

Background and Objectives: False-positive serological results in blood donation screening may arise from antibody cross-reactivity, limitations in assay specificity, or low-level reactivity during early-stage infections, ultimately leading to donor deferral and the discard of viable blood units. This study evaluated the prevalence, time trends, and demographic factors associated with false-positive serologic screening results for transfusion-transmissible infections in a Brazilian blood center.

Materials and Methods: A cross-sectional study was conducted including all blood donations collected between 2013 and 2022 at a private blood center in São Paulo, Brazil. Donations were screened with serologic tests for syphilis, human immunodeficiency virus, hepatitis B virus, and hepatitis C virus. Reactive samples underwent confirmatory testing, where a false-positive result was defined as an initially reactive screening assay followed by a non-reactive confirmatory test. Associations between demographic variables and false-positive or true-positive results compared to true negative results were analyzed using multivariable Poisson regression.

Results: A total of 129,166 donors were included. Most donors (98.7%) had nonreactive results for all serologic tests. Reactive screening results were observed in 0.5% of donations for both syphilis and hepatitis B virus, 0.2% for hepatitis C virus, and 0.1% for human immunodeficiency virus. False positivity occurred more frequently for human immunodeficiency virus (92.0%) and hepatitis C virus (72.7%). Older age, lower education levels, and first-time donation were associated with false-positive results, whereas male sex, non-white race/skin color, lower education levels, and being single or divorced were associated with true-positive status.

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https://doi.org/10.1016/j.htct.2026.106499

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Conclusion: False-positive results were more frequent among first-time and less-educated donors. This study found no consistent overlap between demographic factors associated with false positivity and true positivity. This suggests that false positivity mainly occurs by chance or from antibody cross-reactivity.

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Introduction

Over the past decades, major advances in diagnostic assays for infectious diseases have improved the safety of blood donations. Currently, most blood collection organizations (BCOs) employ highly sensitive laboratory screening tests to detect transfusion-transmissible infections (TTIs), thereby minimizing the risk of transfusing contaminated blood products and making TTIs a rare event [1–3]. Globally, routine TTI screening typically includes tests for human immunodeficiency virus (HIV) [4,5], hepatitis B virus (HBV) [6], hepatitis C virus (HCV), human T-lymphotropic virus (HTLV) [7], and syphilis [8,9]. Additional testing for other TTIs includes Chagas disease, West Nile virus, Babesia, Malaria, and dengue, which are performed according to local epidemiology and risk assessment [10–12].

A major consequence of universal testing with highly sensitive diagnostic assays is the occurrence of false-positive results, which can have several undesirable implications. These include the unnecessary discard of safe blood units, increased laboratory workload and costs related to confirmatory testing, and significant emotional distress for donors, who may face permanent deferral or become reluctant to return for future donations [13–15].

False-positive serologic results may arise through several mechanisms. First, antibody cross-reactivity can occur in the presence of autoimmune disorders, heterophile antibodies, recent vaccinations, or unrelated infections [16,17]. Second, early-stage infections may present with incomplete antibody responses, leading to a reactive screening test but a non-reactive confirmatory result, thus interpreted as false positive [18–20]. Third, test-related technical factors, such as lot-to-lot variations or reduced assay specificity, can contribute to false reactivity [21]. Lastly, false-positive results may occur by chance, without a clearly identifiable underlying cause.

Previous studies have explored factors such as sex, educational attainment, and race/ethnicity as potential predictors of false reactivity in blood donation screening tests [13–15]. Although findings vary across infections and populations, some studies suggest that female sex, lower educational attainment, or African-American race and Hispanic ethnicity are associated with higher odds of false-positive results [13–15].

Despite these findings, recent data addressing how demographic factors influence the frequency of false-positive results using modern, high-performance assays remain limited. A clearer understanding of these associations could improve donor counseling, aid interpretation of discordant results, and encourage donors with previous false-positive findings to continue donating blood.

This study investigated the overall and annual frequencies of positive serological screening and false-positive serological results for syphilis, HIV, HCV, and HBV over a 10-year period at a blood center in São Paulo, Brazil. It also evaluated demographic factors associated with both false-positive and true-positive serologic screening results.

Methods

Study design

In this cross-sectional study, data from all blood donations collected between January 2013 and December 2022 at the BCO of Hospital Israelita Albert Einstein in São Paulo, Brazil, were analyzed. Demographic data, such as sex, age group, self-reported race/skin color, educational attainment, marital status and type of donation were retrieved from the institutional BCO database. For repeat donors, demographic data were extracted from their first recorded donation. Repeat donations were included independently of previous donations. All blood samples underwent routine screening for syphilis, HIV, HCV, and HBV according to institutional protocols focused on serological screening results (Figure 1). Nucleic Acid Testing (NAT) data for HIV, HCV, and HBV were also generated by the BCO but were excluded from this analysis.

Screening routines

Screening for syphilis was performed using a chemiluminescent immunoassay (CLIA) (Alinity i Syphilis TP Reagent Kit; Abbott Diagnostics Division, Abbott Park, IL, USA). Reactive samples were subjected to confirmatory testing using the Fluorescent Treponemal Antibody Absorption assay (FTA-Abs; WAMA Diagnóstica, São Carlos, SP, Brazil) and the Venereal Disease Research Laboratory test (VDRL; WAMA Diagnóstica, São Carlos, SP, Brazil). False-positive results were defined as a reactive chemiluminescence immunoassay (CLIA) result accompanied by nonreactive FTA-Abs and VDRL results.

HIV screening was performed using CLIA-based methods. From 2013 to 2016, assays were conducted on the VIDAS platform (bioMérieux, Marcy-l'Étoile, France) and the VITROS platform (Ortho Clinical Diagnostics, Johnson & Johnson Company, Raritan, NJ, USA). Between 2017 and 2022, testing was changed to the Alinity i HIV Ag/Ab Combo Reagent Kit (Abbott Diagnostics Division, Abbott Park, IL, USA). Reactive results were confirmed using a Western Blot (WB) immunoassay (GS HIV-1 Western Blot; Bio-Rad Laboratories, Hercules, CA, USA). WB results were classified as reactive,

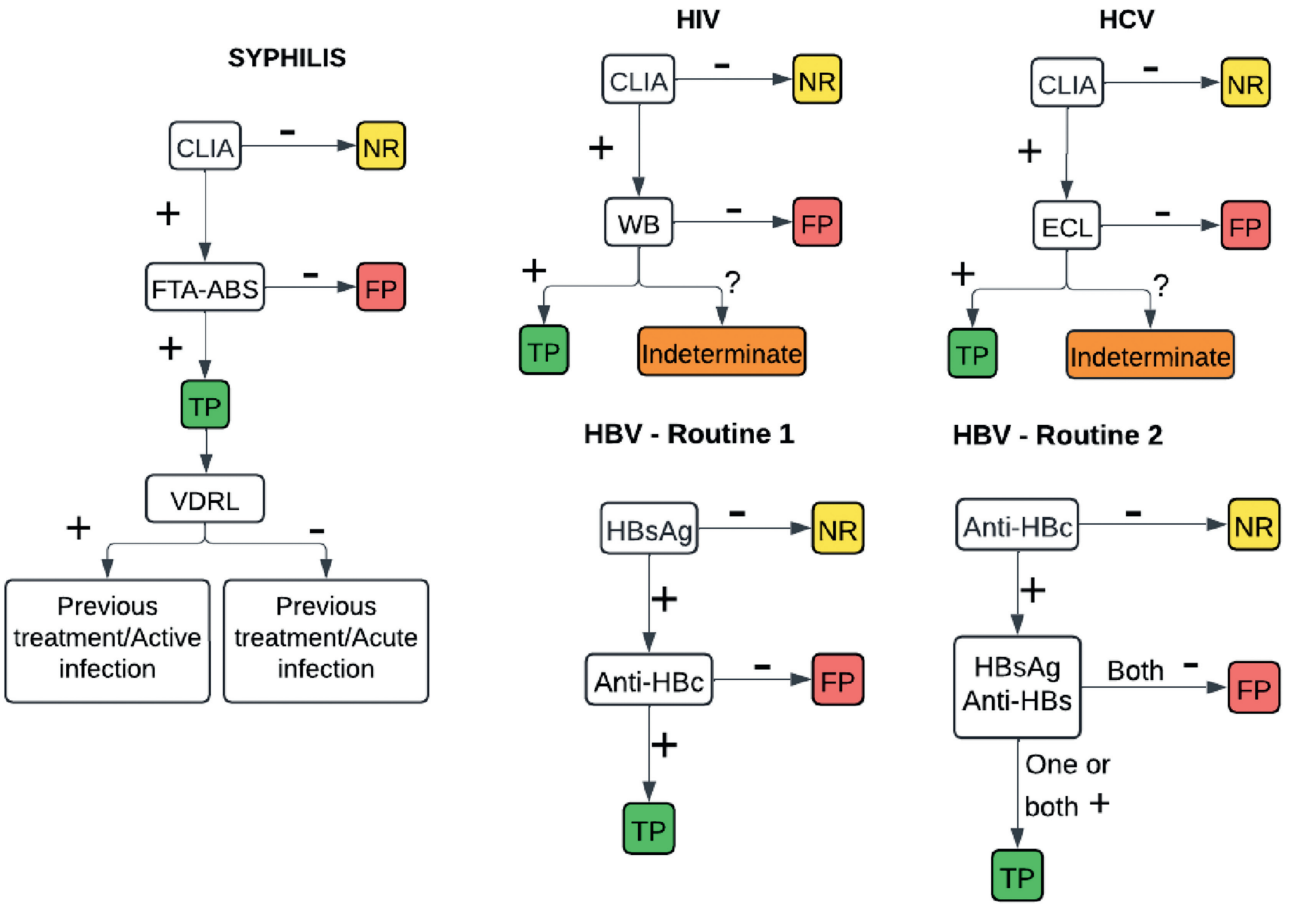

Fig. 1 – Screening routine and interpretations for syphilis, HIV, HBV, and HCV. NR: Non-reactive; FP: False positive; TP: True positive; CLIA: Chemiluminescent immunoassay; FTA-ABS: Fluorescent Treponemal Antibody Absorption; VDRL: Venereal Disease Research Laboratory test; WB: Western Blot; ECL: Electrochemiluminescence assay; HBsAg: Hepatitis B surface antigen; Anti-HBs: Hepatitis B surface antibody and Anti-HBc: Hepatitis B core antibody.

indeterminate or nonreactive. A false-positive result was defined as a reactive CLIA result with a nonreactive WB result.

HCV screening was performed using a CLIA (Alinity i Anti-HCV Reagent Kit; Abbott Diagnostics Division, Abbott Park, IL, USA). Samples that tested reactive on CLIA underwent confirmatory testing using a recombinant immunoblot assay (RIBA) (Deciscan HCV PLUS; Bio-Rad Laboratories, Hercules, CA, USA). RIBA results were classified as reactive, indeterminate, or nonreactive. False-positive donations were defined as a reactive CLIA result with a nonreactive RIBA result.

HBV screening was performed using the Hepatitis B surface antigen (HBsAg) (Alinity i HBsAg Qualitative II Reagent Kit; Abbott Diagnostics Division, Abbott Park, IL, USA), Hepatitis B surface antibody (Anti-HBs) (Alinity i Anti-HBs Reagent Kit; Abbott Diagnostics Division, Abbott Park, IL, USA) and Hepatitis B core antibody (Anti-HBc) (Alinity i Anti-HBc Reagent Kit; Abbott Diagnostics Division, Abbott Park, IL, USA). A false-positive result was defined under two conditions: (1) Reactive HBsAg with nonreactive anti-HBc; or (2) Reactive anti-HBc with nonreactive HBsAg and nonreactive anti-HBs. If either HBsAg or anti-HBs results were reactive, the donation was considered a true positive.

All donations with positive screening results for any infection were immediately discarded according to institutional policy. Donors with false-positive screening results were invited for retesting at approximately one month and five months after the initial donation to assess potential seroconversion. Donors with confirmed seroconversion were referred for clinical follow-up. Those with persistently negative results during the follow-up were eligible for reintegration into the donor pool.

Statistical analysis

Demographic characteristics of donors were described by screening result category. Age was categorized into five groups (≤25, 26–35, 36–45, 46–55, and >55 years). Educational attainment was categorized into three groups (complete college or higher education, incomplete college, and high school or less). Differences between groups were assessed using the chi-squared test. Frequencies and percentages of reactive and false-positive results were calculated and reported as annual distributions. Associations between demographic factors and false-positive screening results (versus nonreactive results) were analyzed using univariable and multivariable Poisson

regression models. Variables included in the multivariable model were sex, age category, self-reported race/skin color, educational level, marital status, and type of donation. Similar analyses were performed for true-positive results when the number of cases permitted. Regression results were presented as prevalence ratios (PRs) with 95% confidence intervals (95% CIs) and p-values.

Analyses were performed separately for each infection and for grouped screening routines. Flowcharts illustrating the testing procedures were generated using Lucidchart (https://www.lucidchart.com). All statistical analyses were conducted in R software, version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical considerations

An Ethics Review Board revised and approved the study (Hospital Israelita Albert Einstein, CAAE Number 61,849,622.3.0000.0071) with no requirement of additional consent for use of de-identified donation data. The study performed no additional tests on donated blood and collected no additional information from participants. The manuscript followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [22].

Results

Participants

Between January 2013 and December 2022, a total of 129,166 donations were received and underwent infectious disease screening at the Hospital Israelita Albert Einstein BCO. Table 1 summarizes the demographic characteristics of participants according to serologic screening results. The median age of donors was 37 years (interquartile range [IQR]: 28.0–46.6). Among all participants, 51,543 (39.9%) were female, 95,065 (73.6%) self-identified as White, 79,313 (61.4%) reported a college-level education or higher, and 56,569 (43.8%) were single. Regarding donation history, 71,808 (56.0%) were classified as repeat donors (≥2 donations within 12 months) or sporadic donors (1 donation every 12 months).

Screening and temporal trends in false-positive results

Overall, 127,457 (98.7%) donations had negative screening results for all infections. During the study period, positive screening results were observed in 677 (0.5%; range: 0.3%–0.8%) for syphilis, 126 (0.1%; range: 0.02%–0.2%) for HIV; 292 (0.2%; range: 0.1%–0.3%) for HCV and 614 (0.5%; range: 0.3%–0.7%) for HBV.

During the study period, among participants with positive screening results, 214 (31.6%; range: 13.6%–43.7%) were false positive for syphilis, 126 (92.0%; range: 80.0%–100%) for HIV, 211 (72.7%; range: 45.5%–90.0%) for HCV and 112 (18.2%; range: 6.4%–43.5%) for HBV. The annual frequencies of reactive and false-positive results for each infection are presented in Figure 2. This study identified a decrease in syphilis-reactive screening results from 2013 to 2019, followed by a gradual increase through 2022. False-positive results for syphilis declined until 2018 but remained stable thereafter. For HIV, a marked decline in screening reactivity occurred in 2017 and then remained stable until 2022. HCV screening frequencies remained relatively constant throughout the study period,

Table 1 – Characteristics of study participants, overall and by screening result, independent of confirmation tests results.

	HBV n = 614	HCV n = 292	HIV n = 126	Syphilis n = 677	TN n = 127,457
Age – years (%)					
≤25	75 (12.2%)	55 (18.8%)	21 (16.7%)	117 (17.3%)	20,718 (16.3%)
26–35	124 (20.2%)	80 (27.4%)	41 (32.5%)	208 (30.7%)	33,736 (26.5%)
36–45	173 (28.2%)	74 (25.3%)	35 (27.8%)	159 (23.5%)	35,474 (27.8%)
46–55	170 (27.7%)	48 (16.4%)	20 (15.9%)	110 (16.2%)	24,801 (19.5%)
>55	72 (11.7%)	35 (12.0%)	9 (7.1%)	83 (12.3%)	12,728 (10.0%)
Sex (%)					
Female	261 (42.5%)	140 (47.9%)	63 (50.0%)	259 (38.3%)	50,820 (39.9%)
Male	353 (57.5%)	152 (52.1%)	63 (50.0%)	418 (61.7%)	76,637 (60.1%)
Race/Skin color (%)					
Non-White	175 (28.5%)	82 (28.1%)	35 (27.8%)	219 (32.3%)	33,196 (26.0%)
White	436 (71.0%)	210 (71.9%)	91 (72.2%)	454 (67.1%)	93,874 (73.7%)
Highest education level (%)					
Complete college or higher	324 (52.8%)	158 (54.1%)	69 (54.8%)	338 (49.9%)	78,424 (61.5%)
Incomplete college	43 (7.0%)	35 (12.0%)	14 (11.1%)	78 (11.5%)	14,552 (11.4%)
High school or less	241 (39.3%)	94 (32.2%)	42 (33.3%)	248 (36.6%)	32,618 (26.0%)
Marital Status (%)					
Divorced/Separated	59 (9.6%)	21 (7.2%)	12 (9.5%)	67 (9.9%)	7886 (6.2%)
Married/Civil Union	316 (51.5%)	140 (47.9%)	62 (49.2%)	258 (38.1%)	62,543 (49.1%)
Single	229 (37.3%)	130 (44.5%)	52 (41.3%)	346 (51.1%)	55,812 (43.8%)
Type of donation (%)					
First time	557 (90.7%)	232 (79%)	91 (72.2%)	549 (81.1%)	55,905 (43.9%)
Repeat/Sporadic	57 (9.3%)	60 (21%)	35 (27.8%)	128 (18.9%)	71,552 (56.1%)

TN: True Negative; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus.

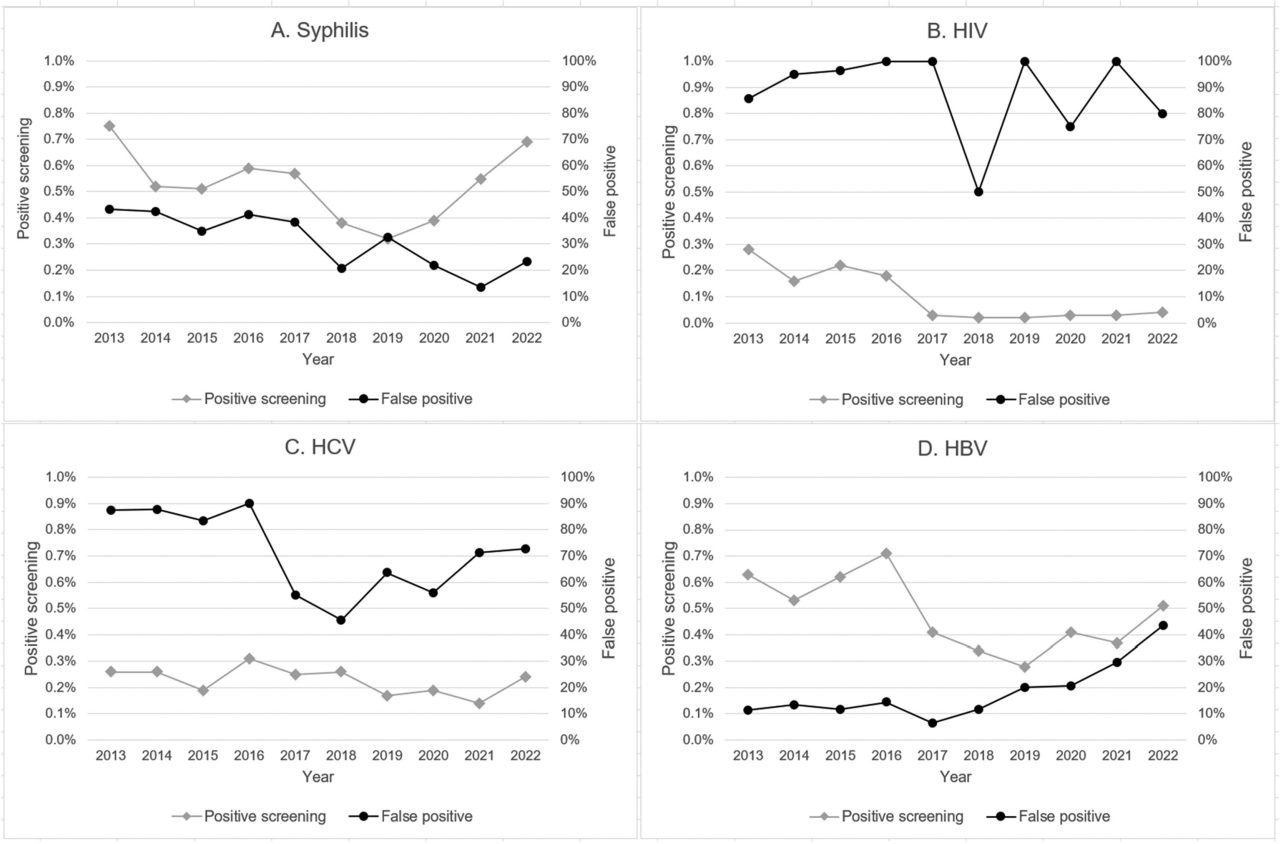

Fig. 2 – Screening and false positive results by year. HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus.

although false-positive results decreased steadily from 2017 to 2022. HBV screening reactivity decreased between 2017 and 2019 and subsequently stabilized, whereas false-positive HBV results increased slightly after 2018.

Poisson regression models assessing factors associated with false-positive and true-positive results

Tables 2–6 show the results of univariable and multivariable models assessing factors associated with false-positive results compared to true negative results. Tables 2–4 show the results of univariable and multivariable models assessing factors associated with true-positive results compared to true negative results for syphilis and HBV. Poisson models for true-positive results were not conducted for HIV and HCV due to the insufficient sample size.

In the multivariable Poisson regression model assessing factors associated with false-positive results across all serologic tests, being aged 36–45 years or >55 years was significantly associated with false positivity compared with ≤25 years. Lower education was associated with a higher likelihood of false-positive results. Additionally, first-time donors were more likely to present false-positive reactivity compared with repeat donors.

In the multivariable model evaluating factors associated with true-positive results across all infections, male sex, older age, non-white race/skin color, lower education, single or divorced/separated status, and first-time donor status were all significantly associated with this outcome (Table 2).

In the model examining factors associated with false-positive results for syphilis, being aged 36–45 years or >55 years, having incomplete college education, and being a first-time donor were independently associated with false positivity. In contrast, for true-positive syphilis results, male sex and older age (except for the 36–45-year group) were associated with true positivity. Additionally, non-white race/skin color, low educational attainment, single or divorced/separated status, and first-time donation were associated with true-positive outcomes (Table 3).

For false-positive HBV results, first-time donation was significantly associated with increased likelihood of false positivity. For true-positive HBV results, all age categories above 25 years, lower educational attainment, single or divorced/separated status, and first-time donation were significantly associated with true positivity (Table 4).

In the model evaluating HIV testing, being of the female sex, having only completed high school or less and being a first-time donor were associated with false-positive results (Table 5).

In the model addressing false-positive results for HCV testing, being aged >55 years, having only high school education or less and being a first-time donor were associated with false-positive results (Table 6).

Table 2 – Univariable and multivariable models addressing factors associated with false-positive and true-positive results for syphilis, HIV, HBV, or HCV.

	FP - PR	FP			TP	
	(95% CI)	p-value	aPR (95% CI)	p-value	aPR (95% CI)	p-value
Sex						
Female	Reference	—	Reference	—	Reference	—
Male	0.82 (0.70–0.96)	0.011	0.95 (0.81–1.11)	0.491	1.33 (1.17–1.52)	<0.001
Age category (years)						
≤25	Reference	—	Reference	—	Reference	—
26–35	0.92 (0.73–1.18)	0.517	1.25 (0.97–1.63)	0.088	1.79 (1.44–2.23)	<0.001
36–45	1.01 (0.80–1.27)	0.948	1.53 (1.15–2.04)	0.004	2.01 (1.58–2.57)	<0.001
46–55	0.78 (0.60–1.02)	0.073	1.30 (0.94–1.81)	0.114	3.44 (2.67–4.43)	<0.001
>55	0.95 (0.70–1.28)	0.746	1.72 (1.18–2.48)	0.004	4.02 (3.00–5.37)	<0.001
self-reported race/skin color						
White	Reference	—	Reference	—	Reference	—
Non-white	1.06 (0.89–1.25)	0.523	0.95 (0.80–1.14)	0.609	1.15 (1.00–1.32)	0.049
Education						
Complete college or higher	Reference	—	Reference	—	Reference	—
Incomplete college	1.32 (1.04–1.67)	0.021	1.49 (1.14–1.92)	0.003	1.01 (0.78–1.29)	0.948
≤ High school	1.45 (1.22–1.72)	<0.001	1.49 (1.24–1.78)	<0.001	1.84 (1.59–2.11)	<0.001
Marital status						
Married/Civil Union	Reference	—	Reference	—	Reference	—
Divorced/Separated	1.00 (0.71–1.37)	0.983	1.00 (0.71–1.37)	0.989	2.03 (1.63–2.52)	<0.001
Single	0.97 (0.83–1.14)	0.696	0.95 (0.78–1.16)	0.625	1.63 (1.38–1.91)	<0.001
Type of donation						
Repeat/Sporadic	Reference	—	Reference	—	Reference	—
First time	3.54 (2.98–4.22)	<0.001	3.57 (2.99–4.29)	<0.001	14.5 (11.7–18.3)	<0.001

FP: False positive; TP: True positive; PR: Prevalence Ratio; aPR: Adjusted Prevalence Ratio; CI: Confidence Interval; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus.

Table 3 – Univariable and multivariable models addressing factors associated with false-positive and true-positive syphilis serologies.

	FP - PR	FP			TP	
	(95% CI)	p-value	aPR (95% CI)	p-value	aPR (95% CI)	p-value
Sex						
Female	Reference	—	Reference	—	Reference	—
Male	0.97 (0.74–1.28)	0.816	1.09 (0.82–1.45)	0.557	1.51 (1.25–1.84)	<0.001
Age category (years)						
≤25	Reference	—	Reference	—	Reference	—
26–35	1.12 (0.71–1.80)	0.634	1.51 (0.92–2.52)	0.105	1.83 (1.38–2.42)	<0.001
36–45	1.60 (1.05–2.51)	0.032	2.43 (1.44–4.17)	<0.001	1.34 (0.95–1.89)	0.096
46–55	1.01 (0.62–1.68)	0.955	1.67 (0.91–3.08)	0.100	1.99 (1.37–2.89)	<0.001
>55	1.39 (0.80–2.40)	0.232	2.66 (1.38–5.12)	0.003	3.65 (2.43–5.44)	<0.001
self-reported race/skin color						
White	Reference	—	Reference	—	Reference	—
Non-white	0.93 (0.68–1.26)	0.652	0.84 (0.60–1.16)	0.308	1.34 (1.09–1.63)	0.004
Education						
Complete college or higher	Reference	—	Reference	—	Reference	—
Incomplete college	1.20 (0.78–1.79)	0.387	1.61 (1.00–2.49)	0.040	1.20 (0.86–1.65)	0.266
≤ High school	1.23 (0.90–1.67)	0.182	1.36 (0.97–1.87)	0.066	1.81 (1.47–2.23)	<0.001
Marital status						
Married/Civil Union	Reference	—	Reference	—	Reference	—
Divorced/Separated	0.91 (0.49–1.56)	0.755	0.93 (0.50–1.60)	0.810	3.05 (2.19–4.17)	<0.001
Single	0.84 (0.63–1.12)	0.235	0.99 (0.70–1.40)	0.974	2.20 (1.72–2.82)	<0.001
Type of donation						
Repeat/Sporadic	Reference	—	Reference	—	Reference	—
First time	2.74 (2.07–3.68)	<0.001	2.98 (2.22–4.05)	<0.001	9.47 (7.22–12.7)	<0.001

FP: False positive; TP: True positive; PR: Prevalence Ratio; aPR: Adjusted prevalence Ratio; CI: Confidence Interval.

Table 4 – Univariable and multivariable models addressing factors associated with false-positive and true-positive HBV serologies.

	FP - PR	FP			TP	
	(95% CI)	p-value	aPR (95% CI)	p-value	aPR (95% CI)	p-value
Sex						
Female	Reference	—	Reference	—	Reference	—
Male	1.02 (0.70–1.51)	0.899	1.26 (0.85–1.88)	0.252	1.17 (0.97–1.40)	0.100
Age category (years)						
≤25	Reference	—	Reference	—	Reference	—
26–35	0.57 (0.32–0.99)	0.045	0.67 (0.36–1.23)	0.198	1.85 (1.30–2.67)	<0.001
36–45	0.72 (0.43–1.22)	0.212	0.87 (0.44–1.71)	0.688	3.11 (2.16–4.53)	<0.001
46–55	0.64 (0.35–1.15)	0.137	0.85 (0.40–1.81)	0.684	5.67 (3.91–8.31)	<0.001
>55	0.63 (0.29–1.26)	0.209	0.64 (0.23–1.65)	0.378	4.60 (2.95–7.17)	<0.001
self-reported race/skin color						
White	Reference	—	Reference	—	Reference	—
Non-white	0.94 (0.60–1.43)	0.787	0.86 (0.55–1.32)	0.506	1.00 (0.82–1.22)	0.965
Education						
Complete college or higher	Reference	—	Reference	—	Reference	—
Incomplete college	1.55 (0.88–2.60)	0.110	1.53 (0.81–2.74)	0.168	0.80 (0.51–1.19)	0.290
≤ High school	1.43 (0.93–2.15)	0.096	1.33 (0.84–2.08)	0.214	1.94 (1.60–2.35)	<0.001
Marital status						
Married/Civil Union	Reference	—	Reference	—	Reference	—
Divorced/Separated	0.82 (0.32–1.75)	0.645	0.74 (0.26–1.68)	0.516	1.52 (1.11–2.04)	0.007
Single	0.89 (0.60–1.31)	0.551	0.70 (0.42–1.16)	0.177	1.29 (1.03–1.61)	0.028
Type of donation						
Repeat/Sporadic	Reference	—	Reference	—	Reference	—
First time	4.02 (2.65–6.32)	<0.001	3.91 (2.54–6.22)	<0.001	25.2 (17.5–37.8)	<0.001

FP: False positive; TP: True positive; PR: Prevalence Ratio; aPR: Adjusted prevalence Ratio; CI: Confidence Interval; HBV: Hepatitis B Virus.

Table 5 – Univariable and multivariable models addressing factors associated with false-positive HIV serology.

	PR (95% CI)	p-value	aPR (95% CI)	p-value
Sex				
Female	Reference	—	Reference	—
Male	0.60 (0.41–0.86)	0.006	0.68 (0.47–0.99)	0.044
Age category (years)				
≤25	Reference	—	Reference	—
26–35	1.20 (0.70–2.12)	0.527	1.64 (0.91–3.03)	0.108
36–45	1.01 (0.58–1.82)	0.961	1.52 (0.77–3.02)	0.229
46–55	0.84 (0.44–1.59)	0.580	1.25 (0.57–2.72)	0.570
>55	0.69 (0.28–1.51)	0.370	1.17 (0.43–2.93)	0.747
Self-reported race/skin color				
White	Reference	—	Reference	—
Non-white	1.08 (0.71–1.60)	0.720	0.90 (0.58–1.36)	0.631
Education				
Complete college or higher	Reference	—	Reference	—
Incomplete college	1.28 (0.69–2.22)	0.409	1.43 (0.74–2.61)	0.258
≤ High school	1.71 (1.15–2.53)	0.008	1.87 (1.23–2.84)	0.003
Marital status				
Married/Civil Union	Reference	—	Reference	—
Divorced/Separated	1.61 (0.80–2.97)	0.147	1.68 (0.82–3.12)	0.124
Single	1.06 (0.72–1.55)	0.772	0.93 (0.58–1.47)	0.755
Type of donation				
Repeat/Sporadic	Reference	—	Reference	—
First time	3.22 (2.17–4.88)	<0.001	3.08 (2.05–4.74)	<0.001

PR: Prevalence Ratio; aPR: Adjusted prevalence Ratio; CI: Confidence Interval; HIV: Human Immunodeficiency Virus.

Discussion

Data from >120,000 blood donors were analyzed in this 10-year cross-sectional study conducted at a major blood center in São Paulo, Brazil, to assess annual trends in positive screening and false-positive results for syphilis, HIV, HCV, and HBV. Demographic factors associated with false-positive and true-positive results were also examined. Nearly all donations had nonreactive screening results (98.7%), with reactive results most frequent for syphilis (0.5%) and HBV (0.5%).

Table 6 – Univariable and multivariable models addressing factors associated with falsely reactive HCV serology.

	PR (95% CI)	p-value	aPR (95% CI)	p-value
Sex				
Female	Reference	—	Reference	—
Male	0.74 (0.56–0.97)	0.026	0.86 (0.65–1.14)	0.293
Age category (years)				
≤25	Reference	—	Reference	—
26–35	0.90 (0.61–1.35)	0.599	1.32 (0.85–2.04)	0.215
36–45	0.76 (0.50–1.14)	0.177	1.31 (0.80–2.16)	0.288
46–55	0.67 (0.42–1.06)	0.090	1.34 (0.76–2.35)	0.304
>55	0.95 (0.57–1.56)	0.851	2.18 (1.16–4.01)	0.013
Self-reported race/skin color				
White	Reference	—	Reference	—
Non-white	1.23 (0.91–1.64)	0.166	1.13 (0.83–1.52)	0.428
Education				
Complete college or higher	Reference	—	Reference	—
Incomplete college	1.40 (0.90–2.08)	0.115	1.44 (0.90–2.23)	0.115
≤ High school	1.58 (1.17–2.13)	0.003	1.53 (1.11–2.10)	0.009
Marital status				
Married/Civil Union	Reference	—	Reference	—
Divorced/Separated	0.87 (0.44–1.55)	0.668	0.86 (0.43–1.54)	0.635
Single	1.12 (0.85–1.48)	0.422	1.07 (0.75–1.51)	0.719
Type of donation				
Repeat/Sporadic	Reference	—	Reference	—
First time	4.71 (3.42–6.62)	<0.001	4.60 (3.31–6.53)	<0.001

PR: Prevalence Ratio; aPR: Adjusted prevalence Ratio; CI: Confidence Interval; HCV: Hepatitis C Virus.

Among positive screening tests, false positivity was most common for HIV (92.0%) and HCV (72.7%).

As for temporal trends, given the relatively small number of reactive samples, year-to-year variability likely reflects random variations rather than true epidemiologic shifts, when considering positive serology and false-positive results for syphilis, HBV, and HCV. For HIV, a marked decline in screening reactivity occurred in 2017 and remained stable until 2022, likely reflecting the replacement of the bioMérieux CLIA platform and Ortho Clinical Diagnostics with assays from Abbott Diagnostics that year. Decreases in false-positive HIV results in 2018, 2020, and 2022 further support an assay-related effect.

In the multivariable Poisson regression models, female sex was associated with a higher prevalence of false-positive results in the HIV model, consistent with previous studies [14]. This pattern may be attributable to higher frequencies of autoantibodies and nonspecific antibody reactivity among women [23]. Such cross-reactivity is a known source of false-positive serologic findings [16,17,24].

Older age was also associated with false-positive results in the overall, syphilis and HCV models though not consistently across infections. This finding aligns with previous research suggesting that older individuals may produce higher titers of nonspecific antibodies after immune stimulation, which can increase assay cross-reactivity [25].

Lower educational attainment was another consistent predictor of false-positive results across most analyses. This association may indirectly reflect socioeconomic status: individuals with lower education may be more exposed to infections or immunologic stimuli, potentially increasing antibody cross-reactivity [13,26]. Lower educational attainment was also associated with true positivity, which may reflect a lower access to healthcare and diagnostic testing [27]. Importantly, limited health literacy associated with lower educational attainment [28] may influence the donor's ability to understand the meaning and implications of false-positive results, potentially increasing the risk of misinterpretation and emotional distress if results are not clearly explained.

First-time donors demonstrated the strongest and most consistent association with false-positive results. Similar findings have been reported previously [7,13,14]. This association may be partially influenced by selection bias, as donors with false-positive results may be discouraged from future donations. Repeat and sporadic donors who have previously tested negative may represent a self-selected population less likely to present false reactivity, resulting in a repeat donor population with inherently lower reactivity rates. First-time donor status was also consistently associated with true-positive results, likely driven by a similar structural mechanism: donors with prior true-positive results are permanently deferred from future donation. Lastly, another possible mechanism is associated with risk behaviors: studies show that repeat donors have fewer high-risk behaviors when compared to first-time donors [29]. Return rates among donors with false-positive results were not evaluated, which could provide further insight into donor retention and behavioral responses following reactive screening.

No significant associations were found between race/skin color or marital status and false-positive results in this study although other reports have described higher false reactivity among non-White donors [7,13,14]. Such discrepancies may reflect regional demographic differences or variations in assay performance.

Due to the small number of true-positive cases, analyses to assess factors associated with true-positive results were not

conducted for HIV and HCV. In the overall, syphilis and HBV models, male sex and older age were associated with higher prevalence of true-positive results, consistent with known epidemiologic patterns of TTIs. Non-white race/skin color was associated with true positivity in the overall and syphilis models but not for HBV. Across all models, lower educational attainment, being single or divorced/separated and first-time donation were consistently related to true positivity.

These findings reinforce the importance of demographic and behavioral screening in donor selection, while highlighting the balance between high assay sensitivity and the occurrence of nonspecific reactivity in maintaining transfusion safety. However, the lack of consistent overlap between variables associated with false-positive and true-positive results suggests that the mechanisms underlying false reactivity differ from those driving true infection risk.

False-positive serologic results are commonly attributed to nonspecific antibody cross-reactivity, which may occur following vaccination, infection, allergic reactions, autoimmune processes or exposure to transplantation antigens [16,17,24]. Differences in assay design and antigen composition can also influence specificity and lead to spurious reactivity [30].

The observed association between female sex and HIV false positivity may reflect immunologic cross-reactivity due to the higher prevalence of autoantibodies in women compared to men [23]. Similarly, the association between lower education and false reactivity may indicate increased immune stimulation from exposure to infections in lower socioeconomic contexts [26]. Older individuals may have accumulated immunologic experiences or demonstrate more pronounced polyclonal antibody responses, contributing to the age-related trend [25].

Although false positivity can also result from incomplete antibody responses during early infection - where screening is reactive but confirmatory testing is negative [18–20] - the lack of consistent overlap between predictors of false and true positivity in the present study suggests that this mechanism was not the predominant cause.

This study has several limitations. Results from subsequent donations of participants who experienced prior false-positive outcomes were not compared. This omission limits the possibility to analyze the underlying mechanisms that drive these occurrences. On false-positive results, additional testing was not conducted to exclude incipient infections, neither was testing to explore cross-reactivity. Although nucleic acid testing (NAT) is routinely performed in the BCO, these data were not included, and some cases classified as false positive may represent early window-period infections. However, given the low prevalence of such window-period infections, this potential misclassification is unlikely to substantially affect the overall findings [31]. Moreover, the change in HIV screening assays in 2017 led to an immediate reduction in reactivity rates, which may limit the interpretation of time trends for this infection. No similar assay transitions occurred for the other infections. Additionally, the study did not collect behavioral or health-related variables, such as sexual activity, history of infections or chronic diseases, which could influence false-positive rates. Nonetheless, this large ten-year dataset provides a robust overview of serologic reactivity trends and associated factors among Brazilian blood donors.

Conclusions

The findings of this study demonstrate that certain demographic characteristics, particularly first-time donation and lower educational attainment, are consistently associated with a higher prevalence of false-positive serologic results in blood donor screening. Older age showed a weaker and less consistent association. The lack of overlap between predictors of false-positive and true-positive results supports the hypothesis that most false-positive findings arise from cross-reactive antibody mechanisms rather than incomplete antibody responses.

These results underscore the need for further studies investigating immune and technical mechanisms underlying false reactivity in blood donation screening. Future research should also explore the psychological and operational impacts of false-positive results and evaluate strategies to support donor retention following false reactivity notifications.

Funding

The authors received no specific funding for this work

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

L.K.S. performed the research, analyzed the data, wrote the first draft of the manuscript and reviewed and edited the manuscript. L.D.S. collected data, designed the study and reviewed and edited the manuscript. V.F.D. collected data, designed the study, supervised the research and reviewed and edited the manuscript. J.M.K. collected data, supervised the research and reviewed and edited the manuscript. C.B.B. designed the study, supervised the research and reviewed and edited the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

We thank Vivian Iida Avelino-Silva for assisting in the idealization and revision of the study.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Decoding *BCR::ABL1* kinase domain mutations in chronic myeloid leukemia: Implications for tyrosine kinase inhibitor resistance in a South Asian population

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ARTICLE INFO

Article history:
Received 31 December 2025
Accepted 21 April 2026
Available online 28 July 2026

Keywords:
Chronic myeloid leukemia
Tyrosine kinase inhibitors
Philadelphia chromosome
Drug resistance

ABSTRACT

Introduction: Treatment resistance has become increasingly common in patients with chronic myeloid leukemia. Lack of response to tyrosine kinase inhibitors is associated with mutations in the *BCR::ABL1* kinase domain. This study aims to report the frequency and types of *BCR::ABL1* kinase domain mutations in Pakistani chronic myeloid leukemia patients.

Materials and methods: This prospective study was conducted from January to June 2025 at the Armed Forces Institute of Pathology in Pakistan. It included adult patients with chronic myeloid leukemia who were non-responsive to treatment based on molecular, hematological, and clinical criteria. Allele-specific real-time polymerase chain reaction was performed to detect four kinase domain mutations: T315I, E255V, E255K and Y253H. Data was analyzed using IBM SPSS v23.

Results: The mean age of the 133 treatment-resistant chronic myeloid leukemia patients included in the study was 47.3 ± 13.6 years. The majority of cases (62.4%) were male and 79 (59.4%) patients were currently on imatinib therapy. The median *BCR::ABL1* level, as measured by quantitative polymerase chain reaction, was 30.73% (range: 1.17–100%) International Scale. *BCR::ABL1* kinase domain mutations were detected in 57 (42.9%) patients. E255K was the most common mutation detected in 45 (33.8%) cases, followed by E255V in 11 (8.3%), T315I in 1 (0.8%) and the Y253H mutation in 1 (0.8%) patient. The presence of kinase domain mutations was significantly associated with higher total leucocyte count ($p = 0.002$), while the E255K mutation was significantly associated with blast crisis ($p = 0.048$).

Conclusion: This study revealed a high prevalence of *BCR::ABL1* kinase domain mutations in Pakistani chronic myeloid leukemia patients. E255K, the most frequently detected mutation, was significantly associated with blast crisis. These findings underscore the significance of molecular testing for personalized treatment.

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https://doi.org/10.1016/j.htct.2026.106502

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Introduction

Chronic myeloid leukemia (CML) is a hematological malignancy characterized by a reciprocal translocation between the *ABL1* gene on chromosome 9 and *BCR* gene on chromosome 22, resulting in the formation of the Philadelphia Chromosome [1]. This *BCR*::*ABL1* fusion gene codes for a protein that has constitutive tyrosine kinase activity, leading to uncontrolled cell proliferation [2]. According to the Global Burden of Disease database, the annual incidence of CML was approximately 35,380 cases in 2021, with a rising trend seen in countries with lower socio-demographic indexes [3]. Currently, CML accounts for approximately 15% of adult leukemias [4].

The advent of specific targeted therapy in the form of tyrosine kinase inhibitors (TKIs) represents a major breakthrough in the treatment of CML, drastically improving outcomes and life expectancy [5]. In 2001, imatinib mesylate was the first TKI to be approved by the Food and Drug Administration (FDA) for the management of newly diagnosed CML [6]. Based on guidelines from the European Leukemia Network (ELN) and National Comprehensive Cancer Network (NCCN), imatinib mesylate remains a standard first-generation TKI and a primary treatment option for treatment-naïve patients with CML [7,8].

However, in recent years, many studies have highlighted an emerging concern of resistance to TKI therapy among CML patients, with poor response to imatinib mesylate seen in approximately one-quarter of patients [9]. Resistance to TKI therapy is multifactorial, related to factors such as patient adherence to therapy, efficacy of drug metabolism and the presence of additional chromosomal abnormalities which confer a poor prognosis [10].

One of the most common causes of TKI resistance in CML is the presence of amino acid substitutions in the *BCR*::*ABL1* kinase domain (KD) [11]. With over 90 different KD mutations reported to date, these mutations cause TKI resistance by impairing binding of the drug to the BCR::ABL1 chimeric protein [12]. The identification of *BCR*::*ABL1* KD mutations has significant clinical implications - each mutation detected has a unique pattern of response to TKIs, therefore patient care should be tailored according to their specific KD mutation profile [13].

In the era of precision medicine, the detection of *BCR*::*ABL1* KD mutations is necessary to provide targeted therapy in CML. Therefore, the aim of this study is to report the frequency and types of *BCR*::*ABL1* KD mutations among Pakistani patients diagnosed with CML.

Methods

This study was conducted from July 2024 to October 2025 at the Armed Forces Institute of Pathology, Pakistan. Adult patients diagnosed with CML who showed a poor response to the first-generation TKI imatinib were prospectively enrolled. Patients were included as confirmed cases of CML on the basis of the diagnostic criteria specified by the World Health Organization (WHO). Patients who failed to achieve a complete hematological response (CHR), complete cytogenetic response (CCR) or major molecular response (MMR) to treatment were included. Molecular response to frontline TKI treatment was defined based on criteria recommended by the European LeukemiaNet (ELN) guidelines [7].

- *BCR*::*ABL1* transcript levels >10% at three months after initiating TKI therapy.
- *BCR*::*ABL1* transcript levels >1% at six months after initiating TKI therapy.
- *BCR*::*ABL1* transcript levels >0.1% at 12 months after initiating TKI therapy.
- Loss of major molecular response or detection of additional cytogenetic abnormalities at any time during treatment.

Patients who were started on imatinib at the time of diagnosis and subsequently empirically switched to second line treatment due to poor tolerance and excessive side effects were also included.

For the detection of *BCR*::*ABL1* KD mutations, a 3-mL peripheral blood sample was collected in an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. From this sample, RNA was extracted using the Thermo Fisher RNA Extraction kit, which works on the principle of silica column-based extraction of total RNA. After RNA extraction, complementary DNA (cDNA) was prepared. The quantity and quality of cDNA was assessed using a Microvolume Spectrophotometer (BioTek Epoch), with target cDNA quantity greater than 10 ng/μL and an A260/A280 ratio of 1.6:2.0 as acceptable DNA purity.

To identify mutations within the *BCR*::*ABL1* KD, allele-specific real-time PCR was carried out using cDNA. Primer sequences for four common KD mutations, namely T315I, E255V, E255K and Y253H, were derived from a previous study by Wang et al. [14]. This study tested for these four mutations due to their significant therapeutic implications and high prevalence reported in previous studies. Initially, the results of twenty samples were further confirmed by Sanger sequencing. Subsequently, aliquots of patient cDNA that tested positive for KD mutations were prepared and stored at -20 °C to be used as positive controls in each run. Normal samples were used as negative controls.

A structured data collection form was designed to collect information regarding patient demographics, laboratory parameters, history of TKI therapy and the presence of *BCR*::*ABL1* KD mutations. Data was entered and analyzed using the IBM Statistical Package for Social Sciences (SPSS) Software v23.

This study was initiated after receiving ethical approval from the Institutional Review Board (IRB) of the Armed Forces Institute of Pathology. Patient confidentiality was maintained throughout the study, and no external funding was received.

Results

A total of 780 diagnosed cases of CML presented to our center during the study period. Among them, 133 patients with poor response to therapy were included in this research. The mean age of the study participants was 47.3 $\pm$ 13.6 years and the

majority (*n* = 83; 62.4%) were male. The median time since diagnosis was 12 months (range: 1–246 months). Most of the patients (*n* = 108; 81.2%) were in the chronic phase of CML at the time of testing for KD mutations, while 12 (0.9%) cases had progressed to blast crises.

The median quantitative PCR for the *BCR::ABL1* transcript level was 30.73% (range: 1.17 – 100%) International Scale. The primary indication for *BCR::ABL1* KD mutational analysis was an inadequate molecular response to TKI treatment (52%; *n* = 69). Additional triggers for testing included a failure to achieve hematological response (13.5%), progression to high-risk CML or blast crisis (12%), and clinical suspicion (22.5%).

Most of the patients (*n* = 79; 59.4%) were on imatinib therapy at the time of KD mutation analysis, while the remaining had been empirically switched to second or third generation TKIs based on clinical suspicion of TKI resistance. Of these, 23 (17.3%) were receiving nilotinib, five (3.8%) dasatinib, two (1.5%) ponatinib and one (0.8%) patient was on asciminib therapy. One patient (0.8%) had undergone hematopoietic stem cell transplantation due to failure of multiple therapies.

Of the 133 patients tested, 57 (42.9%) showed the presence of *BCR::ABL1* KD mutations. E255K was the most frequently detected mutation in 45 (33.8%) patients. Furthermore, E255K was the only KD mutation identified in patients with progression to blast crisis. Figure 1 shows the proportions of *BCR::ABL1* KD mutations detected in the study population.

Table 1 summarizes the clinicopathological characteristics of the study population based on their *BCR::ABL1* KD mutation status.

Discussion

This study assessed the presence of four common *BCR::ABL1* KD mutations in Pakistani CML patients. Based on existing reports, mutations affecting the *ABL* KD have emerged as the predominant driver of TKI resistance in CML [15]. *BCR::ABL1* KD mutation analysis is recommended by both the National Comprehensive Cancer Network (NCCN) and European LeukemiaNet (ELN) guidelines for patients who are not achieving an adequate response to TKI therapy [7,16].

The overall frequency of *BCR::ABL1* KD mutations was 42.9%. There is a wide variation in the literature regarding the frequency of *BCR::ABL1* KD mutations in different patient populations (Table 3) with the reported prevalence ranging from 13%–71%. The prevalence depends on many factors such as the patient demographics, method of detection, TKIs used, phase of disease and other clinicopathological characteristics [17–22].

