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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Clinical characteristics and outcomes of non-tuberculous mycobacterial pulmonary infections after hematopoietic stem cell transplantation: A retrospective cohort study

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ABSTRACT

Introduction: Non-tuberculous mycobacterial infections are rising as complications of bone marrow transplantation with lung disease being the most common clinical presentation. The identification and management of these infections in hematopoietic stem cell transplantation patients remains underrecognized. This study aims to investigate the clinical characteristics and outcomes in patients with post-transplant pulmonary infections.

Methods: The charts of 3,000 adult patients who received transplants over 11 years at the Karmanos Cancer Institute, a tertiary-care cancer center in Detroit, were reviewed. The diagnoses of post-transplant pulmonary non-tuberculous mycobacterial infections of 51 patients were defined as definite, probable or possible based on the American Thoracic Society (ATS) and Centers for Disease Control and Prevention guidelines. The identified organisms were further characterized as rapid- or slow-growing mycobacteria. Clinical characteristics, risk factors, microbiologic data, therapy and outcomes of the patients were collected and analyzed.

Results: About half (n = 26; 51%) of the patients were identified with definite pulmonary infection. There was a trend of cardiovascular and pulmonary comorbidities in these patients. The majority (n = 44; 86.3%) were on steroid and immunosuppressive therapy in the setting of graft-versus-host disease. The most common presenting symptoms were a combination of change in cough and worsening shortness of breath. The most common radiologic pattern was nodular infiltrates in 15 (29.4%) patients. *Mycobacterium avium complex* was identified in 38 (74.5%) patients. The majority of patients with these infections (76.5%) did not receive antimycobacterial therapy. Survival was reported in 42 (82.4%) patients.

Conclusion: Outcomes vary significantly among non-tuberculous mycobacterial pulmonary infections based on mycobacterial species, rate of colonization and degree of

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immunosuppression. The prognosis is overall good due to slow growing mycobacteria. Prospective multicenter studies are required to further guide the management of these patients.

Introduction

Non-tuberculous mycobacterial (NTM) infections are surging as complications of organ transplantation with lung disease being the most common clinical presentation. The identification and management of these infections in patients after hematopoietic stem cell transplantation (HSCT) is underrecognized. This study aims to investigate the clinical characteristics and outcomes of patients with these infections.

Patients and methods

Karmanos Cancer Institute (KCI) is a tertiary-care cancer center in Detroit. Medical records were reviewed to identify all over 18-year-old patients who underwent HSCT at KCI over an 11-year period. Data collection including age, gender, cardiovascular and pulmonary comorbidities, symptoms, radiologic patterns, microbiologic data, immunosuppressive therapy and outcomes.

Microbiology laboratory records were examined to identify patients with positive mycobacterial cultures obtained from sputum, bronchoalveolar lavage, or other clinical specimens throughout the 11-year study period. NTM infections were defined by the American Thoracic Society (ATS) and Centers for Disease Control and Prevention guidelines. Mycobacteria specimens had been cultured, stained and identified by conventional growth in special media and biochemical conditions. All computed tomography scans (CT) of the thorax were reviewed for abnormal radiologic patterns, with findings being characterized as lung nodules, consolidation, bronchiectasis, interstitial pattern, pleural effusion and mediastinal lymphadenopathy. This cohort comprised patients who fulfilled ATS criteria for NTM lung disease, including: (1) chest radiograph or, in the absence of cavitation, chest high-resolution computed tomography (HRCT) scan; (2) three or more sputum specimens for acid-fast bacilli analysis; and (3) exclusion of other disorders. The following criteria applied to symptomatic patients: radiographic opacities (nodular or cavitary) or an HRCT scan that shows multifocal bronchiectasis with multiple small nodules. These criteria fit best with *Mycobacterium avium complex* (MAC), *M. kansasii*, and *M. abscessus*. Not enough is known about most other NTM to be certain that these diagnostic criteria are universally applicable to all NTM respiratory pathogens [1] The NTM organisms were further characterized by their pathogenicity and identified as rapid- growing mycobacteria (RGM) and slow-growing mycobacteria (SGM) [2].

Results

This retrospective study comprised 51 (1.7%) of 3000 adult patients who were diagnosed with NTM infections after HSCT over the 11-year period. All 51 patients met one or more of the ATS clinical, radiologic or microbiologic criteria for NTM lung disease. Thirty-two (62.7%) of the 51 patients were male with a median age of 51 years (range: 20–77). Ten (19.6%) and five (9.8%) had cardiovascular and pulmonary comorbidities, respectively. MAC was identified in 38 (74.5%) patients, *M. gordonae* in seven (13.7%), *M. chelonae* in two (3.9%), and other mycobacteria in four (7.8%) patients. Twenty-six (51%) had definite diagnoses of NTM pulmonary infections with clinical, radiological and culture evidence. Eighteen patients (35.3%) had possible NTM infections with only positive cultures, and one (2%) had probable NTM infection due to radiological and culture evidence without any clinical manifestations. Mycobacterial cultures were positive in 23 (45.1%) bronchoalveolar lavage (BAL) cultures and in 22 (43.1%) sputum cultures (Table 1).

Clinical manifestations were reported in 32 (62.7%) of the 51 patients. Of these, ten (19.6%) had both changes in cough

Table 1 – Clinical characteristics of the patients.

Variable	*n* = 51
Sex - n (%)	
Male	32 (62.7)
Female	19 (37.3)
Age, years – median (range)	51 (20–77)
Clinical manifestations - *n* (%)	32 (62.7)
Worsening shortness of breath	7 (13.7)
Cough and worsening shortness of breath	10 (19.6)
Fever	3 (5.9)
Weight loss	1 (2.0)
Hemoptysis with worsening shortness of breath or weight loss or fever	3 (5.9)
Abnormal radiologic pattern on CT thorax - *n* (%)	
Lung nodules	15 (29.4)
Consolidation	7 (13.7)
Bronchiectasis	4 (7.8)
Interstitial pattern	4 (7.8)
Lung nodule, mediastinal lymphadenopathy and pleural effusion	1 (2.0)
Mycobacterial identification - *n* (%)	
Mycobacterium avium complex	38 (74.5)
Mycobacterium gordonae	7 (13.7)
Mycobacterium chelonae	2 (3.9)
Other mycobacteria	4 (7.8)
Steroid and immunosuppressive therapy - *n* (%)	44 (86.3)
Antimycobacterial therapy for definite NTM infection - *n* (%)	
Complete therapy (*n* = 26)	6 (23.1)
Incomplete therapy (*n* = 26)	20 (76.9)

and worsening shortness of breath, seven (13.7%) had worsening shortness of breath only, three (5.9%) had fever, one (2%) had weight loss and three (5.9%) had hemoptysis with worsening shortness of breath or weight loss or fever. The most common abnormal radiologic finding on thorax CT was lung nodules in 15 (29.4%) patients; seven (13.7%) had consolidation, four (7.8%) had bronchiectasis and four (7.8%) had an interstitial pattern. Only one patient (2%) had lung nodules, mediastinal lymphadenopathy and pleural effusion. Twenty patients (39.2%) had normal thorax CT results.

The median time until diagnosis of NTM pulmonary infections after the HSCT was greater than 180 days in 20 patients (40.8%), and within 90 days in 20 (40.8%) patients. Only nine patients (18.4%) were diagnosed with NTM pulmonary infection between 90 and 180 days after HSCT (Figure 1). The majority, 44 patients (86.3%), were on steroid and immunosuppressive therapy in the setting of graft-versus-host disease (GvHD). Only six (11.8%) completed antimycobacterial therapy, whereas six (11.8%) had incomplete therapy and 39 (76.5%) did not receive any antimycobacterial therapy. Survival was reported in 42 (82.4%) patients with post-HSCT NTM pulmonary infections.

Discussion

This study found that the majority of the patients who were diagnosed with NTM after HSCT were male, had a median age of 51 years, and had pulmonary and cardiovascular comorbidities. In a cohort study by Liu et al., patients aged >45 years with extensive GvHD were at increased risk of mycobacterial infections [3]. Pre-transplant T cell depleting therapy increases the risk of mycobacterial infections [4]. Epidemiologic studies of survivors have shown that HSCT is associated with higher cardiovascular risk partially due to exposure to cardiotoxic chemotherapy and radiation, as well as detrimental direct and indirect effects on the cardiovascular reserve [5]. In the present cohort, the majority (n = 44; 86.3%) were on steroid and immunosuppressive therapy in the setting of GvHD. We believe that the increased incidence of cardiovascular disease in this population is related directly or indirectly to chemotherapy and radiation and its deleterious effects on the immune system in HSCT patients.

In two reviews in 1994 and 1997, NTM infections were rare, occurring in only 0.37% and 0.40% of adult patients who received HSCT, respectively [6,7]. A recent report from other centers indicate that in 2003, the rate of NTM infection was 5 to 20 times higher in a cohort of 50 patients following allogeneic HSCT [4]. In the present cohort, NTM infection occurred in 1.7% of adult HSCT patients. The higher rates of NTM infection may have resulted from an increased recognition and improved identification in mycobacterial cultures [1]. Therefore, NTM infection after HSCT must be recognized as a complication that requires heightened awareness to identify risk factors with timely diagnosis and therapy.

Half (51%) of the patients in the current study had definite pulmonary mycobacterial infections. Of the 25 HSCT patients who had probable or possible NTM infections, 18 (35%) had only positive cultures which solely cannot differentiate between NTM colonization and infection, but can affect the decision to initiate antimycobacterial therapy. Another important factor for treating NTM infections is the identification of NTM species. Most of the identified species in this study were slow-growing mycobacteria. *M. gordonae*, the most frequently isolated mycobacterial, was found in seven (13.7%) patients.

The time to diagnosis after the transplant was either within 90 days (40.8%) or after 180 days (40.8%) in this current study. In a large 2000 retrospective study spanning 20 years, the median time to diagnosis of lower respiratory tract NTM disease following HSCT was 251 days for SGM and 61 days for RGM [6]. The infections could occur early in the pre-engraftment phase of HSCT, or later while receiving immunosuppressive therapy for GvHD [8,9].

The clinical characteristics of pulmonary NTM infections differ between patients with acquired immunodeficiency syndrome (AIDS), and immunocompromised hosts after transplantation and, evidently, these infections different from immunocompetent patients. In the present population, the most common single clinical manifestation of NTM infection after HSCT was deteriorating shortness of breath occurring in seven (13.7%) of the patients, while only three (5.9%) had fever. The combination of change in cough and worsening

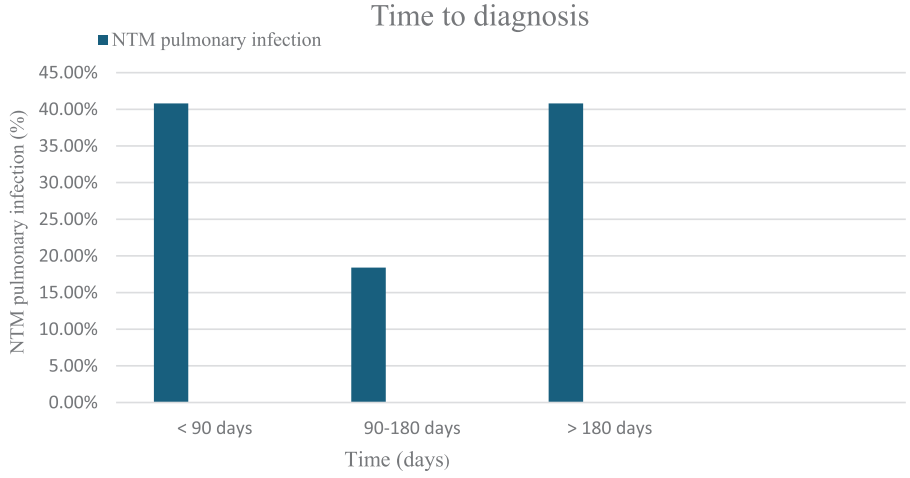

Figure 1 – Time to diagnosis.

shortness of breath was the most common presenting manifestation in this population in ten (19.6%) of 32 patients with three (5.9%) patients having hemoptysis with worsening shortness of breath, weight loss or fever. These clinical characteristics suggest that the majority of patients who lack systemic symptoms of hemoptysis and weight loss do not require antimycobacterial therapy. Clinical suspicion based on symptoms alone is low because NTM infection after HSCT is difficult to diagnose due to lack of specific symptoms and atypical presentation [10].

Studies with high resolution CT of the chest have shown that up to 90% of patients with mid- and lower-lung field noncavitary disease with MAC also have multifocal bronchiectasis [1]. There was no specific radiologic finding in the current cohort although the most common radiologic pattern identified in HSCT patients with NTM infections was lung nodules in 15 (29.4%) of the 51 patients. Seven (13.7%) patients had consolidation and four (7.8%) patients each had bronchiectasis and interstitial pattern. Twenty (39.2%) of the 51 patients in this current study had normal thorax CT.

The most commonly NTM isolated in this population was MAC in 74.5% followed by *M. chelonae* in two (3.9%) patients, and *M. gordonae* in seven (13.7%). These mycobacterial species are all SGM; *M. gordonae* is non-pathogenic and is known as a weak mycobacterium that rarely causes overt disease or requires therapy [1]. However, no RGM, such as *M. abscessus*, which are intrinsically resistant and difficult to treat, were identified in this current study.

Initiation of antimycobacterial therapy is a concern because of multiple factors including 1) adverse effects of antimycobacterial therapy, 2) drug-drug interactions with drug prophylaxis after HSCT, and 3) prolonged duration of therapy until culture conversion [1]. In the present cohort, only six of 26 patients (23.1%) with definite NTM infections completed antimycobacterial therapy, whereas 20 (76.9%) did not complete antimycobacterial therapy. The prognosis of pulmonary NTM infections in transplant patients is overall favorable. Most patients (82.4%) with definite, probable or possible NTM infections in this study are alive and 25 of 51 (49.1%) did not receive any antimycobacterial therapy. A high survival rate is noted in patients with NTM infections after HSCT while only 23.1% completed antimycobacterial therapy. This is related to (1) the nature of the disease due to predominantly SGM MAC which is a less aggressive disease than RGM, (2) the paucity of patients with systemic symptoms (only 5.9% had fever and hemoptysis with worsening shortness of breath or weight loss or fever) and (3) the predominant radiologic patterns of nodularity and bronchiectasis which correlates with less severe disease than cavitary lesions [1].

Our study had several limitations. First, this is a single-center retrospective and uncontrolled study which could affect the validity of the results. Secondly, the study included non-pathogenic SGM which could contribute to a favorable outcome. Third, four of the NTM culture results were not identified as to the specific species. Different species have different clinical impacts with the SGM causing less aggressive mycobacterial disease than the RGM. Lastly, data about potential use of one or more antibiotics of the antimycobacterial therapy such as macrolide agents is lacking in this study. However, it is unlikely that any of our patients received any effective triple antimycobacterial therapy empirically without having a definite NTM infection, and single macrolide therapies are not effective in NTM infections.

Conclusion

Clinical characteristics of NTM pulmonary infections after HSCT are nonspecific and require a high index of suspicion to make the diagnosis of definite NTM pulmonary infections. Overall NTM infections due to SGM in post-HSCT patients have a favorable prognosis. Prospective multicenter studies are required to guide further management of different NTM infections after HSCT.

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Authors contribution

(1) The conception and design of the study: AS
(2) Acquisition of data: Dr Salam, SG
(3) Analysis and interpretation of data, drafting the article or revising it critically for important intellectual content: ZE, AS
(4) Final approval of the version to be submitted: AS, ZE

Conflicts of interest

The authors declare no conflicts of interest.

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Original article

Hematological ratios and cytokine profiles in heterozygous beta-thalassemia

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ABSTRACT

Introduction: β-Thalassemia is defined by a reduced or complete absence of β-globin chain synthesis in hemoglobin, leading to hemolytic anemia. Heterozygous β-thalassemia, also known as β-thalassemia trait (hBTh), the mildest form of this anemia, typically does not cause symptoms in carriers. However, it may lead to changes in the immune system, including an increase in total leukocyte, neutrophil, and lymphocyte counts.
Objective: This study aimed to evaluate various immune and inflammation markers, including neutrophil/lymphocyte, derived neutrophil/lymphocyte, lymphocyte/monocyte, platelet/lymphocyte, neutrophil/platelet ratios, systemic immune-inflammation index, systemic inflammation response index, neutrophil/natural killer cell ratio (NNKR), and inflammatory cytokines in β-thalassemia trait carriers.
Method: A retrospective observational study was conducted, including 50 β-thalassemia trait individuals and 100 healthy controls.

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Results: Leukocyte, neutrophil and reticulocyte counts, and interleukin 6 levels were higher in carriers compared to controls. Notably, the β-thalassemia trait group had increased neutrophil/platelet, neutrophil/lymphocyte and derived neutrophil/lymphocyte ratios, and the systemic immune-inflammation and systemic inflammation response indexes were higher compared to the controls.

Conclusions: β-thalassemia trait shows a more pronounced inflammatory profile as indicated by hematological ratios. These ratios, therefore are potentially cost-effective and easily applicable markers for monitoring patients with the β-thalassemia trait.

Introduction

β-thalassemia is an inherited disease caused by a mutation in the gene responsible for forming the β chain of hemoglobin. The underlying pathophysiological mechanism of β-thalassemia is an imbalance in the production of α-globin and β-globin chains leading to a relative excess of α-globin. Consequently, the surplus α-globin chains produce insoluble aggregates that tend to precipitate within the developing erythroid cells. These aggregates stimulate apoptosis in the erythroid precursors, resulting in ineffective erythropoiesis and hemolytic anemia [1–3]. The clinical manifestations of β-thalassemia vary widely and are related to the difference in the combination of β alleles (a deficient [β^+] or absent [β^0] β-globin subunit in the hemoglobin molecule) [4].

Quantitative and functional immunological abnormalities that alter various components of the immune response are well documented in β-thalassemia major (homozygous β-thalassemia), characterized as transfusion-dependent thalassemia. Moreover, changes in cytokine profiles of innate immunity and an increase in total leukocytes and neutrophil count, which contribute to increased susceptibility to infections in these patients, have been extensively documented [2–7]. Individuals with homozygous β-thalassemia who present milder anemia, typically not necessitating regular transfusions, and those with varying degrees of anemia requiring intermittent transfusions, are classified as β-thalassemia intermedia. These patients exhibit a spectrum of clinical manifestations ranging from severe symptoms necessitating treatment, like in thalassemia major, to mild or asymptomatic conditions, resembling thalassemia minor, according to the presence or absence of modifying genes [4].