This study found a high prevalence of the E255K mutation, seen in approximately one-third of the study population. The E255K point mutation at codon 255 in the *ABL1* KD results in a single amino acid substitution of glutamic acid by lysine in the subsequent protein sequence. This mutation, located in the phosphate binding loop (P-loop) of the *ABL* KD, confers resistance to imatinib and is associated with a poor prognosis [23]. According to the latest ELN guidelines published in 2025, the recommended TKI to use in patients harboring this mutation is ponatinib, while dasatinib or asciminib may also be used [7].

This finding aligns with a study from Iran, which also identified E255K as the most common *ABL1* KD mutation in 20% of patients with TKI resistance [17]. However, while E255K was the most frequently encountered mutation in the present cohort, many studies have identified the T315I mutation as the most common variant within the *BCR::ABL1* KD [18–20]. The T315I mutation comprises a single amino acid substitution of threonine by isoleucine at codon 315 of the *ABL* KD. It is considered a 'gatekeeper' mutation because its location is at the position where TKIs form hydrogen bonds, which enables stable drug binding [24].

In this study, a higher total leucocyte count was significantly associated with presence of *BCR::ABL1* KD mutations. Furthermore, the presence of the E255K mutation was significantly associated with blast crisis in CML patients. A study from China reported several factors that were associated with the development of *BCR::ABL1* KD mutations including young age, male gender and advanced phase of disease [25]. However, these variables did not reach statistical significance in the current cohort. Such discrepancies may be attributed to population diversity; nonetheless, methodological factors, specifically testing techniques and follow-up strategies, must also be considered.

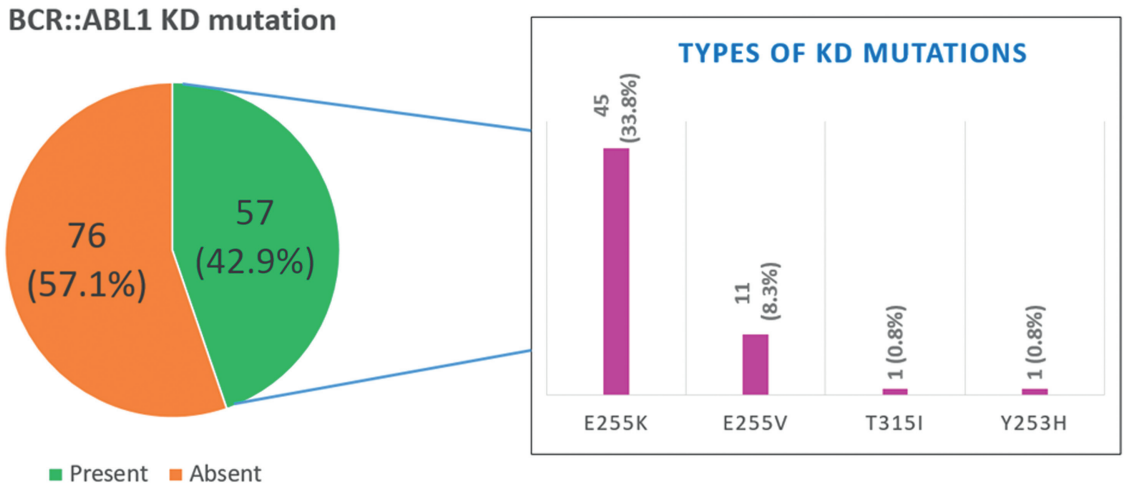

Figure 1 – Frequency and types of *BCR::ABL1* kinase domain mutations in the study population.

Table 1 – Clinicopathological characteristics of the study population according to presence of BCR::ABL1 kinase domain mutations.

	BCR::ABL1 KD Mutation		p-value
	Present (n = 57)	Absent (n = 76)	
Gender			
Male - n (%)	37 (65)	46 (60.5)	0.823
Age (years) - (Mean ± SD)	48.2 ± 14.1	46.6 ± 13.2	0.523
Duration of CML (months) - Median (range)	13.5 (1 – 246)	12.0 (1 – 192)	0.425
BCR::ABL1 qPCR (% IS) - Median (range)	30.73 (1.17 – 100.00)	30.64 (1.53 – 100)	0.553
Hemoglobin (g/dL) - (Mean ± SD)	11.49 ± 2.47	11.54 ± 2.24	0.906
Total leucocyte count (× 10^9/L) - Median (range)	**10.2 (3.6 – 237.0)**	**7.6 (0.9 – 330.0)**	**0.002**
Platelet count (× 10^9/L) - Median (range)	253 (21 – 1173)	215 (11 – 1577)	0.608
TKI used - n (%)			
Imatinib	33 (57.9)	46 (60.5)	0.350
Other	16 (28.1)	15 (19.7)	
Blast crisis - n (%)	7 (12.3)	5 (6.6)	0.240

CML: Chronic myeloid leukemia; TKI: Tyrosine kinase inhibitors.

The presence of *BCR::ABL1* KD mutations was significantly associated with a higher median total leucocyte count (p = 0.002) (Table 1). Further analyses identified factors associated with the presence of E255K, the most frequent type of *BCR::ABL1* KD mutation in this study population (Table 2). The E255K mutation was significantly associated with higher TLC (p = 0.001) and progression to blast crisis (p = 0.048).

Table 2 – Clinicopathological characteristics associated with presence of E255K mutation.

	E255K Mutation		p-value
	Present (n=45)	Absent (n=88)	
Gender			
Male - n (%)	28 (62.2)	55 (62.5)	0.779
Age (years) - (Mean ± SD)	47 ± 14.8	47.4 ± 13.0	0.866
Duration of CML (months) - Median (range)	15 (1 – 246)	10.5 (1 – 192)	0.247
BCR::ABL1 qPCR (%IS) - Median (range)	40.18 (1.17 – 100)	30.08 (1.53 – 100)	0.226
Hemoglobin (g/dL) - (Mean ± SD)	11.16 ± 2.32	11.70 ± 2.33	0.244
Total leucocyte count (× 10^9/L) - Median (range)	**12.8 (3.6 – 237.0)**	**7.7 (0.9 – 330.0)**	**0.001**
Platelet count (× 10^9/L) - Median (range)	237 (21– 1173)	215 (11 – 1577)	0.738
TKI used - n (%)			
Imatinib	25 (55.6)	54 (61.4)	0.182
Other	14 (31.1)	17 (19.3)	
Blast crisis - n (%)	**7 (15.6)**	**5 (5.7)**	**0.048**

CML: Chronic myeloid leukemia; TKI: Tyrosine kinase inhibitors.

Table 3 – Summary of previous studies reporting the frequency of BCR::ABL1 kinase domain (KD) mutations.

Author	Year	Country	n	Frequency of KD mutations	Most common mutations
Mahboobeh S et al. [17]	2021	Iran	50	13 (26.0%)	E255K (20%)
Hasanova et al. [18]	2023	Azerbaijan	163	22 (13.4%)	T315I (5%)
Elias et al. [19]	2014	Malaysia	125	28 (22.4%)	T315I (7.2%) E255K (3.2%)
Datta et al. [20]	2024	India	108	62 (71%)	T315I (46%)
Kizilors et al. [21]	2019	UK	121	25 (21%)	F317L (20%) M244V (14%)
Jabbour et al. [22]	2009	USA	169	86 (51%)	G250E (16%)

Table 3 summarizes the results of previous studies that have reported the frequency and types of *BCR::ABL1* KD mutations in different patient populations.

Allele-specific RT-PCR was used for detecting these mutations; this is a cost-effective strategy that is feasible in low-resource settings. A recent study which compared the diagnostic utility of RT-PCR with Next Generation Sequencing (NGS) found that PCR gave highly sensitive and reliable results, detecting mutations at an estimated variant allele frequency as low as 0.01% [26]. Despite its high sensitivity, this methodology is restricted to a predefined panel of mutations. Unlike Sanger sequencing or NGS, which can screen the entire

kinase domain, allele-specific PCR is limited by the specificities of the allele-specific primers employed [27].

The findings of this study highlight the importance of *BCR*::*ABL1* KD mutation testing and provide the foundation for personalized treatment strategies. However, future studies should assess the presence of these mutations in a large sample employing NGS, which would allow the detection of the full spectrum of *BCR*::*ABL1* KD mutations.

Conclusion

This study identified a high frequency of *BCR*::*ABL1* KD mutations within this CML population; notably, E255K was the most prevalent variant and was significantly associated with blast crisis. This report of *BCR*::*ABL1* KD mutations in Pakistani CML patients is essential to provide targeted treatment. These findings should be validated in a larger cohort using NGS to capture a broader spectrum of *BCR*::*ABL1* KD mutations.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

None of the authors have any conflicts of interest to declare.

Editor

Erich Vinicius De Paula

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Original Article

Immature platelets: A useful tool for thrombocytopenia etiology classification in adults

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ARTICLE INFO

Article history:
Received 20 February 2026
Accepted 17 April 2026
Available online 21 May 2026

Keywords:
Immature platelet fraction
Reticulated platelets
Thrombocytopenia
Laboratory automation

ABSTRACT

Introduction: The etiology of thrombocytopenia is diverse with heterogenous clinical manifestations which demand the correct diagnosis and appropriate management. The immature platelet fraction is associated with new platelet release from the bone marrow by thrombopoiesis. The clinical utility of this low-cost, feasible measurement has been demonstrated across various conditions. This study aimed to assess the immature platelet fraction and count as diagnostic tests to distinguish between the two main causes of thrombocytopenia in selected hematology disorders.

Methods: A total of 444 participants were evaluated: Group 1 (n = 179; healthy blood donors), Group 2 (n = 131; peripheral platelet destruction or consumption, including immune thrombocytopenia and thrombotic thrombocytopenic purpura), and Group 3 (n = 134; bone marrow failure secondary to aplastic anemia, chemotherapy, acute leukemia, or multiple myeloma).

Results: Median immature platelet fraction (%) and immature platelet count ($\times 10^9$/L) values were 3.3/8.1 (Group 1), 16.5/6.1 (Group 2), and 5.0/1.8 (Group 3), with significant intergroup differences between Groups 2 and 3 (p-value <0.001). Optimal diagnostic cut-offs were 8.3% for immature platelet fraction (AUC = 0.8585) and 3.1 $\times$ 10^9/L for immature platelet count (AUC = 0.7687). The immature platelet fraction was highest in immune thrombocytopenia patients and showed significant inverse correlations with platelet counts in Groups 1 and 2 (p-value <0.001).

Conclusion: Immature platelet fraction and immature platelet count can be useful to discriminate between the main etiologies and should be included in the routine of hematology centers for screening thrombocytopenia.

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https://doi.org/10.1016/j.htct.2026.106469

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Introduction

Thrombocytopenia is a clinical condition defined by a platelet count (PLT) below 150 × 10^9/L. Since platelets are responsible for primary hemostasis and the maintenance of the integrity of the blood vessel walls, platelet counts lower than 20 × 10^9/L represent a tendency of spontaneous bleeding. Despite the intensity of the thrombocytopenia, the clinical heterogeneity observed in patients is elevated and the bleeding tolerance varies according to its etiology. The main mechanisms for a low PLT are increased destruction or consumption of platelets and decreased production. Typical examples of the latter are the bone marrow (BM) failure syndromes (e.g., aplastic anemia [AA], myelodysplastic syndromes [MDS], and chemotherapy-induced thrombocytopenia), or marrow-infiltrative processes (e.g., acute leukemia [AL], multiple myeloma [MM]). Increased destruction or consumption, on the other hand, is seen in conditions such as immune thrombocytopenia (ITP) and the thrombotic microangiopathies (TMA) [1–3].

Thrombocytopenia management is tailored to the care setting, typically categorized by outpatient, inpatient, or emergency department requirements [2]. In addition, an understanding of the underlying pathophysiological processes involved in thrombocytopenia and the differential diagnoses is key to appropriate management. Platelet transfusions, for example, are not helpful for ITP yet they remain a mainstay of prophylactic care in AA [1,4].

Establishing the cause of thrombocytopenia has obvious clinical repercussions but is sometimes quite challenging. Beyond the clinical history and physical examination, the laboratory plays a critical role in the diagnostic workup. This includes an extensive investigation of biomarkers for coagulation screening, biochemical profiles, and peripheral blood smear examination. If the etiology of thrombocytopenia is unclear, a BM aspirate and biopsy should be performed to preclude a primary BM disorder [2]. Quantification of plasma glycocalicin and thrombopoietin levels via enzyme-linked immunosorbent assay (ELISA) can be helpful to assess platelet turnover [5]. Platelet indices have also been useful for this assessment but are not standardized and their analysis is affected by storage time [6]. However, 'young platelets' have been reported to be an effective biomarker for distinguishing between increased destruction or consumption of platelets and decreased production [7–10].

Initially identified as reticulated platelets and quantified via flow cytometry, these cells were difficult to adopt in routine practice due to significant technical challenges and a lack of international standardization [11,12]. Advances in fluorescence-based platelet detection on fully automated hematology analyzers have made it possible to quantify the fraction of RNA-rich immature platelets, known as the immature platelet fraction (IPF). This parameter is now available as part of a routine complete blood count (CBC), providing rapid results in approximately one minute at a low cost. It is analogous to the reticulocytes observed in erythropoiesis due to recent release of platelets into the bloodstream from the BM and represents the rate of thrombopoiesis, that is, a surrogate marker for the assessment of BM function [8].

Elevated IPF levels have been reported in cases of thrombocytopenia secondary to increased peripheral destruction, reflecting a compensatory BM response characterized by accelerated thrombopoiesis. Diminished IPF has also been found in cases of 'hypoproductive' thrombocytopenia [9,10]. This study assessed the IPF and immature platelet count (IPC) in selected hematological disorders to establish their clinical utility in the diagnosis of patients with thrombocytopenia and contribute to the standardization of these parameters in the differentiation of two main etiologies.

Method

Study design

This cross-sectional diagnostic accuracy study was developed in the Hematology and Hemotherapy Foundation of Pernambuco, northeastern Brazil from June 2023 to July 2024. The study population consisted of three adult cohorts: healthy blood donors (Group 1, n = 179); patients with peripheral platelet destruction or consumption (Group 2, n = 131), including various clinical phases of ITP and acute thrombotic thrombocytopenic purpura (TTP); and patients with impaired platelet production (Group 3, n = 134), encompassing AA, MM, and AL at diagnosis or during chemotherapy.

Inclusion criteria were defined as: (i) adults aged >18 years; (ii) patients with a platelet count <150 × 10^9/L; (iii) a confirmed diagnosis of ITP, TTP, AL, AA, or MM; (iv) patients with acute myeloid leukemia on day 14 or 15 post-induction therapy with daunorubicin and cytarabine (DA 3+7); and (v) healthy blood donors with a normal CBC. Exclusion criteria were the presence of conditions which cause thrombocytopenia such as infection, liver diseases, folate or vitamin B12 deficiency and pseudo thrombocytopenia observed by the microscope evaluation of blood smears.

This study commenced after the approval of the local Ethics and Research Committee (project registration number CAAE 67609023.4.0000.5195) and approval (number 6.077.121). The blood samples of the patients were part of the routine diagnostic workup. All patients with AL and TTP came from the emergency department, while most of the patients with ITP and MM came from the outpatient clinic and all chemotherapy patients came from the hematology ward. All blood donors and patients who were invited for the research signed an informed consent form.

Sample collection

Samples of 4 mL of peripheral blood were obtained through venipuncture using vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) K3 (Cral, São Paulo, Brazil). Blood samples were gently inverted to ensure homogenization, stored at room temperature, and transported for analysis within 12 h.

Measurement of immature platelet fraction and other variables

The PLT was performed in a Sysmex XN-1000 full-automated hematology analyzer, (Sysmex Corporation, Kobe, Japan) employing the fluorescent method with a dedicated channel

Table 1 – Descriptive analysis of healthy individuals and patient groups.

	Group 1 (n = 179)	Group 2 (n = 131)	Group 3 (n = 134)
Age (years) – median (range)	34 (27–46)	45 (28–57)	52.5 (35–68)
Male – n (%)	125 (69.8)	29 (22.1)	59 (44.0)
Female – n (%)	54 (30.2)	102 (77.9)	75 (56.0)
Hemoglobin (g/dL) - median (IQR)	14.8 (13.7–15.7)	13.5 (12.3–14.4)	8.2 (7.1–9.6)
WBC ($x10^9$/L) - median (IQR)	7.0 (6.0–8.2)	8.1 (6.6–10.6)	5.4 (2.3–17.1)
PLT ($x10^9$/L) - median (IQR)	256 (230–293)	38 (14–89)	40 (17–78)
		39 (15.0–81.0) *	
IPF (%) - median (IQR)	3.3 (2.2–4.7)	16.5 (9.7–27.1)	5.0 (2.8–9.0)
		9.2 (4.4–18.4) *	
IPC ($x10^9$/L) - median (IQR)	8.1 (5.9–11.8)	6.2 (2.9–9.9)	1.8 (0.7–3.8)
		3.4 (1.3–7.6) *	

PLT: Platelet Count; WBC: (White blood cells; IPF: Immature platelet fraction; IPC: Immature platelet count; IQR: interquartile range.

* Measurements were performed on all thrombocytopenic patients, regardless of underlying etiology.

for this purpose. Fluorescence flow cytometry utilizes an oxazine-based fluorescent dye and a semiconductor laser to identify and quantify platelets. The dye penetrates the cell membrane to stain nucleic acids within the rough endoplasmic reticulum and mitochondria, allowing for the differentiation of immature platelets based on their higher fluorescence intensity. Two-dimensional scattergrams were plotted based on the collected data, with forward scattered light (FSC) and side fluorescent light (SFL) representing the Y-axis/cell volume and X-axis/cytoplasmic RNA, respectively. Thus, the IPF is determined by an algorithm which discriminates the larger platelets with intense RNA content from mature platelets. The IPC was calculated by multiplying the IPF by the PLT and dividing by 100. White blood cells (WBC) and hemoglobin were measured in the same analyzer. To assure the performance of the analyzer, three commercial internal quality control samples (Sysmex e-Check) were run before beginning the laboratory routine. Demographic data (age and sex) were obtained from electronic medical records for patients and electronic blood bank records of blood donors.

Statistical analysis

Data were analyzed using the STATA SE-64 statistical software package (Stata Corp LP, Texas, USA). The normality of the quantitative variables was assessed using the Shapiro-Wilk's test. As the distribution of PLT, IPF, and IPC deviated from normality across all groups, these variables are expressed as medians and interquartile ranges (IQR). Differences of these variables between the groups were evaluated by the Kruskal-Wallis and Dunn's post hoc tests and then expressed as box plots. Receiver operating characteristic (ROC) curves were constructed to determine the cut-off value for IPF and IPC to differentiate Group 2 from Group 3 with the area under curve (AUC) being calculated with a 95% confidence interval (95% CI). Correlation analyses between PLT and IPF for the three groups used the Spearman correlation test and are expressed as scatter plots. A p-value ≤ 0.05 was considered statistically significant in all analyses.

Results

A total of 444 participants were evaluated in this study. Of these, 265 had thrombocytopenia and were allocated to groups according to their diagnosis: elevated destruction/consumption of platelets (Group 2) and reduced platelet production (Group 3). A predominance of female participants was observed in Group 2, whereas the highest median age was recorded in Group 3. The median IPF of Group 1 was 3.3% (Range: 0.6%–14.1%). Demographic characteristics of the three groups are summarized in Table 1. The analysis based on the patient diagnosis shows that the median IPF was highest in ITP (16.5%) and lowest in AA. Regarding Group 3, the highest median IPF was in AL (6.4%), followed by MM (4.6%), AA (3.4%) and chemotherapy (1.5%). Data of the immature platelet parameters are shown in Table 2.

There was no significant difference between PLT in the thrombocytopenic groups. Patients in Group 2 had significantly higher median IPF compared to the other groups. Additionally, the IPC was significantly different between the groups (Figure 1).

Table 2 – Platelet parameters of patient groups according to the blood disorders.

	Group 2 (n = 131)		Group 3 (n = 134)			
	ITP (n = 122)	TTP (n = 9)	AL (n = 84)	AA (n = 25)	AML + chemotherapy (n = 8)	MM (n = 17)
PLT ($x10^9$/L)	41 (16–93)	14 (9–17)	31 (11.5–60)	47 (23–116)	22 (14.5–44)	82 (66–122)
IPF (%)	16.9 (10.5–28)	9 (6.8–18.1)	6.4 (3.3–12)	3.4 (2.6–9.2)	1.5 (1.0–2.0)	4.6 (2.8–7.3)
IPC ($x10^9$/L)	6.5 (3.5–9.8)	1.2 (0.6–2.3)	1.7 (0.7–3.3)	2 (1.2–3.4)	0.4 (0.2–0.8)	4.2 (2.3–5.2)

PLT: Platelet Count; IPF: Immature platelet fraction; IPC: Immature platelet count; ITP: Immune thrombocytopenia; TTP: Thrombotic thrombocytopenic purpura; AA: Aplastic anemia; AL: Acute leukemia; AML Acute myeloid leukemia; MM: Multiple myeloma.
Data expressed as median and interquartile range (IQR).

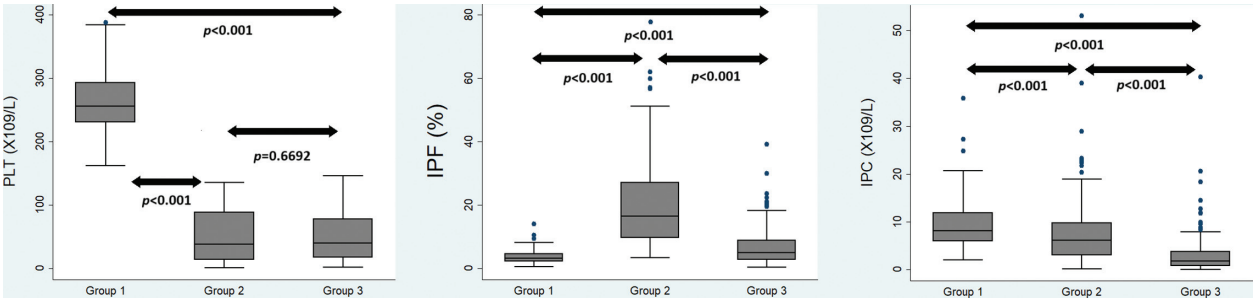

Figure 1 – Comparison of platelet count (PLT) and immature platelet parameters across the study cohorts: Group 1 (healthy controls), Group 2 (thrombocytopenia secondary to increased destruction or consumption), and Group 3 (thrombocytopenia secondary to bone marrow failure). IPF: Immature platelet fraction; IPC: Immature platelet count.

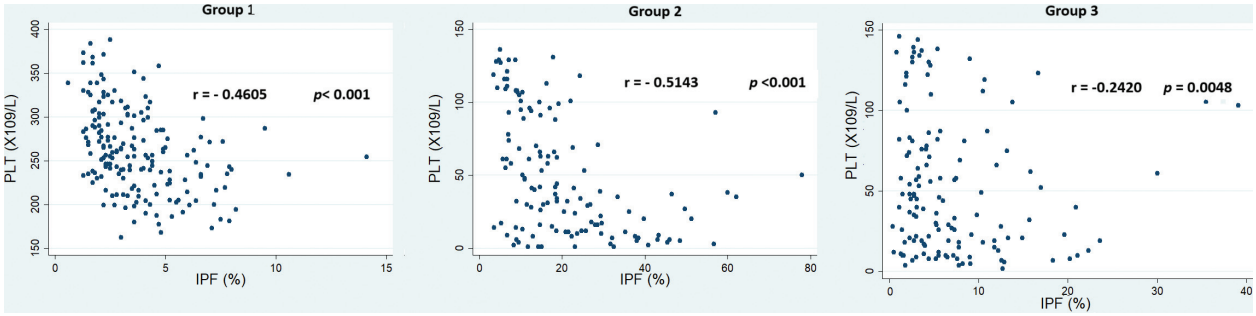

Figure 2 – Distribution of the immature platelet fraction (IPF%) in relation to the platelet count (PLT) across the three study groups: Group 1 (healthy individuals); Group 2 (thrombocytopenia secondary to increased platelet destruction or consumption); and Group 3 (thrombocytopenia secondary to bone marrow failure).

The immature platelet fraction in thrombocytopenic patients was nearly 300% higher than in healthy subjects. This finding does not indicate that the decrease in the PLT always correlates with an increase in immature platelets. Figure 2 illustrates an inverse correlation between IPF and PLT across all groups. This relationship was moderate in Groups 1 and 2, whereas Group 3 exhibited a significantly weaker association.

The diagnostic accuracy of the IPF and IPC measurements for the etiological distinction of the thrombocytopenia was estimated using ROC analysis (Figure 3). The AUC for the differentiation between Groups 2 and 3 were 0.8585 (95% CI: 0.8153–0.9017; p-value <0.001) for IPF and 0.7687 (95% CI: 0.7101–0.8273; p-value <0.001) for IPC. Both parameters were statistically higher in cases of increased destruction or consumption of platelets than in diminished production of platelets. The optimal IPF and IPC values for discriminating between the groups were determined using the ROC curve. For IPF, a cut-off set at 8.3% yielded a sensitivity of 83.2% and a specificity of 73.1%. For IPC, a cut-off set at 3.1×10^9/L yielded a sensitivity of 72.5% and a specificity of 67.9%.

Discussion

Immature platelets are newly produced platelets from BM with a high RNA content with its quantity mirroring thrombopoiesis. These platelets were photographed by electron microscopy over a dozen years ago; they have been shown to have longevity and to be highly biosynthetically active [13]. In clinical conditions characterized by increased platelet turnover and megakaryocyte activity, the IPF can be rapidly quantified via fully automated hematology analyzers. This parameter is measured simultaneously with the standard CBC.

Over the last twenty years, a great number of studies have been developed demonstrating the change in the IPF in different clinical conditions including thrombocytopenia [8].

In Group 1, the median IPF was comparable to the values reported by Ali et al. [14]; despite this cohort being significantly smaller than theirs (n = 2292) and not exclusively limited to blood donors for healthy subject selection, both studies utilized analyzers from the same manufacturer. Furthermore, the same methodology for outlier removal was employed utilizing standard CBC reference ranges. Imperiali et al. followed the Clinical and Laboratory Standard Institute (CLSI) guidelines to enroll a normal group with a similar sample size (n = 153) and mean PLT and found that the median IPF and IPC were comparable with the present study [15]. A lower median IPF and IPC were observed in another study with a smaller sample size (n = 105) carried out using a different series of the analyzer within 30 min of sample collection [9]. Although the current study analyzed blood samples stored at room temperature for a maximum of 12 h, no significant variation had been reported for the stability of assays during 48 h of observation in previous studies [7,16].

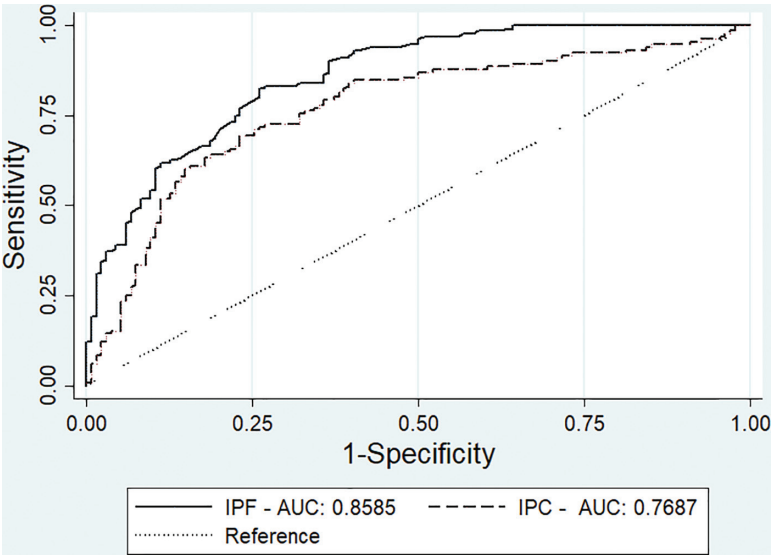

Figure 3 – Receiver operating characteristics (ROC) curves and areas under the curve for immature platelets. IPF: Immature platelet fraction; IPC: Immature platelet count.

Given the high frequency of ITP patients in Group 2, the findings of this study can be compared with research that only included ITP patients. Similar to the present study, the median IPF in these studies was significantly higher than in other clinical groups [9,17,18]. ITP is characterized by immune mechanisms that boost platelet destruction thereby shortening platelet lifespan, consequently causing the percentage of immature platelets to increase. The diagnosis is still reached by the exclusion of other causes of thrombocytopenia, a process that carries a considerable risk of misdiagnosis [19]. Some previous diagnostic criteria warranted revision, such as the lack of specificity of platelet-associated IgG and the absence of specific findings in the BM examination [20]. Since there is no gold standard laboratory marker for this disorder, IPF is potentially useful to differentiate ITP from hereditary macrothrombocytopenia [21] and AA [9,18]. Recent studies proposed that, despite the limited usefulness of IPF alone, its combination with other laboratory evidence can significantly improve the accuracy of the diagnosis of ITP from other causes of thrombocytopenia [20,22]. As for the acute TTP subjects in Group 2, the increased IPF and decreased PLT compared to Group 1 illustrate the higher consumption of platelets due to pathogenic mechanisms. This parameter might have an important role in patient monitoring during plasmapheresis since a previous study observed a decreased IPF in TTP after remission [7]. Kier et al. described an IPF value which guided the therapy in a single thrombotic microangiopathic case, but this study demands prospective clinical trials to validate the finding [23].

Group 3 comprised diverse hematological disorders sharing a common etiology of central thrombocytopenia, albeit with differing underlying mechanisms of BM failure. Diminished platelet production caused by BM infiltration due to hematological malignances such as AL and MM showed median IPFs above that of the healthy subjects. Ali et al. evaluated this parameter using a different analyzer series from the same manufacturer in a larger cohort that included lymphoma, chronic leukemia, and AL. Their reported median IPF of approximately 5.0% is in agreement with the findings of the present study. However, another study that included patients with lymphoblastic and acute myeloid leukemia found a higher IPF (around 10.0%) [17]. In accordance with the present study, all previously reported research demonstrated a significantly higher IPF compared to control groups. Malignant precursor cell infiltration is a dynamic process in which these cells exert a proliferative advantage over other lineages, ultimately leading to bone marrow suppression and subsequent thrombocytopenia [24]. The variation in increased immature platelets may be explained by the progressive infiltration of the bone marrow by malignant blasts or plasma cells. In this model, thrombopoiesis is proportionally impaired as healthy tissue is displaced; however, further correlation studies between IPF and blast percentages are warranted to confirm this relationship. Prospective studies of immature platelets in malignant disorders have been performed by monitoring platelet recovery after chemotherapy and BM transplantation [7,9]. Regarding the clinical utility of this parameter in hematological malignancies, a recent study utilized artificial neural network predictive modeling to demonstrate that the IPF, alongside two other analyzer-derived parameters, may effectively flag cases of acute promyelocytic leukemia [25].

The lower medians of IPF and IPC (approximately three times less) observed in Group 3 compared with Group 2 illustrate the impaired thrombopoiesis which results in thrombocytopenia. This finding agrees with Ashraf et al. who also studied ITP, TTP, AA and AL subjects in their research evaluating IPF [26]. The median IPF was similar to the results of Goel et al. (4.6%) [10] but higher than those of Jeon et al. (1.8%) [22]. This result is expected since 85.7% of the patients in Group 3 in the latter study were cancer patients undergoing chemotherapy; previous studies show that chemotherapy patients have substantially lower IPF values [7,27]. Thrombocytopenia secondary to chemotherapy is a frequent

hematologic complication of myelosuppressive cancer therapy the main mechanisms of which are the inhibition of platelet-derived growth factor and apoptosis of megakaryocytes [28].

In the present study, an inverse correlation between IPF and PLT was observed across all groups; however, this association was not statistically robust in Group 3. This lack of significance may be attributed to the subset of AL patients, who exhibited the highest median IPF within that cohort, consistent with previous reports. The correlation in Group 2 was also observed by Ferreira et al. ($r = -0.70$) whose patient population included ITP patients [21]. Hence, the increase in IPF is related to the reduced PLT in Groups 1 and 2.

Regarding diagnostic performance, the IPF demonstrated high discriminatory power in distinguishing Group 2 from Group 3. The findings of this study are consistent with the AUC values reported by Goel et al. (0.8680), Jeon et al. (0.9310), and Sakuragi et al. (0.9560) [9,10,18].

The IPC for the control group observed here had a similar range compared to previous studies. While IPC demonstrated good discriminatory power between groups, its diagnostic accuracy was slightly inferior to that of the IPF, a finding that aligns with prior research [9]. In terms of clinical utility, the IPC may be preferable to the IPF in scenarios where the fraction does not accurately reflect the physiological state. This includes determining the appropriateness of platelet transfusions in thrombocytopenic patients at high risk of bleeding, as well as monitoring treatment response in ITP subjects [29,30].

Despite rigorous selection criteria aimed at excluding external influences, such as infectious or nutritional factors, and recruiting subjects from a specialized hematology and hemotherapy center, this study has limitations. Specifically, the sample sizes of the chemotherapy and TTP subgroups were small. Furthermore, the clinical stage of disease and the specific treatment regimens were not investigated. As both IPF and IPC reflect active thrombopoiesis, the potential influence of these variables cannot be entirely ruled out.

Conclusions

This study demonstrates that IPF and IPC are substantially lower in patients with BM failure compared with those with conditions involving peripheral platelet destruction and consumption. Thus, the clinical utility of immature platelets for differentiating thrombocytopenia due to distinct pathogenesis with acceptable accuracy in agreement with previous studies involving adults is evident. Since this test is cheap and automated, it should be a standard part of initial blood work for patients with low platelet counts.

Ethics of approval statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the national research committee.

Patient consent statement

Informed consent was obtained from all invited participants included in the study.

Permission to reproduce material from other sources

For this type of study consent for publication is not required.

Clinical trial registration

For this type of study clinical trial registration is not required.

Author contributions

Raphael Ferreira Pimentel conceived and designed the study, recruited patients, updated the clinical data, performed experiments, performed statistical analyses, analyzed and interpreted data, and drafted the manuscript. Tayná Regina Leal dos Santos performed experiments, recruited patients and updated the clinical data. Marcos André Cavalcanti Bezerra, Betânia Lucena Domingues Hatzlhofer and Maria Tereza Cartaxo Muniz, analyzed and interpreted data and reviewed the manuscript. Maria Tereza Cartaxo Muniz gave the final approval of the version to be submitted.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors would like to thank the entire Hemope Foundation (Hematology and Hemotherapy Foundation of Pernambuco), University of Pernambuco, Recife, Brazil, for providing us with the data and for allowing us to carry out the research.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Review article

Harnessing the power of natural killer cells in Lymphoma immunotherapy: Overcoming challenges and enhancing treatment options

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ARTICLE INFO

Article history:
Received 11 December 2024
Accepted 30 November 2025
Available online 4 May 2026

Keywords:
Immunotherapy
Lymphoma treatment
NK cell therapy
Tumor microenvironment

ABSTRACT

Immunotherapy, particularly natural killer cell therapy, is garnering attention for treating lymphoma, due to the unique properties of natural killer cells, most importantly rapid and potent antitumor responses without prior sensitization. However, the tumor microenvironment and immune escape mechanisms can impair natural killer cell function in lymphoma.

To address these challenges, researchers are exploring therapeutic interventions to restore or enhance their activity. Of the various approaches of cell therapy, such as allogeneic- or autologous-natural killer cell infusions, chimeric antigen receptor natural killer cells, and combination therapies, allogeneic natural killer cell transplantation has shown promise in specific subtypes of lymphoma, offering a reduced risk of graft-versus-host disease, improved response rates, prolonged remissions, and increased overall survival. Combining natural killer cell therapy with standard treatments such as chemotherapy or immune checkpoint inhibitors holds potential for synergistic effects. Nevertheless, addressing challenges such as cell persistence, the immunosuppressive tumor microenvironment, and optimal delivery methods is crucial to improve efficacy.

Further investigations are required to gain a better understanding of natural killer cell-mediated responses, refine genetic engineering approaches, identify predictive biomarkers, and optimize combination strategies. Continued research and clinical trials will play a vital

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https://doi.org/10.1016/j.htct.2026.106352
2531-1379/© 2026 Published by Elsevier España, S.L.U. on behalf of Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

role in optimizing cell therapy and expanding treatment options for lymphoma patients.

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Introduction

Lymphoma is a general category of hematological malignancies that arise from aberrant lymphocytes, which are part of the immune system. Lymphomas are typically divided into Hodgkin's lymphoma (HL) and non-Hodgkin lymphoma (NHL), the latter being much more common than the former. Among the subtypes of NHL, the most common are B-cell lymphomas, which include diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, and mantle cell lymphoma. In contrast, T-cell lymphomas and HL are less common but have important clinical subtypes with unique pathologic and clinical features [1]. Despite advances in chemotherapy and targeted therapy regimens, the outlook for the vast majority of patients with relapsed or refractory B-cell lymphoma remains unfavorable. Standard first-line treatment regimens, including R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) have dramatically increased survival rates in B-cell lymphomas, particularly diffuse large B-cell lymphoma (DLBCL). However, these regimens have limited long-term benefits in patients with high-risk or advanced disease. In addition, autologous stem cell transplantation, although beneficial for some, is not an option in all cases. Similarly, resistance to treatment and relapse are often seen in other types of lymphoma, such as some T-cell lymphomas and HLs [2]. These limitations highlight the need for more robust and individualized treatment approaches across the spectrum of lymphatic malignancies.

Cell-based immunotherapy has revolutionized cancer treatment by leveraging immune system cells to identify and eradicate tumor cells. Among the diverse cell-based immunotherapeutic strategies, the utilization of natural killer (NK) cells has emerged as a promising therapeutic approach in lymphoma. As innate immune effectors, NK cells possess distinctive properties that render them attractive candidates for immunotherapy, notably their capacity to recognize and eliminate tumor cells without prior sensitization. NK cells play a critical role in the immune surveillance against tumor development and viral infections. They possess a delicate balance of activating and inhibitory receptors that allows them to distinguish between healthy cells and those that exhibit altered self-characteristics, such as infected or malignant cells [3].

However, in the context of lymphoma, aberrations within the tumor microenvironment can suppress NK cell activity, compromising their ability to effectively target and eliminate tumor cells. Thus, strategies to enhance NK cell function and overcome immunosuppressive mechanisms are being explored to unleash the full potential of these cells in lymphoma treatment. These strategies include targeting immunosuppressive factors, modulating cytokine levels, and overcoming NK cell exhaustion. By understanding the complex interplay between NK cells and the lymphoma microenvironment, novel immunotherapeutic strategies can be developed to improve anti-tumor responses and enhance NK cell-mediated immunity in lymphoma.

Overview of natural killer cell immunotherapy in lymphoma

Natural killer cell phenotypic changes during lymphomagenesis

NK cells are a type of $CD16^+$ $CD56^+$ cytotoxic lymphocyte that belong to the innate immune system. These cells have the ability to induce apoptosis even in the absence of antibodies and major histocompatibility complexes. NK cells recognize tumor antigens through killer-cell immunoglobulin-like receptors (KIRs), which have both inhibitory or activating functions depending on the intracytoplasmic region of the receptor. The presence and behavior of NK cells can be altered in the site where lymphoma is initiated. Lymphomas develop complex interactions with the immune system, including alterations in the microenvironment where lymphomas develop, that impact NK cell function and behavior. Multiple modifications occur in the lymphoma microenvironment, including changes in immunoregulatory cells, the cytokine milieu, stromal cells, expression of activating and inhibitory receptors, and functional NK cells [4]. Variations in NK cells within the lymphoma microenvironment differ based on the particular subtype of lymphoma. Classic HL (cHL) is characterized by a potent immunosuppressive tumor microenvironment (TME) that inhibits NK cells. The immunosuppressive nature of the TME in cHL specifically inhibits the proliferation and activity of NK cells, thereby contributing to the tumor immune-escape mechanisms. This deficiency of NK cells begins at the tumor site and progresses systemically in patients with advanced disease or adverse prognostic factors. Several facets of cHL account for this effect on NK cells. Locally, malignant Reed-Sternberg cells and cells from the TME express ligands for inhibitory receptors on NK cells, including Human Leukocyte Antigen (HLA)-E, HLA-G, and programmed death-ligand 1. The secretion of chemokines and cytokines, including soluble interleukin (IL)-2 receptor (sCD25), transforming growth factor-β, IL-10, CXCL9, and CXCL10, mediates the systemic immunosuppression [5]. However, generally in the lymphoma spectrum in terms of immunoregulatory cells, the lymphoma microenvironment often contains regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). These cells suppress NK cell activity and impair their antitumor functions, leading to immune evasion by lymphoma cells [6]. Besides immunoregulatory cells, stromal cells in the lymphoma microenvironment, such as cancer-associated fibroblasts (CAFs), secrete factors that

suppress NK cell function. They may also contribute to the formation of a physical barrier, limiting NK cell infiltration into the tumor site [7]. In addition to cells, the presence of certain cytokines within the lymphoma microenvironment influence NK cell behavior. Elevated levels of transforming growth factor-beta (TGF-β) and IL-10 inhibit NK cell function, impairing their cytotoxicity and cytokine production. The well-characterized inhibitory effect of TGF-β on the effector functions and cytotoxic activity of NK cells has been demonstrated. It has been shown that the IL-10 signaling pathway can be promoted to inhibit the TGF-β-induced suppression of NK cell cytotoxic function [8]. NK cells also express an array of activating and inhibitory receptors that regulate their activity. In lymphomas, the expression of these receptors on NK cells can be altered, affecting their ability to recognize and kill tumor cells. For example, the downregulation of NKG2D receptor ligands on lymphoma cells impair NK cell activation [9]. Notably, prolonged exposure to the lymphoma microenvironment leads to NK cell exhaustion or dysfunction. This exhaustion is characterized by reduced cytotoxicity, impaired cytokine production, and altered receptor expression. Exhausted NK cells exhibit decreased responsiveness to stimulation and diminished tumor-killing capacity [10]. Understanding the complex interplay between NK cells and the lymphoma microenvironment is crucial for developing novel immunotherapeutic strategies to enhance NK cell function and improve anti-tumor responses.

How natural killer cells are suppressed in lymphoma

Despite the importance of NK cells in the immune response against cancer cells, lymphomas employ various mechanisms to evade or suppress NK cell activity. The most common suppressive factors are tumor-derived soluble factors, which are released by lymphoma cells and have immunosuppressive effects on NK cells. These factors include cytokines, chemokines, and growth factors, such as TGF-β and IL-10, which inhibit the activity of NK cells and impair their cytotoxic functions [11]. The interaction between inhibitory receptors expressed on NK cells and their corresponding ligands on lymphoma cells represents another mechanism that suppresses NK cell activity. Inhibitory receptors recognize specific ligands on target cells, and lymphoma cells can upregulate the expression of ligands for these inhibitory receptors, such as Major Histocompatibility Complex class I molecules. These ligands engage with inhibitory receptors like KIRs and NKG2A, transmitting inhibitory signals to the NK cells and dampening their cytotoxicity against lymphoma cells [12]. In addition, immunoregulatory cells, such as Tregs and MDSCs, that often exist in the lymphoma microenvironment also suppress NK cell function. These cells produce immunosuppressive molecules and inhibit NK cell activity, preventing effective anti-tumor responses [13]. Furthermore, extracellular matrix components and stromal cells in the lymphoma microenvironment, such as cancer-associated fibroblasts (CAFs), secrete factors and remodel the extracellular matrix, creating a physical barrier that hampers NK cell infiltration into the tumor site. This limited access to malignant lymphocytes imposes spatial constraints that impede NK cell recognition and subsequent mediated cytotoxicity [14]. Metabolic reprogramming derived from lymphoma cells also leads to alterations in the tumor microenvironment that affect NK cell function. For example, increased consumption of nutrients and competition for resources within the microenvironment impair the NK cell metabolism and compromise their effector functions [15]. Immune checkpoint molecules, such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), expressed in NK cells are upregulated in the lymphoma microenvironment [16]. Engagement of these checkpoints by their ligands on lymphoma cells leads to NK cell exhaustion and functional suppression. These immune checkpoint molecules are negative regulators of T-cell and NK cell function that limit the amplitude of T cell and NK cell activation. The blockade of immune checkpoints, such as PD-1 and CTLA-4, has shown promising results in enhancing antitumor immunity and producing durable clinical responses [17]. Moreover, genetic and epigenetic changes within lymphoma cells contribute to NK cell suppression. These alterations affect the expression of molecules involved in NK cell recognition and activation, impairing their ability to effectively target and eliminate lymphoma cells. It has been observed that promoter methylation/hypermethylation of tumor suppressor genes, miRNA expression or methylation level, and Long noncoding RNA (LncRNA) expression were associated with lymphoma disease progression [18].

How medications affect the activation of natural killer cells

The treatment of lymphoma relies heavily on medication, which varies depending on the type and stage of lymphoma, as well as individual patient factors. Common types of medication used in lymphoma treatment include chemotherapy, monoclonal antibodies, immunomodulatory drugs, and targeted therapies [19].

Chemotherapy

Some chemotherapy agents have suppressive effects on NK cell function. Investigations have been conducted on the effects of the chemotherapy agents commonly used in lymphoma treatment on NK cell proliferation and cytotoxicity. The findings indicate that NK cell activation is modulated by chemotherapy-induced DNA damage and DNA damage response pathways, leading to alterations in the NK cell phenotype and impaired cytotoxicity [20]. Research has been carried out on the impact of various chemotherapy drugs, such as cyclophosphamide and methotrexate, on the ability of NK cells to destroy cancer cells. These investigations have focused on the role of a protein called NKG2D ligand in mediating this process. The findings have shown that specific chemotherapy agents cause a decrease in the expression of NKG2D ligands on lymphoma cells. As a result, NK cells have a reduced ability to recognize and eliminate these cancer cells [21]. Moreover, when both dendritic cells (DCs) and NK cells were treated with chemotherapy such as ifosfamide, DCs

were able to overcome the negative effect of ifosfamide on NK cytotoxic function. It has been reported that Ifosfamide treatment of DCs resulted in a reduction in indirect DC stimulation of NK cell proliferation via T cells and T cell-derived IL-2. While the negative impact on NK cytotoxic function can be overcome by DC, the restoration of NK cell interferon-gamma (IFN-γ) production is not as effective [22].

Monoclonal antibodies

While chemotherapy may have suppressive effects on NK cell function, monoclonal antibody therapies enhance NK cell-mediated cytotoxicity. Rituximab, an anti-CD20 chimeric monoclonal antibody, targets the CD20 protein found on the surface of B cells, including malignant B cells in some lymphomas. Studies suggest that rituximab enhances NK cell-mediated cytotoxicity through the CD16 signaling pathway, which is involved in antibody-dependent cellular cytotoxicity (ADCC). Rituximab-mediated binding to CD20 on lymphoma cells triggers NK cell activation and ADCC, ultimately resulting in the targeted elimination of malignant cells. The results of a meta-analysis confirmed that NK cell-mediated cytotoxicity against lymphoma cells through ADCC is significantly enhanced by rituximab treatment thereby contributing to improved clinical outcomes [23].

Immunomodulatory drugs

Between 2012 and 2014, research was focused on immunomodulatory medications in lymphoma, including lenalidomide. Lenalidomide has immunomodulatory effects, including the activation and enhancement of NK cell function, stimulating NK cell activity and cytokine production, potentially contributing to antitumor immune responses. Lenalidomide treatment increases CD20 expression on lymphoma cells, leading to enhanced NK cell-mediated lysis of tumor cells. It also enhances NK cell activity, including increased cytotoxicity and IFN-γ production and modulates cytokine production by ovarian cancer cells. These studies provide evidence that lenalidomide can activate and enhance NK cell function, contributing to antitumor immune responses in lymphoma treatment [23]. The immunomodulatory effects of lenalidomide and its potential contribution to antitumor immune responses in lymphoma treatment are supported by these findings. Furthermore, recent studies suggest that the combination of lenalidomide and rituximab may have complementary mechanisms of action as this unique combination is characterized by immune enhancement, not immunosuppression. This benefit was demonstrated in the Phase III AUGMENT trial, which evaluated rituximab with or without lenalidomide in relapsed rituximab-sensitive follicular lymphoma, and found a significant progression-free survival benefit with the combination. Results demonstrated that lenalidomide has synergy with anti-CD20 antibodies such as obinutuzumab and rituximab, via reconstitution of a functional immune synapse, improved NK cell function, and greater antibody-dependent cellular cytotoxicity [24]. In animal models and *in vitro* studies, the effect of rituximab in combination with lenalidomide on ADCC and direct apoptosis was assessed in B-cell lymphoma. The results showed that the lenalidomide and rituximab combination therapy enhanced ADCC and direct apoptosis in B-cell lymphoma [25]. Multiple phase II studies showed that lenalidomide and rituximab therapy achieves clinical synergy in treatment naïve, relapsed and refractory settings [26].

Targeted therapy

Unlike chemotherapy, which can affect both cancerous and healthy cells, targeted therapies are more selective in their action and can minimize damage to healthy cells. They specifically target molecules or pathways involved in the immunosuppressive microenvironment and be used alone or in combination with other treatments, such as chemotherapy or immunotherapy. Various targeted therapies, such as kinase inhibitors, are now used in the treatment of specific lymphoma subtypes including venetoclax, duvelisib, copanlisib, polatuzumab vedotin, and ibrutinib. Venetoclax, duvelisib, and copanlisib inhibit some factors that are involved in the growth and survival of cancer cells [27]. Similarly, ibrutinib disrupts B-cell receptor (BCR) signaling by inhibiting Bruton's tyrosine kinase (BTK), thereby impairing the essential growth and survival mechanisms of malignant B cells. By inhibiting this signaling pathway, ibrutinib helps to slow down or stop the growth of lymphoma cells. Brentuximab vedotin and polatuzumab vedotin target proteins found on the surface of some lymphoma cells. These targeted therapies may indirectly facilitate NK cell activation by disrupting tumor-mediated immune evasion pathways and reducing the overall suppressive burden within the microenvironment [28].