Individuals who have inherited a single β-thalassemia allele, whether β° or β^+, are considered to have the heterozygous thalassemia, also known as β-thalassemia trait (hBTh), and are non-transfusion-dependent. They typically exhibit low hemolysis ratios and frequently are clinically asymptomatic [3,7]. However, some hBTh individuals may display mild anemia, characterized by hypochromic and microcytic red blood cells, elevated levels of hemoglobin (Hb) A_2, and variable increases in Hb F. In some cases, hBTh may manifest a variety of symptoms including headache, lethargy, fatigue, dizziness, and exercise intolerance, despite having hemoglobin levels within the normal range [4,8].

Despite a relatively benign presentation or even an absence of symptoms, the hBTh profile regarding inflammatory biomarkers is not well defined. Therefore, it is crucial to analyze inflammatory biomarkers, such as hematological ratios obtained from blood counts [9–12].

Given the potential contribution of these indices to a more nuanced understanding of hBTh, ultimately leading to more effective patient care, this study aimed to analyze the hematological ratios obtained from blood counts of individuals with hBTh compared to a healthy control group.

Material and methods

This retrospective observational study was approved by the Research Ethics Committees of the School of Pharmaceutical Sciences of the University of São Paulo (protocol no. 69/2012), the Federal University of São Paulo (protocol no. 69574), and Irmandade Santa Casa de Misericórdia de São Paulo Hospital (protocol no. 230.882).

Fifty patients diagnosed with heterozygous β-thalassemia (hBTh) aged from 19–84 years of both sexes were recruited from various sources, including the Hemoglobinopathies Laboratory of the Clinical Pathology Division of Clinics Hospital of State University of Campinas, the Anemia Ambulatory of the Federal University of São Paulo (UNIFESP), the Irmandade Santa Casa de Misericórdia de São Paulo Hospital, and the Hematological Ambulatory of the Faculty of Medicine and Health Sciences at the Pontifical Catholic University of São Paulo. The initial diagnosis of thalassemia was made using a complete blood count and hemoglobin electrophoresis and later confirmed by mutation type evaluations, as previously described.[13] Individuals with β-thalassemia intermedia were excluded from this study, with only those presenting β-thalassemia minor being included. The Control Group comprised 100 healthy individuals aged from 20–82 years from the Faculty of Pharmaceutical Sciences at the State University of São Paulo, and UNIFESP, and volunteers from the city of São Paulo recruited through convenience sampling. Individuals with chronic alcoholism, active infections, pregnancy, chronic diseases, or immunosuppressive medication use, or those who had donated blood in the six months prior to the study, were excluded. Control Group individuals who consumed multivitamins, folic acid, vitamin B12, or iron were also excluded.

The data set included blood count (including natural killer [NK] cells and reticulocytes), high-sensitive C-reactive protein (CRP), interleukin (IL)-6 and IL-10, lactate dehydrogenase (LDH) activity, body mass index (BMI), age, sex, smoking, and folic acid use as previously described.[13] Briefly, venous blood samples were obtained from each participant after an overnight fast (8–10 h). Complete blood and reticulocyte counts were determined in ethylenediaminetetraacetic acid (EDTA) whole-blood samples using a Pentra 120 Hematology Analyzer (Horiba). High-sensitivity CRP was determined by an immunoturbidimetric assay using the Roche-CRPL kit on the Cobas 8000 analyzer (Roche Diagnostics). LDH activity was determined by an enzymatic assay using the Vitros 250 analyzer (Ortho Clinical Diagnostics). IL-6 and IL-10 were determined by a multiplex immunoassay, the high-sensitivity panel T Cell Magnetic Bead Milliplex Map (EMD Millipore Corporation) on the Bio-PLex 200 analyzer (Bio-Rad Laboratories, Inc.), following the manufacturers' protocols. The following hematological ratios were calculated from blood count data: neutrophil/lymphocyte (NLR), derived neutrophil/lymphocyte (d-NLR), lymphocyte/monocyte (LMR), platelet/lymphocyte (PLR) and neutrophil/platelet (NPR) ratios, and SII (multiplication of platelets by total neutrophils, divided by total lymphocytes), SIRI (multiplication of neutrophils by monocytes/lymphocytes) indexes, and the NNKR.

Analyses were conducted using the Statistical Package for the Social Sciences version 22.0 and GraphPad Prism software. Variables are expressed as medians and interquartile ranges, and the Mann-Whitney test was employed to compare the groups. A significance level of 5% (p-value <0.05) was used.

Results

The general data for the groups analyzed are presented in Table 1. The BMIs of hBTh patients were similar to those of the Control Group, as well as the percentages of smokers and females, and the age.

The hBTh Group exhibited higher leukocyte, neutrophil, and reticulocyte counts compared to the Control Group. Regarding the interleukins evaluated, IL-6 showed higher levels in the hBTh Group than in the Control Group. The LDH activity was comparable between the hBTh patients. The detailed results of these analyses are presented in Table 2.

Furthermore, data distribution of each hematological ratio in hBTh patients and controls are shown in Figure 1.

Discussion

To our knowledge, this is the first study to compare hematological ratios in a group of apparently healthy subjects with carriers of usually asymptomatic disorders of hematopoiesis (e.g., hBTh). Despite sometimes being considered healthy, this study demonstrates that hBTh subjects have a greater inflammatory profile compared to the Control Group.

Hematologic ratios such as NLR, D-NLR, LMR, PLR, and SII are inflammatory markers previously described as diagnostic aids for other diseases.[14–18] Recent data suggest that NLR is associated with various inflammatory conditions, including diabetes mellitus, irritable bowel disease, and thyroiditis, both with subtle and overt inflammation.[18–21] Similarly, PLR is associated with inflammatory conditions such as liver fibrosis and cancer.[22,23] SII, an inflammatory marker, has been used as a prognostic indicator in the follow-up of sepsis and cancer patients.[24,25] Furthermore, SII has been studied as an aid in the diagnosis and prognosis of other diseases, including COVID-19.[18,26–28] Most of these hematologic ratios, including SIRI, were associated with length of hospital stay and independent predictors of in-hospital mortality of patients undergoing on-pump cardiac surgery.[29]

Regarding NLR, D-NLR, and SII, there were notable differences between the hBTh and Control Group. In individuals with hBTh, there was a significant increase in the total leukocyte and neutrophil counts compared to the control subjects. Conversely, no statistically significant differences were observed in the total number of lymphocytes and platelets between the groups. Therefore, the elevated neutrophil count observed can be attributed to an inflammatory process with a subsequent increase in NLR, D-NLR, and SII ratios. These ratios may serve as potential inflammatory markers for individuals with hBTh, especially when considering neutrophil values alone.

The NLR, D-NLR, and SIRI ratios showed significant differences on comparing the Control Group and patients with hBTh, as this condition results in a chronic inflammatory

Table 1 – General data of the study participants.

Variable	Control ($n = 100$)	hBTh ($n = 50$)	p-value
Age (y)	45.5 (32.2–58.0)	51.0 (37.0–59.7)	0.228
BMI (kg/m^2)	25.5 (23.5–28.4)	25.5 (24.0 –29.4)	0.440
Female	67 (67.0)	35 (70.0)	0.710[a]
Smoker	14 (14.0)	5 (10.0)	0.487[a]
Folic acid supplementation	0	13 (26.0)	<0.001[b]
Anemia	0	38 (76.0)	<0.001[b]

hBTh, heterozygous β-thalassemia; BMI, body mass index.

[a] Pearson's Chi-square.
[b] Likelihood ratio.
Continuous variables are presented as medians with interquartile ranges. Categorical variables are expressed as the number of subjects and corresponding percentage (in parentheses). The data were subjected to the Mann-Whitney test for comparison.

Table 2 – Hematological ratios and other laboratory data of the study participants.

Variable	Control (n = 100)	hBTh (n = 50)	p-value
NLR	1.38 (1.19–1.83)	1.70 (1.33–2.18)	0.010
d-NLR	1.04 (0.92–1.33)	1.33 (1.07–1.56)	0.001
LMR	4.27 (3.00–7.82)	4.14 (2.80–7.04)	0.614
PLR	0.09 (0.07–0.12)	0.09 (0.08–0.10)	0.616
NPR	16.22 (12.74–19.42)	19.87 (14.72–25.18)	0.001
SII	318 (242–395)	361 (288–459)	0.038
SIRI	691 (420–1189)	928 (507–1618)	0.056
NNKR	7.24 (4.89–8.94)	7.62 (4.30–9.01)	0.909
Leukocytes (/mm^3)	6800 (5625–7600)	7200 (6250–8825)	0.011
Neutrophils (/mm^3)	3456 (2866–4091)	3977 (3334–5303)	<0.001
Lymphocytes (/mm^3)	2408 (1863–2921)	2448 (2086–2811)	0.652
Monocytes (/mm^3)	489 (358–718)	545 (379–797)	0.304
Eosinophils (/mm^3)	130 (70–216)	99 (69–166)	0.307
Basophils (/mm^3)	0 (0–54)	0 (0–0)	0.044
Platelets (x 10^3/mm^3)	220 (187–246)	211 (179–278)	0.674
Hemoglobin (g/dL)	14.0 (13.3–15.0)	11.6 (10.6–12.4)	<0.001
Reticulocytes (%)	0.85 (0.70–1.17)	1.10 (0.85–1.75)	0.001
LDH (U/L)	426 (391–497)	428 (382–492)	0.851
CRP (mg/dL)	0.18 (0.07–0.40)	0.24 (0.10–0.45)	0.323
IL-6 (pg/mL)	0.94 (0.53–1.38)	1.19 (0.84–1.76)	0.016
IL-10 (pg/mL)	2.41 (1.07–4.46)	2.67 (1.04–4.58)	0.778

hBTh, heterozygous β-thalassemia; NLR, neutrophil/lymphocyte ratio; D-NLR, derived neutrophil/lymphocyte ratio; LMR, lymphocyte/monocyte ratio; PLR, platelet/lymphocyte ratio; NPR, neutrophil/platelet ratio; SII, systemic immuno-inflammation index; SIRI, systemic inflammation response index; NNKR, neutrophil/natural killer cells ratio; LDH, lactate dehydrogenase; CRP, C-reactive protein; IL, interleukin.
Variables are presented as median and interquartile range, and the groups were subjected to the Mann-Whitney test for comparison.

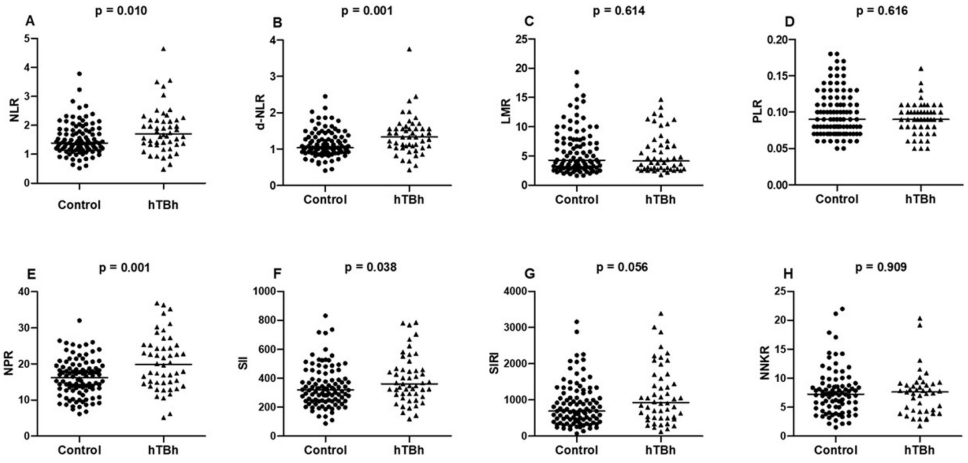

Figure 1 – (A) neutrophil/lymphocyte ratio (NLR); (B) derived neutrophil/lymphocyte ratio (d-NLR); (C) lymphocyte/monocyte ratio (LMR); (D) platelet/lymphocyte ratio (PLR); (E) neutrophil/platelet ratio (NPR); and (F) systemic immuno-inflammation index (SII), (G) systematic inflammatory response index (SIRI); (H) neutrophil/natural killer cell ratio (NNKR) in heterozygous β-thalassemia (hBTh) subjects and the Control Group. The groups were subjected to the Mann-Whitney test for comparisons.

process and a mild form of anemia.[7,9] The NPR in the hBTh group was higher than in the Control Group, confirming an inflammatory state. This observation was made because the neutrophil count was increased in the hBTh Group, while the platelet count did not show any significant variation between the groups. Thus, the NPR ratio can be used as an inflammatory marker for hBTh conditions with the NPR being more indicative than its isolated parameters. In contrast, the LMR did not demonstrate any significant difference between the groups, suggesting that it may not be a reliable biomarker for monitoring inflammatory processes.

Analyses of the reticulocyte counts showed significant differences between both groups, with the findings of this study confirming that the disease involves clear hemolysis mechanisms. In hBTh, the hemolysis is less pronounced, as many patients do not present anemia, indicating hemolysis with

erythropoietic compensation. One study demonstrated that the moderate reticulocytosis resulting from a mild erythrocyte response is typically sufficient to compensate for the hemolysis observed in this condition.[30] These considerations support the view that in hBTh, the hemolysis mechanism is less severe and the erythropoietic response is more effective.

These findings are corroborated by the LDH activity. Under hemolytic conditions, evidence has shown that the quantity of this enzyme increases in the plasma.[31] The LDH activity in both the Control and hBTh Groups was found to be comparable, a result that is consistent with the literature since hBTh is characterized by mild hemolysis.[8]

Other inflammatory markers, such as IL-6 and IL-10 and C-reactive protein, were also analyzed. The hBTh Group exhibited elevated IL-6 activity compared to the Control Group. This phenomenon can be explained by the action of macrophages that phagocytize defective red blood cells, resulting in their production of IL-6.[32] Therefore, this result suggests that IL-6 plays a relevant role in the inflammatory response observed in this disease.

Conversely, IL-10, an anti-inflammatory cytokine that limits the immune response to pathogens,[33] and C-reactive protein, a non-specific marker of systemic inflammatory processes,[34] showed no significant differences between the groups analyzed. Therefore, C-reactive protein lacks prognostic value for hBTh and cannot be used as an inflammatory marker in these conditions.

It is known that in the most severe forms of β-thalassemia (major and intermedia), excess alpha chains aggregate, forming inclusions that damage cell and organelle membranes. These aggregates also induce reactive oxygen species formation, further damaging membrane proteins and lipids. Hemichromes, one of the most toxic products of unpaired α chains, binds to membranes and promotes band 3 clustering, a key membrane constituent, leading to cellular apoptosis.[4] In patients with hBTh, hemolysis, though less intense, is still present as indicated by increased reticulocytes. This suggests that these patients undergo a similar inflammatory process to those with more severe forms of the disease, but to a lesser extent. The milder inflammatory response is likely associated to iron overload, ineffective erythropoiesis, and oxidative stress, collectively contributing to a pro-inflammatory state.

The major limitation of this study is the relatively small sample size. In cases of hBTh, most individuals are asymptomatic and do not seek medical attention. Another important limitation is the lack of clinical follow-up for hBTh individuals. However, we demonstrated that hematological ratios in this form of hemolytic anemia are more informative markers of inflammation than their isolated parameters and are more useful than traditional markers. These ratios could contribute to the management of hBTh, being easily applicable, widely available, and cost-effective.

Conclusion

In hBTh, hematological ratios such as NLR, D-NLR and NPR and SII index demonstrated higher values than those in the Control Group, indicating a more inflammatory profile. These ratios (NLR, D-NLR, NPR, and SII) showed significant potential when applied to the hBTh Group, exhibiting a good capacity to serve as inflammatory markers when compared to isolated parameters from hemogram. Furthermore, these hematological ratios may prove valuable in managing and understanding hBTh manifestations, offering a convenient, accessible, and cost-effective alternative, since this information is calculated from blood count data, without additional analysis costs.

Further studies are required to substantiate this hypothesis, enabling the routine application of hematological indices to support medical decision-making regarding the optimal treatment strategy for individuals with hBTh, considering their chronic inflammatory state. Moreover, hematological ratios could serve as an additional tool for the preliminary assessment of individuals with suspected hBTh, a condition to be confirmed later.

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Author Contribution

ACMC, LEO, and ALR contributed to the study design, data organization, analysis, and manuscript drafting. JAMC performed data analysis and critically revised the manuscript. MRL and GWG were responsible for sample collection, laboratory assessments, data analysis, and manuscript writing. MSF, MNNS, VLNBD, and RDC facilitated patient inclusion, collected clinical data, and reviewed the manuscript. EMGS contributed to the study design, data analysis, statistical evaluation, manuscript writing, and expert review. CP participated in study design, sample and data collection, laboratory determinations, data analysis, manuscript writing, and final review.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Long-term follow-up results of ruxolitinib as salvage therapy for chronic graft-versus-host disease

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ABSTRACT

Introduction: Chronic graft-versus-host disease poses a significant challenge after allogeneic hematopoietic stem cell transplantation with initial treatment often relying on high-dose steroids. However, managing steroid-refractory disease remains daunting. Recent insights into the mechanisms have unveiled new treatment targets, with ruxolitinib, a selective JAK1/2 inhibitor, emerging as a promising and safe therapy for chronic graft-versus-host disease patients.

Methods: This retrospective study describes the long-term outcomes of 23 chronic graft-versus-host disease patients treated with ruxolitinib.

Results: Most patients presented with severe chronic graft-versus-host disease (15/23; 65.2%). The overall response rate was 78.3% (18/23) after a median treatment duration of four weeks, with 55.6% (10/18) achieving complete response. At follow-up, 13 of the 18 responders (72.2%) sustained complete remission. Patients had a median of two previous lines of therapy, with a median follow-up of 14 months (range: 2–46 months) after starting ruxolitinib. Of the patients who were responsive to ruxolitinib, median follow-up extended to 26.5 months. Notably, for the patients who were responsive to ruxolitinib, the 1-year, 2-year, and 3-year overall survival was 83.3% (95% CI: 64.2%-102%), 56.1% (95% CI: 30.1%-80.9%), and 33.3% (95% CI: 9.2%-57.4%), respectively. Malignancy relapse occurred in 17.4% (4/23) of patients, with 34.7% (8/23) experiencing cytopenias, albeit mostly mild. Reactivation rates for cytomegalovirus were nil.