Recent studies have investigated the potential of various inhibitors to enhance NK cell-mediated cytotoxicity against lymphoma cells. One study found that Bruton's tyrosine kinase (BTK) inhibitors, such as ibrutinib, can enhance NK cell-mediated cytotoxicity by targeting signaling pathways involved in immune evasion and tumor cell survival. BTK, an essential component of the B-cell receptor pathway, has emerged as a novel target in the treatment of B-cell malignancies [29].

In addition, selinexor, a selective inhibitor of the nuclear export protein exportin-1 (XPO1), enhances NK cell activation against malignant B cells via downregulation of HLA-E. The NKG2A:HLA-E axis is a novel immune checkpoint target; it has been found that selinexor sensitizes lymphoma cells to NK cell-mediated killing via disruption of this interaction. The study also provides preliminary evidence suggesting an association between NK cell activity and clinical response to selinexor. These data indicate that NK cells may contribute to the therapeutic efficacy of selinexor, potentially providing a rationale for its synergistic use with NK cell-targeted therapies in lymphoma treatment [30].

Natural killer cell role as a therapy for lymphoma

NK cells are crucial in host immunity against cancer, but cancers develop mechanisms to escape NK cell attack or induce defective NK cells. NK cell-based cancer immunotherapy aims to overcome NK cell paralysis using several approaches. These approaches include expanded allogeneic NK cells,

Table 1 – Diverse therapeutic strategies utilizing natural killer cell therapy for lymphoma.

Therapy Approach	Description	Advantages	Challenges
Allogeneic NK Cell Transplantation	Infusion of NK cells from a healthy donor	Reduced risk of graft-versus-host disease (GvHD)	Donor availability and compatibility
Autologous NK Cell Infusion	Infusion of patient's own NK cells	Lower risk of immune rejection	Limited NK cell numbers and quality
CAR-NK Cell Therapy	Genetic engineering of NK cells to express CARs	Enhanced specificity and potency	Optimization of CAR design and manufacturing
Combination Therapies	Co-administration of NK cell therapy with other treatments	Synergistic effects for improved outcomes	Identifying optimal combination strategies
Cytokine Stimulation	Administration of cytokines (*e.g.*, IL-2, IL-15)	Promotes NK cell proliferation and cytotoxicity	Potential systemic side effects
Antibody Stimulation	Administration of antibodies (*e.g.*, anti-CD20)	Enhances NK cell-mediated antibody-dependent cytotoxicity (ADCC)	Resistance or downregulation of target antigens
Gene Editing Techniques	CRISPR/Cas9-mediated gene editing in NK cells	Improved persistence and resistance to immunosuppression	Off-target effects and safety concerns

stable allogeneic NK cell lines, and genetic modification of fresh NK cells or NK cell lines. Therapeutic NK cells can be derived from various sources, including peripheral or cord blood cells, stem cells, or even induced pluripotent stem cells (iPSCs) [31]. A variety of stimulators can be used for large-scale production in laboratories or good manufacturing practice facilities, including soluble growth factors, immobilized molecules or antibodies, and other cellular activators. In the first approach, expanded allogeneic NK cells are not inhibited by self-histocompatibility antigens like autologous NK cells, making them suitable for adoptive cellular immunotherapy. In the second approach, stable allogeneic NK cell lines are more practical for quality control and large-scale production. Thirdly, genetic modification of fresh NK cells or NK cell lines to highly express cytokines, Fc receptors, and/or chimeric tumor-antigen receptors is another approach [31]. In the context of lymphoma, the objective of NK cell therapy revolves around exploiting the inherent anti-tumor attributes possessed by these cells, with the specific aim of selectively targeting and eradicating lymphoma cells. The implementation of this therapy entails the introduction of *ex vivo* expanded and activated autologous (derived from the patient themselves) or allogeneic (derived from a donor) NK cells into the patient's system [32], as illustrated in Table 1. The ultimate goal lies in bolstering the immune response against lymphoma cells, thereby promoting regression of the tumor. Clinical trials investigating allogeneic NK cell therapies in patients with relapsed or refractory hematologic malignancies, including those with B-cell lymphoma, have supported the significant anti-tumor activity of these cells. These therapies may provide a superior safety profile, as they are associated with a lower incidence of cytokine release syndrome (CRS) and neurologic toxicity compared to T-cell therapies. Nevertheless, the use of allogeneic NK cell therapies is hampered by several limitations, such as the scarcity of appropriate donors, the relatively short *in vivo* persistence, and the manufacturing constraints that hinder the consistent administration of multiple doses [33].

NK cell therapy for lymphoma can be administered either as a standalone treatment or in conjunction with other therapeutic modalities such as chemotherapy, radiation therapy, or targeted therapies. The intention behind combination approaches is to amplify the effectiveness of standard treatments while concurrently enhancing the immune response against lymphoma [34]. Table 2 outlines selected clinical studies using allogeneic NK cells in lymphoma, while Table 3 provides preclinical data supporting their potential efficacy.

Chimeric antigen receptor-natural killer cell therapy in lymphoma

Chimeric Antigen Receptors (CARs) are genetically engineered to recognize and bind to antigens on the cell surface. Receptors allow cells to recognize specific targets, for example, tumor cells, and trigger potent cytotoxic activity against them. CARs are usually designed to bind to particular antigens on the surface of cancer cells and therefore provide targeted therapies against tumors. While the earliest cancer treatments were mainly based on CAR-T cell therapy, recent studies have also proposed CAR-NK cell therapy (CAR-engineered NK cells) as a promising candidate for tumor treatment [35].

These treatments make use of NK cells, which already have the innate ability to recognize and eradicate cancer cells. By using CAR technology, the cells are engineered to recognize and eliminate specific tumor antigens. These cells, due to their unique features, *i.e.*, not requiring HLA matching and possessing allogeneic potential, can effectively target cancer cells. Furthermore, the ability of CAR-NK cells to exert potent antitumor effects without inducing severe toxicities (specifically CRS, neurotoxicity, or graft-versus-host disease [GvHD]) establishes this modality as a safe and efficacious alternative to traditional cellular therapies [36].

The efficacy of CAR-NK cells in treating lymphoma and other hematologic malignancies is being investigated in several studies. A pivotal study by Liu et al., published in The New England Journal of Medicine, demonstrated that cord blood-derived CAR-NK cells targeting CD19 induced high response rates without CRS, neurotoxicity, or GvHD; this

Table 2 – Clinical trials of allogeneic natural killer cell therapy in lymphoma.

Clinical Trial ID	Phase	Sponsor	Product Name	CAR Target	NK Cell Source	Indication	Outcome Summary
NCT04074746	I/II	MD Anderson Cancer Center	AFM13-NK	CD30	Cord blood	Relapsed/refractory Hodgkin's and non-Hodgkin lymphomas	ORR: 89 %; CR: 53 %; No major adverse events
NCT05883449	I/II	MD Anderson Cancer Center	Precomplexed AFM13 + CIML NK Cells	CD30	Cord blood	Relapsed/refractory $CD30^+$ lymphomas	ORR: 93 %; CR: 66 %; Favorable safety profile
NCT04245722	I	Fate Therapeutics	FT596	CD19 + IL-15	iPSC-derived	Non-Hodgkin Lymphoma	Early-phase study; outcomes pending
NCT05020015	II	Takeda	TAK-007	CD19	Cord blood	Relapsed/refractory LBCL and iNHL	ORR: 78 % in iNHL; CR: 56 %; No severe CRS or ICANS

CAR: chimeric antigen receptor; CD: cluster of differentiation; CR: complete response; ORR: overall response rate; NK: natural killer; CIML: cytokine-induced memory-like; iPSC: induced pluripotent stem cell; LBCL: large B-cell lymphoma; iNHL: indolent non-Hodgkin lymphoma; CRS: cytokine release syndrome; ICANS: immune effector cell-associated neurotoxicity syndrome.

represents a significant milestone in NK cell therapy development (Clinical Trial: NCT03056339) [37].

Building upon this foundational work, Rezvani et al. reported the successful application of off-the-shelf allogeneic CAR-NK cells with sustained efficacy and favorable safety profiles in patients with relapsed or refractory lymphoid malignancies, including lymphoma. These findings further highlighted the feasibility and clinical relevance of CAR-NK cells as a scalable, low-toxicity alternative to CAR-T cell therapies [38]. One of the most promising clinical trials was conducted by Fate Therapeutics, in which iPSC-derived CAR-NK cells (FT596) were administered to treat relapsed and refractory B-cell lymphomas. FT596 was designed to target three antigens, CD19, CD16, and IL-15 in order to increase antitumor activity and persistence. The trial enrolled 86 patients across nine US research sites. The outcomes revealed that 50 % of the patients who received a high dose of FT596 (900 million cells) and 33 % of the patients receiving lower doses achieved a complete response. Side effects were low, primarily including mild CRS that resolved without intensive care. Notably, no cases of immune effector cell-associated neurotoxicity syndrome (ICANS) or GvHD were observed (Clinical Trial: NCT04245722) [39]. Further advancements were made by Nkarta Therapeutics, who conducted trials using their allogeneic CAR-NK product, NKX019, targeting CD19 antigens. This trial demonstrated an impressive overall response rate (ORR) of approximately 80 % and a complete response (CR) of 70 %. Most importantly, no CRS or neurotoxicity occurred, confirming both the efficacy and safety (Clinical Trial: NCT05020678) [40]. Similarly, the TAK-007 (ARC-TCR) trial employed umbilical cord blood-derived CAR-NK cells for the treatment of indolent NHL (iNHL) and large B-cell lymphoma (LBCL). Preliminary results indicated an ORR of 78 % in iNHL and 50 % in LBCL, again without major side effects such as CRS and neurotoxicity (Clinical Trial: NCT05020015) [41]. A more recent approach by Allogene Therapeutics involved the use of allogeneic CAR-NK cells (ALLO-316) specifically engineered to target CD19 antigens in refractory B-cell lymphomas. This study reported a 60 % ORR, with no high-grade toxicities like CRS or neurotoxicity, positioning ALLO-316 as a promising novel therapy in the management of difficult-to-treat lymphomas (Clinical Trial: NCT04263594) [42]. Key clinical trials evaluating CAR-NK cell therapy in B-cell lymphoma are summarized in Table 4.

Table 3 – Preclinical studies of allogeneic natural killer cells in lymphoma.

NK Cell Source	Engineering	Disease Model	Strategy	Key Findings
Cord Blood	CIML NK Cells + AFM13	CD30+ Lymphoma (Mouse Model)	Combination Therapy	Enhanced cytotoxicity; maintained efficacy in suppressive tumor microenvironment
Peripheral Blood	IL-12/15/18 Activated NK Cells	Lymphoma/Melanoma (Xenograft)	Cytokine-Induced Memory-Like NK Cells	Increased IFN-γ production; improved persistence and antitumor activity

NK: Natural killer cell; CIML: Cytokine-induced memory-like; CAR: Chimeric antigen receptor; TME: Tumor microenvironment; IFN-γ: Interferon gamma.

Table 4 – Clinical trials of chimeric antigen receptor-natural killer cell therapy in lymphoma.

Clinical Trial ID	Phase	Sponsor	Product Name	CAR Target	NK Cell Source	Indication	Outcome Summary
NCT05020015	II	Takeda	TAK-007	CD19	Cord Blood	Relapsed/refractory LBCL and iNHL	ORR: 78 % (iNHL); CR: 56 %; No CRS or ICANS
NCT04245722	I	Fate Therapeutics	FT596	CD19 + IL-15	iPSC-derived	Relapsed/refractory B-cell NHL	CR in 50 % (high dose); safe profile
NCT05020678	I	Nkarta Inc.	NKX019	CD19	Peripheral Blood	Relapsed/refractory B-cell lymphoma	ORR: 73 %; CR: 70 %; well tolerated
NCT03056339	I/II	MD Anderson	iC9/CAR.19/IL15	CD19	Cord blood	Relapsed/refractory $CD19^+$ lymphomas	High response rates; no CRS/GvHD

CAR: chimeric antigen receptor; NK: natural killer; CR: complete response; ORR: overall response rate; GvHD: graft-versus-host disease; CRS: cytokine release syndrome; ICANS: immune effector cell-associated neurotoxicity syndrome; CD: cluster of differentiation; iNHL: indolent non-Hodgkin lymphoma; LBCL: large B-cell lymphoma; iPSC: induced pluripotent stem cell.

AFM13 and natural killer cell combination therapy

Presently, ongoing research endeavors and clinical trials are dedicated to refining these aforementioned aspects of NK cell therapy with a primary focus on evaluating the safety, efficacy, and long-term outcomes in lymphoma patients. Affimed (AFMD), a clinical-stage immuno-oncology company, focuses on developing targeted immuno-oncology therapies. Affimed has three programs (AFM13, AFM24, and AFM26). AFM13 (acimtamig) is a first-in-class tetravalent bispecific innate cell engager, engineered as a tandem antibody (TandAb) construct to treat CD30-expressing malignancies. It facilitates targeted tumor lysis by providing bivalent binding to CD30 on malignant cells and CD16A on NK cells and macrophages. It recruits NK (NK) cells by binding to CD16A as immune effector cells [31]. AFM13 induces NK cell-mediated cytotoxicity, resulting in tumor cell lysis. Results of a Phase 1 dose-escalation study conducted on 28 patients with heavily pretreated relapsed or refractory HL show that AFM13 is well-tolerated and active, with an objective response rate of 50 %. Hence, AFM13 represents a new, feasible, targeted immunotherapy for heavily pretreated patients with HL. The dose regimen of AFM13 has to be optimized and the treatment duration has to be prolonged in order to increase the clinical efficacy [43]. The second study is an ongoing Phase II clinical trial that aims to evaluate the antitumor activity and safety of AFM13 in patients with CD30-positive peripheral T-cell lymphoma (PTCL) or transformed mycosis fungoides (tMF). The trial is investigating the efficacy of AFM13 as monotherapy in patients with relapsed or refractory CD30-positive T-cell lymphoma. The results of the trial are not yet available [44]. Peripheral T-cell lymphomas are highly aggressive and one of the most difficult-to-treat forms of lymphoma. Affimed, after underwhelming monotherapy data, has decided to focus on testing AFM13 in combination with the AB-101 NK cell product of Artiva. AB-101 NK is an allogeneic, non-genetically modified cord blood-derived NK cell. It is a cryopreserved NK cell therapy that has been optimized for combination with monoclonal antibodies to enhance ADCC and anti-tumor responses. The combination of AFM13 and AB-101 NK cells has the potential to provide a more durable and effective response compared to monotherapy with AFM13 [45].

AFM13 and umbilical cord-derived natural killer cell combination

AFM13 is a tetravalent bispecific antibody engineered to engage simultaneously CD30 on cancer cells and CD16A on NK cells, thereby enhancing ADCC. To further improve its efficacy, more recent studies have focused on combining AFM13 with umbilical cord blood-derived NK cells, particularly those activated with cytokines to enhance their persistence and cytotoxic activity. Kerbauy et al. demonstrated in preclinical models that cytokine-induced memory-like NK cells derived from cord blood, which were precomplexed with AFM13, exhibited enhanced antitumor activity against CD30-positive lymphoma cells. The NK cells were hyperactivated and cytotoxic despite the presence of immunosuppressive cytokines such as TGF-β and IL-10, which are typically upregulated within the tumor microenvironment [46]. Thereafter, a Phase I/II first-in-human trial by Nieto et al. at MD Anderson Cancer Center evaluated the safety and efficacy of this combination in relapsed or refractory CD30-positive lymphomas. The trial consisted of *ex vivo* AFM13 precomplexing with cytokine-preactivated cord blood-derived NK cells before infusion. Preliminary outcomes report an ORR of 93 % and CR of 66 % with favorable safety outcomes [47]. These findings underscore the effectiveness of AFM13 combined with NK cells derived from umbilical cord blood as a potent method in the treatment of CD30-positive lymphomas, particularly in relapsed or refractory disease, and implicate the utility of innate immune effectors in next-generation cancer immunotherapies.

Cytokine-induced memory-like natural killer cells in lymphoma therapy

One of the most important advances in NK cell-based immunotherapy is the generation of cytokine-induced memory-like NK (CIML-NK) cells. The cells are generated by a temporary

pre-activation with IL-12, IL-15, and IL-18, which are capable of inducing memory-like functional properties. CIML-NK cells exhibit increased IFN-γ production, increased cytotoxicity, and increased proliferative capacity upon secondary stimulation. Phenotypically, they are $CD56^{bright}CD16^{dim}$ with strong expression of activating receptors (NKG2D, NKp30, and NKp46) and transcription factors (T-bet and Eomes) [48]. Epigenetic reprogramming following cytokine stimulation is a hallmark of CIML-NK cells, leading to lasting changes in phenotype and function [49]. The epigenetic modifications involve demethylation of regulatory elements in the IFNG locus (*e.g.*, CNS1) and regulation of master genes such as *PRDM1/BLIMP1* and *ZBTB32*, which together allow long-term IFN-γ expression in progeny cells as well as enhanced cytotoxic activity. This long-term reprogramming makes CIML-NK cells attractive candidates for cancer immunotherapy, particularly for treating refractory and relapsed lymphomas [50].

Despite their tremendous potential, there are currently few clinical trials evaluating CIML-NK cells in the context of lymphoma, and no specific studies have been formally published yet. Most clinical data come from a Phase I study (Clinical Trial: NCT01898793) that focuses primarily on patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). In this study, NK cells were preactivated with IL-12, IL-15, and IL-18, and infused into patients to assess safety and preliminary efficacy. Although lymphoma was not the primary target, the study illustrated the broad potential of CIML-NK cells for the treatment of refractory hematological malignancies [43].

Preclinical research has further proved the efficacy of CIML-NK cells against lymphoma and melanoma models. In such experimental models, CIML-NK cells, which had been activated with IL-12, IL-15, and IL-18, were infused into mice with established tumors. The results demonstrated that CIML-NK cells effectively controlled tumor growth and increased survival rates, thus confirming their notable therapeutic value in treating tumor resistance to NK cells [51]. However, specialized clinical trials are needed to confirm these results in lymphoma patients.

Various combinatorial approaches have been established to further improve the activity of CIML-NK cell therapy. A combination of CIML-NK cells with monoclonal antibodies like rituximab (anti-CD20) has augmented ADCC and clinical responses considerably [52]. Moreover, strategic targeting of the immune checkpoint pathways suppressing NK cell function, specifically NKG2A and PD-1/PD-L1, is a novel approach in reconstituting CIML-NK cytotoxicity for the immunosuppressive tumor microenvironment [53]. Cytokine support strategies, specifically using IL-15 superagonists such as ALT-803 (*n* - 803), have been found to augment the *in vivo* stability, expansion, and activation of CIML-NK cells, resulting in durable antitumor activity [54].

Moreover, genetic modification of CIML-NK cells through the use of CARs has significantly enhanced their therapeutic efficacy. A recent preclinical study demonstrated that CIML-NK cells transduced with CD19-targeting CAR exhibited potent antitumor activity against CD19-expressing malignancies. In comparison to the conventional CAR-NK cells, CAR-CIML-NK cells produced more IFN-γ and exhibited greater degranulation against $CD19^+$ target cells, resulting in greater cytotoxicity against leukemic and lymphomatous cells. Furthermore, the IL-12, IL-15, and IL-18 induced memory-like characteristics enhanced the *in vivo* persistence of CAR-CIML-NK cells and effectively inhibited tumor growth in $CD19^+$ lymphoma mouse models, thereby resulting in extended survival [55]. For a succinct overview of the role of NK cells as a therapeutic intervention in lymphoma, pertinent studies and evidence are summarized in Table 2.

Future trends

Various preclinical and clinical studies have demonstrated that improving the clinical efficacy of NK cell therapy for hematopoietic malignancies, such as lymphoma and leukemia, requires overcoming significant challenges related to optimal proliferation, long-term persistence, and effector function.

Appropriate selection of cell sources, advances in expansion methods and genetic modification techniques (CAR, Bispecific Killer Cell Engagers [BiKEs], high-affinity, non-cleavable CD16 [hnCD16]), cytokine adjuvants, antibody design and stem cell biology have been effective in overcoming these limitations.

Recently, induced pluripotent stem cells (iPSCs) and the NK-92 cell line have emerged as promising substrates for CAR engineering, owing to their inherent homogeneity and virtually unlimited proliferative capacity. However, due to the lymphoma origin of NK-92 cell lines, there are some limitations. Furthermore, technical complexities inherent to iPSC differentiation have hindered the large-scale manufacturing of NK cells from this progenitor source. However, regarding the potential and ability of NK 92 lines and iPSCs as an off-the-shelf therapy, advances in the use of these cells, expansion methods, and specific targeting employing genetic engineering have been made and are being investigated. These advances promise to overcome many challenges, thus bringing the application of NK 92 lines and iPSC-derived NK cells closer as clinically approved options for the treatment of hematologic and solid malignancies. Until now, the NK92 cell line has not been used in any registered trials, however, iPSC-derived NK cells have been used in Phase I clinical trials of lymphoma and other malignancies (Clinical Trials: NCT04245722; NCT04023071; NCT04630769; NCT0455188; NCT03841110). In recent years, engineered iPSC-derived NK cells have demonstrated promising results in preclinical lymphoma models. Specifically, FT516 was engineered to express a high-affinity CD16 receptor for targeting both CD19 and CD20 antigens. This platform was further optimized in FT596 as a triple-gene-engineered product incorporating these modifications alongside a membrane-bound 15/IL-15Rα fusion protein. Subsequent iterations have utilized transgenic overexpression of *ARID5B* or CRISPR-mediated knockout of *CD38* to enhance *in vivo* persistence and effector function.

Regarding expansion methods, it was observed that the *ex vivo* incubation of NK cells with IL-15 and nicotinamide (NAM) exhibited improved proliferation, persistence, cytotoxicity, and inflammatory cytokine production (*e.g.*, Gamida cell; GDA-201 - Clinical Trial: NCT05296525) [56]. In addition to

cytokine-based approaches, feeder cell-based expansion has shown significant promise. For example, irradiated K562 feeder cells genetically engineered to express membrane-bound IL-15 (mbIL-15), IL-21 (mbIL-21), and 4−1BBL have been extensively used to promote robust NK cell expansion. These feeder-based protocols can achieve over 1000-fold expansion within two to three weeks and improve NK cell cytotoxicity and IFN-γ secretion. Feeder-supported methods have been used in good manufacturing practice-compliant protocols for clinical-grade NK cell production and are currently under evaluation in clinical trials (*e.g.*, NCT00995137, NCT02028407) [57]. Nevertheless, safety concerns regarding the tumor origin of feeder cell lines have led to parallel development of feeder-free systems and artificial antigen-presenting cells (aAPCs).

In recent years, CAR- and BiKE-NK cells have been used as alternative safer cells compared to CAR-T cells, for treating hematological malignancies. NK-92 cell lines represent one of the most widely utilized sources for CAR-NK cells; however, due to their limited *in vivo* expansion, umbilical cord blood (UCB) has emerged as a viable alternative. UCB-derived NK cells exhibit a higher proliferative rate and enhanced *in vivo* persistence compared to cell line-based models. Recently, several CAR-NK cell products have entered Phase I/II clinical trials for solid tumors and hematologic malignancies (Clinical Trials: NCT05654038, NCT05528341). Furthermore, promising Phase II trials are currently underway for patients with relapsed or refractory lymphoma who have previously failed brentuximab vedotin and PD-1 inhibitors. These studies, including LuminICE-203 (Clinical Trial: NCT05883449) and AFM13−104 (Clinical Trial: NCT04074746), utilize allogeneic cord blood-derived NK cells pre-complexed with AFM13, followed by multiple infusions of AFM13 monotherapy. These trials assess dose-escalation of pre-complexed, off-the-shelf NK cells, followed by expansion cohorts in Phase I/II for relapsed or refractory lymphoma, particularly $CD30^+$ peripheral T-cell lymphoma.

Recent studies on the treatment of relapsed and refractory lymphoma suggest that, in addition to selecting optimal NK cell sources and enhancing *in vivo* persistence, combinatorial strategies, including the use of anti-lymphoma monoclonal antibodies, BiKEs, or checkpoint inhibitors, yield superior efficacy compared to monotherapeutic cell therapy. These improved outcomes are primarily mediated through more precise tumor targeting and the blockade of inhibitory regulators.

Preclinical trials using new NK products with improved expansion, demonstrate increased anti-lymphoma activity due to enhanced persistence and potency of transferred NK cells. The findings from these trials will impact the design of future clinical protocols; when integrated with multimodal strategies and the optimization of dosage and administration schedules, these advancements may lead to superior therapeutic outcomes in the treatment of lymphoma.

Conclusion

NK cells are garnering significant attention in lymphoma therapy due to their unique properties, most notably their ability to exert rapid and potent antitumor responses without prior sensitization. However, the complex interplay between NK cells and the lymphoma microenvironment, coupled with immune escape mechanisms and the suppressive effects of certain medications, can impair NK cell function. Integrating NK cell therapy with chemotherapy or immune checkpoint inhibitors holds significant potential for synergistic effects and may overcome these hurdles. While limitations regarding optimal proliferation, long-term persistence, and effector function remain, recent advances are addressing these challenges. Specifically, the selection of appropriate cell sources, progress in expansion protocols, and genetic modifications, including CAR, BiKEs, and hnCD16, alongside cytokine adjuvants and stem cell biology, are proving effective in enhancing the therapeutic efficacy of NK cells.

Funding

This project was supported by Hematopoietic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome

Acknowledgement

We would like to express our deepest gratitude to the staff members of Hematopoietic stem cell research center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Editor

Eduardo Rego

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Review article

Exploring the impact of haptoglobin gene polymorphisms and severity of sickle cell disease: meta-analysis and gene set enrichment study

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ARTICLE INFO

Article history:
Received 15 July 2025
Accepted 8 March 2026
Available online 15 May 2026

Keywords:
Sickle cell anemia
Haptoglobin
Oxidative stress
Meta-analysis
Gene polymorphism

ABSTRACT

Background: Sickle cell disease, a genetic blood disorder marked by vaso-occlusive crises and severe pain, presents a major global health challenge. Haptoglobin, a glycoprotein that binds free hemoglobin in the blood, protects tissues from oxidative damage. This study aims to explore the link between variations in the *haptoglobin* (*HP*) gene and susceptibility to more severe sickle cell disease.

Methods: A comprehensive literature search was performed across Scopus, Embase, Google Scholar, Web of Science, and PubMed. Methodological quality was evaluated using the RoB 2 tool. Statistical analyses employed MetaGenyo software with significance being set at a p-value <0.05. Functional enrichment, gene ontologies, and protein-protein interaction networks were mapped using the Enrichr and STRING web platforms.

Results: Eight studies were evaluated to determine the association between *HP* gene polymorphisms and susceptibility to sickle cell disease complications. The results indicate a significant association between *HP* gene polymorphisms and sickle cell disease severity under the dominant model (odds ratio = 0.76; 95% confidence interval: 0.61–0.94; p-value = 0.01). Additionally, a subgroup analysis based on ethnicity demonstrated associations in the African and South American populations (p-value <0.05) but the Asian population showed no correlation between *HP* gene polymorphisms and sickle cell disease severity (p-value >0.05).

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https://doi.org/10.1016/j.htct.2026.106468
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Conclusions: This meta-analysis demonstrates a significant association between *HP* gene polymorphisms and increased severity of sickle cell disease, suggesting that the *HP* genotype may serve as a valuable predictive biomarker for clinical outcomes.

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Introduction

Sickle cell disease (SCD) encompasses hemoglobinopathies caused by single-point mutations in hemoglobin (Hb) variants, resulting in morphological abnormalities in red blood cells [1]. Sickle cell anemia (SCA), the most common and severe form, arises from homozygosity for Hb S [2]. SCD is the most prevalent monogenic disorder worldwide, with the highest burden in sub-Saharan Africa. In these regions, limited newborn screening contributes to 50–90 % mortality among affected children, whereas in high-income countries with universal screening and care, childhood mortality is rare and survival extends into midlife [3]. Between 2000 and 2021 the incidence of sickle cell disease (SCD) remained stable, but the number of affected newborns rose by 13.7 % to 515,000, mainly in sub-Saharan Africa and the Caribbean. During the same period, the global SCD population increased by 41.4 % to 7.74 million. In 2021, SCD was responsible for 376,000 deaths, including 81,100 in children under five. According to Global Burden of Disease estimates, it now ranks as the 12th leading cause of death worldwide among children under five years old [4]. SCD occurs due to genetic mutations affecting the β-globin subunits of Hb, resulting in abnormal Hb and the development of sickle-shaped red blood cells [5]. SCD is a hereditary hemolytic anemia characterized by accelerated hemolysis, which precipitates oxidative stress, free radical formation, and chronic pain. These processes drive the wide spectrum of systemic complications and end-organ damage associated with the disease. The resulting by-products of hemolysis are especially worrisome due to their high toxicity to vital organs [6].

Individuals with SCD face a variety of acute and chronic complications, many of which can be life-threatening. However, except for certain cases, like stroke in children, most complications are unpredictable [7]. The clinical presentation of SCD is highly variable; while some patients are nearly asymptomatic, others may experience severe complications resulting from vaso-occlusion, red cell damage leading to hemolysis, and inflammation caused by factors such as intravascular hemolysis and ischemia-reperfusion injury [8]. The most common symptoms and complications are acute chest syndrome, stroke, pulmonary hypertension, priapism, pain crises, and severe hemolytic anemia [9,10]. Infants may initially be asymptomatic, but over time, they generally have complications that intensify with age [11]. Environmental and lifestyle factors, including infections, dehydration, physical stress, and hypoxia, also contribute to the complications [12].

To inherit SCA, an individual must receive two copies of the sickle cell gene, one from each parent. Those who inherit only one gene have sickle cell trait (SCT), which usually does not cause symptoms [13]. Traditional markers of hemolysis, along with newer indicators like red blood cell microparticles, are effective predictors of the clinical course in patients with SCD [14]. However, none of these markers can fully account for the clinical variability observed in SCA. As a result, the search for potential disease markers continues.

Haptoglobin (Hp), an acute-phase reactant protein, is determined by two primary codominant alleles, *HP*1 and *HP*2. This results in three possible genotypes (*HP*1–1, *HP*1–2, and *HP*2–2) all associated with the *HP* gene located on chromosome 16q22 [15]. This protein, found in both mammals and humans, is synthesized by hepatocytes. It has strong antioxidant properties and demonstrates varying levels of antioxidant activity based on its different genotypes [16]. The interaction of Hp with extracellular Hb helps mitigate the oxidative stress caused by cell-free Hb on tissues. This suggests that the severity of major SCD phenotypes may be influenced by specific *HP* genotypes [17]. Consequently, specific *HP* genotypes are associated with increased susceptibility to certain diseases and may serve as determinants of clinical prognosis [18].

To date, the determinants of the association between the *HP* genotype and the clinical and biological manifestations of SCD are unclear [19]. Therefore, it is interesting to know the influence of *HP* gene polymorphisms on the severity of SCD. There have been several case-control studies published on *HP* gene polymorphisms and severity of SCD, but the results are conflicting [20–22]. Thus, additional validation is necessary to establish definitive findings.

This meta-analysis was performed to investigate the association of *HP* genetic variants on the severity of SCD. Additionally, Gene Set Enrichment Analysis (GSEA) was employed to identify biologically significant pathways linked to these polymorphisms, providing a more robust and comprehensive evaluation of their potential role in SCD severity.

Materials and methods

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive literature search was performed based on predefined inclusion and exclusion criteria. The study protocol was registered with PROSPERO (ID: CRD42024577211) to ensure methodological transparency and reliability.

Literature search

An extensive literature search was conducted using the following databases: Scopus, Embase, Google Scholar, Web of science, and PubMed. Articles published over a 15-year period

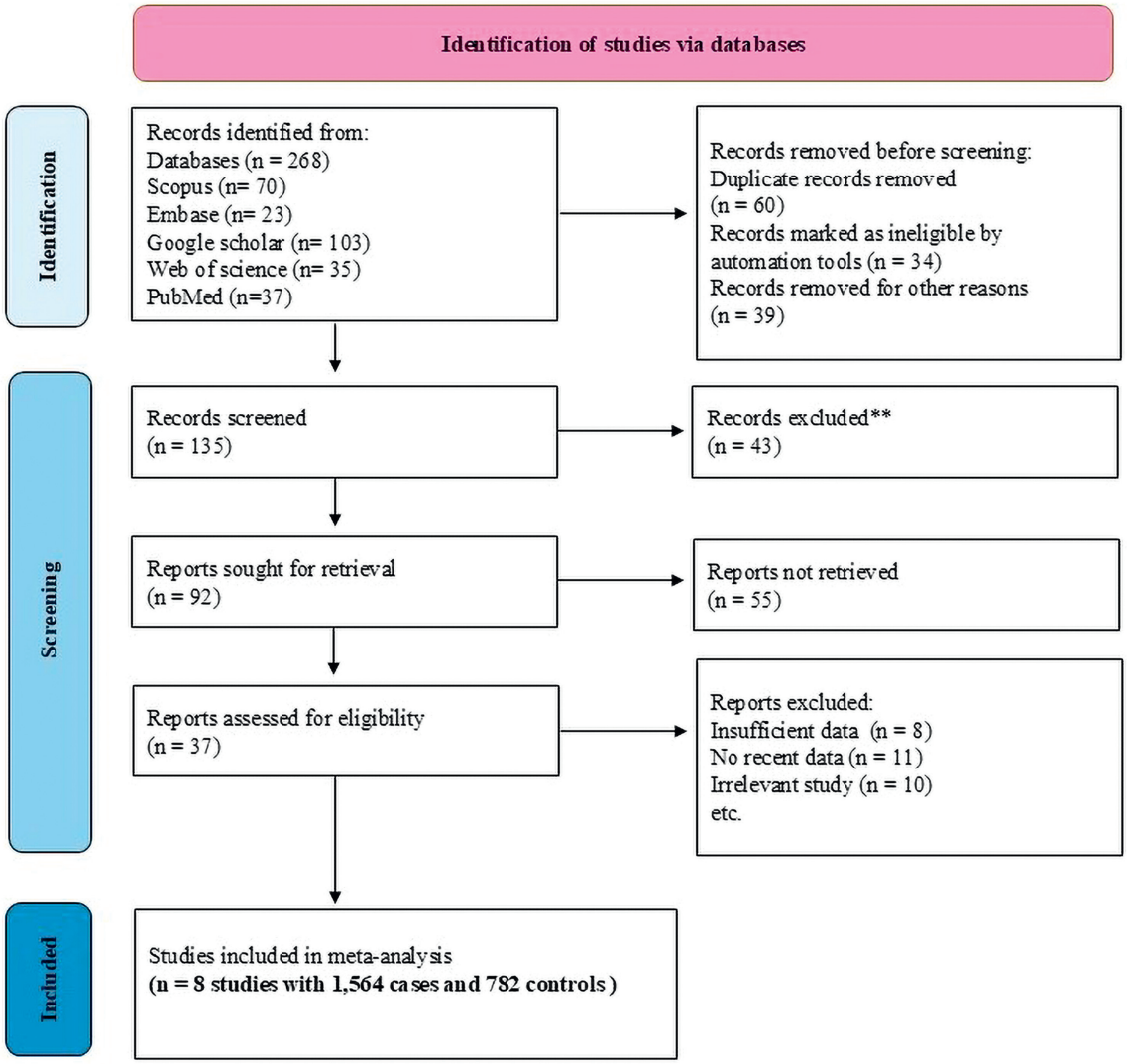

Fig. 1 – PRISMA flow diagram of the literature search and study selection process for *HP* gene polymorphisms and sickle cell disease (SCD) severity.

from 2010 to 2025 were analyzed. The search employed specific keywords like "Haptoglobin", "Sickle Cell Anaemia", "Sickle Cell Anemia", "Sickle Cell Disease", "gene polymorphism", "case vs. control", "*HP* gene", and "Haemoglobin S", combined with the Boolean operators (OR/AND). Both American and British spellings of key terms were included to ensure comprehensive coverage. The database was systematically updated, and duplicates were removed by manual screening. Reference lists of all included articles, relevant reviews, and meta-analyses were also screened to identify additional eligible studies not captured by the database search. No restrictions were placed on geographic origin, ethnicity, or publication status, provided the study met the inclusion criteria. The search strategy and study selection process followed the PRISMA guidelines to ensure methodological rigor and transparency (Figure 1).

Selection criteria

This meta-analysis encompassed studies that fulfilled the following inclusion criteria: (i) Case control studies investigating associations between *HP* gene polymorphisms and the severity of SCD, (ii) Availability of allele or genotype frequency data for both case and control groups, (iii) Studies conducted on human subjects, and (iv) The full text is accessible in English. Cases were defined as individuals diagnosed with SCD according to standard clinical and laboratory criteria, while controls were individuals without SCD. The exclusion criteria were: (i) The study did not explore the association between *HP* gene polymorphisms and the severity of SCD, (ii) Studies with overlapping or duplicate datasets, and (iv) Articles lacking extractable or complete data necessary for meta-analysis.

Data extraction

Investigators gathered relevant articles based on the criteria. Subsequently, after resolving any discrepancies with co-investigators, the articles were carefully scrutinized to extract the genotype and allelic frequencies of both the case and control participants. Some studies lacked complete genotypic information, and missing data were filled in using available allelic frequencies. Studies that did not provide essential data from the control and case groups were eliminated. Data were extracted from all articles including data on the Hardy-Weinberg Equilibrium (HWE), study design, the lead author's name, year of publication, ethnicity, sample size, and genotype frequencies of cases and controls. Strict guidelines were followed to check the eligibility of articles in respect to the screening criteria.

Methodological quality assessment

A thorough evaluation of bias in research is essential for accurately assessing the methodological quality of studies included in a meta-analysis. The Cochrane risk of bias tool (RoB 2) software was used to assess bias. The studies were categorized as three levels of bias: 'high risk,' 'moderate risk,' and 'low risk.'

Protein-protein interactions

The STRING database (v11.0) was used to predict functional interactions between proteins encoded by genes associated with SCD-linked polymorphisms. Differentially expressed genes (DEGs) were identified from public SCD datasets using a threshold of fold change >1.5 and p-value <0.05. These DEGs were subsequently utilized as input for the STRING database to construct protein-protein interaction (PPI) networks. This database predicts both direct (physical) and indirect (functional) interactions between proteins. A confidence score ≥0.4 was applied to define interactions, and up to ten additional interactors per node were included to extend the network. The resulting PPI network was evaluated for statistical enrichment (p-value $<1 \times 10^{-16}$) to confirm that the observed interactions were significantly higher than those expected by chance. This network analysis specifically highlighted potential interactions involving Hp, linking bioinformatic findings to the genetic associations observed in the present meta-analysis.

Enrichr-Gene ontology (GO) annotations of cluster networks

The Enrichr Web platform (https://maayanlab.cloud/Enrichr/) was employed to functionally annotate the proteins/genes identified in the PPI network. Genes were categorized according to: GO Biological process (BP), GO Cellular Component (CC), GO Molecular function (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. Differential expression data were used to classify genes as upregulated or downregulated, and pathway enrichment analyses were conducted separately for each group. The analyses identified biological pathways and processes potentially influenced by Hp, providing a mechanistic context for the associations observed in this meta-analysis.

Statistical analysis

The study investigated genetic variations of *HP* gene polymorphisms and their association with the severity of SCD using various statistical methods. Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated to evaluate the strength of the association, with statistical significance defined as p-value <0.05. To evaluate inter-study heterogeneity, the I^2 statistic was employed, with values ranging from 0 % to 100 % to represent the degree of variability. Statistical heterogeneity was assessed using the I^2 statistic and Cochrane's Q test. A fixed-effects model was employed when $I^2 \leq 50$ %, whereas a random-effects model was utilized for $I^2 > 50$ %. Significant inter-study heterogeneity was determined by a Q-statistic p-value <0.10. Summary ORs were assessed with a Z-test (p-value <0.05). Additionally, a sensitivity analysis was conducted to assess the impact of excluding specific studies, particularly those in which control groups deviated from the HWE. Egger's regression method was utilized to detect potential publication bias. All statistical analyses were performed using the MetaGenyo software.

Results

Search results

This research aimed to investigate *HP* gene polymorphisms related to the severity of SCD. The study utilized a comprehensive search using multiple databases and identified eight studies involving 1564 cases and 782 controls for the *HP* variant (Figure 1) [23–30]. Among these studies, three were conducted in the African population, three in the South American population, and one in the Asian population, along with an additional study performed in both Asian and African populations. The findings from these case-control studies are summarized in Table 1.

Risk bias

The accuracy of the methodologies used in the studies included in this meta-analysis was carefully assessed using the RoB 2 tool. The majority of the studies analyzed exhibited minimal bias (Figure 2).

Quantitative data analysis of HP gene polymorphisms with severity of sickle cell disease

The association between *HP* gene variations and the severity of SCD was explored in eight case-control studies. The results revealed a strong association between *HP* gene polymorphisms and the severity of SCD under the dominant model: *HP*2–1 + *HP*2–2 versus *HP*1–1 (odds ratio = 0.76; 95 % confidence interval: 0.61–0.94; p-value = 0.01). However, no substantial association was found in the allelic (*HP*2 versus *HP*1), recessive (*HP*2–2 versus *HP*2–1 + *HP*1–1) or the overdominant (*HP*2–1 versus *HP*1–1 + v2–2) models. Additionally, a

Table 1 – Characteristics of selected case-control studies for an association of *HP* gene polymorphism with sickle cell disease (SCD) and HWE score.

Study	Continental Identity / Country	Genotypic Distribution of Cases	Genotypic Distribution of Controls				HWE-p-value	HWE-adjusted p-value	Total Cases	Total Controls
		Hp1–1	Hp2–1	Hp2–2	Hp1–1	Hp2–1	Hp2–2				
Tuono et al., 2024	African/Cameroon	0	0	21	80	48	0	0.009	0.0405	21	128
Kengne et al., 2022	African/Cameroon	32	15	55	21	23	16	0.0774	0.1494	102	60
Meher et al., 2021	Asian/India	94	57	146	4	72	22	0	0	297	98
Bernard et al., 2021	African/Cameroon	32	15	55	21	23	16	0.0774	0.1494	102	60
Olatunya et al., 2020	African/Nigerian	43	40	18	22	20	3	0.5835	0.6564	101	45
Pierrot-Gallo et al., 2015	South America/Brazil	10	34	16	25	31	18	0.1836	0.2361	60	74
Santos et al., 2011	South America/Brazil	202	402	141	52	117	67	0.9455	0.9455	745	236
Adekile et al., 2010a	Asian/Kuwait	4	35	43	2	24	23	0.1615	0.2361	82	49
Adekile et al., 2010b	African/Nigerian	21	24	9	12	11	9	0.083	0.1494	54	32

HWE: Hardy Weinberg Equilibrium.

subgroup analysis based on ethnicity was conducted, demonstrating a notable association was found in the African and South American populations (p-value <0.05) (Table 2). Conversely, no association was observed for the Asian population (p-value >0.05), indicating that these differences are likely influenced by additional factors such as population genetics and healthcare access, rather than SCD prevalence alone. The heat map displays the results of multiple studies examining the relationship between *HP* genetic variants and SCD (Figure 3).

Heterogeneity, sensitivity analysis, and publication bias

The forest plot represents the heterogeneity of each genetic model (Figures 4 & 5). An evaluation of the sensitivity was conducted to investigate discrepancies in the HWE across different studies. Studies that demonstrated deviations from the HWE or intervention were omitted. Interestingly, the sensitivity analysis showed that the exclusion of studies did not notably impact the final p-value (Figure 6). A funnel plot was utilized to detect potential publication bias and validate the results. The results from the funnel plot did not reveal any significant bias (Figure 7).

Analysis of the string protein-protein interaction network

The PPI of recognized polymorphic proteins related to SCD was mapped and examined using STRING to determine hub genes. The Hp protein network has 54 edges and 21 nodes, with a PPI enrichment p-value $<4.24 \times 10^{-10}$ and a clustering coefficient of 0.72. The average node degree is 5.14 (Figure 8). The STRING analysis revealed two major functional clusters. The first cluster consisted of Hb subunits (HBA1, HBA2, HBB, HBD, HBE1, HBG2) and associated regulators (AHSP, HPR), which were strongly interconnected and central to oxygen transport and hemolysis. The second cluster included plasma and acute-phase proteins such as Hp, HPX, ALB, APOA1, ORM1/2, SERPINA1/3, A2M, and CD163. Notably, Hp emerged as a hub protein, linking the Hb cluster with plasma proteins, highlighting its role in Hb binding, heme clearance, and modulation of inflammatory responses in SCD. Together, these interactions suggest that Hb subunits and plasma proteins form a coordinated network to mitigate hemolysis-induced oxidative stress and vascular damage.

Gene set enrichment analysis

The present study employed GO enrichment analysis to gain a deeper understanding of how cluster genes in the network are affected in the SCD condition. Co-expressed genes were retrieved from the STRING database and input into the Enrichr database. These co-expressed genes were found to contribute to 116 BP, 22 CC, 19 MF, and 9 KEGG pathways. The most significant terms (p-value <−0.05) were oxygen transport (BP), endocytic vesicle lumen (CC), heme binding (MF), and African trypanosomiasis and malaria (KEGG) (Figures 9 & 10).

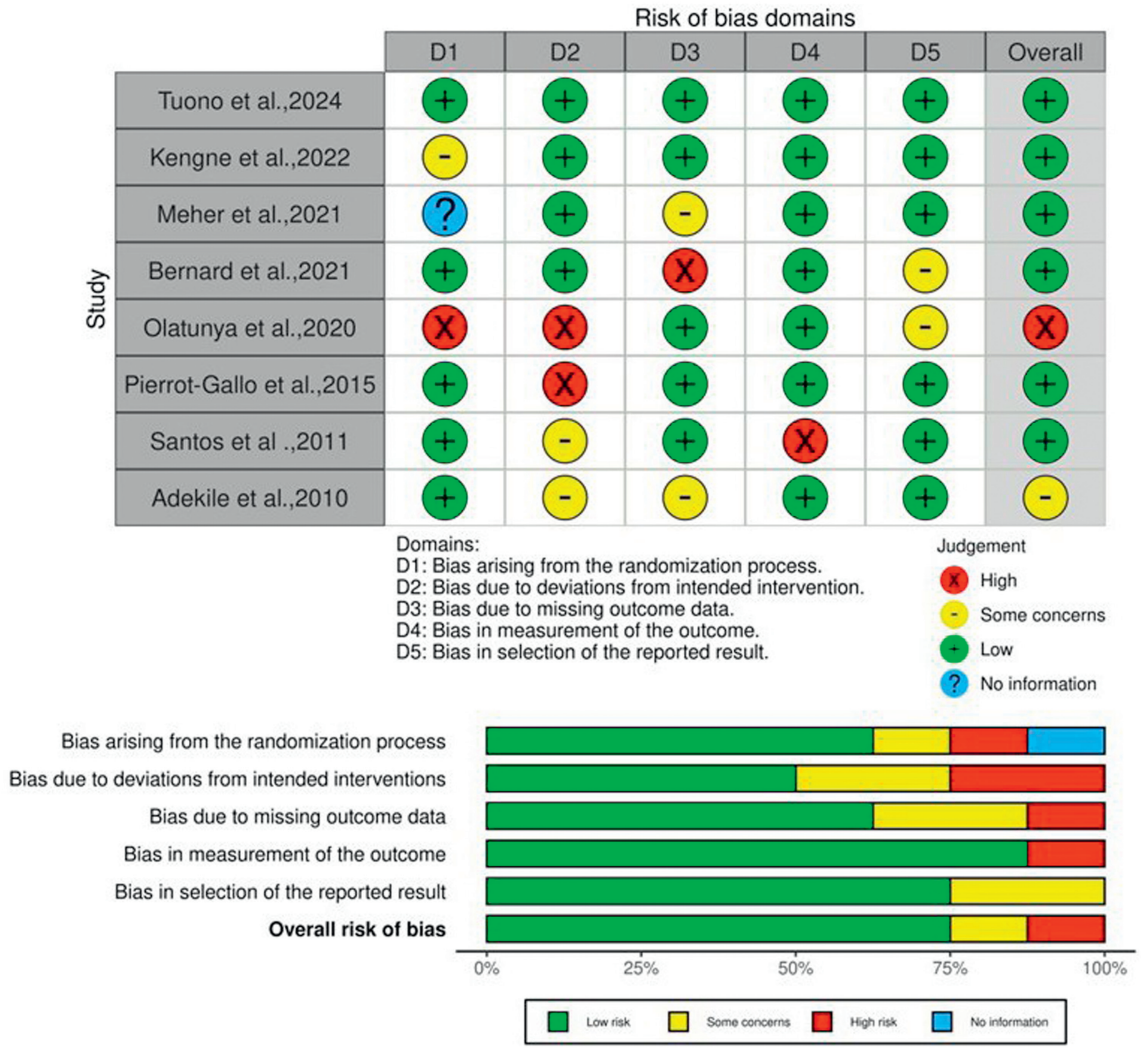

Fig. 2 – Methodological quality assessment of included studies using the Risk of Bias (RoB 2) tool.

Discussion

The present meta-analysis and gene set enrichment analysis provide the first comprehensive evaluation of the association between *HP* gene polymorphisms and SCD. A significant association was observed under the dominant genetic model (OR = 0.76; 95 % CI: 0.61–0.94; p-value <0.01), suggesting a potential protective role of certain *HP* genotypes in respect to the severity of SCD. In contrast, no significant associations were found under the allelic, recessive, or overdominant models. Subgroup analysis by ethnicity revealed a significant association in African and South American populations, however no association was observed in Asian populations. These regional differences may reflect genetic diversity and population-specific variations rather than differences in SCD.

Hp binds free Hb released during hemolysis, preventing kidney damage and oxidative stress [31]. The *HP* gene has three major genotypes (*HP*1–1, *HP*2–1, and *HP*2–2), which differ in Hb-binding capacity and may influence SCD manifestations [32]. Beyond Hb binding, Hp functions as an antioxidant, exhibits antibacterial activity, and regulates the acute phase response [17]. Among the variants, the *HP*1–1 phenotype exhibits the greatest activity, followed by *HP*2–1, while *HP*2–2 demonstrates the weakest activity [33]. Due to this genetic variation, the *HP* gene is a key candidate to explore potential associations with the pathophysiology of SCD and its subtypes [34]. Because of genetic diversity, it is proposed that multiple SNPs play a significant role in the clinical variability of SCD, therefore, identifying polymorphic genetic sites could assist in understanding the genetic complexity involved [35].

The results of the present study indicate that *HP* polymorphisms may influence oxidative stress and inflammation in SCD. This aligns with prior findings showing that an imbalance of the glutathione metabolism exacerbates oxidative stress in SCD [36]. and that *HP*2–2 carriers have higher iron-induced oxidative stress compared to some individuals with thalassemia [37]. The sensitivity analysis demonstrates that no single study has a major effect on the overall results. This meta-analysis confirms that variations of the *HP* gene conform to the HWE.

Table 2 – Subgroup meta-analysis of the association between *HP* gene polymorphisms with sickle cell disease (SCD) severity.

Model	Continental Identity	Number of studies	Test of association		Test of heterogeneity		Publication bias
			OR	95 % CI	p-value	p-value	I^2	p-value (Egger's test)
Allele contrast (A versus a)	Overall	9	0.9549	[0.8352; 1.0918]	0.499870532	0	0.8182	0.0069
	African	4	0.6092	[0.4580; 0.8104]	**0.000664356**	0.0001	0.854	0.3503
	Asian	2	0.9831	[0.7406; 1.3049]	0.905822671	0.6811	0	NA
	South America	3	1.1281	[0.9425; 1.3502]	0.188838144	0.0054	0.8082	0.0624
Recessive model (AA versus Aa +aa)	Overall	9	1.0858	[0.8619; 1.3679]	0.484800928	0	0.7848	0.4974
	African	4	0.8143	[0.5364; 1.2361]	0.334682089	0.0403	0.6384	0.0957
	Asian	2	6.1341	[2.5296; 14.8747]	5.98293E-05	0.0326	0.781	NA
	South America	3	1.0356	[0.7734; 1.3868]	0.814188379	0.0212	0.7406	0.1762
Dominant model (AA+Aa versus aa)	Overall	9	0.7632	[0.6161; 0.9456]	0.013428332	0	0.8885	0.0653
	African	4	0.3879	[0.2496; 0.6028]	2.55726E-05	0	0.8894	0.4663
	Asian	2	0.4249	[0.2785; 0.6484]	7.19307E-05	0.0286	0.7912	NA
	South America	3	1.4073	[1.0417; 1.9012]	**0.025994756**	0.0233	0.7341	0.0549
Over-dominant (Aa versus AA + aa)	Overall	9	0.6657	[0.5473; 0.8097]	4.65624E-05	0	0.9183	0.3783
	African	4	0.4048	[0.2570; 0.6376]	9.55732E-05	0.005	0.7663	0.723
	Asian	2	0.1896	[0.1238; 0.2905]	0	0	0.9576	NA
	Brazilian	3	1.2030	[0.9349; 1.5479]	0.150762965	0.2878	0.1971	0.9717

OR: Odds Ratio; 95 % CI: 95 % Confidence Interval; I^2: Heterogeneity.

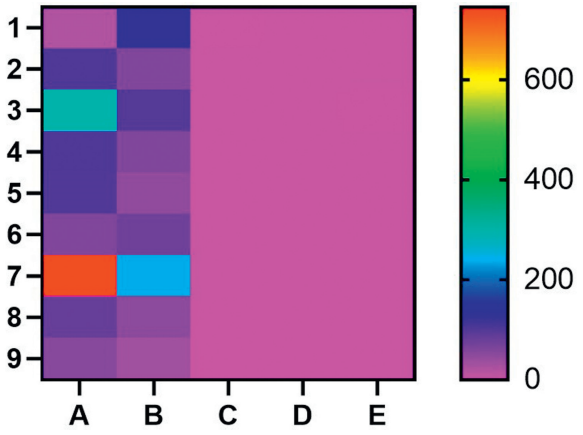

Fig. 3 – Heat map of study results. Each row represents an individual study, while the columns represent the following variables: A: Case (number of individuals with sickle cell disease) B: Control (number of healthy individuals) C: Frequency of the Hp-1 genetic variant D: Frequency of the Hp-2 genetic variant E: Measure of statistical significance. The color scheme represents the magnitude of the values in each column, with darker colors indicating higher values. Studies with significant findings (p-value < 0.05) are shown in red.

Funnel plots and Egger's test were employed to identify publication bias and show its absence. The validity of the findings was confirmed by evaluating the methodological quality using the RoB 2 tool, demonstrating decreased risk of bias of the studies. Thus, statistical evidence strongly supports proposed perspectives. Rigorous protocols were followed for data extraction and analysis. The gene enrichment analysis of *HP* gene polymorphisms and the meta-analysis of SCD risk provide crucial insights into the genetic factors influencing SCD severity. This analysis helps identify key genetic markers and pathways thereby opening the path for the creation of tailored treatments and focused interventions meant to reduce severity and improve patient outcomes [38]. The STRING PPI network analysis plays a pivotal role in elucidating the complex web of interactions between proteins involved in various biological processes. This analysis helps reveal key protein interactions, signaling cascades, and molecular mechanisms, facilitating a deeper understanding of disease etiology, drug targets, and therapeutic strategies [39]. The PPI analysis results identified Hp as a central hub protein, linking Hb subunits with plasma and acute-phase proteins. This network suggests that Hp, together with the HPX, CD163, and Hb subunits, plays a coordinated role in Hb binding, heme clearance, and modulation of inflammation in SCD.

Recent studies have underscored the significant role of *HP* gene polymorphisms in the pathophysiology of SCD, particularly concerning inflammation and iron metabolism. The study conducted by Tuono et al. demonstrated that SCD patients with the *HP*2–2 genotype exhibited higher levels of C-reactive protein (CRP), ferritin, and interleukin-6 (IL-6), indicating a pronounced inflammatory response and disrupted

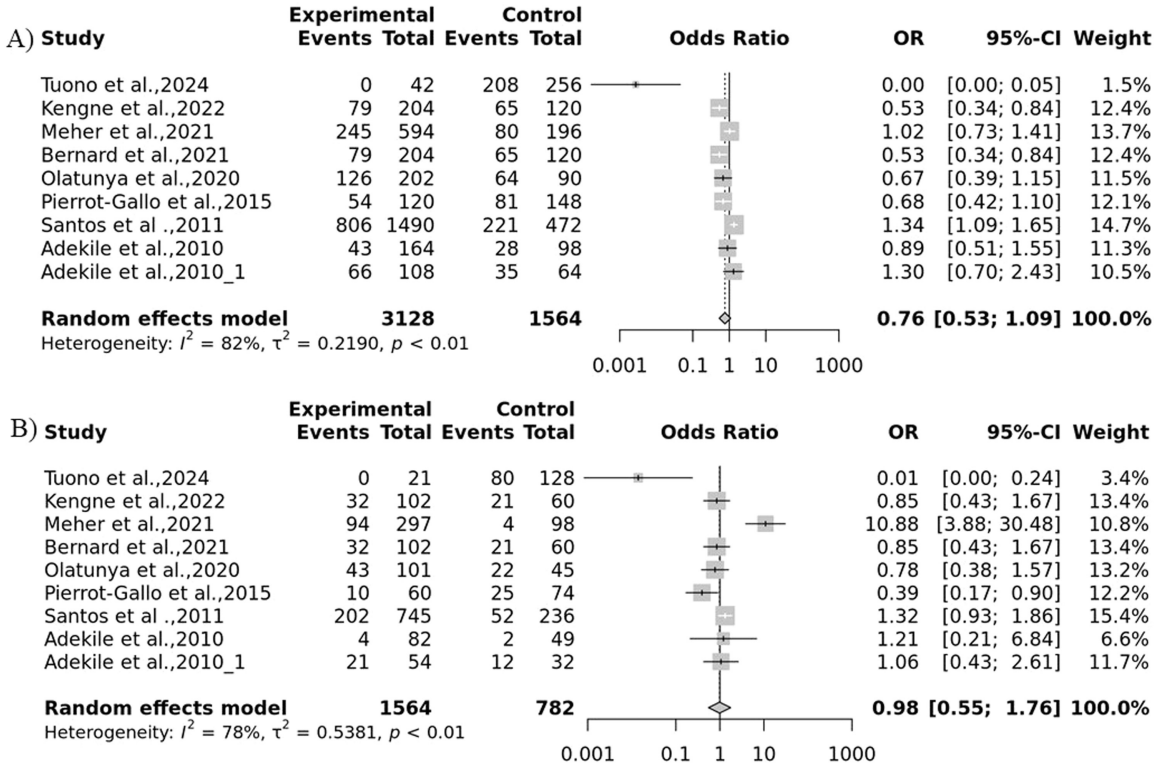

Fig. 4 – Forest plot for the association of *Hp* gene polymorphisms with severity of sickle cell disease (SCD) by (A) Allelic and (B) Recessive models.

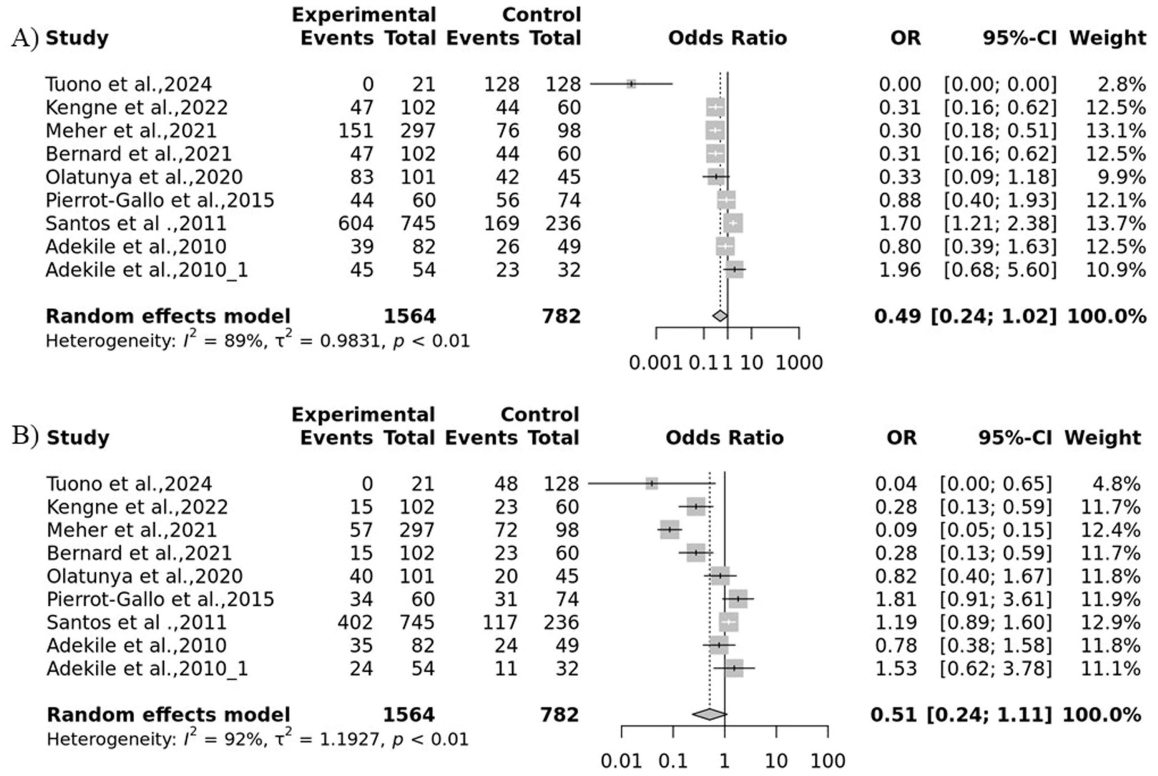

Fig. 5 – Forest plot for the association of *Hp* gene polymorphisms with severity of sickle cell disease (SCD) by (A) Dominant and (B) Overdominant models.

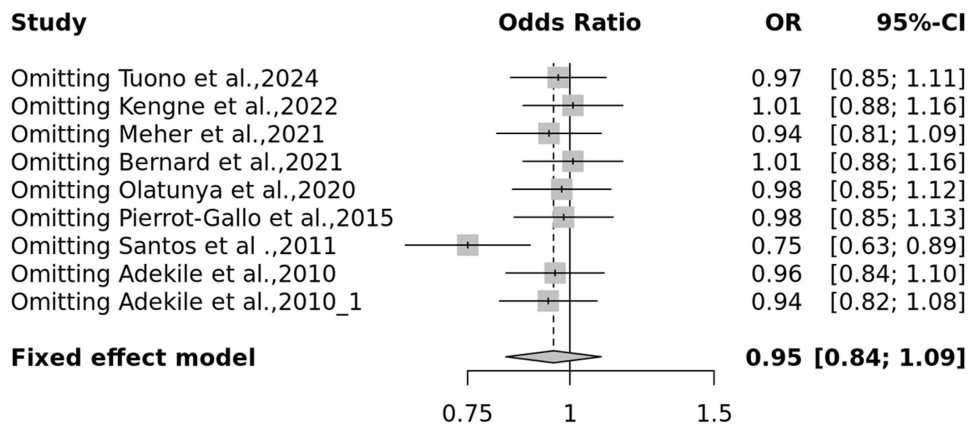

Fig. 6 – Sensitivity analysis on the association of *Hp* gene polymorphisms with increased severity of sickle cell disease (SCD).

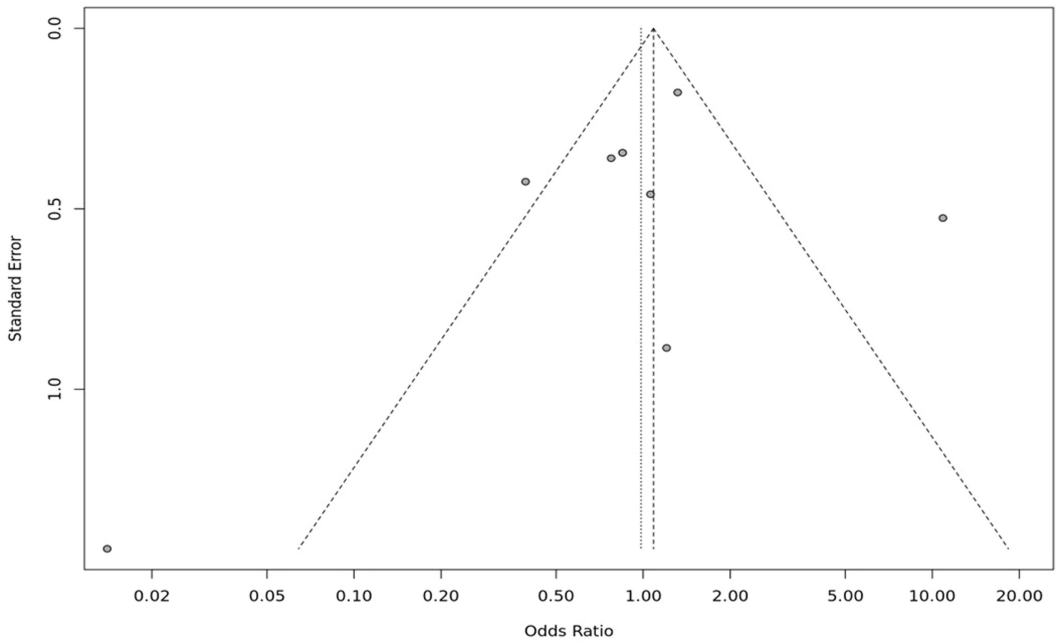

Fig. 7 – nnel plot assessing publication bias for the association between *HP* gene polymorphisms and sickle cell disease (SCD) severity.

iron homeostasis [23]. Similarly, research from West Cameroon highlighted a higher frequency of the *HP*1−1 genotype among SCD patients, suggesting regional variations in *HP* genotype distributions [24]. These findings reinforce the idea that the biological impact of *HP* polymorphisms is context-dependent, shaped by ethnic background, environmental exposure, and genetic interactions. Due to this variability, it is not currently appropriate to generalize *HP* polymorphisms as a universal predictive biomarker for SCD.

*HP*1−1 has the strongest Hb-binding and antioxidant activity and *HP*2−2, the weakest. Knowing a patient's *HP* genotype helps predict their ability to handle the 'hemolytic stress' in SCD. Identifying patients with the *HP*2−2 genotype (who have higher CRP and ferritin levels) could allow clinicians to provide earlier, more aggressive interventions for those at higher risk of iron-induced oxidative damage. The applicability of such biomarkers requires validation in larger, ethnically diverse cohorts using standardized protocols. This limitation is crucial to address before considering clinical translation.

Genetic heterogeneity across human populations involves variations in allelic architecture that significantly influence gene frequencies and disease severity. Such diversity, which varies across genomic regions and traits, results in distinct population-specific genetic structures [40]. This diversity can manifest in differences in linkage disequilibrium patterns and allele frequencies, which influence how genetic variants

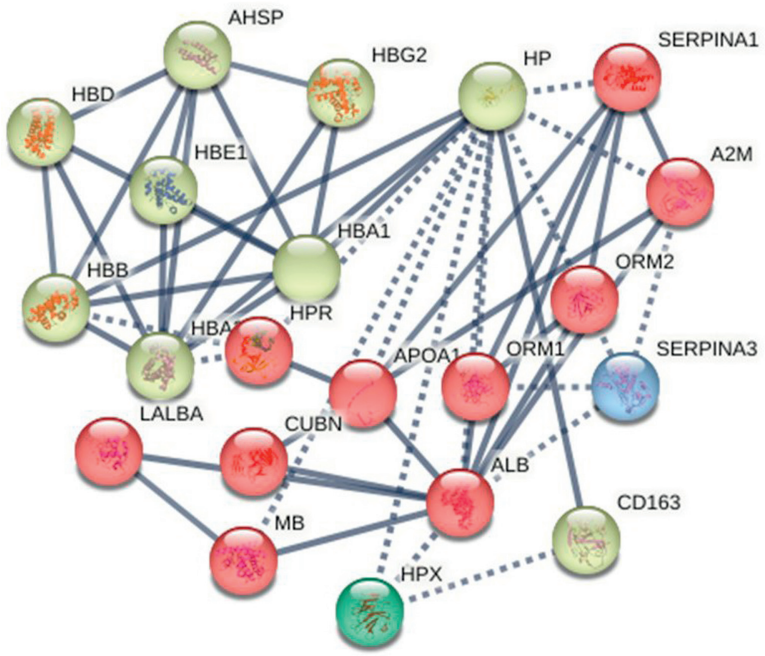

Fig. 8 – Protein-protein interaction (PPI) network analysis. STRING database visualization of functional interactions among differentially expressed genes (DEGs) associated with sickle cell disease (SCD). Inclusion criteria for the network were defined as p-value ≤0.05 and fold change (FC) ≥1.5 or ≤0.67.

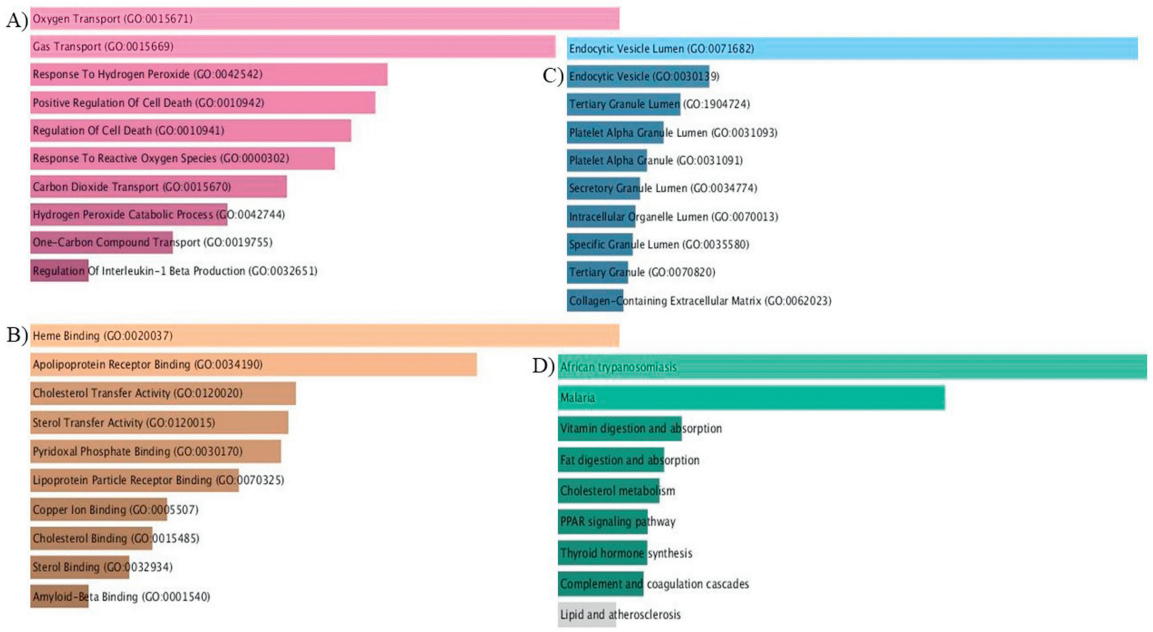

Fig. 9 – Functional enrichment analysis of differentially expressed proteins. Top ten enriched Gene Ontology (GO) terms - comprising Biological Processes (BP), Cellular Components (CC), and Molecular Functions (MF) - and KEGG pathways associated with the HP locus and SCD. Proteins were selected based on a fold change (FC) ≥1.5 or ≤0.67 and p-value ≤0.05. Bar length indicates the enrichment score, while color intensity represents statistical significance.

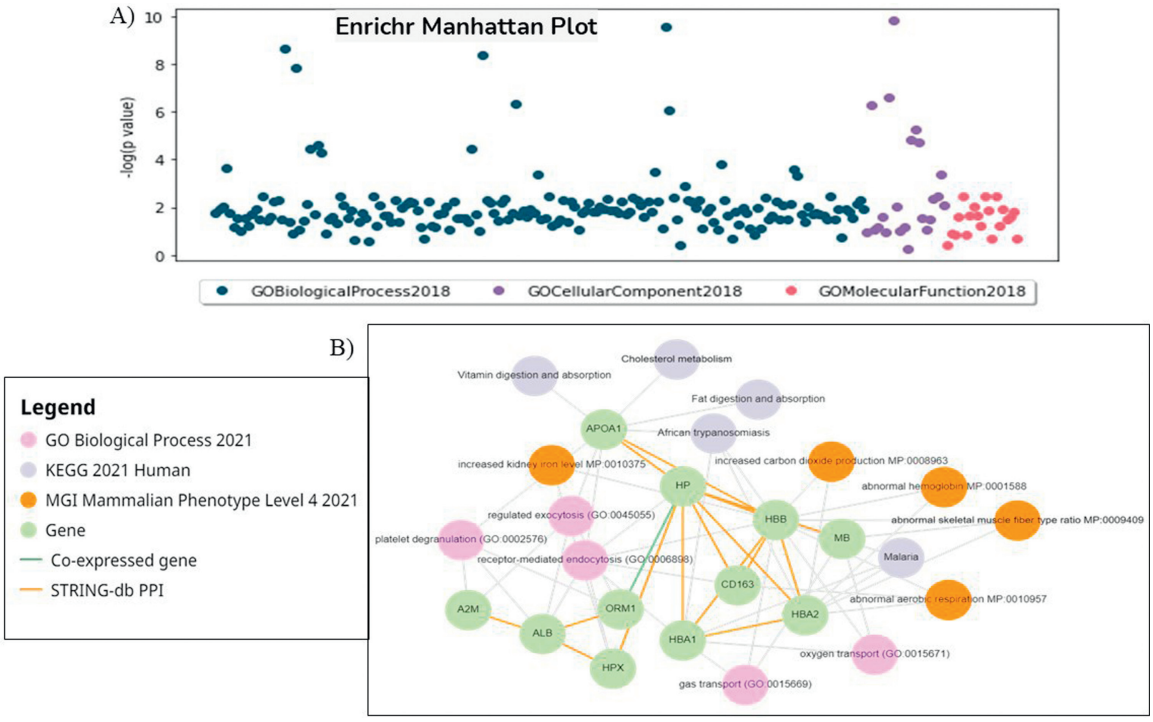

Fig. 10 – (A) Manhattan plot showing the enrichment analysis of gene ontology (GO) terms categorized as Biological Process, Cellular Component, and Molecular Function. Each dot represents a GO term with its position on the Y-axis reflecting the -log10(p-value) of its enrichment, indicating the statistical significance. The X-axis denotes the various GO terms across the three categories. (B) The network wiring shows interconnectivity between different pathways enriched by hub genes.

relate to traits or diseases across populations [41]. Genetic heterogeneity has critical implications for the discovery, development, and validation of biomarkers. Specifically, a marker identified in one population may lack universal validity due to inter-population differences in allele frequencies and underlying genetic architecture [42]. Although the findings support an association between *HP* gene polymorphisms and severity of SCD, the genetic heterogeneity among global populations may influence the predictive value of this biomarker. While *HP* genetic variants may serve as a viable biomarker in specific populations (particularly those of South American and African descent) its universal applicability as a predictive marker for SCD clinical outcomes remains to be established. Further studies are needed in genetically diverse cohorts to assess the broader relevance of *HP* polymorphisms as a global prognostic biomarker.

Limitations

This study has some limitations. It did not account for potential impacts from gene-environment interactions or other demographic factors. Additionally, the small sample size may have influenced the findings. Since the research was conducted in a limited population, definitive conclusions cannot be drawn. Therefore, a larger and more diverse sample size, involving various ethnic groups, is necessary. Further investigation with various demographics is recommended to improve the relevance and applicability of the findings. This study did not include Trial Sequential Analysis (TSA), due to the limited number of available studies, making TSA application impractical. This should be addressed in future research with larger datasets.

Conclusion

This meta-analysis demonstrates a significant association between *HP* gene polymorphisms and the severity of SCD phenotypes under the dominant model, particularly within African and South American cohorts. While no significant correlation was found in Asian populations, these findings suggest that *HP* variants serve as ethnic-specific modifiers of disease progression rather than primary risk factors. The correlation between increased severity of disease due to *HP* gene polymorphisms may not be the same in different cultural settings, and genetic correlations might differ greatly across ethnic groups. Such investigations into genetic polymorphisms are vital for improving risk stratification and informing the development of personalized treatment approaches. This study underscores the need for interdisciplinary research that integrates genetic modifiers into the broader understanding of the SCD pathology.

Acknowledgement

The authors would like to thank the management of Chettinad Academy of Research and Education (Deemed to be University) for providing facilities to perform this study.

Ethical approval

This study does not involve experiments with animals or human subjects.

Consent for publication

Not applicable.

Availability of data and materials

Data and materials used/analyzed during the current study are available from the corresponding author upon reasonable request.

Funding

Not Applicable.

Author's contributions

SV conducted literature searches, collected data, developed the methodology, contributed to writing, and performed data analysis. **NG, KG** and **PG** performed data analysis and created tables and figures. **LK** has done validation and data curation; **GK** conducted investigations, provided editing assistance, supervised, and conceptualized. All authors have thoroughly reviewed and approved the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Editor

Erich Vinicius De Paula

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Review article

Patient blood management in Brazilian cardiovascular surgery: An exploratory survey of surgeons' perceptions and reported practices

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ARTICLE INFO

Article history:
Received 16 December 2025
Accepted 1 June 2026
Available online 20 July 2026

Keywords:
Patient blood management
Cardiovascular surgical procedures
Anemia
Transfusion practices
Health care disparities

ABSTRACT

Introduction: Although the clinical benefits of patient blood management are internationally recognized, data regarding its implementation in Brazilian cardiovascular surgery remain limited. This study evaluated knowledge on patient blood management, reported practices, and perceived barriers among Brazilian cardiovascular surgeons.

Methods: This cross-sectional survey was conducted among 1,105 cardiovascular surgeons registered with the Brazilian Society of Cardiovascular Surgery. A 30-item questionnaire assessing professional characteristics, preoperative anemia management, perioperative transfusion practices, and patient blood management adoption was distributed via email. Descriptive and inferential statistical analyses were performed.

Results: The response rate was 19.4% (n = 205). Most respondents were male (91%) and based in the southeastern region. Preoperative anemia screening and management were heterogeneous. Thirty-one percent estimated the prevalence of preoperative anemia at 5%–15% in their institutions, and 43% reported hemoglobin testing 1–2 days before surgery. Approximately 25% routinely calculated erythrocyte mass and blood volume. Institutional transfusion risk protocols were reported by 48% of respondents, and 62% indicated standardized blood component request parameters. Antifibrinolytics and hemostatic agents were the most frequently reported coagulation strategies. Interest in patient blood management implementation was reported by 52%; perceived barriers included cultural resistance (43%), financial constraints (24%), and administrative challenges (21%). A statistically significant association was observed between geographic region and reported interest in patient blood management implementation.

Conclusion: Patient blood management implementation was reported as heterogeneous across institutions. Respondents identified cultural, economic, and structural barriers to patient blood management implementation. These findings reflect surgeons' self-reported

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https://doi.org/10.1016/j.htct.2026.106500

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perceptions and should be interpreted within the limitations of a cross-sectional survey with limited regional representativeness.

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Introduction

Annually, approximately two million people die from hemorrhagic causes related to trauma or surgical complications [1]. Effective blood management is therefore essential to ensure patient safety. Patient blood management (PBM) is an evidence-based strategy designed to optimize patient care and outcomes and has been recognized by the World Health Organization (WHO) as an important component of clinical practice [2,3].

PBM is a medical model that promotes the preservation and optimization of a patient's own circulating blood, treating it with the same consideration afforded to any other organ system. It is structured around three core pillars. In the preoperative phase, the focus is on identifying and treating conditions such as anemia and coagulation disorders [4]. During surgery, the objective is to minimize blood loss through various strategies, including the use of hemostatic agents, intraoperative blood salvage, and surgical techniques that reduce bleeding [5]. Postoperatively, rigorous monitoring is performed to optimize the patient's physiological tolerance to anemia and avoid unnecessary transfusions.

Despite its demonstrated effectiveness, PBM implementation may encounter several barriers, including limited awareness, financial constraints, and structural limitations. The transition to this care model requires cultural transformation within healthcare teams and a patient-centered approach supported by adequate institutional infrastructure. Several countries, including Australia, the United States, and members of the European Union, have implemented structured PBM programs [6,7].

Cardiovascular surgery is a major contributor to the demand for blood components, accounting for a substantial proportion of perioperative transfusions. In high-volume cardiac surgery centers, annual blood component utilization may reach 10,000 units. Although demand is lower in medium- and small-volume centers, ensuring patient safety remains equally critical [8].

In Brazil, PBM has gained increasing attention within the medical community; however, comprehensive national data regarding its implementation in cardiovascular surgery remain limited. We hypothesized that PBM knowledge and implementation among Brazilian cardiovascular surgeons would be heterogeneous and associated with regional and institutional factors, as well as perceived implementation barriers. This study aims to evaluate PBM knowledge, implementation, and reported practices among Brazilian cardiovascular surgeons, identify perceived challenges, and provide exploratory data to inform future structured assessments.

Methods

This cross-sectional, descriptive survey adhered to the ethical and legal standards established by Resolution No. 466/2012 of the Brazilian National Health Council and the guidelines of the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The protocol was approved by the Research Ethics Committee of the institution (No. 6.752.162; CAAE: 78128824.2.0000.0068), and data confidentiality was ensured in accordance with the Brazilian General Data Protection Law.

A survey was distributed to 1,105 cardiovascular surgeons registered with the Brazilian Society of Cardiovascular Surgery (SBCCV) to evaluate knowledge and reported practices related to PBM.

Of the 1,105 registered surgeons, 1,058 had valid email addresses and were invited to participate.

A 30-item questionnaire was adapted from a previously published instrument by Frietsch et al. [9] and included items addressing demographic and professional characteristics, preoperative anemia management, transfusion practices, coagulation management, and PBM implementation and barriers (Table 1). The questionnaire was distributed via email on three occasions between August and December 2024. Only one response per participant was accepted. Respondents were instructed to base their answers on the institution with which they had the strongest professional affiliation and to consider their primary institutional practice, including elective and emergency procedures as well as adult and pediatric cardiovascular surgeries.

The questionnaire was reviewed by specialists in cardiovascular surgery and hemotherapy to ensure content adequacy prior to dissemination. However, formal psychometric validation, including construct validity and reliability testing, was not performed.

Data were collected using Google Forms® and exported to Microsoft Excel (version 16.16.4) for organization and subsequent statistical analysis. Descriptive statistics included categorical variables expressed as absolute frequency (n) and relative percentage (%). Associations between categorical variables were assessed using Pearson's chi-square test (χ^2) or Fisher's exact test, with Monte Carlo simulation when appropriate. When statistically significant associations were identified (p-value <0.05), effect size was calculated using Cramér's V to estimate the magnitude of the observed association. Statistical analyses were conducted using JAMOVI statistical software version 2.2.5® and RStudio version 2024.04.2+764.

Table 1 – Survey - "situational diagnosis of patient blood management (PBM) in cardiological care in Brazil".

Q1. What is your self-identified gender?
Q2. What is your age group?
Q3. What is highest level of education you have completed?
Q4. In your current professional practice, what is the primary area of your surgical activity?
Q5. In which sector do you practice medicine: public sector, private sector, or both?
Q6. In which Brazilian state is your institution located?
Q7. In which Brazilian city is your institution located?
Q8. Approximately how many beds does your institution have?
Q9. What is the average annual volume of cardiac surgeries performed at your institution?
Q10. What is the average prevalence of preoperative anemia among patients in your institution? *Hemoglobin (Hb) <12g/dL in women and Hb <13g/dL in men.
Q11. What is the time frame for preoperative hemoglobin screening in your institution?
Q12. Where are preoperative exams usually conducted in your patients?
Q13. Are all preoperative exams available in the electronic medical records at your institution?
Q14. If anemia is suspected during the preoperative period, which additional tests are usually requested?
Q15. Does your institution provide follow-up and treatment for patients with preoperative anemia?
Q16. How is the preoperative anemia managed in your institution?
Q17. Do you assess erythrocyte mass and blood volume in your patients prior to surgery?
Q18. How do you evaluate the risk of transfusion, blood loss, and the required number of transfusion units?
Q19. Is a hemostasis or hemotherapy specialist available at your institution?
Q20. Which coagulation management tools are routinely used at your institution?
Q21. How often are two or more units of red blood cell concentrates requested for elective procedures?
Q22. Does your institution have a standardized guideline for requesting blood components based on specialty and/or surgical procedure?
Q23. Are the indications for blood transfusion properly documented at your institution?
Q24. How frequently does the transfusion committee at your institution convene?
Q25. Do you have knowledge of PBM principles and practices?
Q26. What clinical blood management practices are implemented at your institution?
Q27. Is your institution interested in implementing PBM?
Q28. What are the main challenges in implementing and managing PBM at your institution?
Q29. Which PBM practices are implemented for elective patients at your institution?
Q30. Which PBM practices are implemented for emergency patients at your institution?

Note: The questionnaire comprised primarily multiple-choice questions, with questions 7, 14, 18, and 28–30 being open-ended narrative questions.

Results

Demographic and professional profile of cardiovascular surgeons

The overall response rate was 19.4%, corresponding to 205 participants out of 1,058 valid email addresses of SBCCV members. Forty-seven registered members did not receive the survey due to invalid email addresses. The respondents were predominantly male (91%) (Figure 1A), most frequently aged between 46 and 55 years (Figure 1B). The highest level of education reported was residency or specialization (45%) (Figure 1C). Most respondents were affiliated with hospitals located in the southeastern region of Brazil (56%) (Figure 1D), particularly in São Paulo (38%), Minas Gerais (8%), and Rio de Janeiro (7%). No responses were received from surgeons located in the northern states of Amazonas, Roraima, and Acre, or from Sergipe in the northeastern region. A substantial proportion of respondents reported practicing in both the public and private sectors (39%) or exclusively in the public sector (36%) (Figure 1E). Regarding hospital size, 68% indicated that their institutions had between 101 and 500 beds (Figure 1F), and 75% reported performing fewer than 1,000 cardiac surgeries annually (Figure 1G). Additionally, 84% of respondents indicated that their primary surgical practice involved adult cardiovascular surgery.

Preoperative anemia management and screening protocols

The survey assessed strategies related to preoperative anemia screening and perioperative blood management. Thirty-one percent of respondents estimated the prevalence of preoperative anemia in their institutions to range between 5% and 15% (Figure 2C).

Among patients with suspected anemia, 43% of respondents reported measuring hemoglobin levels 1-2 days prior to surgery (Figure 2A). Additional laboratory tests were commonly requested for diagnostic evaluations and therapeutic planning. A complete blood count was the most frequently requested test (78%), followed by serum iron (56%) and ferritin (54%) (Figure 2D).

Sixty-nine percent of respondents indicated that their institutions provided treatment and follow-up for preoperative anemia, most commonly on an outpatient basis (34%) (Figure 2E). Approximately 25% of respondents reported routinely calculating erythrocyte mass and blood volume prior to surgical procedures (Figure 2F).

Perioperative blood transfusion and coagulation management

Forty-eight percent of respondents reported that their institutions followed a standardized protocol for transfusion risk assessment and perioperative blood loss management (Figure 3A). Sixty-seven percent reported the availability of a hemostasis or hemotherapy specialist for perioperative support (Figure 3B).

The frequency of requesting two or more units of red blood cell concentrates for elective procedures varied: 32% of respondents reported ordering them in more than 80% of surgeries, whereas 30% reported doing so in fewer than 20% of cases (Figure 3C). Additionally, 62% indicated that their institutions used standardized criteria for blood component requests based on specialty or procedure (Figure 3D).

Regarding coagulation management strategies, 42% of respondents reported the use of blood component transfusions, and 38% reported the use of antifibrinolytic agents (Figure 3E). Seventy-two percent reported that indications for

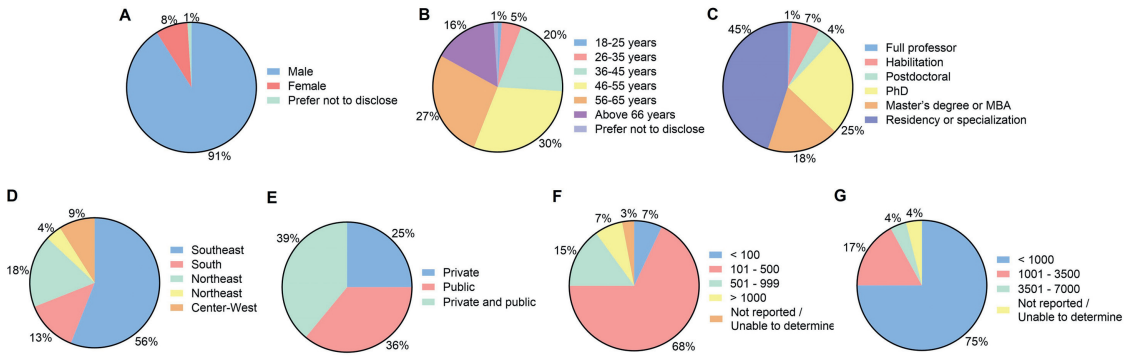

Figure 1 – Demographic and professional profile of cardiovascular surgeons.

(A) Gender distribution. (B) Age groups. (C) Highest level of academic education. (D) Geographic distribution by Brazilian region. (E) Type of hospital affiliation (public, private, or both). (F) Number of hospital beds. (G) Annual volume of cardiovascular surgeries. (H) Type of cardiovascular surgical practice (adult, pediatric, or both).

All percentages are based on respondents (n = 205), unless otherwise specified. Percentages may not total 100% due to rounding.

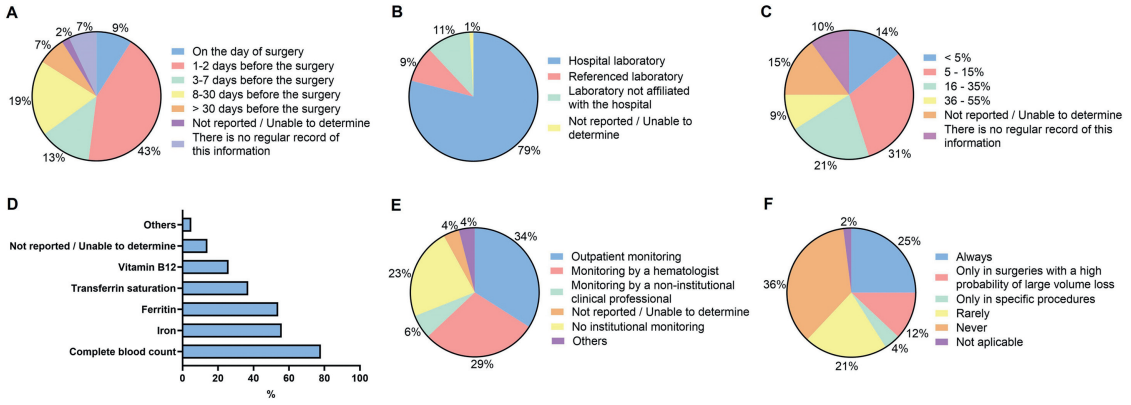

Figure 2 – Preoperative anemia screening practices.

(A) Timing of hemoglobin testing before surgery. (B) Location where the preoperative test is performed. (C) Estimated prevalence of preoperative anemia among surgical patients. (D) Laboratory tests used to investigate suspected anemia. (E) Settings where anemia treatment is conducted. (F) Frequency of erythrocyte mass and blood volume calculations. Preoperative anemia was defined as hemoglobin <12 g/dL in women and <13 g/dL in men.

All percentages are based on respondents (n = 205), unless otherwise specified.

blood transfusion were documented in their institutions (Figure 3F).

The frequency of transfusion committee meetings varied across institutions: 30% reported no regular meetings, 15% reported monthly meetings, and 2% reported weekly meetings (Figure 3G).

Adoption and challenges of patient blood management practices in Brazilian institutions from the respondents' perspective

Fifty-three percent of respondents reported being familiar with the concept of PBM (Figure 4A). The most commonly reported transfusion management strategy was the restrictive approach (45%), followed by the liberal approach (30%). Sixteen percent of respondents indicated that PBM guidelines were incorporated into routine clinical practice at their institutions (Figure 4B).

Regarding willingness to adopt PBM practices, 52% of respondents expressed interest in implementing PBM in their institutions, 1% reported no interest, and 13% stated that PBM protocols had already been adopted (Figure 4C).

Figure 5 illustrates the geographic distribution of respondents who expressed interest in implementing PBM. Among these respondents, 50.9% were based in the state of São Paulo. Reported interest in PBM implementation across other states ranged from 0.9% to 6.5%, with no respondents expressing interest in certain regions. A statistically significant

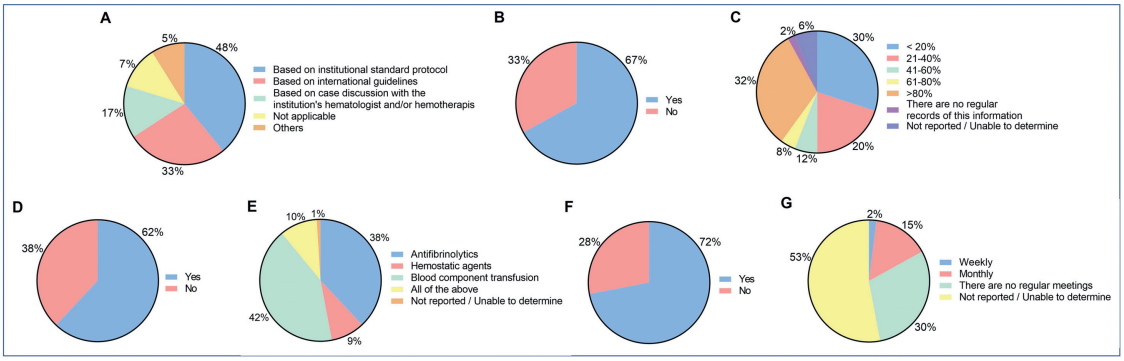

Figure 3 – Blood transfusion and coagulation management practices.

(A) Existence of institutional protocols for transfusion risk and perioperative blood loss. (B) Availability of hemostasis/hemotherapy professionals for surgical support. (C) Frequency of red blood cell requests for elective procedures. (D) Use of standardized parameters for blood component requests. (E) Most commonly used tools for coagulation management. (F) Documentation of blood transfusion indications. (G) Frequency of transfusion committee meetings.

All percentages are based on respondents (n = 205), unless otherwise specified. For multiple-response questions, percentages reflect the proportion of respondents selecting each option. Percentages may not total 100% due to rounding.

association was observed between geographic region and reported interest in PBM implementation (chi-square (22) = 33.3; p-value = 0.020). The effect size, calculated using Cramér's V, was 0.403, indicating a moderate association.

Among respondents reporting PBM implementation, the most frequently utilized interventions in elective surgical patients included antifibrinolytics (63%), hemostatic agents (61%), and point-of-care testing (31%) (Figure 4D). Strategies reported in emergency settings included antifibrinolytics (57%), hemostatic agents (58%), and intraoperative blood salvage (55%) (Figure 4E).

The most frequently reported barriers to PBM implementation were cultural resistance (43%), financial constraints (24%), and administrative challenges (21%) (Figure 4F).

Discussion

The present study provides a cross-sectional situational assessment of knowledge on PBM, reported practices, and perceived barriers among Brazilian cardiovascular surgeons. By exploring surgeons' perspectives, the findings offer exploratory insights into the heterogeneity of perioperative blood management strategies and the institutional variability surrounding PBM implementation.

The geographic distribution of responses demonstrated greater representation from the southeastern region, particularly São Paulo, while certain northern states and parts of the Northeast were not represented in the sample. Although this may reflect the concentration of high-volume cardiovascular

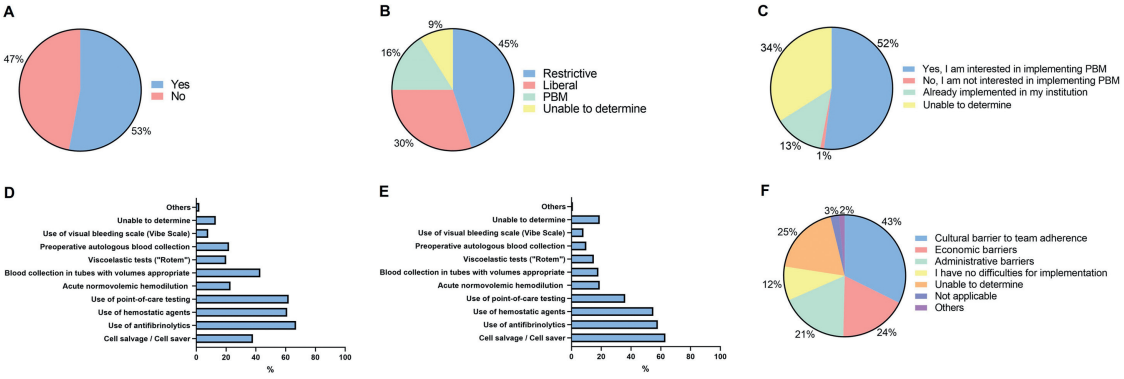

Figure 4 – Surgeons' knowledge, practices, and perceptions regarding patient blood management.

(A) Self-reported familiarity with patient blood management (PBM). (B) Transfusion strategies adopted in clinical practice. (C) Surgeons' interest in implementing PBM in their institutions. (D) PBM interventions most commonly used in elective procedures. (E) PBM strategies used in emergency settings. (F) Main reported barriers to PBM implementation.

All percentages are based on respondents (n = 205), unless otherwise specified.

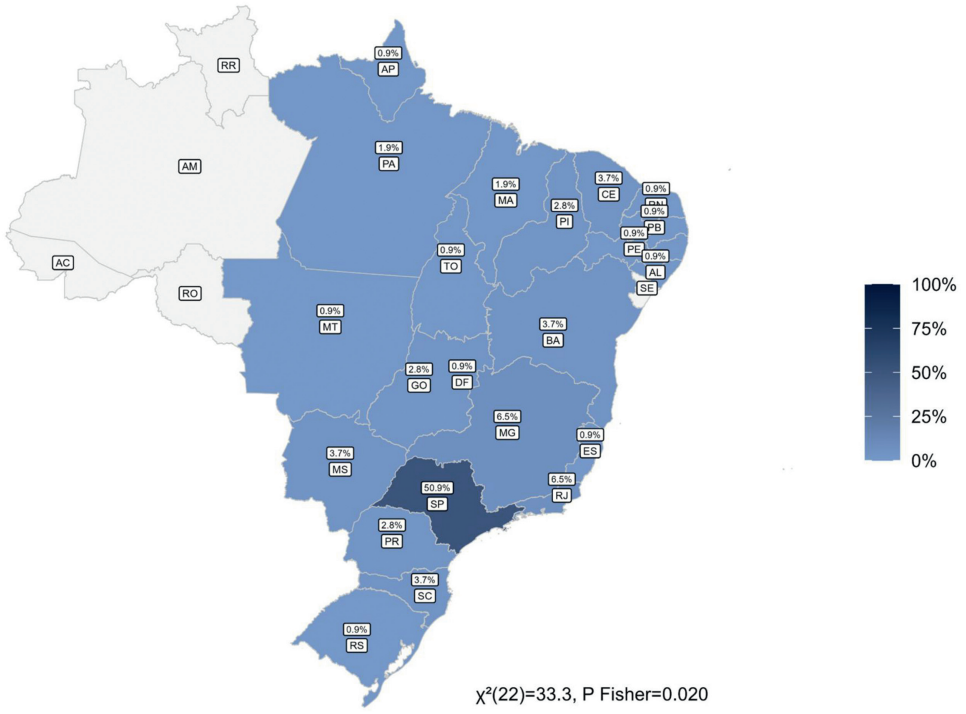

Figure 5 – Geographic distribution of respondents interested in implementing patient blood management.