Conclusion: The long-term follow-up in this study supports ruxolitinib as an effective salvage therapy for chronic graft-versus-host disease with a 78.3% overall response rate and 55.6% complete remission rate. However, large prospective studies are warranted to validate these findings

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a pivotal treatment for patients afflicted with hematological malignancies and non-malignant diseases.[1] The success of allo-HSCT hinges on two primary factors: the management of transplant-related complications and disease relapse.[2] Notably, chronic graft-versus-host disease (cGvHD) stands out as a significant contributor to procedural morbidity and relapse-free mortality, arising in 35–70% of allo-HSCT recipients.[1,2] cGvHD is a multisystem clinical syndrome caused by donor-mediated immune reactions in HSCT recipients[3] with corticosteroids being the mainstay treatment. However, approximately half of cGvHD patients exhibit resistance to corticosteroid therapy, and more than half require second-line treatment within two years.[4] For cGvHD, second-line therapies include calcineurin inhibitors, extracorporeal photopheresis, ibrutinib, Janus kinases (JAK) inhibitors, mycophenolate mofetil, rituximab, mammalian target of rapamycin inhibitors, pentostatin, proteasome inhibitors, and tyrosine kinase inhibitors.[5,6]

Among the array of treatments or interventions available, a consensus has yet to be reached regarding the optimal salvage therapy for steroid-refractory (SR)-cGvHD. For years, the intricate pathophysiology of cGvHD has posed a formidable challenge in its management.[7] However, advancements in understanding the underlying pathways have paved the way for novel treatment modalities targeting these mechanisms.[7,8] Among these, interventions aiming at kinase activity have emerged as promising strategies, showing encouraging outcomes in both preclinical models and clinical trials.[9]

JAK1 and 2 (JAK1/2) have garnered significant attention in GvHD research due to their pivotal roles in cytokine production and activation of inflammatory cells.[10] Ruxolitinib, a selective oral inhibitor targeting JAK1/2-signal transducer and activator of transcription (STAT) signaling, holds promise in mitigating these pathways.[11] JAKs facilitate signaling from various cytokine receptor family members and play a critical role in the inflammatory cascade, leading to tissue damage and fibrosis in cGvHD.[10] By targeting JAK1/2 signaling, inhibitors like ruxolitinib may impede multiple facets of T-cell activation, including donor T-cell expansion, cytokine production, and B-cell differentiation while promoting regulatory T-cell (Treg) function.[9,12] This multifaceted inhibition could potentially alleviate disease severity by suppressing proinflammatory cytokines.[9]

Moreover, unlike conventional immunosuppressive agents that primarily affect T-cell function, ruxolitinib has been shown to disrupt dendritic cell differentiation, maturation, and cytokine production, potentially enhancing its efficacy against GvHD.[12,13] Building on this foundation, Zeiser et al.[14] documented successful ruxolitinib therapy for human GvHD in 2015. A retrospective review of ruxolitinib use in Chinese patients with GvHD revealed an overall response rate (ORR) of 82.1% for cGvHD.[15] Another study assessing the long-term outcomes of ruxolitinib treatment in 35 patients with SR-cGvHD documented an ORR of 89%, with 26% achieving a complete response (CR).[16]

Recently, Zeiser et al.[17] presented findings from a prospective study that compared ruxolitinib with the current optimal treatment, yielding a noteworthy best ORR of 76%. This study holds significance as it provides a prospective evaluation of the efficacy of ruxolitinib. Following this trial, in September 2021, the Food and Drug Administration (FDA) approved ruxolitinib to treat patients aged 12 years and above with cGvHD who have experienced treatment failure with one or two lines of systemic therapy.[6]

Retrospective studies assessing the effectiveness of ruxolitinib in SR-cGvHD often need more median follow-up durations, hampering accurate assessments of response duration and long-term outcomes.[17–22] Hence, investigations with extended follow-up periods are crucial for comprehensive understanding. This paper presents the long-term outcomes of ruxolitinib treatment in 23 patients with cGvHD.

Materials and methods

In this retrospective analysis conducted at a single center, 23 recipients of allo-HSCT with cGvHD who underwent salvage therapy with ruxolitinib between December 2018 and December 2022 were examined. The initial ruxolitinib dosage (5 or 10 mg twice daily) was determined based on individual hematological parameters. Basic transplant-related information was gathered and is summarized in Table 1. Additionally, the time intervals from transplantation to the onset of cGvHD and from cGvHD onset to the initiation of ruxolitinib treatment were recorded.

Prior to commencing ruxolitinib therapy, the affected organ sites were stratified and cGvHD was graded as per the National Institutes of Health (NIH) 2015 criteria.[23] Response assessment adhered to NIH criteria, delineating responses as CR, partial response (PR), or lack of response (unchanged, mixed response, or progression). CR signified the complete resolution of all disease manifestations across all involved organs or sites, whereas PR indicated improvement in at least one organ or site without progression. Lack of response encompassed disease progression in any organ, site, or outcomes not meeting CR or PR criteria. The ORR is the proportion of patients achieving CR and PR. Overall survival (OS) was determined as the time elapsed from the initiation of ruxolitinib treatment to the last follow-up or death. This study diligently documented prevalent adverse events linked with ruxolitinib, including cytopenias and infections, and categorized toxicities based on the grading of the National Cancer Institute Common Terminology Criteria for Adverse Events.

Approval for the study was granted by the Erciyes University Faculty of Medicine Ethics Committee (Date: 26–04–2023, Decision No: 2023/311). All procedures adhered to ethical guidelines and the principles outlined in the Helsinki Declaration.

Patient characteristics are summarized using descriptive statistics. OS was determined using the Kaplan-Meier method. Descriptive analyses are presented as numbers (n), percentages (%), and 95% confidence intervals (95% CIs).

Table 1 – Patient characteristics at the start of ruxolitinib therapy.

Variable	Result
Patients – n	23
Age, years - median (range)	46 (30–67)
Gender (male/female) - n (%)	10 (43.5)/13 (56.5)
Diagnosis - n (%)	
Acute myelogenous leukemia	13 (56.5)
Acute lymphoblastic leukemia	6 (26)
Myelodysplastic syndrome	1 (4.3)
Lymphoma	3 (14)
Conditioning regimen - n (%)	
Myeloablative	17 (73.9)
Reduced intensity or nonmyeloablative	6 (26.1)
Donor - n (%)	
Matched related donor	20 (87)
Unrelated donor	1 (4.3)
Haploidentical donor	2 (8.7)
CMV serostatus	
R-/D-	5 (21.7)
R-/D+	3 (13)
R+/D-	4 (17.4)
R+/D+	11 (47.8)
GvHD prophylaxis - n (%)	
Cyclosporine + Mtx	20 (87)
Cyclosporine + Mtx + ATG	1 (4.3)
PT-Cy + Cyclosporine + MMF	2 (8.7)
cGvHD severity	
Moderate	8 (34.8)
Severe	15 (65.2)
Organ involvement of cGvHD - median (range)	1 (1–3)
Previous therapies before ruxolitinib - median (range)	2 (2–5)
Time from cGvHD to start of ruxolitinib treatment (days) - median (range)	40 (60–180)
Duration of ruxolitinib treatment (months) - median (range)	14 (2–46)
Follow-up after ruxolitinib treatment initiation (months) - median (range)	14 (2–46)
Time to response (weeks) - median (range)	4 (1–21)

cGvHD, chronic graft-versus-host disease; HLA, human leukocyte antigen; PT-Cy, post-transplant endoxan; MTX, methotrexate; MMF, mycophenolate mofetil; ATG, anti-thymocyte globulin; R+, recipient CMV positive; R-, recipient CMV negative; D-, donor CMV negative; D+, donor CMV positive.

Results

The cohort of this study consisted of 23 patients who underwent salvage therapy with ruxolitinib; their characteristics are outlined in Table 1. The median age was 46 years (range: 30–67 years), with a male-to-female ratio of 10/13 (43.5/56.5%). The most prevalent diagnoses were acute myeloid leukemia (AML) in 13 patients (56.5%) and acute lymphoblastic leukemia (ALL) in six patients (26%). Graft sources included human leukocyte antigen (HLA)-matched related donors in 20 patients (87%), HLA-matched unrelated donors in one patient (4.3%), and HLA haploidentical donors in two patients (8.7%). The majority of patients underwent myeloablative conditioning regimens (73.9%).

Regarding cGvHD severity, eight patients (34.8%) had moderate cGvHD, while 15 patients (65.2%) had severe cGvHD. The affected organs included the liver in 52.2% (12/23) of patients, lung in 8.7% (2/23), oral mucosa in 30.4% (7/23), gastrointestinal system in 13% (3/23), and skin in 43.5% (10/23). The median number of prior therapy lines was two (range: 2–5), with ruxolitinib administered as the third line in ten patients, fourth line in seven patients, fifth line in three patients, and sixth line in three patients.

The median duration from the onset of cGvHD to the commencement of ruxolitinib therapy was 60 days (range: 40–180 days), with a median response time of four weeks (range: 1–21 weeks) after initiation of ruxolitinib. Of the 23 patients, 18 exhibited a response to ruxolitinib, resulting in an ORR of 78.3%. Of these responders, the majority (55.6%) achieved CR at a median of four weeks into treatment. Eight patients (45.4%) achieved PR, with three of them switching to CR during follow-up, culminating in a total of 13 patients (72.2%) reaching CR during ruxolitinib therapy. Of the patients who were responsive to ruxolitinib, prednisone was successfully tapered to physiologic doses in three patients (16.8%) and discontinued in 15 patients (83.2%) at a median of 51 days (range: 10–90 days) after ruxolitinib initiation.

Five patients (21.7%) exhibited no response to ruxolitinib, as outlined in Table 2. All five patients presented with severe cGvHD; one had pulmonary involvement, resulting in significant sequelae and pleuroparenchymal fibroelastosis, three had mouth involvement and all five patients manifested sclerotic changes with skin involvement. Of the patients who were not responsive to ruxolitinib, two patients experienced relapse and subsequent mortality, one within the second month of ruxolitinib treatment and the other within the third month of ruxolitinib treatment.

During follow-up, 15 patients (85.2%) remained alive, while eight patients (34.8%) died. The causes of death included coronavirus disease-2019 (COVID-19) in three patients, refractory cGvHD in one patient, and relapse in four patients.

The median follow-up duration after initiation of ruxolitinib was 14 months (range: 2–46 months) for all 23 patients and extended to 26 months (range: 2–46 months) for the 18 patients who were responsive to ruxolitinib. In the entire cohort of 23 patients, the OS was 73.9% (95% CI: 54.5–93.3%) at 1 year, 43.4% (95% CI: 21.6–65.4%) at 2 years, and 26.1% (95% CI: 6.6–45.5%) at 3 years (Figure 1). For the 18 patients who were responsive to ruxolitinib, the OS was 83.3% (95% CI: 64.2–102.4%) at one year, 56.1% (95% CI: 30.1–80.9%) at two years, and 33.3% (95% CI: 9.2–57.4%) at three years (Figure 2).

The median treatment duration spanned 14 months (range: 2–46 months) for all 23 patients and 20 months (range: 2–46 months) for the 18 patients who were responsive to ruxolitinib. After the follow-up period, of the patients who were responsive to ruxolitinib, nine (50%) relied solely on ruxolitinib as an immunosuppressive agent and maintained either PR (n = 2) or CR (n = 7), while three patients (16.7%) supplemented ruxolitinib with additional immunosuppressants (2 in CR and 1 in PR). Six patients (33.3%) discontinued ruxolitinib upon achieving sustained response, with a median treatment duration of 25.5 months (range: 17–36 months). Of these, four attained CR, and two met the criteria for PR. In cases of PR, residual cGvHD involvement was considered, and

Table 2 – Details of patients.

#	Age	Gender	cGvHD onset[a]	Donor	Global cGvHD score	Involved sites	Prior therapies[b]	Day for RUX[c]	Response to RUX[d]	Duration m[e]	Response to RUX[f]	Status/IS	Follow-up, m	Stopping RUX[g]
1	61	female	9	MRD	mild	liver	Steroids, CSP	45	PR	15	PR	Death from covid-19/ RUX, CSP	15	–
2	38	female	5	MRD	mild	liver	Steroids, CSP, MSC, ibrutinib	180	CR	17	CR	Alive/-	31	+
3	45	female	4	MUD	severe	liver	Steroids, CSP	45	PR	29	CR	Death from covid19/ RUX, CSP	29	–
4	46	female	4	MRD	severe	skin, mouth	Steroids, CSP	120	PR	27	CR	Alive/-	36	+
5	46	male	4	MRD	severe	liver	Steroids, CSP	45	CR	36	CR	Alive/-	41	+
6	67	female	14	MRD	severe	mouth	Steroids, CSP	90	PR	24	PR	Alive/-	31	+
7	30	female	4	HID	mild	skin, gut	Steroids, CSP, MMF, MSC, ECP	60	PR	32	CR	Alive/RUX	38	–
8	41	male	11	MRD	severe	lung	Steroids, CSP, MMF	90	PR	24	PR	Alive/-	37	+
9	57	male	7	MRD	mild	liver	Steroids, CSP, MMF, ECP	45	CR	33	CR	Alive/-	39	+
10	57	male	9	MRD	severe	liver	Steroids, CSP, MMF	120	PR	46	PR	Alive/RUX	46	–
11	51	female	7	MRD	severe	Lung skin, mouth	Steroids, CSP, MMF	90	Lack of response lung: unchanged others: PR	14	lung: Lack of response others: PR	Death from refractory cGvHD/RUX, Steroid, CSP, MMF	14	–
12	55	female	4	MRD	severe	skin	Steroids, CSP	40	Lack of response	3	Lack of response	Death from relapse/Rux-olitinib, CSP	3	–
13	37	female	10	MRD	severe	liver	Steroids, CSP, MMF, MSC, ECP	120	CR	23	CR	Death from relapse/RUX	23	–
14	32	male	9	MRD	severe	Skin mouth	Steroids, CSP, imatinib, rituximab, ECP	150	Lack of response	2	Lack of response	Death from relapse/ RUX, ECP	2	–
15	59	female	20	MRD	severe	skin	Steroids, CSP	110	Lack of response	5	Lack of response	Death from covid-19/ RUX, CSP, ECP	5	–
16	54	male	5	MRD	mild	Liver mouth	Steroids, CSP, MMF	60	CR	2	CR	Death from relapse/RUX	2	–
17	32	male	3	MRD	severe	Liver skin	Steroids, CSP, İmatinib	90	CR	12	CR	Alive/RUX	12	–
18	43	male	4	MRD	mild	Liver skin	Steroids, CSP	60	CR	13	CR	Alive/RUX	13	–
19	33	female	4	MRD	mild	skin	Steroids, CSP, MMF	60	PR	6	PR	Alive/RUX, MMF	6	–
20	48	female	4	MRD	severe	gut	Steroids, CSP, MSC, ECP	50	CR	12	CR	Alive/RUX	12	–
21	63	female	11	MRD	mild	Liver mouth	Steroids CSP, ECP	45	CR	10	CR	Alive/RUX	10	–
22	38	male	4	MRD	severe	Skin mouth	Steroids, CSP	60	Lack of response	12	Lack of response	Alive/RUX, ECP, MMF	12	–
23	56	male	3	HID	severe	Liver gut	Steroids, MMF	40	CR	14	CR	Alive/RUX	14	–

MUD, matched-unrelated donor; MRD, matched-related donor; HID, haploidentical donor; RUX, Ruxolitinib; PR, partial response; CR, complete response; cGvHD, chronic graft-versus-host disease.

[a] posttransplant month, the onset of GvHD attack in which ruxolitinib treatment was started.
[b] Therapies administered in the treatment of cGvHD before RUX.
[c] day from onset of cGvHD to initiation of ruxolitinib treatment.
[d] response after a median 4 weeks of ruxolitinib treatment; m:month.
[e] Total duration of ruxolitinib administration.
[f] response to ruxolitinib treatment at the last follow-up.
[g] Whether or not RUX was discontinued after RUX treatment MMF, mycophenolate mofetil; CSP, cyclosporine A; MSC, mesenchymal stem cells; ECP, extracorporeal photopheresis; IS, Immunosuppressants administered for cGvHD treatment at the last follow-up.

Patient 1 diagnosed with mild liver cGvHD achieved a PR after a median of four weeks of ruxolitinib treatment. The patient passed away from COVID-19 while the PR continued after 15 months of treatment. Follow-up period was 15 months.

Patient 2 diagnosed with mild liver cGvHD achieved a CR after a median of four weeks of treatment. The CR persisted for 17 months of treatment and ruxolitinib was discontinued. No recurrence of cGvHD was observed during 14 months of drug-free follow-up. Follow-up period was 31 months.

Patient 3 diagnosed with severe liver cGvHD achieved a PR after a median of four weeks of treatment. The response converted to a CR by the 6th month of treatment. The patient passed away from COVID-19 while the CR continued after 29 weeks of treatment. Follow-up period was 29 months

Patient 4 diagnosed with severe skin and mouth cGvHD achieved a PR after a median of four weeks of treatment. The response converted to a CR within the first year of treatment. CR was maintained after 27 months of treatment, and ruxolitinib was discontinued. No cGvHD recurrence was observed during 9 months of drug-free follow-up. Follow-up period was 36 months

Patient 5 diagnosed with severe liver cGvHD achieved a CR after a median of four weeks of treatment. The CR was sustained after 36 months of treatment, and ruxolitinib was discontinued. No cGvHD recurrence was observed during five months of drug-free follow-up. Follow-up period was 41 months.

Patient 6 diagnosed with severe mouth cGvHD achieved a PR after four weeks of treatment. The PR was maintained after 24 months of treatment, and ruxolitinib was discontinued. No cGvHD recurrence was observed during seven months of drug-free follow-up. Follow-up period was 31 months.

Patient 7 diagnosed with mild skin and gut cGvHD achieved a PR after a median of four weeks of treatment. The response converted to a CR within the first year of treatment and maintained after 32 months of treatment, even after ruxolitinib was discontinued. No cGvHD recurrence was observed during six months of drug-free follow-up. Follow-up period was 38 months.

Patient 8 diagnosed with severe lung cGvHD achieved a PR after a median of four weeks of treatment. The PR continued after 24 months of treatment, and ruxolitinib was discontinued. No cGvHD recurrence was observed during 13 months of drug-free follow-up. Follow-up period was 37 months.

Patient 9 diagnosed with mild liver cGvHD achieved a CR after a median of four weeks of treatment. The CR continued after 33 months of treatment, and ruxolitinib was discontinued. No cGvHD recurrence was observed during six months of drug-free follow-up. Follow-up period was 39 months.

Patient 10 diagnosed with severe liver cGvHD achieved a PR after a median of four weeks of treatment. The PR continued after 46 months of treatment, and the patient remains on medication. Follow-up period was 46 months.