Map of Brazil indicating the percentage of surgeons expressing interest in adopting PBM by state. All percentages are based on respondents (n = 205), unless otherwise specified.

centers and specialized infrastructure in more developed regions, it also underscores the uneven distribution of specialized surgical services across Brazil [10,11]. Importantly, because participation was voluntary and response rates varied regionally, these findings should be interpreted as reflective of the responding cohort rather than definitive national patterns. Nevertheless, regional variations in reported engagement with PBM may suggest differences in access to infrastructure, continuing education opportunities, and institutional support mechanisms.

A central finding of this study was the heterogeneity in the reported management of preoperative anemia. Although anemia is widely recognized as an independent risk factor for perioperative transfusion, postoperative complications, prolonged hospitalization, and mortality [12], structured evaluation and optimization pathways were inconsistently reported. A substantial proportion of respondents indicated that hemoglobin testing was performed only 1–2 days prior to surgery, which significantly limits the therapeutic window for effective intervention. From a clinical standpoint, early identification of anemia, ideally several weeks prior to elective procedures, is essential to allow adequate diagnostic clarification and targeted treatment.

Furthermore, although the complete blood count, serum iron, and ferritin were commonly requested, the reliance on isolated ferritin measurements may be insufficient in cardiac surgical populations. Anemia of inflammation and functional iron deficiency are highly prevalent among patients with chronic cardiovascular disease [13,14]. In such contexts, ferritin levels may be normal or elevated despite depleted bioavailable iron. A comprehensive diagnostic assessment, including transferrin saturation and evaluation of inflammatory markers, is often necessary to differentiate absolute iron deficiency from anemia of chronic disease. Without adequate diagnostic stratification and sufficient preoperative optimization time, opportunities for targeted interventions such as intravenous iron supplementation may be missed, potentially limiting the effectiveness of PBM strategies.

Transfusion practices also demonstrated variability among participating institutions. Approximately half of respondents reported the existence of institutional protocols for transfusion risk assessment and perioperative blood loss management. Although a majority indicated the availability of hemostasis or hemotherapy specialists, not all institutions reported regular transfusion committee meetings or consistent documentation practices. Structured transfusion oversight and institutional protocols have been associated with improved compliance with evidence-based transfusion thresholds and reduced variability in clinical decision-making [15,16]. The variability observed in this survey suggests differing levels of organizational maturity in transfusion governance across the institutions represented in the sample.

Regarding PBM awareness and implementation, slightly more than half of respondents reported familiarity with the PBM concept, yet only a minority indicated formal integration of PBM guidelines into routine institutional practice. This discrepancy between conceptual awareness and structured implementation is consistent with international experiences,

where knowledge dissemination alone is insufficient to ensure systemic adoption [17,18]. The most frequently reported barriers (cultural resistance, financial constraints, and administrative challenges) mirror those described in other healthcare systems undergoing transition toward PBM-based models.

The statistically significant association between geographic region and reported interest in PBM implementation further supports the presence of regional variation in engagement. However, this association must be interpreted cautiously, given the response distribution and the cross-sectional design. The findings reflect reported interest rather than objectively verified implementation levels.

In the global context, structured PBM programs implemented in the United States and several European countries have been associated with reductions in transfusion rates, improved adherence to restrictive transfusion strategies, decreased postoperative complications, and cost savings [19,20]. In countries such as Canada and Australia, governmental policies and institutional incentives have facilitated broader adoption of PBM frameworks, including reimbursement models aligned with evidence-based practices [21,22]. While these international experiences provide a relevant benchmark, the present study did not evaluate clinical outcomes, transfusion rates, or economic impact; therefore, direct extrapolation to the Brazilian context is not possible.

Technological infrastructure may represent a facilitating factor for PBM expansion. The high proportion of respondents reporting availability of electronic health records for preoperative laboratory data suggests potential capacity for protocol integration, automated alerts, and data-driven decision support [23,24]. Integration of electronic systems with standardized PBM pathways may support compliance monitoring and quality improvement initiatives.

Taken together, these findings provide an exploratory overview of surgeons' perceptions regarding PBM implementation in Brazilian cardiovascular surgery. Rather than establishing definitive national patterns, the study delineates areas of variability in anemia management, transfusion governance, and institutional readiness. By identifying perceived structural and cultural barriers, this survey offers a foundation for future multicenter evaluations, objective institutional audits, and the development of targeted educational and policy-driven initiatives.

Limitations

Several limitations must be acknowledged. First, the response rate of 19.4% introduces the possibility of non-response bias. Surgeons with greater interest or engagement in PBM may have been more likely to participate, potentially overestimating reported familiarity and implementation. Second, all data were self-reported and not independently verified at the institutional level, limiting the ability to confirm actual practice patterns. Third, certain geographic regions were underrepresented, restricting national generalizability. Fourth, although the questionnaire was adapted from a previously published instrument and reviewed by experts for content adequacy, it did not undergo formal psychometric validation, such as the assessment of reliability and construct validity. Finally, the cross-sectional design precludes evaluation of temporal changes, causal relationships, or outcome associations.

Despite these limitations, this study provides exploratory baseline data regarding surgeons' perceptions of PBM implementation in Brazilian cardiovascular surgery. The findings highlight areas of variability in anemia management, transfusion governance, and institutional readiness, which may inform future structured national assessments and targeted educational initiatives.

Conclusion

Among participating cardiovascular surgeons, PBM implementation was reported as heterogeneous across institutions. Respondents identified perceived cultural, economic, and structural barriers to implementation, in addition to variability in anemia management, transfusion governance, and institutional readiness for structured PBM integration. Variability was observed in preoperative anemia management, transfusion governance, and institutional readiness for structured PBM integration. These findings provide exploratory baseline data regarding surgeons' perceptions and may inform future multicenter evaluations, objective institutional assessments, and targeted educational initiatives. Interpretations should remain within the context of a perception-based cross-sectional survey with limited regional representativeness.

Funding

CSL Vifor Brazil.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

CSL Vifor Brazil Sociedade Brasileira de Cirurgia Cardiovascular (SBCCV) Prof Alfredo Fiorelli, Prof Marcelo Jatene, Prof Carlos Manuel Brandão.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Special article

Brazilian consensus recommendations on the diagnosis and treatment of light chain amyloidosis

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ARTICLE INFO

Article history:
Received 30 April 2026
Accepted 30 April 2026
Available online 2 June 2026

Keywords:
Amyloidosis
Light chain
Brazil
Consensus
Recommendations

ABSTRACT

Introduction: Systemic immunoglobulin light chain amyloidosis is characterized by the extracellular deposition of misfolded light chains, leading to progressive dysfunction of multiple organs. Diagnosis remains a significant clinical challenge and is frequently delayed due to the presence of nonspecific symptoms, coupled with limited access to specialized diagnostic tools and expertise. Currently, no established national recommendations for the diagnosis and management of this condition exist in Brazil.

Methods: These expert recommendations were developed following a structured advisory meeting in May 2025. The guidelines were formulated after a national survey of 96 Brazilian institutions (50 private and 46 public). Key diagnostic and therapeutic challenges were identified, including delayed diagnosis, limited access to biopsy techniques and biomarker testing, and uneven availability of treatment options. Recommendations focused on areas where consensus would benefit healthcare professionals in establishing best practices.

Results: The recommendations included: 1) Early detection: Educational initiatives for patients and healthcare professionals and the establishment of multidisciplinary specialized centers are needed to improve time to diagnosis and subsequent prognosis. 2) Accurate diagnosis: Clear, standardized procedures for tissue biopsy and timing are necessary to ensure accurate diagnosis and efficient testing. Improved access to Congo red staining and polarized light microscopy is critical. 3) Evaluation and classification: Biomarker testing should be used at all stages. Validated biomarker cut-offs and scoring systems should be

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https://doi.org/10.1016/j.htct.2026.106482

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implemented to standardize patient evaluation and risk stratification. 4) Optimal management and treatment: A multidisciplinary approach is crucial. Daratumumab should be added, if available, to bortezomib, cyclophosphamide and dexamethasone. Bortezomib and melphalan based regimens are an alternative.

Conclusion: These expert-guided recommendations aim to support earlier diagnosis, standardized evaluation, and optimized treatment of light chain amyloidosis in Brazil.

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Introduction

Amyloidosis is a heterogeneous disorder of protein misfolding and metabolism in which insoluble fibrils are deposited in various tissues (Supplementary Table 1), causing organ dysfunction and eventually death [1,2]. Immunoglobulin light chain amyloidosis (AL) is characterized by the excessive production of abnormal and unstable antibody light chains by a proliferative plasma cell or B cell clone. In the systemic disease, light chains produced by clonal cells in the bone marrow accumulate in multiple organs, particularly in the heart and kidneys [1,3,4]. Systemic AL amyloidosis is progressive and has high morbidity and mortality rates, particularly when diagnosis is delayed and significant organ damage has occurred [5,6]. Diagnosing and treating AL amyloidosis early is therefore critical to reduce or eliminate the light chain-producing plasma cell clone and halt organ damage [4,7–9].

Epidemiology and patient journey to diagnosis

AL amyloidosis is a relatively rare condition, with a worldwide incidence of 5.1–12.8 cases per million population [1,7]. Systemic disease is more common, with localized disease representing just 7–12% of all AL amyloidosis cases [5]. Median age at diagnosis is 64 years with incidence increasing with age; however there is no established association with ethnicity or geography [1]. Pre-existing plasma cell dyscrasias, including MGUS and multiple myeloma, carry a significant risk of progression to AL amyloidosis [10,11]. In 2025, a new definition of light chain MGUS was proposed, with the aim of decreasing the overdiagnosis of monoclonal gammopathies often linked to using the standard reference intervals for serum free light chains (sFLCs) [12]. With the new definition of light chain MGUS based on these revised, more accurate reference intervals, the false-positive rate of free light chain (FLC) testing decreased by 82% [12]. New reference intervals for sFLCs in individuals with chronic kidney disease have been created from the large prospective population-based cohort of the iStopMM study. Using the current reference intervals, 60% and 21% of patients had abnormal kappa and lambda sFLC values, respectively. Novel reference intervals (based on central 99% reference intervals) have been proposed for serum kappa FLC (mg/L), serum lambda FLC (mg/L), and the sFLC ratio in individuals with estimated glomerular filtration rates (eGFR) of 45–59, 30–44, and < 30 mL/min/1.73 m^2. However, it should be noted that these new ranges were established in a homogeneous cohort; therefore, they may not be fully generalizable to more diverse or wider populations [13]. These updates to the definition of MGUS will directly impact the diagnostic workflow (detection of the monoclonal component) and, in turn, the need for biopsies to detect amyloid deposits [14].

Light chain infiltration leads to multiorgan damage and dysfunction [7]. There are two forms of organ involvement: deposition of insoluble protein leading to organ dysfunction (primarily in the heart and kidneys) and direct tissue toxicity from circulating soluble-free light chains (e.g. elevated N-terminal pro-brain natriuretic peptide [NT-proBNP] values indicative of cardiac stress dissociated from structural alterations) [15]. Symptoms appear after organ damage has already occurred and are related to the organs involved; most patients have multi-organ involvement at the time of diagnosis [7,16,17]. One-year mortality is high (12%–30%), reflecting the failure to diagnose prior to irreversible end organ damage [9,18]. Heart involvement is the major determinant of prognosis and mortality [4,7]. Cardiac dysfunction results from proteotoxicity of the light chains and amyloid deposits that cause widespread disruption of tissue architecture [19].

Current treatment landscape

Advances in treatments of AL amyloidosis in the last 40 years have increased the median overall survival from 1.4 years in the 1980s to 4.6 years in the 2010s, with a growing number of patients achieving survival of ten years or longer [9,20,21]. Improvements in survival have lagged in patients with cardiac involvement. Deaths result from disease-related factors with cardiac failure (32%) and sudden unexpected death (23%) being the leading causes [9]. Overall survival in the 2010s was 8.8 years for patients with no cardiac involvement compared with 2.6 years in patients with cardiac involvement [9]. With improved long-term survival (29% of deaths occurring > 10 years after diagnosis), non-AL amyloidosis-related death has increased from 3% to 16% of the total deaths [9]. However, most patients who survived at least ten years required one or more subsequent lines of therapy [20], and patients continued to experience significant and persistent symptom burden after diagnosis, particularly while on active treatment [22].

Developing practical guidance for diagnosing light chain amyloidosis in Brazil

The incidence of AL amyloidosis in Brazil is estimated to be 6.72 cases per million population [23], though data on AL

amyloidosis are scarce. However, physicians are increasingly aware of the disease because of the growing number of publications, mainly from American, European, and Asian centers [24]. In Brazil, amyloidosis referral centers have emerged in recent years, with an aim to improve the diagnosis and treatment of patients [24,25]. Several guidelines and consensus recommendations exist for the diagnosis and management of AL amyloidosis worldwide, including regional versions, e.g., for the UK and Singapore [4,26–30]. However, there are no Brazilian guidelines or recommendations.

Objective

The purpose of this article is to provide expert-guided practical guidance and recommendations for the optimal diagnosis, evaluation and classification, and treatment of AL amyloidosis in Brazil. This article aims to educate and aid specialists and community physicians in Brazil who are involved in the treatment of patients with AL amyloidosis, as well as wider clinical teams who may support the early diagnosis of patients (including specialists such as cardiologists, nephrologists and neurologists).

Methods

These expert recommendations were formulated following a virtual structured advisory exchange on May 21, 2025. The objective of the meeting was to discuss the latest and most significant data for AL amyloidosis. Advisors in Brazil were selected based on their significant expertise in hematology and extensive experience managing AL amyloidosis within both academic and real-world settings. Selection criteria also included a distinguished record of peer-reviewed publications and active participation in AL amyloidosis clinical trials.

During the meeting, leading experts shared practical, real-world insights regarding the management of AL amyloidosis in Brazil, specifically focusing on: diagnosis (clinical manifestations and investigations); evaluation and classification (techniques, staging, and prognosis); and therapeutic strategies (treatment principles, the current landscape, and response evaluation). The topics selected were areas where consensus recommendations would be highly beneficial to healthcare professionals (HCPs) managing patients with AL amyloidosis and where alignment is required to establish best practices. In particular, the consensus topics and recommendations were informed by the results of the survey conducted at 96 institutions across Brazil (North, Northeast, Central-West, Southeast, and South) to identify the diagnostic and therapeutic challenges in AL amyloidosis; the survey questions are summarized in Table 1.

Expert consensus and recommendations were obtained using elements of the Nominal Group Technique (NGT), which allows simplicity, structured discussion, and immediate consensus [31]. To implement the NGT, the meeting was structured to first review key data around each topic. This review was followed by a discussion in a round robin format to allow sequential view sharing and equal contribution from all advisors. Group discussion occurred at the end of the round robin to allow time for questions, clarification, and consolidation of key recommendations.

Recommendations generated are based on the available clinical evidence, as well as clinical experience and knowledge gained through daily practice of the participating experts in the field of AL amyloidosis. Although the best-practice recommendations are intended to be a flexible tool to assist timely and informed decisions, they should not replace sound clinical judgment or be used as a legal resource.

Light chain amyloidosis diagnosis

Difficulties in early detection

AL amyloidosis is a heterogeneous disease with the potential to affect multiple organs, resulting in broad and variable clinical presentations that complicates diagnostic characterization [32]. The diagnosis is delayed as symptoms typically emerge only after organ damage has accumulated to the point of dysfunction. Increased serum FLC levels may be present for at least four years before the onset of clinical manifestations [7,33]. Early symptoms of AL amyloidosis are vague (i.e., neurologic symptoms, fatigue, dizziness, abdominal pain) and may be misattributed by patients and HCPs to other, more common conditions or simply as part of aging [16,34,35]. Once symptoms become more serious, their heterogenous nature may further contribute to misdiagnosis and diagnosis delay [36]. In a retrospective study of patient medical records, patients had a median of five precursor diagnoses within the two years prior to diagnosis [35]. The median time from symptom onset to AL amyloidosis diagnosis is approximately six months to 2.7 years with 50% of patients visiting five or more physicians of different medical specialties before diagnosis [16]. HCPs involved in diagnosis include, but are not limited to, primary care providers, medical internists, cardiologists, nephrologists, neurologists, gastroenterologists and hematologists [34]. Notably, patients with renal involvement often experience a more direct diagnostic pathway and are frequently diagnosed within six months of symptom onset, likely reflecting the routine use of kidney biopsy in patients with proteinuria. In contrast, delayed diagnosis is particularly consequential in patients with cardiac involvement, for whom early recognition is critical to improving outcomes [32].

The clinical presentation of AL amyloidosis depends on organ involvement, most commonly the heart, kidneys, nervous system, soft tissues, and liver [37] (Supplementary Figure 1, Supplementary Table 2). Symptoms more specific to AL amyloidosis are not common and present later in the disease, further complicating diagnosis [4,16,34]. Symptoms that should immediately raise suspicion of possible AL amyloidosis include the involvement of soft tissues with macroglossia, periorbital purpura, submandibular gland swelling, and the shoulder pad sign; however, these highly specific symptoms are rare and occur only in a subset of individuals [37,38]. In the absence of more specific symptoms, physicians should suspect AL amyloidosis when patients present with heart failure with preserved ejection fraction, nephrotic-range proteinuria in the absence of diabetes, peripheral neuropathy, unexplained hepatomegaly, and diarrhea, especially when

Table 1 – Questions from a survey conducted at 96 institutions across Brazil to identify the diagnostic and therapeutic challenges in light chain amyloidosis.

Survey Title: Diagnosis and Treatment of light chain Amyloidosis (AL) in Brazil – Mapping Challenges and Possible Pathways	
Question	Possible responses
1) How many cases of AL amyloidosis are diagnosed annually at your institution?	0–5 6–10 11–20
2) In your clinical practice, what is the average time between the onset of the first signs/symptoms and the diagnosis of AL amyloidosis?	< 6 months 7–12 months 13–24 months > 24 months
3) Do you have easy access to different specialties (such as cardiology, nephrology, neurology) for multidisciplinary diagnostic and therapeutic management of patients with AL amyloidosis?	Yes No
4) In your clinical practice for the multidisciplinary diagnostic and therapeutic management of patients with AL amyloidosis, which specialties do you have easy access to?	Cardiologist Nephrologist Neurologist Dermatologist Gastroenterologist Gastrointestinal surgeon Hepatologist Interventional radiologist Quaternary hospital
5) Which of the following tests for detecting the monoclonal components are available in your clinical practice for investigating AL amyloidosis?	Serum protein electrophoresis Serum immunofixation Urinary immunofixation dFLC None of the above
6) Which of the following tissues are available for collection in your clinical practice for the purpose of identifying amyloid material?	Bone marrow Abdominal fat Tongue Myocardium Kidney Peripheral nerve Gastrointestinal tract Salivary gland Liver
7) Which technique is used for abdominal fat collection?	Incisional biopsy Punch biopsy Core needle biopsy Fine needle aspiration of abdominal fat None of the above
8) Which of the following techniques are available in your clinical practice for anatomopathological analysis and identification of amyloid material?	Congo red staining Polarization with polarized light (birefringence) None of the above
9) Which of the following amyloid protein typing techniques are available in your clinical practice?	Immunohistochemistry Immunofluorescence Immunoelectron microscopy Mass spectrometry None of the above
10) Is mass spectrometry performed at your institution or are the samples sent to a reference center?	Performed internally Sent to national reference laboratory Sent to international center I do not know
11) Which of the following tests are available in your clinical practice for prognostic and organ dysfunction assessment in patients with AL amyloidosis?	NT-proBNP Troponin (T or I) Echocardiogram with GLS Echocardiogram without GLS Cardiac MRI Scintigraphy 24-hour proteinuria None of the above
12) Which of the following therapies are available in your clinical practice for the treatment of patients with AL amyloidosis?	Autologous HSCT Melphalan Cyclophosphamide Proteasome inhibitors (e.g., bortezomib, carfilzomib) Anti-CD38 antibody (e.g., daratumumab)

Table 1 (*continued*)

Survey Title: Diagnosis and Treatment of light chain Amyloidosis (AL) in Brazil – Mapping Challenges and Possible Pathways	
Question	Possible responses
	BCL-2 inhibitors (e.g., venetoclax) Thalidomide Lenalidomide None of the above (referral to relevant service, we do not perform any chemotherapy)

AL: immunoglobin light chain amyloid protein; BCL-2: B-cell lymphoma 2; CD: cluster of differentiation; dFLC: difference between involved and uninvolved free light chains; GLS: global longitudinal strain; HSCT: hematopoietic stem cell transplantation; MRI: magnetic resonance imaging; NT-proBNP: N-terminal pro-brain natriuretic peptide.

multiple organ systems are involved [38] (Supplementary Figure 1, Supplementary Table 2).

Diagnostic testing

Assessment for AL amyloidosis is indicated when there is a clinical suspicion of the disease due to the presence of common symptom patterns (Supplementary Figure 1, Supplementary Table 2). Patients with pre-existing conditions that increase the risk of acquiring AL amyloidosis would benefit from regular screening for biomarkers of the disease that may increase before symptoms appear. Biomarkers, including alkaline phosphatase (ALP), proteinuria, and NT-proBNP, should be monitored regularly in patients with MGUS to detect early signs of organ involvement [39]. Patients are assessed for AL amyloidosis using a 4-step algorithm: 1) Screening for clinical suspicion of AL amyloidosis, 2) identification of monoclonal light chains, 3) tissue diagnosis, and 4) typing of tissue employing adequate technologies (Figure 1) [7,8,40]. The presence of plasma cell dyscrasia in patients with suspected AL amyloidosis is determined by the identification of monoclonal light chains by three tests: serum protein immunofixation, urinary immunofixation, and the serum kappa/lambda ratio [4,8]. Because the clonal plasma cell population in AL amyloidosis is often small (< 10% of total bone marrow cells), protein electrophoresis is frequently negative and therefore usually omitted from the diagnostic algorithm [4,8]. Once a monoclonal component has been detected, the presence of AL amyloidosis requires the identification of amyloid fibrils in a tissue biopsy. This confirmation is based on the demonstration of amorphous tissue material that stains positive with Congo red and exhibits the characteristic apple-green birefringence under polarized light, a critical step to avoid both false-positive and false-negative interpretations in the routine practice [4,41,42]. Congo red is a relatively inexpensive dye with a long shelf-life that rebinds all forms of amyloid [41]. The initial evaluation typically relies on combined sampling of peripheral tissues, such as abdominal fat aspirate or biopsy, bone marrow, or salivary gland, which are more accessible and carry lower procedural risk. However, negative fat and bone marrow biopsies do not rule out AL amyloidosis; there is still an approximate 15% chance of disease and hence further biopsies of the appropriate organs are recommended [18]. Biopsies of clinically affected organs provide higher sensitivity and specificity but are less readily available and associated with a higher risk of complications, such as perioperative bleeding of which kidney biopsies pose a significant risk [4,18,43]. The sensitivity of peripheral tissues in detecting amyloid deposits can be lower in the clinical practice than that demonstrated by the literature (e.g., abdominal fat pad biopsy). In individuals who are highly suspected of having AL amyloidosis and have an absence of amyloid deposits in peripheral tissues, biopsy of an affected organ should be prioritized. According to the American Society of Hematology (ASH) guidelines on the diagnosis of AL amyloidosis, the combination of fat pad sampling and bone marrow biopsy yields a high sensitivity (89%). When performed individually, however, sensitivity is significantly lower (76.6% and 55.1%, respectively), whereas endomyocardial biopsy demonstrates a sensitivity of 93.5%. However, these guidelines recognize that proceeding with one approach over the other is dependent on several external factors including resource access, available expertise, clinical presentation, and associated costs [13]. Once amyloid deposits have been confirmed with Congo red testing, the type of amyloid protein must be identified in a tissue biopsy using more specific methods such as immunological techniques or mass spectrometry. Genetic testing may also contribute to subtyping the amyloidosis in hereditary cases [44]. The choice of the subtyping technique depends on ease of referrals, funding availability, cost, and access to specialized resources, including specific antibodies and mass spectrometry expertise [44].

In the absence of a monoclonal component, patients with suspected cardiac amyloidosis should be tested for transthyretin (ATTR) amyloidosis using cardiac scintigraphy with bone-seeking tracers, notably ^{99m}Tc-diphosphono-propanodicarboxylic acid, ^{99m}Tc-pyrophosphate, and ^{99m}Tc-hydroxymethylene diphosphonate [4,45]. A DNA analysis is necessary to differentiate between hereditary and wild-type ATTR amyloidosis and to dismiss other rarer hereditary forms [4,45].

Risk stratification

Patients diagnosed with AL amyloidosis undergo risk stratification and disease staging based on the type and extent of organ involvement to help determine prognosis and guide treatment decisions. Over recent decades, improved diagnostic accuracy and therapeutic advances have led to a better understanding of the natural history of AL amyloidosis and

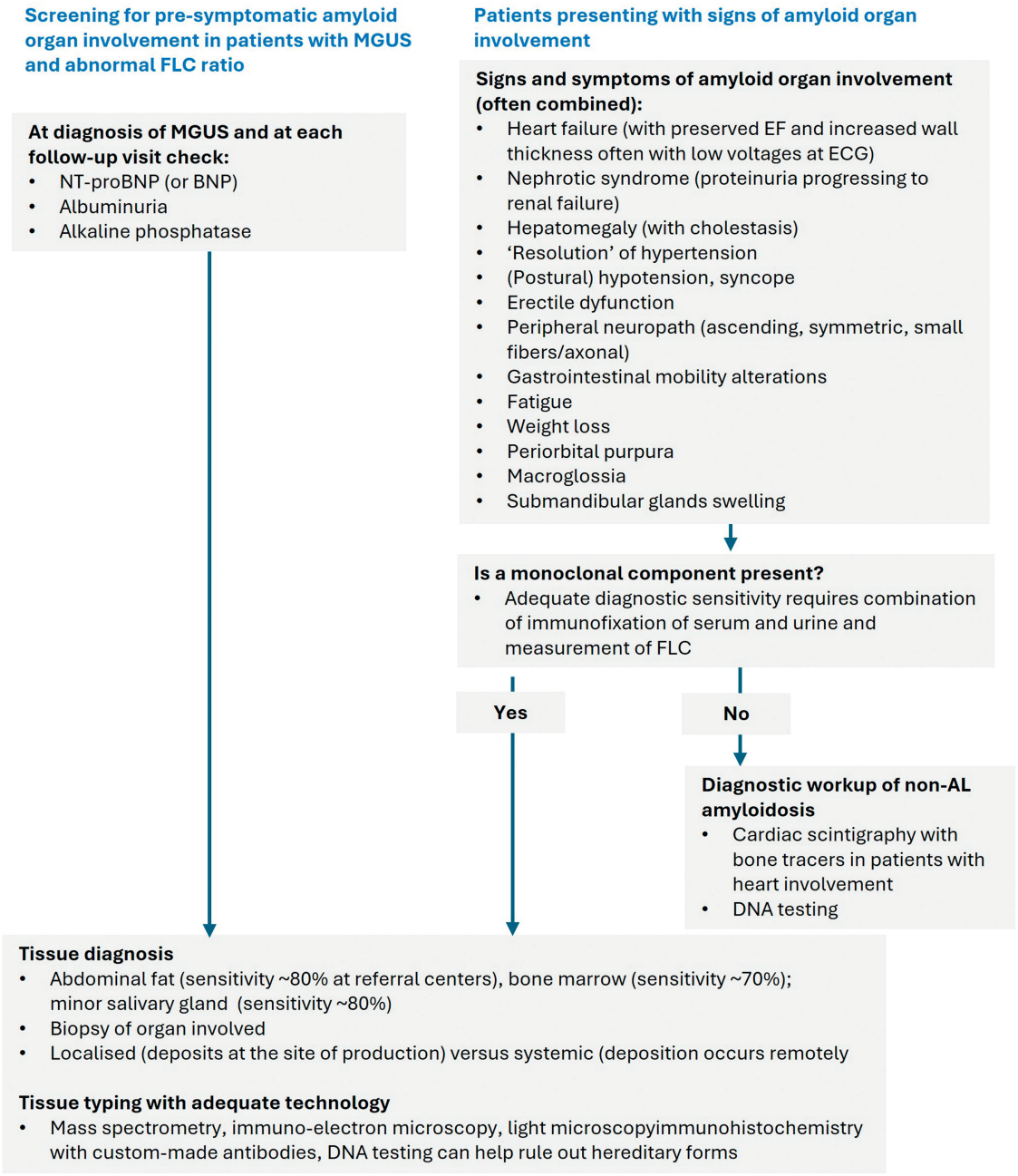


Figure 1 – Diagnostic algorithm for AL amyloidosis

Adapted from Palladini [4]

AL: Immunoglobin light chain amyloid protein; BNP: B-type natriuretic peptide; ECG: electrocardiogram; EF: Ejection fraction; FLC: Free light chain; MGUS: Monoclonal gammopathy of undetermined significance; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

contributed to better overall outcomes [9]. Nevertheless, prognosis remains strongly influenced by the diagnostic timing and the presence and severity of cardiac involvement – a key determinant of both treatment feasibility and survival. Cardiac complications, particularly progressive heart failure and malignant arrhythmias, remain the leading causes of morbidity and mortality in AL amyloidosis [9]. The current staging systems therefore rely heavily on measures of cardiac function and circulating biomarkers of cardiac damage [8,29]. Cardiac biomarkers assessed in these models include levels of troponin T (TnT) and NT-proBNP or brain natriuretic peptide (BNP) [46]. Additional criteria used for disease staging include an assessment of clonal disease based on the difference between involved and uninvolved circulating FLC levels (dFLC); however, in the daratumumab era, this may not be as prognostically important [18]. Cardiac biomarkers alone

might be more suitable to stage AL amyloidosis as baseline FLC showed no effect on the depth of response in newly diagnosed patients [47]. A renal staging system, in which renal involvement is assessed as the eGFR level and degree of proteinuria, helps predict the risk of progression to dialysis [46].

Staging models that utilize soluble cardiac biomarkers include the Mayo 2004 model, the European 2015 modification of Mayo 2004 model, the Mayo 2012 model, and the Boston University (BU) model [7,48,49]. The Mayo 2004 model and its European modification rely on TnT and NT-proBNP levels to assign risk and are most widely used to determine patient management, while the Mayo 2012 and BU models incorporate dFLC and BNP, as additional markers of disease burden, respectively [4,7,8,48]. The European modification of Mayo 2004 more accurately predicts early death than Mayo 2012; the Mayo 2012 more accurately predicts late survival than Mayo 2004 [48]. In 2024, Khwaja et al. found that incorporating myocardial global longitudinal strain (GLS) into the European modification of Mayo 2004 allowed the identification of patients at highest risk of death ("Stage IIIc"), though this model has not yet been externally verified [50]. In addition to the cardiac-based models, a renal staging model has been developed to predict the risk of progression to dialysis [8,48] (Supplementary Table 3). Additional biomarkers with predictive qualities that have not yet been incorporated into staging systems include von Willebrand factor, D-dimer, and growth differentiation factor 15 [8,48].

Summary of the existing literature on light chain amyloidosis in Brazil

Data on AL amyloidosis diagnosis and classification in Brazil are limited, but it is clear that diagnosis is often delayed. The main causes of delayed diagnosis are that AL amyloidosis is a rare and underdiagnosed heterogeneous disease that presents with nonspecific signs and symptoms, often mimicking other diseases, as well as difficulties in detecting amyloid deposits, identifying the amyloidogenic protein, and detecting the monoclonal component. In a single center study of patients at the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil, most patients (56%) were seen by ≥3 physicians before obtaining a diagnosis. Healthcare specialists visited by patients were most often general practitioners (65%), nephrologists (52%), and cardiologists (34%) [25]. The median time from symptoms onset to diagnosis was 9.0 months with 90% of patients having at least two organs involved at the time of diagnosis [25]. In 72% of cases, two or more biopsies were required to reach a diagnosis, most commonly obtained from bone marrow (57%), kidney (42%), and fat pad (38%) [25]. Techniques to identify the clonal protein were used in 80 patients (56%); within this subgroup, 66% underwent indirect immunofluorescence, 36% immunohistochemistry, and 6% mass spectrometry [25]. Standard Mayo Clinic staging was assessed in 69% of patients. Most patients (66%) were Stage III (55% were Stage IIIb) according to the European staging of advanced cardiac involvement [25]. Patients with advanced cardiac stage had the worst outcomes (median overall survival was 8.6 months for Stage III versus 52.3 months for Stage I–II – p-value < 0.001) [25]. Most patients (84%) received chemotherapy, with a median of four cycles administered; autologous stem cell transplantation (ASCT) was performed in 14% of the cohort. Only one patient (1%) underwent kidney transplantation [25]. Of the 55 patients evaluated for hematological response, 15% achieved complete response, 9% very good partial response, 25% partial response, 25% no response, and 25% had disease progression [25].

Questionnaire survey of light chain amyloidosis in Brazil

Responses were received from 96 institutions across the different regions of Brazil (North, Northeast, Central-West, Southeast, and South). Of this total, 52.1% were from private and 47.9% were from public institutions. The results of the questionnaire are summarized in Figure 2.

The majority (80.2%) of the institutions performed 0–5 AL amyloidosis diagnoses per year with no institution performing > 20. Regarding the interval between symptom onset and AL amyloidosis diagnosis, 7.5% of the institutions reported a timeframe of < 6 months, while 15.1%, 26.9%, and 50.5% reported delays of 7–12 months, 13–24 months, and > 24 months, respectively. Access to multidisciplinary specialties were reported by 70.7% of the institutions; the main specialties being cardiology (98.5%), nephrology (94.1%), neurology (73.5%), dermatology (57.4%), and gastroenterology (51.5%).

Most institutions (79.1%) had combined access to FLC measurement, serum immunofixation, and urinary immunofixation to detect the monoclonal component; however, access varied significantly between public (58%) and private (98%) institutions. Furthermore, 95.8% of institutions had access to serum protein electrophoresis, 92.7% to serum immunofixation, 86.5% to urinary immunofixation, and 83.3% to FLC assays; 3.1% did not have access to any of these diagnostic tools. In terms of anatomopathological analysis and identification of amyloid material, 97.9% of the institutions were capable of performing Congo red staining, 42.7% utilized polarized light microscopy to identify an apple-green birefringence; however, 2.1% did not have access to either technique. Regarding the tissue types available for amyloid detection, bone marrow biopsies were available at 100% of institutions, followed by abdominal fat pad (86.5%), salivary gland (36.5%), and tongue (31.3%) biopsies. Regarding amyloid subtyping, 76.0% of institutions had access to immunohistochemistry, 53.1% to mass spectrometry, 35.5% to immunofluorescence, and 15.6% to electron microscopy. Notably, 9.4% of institutions lacked access to any of these identification techniques.

In terms of prognostic biomarker assessment, access rates were 89.6% for NT-proBNP, 91.7% for troponin T (TnT), and 83.3% for echocardiography with GLS analysis. Combined access to all three assessments was available in 71.9% of the institutions, though a significant disparity existed between private (92%) and public (50%) centers. Notably, while NT-proBNP was available in all private institutions (100%), access dropped to 78% in the public sector.

Regarding therapeutic access for AL amyloidosis, bortezomib (97.9%), cyclophosphamide (94.8%), and melphalan (90.6%) were widely available. However, access to

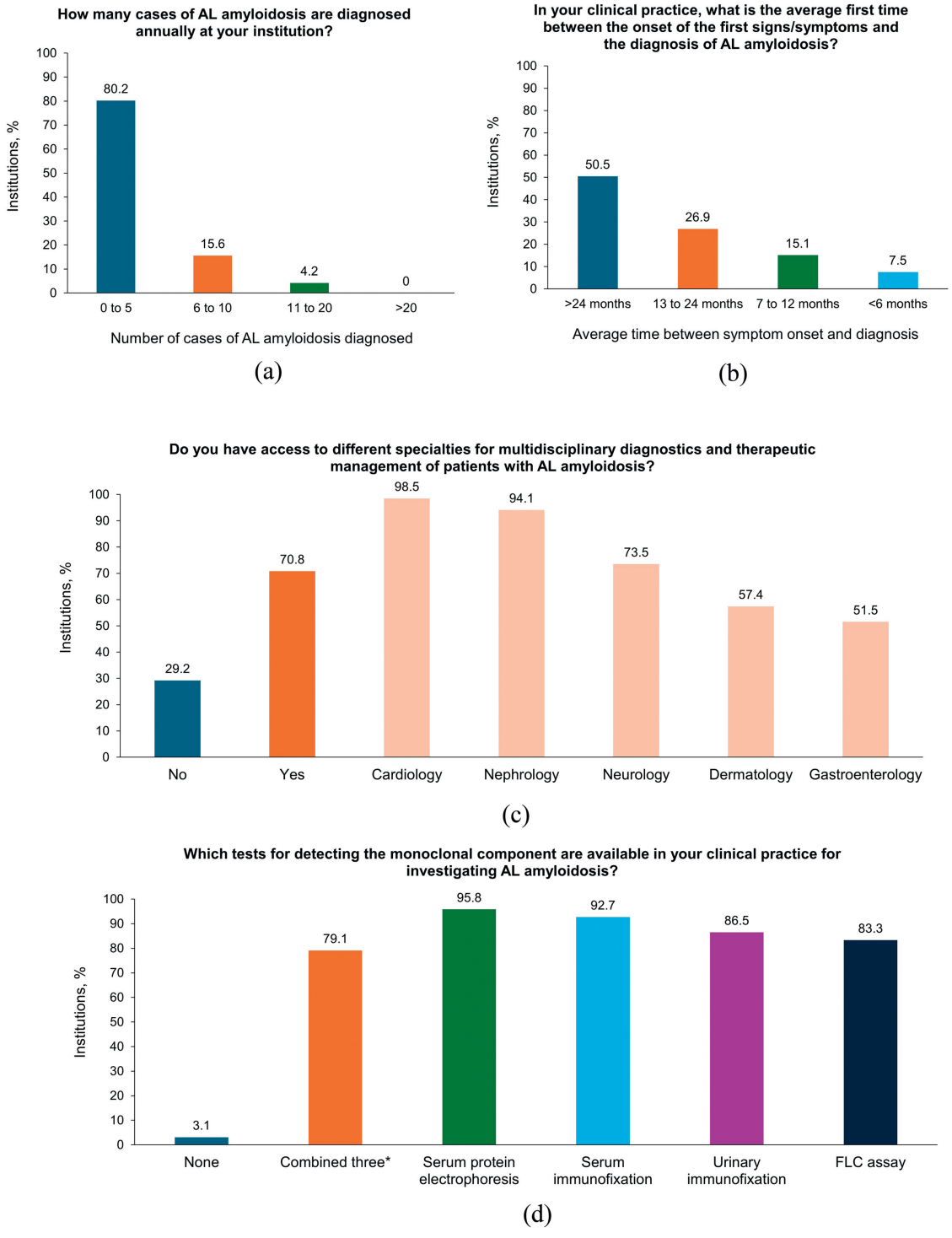
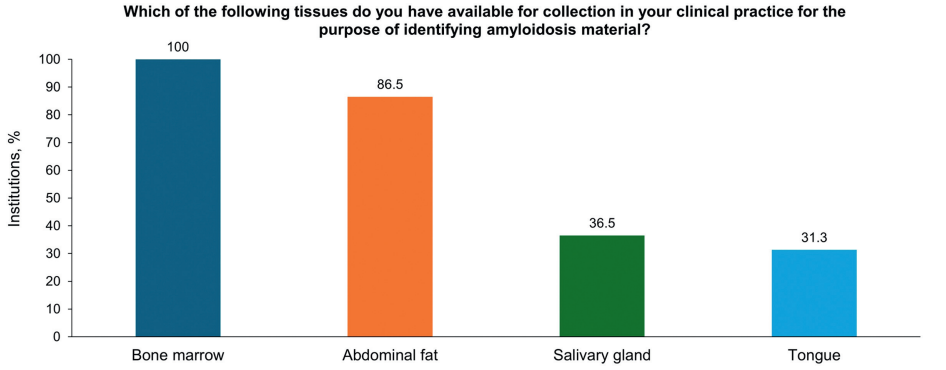


Figure 2 – Survey results of 96 institutions across Brazil to identify the diagnostic and therapeutic challenges in AL amyloidosis

*FLC assay, serum immunofixation, and urinary immunofixation.

AL: Immunoglobin light chain amyloid protein; ASCT: Autologous stem cell transplant; FLC: Free light chain; GLS: Global longitudinal strain; NT-proBNP: N-terminal pro-B-type natriuretic peptide.



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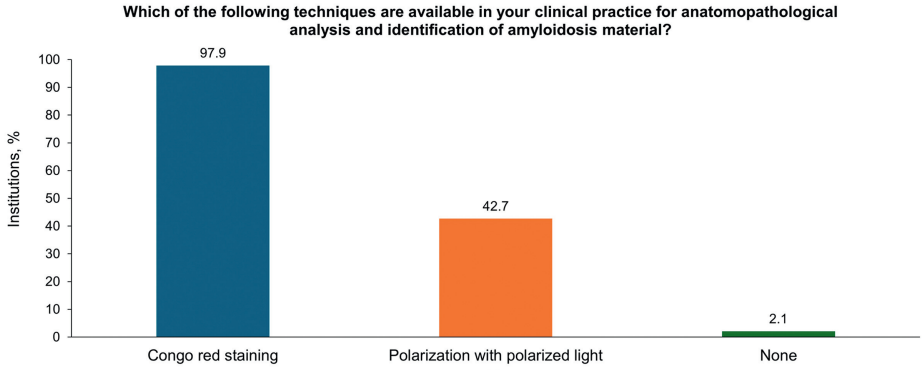

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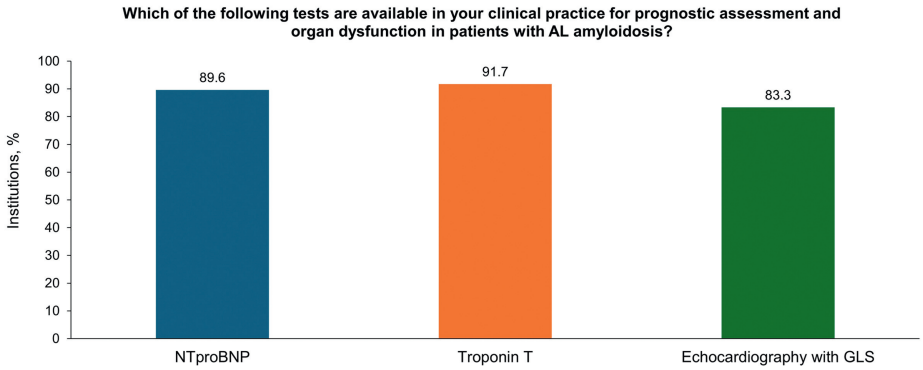

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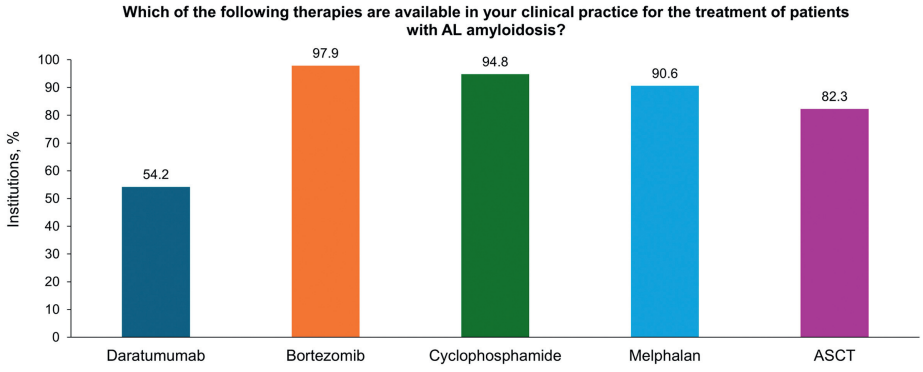

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Figure 2 Continued.

daratumumab was limited to 54.2% of the institutions. Furthermore, only 82.3% of the centers had the infrastructure to perform ASCT.

Recommendations

It is clear that the management of AL amyloidosis faces a number of challenges in Brazil, including the evident disparity between public and private institutions, the need to create collaborative networks for data collection and multidisciplinary action, the long delay between clinical suspicion and diagnostic confirmation, the unavailability and low accuracy of diagnostic and prognostic tests, and limited access to therapies. Based on the available clinical evidence as well as clinical experience of the experts, a diagnostic algorithm for AL amyloidosis in Brazil is therefore proposed in Figure 1. Best-practice recommendations for the evaluation and classification of AL amyloidosis in Brazil and unanswered questions are summarized in Tables 2 and 3.

Early detection

Patients with AL amyloidosis in Brazil face significant diagnostic delays, often consulting multiple HCPs including general practitioners, nephrologists, and cardiologists and at the time of diagnosis they typically present with multi-organ involvement. Early diagnosis of AL amyloidosis is critical to improve outcomes and prevent organ damage. Educational initiatives for patients and HCPs and the establishment of multidisciplinary specialized amyloidosis centers are needed in Brazil to improve the time to diagnosis and subsequent prognosis of patients. In line with recent International Society of Amyloidosis (ISA) recommendations, such centers should be structured to include a multidisciplinary clinical team; adequate infrastructure and access to diagnostic tools; expertise across different amyloidosis subtypes; access to disease-

Table 2 – Overview of expert consensus and best-practice recommendations on the diagnosis, evaluation and classification, and treatment of AL amyloidosis in Brazil.

Theme	Recommendation/consensus
Early detection	Early diagnosis of AL amyloidosis is critical to improve outcomes
	Educational initiatives need to be improved among patients, primary care physicians, and other disciplines (such as dentists and head and neck surgeons) to improve the time to diagnosis and subsequent prognosis
	The establishment of multidisciplinary specialized amyloidosis centers would allow more collaborative working to improve diagnosis
Diagnostic testing	The availability and use of diagnostic tests is variable in Brazil. This should be standardized where possible
	Procedures for what tissues to biopsy and at which timepoints need to be clear
	Transportation protocols for laboratory analysis of tissue samples should be improved and aligned to protect samples
	Training of pathology centers and the standardization of techniques in how to perform Congo red and polarized light testing consistently are required
	Involvement of the multidisciplinary approach to support biopsies is recommended. Cardiac evaluation is important to assess disease stage
	Rapid and structured diagnostic approach should follow the diagnostic algorithm in Figure 1
Evaluation and classification	Cardiac biomarker testing should be used at diagnosis, evaluation, patient follow-up, and in assessing suitability for transplant
	The tests performed at diagnosis should be used at every follow-up stage
	It is recommended to follow validated models of biomarkers and cut-offs, as well as scoring systems
	It is important to distinguish between involved and non-involved free light chains
Optimal management	A multidisciplinary approach is crucial due to the many challenges that AL amyloidosis patients experience
	The establishment of specialized amyloidosis centers should include a multidisciplinary clinical team with experience across different amyloidosis subtypes and access to disease-specific diagnostic tools and therapies
	Specialized amyloidosis centers should also include adequate infrastructure to deliver patient support services, research and educational activities, telemedicine, and collaborative networks
	The recommended treatment is the D-VCD combination, if available. or VCD where this combination is unavailable. Other options are available in low- and middle-income countries.
	The decision to use transplant should be approached with caution and with consideration of the patient's condition. There should be alignment in the eligibility criteria for transplantation (EHA-ISA guidelines). Access to transplantation may be lower in the real-world, and eligibility is dynamic
Treatment landscape	The incorporation of new therapeutic mechanisms, such as immunotherapies (anti-BCMAs, BsAbs, and CAR-T therapy), will potentially improve therapeutic options for patients

AL: immunoglobin light chain amyloid protein; BCMA: B-cell maturation antigen; BsAbs: bispecific antibodies; CAR-T: chimeric antigen receptor T-cell; D-VCD: daratumumab with bortezomib, cyclophosphamide, and dexamethasone; EHA-ISA: European Haematology Association – International Society of Amyloidosis; VCD, bortezomib, cyclophosphamide, and dexamethasone.

Table 3 – Unanswered questions/challenges and potential solutions/future research for the management of AL amyloidosis in Brazil.