Patient 11 diagnosed with severe lung, skin, and mouth cGvHD showed no response after a median of four weeks of treatment. The lack of response persisted after 14 months (lung: unchanged, skin: PR, and mouth: PR) and the patient eventually passed away due to cGvHD. Follow-up period was 14 months.

Patient 12 diagnosed with severe skin cGvHD showed no response after a median of four weeks of treatment. The lack of response persisted after three months of treatment, and the patient passed away due to a relapse of the primary disease. Follow-up period was three months.

Patient 13 diagnosed with severe liver cGvHD achieved a CR after a median of four weeks of treatment. The CR continued after 23 months of treatment, but the patient passed away due to a relapse of the primary disease. Follow-up period was 24 months.

Patient 14 diagnosed with severe skin and mouth cGvHD showed no response after a median of four weeks of treatment. The lack of response continued after two months of treatment and the patient passed away due to a relapse of the primary disease. Follow-up period was two months.

Patient 15 diagnosed with severe skin cGvHD showed no response after a median of four weeks of treatment. The lack of response persisted after five months, and the patient passed away due to COVID-19. Follow-up period was five months.

Patient 16 diagnosed with mild liver and mouth cGvHD achieved a CR after a median of four weeks of treatment. The CR continued after two months of treatment, but the patient passed away due to a recurrence of the primary disease. Follow-up period was two months.

Patient 17 diagnosed with severe liver and skin cGvHD achieved a CR after a median of four weeks of treatment. The CR continued at the end of 12 months of treatment. Follow-up period was 12 months.

Patient 18 diagnosed with mild liver and skin cGvHD achieved a CR after a median of four weeks of treatment. The CR continued at the end of 13 months of treatment. Follow-up period was 13 months.

Patient 19 diagnosed with mild skin cGvHD achieved a PR after a median of four weeks of treatment. The PR continued at the end of six months of treatment. Follow-up period was six months.

Patient 20 diagnosed with severe gut cGvHD achieved a CR after a median of four weeks of treatment. The CR continued after 12 months of treatment. Follow-up period was 12 months.

Patient 21 diagnosed with mild liver and mouth cGvHD achieved a CR after a median of four weeks of treatment. The CR continued after ten months of treatment. Follow-up period was ten months.

Patient 22 diagnosed with severe skin and mouth cGvHD showed no response after a median of four weeks of treatment. The lack of response persisted after 12 months of treatment. Follow-up period was 12 months.

Patient 23 diagnosed with severe gut and liver cGvHD achieved a CR after a median of four weeks of treatment. The CR continued after 14 months of treatment. Follow-up period was 14 months.

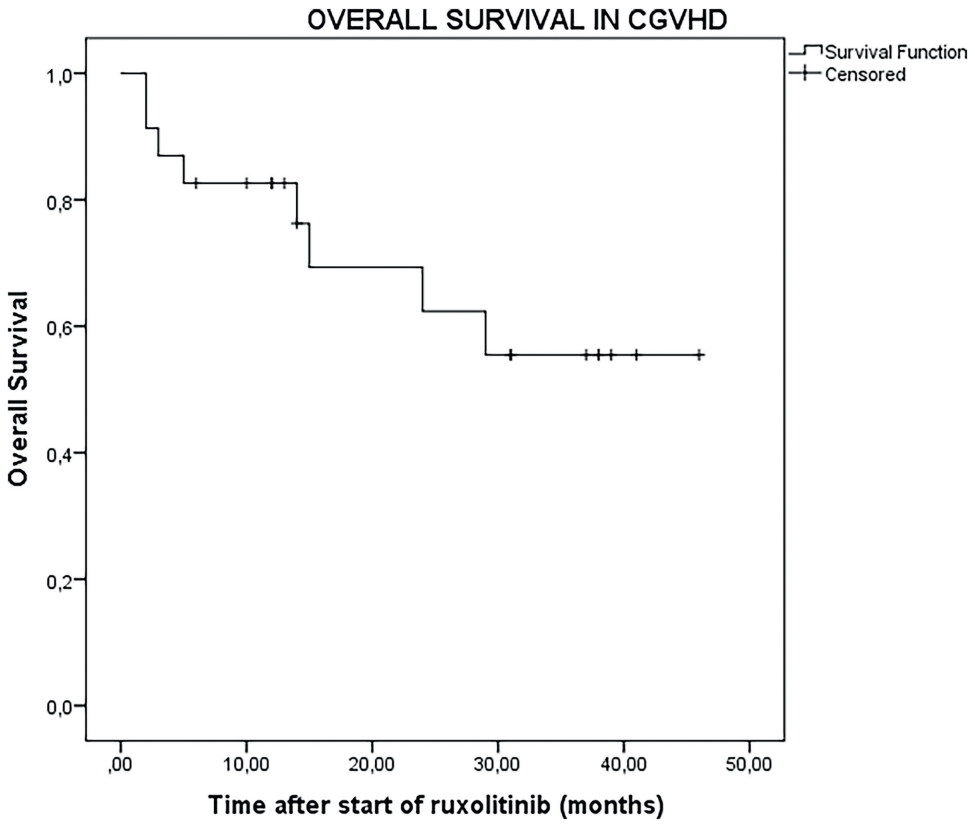

Figure 1 – Kaplan-Meier curve showing overall survival for all patients after initiation of ruxolitinib treatment.

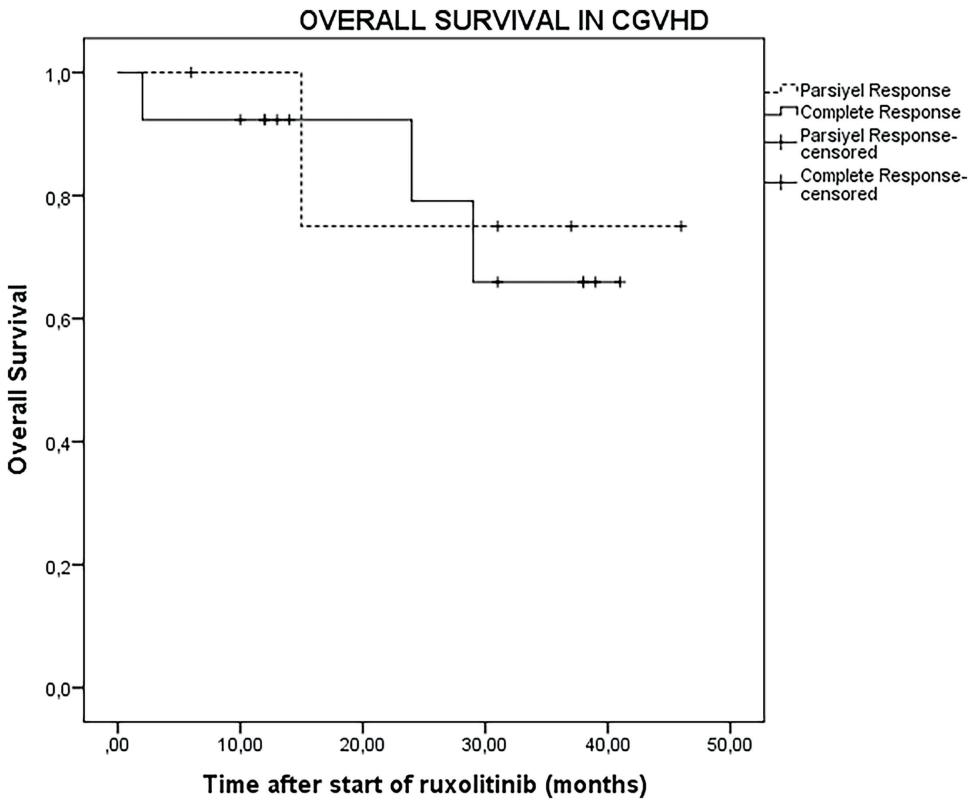

Figure 2 – Kaplan-Meier curve showing overall survival for patients who responded to ruxolitinib treatment.

Table 3 – Adverse events (n = 19)/.

Event	n (%)
Infections	
Pneumonia and herpes zoster	1 (5.3)
Peripheral edema	2 (10.5)
CMV colitis	1 (5.3)
Vomiting	2 (10.5)
Severe cytopenia (Grade 3 and 4)	
Thrombocytopenia	1 (5.3)
Mild cytopenia (Grade 1 and 2)	
Neutropenia	2 (10.5)
Malignancy relapse	1 (5.3)

no further benefit was anticipated from maintaining the drug. Notably, cGvHD relapse was absent within a median of nine months (range: 5–14 months) following drug discontinuation.

Adverse events

Table 3 shows the adverse events documented during ruxolitinib treatment. Hematologic toxicities were prevalent within this cohort, with eight patients (34.7%) experiencing cytopenias, including Grade 3 anemia in one patient and Grade 3 thrombocytopenia in another. Dose reduction resolved the issue in both cases of Grade 3 cytopenias, while in six patients, no alterations were made to avoid compromising the clinical benefits of the drug, with close monitoring for cytopenia-related symptoms.

Throughout the follow-up period on ruxolitinib, bacterial infections affected 13% of patients (3/23), while viral infections affected 30.4% (7/23). Of the viral infections, there were two cases (8.7%) of herpes zoster, one case (4.3%) of human polyomavirus 1 (BK) virus, three cases (13%) of COVID-19, and one case (4.3%) had herpes simplex with oral lesions. Notably, severe BK-viral hemorrhagic cystitis was not observed, and no fungal events were diagnosed among patients undergoing cGvHD treatment.

No instances of cytomegalovirus (CMV) reactivation were detected in the 23 patients, suggesting that ruxolitinib may not significantly elevate the risk of CMV reactivation. Plasma CMV polymerase chain reaction (PCR) monitoring was conducted for all recipients.

Relapse of the underlying malignancy occurred in four patients (17.4%), with two being non-responsive to ruxolitinib. Of the patients who were responsive to ruxolitinib, two (11.1%) experienced relapses, one with refractory AML and the other with ALL. Both patients were on ruxolitinib at the time of relapse (approximately two months and 23 months, respectively), having achieved CR of cGvHD.

Discussion

CGvHD remains the primary long-term complication after allo-HSCT, yet significant transformations have unfolded over the past decade. Novel strategies for managing cGvHD have shifted from broad, protracted immunosuppression with high-dose corticosteroids to therapies pinpointing specific mechanistic pathways relevant to cGvHD pathophysiology. By inhibiting JAK1/2, ruxolitinib addresses various facets of the immune response implicated in cGvHD, including allogeneic T cell proliferation and inflammatory cytokine generation.[9,24,25] The favorable clinical outcomes of ruxolitinib in refractory cGvHD were initially highlighted by Zeiser et al.[14] in 2015, with subsequent retrospective studies consistently corroborating its efficacy. Notably, the REACH3 trial has recently furnished robust evidence further advocating the utilization of ruxolitinib in this setting.[15–17]

In the current investigation, significant responses to ruxolitinib treatment were observed in cases of moderate and severe cGvHD. The analysis of the present study revealed an ORR of 78.3% after a median treatment duration of four weeks, with the majority of responses being CR (55.6%). These findings closely parallel those reported by Ferreira et al.[16] in 2021, who conducted a long-term follow-up study of ruxolitinib in 35 cGvHD patients, demonstrating an ORR of 89% (with CR accounting for 26%) after a similar median treatment duration. Similarly, Wu et al.[27] reported an ORR of 70.7% in 41 cGvHD patients treated with ruxolitinib. Furthermore, a Phase 3 randomized controlled study showcased favorable outcomes for ruxolitinib in cGvHD compared to the best available treatment, with an ORR of 50% versus 26% at Week 24.[17] Notably, the current patient cohort exhibited a higher proportion of severe cGvHD cases (65.2%) compared to the REACH3 study (59%) and demonstrated a superior ORR (78.3%).

In the systematic review and meta-analysis conducted by Zang et al.,[5] the ORR for cGvHD was documented as 73.1%. Additionally, a meta-analysis encompassing 26 studies investigating ruxolitinib in SR-cGvHD reported an ORR of 0.78 (95% CI: 0.74–0.81) at any time, with a two-year OS of 75.3% (95% CI: 68.0–82.7%).[4] Examination of ORRs across studies focusing on ruxolitinib treatment for cGvHD reveals a wide range, varying from 45% to 89%.[16,20,21,28]

While the majority of patients in the studies by Ferrari et al.[16] and Abedin et al.[22] presented with moderate cGvHD, the current study predominantly included patients with severe cGvHD (65.2%). Consequently, achieving a high ORR in severe cGvHD patients is a significant outcome. Moreover, the majority of patients were responsive to ruxolitinib in this study achieving a CR rate of 72.2% at follow-up, representing the highest CR rate reported to date, whereas lower CR rates ranging from 3.5% to 36.6% were reported in other studies.[16,21,27–29]

Long-term follow-up reports of ruxolitinib treatment in cGvHD patients are largely confined to small retrospective analyses, with the majority of studies featuring a short-term follow-up ranging from 12 to 19 months.[18–22] Moisev et al.[29] documented a median follow-up time of 28 months and a median ruxolitinib duration of 23 months, reporting a one-year OS rate of 81%. In another study, Ferreira et al.[16] reported a median follow-up of 43 months in 35 cGvHD patients. The present study contributes to this limited pool as one of the few investigations providing long-term follow-up data on ruxolitinib treatment in cGvHD patients.[16,26,27,29] In this study, the median follow-up duration after the initiation of ruxolitinib was 14 months for all 23 patients and 20.5 months for the 18 patients who were responsive to ruxolitinib. Of the patients who were responsive to ruxolitinib, 33.3% discontinued the drug, 50% received ruxolitinib as the sole immunosuppressive therapy, and no cGvHD relapse was observed. On

the other hand, the study of Ferreira et al.[16] reported that 15 patients had discontinued the drug, with only 22% receiving ruxolitinib as the sole immunosuppressive therapy.

In existing literature, studies have reported rates of steroid dose reduction to physiological levels or discontinuation of prednisone ranging from 57 to 89%.[16,19,21] The primary objective in treating cGvHD is to alleviate the adverse effects associated with steroids and significantly improving the patient's quality of life by discontinuing steroids as early as possible. In the current study, all the patients who were responsive to ruxolitinib successfully reduced their steroid dose or discontinued it altogether. Our steroid discontinuation rate (83.2%) closely mirrors that reported by Ferreira et al.[16] (81%), likely reflecting the high CR rate (55.6%) we achieved.

The optimal duration of ruxolitinib use in responsive patients, particularly after achieving CR, remains uncertain. Notably, the heightened immunosuppression resulting from the mechanism of action of ruxolitinib may increase the risk of relapse of the underlying malignancy.[13] Only two relapses (11.1%) were observed in this study, both of which responded to ruxolitinib treatment. One relapse occurred in the second month of ruxolitinib treatment in a patient diagnosed with AML, while the other occurred in the twenty-third month of treatment in a patient with ALL. We did not consider the AML relapse to be treatment-related, as it occurred early during ruxolitinib treatment. Wu et al.[27] reported a relapse rate of 14.6% in their study, while Zeiser et al.[14] reported a low incidence of disease relapse (2.4%) during ruxolitinib treatment. Similarly, Ferreira et al.[16] observed a low relapse rate (6%). Based on the findings of this study, it appears that ruxolitinib treatment does not increase the risk of disease relapse. However, it is imperative to emphasize the need for further studies with prolonged follow-up periods similar to validate these findings.

Cytopenias were observed as the most prevalent treatment-related toxicity (34.8%), with only two patients experiencing Grade ≥3 cytopenias, both of which resolved upon dose reduction. Ferreira et al.[16] reported a similar general cytopenia rate of 31%, consistent with these findings. Moisev et al.[29] noted Grade 4 cytopenias in less than 15% of cGvHD patients. Given that JAK-STAT pathways play a crucial role in cytokine-mediated hematopoiesis, it is unsurprising that thrombocytopenia or anemia emerge as common side effects in studies investigating ruxolitinib use.[5,9,17,29]

According to the findings of this study, CMV reactivation was not observed during cGvHD treatment despite a high proportion of donor or recipient CMV seropositivity (78%). Similarly, Dang et al.[15] did not observe CMV reactivation in their study. In contrast, Zeiser et al.[14] reported CMV activation rates of up to 14.6% in cGvHD patients, while Modi et al.[20] observed a lower rate of CMV infection (8.6%). Given reported cases of CMV reactivation, frequent monitoring of CMV copy numbers in patients receiving ruxolitinib treatment remains important.[5] Within the current cGvHD patient cohort, herpes zoster infections were recorded in 8.7% and COVID-19 infections in 13% of cases. A prior study documented a herpes zoster infection rate of 7.1% in cGvHD patients.[15] Notably, the heightened COVID-19 infection rate may be attributed to the ongoing COVID-19 pandemic during the observation period.

Regarding bacterial infections, this study observed a lower occurrence rate (13%) than literature reports. Abedin et al.[22] identified bacterial infections in 21% of cGvHD patients, while Modi et al.[20] reported a 52% infection rate during ruxolitinib treatment. The relatively low infection rate reported here might be associated with the absence of severe Grade 3–4 neutropenia. Additionally, reducing or discontinuing steroid doses in all patients may have contributed to this outcome. Based on these data, it seems that ruxolitinib treatment does not significantly increase the risk of severe infection.

Examining the biology of cGvHD development reveals a progression through three stages. Initially, cytotoxic tissue damage triggers the activation of innate immune system cells, fibroblasts, and endothelial cells. Subsequently, the adaptive immune system becomes hypersensitive while immune regulators decrease. The final stage is characterized by abnormal tissue repair and fibrosis, driven by activated macrophages producing transforming growth factor beta and platelet-derived growth factors, promoting fibroblast activation.[30] Ruxolitinib may exhibit greater efficacy during the second phase of disease progression and less efficacy during the fibrosis-dominated third phase. Patient selection could play a pivotal role in enhancing treatment responses. Huravelle et al.[14] reported that ruxolitinib treatment softened the skin in eight out of 12 patients with a scleroderma pattern of cGvHD but did not reduce the affected skin area. Similarly, Xue et al.[28] found that ruxolitinib treatment did not yield significant improvement in patients with fasciitis, a sclerotic-type of cGvHD of the skin. Of this cohort, five patients exhibiting severe skin involvement in cGvHD, characterized by notable sclerotic changes and fibrosis, did not respond to ruxolitinib treatment, potentially attributable to the advanced stage of their cGvHD.

Limitations of this study include the small patient cohort and its retrospective nature.

This study underscores ruxolitinib as an effective and safe salvage treatment option for cGvHD patients, evidenced by an ORR of 78.3% and a high CR rate of 72.2% of the responders. Given the often prolonged duration of cGvHD treatment, assessing the long-term sustainability of response and potential consequences of ruxolitinib therapy is crucial. As the number of long-term follow-up studies increases, the impact of this treatment on cGvHD will become more evident. However, prospective multicenter studies are merited in confirming our findings.

Author contribution

Neslihan Mandaci Sanli: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration. Esen Karakuş: Investigation, Data curation, Resources, Writing - Review & Editing, Visualization.

Conflicts of interest

The authors declare no conflicts of interest.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

The impact of pathogen reduction on ABO isoagglutinin titers in apheresis platelets

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ABSTRACT

Background: Platelet transfusions are a cornerstone of modern medical care, used across various clinical contexts. Ensuring the compatibility of blood products, especially regarding ABO isoagglutinins, is critical to minimize adverse reactions. Pathogen reduction technologies have been widely adopted to enhance the safety of blood products, however, the impact of such treatments on ABO isoagglutinin titers in platelet products remains unclear.
Methods: This study analyzed 60 apheresis platelet donations, including type O, A, and B donors, using the INTERCEPT® Blood System for pathogen reduction. Samples were collected both from donor whole blood at the time of apheresis (Retention) and from the final pathogen-reduced platelet product after it had passed through the compound adsorption device (Post-CAD). ABO isoagglutinin titers, including both IgM and IgG classes, were measured using solid-phase technology on the NEO Iris platform.
Results: This study found a significant reduction in IgM isoagglutinin titers in Post-CAD samples, with 99 % of Retention titers being greater than or equal to their Post-CAD counterparts. IgG titers exhibited more variability, with 9 % of Post-CAD samples displaying higher titers than Retention samples. Statistical analysis confirmed differences between Retention and Post-CAD samples for both IgM and IgG titers, with p-values <0.05 in most comparisons.
Conclusion: Pathogen reduction using the INTERCEPT® Blood System effectively reduces ABO isoagglutinin titers in apheresis platelets, potentially lowering the risk of hemolytic transfusion reactions. This reduction is beneficial for safer out-of-group platelet transfusions, especially in vulnerable populations such as pediatric patients. These findings support the continued use of pathogen-reduced platelets in transfusion medicine to enhance both safety and availability of blood products.

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Introduction

Platelet transfusions are a vital component of modern medical care, utilized across various clinical settings ranging from trauma and surgery to the management of hematological disorders. The compatibility of blood products, including platelets, is paramount to ensure patient safety and the efficacy of treatment [1]. The ABO blood group system, characterized by the presence or absence of A and B antigens on red blood cells, plays a central role in blood transfusion compatibility [2]. In addition to these antigens, individuals also possess naturally occurring antibodies known as isoagglutinins, which are directed against the ABO antigens absent from their own blood [3]. ABO antibodies develop in individuals at 3–6 months of age and reach adult levels at 5–10 years [4]. They are primarily IgM, and IgG antibodies often belonging to the IgG_2 subclass. Historically, ABO matching has been considered less critical for platelet transfusions compared to red blood cell transfusions due to the lower expression of ABO antigens on platelets and the shorter lifespan in circulation [5]. As a result, out-of-group platelet transfusions, where the donor and recipient have different ABO blood groups, have been commonly practiced, especially in situations where ABO-matched platelets are unavailable or in high demand [6].

Despite the prevailing acceptance of out-of-group platelet transfusions, concerns regarding the potential risks associated with ABO-incompatible platelet transfusions have prompted a reevaluation of transfusion practices [7]. One significant development in this regard is the recognition of ABO titers as a valuable tool in assessing the suitability of out-of-group platelet transfusions [8]. The semi-quantitative assessment of ABO isoagglutinin titers is valuable for evaluating the compatibility of blood products with the recipient and for minimizing the risk of adverse reactions such as hemolytic transfusion reactions [9]. Traditional methods for titration involve labor-intensive techniques such as tube agglutination or gel centrifugation. However, advancements in technology have introduced automated methodologies, offering high-throughput and standardized approaches for ABO isoagglutinin titration [10].

In addition, pathogen reduction of platelets represents an important advancement in blood safety technology and is increasingly being adopted by blood centers and hospitals worldwide [11]. Psoralen is a photosensitive compound that, when activated by ultraviolet (UV) light, forms cross-links between nucleic acids, thereby preventing replication and transcription of DNA and RNA in pathogens such as bacteria, viruses, and parasites [12]. Residual psoralen and byproducts are removed by adsorption via the compound adsorption device (CAD) to reduce toxicity [13].

Psoralen treatment of platelets is an effective method of pathogen reduction, yet its impact on the levels of ABO isoagglutinins in the plasma of the final treated product is unknown. This study aims to evaluate ABO isoagglutinin titers (both IgM and IgG) in platelet donations using automated solid-phase technology on the NEO Iris platform (Werfen, previously Immucor, Inc). By comparing titers between donor whole blood samples collected at the time of apheresis and final pathogen-reduced platelet product samples, this study seeks to identify any changes in isoagglutinin levels that may occur during the manufacturing process.

Material and methods

Sixty apheresis platelet donations from 30 type O, 15 type A, and 15 type B donors collected using a Trima Accel Automated Blood Collection System were analyzed. Each donation provided two samples: donor whole blood retention samples (Retention) collected in EDTA tubes at the time of apheresis, and final platelet product post-CAD samples (Post-CAD) collected after processing.

All platelets were treated with the pathogen reduction INTERCEPT® Blood System for Platelets System (Cerus Corp.). In the manufacturing process, platelets are sterilely transferred into a single-use processing set containing amotosalen solution. The platelets are placed in an illumination device which delivers a controlled dose of ultraviolet A (UVA) light for each treatment, lasting approximately four minutes. After illumination, platelets are transferred to the bag containing the CAD and agitated for 6–24 h at room temperature. At completion of the CAD incubation, the platelets are transferred by gravity flow to the storage container in their final state as INTERCEPT platelets. The Post-CAD sample is then collected for testing.

Samples were tested for IgM and IgG classes of anti-A, anti-B, and anti-A/B isoagglutinins, totaling 360 individual tests split evenly between Retention and Post-CAD samples. ABO Isoagglutinin titers of both IgM and IgG classes were determined using solid-phase technology on the NEO Iris platform (Werfen, previously Immucor, Inc.) [14]. Initial IgM and IgG results were measured up to a dilution of 1:128. For IgG isoagglutinin titer results exceeding 128, reflex testing was conducted to establish titers up to a dilution of 1:2048. Automated protocols were unavailable for IgM titers above 128. In the event of invalid automation results, the test was repeated up to two times and excluded from the study upon the third invalid result.

In the automated IgM protocol, 50 μL of sample was serially diluted to a dilution of 1:128, then incubated with 15 μL of pooled A or B cells (2–4 %, Immucor) for 10 min at 20 °C. The IgG protocol utilizes Capture-R® technology to detect IgG red blood cell antibodies. Pooled A or B cells (2–4 %) were added to each well of Capture-R Select strips, followed by mixing with 50 μL of system fluid. After centrifugation and washing steps, 50 μL of system fluid and 100 μL of sample were added and serially diluted up to 1:128. Subsequently, 100 μL of Low Ionic Strength Saline (LISS) was added and incubated for 15 min at 39 °C, followed by washing. Capture-R® Indicator cells (55 μL) were added, and the plate was centrifuged, and read. Reflex testing employed the same IgG protocol at higher dilutions.

Statistical analysis

Since ABO titers represent non-continuous data, the results were transformed into titer steps using a logarithmic (log_2)

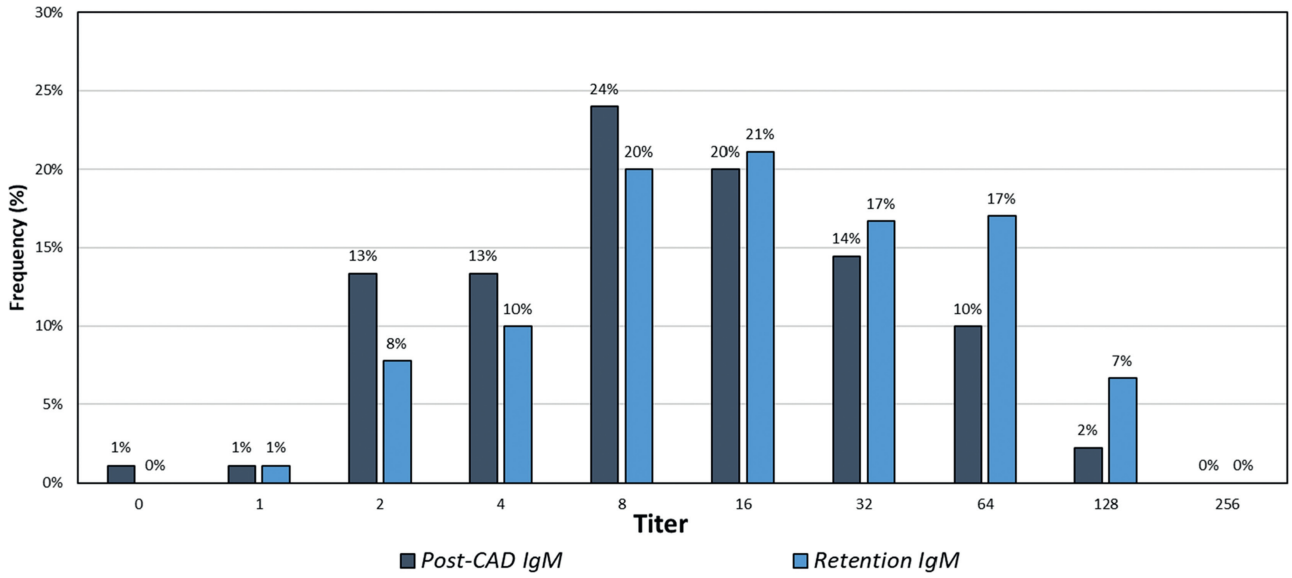

Figure 1 – Distribution of ABO IgM isoagglutinin titers for retention and post-CAD samples.

transformation. Specifically, a titer of 0 corresponded to −1, a titer of 1 corresponded to 0, and so forth, with each doubling dilution represented by a single digit increase in titer steps. The differences in titer steps between Retention and Post-CAD samples were then assessed to demonstrate if one sample type produced higher or lower results. Meanwhile, the absolute difference was used to determine the percentage of concordance within ±1 and ±2 titer steps. To evaluate the statistical significance between Retention and Post-CAD IgM and IgG results, a non-parametric Wilcoxon paired signed-rank test (using Z distribution) was employed, with a significance level set at 0.05.

Results

The distribution of titer results for IgM class isoagglutinin titers was close to symmetrical (Figure 1): Post-CAD results had a median titer of 8, mode of 8, and a sample coefficient of variation (CV) of 0.50 (in titer steps), and the Retention results had a median titer of 16, mode of 16, and CV of 0.42.

The distribution for IgG class isoagglutinin titers (Figure 2) was asymmetrical: Post-CAD results had a median titer of 16, mode of 128, and CV of 0.65, and Retention results had a median of 32, mode of 128, and CV of 0.61. The data for IgG

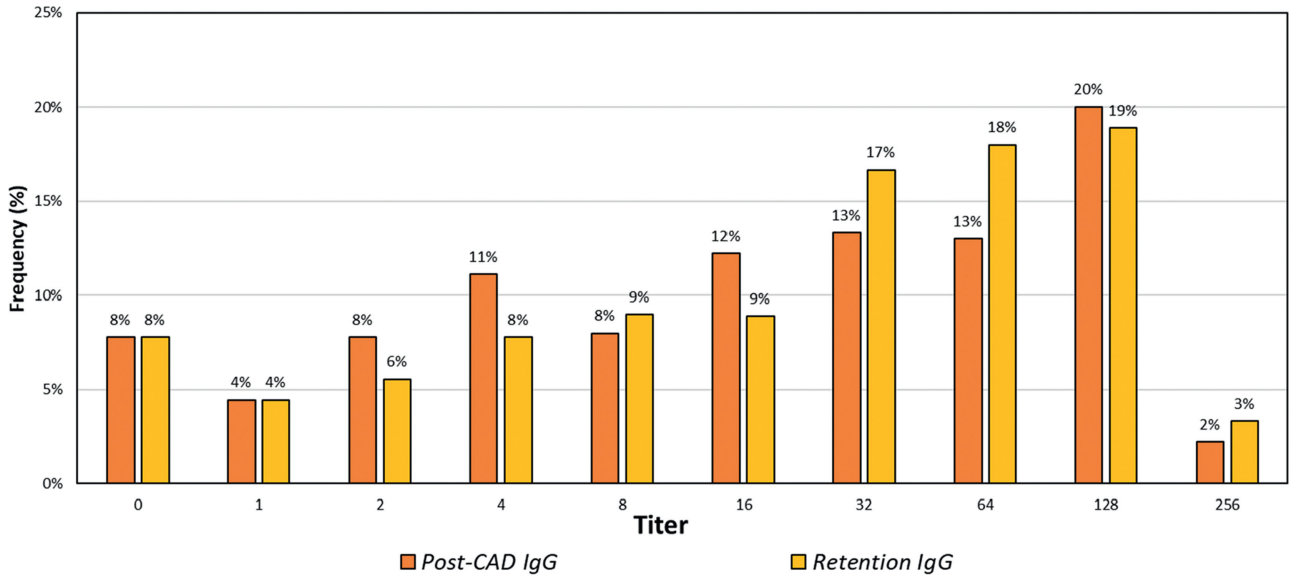

Figure 2 – Distribution of ABO IgG isoagglutinin titers for retention and post-CAD samples.

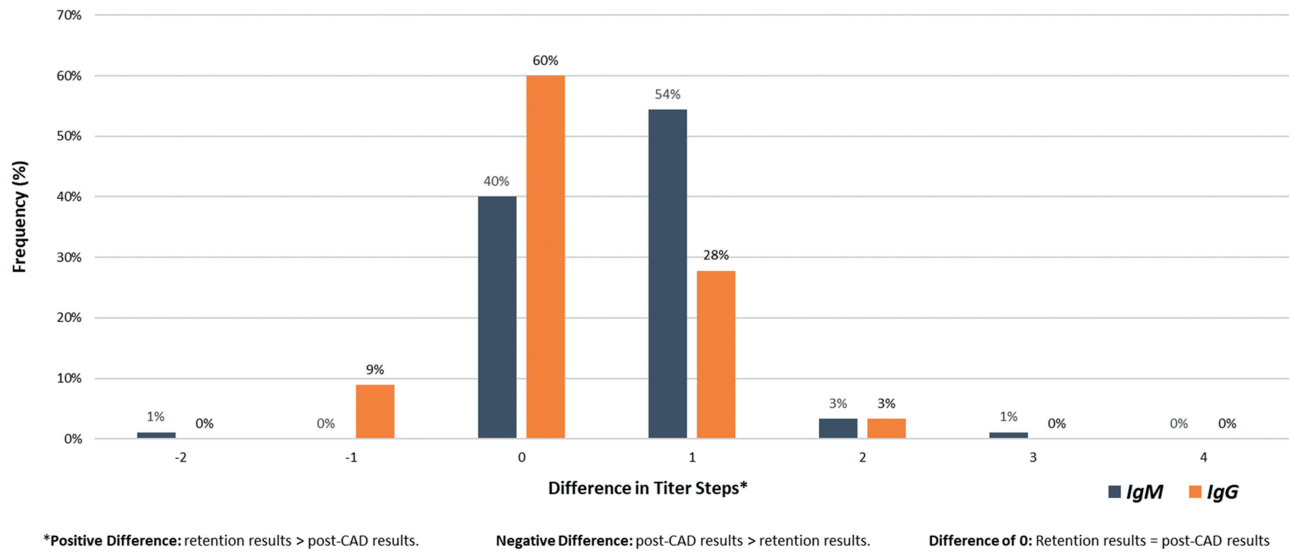

Figure 3 – Distribution of titer step differences (%) (retention − Post-CAD).

titers was more evenly distributed between titers 0 to 256, and skewed towards higher results.

Comparing IgM Post-CAD and Retention samples, 99 % of all Retention titer results were greater than or equal to their Post-CAD counterparts (Figure 3). An exception to this was a single outlier (1 %) where the Post-CAD IgM result was two titer steps greater than the corresponding Retention result. In contrast to IgM results, IgG data had 9 % of its Post-CAD samples yield higher titer results than the paired Retention samples.

Retention and Post-CAD samples were compared according to type and isoagglutinin class (IgM or IgG) using Wilcoxon paired signed-rank test. Overall, the p-values for both IgM and IgG titers were <0.05, indicating significant differences between the Post-CAD and Retention samples. Upon breaking down the antibody classes by blood type, the p-values for most comparisons indicated statistically significant differences between Post-CAD and Retention results (Table 1).

There were three cases in which the p-values exceeded 0.05: anti-A IgG for group O, anti-B IgG for group A, and anti-A IgM for group B. In the last case, the high p-value was attributed to a single outlier which, when excluded (n = 14 for type B) from the statistical test, yielded a p-value of less than 0.05 (p-value = 0.006). The percentage concordance between Retention and Post-CAD samples was calculated using titer steps. All comparisons had 100 % concordance within ±2 titer steps.

Distribution of titers from all blood group results utilizing maximum titer per sample was determined (Figure 4). Since blood type O contains antibodies of both anti-A and anti-B specificity, the higher of the two results was used to determine the maximum titer. For IgM, 83 % of all Post-CAD samples and 69 % of all Retention samples yielded titer results ≤32. For IgG, 65 % of Post-CAD and 62 % of Retention samples yielded titer results ≤32.

The distribution of the differences between IgG and IgM (IgG-IgM) results was similar when comparing Post-CAD and Retention samples. However, in both cases IgG isoagglutinin testing generally yielded higher titer results than the IgM counterparts. Refer to Table 1 to compare median titer results between IgM and IgG class isoagglutinin titers.

Discussion

The ABO blood group system plays a pivotal role in transfusion medicine, dictating the compatibility between donor and recipient blood types [15]. In vulnerable patient populations such as pediatric patients, ensuring compatibility is essential to mitigating the risk of adverse reactions during platelet transfusions [16]. This study investigated the quantitative assessment of ABO isoagglutinin titers, focusing on both IgM and IgG classes, in platelet donations using solid-phase technology [17]. By comparing titers between donor Retention samples and final platelet Post-CAD samples, this study aimed to evaluate the impact of the pathogen reduction manufacturing process on isoagglutinin levels.