Theme	Unanswered questions and challenges
Diagnosis	Epidemiologic data on AL amyloidosis are limited due to the absence of comprehensive population databases
	Adequate monitoring of light chain MGUS using biomarker tests (e.g., alkaline phosphatase, proteinuria, and NT-proBNP) is required for the early detection of AL amyloidosis – to allow prompt recognition of 'red-flags', such as fatigue, weight loss, cardiomyopathy, proteinuria, and peripheral neuropathy
	Delayed diagnosis is often due to low clinical suspicion; additional education is required
	Diagnostic resources for amyloid detection and protein characterization are scarce in many regions (e.g., abdominal fat biopsy and endomyocardial biopsy)
	Sensitivity of peripheral tissues in detecting amyloid deposits can be low in clinical practice (e.g., abdominal fat pad biopsy)
	There is inconsistency in the laboratory screening process across centers and regions
	There is limited access across different specialties
Evaluation and classification	There is inconsistency among centers and regions on what tests are being performed and at which timepoints
	There is limited access to of cardiac necrosis biomarker testing – different troponin subtypes (new generations)
	Limited access to and standardization of ECO with global longitudinal strain
Treatment and response assessment	Limited access to the best first-line treatments such as stem cell transplantation and daratumumab
	Lack of effective therapeutic options in the context of relapsed and refractory AL amyloidosis
	There is a need for a multidisciplinary approach for adequate supportive care
	Further clinical trials are needed to support future therapies in Brazil

AL: immunoglobin light chain amyloid protein; ECO: echocardiography; MGUS: monoclonal gammopathy of undetermined significance; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

specific therapies; patient support services; research and educational activities; telemedicine capabilities; and integration within collaborative networks [51].

Diagnostic testing

Patients in Brazil often require ≥2 biopsies for the diagnosis of amyloidosis. Improving the timeliness and accuracy of amyloidosis diagnosis requires expanded availability of monoclonal protein screening, prompt access to tissue biopsy, and standardized recommendations for biopsy site selection and timing. Additional training of pathology centers in how to consistently perform Congo red and polarized light testing is required. The use of a multidisciplinary team approach to support biopsies is recommended. Furthermore, standardization is needed for transportation protocols, for laboratory analysis of tissue samples, and for the availability and use of diagnostic tests.

Evaluation and classification

Cardiac involvement in AL amyloidosis is associated with the worst outcome. Cardiac biomarker testing should be used at every stage, including diagnosis, prognostic evaluation, patient follow-up, and assessing suitability for transplantation. Validated models of biomarkers and cut-offs, as well as scoring systems, should be followed to standardize evaluations. Identification and quantification of involved FLC is important for biomarker testing and prognostic classification and response assessment.

Optimal management and treatment

A multidisciplinary approach is crucial to support AL amyloidosis patients through the many challenges they experience. The main goal of treatment is to achieve a rapid and deep hematological remission by eliminating or reducing the amyloid-producing clonal cell population [4,52]. A degree of recovery in organ function occurs after circulating FLCs have been significantly reduced; recovery can continue to improve for up to two years after treatment, even when the amount of amyloid deposit remains unchanged [53–56]. Given this, if an adequate hematological response (very good partial response or higher) is achieved, it is recommended to avoid premature therapeutic changes despite a lack of organ response within the first few months. Organ non-response is more common with more advanced disease and when more than one organ is involved [53,54].

Management of AL amyloidosis involves supportive care and plasma cell-targeting therapies derived from multiple myeloma protocols, utilizing a similar risk-adapted approach [52]. The current standard of care for newly diagnosed AL amyloidosis is the quadruplet regimen consisting of daratumumab, bortezomib, cyclophosphamide, and dexamethasone. Approved by the Brazilian Health Regulatory Agency (ANVISA), this is also the only treatment approved by the Food and Drugs Administration (FDA) in the United States [57,58]. Standard treatment for AL amyloidosis from the late 1970s through the early 2000s involved the alkylating agent melphalan initially in combination with the corticosteroid prednisone and later with dexamethasone [59]. While

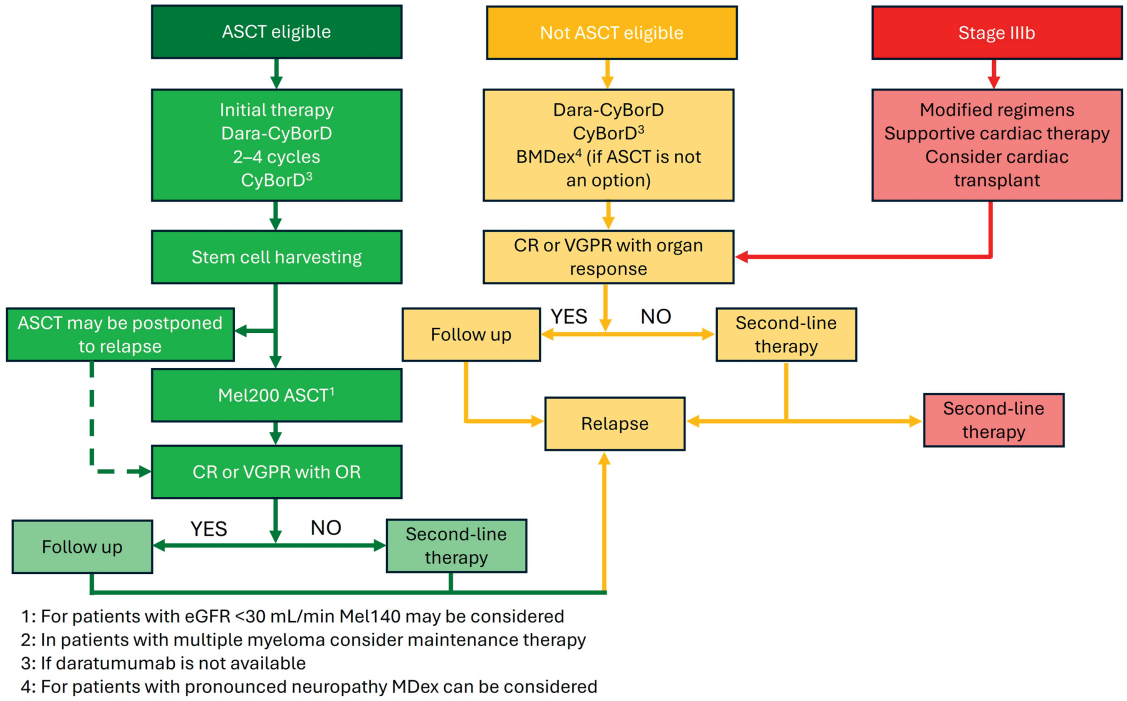

Figure 3 – Therapeutic algorithm for the treatment of AL amyloidosis*

*In cases where daratumumab is unavailable, the bortezomib-cyclophosphamide-dexamethasone should be chosen.

ASCT: Autologous stem cell transplant; BMDex: Bortezomib, melphalan, and dexamethasone; CR: Complete response; CyBorD: Cyclophosphamide, bortezomib, dexamethasone; Dara: Daratumumab; eGFR: Estimated glomerular filtration rate; MDex: Melphalan and dexamethasone; Mel140: melphalan 140 mg/m^2; OR: Organ response; VGPR: Very good partial response.

melphalan-dexamethasone continues to be an effective treatment [60], hematological response rates and overall survival improve when bortezomib is added [46,61]. Results of the ANDROMEDA study show that adding daratumumab to the combination of bortezomib-cyclophosphamide-dexamethasone regimen further improved the speed and depth of hematological response, resulting in a clinically meaningful and significant improvement in overall survival and survival free from major organ deterioration [62,63]. An upfront treatment algorithm for AL amyloidosis is shown in Figure 3 [64,65]. In cases where daratumumab is unavailable, the bortezomib-cyclophosphamide-dexamethasone should be used. As mentioned, in Brazil, daratumumab is approved for the treatment of AL amyloidosis, but access is only available to patients with supplementary health assistance [66]. Most patients from public institutions receive bortezomib, cyclophosphamide, melphalan and dexamethasone; the addition of daratumumab, when available, is recommended.

Due to the high risk of mortality, ASCT is generally restricted to younger individuals with limited cardiac involvement, although evidence is lacking for its superiority over chemotherapy alone [57,67]. Indeed, only a small proportion of patients (around 20%) with AL amyloidosis are eligible for ASCT [4]. Furthermore, major shifts in ASCT utilization have occurred since the introduction of daratumumab, with some centers experiencing an 80% decline in treatment volume [68]. The decision to use ASCT should be approached with caution and should take into account the patient's condition and the experience of the centers. Patient selection for ASCT should ideally follow risk-adapted recommendations, such as the European Hematology Association–International Society of Amyloidosis (EHA-ISA) guidelines for stem cell transplantation in systemic AL amyloidosis [69]. ASCT should not be offered to patients with: advanced cardiac involvement (particularly Stage IIIb disease), medically refractory pleural effusions, refractory orthostatic hypotension, active gastrointestinal bleeding, or acquired factor X deficiency with active bleeding. Conditioning melphalan should be dose-adjusted (200 mg/m^2 or 140 mg/m^2) according to age, cardiac status, renal function, and overall condition. Similarly stringent eligibility criteria have been published by the Mayo Clinic [32]. Solid organ transplantation may be an option for patients with more advanced disease, particularly with renal involvement, but again this is not well supported by available data [70,71]. Use of immunomodulatory drugs, such as lenalidomide, or proteasome inhibitors (e.g., carfilzomib, ixazomib) alone or in combination is limited, due to the increased potential for cardiotoxicity and lack of evidence of efficacy [26]. Agents that directly target fibril formation and amyloid deposits at early stages of clinical development [72] are not currently available for clinical use. Although Phase 3 studies have been conducted for anti-amyloid agents such as birtamimab and anselamimab, these trials did not meet their primary endpoints [73,74].

Regarding second-line treatment, daratumumab rechallenge can be considered, with bortezomib re-exposure also being an option in certain circumstances. Patients who achieve a durable, well-tolerated response to a bortezomib-containing regimen, particularly in the absence of neuropathy, may be considered for proteasome inhibitor re-exposure, ideally employing a different combination. For patients who have not previously received anti-CD38 therapy, daratumumab-based regimens represent a reasonable option [75]. Also, enrollment in clinical trials should be encouraged if feasible [76,77]. While not currently FDA approved, venetoclax is an investigational second-line treatment option for patients with AL amyloidosis with the t(11;14) translocation [78]. Venetoclax has demonstrated good efficacy in patients with relapsed or refractory AL amyloidosis harboring the t(11;14), although its availability remains largely restricted to clinical trials [78,79]. In a multicenter, retrospective analysis of 22 patients with AL amyloidosis treated with venetoclax, the median number of prior lines of therapy was one (range: 1–3). The most common frontline regimen was daratumumab, bortezomib, cyclophosphamide, and dexamethasone (68%), with 22% of patients having previously undergone ASCT. All 22 patients achieved a complete response or very good partial response with venetoclax, though nine patients eventually progressed before or after venetoclax discontinuation [80].

Assessing response to therapy

Response to therapy is assessed in terms of hematological response and organ-specific responses, with cardiac involvement being the most important factor in early mortality [46]. Hematological response is measured as the difference between involved and uninvolved free light chains (dFLC) [26,46] and organ response is assessed by measuring organ-specific biomarkers [26,46]. Supplementary Table 4 summarizes the criteria for evaluating hematologic response and organ response to therapy [48]. A significant decrease in dFLC is associated with improved clinical outcomes; specifically, achieving a dFLC < 10 mg/L predicts superior overall survival, higher organ response rates, and a longer time to next treatment [26,46]. Assessment of hematological response should be conducted at least every three months while on active treatment and after completion of treatment; proper monitoring should begin early, especially in the initial treatment phase [26]. As rising dFLC levels per se do not uniformly mandate the initiation of second-line therapy, an individualized approach is needed; some patients may experience prolonged periods without organ progression after hematologic relapse. In a Mayo Clinic study, 23% of patients had no evidence of organ disease progression for a median of 14 months and up to 8.3 years [81]. However, the optimal timing of treatment at relapse remains an area of active debate. Some experts advocate earlier intervention to prevent organ damage [82]. Accordingly, more recent consensus recommendations suggest that, in patients who previously achieved a very good partial response defined as the dFLC < 40 mg/L or better, salvage therapy should be considered in the presence of rising dFLC levels before overt organ progression develops, even if hematologic progression is not seen [32].

In cases of relapsed and refractory AL amyloidosis, treatment options are highly individualized with no universally accepted treatment sequence. While some patients experience slow biochemical relapse without immediate organ deterioration, management must be tailored to prior organ involvement, patient frailty, and the kinetics of light-chain progression. This is best captured by the concept of 'high-risk dFLC progression' (dFLC > 20 mg/L, representing $> 20\%$ of the baseline and a $> 50\%$ increase from the nadir) [83]. Such a personalized strategy is preferred over a uniform 'watch and wait' approach. Guidelines developed by the EHA-ISA working group emphasize balancing early treatment to prevent serious organ damage, particularly in patients with significant cardiac, renal, or autonomic involvement, against the risks of premature re-initiation of chemotherapy in frail patients, underscoring the need for individualized clinical judgment [84]. While the optimal sequencing of therapies in relapsed or refractory AL amyloidosis cannot be prescriptive, treatment decisions should be guided by the depth and duration of the initial hematologic response, prior exposure to specific drug classes, and the constraints related to the patient's condition and the extent of end-organ involvement.

Discussion and future direction

Data on AL amyloidosis are generally scarce in middle- and low-income countries, and there are currently no Brazilian guidelines for the treatment of AL amyloidosis. In contrast, real-world evidence exists across Europe [85] and several region-specific consensus recommendations or guidelines exist to optimize the management of AL amyloidosis [4,26–30,32,84]. Here, we summarize the available evidence for AL amyloidosis in Brazil from the literature, report contemporary data from a recent survey of Brazilian institutions, and provide expert-guided practical guidance and recommendations for the optimal diagnosis, evaluation and classification, and treatment of AL amyloidosis in Brazil.

The results from the recent survey provide contemporary insights and highlight gaps in the diagnosis and management of AL amyloidosis in Brazil. First, the low diagnosis rates likely reflect the epidemiological rarity of AL amyloidosis as well as challenges in its recognition, suggesting that the disease is underdiagnosed in Brazil. In Brazil, the low identification rates and diagnostic delays in AL amyloidosis are likely driven by clinical heterogeneity alongside systemic barriers, such as the complexity of the workup and limited access to specialized tools. Given the clinical heterogeneity of AL amyloidosis, a multidisciplinary approach is critical for accurate diagnosis and to reduce the delay; however, 29.2% of institutions in Brazil did not have access to different specialties in diagnostic and therapeutic management. The survey also highlights the gaps in the fundamental diagnostic testing of AL amyloidosis in Brazil: 20.9% of institutions did not have access to all three tests (FLC assay, serum immunofixation, and urinary immunofixation) to detect the monoclonal component and 3.1% did not have access to any of these tests. Moreover, 2.1% did not have access to any of the anatomopathological techniques for identifying amyloid material, and 9.4% did not have access to amyloid protein typing techniques. These missing diagnostic

techniques pose a significant limitation to the accurate and timely diagnosis and staging of AL amyloidosis in Brazil. Because the prognosis of AL amyloidosis is directly linked to the timing of interventions, a delayed diagnosis often results in advanced cardiac involvement and, consequently, inferior survival outcomes [86]. Finally, the survey highlighted that there is still considerable heterogeneity in access to new AL amyloidosis therapies across Brazilian institutions, chiefly for those within the public health system. In particular, only 54.2% of institutions had access to daratumumab for AL amyloidosis, despite daratumumab plus bortezomib-cyclophosphamide-dexamethasone being the standard of care endorsed by the ANDROMEDA study [62,63].

The expert recommendations provided here aim to educate and aid specialist and community physicians in Brazil who are involved in the treatment of patients with AL amyloidosis, as well as the wider clinical teams who may support the early identification and diagnosis of patients (such as cardiologists and nephrologists). Despite the practical guidance provided here, there remain unanswered questions and challenges that impede the optimal diagnosis and management of AL amyloidosis in Brazil (Supplementary Table 4).

A key strength of this survey is the inclusion of 96 institutions spanning all Brazilian regions and both public and private healthcare sectors, providing a comprehensive view of the diverse challenges in managing AL amyloidosis. Furthermore, these consensus recommendations consolidate the collective expertise of a multidisciplinary group of specialists from across the country. Elements of the NGT were implemented to capture expert consensus on AL amyloidosis in a structured, balanced, and clear manner. A limitation of the survey and the consensus recommendations is that they are representative of the management of AL amyloidosis in Brazil and therefore may not be applicable to countries with different treatment options or healthcare systems.

Diagnosis and treatment of localized light chain amyloidosis

Localized AL amyloidosis accounts for around 10% of all amyloidosis cases [87]. In the clinical practice, localized AL amyloidosis may be misdiagnosed as systemic AL amyloidosis, leading to chemotherapy treatment that is not clinically indicated. Correct staging and diagnosis are therefore essential to avoid intensive treatment for what is often a curable condition. Localized AL amyloidosis presentation is variable, depending on the specific organ involved [88]. Diagnosis of localized AL amyloidosis differs from the systemic form in that it only requires detection of amyloid deposits and identification of the amyloid protein. An initial hematologic evaluation for a monoclonal protein is typically sufficient to rule out systemic AL amyloidosis [88]. In localized AL amyloidosis, there is typically no evidence of a circulating monoclonal protein or systemic organ involvement. Consequently, these patients must be accurately distinguished from those with systemic disease to ensure they receive an appropriate site-specific therapy.

Management of localized disease can be challenging [89]. The goal of treatment for patients is symptom control [88]. Surgical resection is the most common first-line treatment [88,89], while radiotherapy can stabilize refractory and locally advanced disease that is not suitable for surgery. In some cases, a watch and wait strategy may be appropriate. Localized AL amyloidosis usually does not progress to systemic AL amyloidosis [90], which should directly impact how clinical and laboratory follow-up is performed in practice [91].

Conclusions

Developed through structured virtual advisory exchanges, this article provides expert consensus and practical recommendations for the optimal diagnosis, evaluation, and treatment of AL amyloidosis in Brazil. These guidelines serve as a primary reference for healthcare professionals (HCPs) and outline strategic solutions and research priorities aimed at strengthening the evidence base and improving patient outcomes in Brazil.

Author contributions

All authors were responsible for idea generation, critical review, and input into the manuscript drafts, as well as approval of the final draft for submission.

Data availability

All data are available within the text.

Conflicts of interest

Pedro Manoel Marques Garibaldi: Honoraria from GSK, Johnson & Johnson, Pfizer

João Tadeu Damian Souto Filho: Honoraria from AbbVie, AstraZeneca, BeOne, Johnson & Johnson, Sanofi

Roberto Jose Pessoa de Magalhães Filho: Honoraria from Johnson & Johnson

Roberta Shcolnik Szor: Honoraria from Johnson & Johnson

Carlos Miguel: Honoraria from Johnson & Johnson

Edvan Crusoe: Honoraria from Amgen, Bristol Myers Squibb, GSK, Johnson & Johnson, Natcofarma, Sanofi, Takeda

Carolina Tosin Bueno and Thiago Queiroz: Johnson & Johnson employees

Vânia Hungria: Honoraria from Amgen, AbbVie, BMS, GSK, Johnson & Johnson Pfizer, Regeneron, Roche, Sanofi, Takeda

Acknowledgments

The Brazilian Association of Hematology (ABHH) has endorsed this project.

The virtual structured advisory exchange was supported by Johnson & Johnson.

Editorial support was provided by Morgan O'Hagan and Patrick Hoggard of Ashfield MedComms, and was funded by Johnson & Johnson.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.htct.2026.106482.

Editor

Erich Vinicius De Paula

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Special article

Prophylaxis of graft-versus-host-disease: Systematic evidence-based recommendations of the Brazilian association of hematology, hemotherapy, and cell therapy (ABHH) and the Brazilian society of cellular therapy and bone marrow transplantation (SBTMO)

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ARTICLE INFO

Article history:
Received 1 April 2026
Accepted 20 May 2026
Available online 9 July 2026

Keywords:
Bone marrow transplantation
Graft vs host disease
Practice guideline
Systematic review
Prevention and control

ABSTRACT

Graft-versus-host disease, involving from 20%–80% of patients, is one of the main complications of allogeneic hematopoietic cell transplantation contributing significantly to mortality and morbidity. The methodological basis of these graft-versus-host disease prophylaxis guidelines for allogeneic hematopoietic cell transplantation, developed by the Brazilian Association of Hematology, Hemotherapy, and Cell Therapy (ABHH) and the Brazilian Society of Cellular Therapy and Bone Marrow Transplantation (SBTMO), is a review which applied the GRADE process to eleven PICO (population, intervention, comparator, and outcome) questions. These questions address graft-versus-host disease prophylaxis in allogeneic hematopoietic cell transplantation across various settings, including myeloablative and non-myeloablative/reduced-intensity conditioning. The scope encompasses related matched, unrelated matched or mismatched, and haploidentical donors, utilizing both bone marrow and peripheral blood as stem-cell sources. The combination of a

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https://doi.org/10.1016/j.htct.2026.106494
2531-1379/© 2026 Published by Elsevier España, S.L.U. on behalf of Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

calcineurin inhibitor (e.g., cyclosporine or tacrolimus) plus methotrexate or mycophenolate mofetil (MMF) has been the standard graft-versus-host disease prophylaxis regimen for allogeneic hematopoietic cell transplantation. The excellent results of post-transplant cyclophosphamide in haploidentical related donor hematopoietic cell transplantation have led to broader use in human leukocyte antigen-(mis)matched related and unrelated donor grafts. Sirolimus, low-dose antithymocyte globulin (4–6 mg/kg) and more recently abatacept are other drugs used. Hence, this review is meant to be a useful reference tool for clinicians who are dealing with this complex complication.

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Introduction

Graft-versus-host disease (GvHD) can occur after allogeneic hematopoietic cell transplantation (allo-HCT) when immune cells from a donor (the graft) initiate an immune reaction against a transplant recipient (the host). According to National Institutes of Health (NIH) consensus criteria, acute GvHD (aGvHD) and chronic GvHD (cGvHD) are multisystem disorders that are distinguished by clinical findings [1–3].

Despite prophylactic treatment with immunosuppressive agents, 20%–80% of allo-HCT recipients develop aGvHD [4]. The main risk factors for aGvHD are human leukocyte antigen (HLA)-mismatching between donor and recipient, use of an unrelated donor, gender disparity (specifically female donor to male recipient), myeloablative conditioning, total-body-irradiation-based conditioning, progenitor stem cell source (peripheral blood > bone marrow), and older donor age [5–6].

With a prevalence of 30%–70% among allo-HCT recipients, cGvHD remains the main cause of long-term post-transplant morbidity and mortality in this population [7]. Risk factors associated with cGvHD are hematopoietic cell transplantation (HCT) with HLA-mismatched or unrelated donor, use of a female donor for a male recipient, grafting with mobilized blood, prior aGvHD, and older donor or recipient age [5].

The success of allo-HCT depends on the prophylaxis of GvHD without relapse of the underlying disease, which can occur when the alloimmune response is blunted by excessive immunosuppressive GvHD prophylaxis [8].

The combination of a calcineurin inhibitor (CNI: e.g., cyclosporine or tacrolimus) plus methotrexate or MMF has been the standard GvHD prophylaxis regimen for allo-HCT. However, the excellent results of post-transplant cyclophosphamide (PTCy) in haploidentical related donor HCT have led to broader use in cases of HLA-(mis)matched related and unrelated donor grafts [9–12]. Sirolimus, low-dose antithymocyte globulin (ATG) (4–6 mg/kg), and more recently abatacept are other drugs used [13].

These evidence-based guidelines, developed using the PICO (Population, Intervention, Comparator, and Outcome) methodology, address GvHD prophylaxis in allo-HCT performed with non-myeloablative/reduced-intensity or myeloablative conditioning regimens and using related matched, unrelated matched or mismatched, and haploidentical donors, with bone marrow or peripheral blood as the progenitor stem cell source.

Methods

The methodological basis of these GvHD prophylaxis guidelines for allo-HCT, developed by the Brazilian Association of Hematology, Hemotherapy, and Cell Therapy (ABHH) and the Brazilian Society of Cellular Therapy and Bone Marrow Transplantation (SBTMO), is a formal and explicit systematic review that applied the GRADE process to eleven PICO (Population, Intervention, Comparator, and Outcome) questions. For all questions there are common eligibility criteria related to study design (observational or experimental studies), search period (no restriction), language (Portuguese, Spanish, English) and the inclusion of full texts or abstracts with data. The other eligibility criteria varied by question and were directly related to the specified patients, interventions, comparisons, and outcomes. In general, the methodology followed the steps outlined below:

- **Information Sources:** The following electronic databases were searched: Medline, Embase, Clinical Trials, Google Scholar. Additionally, a manual search of relevant reference lists was performed. The following search terms were used:

#1 (Bone Marrow Transplantation OR Bone Marrow Grafting)

#2 (Hematopoietic Stem Cell Transplantation OR Peripheral Blood Stem Cell Transplantation)

#3 (Graft vs Host Disease OR Graft-Versus-Host Disease OR Graft Versus Host Disease OR Runt Disease OR Graft-vs-Host Disease OR Graft-vs-Host Diseases)

#4 (Abatacept OR “Antilymphocyte Antibody” OR “Antilymphocyte Globulin” OR “Antilymphocyte Globulins” OR “Antilymphocyte Immunoglobulin” OR “Antilymphocyte Serum” OR “Anti-T lymphocyte globulin” OR Antithymoglobulin OR “Antithymocyte Globulin” OR “Anti-Thymocyte Globulin” OR “Anti Thymocyte Globulin” OR “Antithymocyte immunoglobulin” OR “Anti-human thymocyte globulin” OR “Anti-T-cell globulin” OR ATGAM OR ATG OR ATLG OR “Calcineurin Antagonists” OR “Calcineurin Blockers” OR “Calcineurin Inhibitor” OR “Calcineurin Inhibitors” OR Ciclosporin OR Cy OR Cyclosporin OR Cyclosporine OR Cyclosporins OR Cyclophosphamide OR CsA OR CyA OR CSP OR Everolimus OR Methotrexate OR “Mycophenolic Acid” OR “Mycophenolate Mofetil” OR PTCy OR Sirolimus OR Tacrolimus OR Thymoglobulin)

#5 ("Comparative study" OR "Comparative studies" OR ((clinical [Title/Abstract] AND trial [Title/Abstract]) OR clinical trials as topic [MeSH Terms] OR clinical trial [Publication Type] OR random* [Title/Abstract] OR random allocation [MeSH Terms] OR therapeutic use [MeSH Subheading]) OR "Epidemiologic methods" OR "Multicenter study")

#6 = #1 OR #2 OR #3

#7 = #6 AND #4 AND #5 (global search)

- **Data Extraction and Presentation of Results:** The extracted outcomes were clinical rather than intermediate and included aGvHD, cGvHD, overall survival (OS), relapse, non-relapse mortality (NRM), disease-free survival (DFS), GvHD-Free and relapse-free survival (GRFS), event-free survival (EFS: such as disease, progression, graft failure, death), invasive fungal disease, bacterial infections, and cytomegalovirus and Epstein-Barr virus reactivation. Results were expressed as the absolute risk of events or outcomes in each group, risk differences, 95% confidence intervals (95% CI), and the number needed to treat (NNT) or the number needed to harm (NNH).
- **Risk-bias assessment and quality of evidence:** The risk of bias of the studies was assessed using the expanded Rob 2 instrument. The variables included were randomization, allocation concealment, blinding (double-blinding and blinded outcome assessment), prognostic characteristics, losses, outcomes (timing, appropriateness, and measurement), intention-to-treat analysis, sample calculation, and early interruption. The quality of evidence was assessed using the GRADE classification as very low, low, moderate, or high based on the risk of bias (when meta-analysis was not possible).
- **Data analysis, evidence synthesis and recommendation:** If there were common outcomes reported by the selected works, these were aggregated in a meta-analysis using software and utilizing a consistent effect measure (difference in risk and 95% CI), a priori fixed or random model (depending on the heterogeneity calculated by I2). A random-effects model was used when the I2 exceeded 50%, otherwise a fixed-effect model was applied. Finally, a summary of findings (SoF) table was

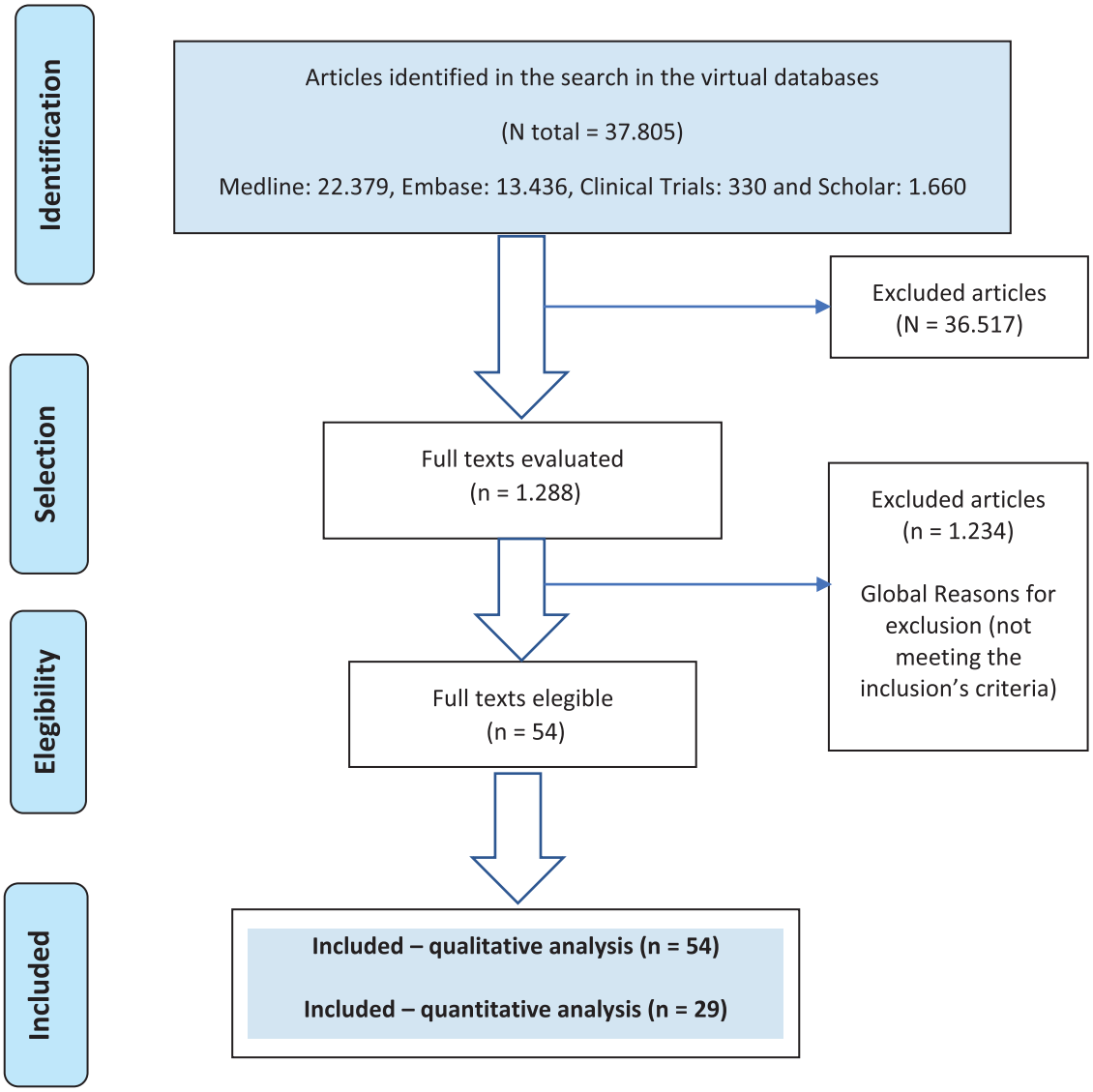

Figure 1 – PRISMA flow diagram of study selection for graft-versus-host disease prophylaxis.

generated to support the recommendations, which were designed to answer the initial clinical questions.
- All the steps were monitored, evaluated, and validated by a panel of experts in allo-HCT from the ABHH and SBTMO. Disagreements were resolved by consensus, and no formal adjudication by a third reviewer was required.

Results

The search process retrieved a total of 37,805 articles (Medline: 22,379; Embase: 13,436; ClinicalTrials.gov: 330; and Google Scholar: 1660). Based on the inclusion criteria, 1288 articles were selected for full-text or abstract review. Ultimately, 54 studies were selected to support the recommendations. Data from 29 publications were aggregated in the meta-analyses for Questions 6-8. Meta-analyzes was performed for Questions 3-8 (Figure 1). Reasons for exclusion depended on the specific criteria of each question and are presented as general reasons for not meeting the inclusion criteria.

CLINICAL QUESTION 1

What are the efficacy and risks of the cyclosporine plus methotrexate regimen in patients undergoing myeloablative allogeneic hematopoietic cell transplantation (allo-HCT) with related or unrelated matched donors?

Evidence synthesis

- Tacrolimus plus methotrexate showed superiority over cyclosporine plus methotrexate in the prophylaxis of Grade II–IV aGvHD with no significant difference in the DFS or OS in patients with non-advanced disease [14].
- The combination of tacrolimus plus methotrexate was more effective than cyclosporine plus methotrexate for the prophylaxis of aGvHD, without any significant increase in graft failure, relapse, or toxicity [15].
- GvHD prophylaxis with cyclosporine plus MMF following a myeloablative allogeneic preparative regimen was associated with faster hematopoietic engraftment and a decreased incidence of mucositis compared to cyclosporine plus methotrexate. The incidence of aGvHD and survival rates remained comparable [16]. With a longer follow-up, there were no significant differences in infection rates or late mortality [17].

Recommendation

For patients undergoing myeloablative allo-HCT with related or unrelated matched donors, the use of cyclosporine plus methotrexate is a therapeutic option for reducing the risk of aGvHD. However, this has not been directly associated with a reduction in mortality.

Quality of Evidence: Very Low

CLINICAL QUESTION 2

What are the efficacy and risks of the tacrolimus plus methotrexate regimen in patients undergoing myeloablative allogeneic hematopoietic cell transplantation (allo-HCT) with related or unrelated matched donors?

Evidence synthesis

The use of tacrolimus plus methotrexate had the following effects compared with:

Cyclosporine plus Methotrexate

- Tacrolimus plus methotrexate showed superiority over cyclosporine plus methotrexate in the prophylaxis of Grade II-IV aGvHD with no significant difference in the DFS or OS in patients with non-advanced disease [14].
- The combination of tacrolimus plus methotrexate was more effective than cyclosporine plus methotrexate for the prophylaxis of aGvHD, without any significant increase in graft failure, relapse, or toxicity [15].

Tacrolimus plus mini-Methotrexate and Mycophenolate Mofetil

- Compared with the full-methotrexate regimen, mini-methotrexate plus MMF was associated with no significant difference in Grade II-IV aGvHD and a more favorable toxicity profile; however, a higher incidence of Grade III-IV aGvHD was seen with mini-methotrexate plus MMF. Patients who received the mini-methotrexate plus MMF regimen had less Grade 3 or 4 mucositis and faster engraftment. There were no significant differences in moderate-to-severe cGvHD at one year or infection rates. Patients who received the mini-methotrexate plus MMF regimen had less nephrotoxicity and respiratory failure. There were no significant differences in the one-year relapse and OS rates [18].

Tacrolimus plus Sirolimus

- Patients who received sirolimus plus tacrolimus had significantly less moderate-severe cGvHD and late aGvHD. Patients who received sirolimus plus tacrolimus had lower prednisone exposure and earlier discontinuation, but there was no significant difference in the total discontinuation of immunosuppression [19].
- There was no significant difference in the GvHD-free survival. The sirolimus plus tacrolimus regimen can be considered in patients undergoing total-body-irradiation-based conditioning who are at higher risk of oropharyngeal mucositis, and in patients in whom timely engraftment is required after appropriate screening for risks of excessive hepatotoxicity are excluded [20].
- Sirolimus plus tacrolimus prevents Grade II-IV aGvHD and moderate-severe cGvHD more effectively than methotrexate plus tacrolimus [21].

Recommendation

For patients undergoing myeloablative allo-HCT with related or unrelated matched donors, the tacrolimus plus methotrexate regimen is a therapeutic option to reduce the risk of aGvHD and cGvHD, although this has not been directly associated with a reduction in mortality.

Quality of Evidence: Very Low

CLINICAL QUESTION 3

What are the efficacy and risks of a regimen using a calcineurin inhibitor (CNI) and MMF in patients undergoing

myeloablative allogeneic hematopoietic cell transplantation with related or unrelated matched donors?

Evidence Synthesis

The CNI plus MMF and CNI plus methotrexate regimens were compared. Quantitative data were presented as the number of events for each outcome, and the absolute risk for each group (MMF and methotrexate) was calculated as the ratio between the number of events and the total number of patients. The results of the meta-analysis expressed the difference in risk between the two groups compared as well as the 95% confidence interval (95% CI).

- There was no significant difference in the risk of aGvHD when comparing the combination of CNI plus MMF versus the combination with methotrexate, regardless of the use of tacrolimus or cyclosporine (Supplementary Figure 1). Very low quality of evidence [16,18,22].
- There was no significant difference in the risk of cGvHD when comparing the combination of CNI plus MMF versus the combination with methotrexate, regardless of the use of tacrolimus or cyclosporine (Supplementary Figure 2). Low quality of evidence [16,18].
- There was no significant difference in mortality when comparing the combination of CNI plus MMF versus the combination with methotrexate, regardless of the use of tacrolimus or cyclosporine (Supplementary Figure 3). Low quality of evidence [16,18,22].
- There was no significant difference in relapse when comparing the combination of CNI plus MMF versus the combination with methotrexate, regardless of the use of tacrolimus or cyclosporine (Supplementary Figure 4). Very low quality of evidence [16,18].
- There was a 30% reduction in the risk of mucositis (95% CI: 15%–45%) with the use of CNI plus MMF compared to the methotrexate combination (Supplementary Figure 5). The number needed to treat to prevent one case of mucositis was 3 (NNT: 3). In the subgroup analysis, the risk reduction was greater for the cyclosporine-based regimen (44%) than for the tacrolimus-based combination (24%) (Supplementary Figure 5). Moderate quality of evidence [16,18].
- There was no significant difference in the risk of infection when comparing the combination of CNI plus MMF versus the combination with methotrexate, regardless of the use of tacrolimus or cyclosporine (Supplementary Figure 6). Low quality of evidence [16,18].
- There was a 20% reduction in the risk of toxicity, specifically nephrotoxicity and respiratory failure (95% CI: 4%–36%) with the use of tacrolimus plus MMF compared to tacrolimus plus methotrexate (Supplementary Figure 7). The number needed to treat to prevent one toxicity event was 5 (NNT: 5). Moderate quality of evidence [18].

Recommendations

In patients undergoing myeloablative allo-HCT with related or unrelated matched donors, the efficacy of GvHD prophylaxis using a regimen containing CNI (cyclosporine or tacrolimus) plus MMF is comparable to the combination with methotrexate in respect to aGvHD, cGvHD, mortality, relapse, and infection rate. However, the combination with MMF can be recommended due to lower toxicity (nephrotoxicity and respiratory failure), particularly with tacrolimus and the lower incidence of mucositis (regardless of cyclosporine and tacrolimus), when compared to the combination with methotrexate.

Quality of Evidence: varied between very low and moderate, depending on the analyzed outcome.

CLINICAL QUESTION 4

What are the efficacy and risks of regimens using low-dose (<6 mg/kg) or high-dose (≥6 mg/kg) rabbit anti-thymocyte globulin in patients undergoing allogeneic hematopoietic cell transplantation using matched sibling or unrelated peripheral blood donors?

Evidence Synthesis

Quantitative data were presented as the number of events for each outcome, and the absolute risk for each group, intervention (ATG) and control (no ATG), was calculated as the ratio of the number of events to the total number of patients. The results of the meta-analysis expressed the risk difference for the outcome between the two groups, along with the 95% CI. A subgroup analysis was performed based on the doses used: low-dose (<6 mg/kg) or high-dose (≥6 mg/kg).

- In patients undergoing allo-HCT, the use of rATG (regardless of dose) in combination with standard GvHD prophylaxis, compared with standard prophylaxis alone, was associated with a 15% reduction in the risk of aGvHD (95% CI: 10% –20%). In the subgroup analysis, the reduction risk ranged from 13% (low-dose) to 17% (high-dose) (Supplementary Figure 8) [23–29]. Moderate quality of evidence.
- In patients undergoing allo-HCT, the use of rATG in combination with standard GvHD prophylaxis, compared with standard prophylaxis alone, was associated with an 18% reduction in the risk of cGvHD (95% CI: 13%–23%); however, this effect was significant only in the high-dose subgroup. In the subgroup analysis, the reduction ranged from 9% (low-dose) to 25% (high-dose) (Supplementary Figure 9) [23 –29]. Low quality of evidence.
- In patients undergoing allo-HCT, the use of rATG (regardless of dose) in combination with standard GvHD prophylaxis, compared with standard prophylaxis alone, showed no significant difference in OS (Supplementary Figure 10) [23–29]. Low quality of evidence.
- In patients undergoing allo-HCT, the use of rATG (regardless of dose) in combination with standard GvHD prophylaxis, compared with standard prophylaxis alone, showed no significant difference in DFS (Supplementary Figure 11) [23 –27,29]. Very Low quality of evidence.
- In patients undergoing allo-HCT, the use of rATG (regardless of dose) in combination with standard GvHD prophylaxis, compared with standard prophylaxis alone, showed no significant difference in NRM (Supplementary Figure 12) [23 –25,27,29]. Moderate quality of evidence.
- In patients undergoing allo-HCT, the use of rATG in combination with standard GvHD prophylaxis, compared with standard prophylaxis alone, showed an 8% reduction in relapse risk (95% CI: 1%–15%) in the high-dose subgroup, and a 6% reduction (95% CI: 1%–10%) in the overall analysis

(low- and high-risk). There was no significant difference in the low-dose subgroup (Supplementary Figure 13) [23–25,27,29]. Moderate quality of evidence.

- In patients undergoing allo-HCT, the use of rATG (regardless of dose) in combination with standard GvHD prophylaxis, compared with standard prophylaxis alone, showed no significant difference in the risk of bacterial infection (Supplementary Figure 14) and invasive fungal infection (Supplementary Figure 15) [23–25,27,28]. Low quality of evidence.
- In patients undergoing allo-HCT, the use of rATG (regardless of dose) in combination with standard GvHD prophylaxis, compared with standard prophylaxis alone, showed no significant difference in the risk of CMV reactivation (Supplementary Figure 16) [23–25,27,28]. Very Low quality of evidence.
- In patients undergoing allo-HCT, the use of rATG in combination with standard GvHD prophylaxis, compared with standard prophylaxis alone, was associated with a 2% increased risk of EBV reactivation (95% CI: 0%–4%) in the high-dose subgroup, with no significant difference observed in the overall analysis (combined low- and high-risk groups) or in the low-dose subgroup (Supplementary Figure 17) [23–25,27–29]. Very low quality of evidence.

Recommendation

In patients undergoing allo-HCT, the use of rATG in combination with standard GvHD prophylaxis is recommended over standard prophylaxis alone. This recommendation is based on reduced risks of aGvHD (regardless of dose), cGvHD (specifically in the high-dose subgroup), and relapse (regardless of dose).

Quality of Evidence: ranged from very low to moderate, depending on the outcome assessed.

CLINICAL QUESTION 5

What are the efficacy and risks of a CNI plus MMF regimen in patients undergoing reduced intensity and non-myeloablative conditioning allogeneic hematopoietic cell transplantation?

Evidence Synthesis

Quantitative data were presented as the number of events for each outcome. The absolute risks for the intervention group (CNI plus MMF) and the comparison groups (CNI alone or CNI plus methotrexate) were calculated as the ratio of the number of events to the total number of patients.

The results of the meta-analysis expressed the risk difference for the outcome between the groups, along with the 95% CI.

- There was a 16% increase (range: 7%–26%) in aGvHD-free survival with the use of CNI plus MMF compared with the control groups (CNI alone or CNI plus methotrexate). It was necessary to treat approximately seven patients to cause one additional harm (NNH: 7) (Supplementary Figure 18). Low quality of evidence [30–33].
- There was an 8% increase (95% CI: 5%–11%) in aGvHD with the use of CNI plus MMF compared with the control groups (CNI alone or CNI plus methotrexate) (Supplementary Figure 19). Approximately 13 patients need to be treated to cause one additional harm (NNH: 13). Moderate quality of evidence [30–33].
- There was a 12% increase (range: 8%–16%) in cGvHD risk with the use of CNI plus MMF when compared with the control groups (CNI alone or CNI plus methotrexate) (Supplementary Figure 20). It was necessary to treat approximately nine patients to cause one additional harm (NNH: 9). Moderate quality of evidence [30–33].
- There was a 17% reduction (range: 6%–29%) in mortality risk with the use of CNI plus MMF when compared to the control groups (CNI alone or CNI plus methotrexate) (Supplementary Figure 21). It was necessary to treat approximately six patients to prevent one additional death (NNT: 6). Low quality of evidence [30–33].
- There was no significant difference in the relapse or progression with the use of CNI plus MMF compared with the control groups (CNI alone or CNI plus methotrexate) (Supplementary Figure 22). Very Low quality of evidence [30–33].
- There was a 3% increase (95% CI: 1%–6%) in the probability of GRFS with the use of CNI plus MMF compared with the control group (CNI alone or CNI plus methotrexate) (Supplementary Figure 23). It was necessary to treat 33 patients to achieve one additional favorable GRFS outcome (NNT: 33). Moderate quality of evidence [30–32].

Recommendation

In patients undergoing reduced intensity and non-myeloablative conditioning allo-HCT, the use of CNI plus MMF compared with a CNI alone or CNI plus methotrexate is associated with the following outcomes: an increase in the probability of GRFS and an increased risk of both aGvHD and cGvHD. However, the CNI plus MMF regimen can be recommended because it is associated with a reduction in death risk, and no difference in the relapse or progression, despite the higher risks of aGvHD and cGvHD.

Quality of evidence: varied between very low and moderate, depending on the analyzed outcome.

CLINICAL QUESTION 6

What are the efficacy and risks of a regimen with high-dose post-transplant cyclophosphamide (PTCy) regimen (50 mg/kg on Day +3 and Day +4) plus a CNI with or without MMF in patients undergoing haploidentical allogeneic hematopoietic cell transplantation for malignant diseases, using either myeloablative or non-myeloablative conditioning and bone marrow or peripheral blood grafts?

Evidence Synthesis

In patients undergoing haploidentical allo-HCT for malignant diseases, using either myeloablative or non-myeloablative conditioning and bone marrow or peripheral blood grafts, the regimen with high-dose PTCy plus CNI with or without MMF produced the following effects, when compared with high-dose PTCy plus sirolimus and MMF [34–36]:

- There was no significant difference in Grade II-IV aGvHD risk (Supplementary Figure 24).

- There was no significant difference in Grade III-IV aGvHD risk (Supplementary Figure 25).
- There was no significant difference in cGvHD risk (Supplementary Figure 26).
- There was no significant difference in OS (Supplementary Figure 27).
- There was no significant difference in EFS (Supplementary Figure 28).
- There was no significant difference in NRM risk (Supplementary Figure 29).
- There was no significant difference in CMV reactivation (Supplementary Figure 30).
- There was a 21% reduction (95% CI: 12%−31%) in hemorrhagic cystitis risk with the use of high-dose PTCy plus CNI with or without MMF compared with high-dose PTCy plus sirolimus and MMF (Supplementary Figure 31).
- There was a 11% reduction (95% CI: 5%−17%) of sinusoidal obstructive syndrome risk with the use high-dose PTCy plus a CNI with or without MMF compared with high-dose PTCy plus sirolimus and MMF (Supplementary Figure 32).

Recommendations

In patients undergoing haploidentical allo-HCT for malignant diseases, using either myeloablative or non-myeloablative conditioning and bone marrow or peripheral blood grafts, a regimen with high-dose PTCy plus CNI with or without MMF, compared with high-dose PTCy plus sirolimus and MMF, reduces hemorrhagic cystitis and sinusoidal obstructive syndrome risks, despite showing no significant differences in the risk of Grade II–IV or III–IV aGvHD, cGvHD, NRM, OS, or EFS.

CLINICAL QUESTIONS 7a and 7b

7a. What are the efficacy and risks of regimens involving high-dose PTCy alone, or high-dose PTCy plus a CNI (with or without MMF), in patients undergoing allo-HCT with matched related or unrelated donors?

Evidence Synthesis

In patients undergoing allo-HCT with related or unrelated matched donors, regimens utilizing high-dose PTCy alone or high-dose PTCy plus a CNI (with or without MMF) demonstrated the following effects when compared with regimens without high-dose PTCy:

Randomized controlled trials [12,37,38]

- There was no significant difference in Grade II-IV aGvHD risk (Supplementary Figure 33).
- There was no significant difference in Grade III-IV aGvHD risk (Supplementary Figure 34).
- There was no significant difference in OS (Supplementary Figure 35).
- There was no significant difference in EFS (Supplementary Figure 36).
- There was no significant difference in NRM risk (Supplementary Figure 37).
- There was no significant difference in CMV reactivation (Supplementary Figure 38).
- There was no significant difference in EBV reactivation (Supplementary Figure 39).
- There was an 11% reduction (95% CI: 3%−18%) in cGvHD risk using regimens involving high-dose PTCy compared with regimens without high-dose PTCy in randomized controlled trials (Supplementary Figure 40).