The results revealed notable differences in the distribution of isoagglutinin titers between Retention and Post-CAD samples. Specifically, the median and mode titers for both IgM and IgG classes differed between the two sample types. For IgM isoagglutinin titers, Retention samples exhibited higher median and mode titers compared to Post-CAD samples. These differences suggest that the manufacturing process influences isoagglutinin levels in platelet products. Specifically, 99 % of IgM Retention titers were greater than or equal to their Post-CAD counterparts, with only a single outlier. In contrast, IgG results showed a more variable pattern, with 9 % of Post-CAD samples exhibiting higher titers than Retention samples, and a greater percentage of equivalent results between Retention and Post-CAD titers compared to IgM.

The observed reduction in isoagglutinin titers in Post-CAD samples can be attributed to the pathogen reduction process using the INTERCEPT® Blood System, which includes psoralen

Table 1 – Statistical results summary table.

Blood type	Sample n	Antibody specificity, class	Specimen source	Median	Mode	Min	Max	CV[a]	*p* value[b]	Concordance (%)[c] +/−1	Concordance (%)[c] +/−2
O	30	Anti-A, IgM	Retention	32	64	2	128	0.37	<0.05	93 %	100 %
			Post-CAD	16	64	2	64	0.41			
O	30	Anti-B, IgM	Retention	16	16	1	64	0.45	<0.05	100 %	100 %
			Post-CAD	8	8	0	64	0.49			
O	30	Anti-A, IgG	Retention	64	128	1	256	0.28	0.066	100 %	100 %
			Post-CAD	64	128	1	256	0.31			
O	30	Anti-B, IgG	Retention	32	128	2	128	0.36	<0.05	100 %	100 %
			Post-CAD	32	32	2	128	0.40			
A	15	Anti-B, IgM	Retention	8	8	2	128	0.50	<0.05	87 %	93 %
			Post-CAD	4	2	1	128	0.73			
A	15	Anti-B, IgG	Retention	1	0	0	8	3.32	0.484	100 %	100 %
			Post-CAD	1	0	0	8	2.50			
B	15[d]	Anti-A, IgM	Retention	16	32	2	128	0.38	0.095[d]	93 %	100 %
			Post-CAD	8	8	2	128	0.43			
B	15	Anti-A, IgG	Retention	8	32	0	64	0.60	<0.05	80 %	100 %
			Post-CAD	4	2	0	32	0.82			
All	60	Anti-A & Anti-B, IgM	Retention	16	16	1	128	0.42	<0.05	94 %	99 %
			Post-CAD	8	8	0	128	0.50			
All	60	Anti-A & Anti-B, IgG	Retention	32	128	0	256	0.65	<0.05	97 %	100 %
			Post-CAD	16	128	0	256	0.61			

[a] Coefficient of variation was calculated using titer steps.

[b] The results were compared using non-parametric Wilcoxon signed ranked test (using Z distribution) with a significance level at 0.05.

[c] Concordance is calculated using absolute difference in titers steps between retention and post-CAD samples.

[d] One of 15 Type B, IgM samples was an outlier with a post-CAD result two titer steps above its corresponding retention sample. This was the only pair of samples out of 60 pairs that had a higher IgM titer on the post-CAD sample than the retention sample. When the outlier is excluded (n = 14, for type B) from the analysis the p value is below 0.05 (p value of 0.006).

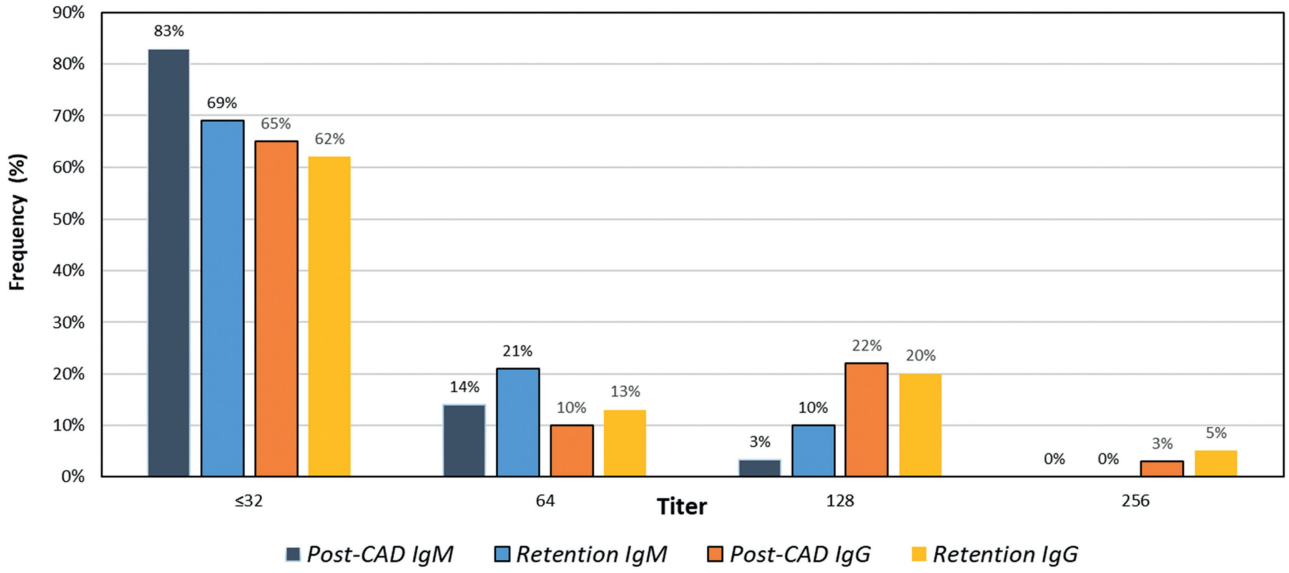

Figure 4 – Distribution of maximum titer results (anti-A or anti-B) per sample tested.

treatment and UV light activation [18]. This process not only inactivates pathogens but may also impact the isoagglutinin levels by binding and possibly denaturing these antibodies, thereby reducing their effective concentration in the final platelet product. This reduction is beneficial in minimizing the risk of hemolytic transfusion reactions, particularly in pediatric patients who are more susceptible to such adverse events [19].

The statistical analysis, using the Wilcoxon paired signed-rank test, indicated significant differences between Retention and Post-CAD samples for both IgM and IgG titers, with p-values <0.05 in most comparisons. This finding underscores the consistent impact of the manufacturing process on reducing isoagglutinin titers across different blood types and antibody classes.

The percentage concordance within ± 2 titer steps was 100 % for all comparisons, indicating a high degree of consistency between Retention and Post-CAD samples, even with the observed reductions in isoagglutinin titers. This high concordance rate supports the reliability of the pathogen reduction process in maintaining relative titer levels while reducing absolute concentrations.

Notably, three comparisons did not reach statistical significance: anti-A IgG for group O, anti-B IgG for group A, and anti-A IgM for group B. The high p-value for anti-B IgG for group A is likely due to the predominance of negative results (mode of 0), which diminishes the potential for detecting significant differences. After exclusion of the outlier in the anti-A IgM for group B, the p-value reached statistical significance. Anti-A IgG for group O had a p-value of 0.066, where a minor increase in the sample size could make it significant.

The reduction in isoagglutinin titers after pathogen reduction highlights the potential for safer out-of-group platelet transfusions with pathogen-reduced platelets, particularly in settings where ABO-matched platelets are scarce [20]. By lowering the risk of hemolytic reactions, pathogen-reduced platelets can be more safely used across different patient populations, including vulnerable groups such as pediatric patients. This aligns with current trends in transfusion medicine that emphasize both safety and availability of blood products [21]. Moreover, the use of solid-phase technology on the NEO Iris platform for titer measurement offers a robust and standardized approach for assessing isoagglutinin levels [22]. This technological advancement facilitates high-throughput, automated testing, enhancing the efficiency and accuracy of compatibility assessments in transfusion services.

While this study provides significant insights, it has limitations that should be addressed in future research. The sample size, although adequate for demonstrating significant differences, could be expanded to include a more diverse range of donor demographics. Additionally, the impact of other variables such as storage duration and donor health status on isoagglutinin titers could be further investigated.

In conclusion, this study highlights the significant reduction in ABO isoagglutinin titers achieved through the pathogen reduction process, enhancing the safety profile of out-of-group platelet transfusions. The use of advanced solid-phase technology for titer assessment ensures precise and reliable measurements, supporting informed clinical decision-making [23]. These findings contribute to the evolving landscape of transfusion medicine, promoting safer and more effective blood product utilization.

Contribution of the author

Mikayel Yeghiazaryan: Contributed to data review, data cleaning, figure generation and supported statistical interpretation.

Yembur Ahmad: Assisted in data analysis and critically revised the manuscript for important intellectual content.

Jessie Singer: Performed literature review, organized and extract data, and contributed to manuscript writing.

Vaanush Nazaryan: Participated in data management and quality control, and supported results interpretation.

Craig Fletcher: Critically revised the manuscript for important intellectual content.

Yamac Akgun: Conceived and designed the study, conducted statistical analysis, interpreted results, supervised the research process, and reviewed the manuscript for final approval.

Conflicts of interest

The authors declare no conflicts of interest.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Efficacy, safety and satisfaction of using emicizumab in hemophilia A patients without factor VIII inhibitors: A systematic review

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ABSTRACT

Background: Hemophilia A is a genetic disorder characterized by deficiency or dysfunction of the factor VIII clotting protein, leading to serious bleeding disorders. Conventional treatment involves the exogenous administration of factor VIII. However, this therapy faces significant challenges, including the development of inhibitors and the need for frequent intravenous administration. Emicizumab, a recombinant bispecific monoclonal antibody that can be administered subcutaneously, offers a novel therapeutic alternative by mimicking the action of factor VIII.

Methods: This systematic review evaluates the efficacy, safety, and patient satisfaction with emicizumab in patients with hemophilia A without inhibitors. A comprehensive literature search was conducted using the MEDLINE, SciELO, and LILACS databases. The included studies were original articles on the use of emicizumab in hemophilia A patients without inhibitors and reviews, short communications, expert comments, and case reports were excluded. Data extraction and analysis were performed using predefined criteria.

Results: A total of 471 articles were identified, with 28 meeting the inclusion criteria. Studies demonstrated robust evidence of the efficacy of emicizumab in reducing bleeding episodes, with significant reductions in the Annualized Bleeding Rate and Annualized Joint Bleeding Rate. Safety profiles were favorable, with mainly minor adverse events reported. High patient satisfaction scores highlighted improvements in quality of life and treatment adherence.

Conclusion: Emicizumab represents a significant advancement in hemophilia A treatment, offering superior efficacy, safety, and patient satisfaction compared to traditional therapies. Future research should focus on long-term outcomes and specific subpopulations to further validate these findings.

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Introduction

Hemophilia A is a genetic disease characterized by deficiency or dysfunction of the factor VIII clotting protein, leading to serious bleeding disorders that can manifest in total (severe) or partial (moderate or mild) form [1] Hemophilia A is inherited in a recessive form that often results in severe disease; approximately 10 % have moderate disease, and approximately 50 % of individuals have mild hemophilia [2].

Factor VIII deficiency leads to impaired blood clotting thereby significantly increasing the risk of spontaneous or prolonged bleeding, which can be fatal especially in cases of internal, intramuscular or joint bleeds [3,4]. Musculoskeletal bleeding can lead to debilitating chronic diseases, such as hemophilic arthropathy [1].

The conventional treatment of hemophilia A has historically been based on the exogenous administration of recombinant or plasma-derived factor VIII, with the aim of restoring deficient coagulation function [5]. This treatment, essential to avoid unnecessary hemorrhages and long-term sequelae, employs prophylactic therapies to reduce the frequency of bleeding and on-demand regimens to treat bleeding as it occurs [2].

In recent decades, the treatment of hemophilia has improved substantially due to the availability of effective and safe clotting factor concentrates that can be administered as long-term prophylaxis from early childhood in the most severe cases [3]. However, this therapeutic approach faces significant challenges. The need for frequent intravenous administration of factor VIII can impose a significant burden on the patient, affecting their quality of life, resulting in logistical difficulties and high costs [4,6].

Furthermore, replacement therapy is also compromised by the development of alloantibodies against FVIII. The development of factor VIII inhibitors is a serious and potentially fatal complication that occurs in 25–40 % of patients with severe hemophilia A within the first 50 days of exposure to FVIII [6]. These inhibitors neutralize the activity of factor VIII, making treatment less effective and increasing the risk of bleeding complications [4].

Faced with these challenges, emicizumab, a recombinant immunoglobulin G subclass 4 (IgG4) bispecific monoclonal antibody, emerges as an innovative therapeutic alternative for patients with hemophilia A.[1] Emicizumab mimics the action of factor VIII, promoting the formation of thrombin and, consequently, effective blood clotting [7]. This medication was initially approved for use in hemophilia A patients with inhibitors, and more recently licensed for use in patients without inhibitors.[1] Unlike conventional treatment, emicizumab can be administered subcutaneously, significantly reducing the frequency and the complexity of therapeutic administrations. Furthermore, clinical studies have demonstrated a lower incidence in the development of factor VIII inhibitors in patients treated with emicizumab, suggesting a significant potential to improve the safety and efficacy of hemophilia A treatment [7]. Although emicizumab represents a promising therapeutic alternative, current evidence regarding its efficacy, safety, and patient satisfaction in specific populations, such as those with hemophilia A without factor VIII inhibitors, is not fully understood [8].

In this systematic review, this study intends to address the effectiveness of emicizumab in preventing bleeding, its safety in terms of serious and non-serious adverse events, as well as patient satisfaction and quality of life. Furthermore, it aims to explore the limitations of existing studies and provide recommendations for future research to fill knowledge gaps and improve the clinical management of hemophilia A in order to significantly contribute to the understanding and advancement of the treatment of this disease.

Methods

The present study is a systematic literature review with the objective of evaluating the efficacy, safety and satisfaction of patients with hemophilia A without inhibitors using emicizumab. The following steps were adopted to prepare it: identification of the problem with definition of the guiding question, objectives, inclusion and exclusion criteria; research of existing literature with pre-established descriptors; data collection and evaluation; critical analysis of included data; and presentation of the integrative review, synthesizing the findings and discussing the results. To define the guiding question, the PICO (population, intervention, comparison and outcome, respectively) method was used. The search terms were developed based on the use of descriptors and free text terms: "Hemophilia A", "Haemophilia A", "Emicizumab" and "without Inhibitors". This study was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under number CRD42024528804.

To select the articles, three electronic databases were used: Medical Literature Analysis and Retrieval System Online (MEDLINE, PubMed), Scientific Electronic Library Online (SciELO) and Latin American and Caribbean Literature in Health Sciences (LILACS). The established inclusion criteria were complete original articles on the efficacy, safety and satisfaction of the use of emicizumab in hemophilia A patients without factor VIII inhibitors. The exclusion criteria were the form of acquired hemophilia, in vitro and animal analysis, and the following article types: review studies, short communications, expert comments and case reports. Two researchers independently selected the studies in several stages: analysis of titles and abstracts and, *a posteriori*, full-text analysis. Disagreements were resolved by a third reviewer.

An extraction form was prepared and previously tested to identify inconsistencies and make appropriate adjustments. The form consisted of the following items: (i) general information about the study; (ii) characteristics of the study and of the participants; (iii) exposure; and (iv) outcomes evaluated.

Results

As indicated in Figure 1, the search resulted in 471 articles related to the topic covered, of which 33 were excluded due to duplication in the databases consulted. After reading the title, 228 additional articles were excluded and, after reading the abstracts, another 135 articles were excluded. Seventy-five articles were selected for full reading, resulting in the inclusion of 28 articles.

When compiling the data for each article, the title, author, year of publication, journal, study objectives, participants and groups, results were recorded, as shown in Table 1 below.

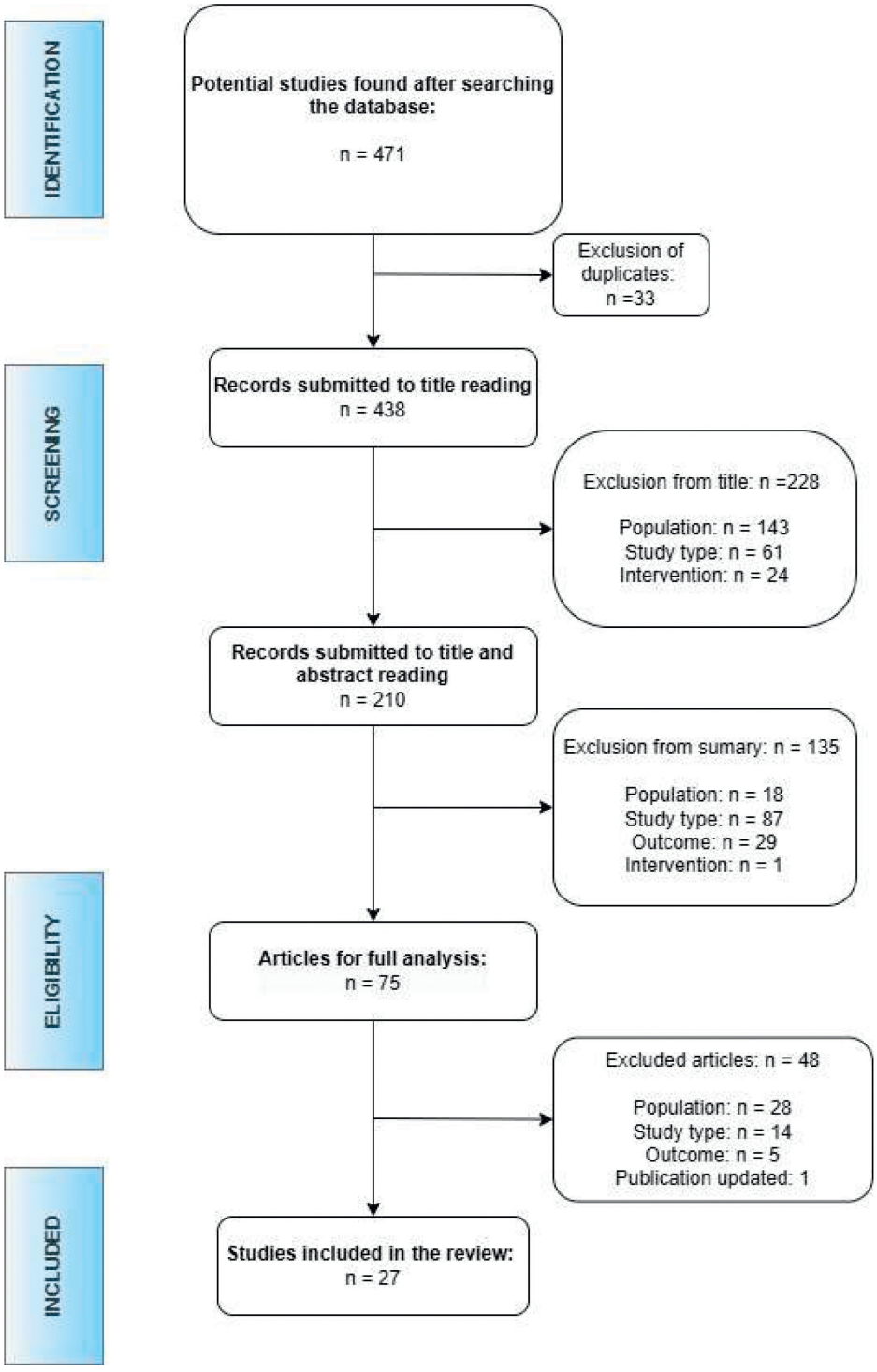

Figure 1 – PRISMA flow for articles selection.

Discussion

Efficacy

The studies reviewed provide robust evidence supporting the efficacy of emicizumab in reducing bleeding episodes in patients with hemophilia A without factor VIII inhibitors. The multicenter open-label study by Shima et al. [9] reported significant reductions in the Annualized Bleeding Rate (ABR) for treated bleeding events, demonstrating the potent hemostatic effect of emicizumab. The study found that patients receiving biweekly (Q2W) and monthly (Q4W) doses of emicizumab had ABRs of 1.3 and 0.7, respectively, indicating a substantial reduction in bleeding frequency compared to traditional treatments.

Furthermore, the retrospective cohort study by Oka et al. [11] corroborated these findings, showing significant improvement

Table 1 – Summary of articles included in the study.

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
A multicentre, open−label study of emicizumab given every 2 or 4 weeks in children with severe haemophilia A without inhibitors	Shima, M. et al., [9]	Haemophilia	Randomized Clinical Trial	Evaluate the efficacy, safety and PK of emicizumab in Japanese paediatric patients aged <12 years with severe haemophilia A without factor VIII (FVIII) inhibitors	*n* = 13 male paediatric patients with severe haemophilia A without inhibitors Q2W (*n* = 6): maintenance doses of 3 mg/kg Q2W Q4W (*n* = 7): maintenance doses 6 mg/kg Q4W	Annualized Bleeding Rate (ABR) for treated BEs: Q2W 1.3 (95 % CI: 0.6−2.9) and Q4W 0.7 (95 % CI: 0.2−2.6). ABRs for all BEs: Q2W 14.1 (95 % CI: 7.6−26.2) and Q4W 21.8 (95 % CI: 9.2−51.8) Non-treated BEs: Q2W = 2 patients (33,3 %), Q4W = 5 (71,4 %), majority traumatic. AES: 133 (62 Q2W and 71 Q4W) = contusion in 10 patients (76.9 %), nasopharyngitis in 5 (38.5 %), and excoriation and fall in 4 (30.8 %), 1 injection site reaction. No TEs or TMAs. All patients tested negative for anti−emicizumab antibodies. All caregivers preferred emicizumab to the patient's previous treatment.
AOZORA: long-term safety and joint health in paediatric persons with haemophilia A without factor VIII inhibitors receiving emicizumab - protocol for a multicentre, open-label, phase IV clinical study	Shima, M. et al., [10]	British Medical Journal	Clinical Trial	Investigate the long-term safety and effects of emicizumab on joint health in patients with hemophilia A aged <12 years without FVIII inhibitors	Approximately 30 patients aged <12 years without FVIII inhibitors will be enrolled at 10 centers in Japan.	Ongoing study
Assessment of the clinical perception, quality of life and satisfaction of patients with severe congenital haemophilia A without inhibitor after 1 year of emicizumab therapy	Oka, G. et al., [11]	Haemophilia	Retrospective Cohort	Assess the perceived clinical evolution, quality of life and treatment satisfaction of patients after 1 year of emicizumab therapy in real-life settings.	*n* = 38, median age 45.50 ± 13.21 years, all without inhibitors	General state of health was significantly improved after emicizumab than before 4.5/6 versus 3.6/6 (p-value = 0.0023) According to the EQ-5D-3 L, the VAS score of the general state of health was 69.6 (±19.4) out of 100 Chronic pains were significantly reduced after starting emicizumab (p-value <0.0431), but the EQ-5D-3 L survey highlighted a persistent chronic pain, being still an issue for 33 (86.8 %) patients. 16 (42.1 %) patients reported AEs, mainly at the infusion site, such as redness, skin rash or local pain (*n* = 14). Patients" satisfaction of emicizumab therapy after 1 year was 9.1 ± 1.0 score (out of 10) and no patient wanted to go back to the previous treatment.

Table 1 (*continued*)

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
Association of physical activity with BEs and safety in patients with haemophilia A starting emicizumab prophylaxis: an interim analysis of the TSUBASA study	Nogami K.et al. [12]	International Journal of Hematology	Prospective cohort	Explore the relationship between physical activity and BEs, as well as safety and QoL, in PwHA initiating prophylactic treatment with emicizumab in a Japanese cohort.	*n* = 107 with congenital HA without FVIII inhibitors, median age 35 (0–73)	The overall median ABR was 0.91 (IQR 0.00 −2.46); the model-based ABR for the overall population was 2.3 53.8 % of the patients had zero bleeds during the observation period 31.1 % of participants reported ≥1 incidence of spontaneous bleeding, and 33.0 % reported ≥1 incidence of traumatic bleeding 2 (0.5 %) exercise events in the same individual were associated with bleeding (running, weight training). 21 (19.8 %) participants experienced a total of 39 AEs. 5 (4.7 %) experienced a serious AE, none of which was emicizumab-related, and 3 (2.8 %) experienced an adverse drug reaction.
Bleeding control improves after switching to emicizumab: Real-world experience of 177 children in the PedNet registry	Zwer, KVDet al. [13]	Haemophilia	Prospective Cohort	Report on bleeding and safety in paediatric patients receiving emicizumab prophylaxis.	*n* = 177 children with congenital haemophilia A with and without inhibitors extracted from the PedNet registry	91 patients without FVIII inhibitors: mean ABR reduced after starting emicizumab from 2.41 (95 % CI: 1.98–2.95) to 1.11 (95 % CI: 0.90–1.36, *p*-value <0.001) AJBR reduced from 0.74 (95 % CI: 0.56–.98) to 0.31 (95 % CI: 0.21–.46; *p*-value <0.001) No life-threatening bleed was reported 5 emicizumab-related AEs were reported: 4 patients reported injection site reactions and One patient developed non-neutralizing ADAs The number of injections of long acting FVIII prophylaxis was reduced to 35/year (*p*-value <0.001) and CFC consumption was reduced by 97.6 %, from median 4847 IU/kg/year to 116 IU/kg/year (p-value <0.001).
Bleeding events and safety outcomes in persons with hemophilia A (PWHA) without inhibitors: non-interventional study (NIS) from a real-world setting	Kruse- Jarres, R. et al., [14]	11th Annual Congress of the European Association for haemophilia and Allied Disorders	Prospective Cohort	Describe bleed and safety outcomes in HA without inhibitors from FVIII therapy as per routine clinical practice	*n* = 94 ≥ 12 years old, severe HA, no FVIII inhibitors, ≥6 months of episodic or prophylactic Prophylactic: *n* = 49 Episodic: *n* = 45	ABR for treated bleeds (95 % CI: 36.1; range: 30.8–42.3 - episodic and 95 % CI: 5.0; range: 3.3–7.5 - prophylactic) ABR for all bleeds (95 % CI: 43.1; range: 36.5 −50.9 and 95 % CI: 6.2; range: 4.2–9.2) Most bleeds were treated (82 %) AEs: viral upper respiratory infection and arthralgia. 5 severe AE (hemarthrosis, GI polyp bleed) in 3/49 (6.1 %) patients on Px and none on episodic regimen

Table 1 (*continued*)

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
Development and testing of the Satisfaction Questionnaire with Intravenous or subcutaneous hemophilia injections and results from the Phase 3 HAVEN 3 study of emicizumab prophylaxis in persons with haemophilia A without FVIII inhibitors	Kempton, C. et al., [15]	Haemophilia	Cross-sectional study	Measure patient satisfaction with emicizumab	*n* = 63 ≥12 years old with moderate or severe congenital HA	Mean „overall satisfaction'' with emicizumab prophylaxis: 8.8 (95 % CI: 8.4–9.3) - week 21/25. Satisfaction with treatment half-life: 8.6 (95 % CI: 8.0–9.2) Reason for satisfaction: efficacy of their treatment (*n* = 14; 74 %) Desires: fewer infusions (*n* = 13; 68 %); a non-injectable treatment (*n* = 7; 37 %); not having to find a vein (*n* = 5; 26 %)
Effect of emicizumab prophylaxis on bone and joint health markers in people with haemophilia A without factor VIII inhibitors in the HAVEN 3 study	Kiialainen A. et al., [16]	Haemophilia	Randomized Clinical Trial	Explore the effect of emicizumab prophylaxis on bone/joint health in people with haemophilia A without FVIII inhibitors enrolled in HAVEN 3.	*n* = 152 patients Group A -C randomized in episodic treatment and Group D in previous prophylaxis with FVIII Group A (*n* = 36): a loading dose of emicizumab of 3.0 mg/kg/week was administered for 4 weeks followed by a maintenance dose of either 1.5 mg/kg per week. Group B (*n* = 35): a loading dose of emicizumab of 3.0 mg/kg/week was administered for 4 weeks followed by a maintenance dose of either 3.0 mg/kg Q2W. Group C (*n* = 18) = no prophylaxis, but after 24 weeks, they could switch to emicizumab 3.0 mg/kg Q2W. Group D (*n* = 63): previously receiving FVIII prophylaxis, received a loading dose of emicizumab 3.0 mg/kg for 4 weeks followed by a maintenance dose of 1.5 mg/kg per week.	Haemophilia Joint Health Score (HJHS): improvements from 95 % CI: −2.13 to (−3.96; −0.29) at Week 49 with at least one target joint at study entry (*n* = 71). Improvements from baseline were also observed for patients aged 12–39 years. Biomarkers of bone resorption/formation, cartilage degradation/synthesis, and inflammation did not change significantly during emicizumab prophylaxis. Biomarkers of bone/joint health did not show significant changes during 72 weeks.

Table 1 (*continued*)

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
Effect of late prophylaxis in hemophilia on joint status: a randomized trial	Manco- Johnson, MJ. et al., [17]	Journal of Thrombosis and Haemostasis	Randomized Clinical Trial	Describe 3-year bleeding, joint health and structure, health-related quality-of-life (HRQoL) in HA patients	*n* = 88 males 12–50 years old with severe hemophilia A, ≥150 factor VIII exposure days, no inhibitors and no prophylaxis for >12 consecutive months in the past 5 years. OD = On-demand (*n* = 42); Prophylaxis (*n* = 42)	BE's: P group (0.7 [0; 1.6], 2.5[SD 4.7]), OD group (37.4 [24.1; 52.6], 37.2[SD 19.9]). P group 93.9 % reduction in bleeding frequency (95 % CI: 89.6 %–96.4 %, p-value <0.0001). Most BEs were of joints (77.4 % [OD] and 75.8 %[P]). Bleed-free: 15 participants of P (35.7 %) and no OD. ABR for joint BEs: P group (0.3 [0; 1.2], 1.9 [SD 4.1]), OD group (27.3 [14.9; 41.1], 28.7 [SD 18.8]). Treatment satisfaction: 42.9 % of P participants and 26.2 % of OD "exceeded their expectations"; 64.3 % of P participants and 42.9 % of OD "very/extremely satisfied with treatment". AEs: 62 patients (73.8 % [P - *n* = 25; OD, *n* = 37]) No inhibitors developed in study.
Effectiveness of emicizumab in preventing bleeding events in severe and moderate hemophilia A: A single-center experience in Bangladesh	Tory, SS. et al., [18]	EJHaem	Prospective cohort	Evaluate the effectiveness of emicizumab in treating hemophilia A	*n* = 30 patients with severe hemophilia A or moderate A with severe bleeding phenotype with ABR of >8, regardless of FVIII inhibitor status	*n* = 22 patients with hemophilia A without inhibitors: There was a significant reduction in ABR ($p < 0.001$) in patients without an inhibitor - ABR before emicizumab prophylaxis 48.0 [33.0–60.0] versus ABR after emicizumab prophylaxis 1.0 [0.0–4.0] After emicizumab prophylaxis, BEs were significantly reduced (p-value <0.001). ABR decreased significantly in patients with and without inhibitors (p-value <0.001), there was no significant difference in ABR between patients with and without inhibitor after emicizumab prophylaxis
Emicizumab Prophylaxis Administered Once-weekly or Every Two Weeks Provides Effective Bleed Prevention in Persons with hemophilia A (PwHA) without Inhibitors - Results from the Phase III HAVEN 3 Study	Oldenburg J., et al., [19]	Hamostaseologie	Clinical Trial	Assess the efficacy, safety, and PK of emicizumab prophylaxis QW and Q2W (Q2W) in adolescent/adult PwHA without inhibitors.	*n* = 152 patients, aged 13–77 years (median: 38), with severe haemophilia A patients without inhibitors aged ≥12 year A: emicizumab prophylaxis 3 mg/kg QW for 4 weeks, followed by 1.5 mg/kg QW B: emicizumab prophylaxis 3 mg/kg QW for 4 weeks, followed by 3 mg/kg Q2W C: No prophylaxis D: previously on FVIII	Statistically significant reductions: ≥94 % reduction in treated, all, spontaneous, joint, and target joint bleeds with QW or Q2W emicizumab versus no prophylaxis. 55 % had zero treated bleeds. 91 % had ≤3 treated bleeds. Intra-Individual comparison: 68 % reduction in treated bleed rate with QW emicizumab compared to prior FVIII prophylaxis. Emicizumab was well tolerated, the most common AE was Injection-site reaction (25 %).

Table 1 (*continued*)

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
					prophylaxis received 1.5 mg/kg QW emicizumab maintenance	No TEs, ADA, or de novo FVIII inhibitors reported. Sustained trough concentrations achieved with both QW and Q2W regimens.
Emicizumab prophylaxis improves long-term physical health scores in persons with haemophilia A (PWHA) with and without inhibitors: update from the HAVEN 3 and HAVEN 4 studies	Skinner, MW., et al., [20]	Research and practice in thrombosis and haemostasis	Clinical Trial	Assess the impact of prophylactic emicizumab on HRQoL of PwHA with/without FVIII inhibitors	HAVEN 3: PwHA ≥12 years without inhibitors previously receiving episodic (n = 88) or prophylactic (n = 63) FVIII were assigned to emicizumab 1.5 mg/kg once weekly or 3 mg/kg once every two weeks.	Questionnaire compliance rates were 94.9 % in HAVEN 3 (up to Week 49) Beyond Week 25, PHS scores improved by ≥10 in over 50 % in HAVEN 3 Mean (SD) total score at baseline was 31.5 (15.0) and improved to 22.8 (15.1) for HAVEN 3 At baseline, 76 % (75/99) of employed patients from HAVEN reported no missed days of work in the prior 28 days. At Week 25, 91 % (88/97) of HAVEN 3 participants reported no missed workdays, this remained stable thereafter.
Emicizumab prophylaxis in infants with hemophilia A (HAVEN 7): primary analysis of a phase 3b open-label trial	Pipe, S.W. et al., [21]	Blood	Clinical Trial	Investigate the efficacy, safety, PK, and pharmacodynamics of emicizumab in those aged ≤12 months with severe HA without factor VIII (FVIII) inhibitors	n = 55 male infants with severe congenital HA (intrinsic FVIII level <1 %) without FVIII inhibitors, mean age 5 months	ABR was 2.0 (95 % CI: 1.49–2.66) for all bleeds, 0.4 (95 % CI: 0.30–0.63) for treated bleeds, 0.0 (95 % CI: 0.01–0.09) for treated joint bleeds, and 0.1 (95 % CI: 0.02–0.12) for treated muscle bleeds. There were no treated spontaneous bleeds, because all 42 treated bleeds were traumatic. 207 bleeds were reported in 46 participants (83.6 %), 87.9 % of which were traumatic No intracranial hemorrhages occurred. All participants experienced an AE, with 63 reported in total. Sixteen participants (29.1 %) experienced a total of 30 serious AEs (SAEs), but none were related to emicizumab. And 9 participants (16.4 %) had ≥1 emicizumab-related AE (all grade 1 injection-site reactions). No AE led to treatment changes. No deaths, TEs, or TMAs occurred. No participant tested positive for ADAs. Two participants were confirmed positive for FVIII inhibitors.