Observational Cohorts [39−44]

- There was a 22% reduction (95% CI: 12%−31%) in the risk of Grade II−IV aGvHD when using PTCy regimens, compared with regimens without PTCy (Supplementary Figure 41).
- In cohorts, there was a 18% reduction (95% CI: 5%−31%) in the risk of aGvHD Grade III-IV, when using PTCy regimens compared with regimens without PTCy (Supplementary Figure 42).
- In cohorts, there was a 18% (95% CI: 12%−24%) reduction in the risk of cGvHD when using PTCy regimens compared with regimens without PTCy (Supplementary Figure 43).
- In cohorts, there was a 13% (95% CI: 6%−19%) increase in OS rate when using PTCy regimens compared with regimens without PTCy (Supplementary Figure 44).
- In cohorts, there was an 8% (95% CI: 0%−16%) increase in EFS Rate when using PTCy regimens compared with regimens without PTCy (Supplementary Figure 45).
- In cohorts, there was a 5% (95% CI: 1%−9%) reduction in NRM when using PTCy regimens compared with regimens without PTCy (Supplementary Figure 46).
- In cohorts, there was a 23% (95% CI: 11%−35%) increase in GRFS rate when using PTCy regimens compared with regimens without PTCy (Supplementary Figure 47).
- In cohorts, there was a 16% (95% CI: 8%−25%) increase in hemorrhagic cystitis risk when using PTCy regimens compared with regimens without PTCy (Supplementary Figure 48).
- There was no significant difference in sinusoidal obstructive syndrome (Supplementary Figure 49).
- There was no significant difference in CMV reactivation (Supplementary Figure 50).

In cohorts, for patients undergoing allo-HCT with related and unrelated matched donors, the regimen with a high-dose PTCy plus CNI, compared with a high-dose PTCy plus CNI plus MMF, produced the following effects [45,46]:

- There was no significant difference in the risk of Grade II−IV aGvHD (Supplementary Figure 51).
- There was no significant difference in the risk of Grade III −IV aGvHD (Supplementary Figure 52).
- There was no significant difference in the risk of cGvHD (Supplementary Figure 53).
- There was no significant difference in the OS rate (Supplementary Figure 54).
- There was no significant difference in the EFS (Supplementary Figure 55).
- There was no significant difference in NRM risk (Supplementary Figure 56).

Recommendation

In patients undergoing allo-HCT with related and unrelated matched donors, the use of high-dose PTCy, either alone or combined with a CNI (with or without MMF), compared

with regimens without high-doses of PTCy is associated with reduced risks of cGvHD, Grade II–IV aGvHD, Grade III–IV aGvHD, and NRM compared with regimens that do not include high-dose PTCy. In addition, PTCy- based regimens improve OS, EFS, GRFS, and increase the risk of hemorrhagic cystitis.

In cohorts, for patients undergoing allo-HCT with related and unrelated matched donors, the regimen with a high-dose PTCy plus CNI, compared with CNI plus MMF, shows no significant difference in Grade II–IV aGvHD, Grade III-IV aGvHD, cGvHD, NRM, OS, and EFS.

7b What are the efficacy and risks of a high-dose PTCy plus CNI and MMF or sirolimus, compared with regimens without PTCy, in patients undergoing allogeneic hematopoietic cell transplantation with unrelated mismatched donors?

Evidence Synthesis

In unrelated mismatched donors a regimen with high-dose PTCy plus a CNI and MMF or sirolimus, compared with regimens without PTCy produced the following effects:

Randomized controlled trials [9,47]:

- There was no significant difference in Grade II-IV aGvHD risk (Supplementary Figure 57).
- There was no significant difference in OS (Supplementary Figure 58).
- There was a 10% reduction (95% CI: 6%–14%) in Grade III-IV aGvHD risk when using regimens with PTCy compared with regimens without PTCy (Supplementary Figure 59).
- There was a 22% reduction (95% CI: 4%–40%) in cGvHD risk, when using PTCy-based regimens compared with regimens without PTCy (Supplementary Figure 60).

Observational Cohorts [48–51]

- There was no significant difference in Grade II-IV aGvHD risk (Supplementary Figure 61).
- There was no significant difference in Grade III-IV aGvHD risk (Supplementary Figure 62).
- There was no significant difference in cGvHD risk (Supplementary Figure 63).
- There was a 23% increase (95% CI: 14%–33%) in OS when using regimens with PTCy compared with regimens without PTCy (Supplementary Figure 64).
- There was no significant difference in EFS (Supplementary Figure 65).
- There was no significant difference in NRM (Supplementary Figure 66).
- In cohorts, there was a 9% reduction (95% CI: 1%–18%) in hemorrhagic cystitis, when using PTCy-based regimens compared with regimens without PTCy (Supplementary Figure 67).
- In cohorts, there was a 25% reduction (95% CI: 2%–47%) in CMV reactivation risk, using PTCy regimens, compared with regimens without PTCy (Supplementary Figure 68).

Recommendation

In unrelated mismatched donor transplants, regimens utilizing high-dose PTCy plus a CNI and MMF (or sirolimus) are associated with a reduced risk of Grade III–IV aGvHD, cGvHD, hemorrhagic cystitis, and CMV reactivation compared to regimens without PTCy. Furthermore, these regimens are associated with improved OS, with no significant differences observed in Grade II–IV aGvHD, NRM, or EFS.

CLINICAL QUESTION 8

What are the efficacy and risks of the regimen with high-dose PTCy plus ATG in patients undergoing allogeneic hematopoietic cell transplantation with haploidentical, related or unrelated matched donors or unrelated mismatched donors?

Evidence Synthesis

In patients undergoing allo-HCT from haploidentical, related or unrelated matched donors or unrelated mismatched donors, the addition of high-dose PTCy plus ATG, compared with regimens lacking this combination, produces the following effects [40,52–59]:

- There was a reduction in the risk of Grade II–IV aGvHD among patients treated with PTCy plus ATG compared with regimens that did not include this combination. The estimated risks were 9% (95% CI: 8%–16%) in the overall analysis (combining the haploidentical and non-haploidentical subgroups) and 13% (95% CI: 5%–21%) in the haploidentical subgroup. This effect was not observed in the non-haploidentical subgroup when analyzed separately (Supplementary Figure 69).
- There was no significant difference in the overall risk of Grade III–IV aGvHD between patients treated with PTCy plus ATG and those receiving regimens without this combination, regardless of donor type (haploidentical or non-haploidentical) (Supplementary Figure 70).
- There was a reduction in the risk of cGvHD among patients treated with PTCy plus ATG compared with regimens that did not include this combination. The estimated risks were 9% (95% CI: 1%–17%) in the overall analysis (combining haploidentical and non-haploidentical subgroups). This effect was not observed in the haploidentical and non-haploidentical subgroups when analyzed separately (Supplementary Figure 71).
- There was no significant difference in the aggregate OS rate between patients treated with PTCy plus ATG and those treated with other regimens, regardless of donor type (haploidentical or non-haploidentical) (Supplementary Figure 72).
- There was no significant difference in the aggregate EFS in patients treated with PTCy plus ATG compared with other regimens, regardless of donor type (haploidentical or non-haploidentical) (Supplementary Figure 73).
- There was no significant difference in the aggregate NRM risk in patients treated with PTCy plus ATG compared with other regimens, regardless of donor type (haploidentical or non-haploidentical) (Supplementary Figure 74).
- There was no significant difference in the aggregate GRFS rate in patients treated with PTCy plus ATG compared with other regimens without this combination, regardless of donor type (haploidentical or non-haploidentical) (Supplementary Figure 75).

- There was no significant difference in the aggregate CMV reactivation risk in patients treated with PTCy plus ATG compared with other regimens, regardless of donor type (haploidentical or non-haploidentical) (Supplementary Figure 76).
- There was no significant difference in the aggregate hemorrhagic cystitis risk in patients treated with PTCy plus ATG compared with other regimens, regardless of donor type (haploidentical or non-haploidentical) (Supplementary Figure 77).
- There was an increase in the EBV reactivation risk in patients treated with PTCy plus ATG compared with other regimes. The estimated risks were 24% (95% CI: 10%–37%), 42% (95% CI: 23%–61%), and 18% (95% CI: 10%–26%), in the overall analysis (combining haploidentical and non-haploidentical subgroups), the haploidentical subgroup, and the -non-haploidentical subgroup, respectively (Supplementary Figure 78).

Recommendations

In patients undergoing allo-HCT with haploidentical, related or unrelated matched donors or unrelated mismatched donors, the regimen combining high-dose PTCy plus ATG is associated with reduced risks of Grade II-IV aGvHD, and cGvHD, as well an increased risk of EBV reactivation when compared with other regimens lacking this combination. Despite these differences, the two regimens show no significant difference in terms of Grade III-IV aGvHD, OS, EFS, NRM, GRFS, CMV reactivation and hemorrhagic cystitis.

CLINICAL QUESTION 9

What are the efficacy and risks of the regimen with tacrolimus plus sirolimus in patients undergoing myeloablative allogeneic hematopoietic cell transplantation with related or unrelated matched donors?

Evidence Synthesis

It was possible to pool the outcomes (aGvHD, cGvHD, and mortality) from three randomized clinical trials included in this analysis [20,21,60].

- There was no significant difference in the risk of aGvHD when comparing the sirolimus plus tacrolimus prophylaxis regimen with tacrolimus plus methotrexate or cyclosporine plus methotrexate.
- There was no significant difference in the risk of cGvHD between the sirolimus plus tacrolimus regimen and the tacrolimus plus methotrexate or cyclosporine plus methotrexate regimens.
- Mortality rates were also similar across the groups, with no significant difference observed between sirolimus plus tacrolimus and either comparator regimen.

Recommendations

In patients undergoing myeloablative allo-HCT with related or unrelated matched donors, GvHD prophylaxis with sirolimus plus tacrolimus does not demonstrate greater benefit than tacrolimus plus methotrexate or cyclosporine plus methotrexate in terms of aGvHD, cGvHD, or mortality.

CLINICAL QUESTION 10

What are the efficacy and risks of a triple-drug prophylaxis regimen consisting of a CNI, MMF, and sirolimus in patients undergoing reduced-intensity or non-myeloablative allogeneic hematopoietic cell transplantation from related or unrelated (matched or mismatched) donors?

Evidence Synthesis

- In patients undergoing non-myeloablative conditioning allo-HCT with an unrelated matched donor, replacing tacrolimus plus MMF with a combination of cyclosporine, MMF, and sirolimus resulted in a lower incidence of Grade II–IV aGvHD and improved two-year OS [61]. Low quality of evidence.
- For GvHD prophylaxis in patients undergoing non-myeloablative conditioning allo-HCT with a related or unrelated mismatched donor, the addition of sirolimus to cyclosporine and MMF resulted in a lower incidence of aGvHD and superior OS [62]. Very Low quality of evidence.
- In patients undergoing non-myeloablative conditioning allo-HCT with an unrelated matched or mismatched donor, adding sirolimus to cyclosporine and MMF resulted in a significantly lower incidence of acute GvHD [63]. Low quality of evidence.

Recommendations

In patients undergoing reduced intensity or non-myeloablative allo-HCT with related or unrelated matched or mismatched donors, cyclosporine plus MMF and sirolimus led to a reduction in the incidence of Grade II-IV aGvHD and an improved two-year OS.

Quality of evidence: varied between very low and low.

CLINICAL QUESTION 11

What is the efficacy and risks of the abatacept plus a CNI and methotrexate regimen in patients undergoing non-myeloablative or myeloablative allogeneic hematopoietic cell transplantation with an unrelated matched or mismatched donors?

Evidence Synthesis

In patients undergoing non-myeloablative or myeloablative allo-HCT with an unrelated matched or mismatched donor, the abatacept plus CNI and methotrexate regimen produced the following effects:

In randomized clinical trials, the use of this regimen was associated with a lower risk of Grade III–IV aGvHD (28% risk reduction) at three months of follow-up and a lower risk of relapse (12% risk reduction) at 24 months of follow-up, with no significant difference in the cGvHD risk and OS compared with a methotrexate plus CNI regimen [64,65].

Recommendations

In patients undergoing non-myeloablative or myeloablative allo-HCT with matched and mismatched unrelated donors, bone marrow or peripheral blood, the abatacept plus CNI and methotrexate regimen produces lower risk of aGvHD III-IV and relapse, with no significant difference in the risk of

cGvHD and OS, when compared to methotrexate combined with a CNI.

Quality of evidence: very low

Conclusions

Preferred approaches to GvHD prophylaxis vary according to transplant characteristics, including donor–recipient matching, conditioning intensity, graft source, and other clinical factors. The choice of GvHD prophylaxis should therefore be guided by donor type (matched sibling, matched unrelated, or haploidentical), conditioning regimen (myeloablative versus non-myeloablative/reduced intensity), and graft source (peripheral blood or bone marrow).

This systematic review of GvHD prophylaxis addressed 11 clinical questions encompassing patients who underwent allo-HCT using myeloablative or non-myeloablative/reduced-intensity conditioning regimens with haploidentical, matched sibling, matched unrelated, or mismatched unrelated donors.

Choosing a graft-versus-host disease prophylaxis regimen

1. Calcineurin Inhibitor plus methotrexate in myeloablative regimens:

For allo-HCT using matched related or matched unrelated donors, cyclosporine, or tacrolimus in combination with methotrexate reduces the risk of acute and chronic GvHD without affecting mortality.

2. Calcineurin inhibitor plus mycophenolate mofetil in myeloablative regimens:

For allo-HCT using matched related or matched unrelated donors, regimens using cyclosporine or tacrolimus plus MMF do not differ from methotrexate-based combinations in terms of aGvHD, cGvHD, mortality, relapse, or infection rates. However, MMF-based regimens can be recommended due to lower toxicity (nephrotoxicity and respiratory failure, particularly when combined with tacrolimus) and the lower incidence of mucositis (regardless of whether cyclosporine or tacrolimus is used).

3. Anti-thymocyte Globulin (rATG) <6 mg/kg (low dose) or ≥6 mg/kg (high dose) in peripheral blood sibling and unrelated donors:

The use of rATG in combination with standard GvHD prophylaxis is recommended over standard prophylaxis alone, as it reduces the risk of aGvHD and relapse (regardless of dose), as well as cGvHD (specifically in the low-dose subgroup).

4. A calcineurin inhibitor plus mycophenolate mofetil in non-myeloablative/reduced intensity conditioning regimens:

The use of a CNI plus MMF compared with a CNI alone or a CNI plus methotrexate is associated with an increase in the probability of GRFS and an increased risk of both aGvHD and cGvHD. The CNI plus MMF regimen can be recommended because it is associated with a reduction in death risk, and no difference in the relapse or progression, despite the higher risks of aGvHD and cGvHD.

5. *Cyclophosphamide*

a. Nonmyeloablative and myeloablative haploidentical allo-HCT

A high-dose PTCy plus CNI regimen (with or without MMF) reduces the risks of hemorrhagic cystitis and sinusoidal obstructive syndrome compared with high-dose PTCy plus sirolimus and MMF, despite having no significant differences in the risk of Grade II–IV or III–IV aGvHD, cGvHD, NRM, OS and EFS.

b. Related and unrelated matched donors

A high-dose PTCy regimen, used alone or combined with a CNI (with or without MMF), is associated with reduced risks of cGvHD, Grade II–IV aGvHD, Grade III–IV aGvHD, and NRM, compared with regimens that do not include high-dose PTCy. In addition, PTCy-based regimens improve OS, DFS, and GRFS, but increase hemorrhagic cystitis risk. In cohort studies, for patients undergoing allo-HCT with related and unrelated matched donors, the regimen with a high-dose PTCy plus CNI, compared with CNI plus MMF, shows no significant difference in Grade II–IV aGvHD, Grade III-IV aGvHD, cGvHD, NRM, OS, and EFS.

c. Unrelated mismatched donors

A high-dose PTCy plus CNI regimen (combined with either MMF or sirolimus) is associated with reduced risks of Grade III –IV aGvHD, cGvHD, hemorrhagic cystitis, and CMV reactivation compared with regimens without PTCy. Furthermore, this approach yielded an OS benefit, notwithstanding the lack of significant differences in Grade II–IV aGvHD, NRM, or EFS.

6. Cyclophosphamide plus antithymocyte globulin (ATG)

High-dose PTCy plus ATG for related or unrelated matched donors or unrelated mismatched donors is associated with reduced risks of Grade II-IV aGvHD, and cGvHD, as well as an increased risk of EBV reactivation when compared with other regimens lacking this combination. Despite these differences, the two regimens show no significant differences in outcomes such as Grade III-IV aGvHD, OS, EFS, NRM, GRFS, CMV reactivation and hemorrhagic cystitis.

7. Tacrolimus plus sirolimus in myeloablative conditioning regimens

The use of sirolimus plus tacrolimus for related or unrelated matched donors does not demonstrate greater benefit than tacrolimus plus methotrexate or cyclosporine plus methotrexate in terms of aGvHD, cGvHD, and mortality.

8. Calcineurin inhibitor plus mycophenolate mofetil and sirolimus in non-myeloablative / reduced intensity conditioning

The use of cyclosporine plus MMF and sirolimus for related or unrelated matched or mismatched donors leads to a reduction in the incidence of Grade II-IV aGvHD and improved two-year OS.

9. Abatacept plus calcineurin inhibitor and methotrexate

For patients undergoing myeloablative or non-myeloablative allo-HCT (using bone marrow or peripheral blood from matched or mismatched unrelated donors), the addition of abatacept to a CNI and methotrexate regimen is associated with a lower risk of Grade III–IV aGvHD and relapse, with no significant difference in the risk of cGvHD or OS, compared to a CNI plus methotrexate alone.

This systematic review of GvHD prophylaxis did not address questions regarding the optimal prophylactic regimen for specific clinical contexts such as myeloablative versus reduced-intensity or non-myeloablative conditioning, related versus unrelated donors, or bone marrow versus peripheral blood graft sources but it represents an important step toward defining preferred GvHD prophylaxis strategies. In addition, despite limited long-term data, abatacept

represents a promising strategy, particularly in unrelated donor transplantation. Furthermore, PTCy has emerged as a novel approach to GvHD prophylaxis beyond the haploidentical transplantation setting.

These guidelines are limited by the predominance of observational studies, heterogeneity of conditioning regimens, and evolving transplant platforms. However, these recommendations are particularly relevant for low- and middle-income countries, where access to novel agents and graft manipulation remains limited.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2026.106494.

Editor

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Case Report

Automated red cell exchange in acute sickle cell crises: Case series and review of the literature

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ARTICLE INFO

Article history:
Received 6 October 2025
Accepted 3 February 2026
Available online 22 April 2026

Key words:
Sickle cell disease
Erythrocytapheresis
Red cell exchange
Vaso-occlusive crisis
Acute chest syndrome

ABSTRACT

Automated red cell exchange (erythrocytapheresis) offers rapid suppression of circulating hemoglobin S without adding iron burden and is increasingly used to escalate care in acute sickle cell disease complications. This study describes four patients with severe vaso-occlusive crisis and/or acute chest syndrome managed with automated red cell exchange in a tertiary-care intensive care unit in India and summarizes Indian reports on its feasibility and on early outcomes. In the Indian scenario, this therapy is underutilized mostly due to the limited-resource setting, non-affordability, and probable lack of awareness of its availability.

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Introduction

Sickle cell disease (SCD) is an autosomal-recessive disorder of the *β-globin* gene that substitutes glutamic acid with valine at codon 6 leading to the production of hemoglobin S (Hb S), which under deoxygenated conditions undergoes polymerization and consequent sickling [1,2]. This change promotes endothelial adhesion, microvascular obstruction, tissue ischemia/infarction, and hemolysis [1,3].

The clinical spectrum of the disease spans acute and chronic complications. Common acute events include painful vaso-occlusive crises (VOC), acute chest syndrome (ACS), stroke, and hepatic or splenic sequestration, while longer-term morbidity reflects cumulative organ damage and growth impairment. Of these complications, VOC remain the clinical hallmark and the most frequent reason for hospitalization [4].

Red blood cell transfusion remains a core supportive therapy aimed at improving oxygen-carrying capacity and reducing circulating Hb S. Depending on indication, simple transfusion is used to raise hemoglobin levels however, automated red cell exchange (RCE; erythrocytapheresis) is preferred when rapid Hb S reduction is required for high-risk complications such as acute stroke or severe ACS [5–7].

Automated RCE offers significant advantages in this regard, as it rapidly reduces the percentage of Hb S, decreases blood viscosity, and improves oxygen delivery without causing excessive iron accumulation [7].

This study presents a case series of sickle cell crises managed with RCE in an intensive care unit (ICU) setting in a tertiary care center. This study also reviewed the literature to

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https://doi.org/10.1016/j.htct.2026.106454

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assess the utilization and clinical efficiency of this therapy in India.

Case 1

A 9-year-old male patient with homozygous SCD presented with severe limb and back pain and a past history of right hemiparesis and inconsistent hydroxyurea. Baseline Hb S was 80.4%. Automated RCE was performed using a continuous-flow system via a left femoral double-lumen catheter; the circuit was primed with packed red blood cells (PRBC). A total volume of 775 mL of leukoreduced PRBCs was exchanged at an inlet flow rate of 10 mL/min. The procedure targeted a final Hb S concentration of ≤30% and a hematocrit (Hct) of approximately 30%. Continuous IV calcium was given. The procedure was uneventful; pain and mobility improved markedly and Hb S fell to 16.6%. He was discharged on hydroxyurea, folate, and calcium. The patient remained ambulatory (able to walk independently) and clinically stable at the time of follow-up.

Case 2

A 26-year-old male patient presenting with VOC and ACS exhibited chest pain, diffuse aches, and a transient altered sensorium. Following initial supportive therapy, automated RCE was initiated due to deteriorating clinical symptoms. The procedure was performed via right femoral access, exchanging 2403 mL of PRBCs at an inlet flow rate of 30 mL/min. The procedure targeted a Hb S level of ≤30% and a Hct of approximately 25%, with concurrent continuous IV calcium supplementation. Following the exchange, the patient's respiratory status and mentation improved significantly; his Hb S level decreased from 64.6% to 25.1% within 24 h. He was subsequently discharged on hydroxyurea and nutritional supplements

Case 3

A 19-year-old female patient with prolonged VOC and ACS was admitted to the ICU with severe multiorgan failure, including acute respiratory distress. Automated RCE, performed via right femoral access, exchanged 905 mL PRBCs at an inlet rate of 30 mL/min. The procedure targeted a Hb S level of ≤30% and a Hct of approximately 25%, with concurrent continuous IV calcium supplementation. Twenty-four hours after the exchange, the Hb S level had dropped from 62.7% to 6.7%. Despite this laboratory response, there was no immediate clinical improvement, and intensive support was continued.

Case 4

A 16-year-old male patient with a history of intermittent hydroxyurea adherence presented with pleuritic chest pain, severe bone pain, fever, and hypoxemia. Chest radiography revealed new basilar opacities consistent with **ACS**. Due to persistent hypoxemia, an automated RCE was performed via right internal jugular access. A total of 1800 mL of PRBCs was exchanged, targeting a Hb S level of ≤30% and a Hct of approximately 25–30%, with concurrent IV calcium supplementation. Post-procedure chills were managed symptomatically. Clinical recovery was rapid, characterized by reduced oxygen requirements and the abatement of chest pain. The Hb S level decreased from 74.5% to 24.1% within 24 h, and the patient was discharged with an incentive spirometry regimen.

The pre- and post-automated RCE hematological parameters and clinical outcomes for all four cases are summarized in Table 1.

Discussion

In this four-patient case series, automated RCE was instituted for severe VOC or ACS refractory to initial supportive measures. Procedurally, all automated RCEs were performed using the Spectra Optia Apheresis System (Terumo BCT) on a continuous-flow platform. The procedures utilized leukoreduced, cross-match-compatible PRBCs, with prophylactic IV calcium administered throughout. Post-exchange targets were a hematocrit of approximately 25–30% and Hb S ≤ 30%; in all cases, Hb S decreased to target levels. Three patients demonstrated prompt clinical improvement, while one intensive-care patient with advanced ACS had laboratory response but no immediate clinical response as automated RCE was

Table 1 – Hematological parameters before and after automated red cell exchange and clinical outcome.

Case	Indication	Exchange volume (mL)		Pre-procedure (%)	Post-procedure (%)	Clinical outcome
1	Severe VOC, high Hb S	775	Hb S	80.4 g/dL	16.6	Rapid pain relief; ambulatory at follow-up
			Hb	7.7 g/dL	10.7	
			Hct	22.9%	32	
2	VOC + ACS; altered sensorium	2403	Hb S	64.6 g/dL	25.1	Improved oxygenation and mentation
			Hb	10.9 g/dL	7.6	
			Hct	31.0%	26	
3	VOC + ACS with respiratory failure	905	Hb S	62.7 g/dL	6.7	No immediate clinical improvement
			Hb	8.7 g/dL	7.8	
			Hct	26.4%	24.7	
4	ACS with hypoxemia	1800	Hb S	74.5 g/dL	24.1	Rapid improvement; discharged with spirometry
			Hb	8.2 g/dL	8.3	
			Hct	22.5%	23.8	

VOC: Vaso-occlusive crisis; ACS: Acute chest syndrome; Hct: hematocrit, Hb: hemoglobin.

performed at an advanced stage and the patient had already severe preexisting multiorgan failure. No serious procedure-related adverse events occurred in all four patients.

Automated RCE is strongly recommended for acute sickle cell complications such as stroke, severe or rapidly progressive ACS with hypoxia, acute multiorgan failure, severe sickle cell hepatopathy, prolonged ischemic priapism, preoperative optimization in moderate to high-risk surgeries, and rapidly evolving severe symptoms [7–9].

As per the American Society for Apheresis (ASFA) guidelines, automated RCE in SCD is used in acute and nonacute conditions [10]. However, the category of indication and Grade of recommendation varies with each condition as listed in Table 2.

Table 2 – Category and grade of recommendation for red cell exchange in sickle cell disease (SCD).

SCD	Indication	Category	Grade
Acute	Acute stroke	I	1C
	Acute chest syndrome, severe	II	1C
	Other complication	III	2C
Non-acute	Stroke prophylaxis	I	1A
	Pregnancy	II	2B
	Recurrent vaso-occlusive pain crisis	II	2B
	Preoperative management	III	2A

Adopted from American Society for Apheresis Guidelines [10].

Review of literature

Published Indian data dedicated to automated RCE for VOC are limited and largely consist of single-centre experiences where VOC represents a minority of indications for acute crises. The Rapid Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines were used for this review. India-based primary studies (case reports/series or cohorts) that used automated RCE for VOC or mixed acute crises that reported at least one outcome (change in Hb S percentage, clinical response, or adverse events) were included. Non-India populations and non-automated RCE interventions were excluded.

PubMed/PMC and Indian transfusion journals (Asian Journal of Transfusion Science; Global Journal of Transfusion Medicine/Medknow; DOAJ) were searched using combinations of 'India', 'automated red cell exchange', 'erythrocytapheresis', 'sickle cell', 'vaso-occlusive crisis', and 'acute chest syndrome' with the titles and abstracts being screened for India-based automated RCE in SCD. Full texts were reviewed for automated RCE and acute indication as shown in Figure 1.

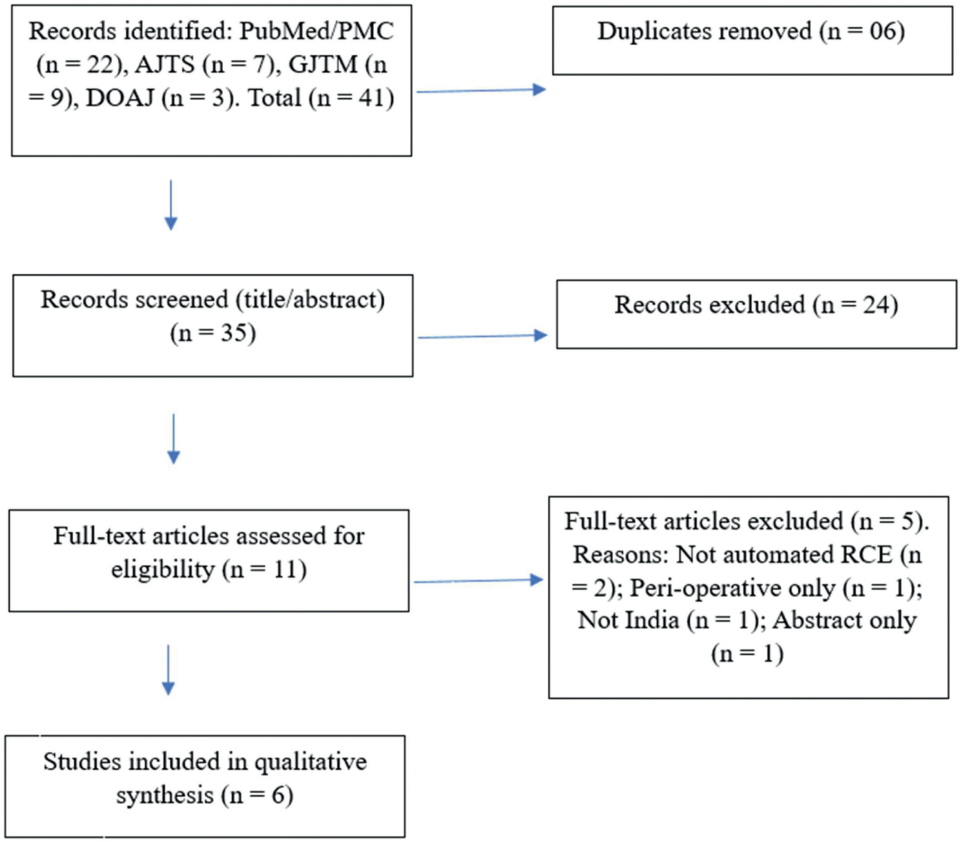

Figure 1 – PRISMA chart showing A summary of data extraction.

AJTS: Asian Journal of Transfusion Science; GJTM: Global Journal of Transfusion Medicine; RCE: Red cell exchange.

Table 3 – Characteristics of the sickle cell disease cases in India managed by automated red cell exchange.

Study (Year)	Design	VOC as indication	Other indications	Target Achieved	Outcomes / Safety
Daniel et al., [11] 2016	Retrospective; 21 exchanges (18 SCD)	No (pre-operative)	AVN optimization	Hb S: 22–29%	Targets met, minimal reactions
Chowdhry et al., [12] 2021	Retrospective series; n = 5 (8 exchanges)	Not separated (mixed acute crises)	Mixed; pre-op 1	FCR: ≤30%	Safe/effective
Iyyapan et al., [13] 2023	Case report; n = 1	Yes (acute bone pain)	—	Hb S: 74% → 18%	Prompt symptom relief
Rajendran et al., [7] 2023	Case report + review; n = 1	Painful crisis with ACS	ACS, hemolytic crisis	Hb S; 16% after 2 RCEs	Rapid clinical recovery under-utilization noted
Kanungo et al., [14] 2024	Retrospective series; n = 9	2 cases of 9	Pre-op 3/9; hepatopathy 2/9; others	Mean post-Hb S: ≈30.7%	Symptoms & lab improvement
Zanzari et al., [15] 2024	Prospective observational; n = 8	1/8 (ACS 7/8; sequestration 1/8)	ACS predominant	Hb S: 68.45% → 16.44%	Improved vitals

VOC: Vaso-occlusive crisis; ACS: Acute chest syndrome; AVN: Avascular necrosis; Hb: Hemoglobin; FCR: Fractional cell residual.

The peri-operative cohort by Daniel et al., although not focused on VOC, included 21 automated RCE procedures in 18 SCD patients undergoing surgery for avascular necrosis. Hb S was reduced from 73 to 85% to 22–29%, thus meeting transfusion targets with minimal adverse reactions. While the indication was surgical optimization, the findings support the technical feasibility and safety of automated RCE in India [11]. The five-patient series (eight exchanges) of Chowdhry et al. included patients with mixed acute crises. VOC-specific data were not separated. A target fractional cell residual of ≤30% was consistently achieved. The presence of alloantibodies in two patients underscored the significant challenges for blood supplies in India [12]. Iyyapan et al. published a single case report of acute bone-pain VOC. After one automated RCE session, the Hb S was reduced from 74% to 18%, with rapid resolution of symptoms demonstrating clear clinical benefit of this procedure in cases of severe VOC [13]. Rajendran et al. managed a patient with VOC and ACS using two successive automated RCE sessions, effectively reducing the Hb S level to 16%. Following clinical recovery, the authors highlighted the underutilization of automated RCE in India, citing limited awareness, constrained resources, and poor donor availability [7]. Kanungo et al. reported a retrospective experience from Eastern India involving nine SCD patients who underwent automated RCE; among these, two presented with VOC. Mean post-procedure Hb S was approximately 30.7%, with no procedure-related complications reported. This demonstrates feasibility and safety of automated RCE even outside major metropolitan centres [14]. Zanzari et al. conducted a prospective observational study of eight patients with acute crises, including one with VOC and seven with ACS. Following automated RCE, Hb S levels fell significantly from 68.5% to 16.4% with parallel improvements observed in oxygenation and vital signs. This highlights the rapid physiological benefits produced by automated RCE [15]. Table 3 describes the various characteristics of the SCA cases in India managed by automated RCE.

Conclusion

Automated RCE is a promising acute treatment for indicated SCD complications. In the Indian context, this therapy remains underutilized, primarily due to resource constraints, high costs, a shortage of trained staff, and the requirement for large volumes of blood. Furthermore, limited awareness of its availability and the challenges of securing compatible units for alloimmunized patients remain significant barriers. Further studies should be conducted in India to explore the evidence of the efficacy of automated RCE in the acute management of SCA crises. Expanded prospective, multicentre studies are needed in India to refine patient selection and assess long-term outcomes.

Funding

The author(s) received no specific funding for this work

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Informed consent

A written informed consent obtained from the patient

Author contribution

VA: writing -original draft, Conceptualization, Formal analysis
SD: writing-review/ and editing
JSC: writing-review/ and editing

RD: Supervision
PF: Supervision
NV: PData curation

Conflicts of interest

None

Editor

Eduardo Rego

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Case Report

Novel *ABCG8* Mutation in Pediatric Sitosterolemia: A Case Report of Siblings with Hemolytic Anemia

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ARTICLE INFO

Article history:
Received 25 July 2025
Accepted 17 March 2026
Available online 23 May 2026

Introduction

Sitosterolemia, also referred to as phytosterolemia (MIM #210,250), is a rare autosomal recessive sterol storage disorder first identified in 1974 by Bhatacharyya & Connor [1]. It results from pathogenic mutations in the *ABCG5* and *ABCG8* genes, which encode sterolin-1 and sterolin-2, respectively. These proteins form a heterodimeric transporter complex responsible for the efflux of dietary plant sterols from enterocytes back into the intestinal lumen and from hepatocytes into the bile, therefore limiting systemic absorption of plant sterols [2]. The hematological phenotype of sitosterolemia was historically described as Mediterranean stomatocytosis with thrombocytopenia [3]. Due to underrecognition and limited access to diagnostic assays, the actual prevalence remains unknown, although the condition is believed to be extremely rare [4]. Sitosterolemia has marked clinical heterogeneity, which often results in delayed diagnosis. Clinical manifestations can range from cutaneous xanthomas, premature atherosclerotic cardiovascular disease, and arthritis to endocrine abnormalities such as thyroid dysfunction. Hematologic features are also common including unexplained hemolytic anemia, macrothrombocytopenia, splenomegaly, and bleeding tendencies [5]. As routine lipid profiles do not measure plant sterols, diagnosis often requires high clinical suspicion and genetic or sterol quantification testing. Here, we report two pediatric siblings with hemolytic anemia and macrothrombocytopenia, ultimately diagnosed with sitosterolemia through genetic analysis.

Case presentations

Case 1

A 12-year-old female, the product of a non-consanguineous union, had reported a three-year history of intermittent jaundice and pallor. The patient has a transfusion history of four units of packed red blood cells (RBCs) over a two years span but no history of fever, rash, bleeding, joint pain or skin lesions. The physical examination demonstrated moderate

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https://doi.org/10.1016/j.htct.2026.106472

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pallor with mild splenomegaly (2 cm below the left costal margin) without any lymphadenopathy. A complete blood count showed anemia (hemoglobin [Hb] 7.6 *g*/dL) with corrected reticulocyte count of 9 %, indicating active hemolysis. Total leukocyte count measured 7600/μL with an absolute neutrophil count of 5320/μL and thrombocytopenia (80,000/μL). RBC indices showed increased Mean Corpuscular Volume (MCV) of 102.5 fl with normal Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Cell Distribution Width (RDW). A peripheral smear revealed stomatocytes and macrothrombocytopenia (Figure 1A), suggestive of an underlying red-cell membrane defect. Iron profile, ferritin, vitamin B12 and folate were normal, ruling out iron overload or nutritional deficiency. Laboratory studies revealed an elevated LDH of 572 IU/L (range: 140 −280 IU/L) and indirect hyperbilirubinemia (2 mg/dL). Serum urea, creatinine and the standard lipid profile were within reference limits. Hemoglobin High-Performance Liquid Chromatography was normal and Direct Coombs test (DCT), Paroxysmal Nocturnal Hemoglobinuria testing by Fluorescent Aerolysin, and an Eosin-5′-Maleimide Binding Test were negative, therefore ruling out other causes of hemolytic anemia. A bone-marrow aspirate demonstrated normocellular marrow with erythroid hyperplasia, with concordant findings in a bone marrow biopsy. Given the constellation of stomatocytic hemolytic anemia and presumed autosomal-recessive inheritance, targeted next-generation sequencing was performed which identified a novel homozygous missense variant in *ABCG8*:c.691C>A; p.(Pro231Thr) in exon 5 (Figure 1B). The mutation was pathogenic and was concordant with sitosterolemia. Management comprised dietary restriction of plant sterols and initiation of ezetimibe (10 mg daily). The subsequent follow-up documented normalization of Hb levels, resolution of hyperbilirubinemia, and obviated the need for transfusional support.

Case 2

The 10-year-old younger sibling of Case 1 underwent evaluation as part of a familial screening protocol following the elder sister's diagnosis of sitosterolemia. She was clinically asymptomatic, with no history of jaundice, bleeding tendencies, or pallor requiring blood transfusions. A physical examination showed mild pallor with no hepatosplenomegaly, lymphadenopathy or xanthomas. Hematological investigations revealed mild anemia (Hb: 9.9 g/dL) with a corrected reticulocyte count of 4%. The MCV was 95.3 fL, with normal MCH, MCHC and RDW. The total leukocyte count was 6100/μL with platelet count mildly reduced (105,000/μL). A peripheral blood smear showed normocytic normochromic red blood cells with occasional stomatocytes. Serum LDH was modestly elevated (480 IU/L), and bilirubin levels were mildly raised (total: 1.6 mg/dL; direct: 0.5 mg/dL). A DCT was negative and a nutritional workup (iron, vitamin B12, folate) was non-contributory. Molecular analysis detected the same homozygous missense mutation - *ABCG8*:c.691C>A; p.(Pro231Thr) - in exon 5 as was observed in the index case, confirming the diagnosis of sitosterolemia. Despite being asymptomatic, she was initiated on a plant sterol-restricted diet and enrolled in a regular follow-up program. Subsequent evaluations showed stable hematologic parameters and no development of clinical symptoms.

Hematological Parameters and six-month follow-up data of both siblings are summarized in Table 1.

Discussion

Sitosterolemia is a rare autosomal recessive disorder of sterol trafficking that remains underrecognized due to its heterogeneous clinical spectrum. Notably, its presentation as

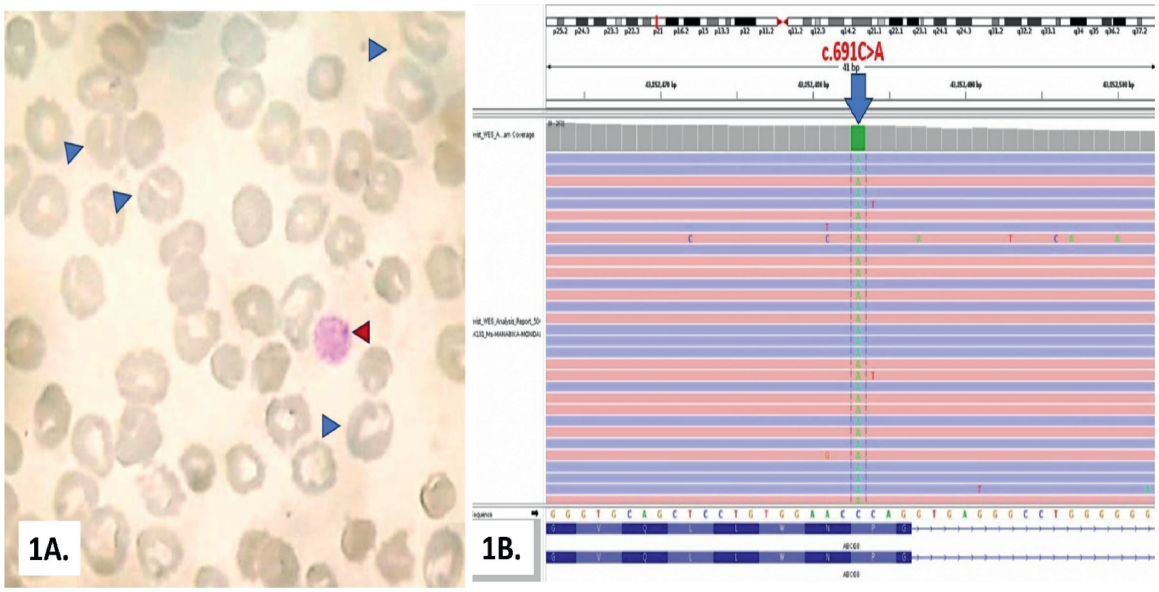

Fig. 1 – A) Peripheral blood smear (Leishman Giemsa stain, 100x) of a 12-year-old girl with hemolytic anemia showing stomatocytes (blue arrow head) and large platelet (red arrow head); B) Integrative genomics viewer visualization of alignments in chromosome 2 is showing C > A variation corresponding to *ABCG8*:c.691C>A;p.(Pro231Thr).

Table 1 – Hematological Parameters and six-month follow-up of two siblings with Sitosterolemia.

Parameter	Case 1 (12-year-old female)	Case 1 Follow-up	Case 2 (10-year-old female)	Case 2 Follow-up	Reference Range
Hemoglobin (g/dL)	7.6	11.5	9.9	11.2	11.5–15.5
Mean corpuscular volume (fL)	102.5	95	95.3	94	80–100
Reticulocyte count (%)	9	2	4	2	0.5–2
Platelets (/μL)	80,000	130,000	105,000	160,000	150,000–450,000
Serum lactate dehydrogenase (IU/L)	572	220	480	260	140–280
Total Bilirubin (mg/dL)	2.0	0.8	1.6	0.9	0.3–1.2
Peripheral smear	Stomatocytes, macrothrombocytopenia	Normalized morphology	Occasional stomatocytes	Normal morphology	–
Therapy	Plant sterol restriction + ezetimibe 10 mg/day	Continued	Plant sterol restriction	Continued	–

hemolytic anemia is even rarer, often mimicking other congenital or immune-mediated hematologic disorders. While roughly 200 cases of sitosterolemia have been documented to date, emerging genetic data suggest the condition is likely underdiagnosed. The estimated frequency of heterozygous loss-of-function variants of the *ABCG5* or *ABCG8* genes is approximately 1 in 220 individuals, suggesting that the prevalence of sitosterolemia caused by homozygous or compound heterozygous mutations could be as high as 1 in 200,000 [6].

Demographic distribution

Both the siblings in the present report are females aged 12 and 10 years. The cohort by Wang et al. [7] comprised adults (aged 23–61 years; n = 13) with a near-equal male-to-female ratio (1:1.16). Desai et al. [8] reported younger patients (aged 7–29 years; n = 4), while Risinger et al. [9] and Zhao et al. [10] reported cases of a 19-year-old male and an eight-year-old male, respectively. The lack of sex predilection across all cases suggests autosomal inheritance, but pediatric-onset cases may display earlier and more severe hematologic manifestations due to prolonged exposure to sterol imbalance.

Genotypic spectrum and phenotypic implications

Wang et al. [7] observed both nonsense and frameshift variants including C18802T, C20896T, and ISV713G>A. Desai et al. [8] identified predominantly truncating variants in *ABCG5* (e.g., p.R243X, p.R408X). The mutation (p.Arg446Ter in *ABCG5*) reported by Zhao et al. [10] reaffirms the severity linked with nonsense variants. The current report adds to the literature with a novel homozygous *ABCG8* variant (p.Pro231Thr, exon 5) found in both siblings. Such variants may contribute to a less severe hematologic phenotype compared to truncating mutations, although individual variability is considerable.

Hematological manifestations

The clinical features of sitosterolemia encompasses a spectrum ranging from being totally asymptomatic to involvement of multiple organ systems. Hematologic abnormalities in sitosterolemia include hemolytic anemia and bleeding episodes as a result of dysfunctional macrothrombocytes. Stomatocytosis is a morphological consequence of abnormal membrane lipid composition, not the direct cause of hemolysis. Hemolysis more likely arises from the incorporation of plant sterols into red cell membranes, rendering them rigid (less deformable) and more susceptible to splenic clearance. Splenic lipid accumulation and sequestration may also contribute to both anemia and thrombocytopenia [11]. Hemolytic anemia with stomatocytes and macrothrombocytopenia was a consistent finding in the present report. Hb ranged from 5.7 g/dL to 14.9 g/dL and platelet counts ranged from 50,000/μL to 66,000/μL, as reported by Zhao et al. [10] and Risinger et al. [9], respectively. Blood smears revealed stomatocytes in both the siblings in the current report. Macrothrombocytopenia, along with elevated mean platelet volume and platelet distribution width, further supports the morphological abnormality in typical cases [2]. These findings were contrasted with dehydrated stomatocytosis; our patients presented with normal iron profiles and no evidence of hepatic steatosis, thereby ruling out potential confounders for hemolysis or hyperferritinemia.

Bleeding tendency

Similarly, attributing hemorrhage solely to dysfunctional macrothrombocytes underestimates the complexity of the platelet phenotype. Mechanistic studies by Kanaji et al. show that phytosterol accumulation induces αIIbβ3 internalization, activation of signaling pathways, microparticle release, and calpain-mediated degradation of GPIbα and filamin A [12]. These events lead to giant platelets, defective aggregation, megakaryocytic abnormalities, and a paradoxical bleeding phenotype resembling inherited platelet function disorders.

Therapeutic interventions and outcomes

Management of sitosterolemia primarily focuses on lowering circulating levels of plant sterols such as campesterol, stigmasterol, and β-sitosterol. This is achieved through dietary restriction of plant sterol intake, complemented by pharmacologic therapy. Oral administration of ezetimibe at a daily dose of 10 mg has demonstrated efficacy in reducing sterol absorption and improving clinical outcomes [8–10]. While in the older reports by Wang et al. [7] cholestyramine was used, newer cases, including the present report, demonstrated

Table 2 – Comparison of hematological parameters and outcomes of the present cases with previously published reports exhibiting pronounced hematological features.

Source / Year	Number of Patients (Age range / Male: Female ratio)	Genetic Mutation (Location)	Hb (g/dL)	Platelet Count (/μL)	Treatment
Present Report	2 siblings, 12- and 10-year-old females	*ABCG8* - homozygous (p.Pro231Thr exon 5 in both)	7.6 / 9.9	80 / 105	Sterol-restricted diet + Ezetimibe 10 mg, Sterol-restricted diet
Wang et al. [7]	13 (23- 61 years, 1: 1.16)	*ABCG5/ABCG8* - homozygous nonsense mutations (e.g., C18802T in exon 1, C20896T in exon 10, G19839A in exon 9, and ISV713G>A splice site), compound heterozygous variants in *ABCG5* (A20883G intron 9 and C20896T exon 10), and in *ABCG8* (del43683–43,727, del43866C-43867G/ ins43866T in exon 3, G18729A exon 11, and 10 bp insertion exon 11)	Mean 10.7	Mean 33,520	Sterol-restricted Diet, + cholestyramine
Desai et al. [8]	4 patients (7–29 years, 1:1)	*ABCG5* - 3 homozygous (p.R243X, p.R408X, p.G111E and 2 heterozygous: p.R408X and p.R419C)	Mean 7.93	Mean 19,250	Sterol-restricted diet ± Ezetimibe 10mg
Risinger et al. [9]	1 19-year-old male	Presumed - *ABCG8* compound heterozygous (c.63_63+53del and c.547del over exon1 and intron1 boundary)	14.9	66	Sterol-restricted diet + Ezetimibe 10mg
Zhao et al. [10]	1 8-year-old male	*ABCG5* - homozygous non sense (p.Arg446Ter in exon 10)	5.7	50	Sterol-restricted diet + Ezetimibe 10mg

effective hematologic recovery with ezetimibe (10 mg/day). A comparison of hematological parameters and outcomes of the present cases with previously published reports exhibiting pronounced hematological features is summarized in Table 2.

This report describes only two patients from the same family, which limits how broadly the findings can be applied. Due to limited resources, plant sterol levels could not be measured, and the long-term effects of treatment are still unclear. A novel missense mutation (p.Pro231Thr) was identified in the present report, however its functional impact biochemically is not validated. Even so, its presence in both siblings and inheritance pattern support its likely role in the disease. Even with the small sample size, this case contributes valuable insight to the limited pediatric literature on sitosterolemia in India. These findings highlight the need to consider sitosterolemia in cases of Coombs-negative hemolytic anemia and unexplained macrothrombocytopenia. Family screening initiated by the index case is crucial; in this report, this investigation facilitated the early diagnosis and management of an asymptomatic sibling, underscoring the clinical utility of timely genetic testing. Despite being a potentially treatable condition, it remains largely underdiagnosed due to its nonspecific clinical features and the lack of routine plant sterol testing. Early molecular confirmation is of utmost importance to avoid diagnostic delays and to initiate targeted interventions, such as dietary sterol restriction and pharmacologic inhibition of sterol absorption using ezetimibe, which can result in substantial clinical improvement and prevention of long-term complications.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

There are no conflicts of interest

Editor

Eduardo Rego

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Letter to the Editor

Exploring the prognostic impact and biological functions of mutant-like *TP53*-related genes in acute myeloid leukemia

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To the editor,

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy characterized by the clonal expansion of myeloid progenitor cells resulting from genetic and epigenetic alterations in hematopoietic stem or progenitor cells. These mutations disrupt normal differentiation and lead to the accumulation of immature myeloid blasts in the bone marrow, peripheral blood, and in other tissues, impairing normal hematopoiesis [1,2]. According to the 2022 World Health Organization (WHO) classification, AML is categorized into two major groups: AML with defined genetic abnormalities, which includes entities characterized by recurrent gene mutations or chromosomal rearrangements, and AML defined by differentiation, which is classified based on the predominant lineage and maturation stage of the leukemic blasts [3]. In parallel, the International Consensus Classification (ICC) proposed an alternative approach, creating new categories: AML with myelodysplasia-related cytogenetic abnormalities, AML with myelodysplasia-related gene mutations, and AML with mutated *TP53* [4]. Mutations in the *TP53* gene are strongly associated with an unfavorable prognosis in AML and represent a challenge in current therapeutic management [5–7]. In the study conducted by Lee et al. [8], using machine learning-based analysis of gene expression, a specific genetic signature was identified in patients with AML harboring wild-type *TP53*, whose transcriptional profile closely mimics that of *TP53*-mutated AML. These patients, referred to as *TP53* mutant-like, also exhibit adverse clinical outcomes comparable to those observed in cases with *TP53* mutations. Given these findings, a detailed characterization of this molecular signature is essential to improve prognostic stratification and enable the selection of more appropriate therapeutic strategies. Such an approach could significantly enhance treatment response and overall quality of life for these patients. This study aims to explore the prognostic potential of *TP53* mutant-like-related genes and to develop a prognostic score based on the expression of key genes, capable of defining risk groups with greater precision than traditional stratification systems that rely solely on molecular and cytogenetic risk factors.

Patients with non-M3 AML who had received potentially curative therapy, had wild-type *TP53* status, and had RNA-seq data available in the TCGA (n = 113) and Beat AML (n = 197) cohorts were included in the analysis [9,10]. Dichotomization of gene expression was performed based on receiver operating characteristic (ROC) curve analysis and the concordance index (C-index). Overall survival (OS) was defined as the time, in months, from diagnosis to the date of last follow-up or death. Survival analyses were performed using Kaplan-Meier curves and compared with the log-rank test and/or Cox proportional hazards regression. For continuous variables, Student's *t*-test, ANOVA, Kruskal-Wallis, or Mann-Whitney tests were applied as appropriate, while categorical variables were analyzed using the chi-square test or Fisher's exact test in GraphPad Prism 8 (GraphPad Software, Inc.), Stata Statistic/Data Analysis 14.1 (Stata Corp., College Station, TX, USA), or SPSS Statistics for Windows, version 21.0 (SPSS, Chicago, IL, USA). All transcripts from the TCGA AML RNA-seq dataset were pre-ranked according to their differential expression by comparing samples stratified by the 3-gene *TP53* mutant-like score (low, intermediate, and high). This analysis was performed using the normalized quantile method and the Limma-Voom package implemented in Galaxy (https://usegalaxy.org/). A heatmap was generated using ClusterVis (https://biit.cs.ut.ee/clustvis), while volcano and correlation plots were produced with SRplot (https://www.bioinformatics.com.cn/srplot). Gene set enrichment analysis (GSEA) was performed with GSEA v.4.0.15, using the Hallmark gene sets curated by MSigDB. Enrichment scores (ES) were calculated based on the Kolmogorov-Smirnov statistic and tested for significance using 1000 permutations. The scores were normalized (NES) to account for the size of each gene set. All differentially expressed genes (p-value <0.05) obtained from

Galaxy were used for Gene Ontology (GO) enrichment analysis through the ShinyGO v0.77 database (http://bioinformatics.sdstate.edu/go/), and the top upregulated and downregulated GO biological processes were illustrated. Statistical significance was defined as p-value <0.05 and/or false discovery rate (FDR) <0.25.

From the analysis, four of the 25 genes initially associated with the *TP53* mutant-like profile showed a significant impact on overall survival (OS) in both cohorts, with three genes, *PEAR1*, *UGCG*, and *NUDT13*, exhibiting consistent effects in the same direction (p-value <0.05) (Figure 1A and B). Based on the hazard ratio (HR) values corresponding to each gene, a prognostic score, termed the "3-gene *TP53* mutant-like score", was developed. This score stratified patients into three distinct risk categories: low, intermediate, and high risk. In the Beat AML cohort, a significant reclassification of patients was observed when comparing the conventional European LeukemiaNet (ELN) 2022 risk classification with the new 3-gene *TP53* mutant-like score-based classification (Figure 1D). The intermediate ELN group was predominantly redistributed into the intermediate and high-risk categories of the new system. Moreover, patients previously classified as favorable risk were almost evenly redistributed across the three new categories (Figure 1D). Compared with the ELN 2022 classification,

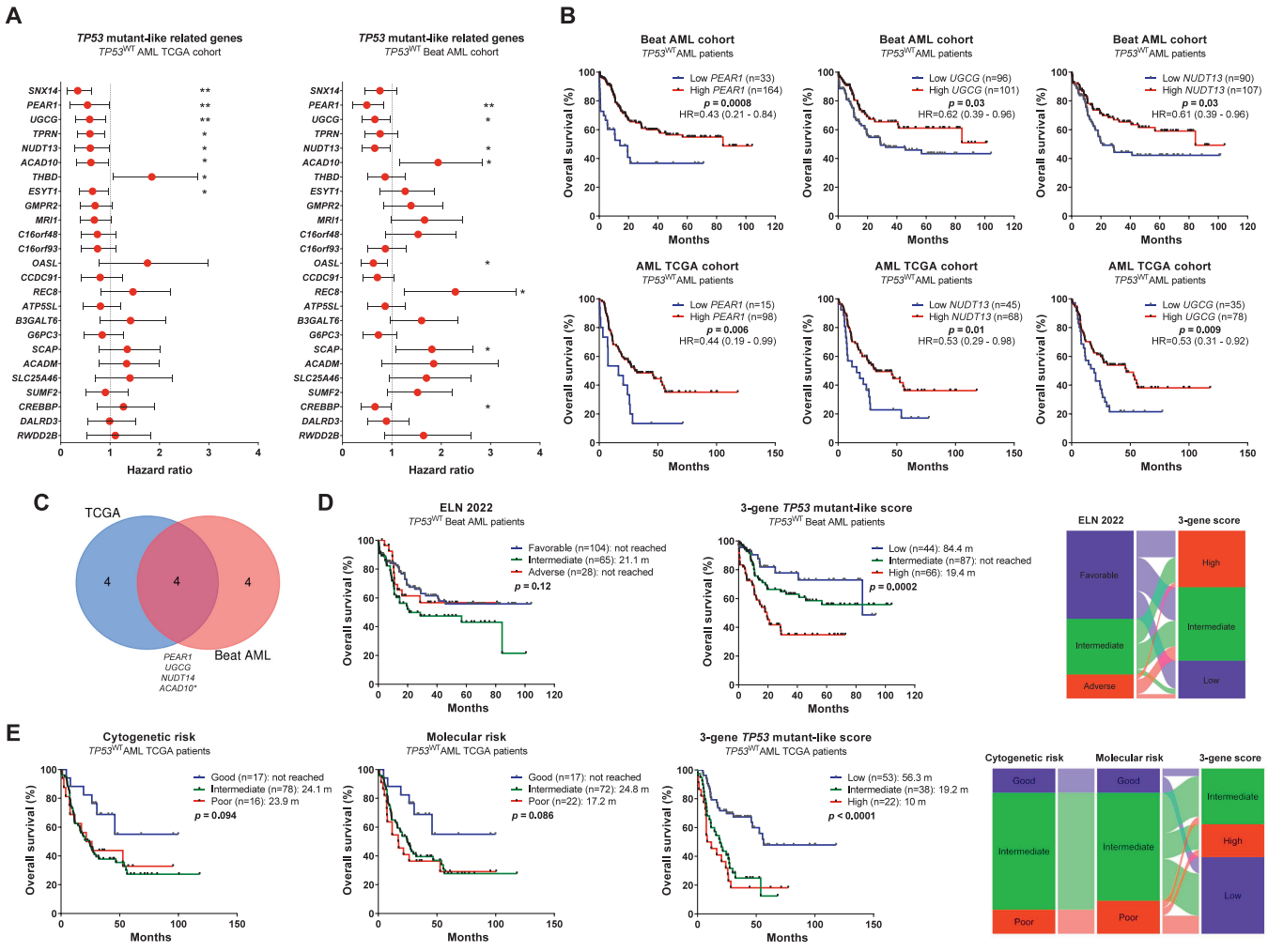

Fig. 1 – Prognostic impact of the 3-gene TP53 mutant-like score in AML. (A) Hazard ratio (HR), 95% confidence interval (95% CI), and p-values (Log-rank test) for overall survival (OS) associated with the 25 genes previously related to mutant-like TP53 in the TCGA and Beat AML cohorts. (B) Kaplan–Meier curves showing OS for AML patients stratified into low and high expression groups for each gene, using the ROC curve-defined cutoff. HR, 95% CI, and p-values (Log-rank test) are shown. (C) Venn diagram showing the overlap of genes significantly impacting prognosis in AML across both cohorts. The genes *PEAR1*, *UGCG*, and *NUDT4* were consistently associated with prognosis in the same direction in both datasets. (D) Kaplan–Meier curves depicting OS for Beat AML patients stratified according to the ELN 2022 classification and the 3-gene TP53 mutant-like score. HR, 95% CI, and p-values (Log-rank test) are shown. The schematic illustrates patient reclassification according to the ELN 2022 and 3-gene TP53 mutant-like score. (E) Kaplan–Meier curves showing OS for Beat AML patients stratified according to cytogenetic risk, molecular risk, and the 3-gene TP53 mutant-like score. HR, 95% CI, and p-values (Log-rank test) are shown. The schematic highlights patient redistribution when applying the 3-gene TP53 mutant-like score compared with conventional risk classifications.

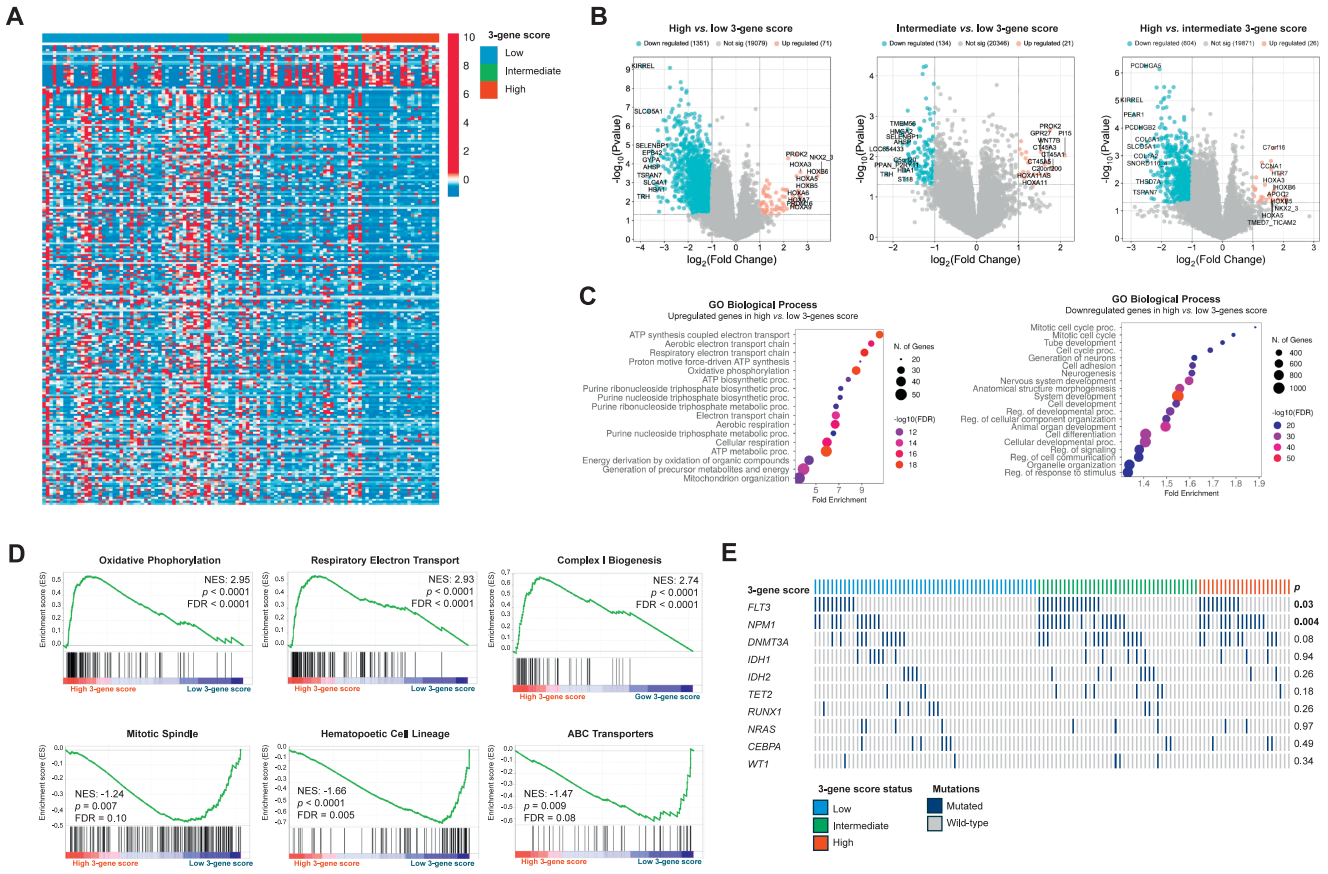

Fig. 2 – Functional genomic characterization of the 3-gene TP53 mutant-like score in AML. (A) Heatmap summarizing the expression patterns of the three genes. Color intensity represents the z-score normalized within each row. (B) Volcano plots illustrate the magnitude (x-axis) and statistical significance (y-axis) of differential gene expression across comparisons of high vs. low, high vs. intermediate, and intermediate vs. low groups according to the 3-gene TP53 mutant-like score. (C) Biological processes enriched among upregulated and downregulated genes in the high vs. low 3-gene TP53 mutant-like score groups. (D) Gene set enrichment analysis (GSEA) in the TCGA AML cohort showing that higher expression levels of the 3-gene TP53 mutant-like score are significantly enriched for pathways related to oxidative phosphorylation, respiratory electron transport, and complex I biogenesis, while being depleted for mitotic spindle organization, hematopoietic cell lineage, and ABC transporter activity. NES, FDR, and *p*-values are indicated. (E) Analysis of mutational enrichment demonstrating the distribution of AML-relevant mutated genes across groups defined by the 3-gene TP53 mutant-like score.

the 3-gene *TP53* mutant-like score identified a greater proportion of patients within the intermediate and high-risk groups (Figure 1D). A similar redistribution pattern was observed in the TCGA cohort when comparing the new score with traditional molecular and cytogenetic risk stratification systems (Figure 1E). In AML patients stratified according to the ELN 2022 criteria, the 3-gene *TP53* mutant-like score provided additional prognostic value within the favorable-risk group (p-value = 0.0002; Supplementary Figure 1). When patients were stratified based on *FLT3* and *NPM1* mutation status, a trend toward reduced overall survival was observed in those with a high 3-gene *TP53* mutant-like score, reaching statistical significance in the *NPM1* mutated Beat AML cohort (p-value = 0.006; Supplementary Figure 2). Details of the included AML patients, as well as the associations between the 3-gene *TP53* mutant-like score and clinical or laboratory features, are presented in Supplementary Tables 1 and 2. In summary, the high-score group showed higher leukocyte counts and was associated with poorer risk stratification categories. Although the score correlated with parameters typically linked to adverse prognosis in AML [11], multivariate analyses indicated that the 3-gene score is an independent prognostic factor (Supplementary Tables 3 and 4) in both analyzed cohorts. A limitation of this study is the heterogeneity of the original datasets in terms of AML subtypes, disease stage, and treatment regimens, which may influence clinical outcomes, as well as the reliance on RNA-seq data that require independent validation by quantitative polymerase chain reaction-based methodologies to support potential clinical application.

From a biological perspective, *PEAR1* participates in intracellular signaling and cell-cell interactions, *UGCG* regulates glycosphingolipid biosynthesis and membrane-associated signaling, and *NUDT13* contributes to mitochondrial nucleotide metabolism and oxidative stress control,

collectively linking signal transduction, membrane biology, and cellular metabolic homeostasis.

Subsequently, using functional genomics, we sought to evaluate the biological and molecular processes differentially regulated according to the risk stratification defined by the 3-gene *TP53* mutant-like score. The most pronounced differences in gene expression signatures were observed between the high and low 3-gene *TP53* mutant-like score groups (Figure 2A and B). Genes upregulated in the high- versus low-score group were predominantly associated with enhanced mitochondrial metabolism, oxidative phosphorylation, ATP production, and purine biosynthesis, whereas those downregulated in the high- versus low-score group were mainly related to mitosis, cell cycle progression, and response to external stimuli (Figure 2C). GSEA analyses further corroborate these findings (Figure 2D). These findings are consistent with recent studies showing that increased mitochondrial metabolism and biomass production are associated with chemoresistance and poor prognosis in AML [12–14]. Finally, when exploring potential associations between the 3-gene *TP53* mutant-like score and specific mutational signatures, we identified an enrichment of *FLT3* and *NPM1* mutations within the intermediate- and high-risk groups defined by the new score (Figure 2E). Overall, the establishment of this score underscores its potential as a robust tool for refining prognostic stratification in AML and may contribute to the development of more personalized therapeutic approaches.

In summary, the present study proposes a model that enables more accurate therapeutic decision-making, offering some patients, previously classified as low risk by conventional systems, a new therapeutic perspective and a potential opportunity for remission. The findings expand current evidence on the clinical relevance of the wild-type *TP53* transcriptional signature in AML, underscoring the importance of adopting more refined risk stratification strategies. The proposed 3-gene *TP53* mutant-like score demonstrates strong potential to overcome the limitations of existing prognostic models, while also providing insights into the underlying biological mechanisms that may inform the development of novel targeted therapies aimed at improving outcomes for patients with AML.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no competing interests.

Acknowledgments

This study was supported by grants 2024/07906-0, 2023/08735-1, 2023/12246-6 from the São Paulo Research Foundation (FAPESP) and grant 303623/2025-0 from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES), Finance Code 001.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.htct.2026.106455.

Editor

Erich Vinicius De Paula

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Received 24 November 2025
Accepted 20 February 2026
Available online 14 April 2026

https://doi.org/10.1016/j.htct.2026.106455
2531-1379/
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Letter to the Editor

Hematogones in CD34+ leukapheresis units: Is it important to quantify and report them?

Check for updates

Dear Sir,

The quantification of $CD34^+$ hematopoietic stem cells (HSCs) plays a critical role in the feasibility of hematopoietic stem cell transplantation (HSCT). In general, most centers collect a minimum of 2.0 and 4.0 $\times$ 10^6 $CD34^+$ HSCs per kilogram of recipient body weight for autologous and allogeneic HSCT, respectively. In this scenario, normal B-cell lymphoid precursors, also known as hematogones, are sometimes identified by flow cytometry in mobilized leukapheresis units. As hematogones are characterized by the expression of the CD34 antigen, their presence in apheresis products scheduled for infusion in patients undergoing HSCT raises the question of whether, in fact, a sufficient number of bona fide $CD34^+$ HSCs has been collected [1].

From January 2020 to January 2024, the Flow Cytometry Section of the Center of Hematology and Hemotherapy of

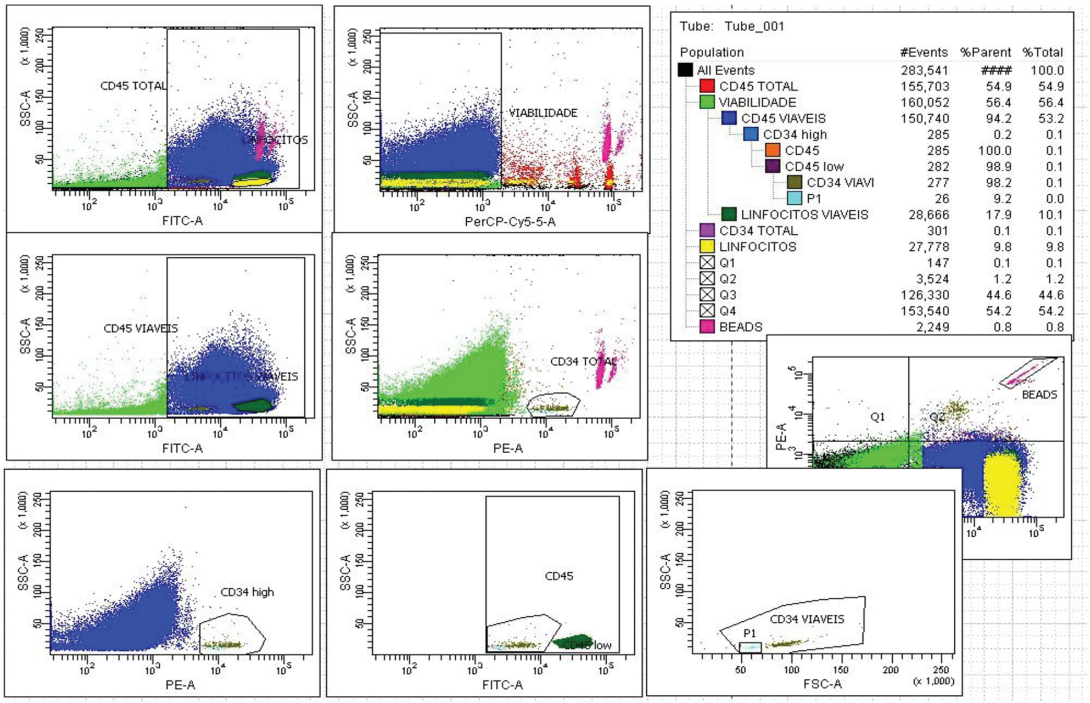

Figure 1 – Enumeration of $CD34^+$ hematopoietic stem cells (HSCs) and hematogones using a single-platform ISHAGE-based protocol.Data were collected on a BD FACS Canto II. An extra-gate (P1) was designed to include hematogones. Notice the very low forward scatter (FSC) and side scatter (SSC) of hematogones (light blue) when compared with $CD34^+$ HSCs (green). Presence of 9.2 % of hematogones

Ceará (CPC–HEMOCE) received a total of 501 leukapheresis units. These samples were systematically evaluated to identify cases in which the detection of hematogones was essential to avoid proceeding with HSCT using a suboptimal dose of $CD34^+$ HSCs. All specimens included in this study were part of the routine activities of the CPC and were handled under the auspices of the HEMOCE. Anonymity and confidentiality of the information were maintained.

The enumeration of $CD34^+$ HSCs was performed in accordance with the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines using three flow cytometers: BD FACSVia (BD Biosciences), BD FACSCanto II (BD Biosciences), and DxFLEX (Beckman Coulter). On the BD FACSVia and BD FACSCanto II, $CD34^+$ HSC quantification was carried out using the single-platform BD Stem Cell Enumeration Kit (BD Biosciences) [2], prepared according to the manufacturer's instructions and following the ISHAGE sequential gating strategy. Briefly, 20 μL of a pooled monoclonal antibody reagent containing anti-CD45-FITC (clone 2D1) and anti-CD34-PE (clone 8G12), along with 20 μL of 7-aminoactinomycin D (7-AAD) for viability assessment, were added to the bottom of a BD Trucount tube. Next, 100 μL of the sample was pipetted onto the side of the tube, just above the bead retainer. Erythrocytes were lysed by adding 2 mL of 1 × ammonium chloride lysing solution. The sample was incubated for 10 min at 20–25 °C, protected from light. For $CD34^+$ HSC enumeration on the DxFLEX, the single-platform Stem-Kit (Beckman Coulter) was prepared according to the manufacturer's recommendations. Briefly, 20 μL of a pooled monoclonal antibody reagent containing anti-CD45-FITC (clone J33) and anti-CD34-PE (clone 581), together with 20 μL of 7-AAD, were pipetted into the bottom of the tube. Erythrocytes were lysed by adding 2 mL of the Stem-Kit lysing solution, followed by incubation for 10 min at 20–25 °C, protected from light. Subsequently, 100 μL of Stem-Count Fluorospheres was added to the tube. Data acquisition and analysis were performed using an ISHAGE-based gating template, with a minimum of 100 $CD34^+$ events and at least 150,000 $CD45^+$ events being acquired per sample.

To detect hematogones, an additional gate was created to include events characterized by CD34 positivity, low-intensity CD45 expression, very low forward scatter (FSC), and very low side scatter (SSC). This profile corresponds to the typical immunophenotype of hematogones (Figure 1).

Hematogones were detected in 61 (12.1 %) of the 501 leukapheresis units analyzed. In three cases, the identification and quantification of hematogones were of paramount importance to avoid the infusion of a suboptimal dose of $CD34^+$ HSCs (Table 1).

Case 1 was a 65-year-old female patient scheduled to undergo autologous HSCT for multiple myeloma. The first leukapheresis collection yielded 2.0×10^6 $CD34^+$ HSC/kg, of which 9.2 % were identified as hematogones. After correction for the hematogone percentage, the effective $CD34^+$ HSCs dose decreased to 1.8×10^6 $CD34^+$ HSC/kg. On this basis, the transplant physician deemed it necessary to perform a second leukapheresis procedure. The second collection yielded 0.8×10^6 $CD34^+$ HSC/kg, resulting in a final infused dose of 2.6×10^6 $CD34^+$ HSC/kg.

Case 2 was a 62-year-old female patient also undergoing autologous HSCT for multiple myeloma. The first leukapheresis

Table 1 – Profiles of three autologous hematopoietic stem cell transplantation patients with borderline $CD34^+$ counts due to hematogones.

Case	Age / gender	Mobilization	Diagnosis	1 °Collection (CD34+ cells/kg)	Hematogones (%)	1 °Collection ($CD34^+$ cells/kg) *	2 °Collection ($CD34^+$ cells/kg) *	Total ($CD34^+$ cells/kg) *
1	65 / female	G-CSF (1.200 mg/d)	Multiple myeloma	2.0×10^6	9.2	1.8×10^6	0.8×10^6	2.6×10^6
2	62 / female	G-CSF (1.500 mg/d)	Multiple myeloma	2.0×10^6	15.8	1.6×10^6	0.9×10^6	2.5×10^6
3	23 / male	G-CSF (1.200 mg/d)	Hodgkin lymphoma	2.6×10^6	21.8	2.0×10^6	0.6×10^6	2.6×10^6

* $CD34^+$ cell counts were adjusted to exclude hematogones.

yielded 2.0 × 10^6 $CD34^+$ HSC/kg, of which 15.8 % were identified as hematogones. After correction for the hematogone percentage, the effective $CD34^+$ HSCs dose decreased to 1.6 × 10^6 $CD34^+$ HSC/kg. The second collection yielded 0.9 × 10^6 $CD34^+$ HSC/kg, resulting in a final infused dose of 2.5 × 10^6 $CD34^+$ HSC/kg.

Case 3 was a 23-year-old male patient scheduled for autologous HSCT for Hodgkin lymphoma. The first leukapheresis collection yielded 2.6 × 10^6 $CD34^+$ HSC/kg, a dose considered acceptable for autologous HSCT. Hematogones accounted for 21.8 % of $CD34^+$ events. After correction for the hematogone percentage, the effective $CD34^+$ HSCs dose decreased to 2.0 × 10^6 $CD34^+$ HSC/kg, representing the minimum value for proceeding with autologous HSCT. Consequently, the transplant physician requested an additional leukapheresis procedure that yielded 0.6 × 10^6 $CD34^+$ HSC/kg, resulting in a final infused dose of 2.6 × 10^6 $CD34^+$ HSC/kg.

To date, there is no definitive clinical evidence in the literature regarding the optimal management of leukapheresis units containing hematogones. Studies by Nadeem et al. [3] and Ondrejka et al. [4] evaluated the relationship between the presence of hematogones in leukapheresis units and neutrophil and platelet engraftment times. However, neither study examined whether reduced CD34+ cell doses, caused by the presence of hematogones, led to delayed or impaired engraftment. In light of this uncertainty, we consider it essential to provide transplant physicians with corrected $CD34^+$ HSCs counts, adjusted for the proportion of hematogones, in order to highlight potentially suboptimal graft doses.

Thus, until definitive evidence is available regarding the relationship between hematogones and engraftment kinetics, we strongly recommend that laboratories involved in $CD34^+$ HSCs enumeration (a) routinely assess the presence of hematogones in all leukapheresis samples and (b) call attention to their presence in laboratory reports, particularly when hematogones substantially reduce the effective $CD34^+$ HSCs dose to a level considered inadequate for HSCT. This practice would alert transplant physicians to the actual number of bona fide $CD34^+$ HSCs in leukapheresis units scheduled for infusion.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

Editor

Erich Vinicius De Paula

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Received 12 September 2024
Accepted 15 April 2026
Available online 23 May 2026

https://doi.org/10.1016/j.htct.2026.106479
2531-1379/
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HTCT Journal

Letter to the Editor

Is time to plasma exchange the right question in suspected thrombotic thrombocytopenic purpura?

To the Editor,

I read with great interest the recent publication by Soares Ferreira Junior et al.: In patients with suspected thrombotic thrombocytopenic purpura, what is the optimal time to therapeutic plasma exchange? [1]

Therapeutic plasma exchange (TPE) remains the cornerstone of management in suspected thrombotic thrombocytopenic purpura (TTP), yet outcomes continue to vary widely despite a guideline-driven emphasis on early initiation [1] Recent population-level analyses using large administrative datasets have highlighted a non-linear relationship between time to TPE and outcomes, with only modest predictive capacity when timing is considered in isolation [1]. While these findings are important, interpreting temporal thresholds in isolation may overlook broader clinical and system-level determinants that influence outcomes in real-world practice.

From a transfusion medicine and apheresis perspective, suspected TTP represents a complex, multi-step care pathway, involving emergency physicians, haematologists, laboratories, blood banks, and apheresis services. Delays frequently arise not only before TPE initiation but also within the diagnostic-treatment interface, particularly related to diagnostic uncertainty, plasma availability, and coordination of apheresis services. These operational delays are rarely captured when time to TPE is defined solely by hospital admission or plasma issue time [2].

Importantly, clinical heterogeneity at presentation complicates the interpretation of time-based metrics. Disease severity, extent of neurologic or cardiac involvement, and comorbid organ dysfunction are well-recognised predictors of the outcome in TTP [3]. Patients with fulminant disease may experience poor outcomes despite early TPE, whereas others with less severe presentations may tolerate modest delays without adverse consequences. This heterogeneity may partly explain why timing alone demonstrates limited prognostic value in contemporary analyses [1].

Risk stratification tools such as the PLASMIC score provide an opportunity to refine early decision-making by identifying patients at highest likelihood of immune-mediated TTP when definitive ADAMTS13 testing is unavailable [4]. Incorporating rapid risk assessment into early workflows may be more clinically actionable than rigid adherence to time thresholds alone.

A complementary strategy is the implementation of a 'TPE Readiness Bundle' for suspected TTP, analogous to sepsis bundles. Such a model emphasises parallel processing rather than sequential delays and includes early haematology-transfusion medicine co-activation, standardised plasma allocation pathways, avoidance of prophylactic platelet transfusions, and predefined escalation for intensive monitoring [3,5]. Readiness-based strategies may reduce care variability even when the absolute time to TPE cannot be uniformly shortened (Table 1).

Furthermore, the evolving therapeutic landscape necessitates reassessment of TPE-centric outcome models. The introduction of caplacizumab has altered early disease biology by rapidly inhibiting microvascular thrombosis and may modify the relationship between TPE timing and outcomes in selected patients [6]. Future studies should therefore evaluate interaction effects between TPE timing, adjunctive therapies, disease severity, and institutional readiness rather than evaluating time as an isolated predictor [1].

From a transfusion systems perspective, quality improvement efforts may benefit from shifting toward process-based indicators, such as time to haematology consultation, time to PLASMIC scoring, plasma availability latency, and appropriateness of component utilisation. These metrics are actionable, auditable, and adaptable across healthcare settings, including resource-limited environments [5].

In conclusion, while timely initiation of TPE remains essential, its impact should be interpreted within a broader, systems-based framework. A readiness-oriented approach that integrates clinical severity, diagnostic tools, transfusion infrastructure, and adjunctive therapies may offer greater potential to improve outcomes in suspected TTP rather than reliance on time thresholds alone.

Table 1 – Moving beyond 'time to plasma exchange (TPE)': a readiness-based framework for suspected thrombotic thrombocytopenic purpura (TTP) care.

Domain	Traditional time-based focus	Readiness-based approach (proposed)	Clinical value
Trigger for action	Fixed time threshold from admission	Early clinical suspicion + PLASMIC score	Reduces diagnostic inertia
Decision pathway	Sequential (diagnosis → plasma → TPE)	Parallel processing across teams	Minimises hidden delays
Risk stratification	Often implicit or delayed	Structured bedside risk scoring	Prioritises high-risk patients
Plasma availability	Reactive allocation	Pre-defined emergency plasma pathways	Prevents logistical delays
Transfusion practices	Variable platelet use	Standardised platelet avoidance protocols	Reduces iatrogenic risk
Apheresis activation	After confirmatory steps	Early co-activation with haematology	Improves workflow efficiency
Adjunctive therapy	TPE-centric	Integrated early immunotherapy/ caplacizumab	Addresses disease biology
Quality indicators	Time to first TPE	Process metrics (consult time, readiness markers)	Enables audit and improvement
Applicability	High-resource settings	Adaptable across resource settings	Improves global relevance

Interpretation

This framework emphasises that outcomes in suspected TTP are influenced not only by when TPE is initiated, but by how prepared the system is to deliver coordinated, risk-adapted care. A readiness-based approach offers actionable targets for quality improvement beyond rigid time threshol.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

NIL.

Editor

Erich Vinicius De Paula

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Received 17 January 2026
Accepted 23 March 2026
Available online 30 May 2026

https://doi.org/10.1016/j.htct.2026.106478
2531-1379/
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HTCT Journal

Letter to the Editor

Patterns of chromosomal translocations in Acute Leukemias: A Brazilian amazon perspective

Dear Editor,

Acute leukemias (AL) are hematological malignancies characterized by the proliferation of progenitor cells in the bone marrow and peripheral blood. Chromosomal translocations represent the primary genetic alterations in these pathologies, serving as critical biomarkers for prognosis and therapeutic management [1]. Despite the high incidence of AL in the state of Amazonas, the frequency of these translocations in this specific population remained unknown.

Ninety patients were analyzed (59 with acute lymphoblastic leukemia [ALL] and 31 with acute myeloid leukemia [AML]) in this descriptive study conducted at the Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas (HEMOAM) between 2021 and 2022. Reverse Transcription polymerase chain reaction (RT-PCR) following the BIOMED-1 Consortium protocol [2] was utilized to identify the most prevalent fusion transcripts. The results of this study revealed that 27 % of ALL patients presented translocations; *BCR*::*ABL1 p190* (8 %) and *ETV6*::*RUNX1* (8 %) were the most frequent (Supplementary Table 1). Among AML patients, the positivity rate was 42 %, with *RUNX1*::*RUNX1T1* (19 %) and *PML*::*RARA* (16 %) being the most frequent translocations (Supplementary Table 2).

The age and sociodemographic distribution exhibit regional particularities (Figure 1). In ALL, the *BCR*::*ABL1 p190* translocation was prevalent in adults (80 % of positive cases >18 years), while *ETV6*::*RUNX1* was concentrated in the pediatric group (60 % of cases <9 years) [3]. In AML, the *PML*::*RARA* translocation was also more frequent in adults (80 % >18 years), whereas *RUNX1*::*RUNX1T1* and *CBFB*::*MYH11* showed a more heterogeneous distribution across age groups (Supplementary Table 3). Overall, we observed a predominance of admixed individuals and residents of the capital, Manaus, reflecting the demographic reality and centralized access to health services in the Amazon region [4].

Clinically, the majority of patients (97 % ALL; 90 % AML) achieved complete remission. However, we highlight a rare case the of co-occurrence of *ETV6*::*RUNX1* and *KMT2A*::*AFF1* translocations in a pediatric patient, who died before the 35th day of treatment, reinforcing the impact of these alterations on clinical outcomes.

Although the sample size was limited by the impacts of the SARS-CoV-2 pandemic, this research establishes an unprecedented epidemiological profile for northern Brazil. The high prevalence of Pardo (admixed ancestry) individuals in our study aligns with the unique demographic mosaic of the northern region, which differs significantly from other Brazilian regions. While no genetic ancestry testing was performed, these sociodemographic findings suggest that regional population characteristics may influence the molecular landscape of acute leukemias. These data justify the implementation of routine molecular surveillance to optimize risk stratification and measurable residual disease monitoring in the Amazon, as recommended by the latest international guidelines.

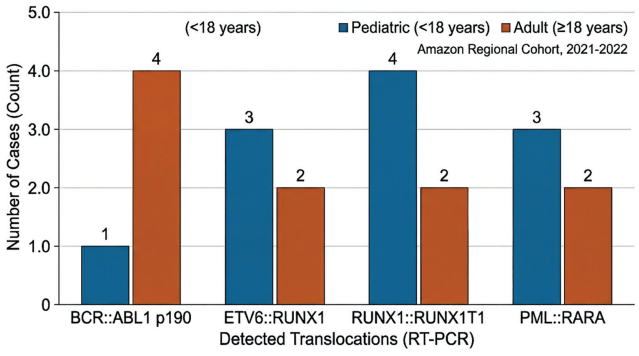

Figure 1 – Frequency of major fusion transcripts in pediatric and adult acute leukemia cohorts from the Brazilian Amazon. The bar chart illustrates the frequency of major fusion transcripts detected by RT-PCR in a cohort of 90 patients (59 ALL and 31 AML) at the HEMOAM Foundation between 2021 and 2022. Data is stratified by age group: Pediatric (blue bars: <18 years) and Adult (terracotta bars: ≥18 years).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

To FAPEAM, CNPq, and CAPES for financial support and to the HEMOAM Foundation team.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2026.106481.

Editor

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Received 26 March 2026
Accepted 15 April 2026
Available online 12 June 2026

https://doi.org/10.1016/j.htct.2026.106481
2531-1379/

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Letter to the Editor

MPL mutations are over-represented in patients with dual driver-mutated myeloproliferative neoplasms: an Irish cohort study

Dear Editor

Singular mutations in either *JAK2, CALR* or *MPL* genes are the most frequent drivers of the myeloproliferative neoplasms (MPN) with variants in other myeloid-associated genes contributing to disease phenotype and clinical course. MPN molecular diagnostic algorithms can be either sequential or simultaneous with each technology employed possessing its own sensitivities, specificities and clinical applicability [1]. An increasingly recognised, minor subgroup of MPN patients, particularly those with essential thrombocythemia (ET) and primary myelofibrosis (PMF), harbor two driver mutations [2]. Prior evidence suggests a second driver mutation occurs more commonly in low variant allele frequency (VAF) *JAK2* V617F-mutated MPN with the presence of two mutations affecting the phenotype such as a higher platelet count [3,4].

In order to evaluate the incidence and molecular diversity of the dual driver phenomenon in the Irish MPN population, an audit was performed on all requests received at a center for molecular diagnosis of MPN. Mutations in *JAK2* (exons 12 and 14), *CALR* (exon 9), and *MPL* (exon 10) were identified using a previously described single-molecule, molecular inversion probe-based next-generation sequencing (NGS) methodology [5,6]. From March 2023 to November 2025, 1275 consecutive, new patients were identified with either *JAK2* V617F (n = 1011; 79.3%), *JAK2* exon 12 (n = 12; 0.9%), *CALR* exon 9 (n = 168; 13.2%) or *MPL* exon 10 (n = 84; 6.6%) mutations. This cohort consisted of 665 females and 610 males with a median age of 68 years (range: 21–97 years). Of these 1275 patients, thirteen (1.0%) were identified with ET (n = 12) or PMF (n = 1) with two driver mutations. This group comprised eight females and five males with a median age at presentation of 71 years (range: 61–89 years) and a median presenting platelet count of 853 $\times$ 10^9/L (range: 562–1244 $\times$ 10^9/L) (Table 1). Of the 26 mutations in 13 patients, eleven (42.3%) were *JAK2* V617F, eleven (42.3%) were in *MPL* exon 10 and four (15.4%) were in *CALR* exon 9. Of the eleven patients with a *JAK2* V617F, seven (63.6%) had a VAF ≤5% (Table 1).

In this European cohort, 1% of MPN patients presented with dual driver mutations, similar to the recently described rate of 1.5% [7]. The association of dual driver mutations in patients with a low *JAK2* V617F VAF is also confirmed [8,9]. In agreement

Table 1 – Myeloproliferative neoplasm patients with dual driver mutations. Largest variant allele frequency (VAF) mutation first.

Case	Sex	Age (years)	MPN	Platelets (x 10^9/L)	Mutation #1	VAF (%)	Mutation #2	VAF (%)
#1	F	64	ET	615	*MPL* p.(Trp515Leu)	27.0	*JAK2* p.(Val617Phe)	2.0
#2	F	84	ET	736	*JAK2* p.(Val617Phe)	47.0	*CALR* p.(Leu367Thrfs*46)	3.9
#3	F	71	ET	1147	*CALR* p.(Lys385Asnfs*47)	3.5	*JAK2* p.(Val617Phe)	1.1
#4	F	70	ET	958	*JAK2* p.(Val617Phe)	6.1	*MPL* p.(Trp515Leu)	2.7
#5	M	67	ET	787	*CALR* p.(Leu367Thrfs*46)	30.0	*JAK2* p.(Val617Phe)	3.2
#6	F	61	ET	1113	*MPL* p.(Trp515Leu)	31.0	*JAK2* p.(Val617Phe)	1.5
#7	F	89	ET	1244	*MPL* p.(Trp515Arg)	4.4	*JAK2* p.(Val617Phe)	1.1
#8	F	85	ET	853	*MPL* p.(Trp515Arg)	9.6	*MPL* p.(Ser505Asn)	9.3
#9	F	86	ET	1100	*JAK2* p.(Val617Phe)	7.2	*MPL* p.(Ser505Asn)	5.4
#10	M	70	PMF	857	*JAK2* p.(Val617Phe)	17.0	*MPL* p.(Trp515Leu)	2.8
#11	M	69	ET	532	*MPL* p.(Trp515Leu)	16.0	*JAK2* p.(Val617Phe)	2.3
#12	M	72	ET	624	*MPL* p.(Trp515Leu)	2.5	*CALR* p.(Glu371Metfs*42)	2.3
#13	M	81	ET	795	*MPL* p.(Trp515Ala)	11.0	*JAK2* p.(Val617Phe)	3.4

with a proposal that a platelet count higher than 700 × 10^9/L acts as a general discriminator for identifying dual driver mutations [4], only three of thirteen patients presented with a lower platelet count. The relatively small size and short follow-up of this cohort preclude any clinical correlation. In MPN patients with a single driver mutation, *MPL* exon 10 mutations are present in approximately 5% and 9% of patients with ET and PMF respectively with the W515L allele being most prevalent. In contrast, *MPL* exon 10 mutations account for >40% of variants in this dual-driver population; these include the less common *MPL* S505N, W515R, and W515A mutations. This study provides confirmation of the prevalence of this molecular subtype in a national MPN cohort and demonstrates a distinct pattern of *MPL* mutations in dual driver MPN. This brief audit further underscores the molecular and clonal complexity of this subset of MPN patients.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval

This non-interventional, observational study was performed under routine standard of care procedures in accordance with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from participants at the referring centers.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

All authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Editor

Erich Vinicius De Paula

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Received 21 January 2026
Accepted 23 March 2026
Available online 9 July 2026

https://doi.org/10.1016/j.htct.2026.106495
2531-1379/
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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Images in Clinical Hematology

Atypical chronic lymphocytic leukemia with biallelic 13Q deletion

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A 73-year-old woman presented with an ulcer of the right foot. Physical examination revealed adenopathies. On admission, a blood test showed hemoglobin 78 g/L, platelets 185 × 10^9/L, leukocytes 451 × 10^9/L (lymphocytes 447.2 × 10^9/L), and lactate dehydrogenase 739 U/L (normal range: 125–264 U/L); without alterations of the other hemolytic parameters. A peripheral blood smear displayed atypical lymphocytes some small with 'turtle-like' chromatin and coarse cytoplasm; but others were of medium-large size with occasional vacuoles, some of which had lobated or flower-shaped nuclei (Figure 1A-C). Flow cytometry confirmed the clonality: positive for CD19, $CD20^{low}$, CD43, $CD79^{low}$, CD22 and CD200 with kappa chain restriction and negative for CD5, CD10 and CD38. Fluorescence in situ hybridization (FISH) demonstrated a biallelic 13q deletion (Figure 1D). The *IGVH* and *TP53* genes were unmutated. A Positron Emission Tomography/Computed Tomography study demonstrated supra- and infradiaphragmatic adenopathies (standardized uptake value [SUV]$_{max}$ 1.5–5.0) and a lytic lesion in the left ilium (SUV_{max} 4.3). Histopathological analysis of the bone biopsy revealed an infiltration of lymphocytes positive for CD19, CD23, and ZAP70, and negative for Cyclin D1, CD10, CD38, and BCL6. These findings are diagnostic of atypical chronic lymphocytic leukemia (aCLL); high-grade transformation to large cell lymphoma was excluded.

aCLL may be defined by morphological (large lymphocytes, nuclear irregularities with or without nucleoli) or phenotypical characteristics (lack of expression of antigens). This is most frequently described in patients with markers associated with poor prognosis like trisomy 12 or CD38 expression [1,2]. Monoallelic 13q deletion is the most common cytogenetic abnormality in CLL; biallelic deletions are rare [3].

We report this case due to its atypia, which raised the possibility of a leukemic phase of a large cell lymphoma, accompanied by elevated LDH levels and adenopathies. This case highlights the importance of an integrated diagnosis, including a pathological examination of suspected lesions to rule out large cell lymphoma.

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https://doi.org/10.1016/j.htct.2026.106457

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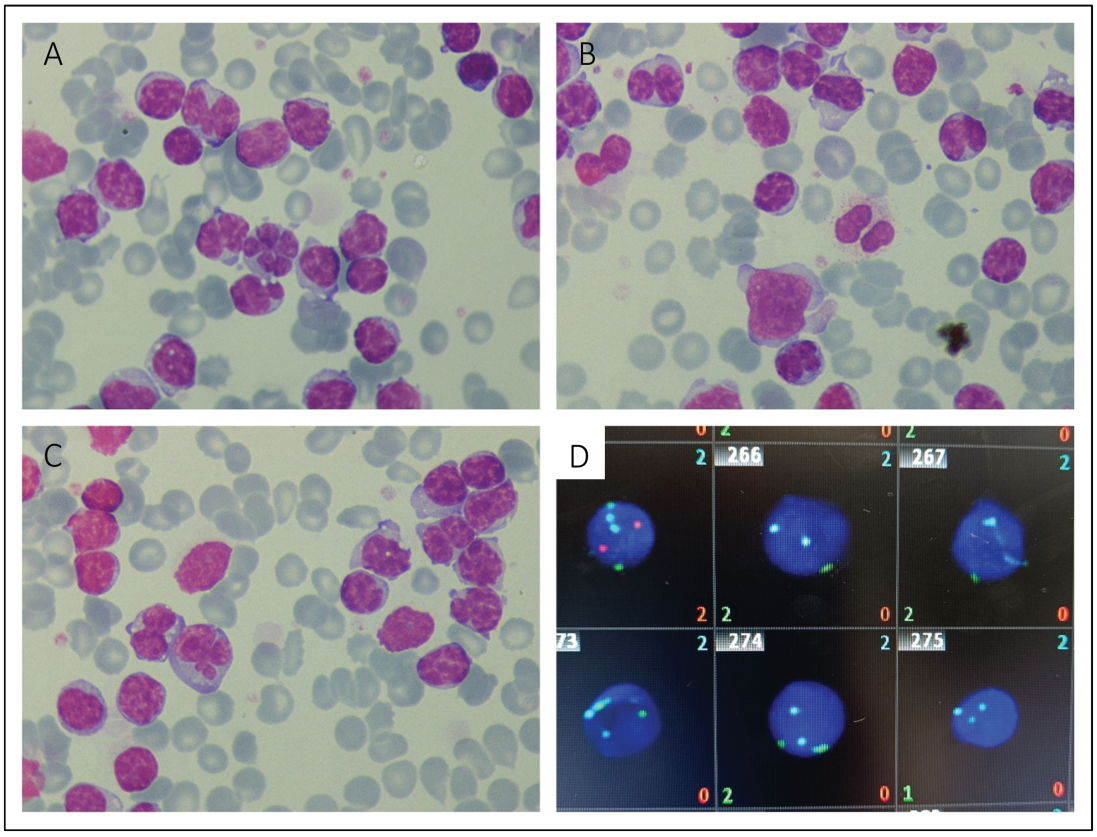

Figure 1 – A-C. Peripheral blood smear, May-Grunwald Giemsa x100. D. FISH, CLL Probe Kit: XL DLEU/LAM/12cen showing 5 abnormal cells with two green and two blue signals; and one with a normal pattern: 2G2R2B-upper left-.

Author contributions

Verónica Roldán Galiacho performed the morphological examination of peripheral blood, FISH analysis, collected the data, carried out the literature research and wrote the manuscript. Leticia Altuna Tellechea contributed with the morphological examination and literature research. Marta Alonso Varela performed the flow cytometry analysis. Ignacio Diez de Lezcano Manrique deLara performed de pathological analysis of the iliac lesion

Funding

None.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors of this paper have no conflicts of interest.

Editor

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