Table 1 (*continued*)

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
Emicizumab prophylaxis in patients who have hemophilia A without inhibitors	langu, J. et al., [22]	The New England Journal of Medicine	Randomized Clinical Trial	Investigate the efficacy, safety, and PK of emicizumab prophylaxis in patients who have hemophilia A without inhibitors	*n* = 152 patients Group A (*n* = 36): 1.5 mg/kg body weight/week. Group B (*n* = 35) = 3.0 mg/kg Q2W Group C (*n* = 18): no prophylaxis. Group D (*n* = 63): previously receiving FVIII prophylaxis, followed by a maintenance dose of 1.5 mg/kg	ABR: 1.5 events (95 % CI: 0.9–2.5) in Group A; 1.3 events (95 % CI: 0.8–2.3) in Group B, 38.2 events (95 % CI: 22.9–63.8) in Group C. Bleeding Rate (BR) 96 % lower in A than in C (rate ratio: 0.04; 95 % CI: 0.02–0.08; p-value <0.001) and 97 % lower in B than in C (rate ratio: 0.03; 95 % CI: 0.02–0.07; p-value <0.001 No treated BEs: 56 % of the participants of A and 60 % of those in B had no treated BEs, 0 % of participants of group C remained without BEs Secondary Bleeding-Related End Points: lower with each emicizumab regimen than with no prophylaxis Patients Preferences: 94 % (95 % CI: 87–98) preferred emicizumab AEs: 543 AEs in 127 participants, especially injection-site reaction (25 %) No TE or TMA No development of ADs, nor development of factor VIII inhibitors
Emicizumab prophylaxis in patients with haemophilia A with and without inhibitors	Ebbert PT. et al., [23]	Haemophilia	Retrospective Cohort	Describe real–world patient experience with emicizumab by retrospective chart review	*n* = 42 HA patients 1.5 mg/kg emicizumab weekly	ABR: 0.9 ± 0.4 ABR joint bleed: 0.1 ± 0.1 At least one BE: 6 patients (33.3 %) Postoperative bleed: 1 (16.7 %) Rating treatment: 5 improved (83.3 %)
Emicizumab prophylaxis in persons with mild or moderate hemophilia A: Results from the interim analysis of the HAVEN 6 study	Négrier C. et al., [24]	Blood	Clinical Trial	Assess the safety, efficacy, PK, and pharmacodynamics of emicizumab prophylaxis in persons with mild or moderate HA without FVIII inhibitors.	*n* = 71, median age 23 (>2 years old), follow-up median 27.5 weeks, *n* = 20 mild HA, *n* = 51 moderate HA, *n* = 37 on FVIII prophylaxis at baseline	*n* = 49 (69.0 %) had ≥1 AE, in which headache was the most common (14.1 %). The majority of AEs (84.5 %) were not emicizumab-related. *n* = 9 with local injection-site reactions (12.7 %), all were emicizumab-related. There were no deaths, AEs leading to treatment withdrawal/modification/interruption. Zero bleeds were reported for 80.3 % (treated bleeds), 46.5 % (all bleeds), 90.1 % (treated joint bleeds), 95.8 % (treated spontaneous bleeds), and 94.4 % (treated target joint bleeds) of participants. *n* = 2 (2.8 %) had ADAs, one of these had ADAs that were neutralizing in vitro, but no clinical impact or impact on emicizumab PK was observed

Table 1 (*continued*)

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
Emicizumab treatment and mo nitoring in a paediatric cohort: real-world data	Barg AA et al., [25]	British Journal of Haematology	Prospective cohort	Give the paucity of information on emicizumab safety, and efficacy and monitoring in paediatric patients	*n* = 40 children with HA with or without inhibitors	*n* = 22 non-inhibitor patients: The ABR was 1 (0–3), and 55 % of these patients did not have bleeds None of the patients encountered either TE or TMA, No signs of renal failure, haemolytic anaemia or thrombocytopenia were noted
Factor VIII–mimetic function of humanized bispecific antibody in hemophilia A	Shima, M. et al., [26]	The New England Journal of Medicine	Nonrandomized Clinical Trial	Evaluate the safety, PK, and pharmacodynamics of weekly emicizumab in patients who had severe hemophilia A with or without factor VIII inhibitors	*n* = 18 Cohort 1 (*n* = 6): 0.3 mg/kg body weight Cohort 2 (*n* = 6): 1.0 mg/kg body weight Cohort 3 (*n* = 6): 3.0 mg/kg body weight	Plasma concentrations of emicizumab: increased in a dose-dependent manner. Activated partial-thromboplastin times: remained short throughout the study. No bleeding in 5 of 7 (71 %) patients without factor VIII inhibitors. Antibodies to emicizumab did not develop. No serious AEs or clinically relevant coagulation abnormalities
Long-term safety and efficacy of emicizumab for up to 5.8 years and patients' perceptions of symptoms and daily life: A phase 1/2 study in patients with severe haemophilia A	Shima, M. et al., [27]	Haemophilia	Clinical Trial	Evaluate further longer-term data (5,8 years) including patients' perceptions	*n* = 18 patients HA with or without inhibitors A: 0,3 mg/kg emicizumab QW B: 1 mg/kg QW. C: 3 mg/kg QW All patients were later switched to the approved maintenance dose of 1.5 mg/kg.	*n* = 7 patients without inhibitors: Reduction in ABR after the use of emicizumab in patients without inhibitors: patient 1–5 of Group A (8.1 before the drug versus 1.6 after 0.3mg/kg of the drug versus 0.3 with 1 mg/kg versus 0.0 with 1.5mg/kg), patient 1–6 of Group A (77.1 versus 59.5 with 0.3 mg/kg versus 29.1 with 1 mg/kg versus 15.4 with 3 mg/kg versus 11.6 with 11.5 mg/kg), patient 2–5 of Group B (14.2 versus 2.6 with 1 mg/kg versus 0.0 with 1.5 or 3 mg/kg), patient 2–6 of Group B (10.1 versus 0.8 with 1 mg/kg versus 0.0 with 1.5 mg/kg), patient 3–4 of Group C (10.1 versus 0.2 with 3 mg/kg versus 4.3 with 1.5 mg/kg), patient 3–5 of Group C (0.0 versus 0.0 with 3 mg/kg), patient 3–6 of group C (8.1 versus 0.4 with 3 mg/kg versus 0.0 with 1.5 mg/kg). Perceptions change in bleeding severity and reduction in time until bleeding stops in 90 % of patients Improvements in carrying out daily activities, physical exercise and anxiety Symptoms such as joint pain and swelling were slightly improved

Table 1 (*continued*)

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
Physical activity limitations in children with severe haemophilia A. Does emicizumab make a difference?	Hassan AS. et al. [28]	Journal of Pakistan Medical Association	Prospective cohort	Assess the effect of emicizumab on physical activity in children with severe haemophilia A by using Paediatric Haemophilia Activities List (PedHAL) score	*n* = 29 children with HA, all boys, with mean age 8.7 ± 3.51 years (range 4 −15 years) Emicizumab in the form of 3 mg/kg QW for 4 weeks, followed by 3 mg Q2W - followed-up for 6 months	*n* = 17 (58.62 %) negative for inhibitors: There was a significant increase (p-value <0.001) in the PedHAL score in these patients − baseline 61.5 (54.3 − 62.2) versus 6 months after introduction of emicizumab 84.4 (80.25 − 86.35)
Real-world data on bleeding patterns of hemophilia A patients treated with emicizumab	Levy- Mendelovich, S. et al. [29]	Journal of Clinical Medicine	Prospective cohort	Compare the occurrence of breakthrough bleeding at different time points, starting from emicizumab initiation	*n* = 70 HA patients (1 month to 74.9 years - median 14.6 years) that completed at least 18 months of follow-up (*n* = 42 without inhibitors)	Patients without inhibitors (*n* = 42): Mean age = 17.2 (9.2−45.9) years old ABR = 4 (1−12) 19 patients had zero bleeds and 23 patients had at least one bleed The proportion of patients who had at least one episode of traumatic bleeding was not significantly different between patients with versus without FVIII inhibitors (*p*-value = 0.057), as well as the proportion of patients who had at least one episode of spontaneous bleeding (p-value = 0.241)
Real-world experience of emicizumab prophylaxis in young children with hemophilia A: retrospective data from China	Liu, G. et al., [30]	Frontiers in Pediatrics	Retrospective cohort	Report the real-world data of our thirteen hemophiliac boys taking emicizumab for prophylaxis in a center in China	*n* = 13 pediatric patients with HA After the first 4 weeks of the loading period with a dosage of 3 mg/kg QW, they went into maintenance (recommended dosage as 1.5 mg/kg weekly, 3 mg/kg Q2W, or 6 mg/kg Q4W)	Patients without inhibitors (*n* = 7): Reduction of ABR [0.5 (0−3) versus 2 (0−6), *p*-value <0.05] Reduction of AJBR [0 (0−0.5) versus 1 (0−6), *p*-value <0.05] Reduction of Annualized Spontaneous Bleeding Rate (ASBR) [0 (0−0.5) versus 2 (0 −6), *p*-value <0.05]
Real-world use of emicizumab in patients with haemophilia A: Bleeding outcomes and surgical procedures	McCary I. et al., [31]	Haemophilia	Prospective Cohort	Report the experience treating patients with emicizumab, including bleeding rates pre- and post-emicizumab, peri-procedural management and outcomes and serious drug-related AE	*n* = 93 patients with haemophilia A using emicizumab. 74 without an active inhibitor	ABR: dropped from 1.6 (95 % CI: 0.9−2.4) in non-inhibitors to 0.4 (95 % CI: 0.2- 0.6) on emicizumab, *p*-value = 0025 No patient discontinued therapy There were no TE, TMA or deaths No patient developed a clinical loss of efficacy''.

Table 1 (*continued*)

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
Study protocol for assessment of the coagulation potential of concomitantly used factor VIII concentrates in patients with haemophilia A with emicizumab prophylaxis (CAGUYAMA Study): a multicentre open-label non-randomised clinical trial	Takeyama, M. et al., [32]	British Medical Journal	Clinical Trial	Evaluate global coagulation function under treatment with emicizumab concomitantly with FVIII concentrates in patients with AH without inhibitor	*n* = 100 patients ≥4 years old with HA without inhibitors will be enrolled in this study for a maximum duration of 1 year	Ongoing study
The effect of emicizumab prophylaxis on long-term, self-reported physical health in persons with haemophilia A without factor VIII inhibitors in the HAVEN 3 and HAVEN 4 studies	Skinner, MW., et al., [33]	Haemophilia	Cohort	The impact of emicizumab on health-related quality of life (HRQoL) in persons with severe HA without factor VIII (FVIII) inhibitors in the phase 3 HAVEN 3 and 4 studies.	*n* = 176, HA patients without FVIII inhibitors >12 years old.	From baseline, mean (SD) pH scores improved by −9.8 (21.08) points (*n* = 157) at Week 25 and by −12.0 (21.26) points (*n* = 113) at Week 73 The mean (SD) Treatment change from baseline was −18.3 (17.48) at Week 25 (*n* = 157) and −17.9 (17.81) at Week 73 (*n* = 113) Mean (SD) TS improved by −8.1 (12.73) points (*n* = 157) at Week 25 and by −8.6 (12.57) points (*n* = 113) at Week 73 54 % had a clinically meaningful improvement at Week 73 In participants who had ≥9 bleeds before the study, the mean change from baseline to Week 73 was −16.9 (21.35), which is a larger physical health improvement than in those with <9 bleeds (−6.2 [19.81]) With emicizumab, fewer employed participants missed workdays than in the 28 days prior to study enrolment (9.1 % versus 25 %). No change over time was detected by the EQ-5D-5 L questionnaire.

Table 1 (*continued*)

Title	Author(s) and year	Journal	Type of study	Objective	Participants and groups	Results**
The emicizumab switch: real-world data of 251 pediatric patients from the PedNet Registry	van der Zwet, K. et al., [34]	64th ASH Annual Meeting	Prospective Cohort	Report on bleeding and safety in children and adolescents with HA receiving emicizumab prophylaxis in a large prospective multicenter cohort study.	*n* = 251 from ongoing PedNet Registry 2022, 94 % severe HA with no other coagulopathies, <18 years old at start of emicizumab in maintenance therapy of emicizumab	The ABR and AJBR significantly improved during emicizumab therapy in patients without inhibitors. In patients without inhibitors the mean ABR reduced from 2.8 prior to emicizumab to 1.1 during emicizumab (*p*-value <0.001) AJBR reduced from 0.8 to 0.3 (*p*-value <0.001). Serious AEs included 1 death unrelated to emicizumab therapy (retroperitoneal bleed in a baby treated with LMWH for CVL thrombosis) 1 patient developed ADAs without breakthrough bleeding, who continued emicizumab therapy. AEs were all related to local injection site reaction in 6 patients No TMA or TE were observed
Untreated bleeds in people with hemophilia A in a noninterventional study and intrapatient comparison after initiating emicizumab in HAVEN 1–3	Callaghan, MU. et al., [35]	Research and Practice in Thrombosis and Haemostasis	Prospective Cohort	Determine incidence of untreated bleeds during a noninterventional hemophilia A study with or without FVIII inhibitors	*n* = 221 Group A (*n* = 103): adults/adolescents (age ≥12 years) with FVIII inhibitors; Group B (*n* = 24): children (aged <12 years) with FVIII inhibitors Group C (*n* = 94): adults/adolescents without FVIII inhibitors.	Untreated bleeds: 433 (26.2 % of bleeds) in Group C, especially ankle (*n* = 72; 30.8 %) Spontaneous bleeds: 35.8 % - Group C Surgery/procedural untreated bleeds: 15.2 % - Group C No change was seen in untreated bleeds in Group C taking prophylaxis (5.9 [2.43 −14.12] for FVIII in the NIS versus 5.7 [2.47 −13.22] for emicizumab in HAVEN 3) A notable increase in untreated bleeds associated with surgeries/procedures in HAVEN 3 compared with Cohort C in the NIS (44.7 % versus 2.7 %).

QW, every week; Q2W, every two weeks; Q4W, every four weeks; 95 % CI, 95 % confidence interval; ABR, annualized bleeding rate; BE, bleeding events; AE, adverse event; AJBR, annualized Joint bleeding rate; CFC, coagulation factor concentrate; ADA, anti-drug antibody; TE, thrombotic event; TMA, thrombotic microangiopathy; PK, pharmacokinetics; LMWH, low molecular weight heparin; CVL, central venous Line.
Source: the authors.

in patients' overall health and a reduction in chronic pain after one year of emicizumab therapy. This study emphasized the real-world effectiveness of emicizumab, as evidenced by the patients' enhanced quality of life and reduced bleeding episodes.

Nogami et al. [12] and Zwer et al. [13] extended these findings to larger cohorts, further substantiating the efficacy of emicizumab. The study by Nogami et al. [12] reported a median ABR of 0.91, with over half of the patients experiencing no bleeding events during the observation period. Whereas the study by Zwer, part of the PedNet registry, highlighted a significant reduction in mean ABR from 2.41 to 1.11 after emicizumab initiation, along with a decrease in the Annualized Joint Bleeding Rate (AJBR). These results consistently demonstrate the superior efficacy of emicizumab in preventing both overall and joint-specific bleeding episodes in pediatric populations.

Safety

Safety is a paramount concern in the management of hemophilia A, and the reviewed studies collectively affirm the favorable safety profile of emicizumab. Shima et al. [9] documented 133 adverse events, primarily minor, such as contusions and nasopharyngitis, with no reports of thrombotic events or thrombotic microangiopathy. The absence of anti-emicizumab antibodies further underscores the drug's safety.

Oka et al. [11] reported adverse events in 42.1 % of the cohort, mainly localized to the infusion site, aligning with findings from Nogami et al. [12] and Zwer et al. [13], where injection-site reactions were the most common adverse events. Importantly, no serious adverse events directly linked to emicizumab were reported, reinforcing its safety.

The PedNet registry also reported minimal adverse events, with no life-threatening bleeds or thrombotic complications [13]. The presence of non-neutralizing anti-drug antibodies in one patient did not result in clinical efficacy loss, indicating the rarity and limited clinical impact of such occurrences.

Patient satisfaction

High patient satisfaction is a critical outcome, influencing adherence and overall treatment success. Oka et al. [11] highlighted a high satisfaction rate among patients, with a mean satisfaction score of 9.1 out of 10 after one year of emicizumab therapy. Patients reported significant improvements in general health and a strong preference for continuing emicizumab over previous treatments, underscoring the positive impact on their quality of life.

Kempton et al. [15] reported similarly high satisfaction scores, with the majority of patients expressing contentment with the efficacy of emicizumab and the reduced need for frequent infusions. This reduced treatment burden is particularly advantageous, as it addresses the logistical and psychological challenges associated with frequent intravenous administrations of factor VIII.

Implications for the clinical practice

This systematic review highlights emicizumab as a transformative treatment for hemophilia A patients without factor VIII inhibitors. Its efficacy in significantly reducing ABR and AJBR, coupled with a favorable safety profile and high patient satisfaction, positions emicizumab as a superior alternative to traditional factor VIII therapies.

The reduction in bleeding episodes not only improves clinical outcomes but also enhances the patient quality of life by alleviating chronic pain and reducing the need for invasive treatments. The ability to administer emicizumab subcutaneously further simplifies treatment regimens, promoting better adherence and reducing healthcare resource utilization.

While the evidence supporting the efficacy and safety of emicizumab is compelling, there are limitations to consider. The variability in study designs, sample sizes, and follow-up durations across the reviewed studies may introduce heterogeneity in the findings. Additionally, long-term safety data, particularly concerning the development of anti-drug antibodies and potential thrombotic complications, require further investigation.

Future research should focus on long-term, real-world studies to monitor the sustained efficacy and safety of emicizumab over extended periods. Studies exploring the impact of emicizumab on specific subgroups, such as infants and elderly patients, would also provide valuable insights into its broader applicability.

Conclusion

In conclusion, emicizumab represents a significant advancement in the treatment landscape for hemophilia A patients without factor VIII inhibitors. This systematic review confirms its efficacy in reducing bleeding episodes, its favorable safety profile, and high patient satisfaction. These findings position emicizumab as a superior alternative to traditional factor VIII therapies, offering a more convenient and effective treatment option. As research continues to elucidate its long-term impact, emicizumab is poised to become a cornerstone in the management of hemophilia A, transforming the lives of patients worldwide.

The insights gained from this systematic review provide valuable guidance for clinicians and researchers, emphasizing the importance of continued evaluation and optimization of hemophilia A therapies. The success of emicizumab underscores the potential for innovative therapeutic approaches to significantly improve patient outcomes and quality of life in rare bleeding disorders.

Conflicts of interest

The authors declare no conflicts of interest.

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Original article

Effect of testosterone on blood-clotting markers in transsexual men

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ABSTRACT

Background: The use of testosterone in gender-affirming hormone therapy for trans men is associated with several adverse effects. However, research on the risk of venous thromboembolism in this treatment remains limited and inconclusive. This study aimed to assess the impact of intramuscular testosterone on specific direct and indirect blood-clotting markers in trans men.

Method: Treatment of trans men without previous use of testosterone was followed up in a prospective observational study in a trans people healthcare service. Gender-affirming hormone therapy was initiated with intramuscular testosterone cypionate (Depo-Testosterone). The blood-clotting markers prothrombin time, activated partial thromboplastin time, D-dimer, antithrombin, and factors VIII and VII were evaluated before and 12 weeks after starting the medication.

Results: Nineteen trans men with a mean age of 23.7 ± 3.7 years were enrolled. After 12 weeks of hormone therapy, significant increases in weight (p-value = 0.002) and body mass index (p-value = 0.007) were observed in patients. Furthermore, there were significant increases of 830 % in serum testosterone (p-value = 0.000), 7 % in hemoglobin (p-value = 0.000) and 10 % in hematocrit (p-value = 0.001). Conversely, a 10 % decrease in high density lipoprotein cholesterol levels (p-value = 0.000), and 15 % decrease in Factor VII (p-value = 0.000) were detected.

Conclusion: Intramuscular testosterone in trans men was associated with increases in hematocrit, hemoglobin, and the body mass index, and decreases in high density lipoprotein cholesterol and Factor VII. Nevertheless, these variables remained within normal reference values. Long-term follow-up studies evaluating gender-affirming hormone therapy with testosterone are needed to determine adequate risk management of venous and arterial thromboembolism in this population.

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Introduction

Gender-affirming hormone therapy (GAHT) for transgender men involves the administration of exogenous testosterone to promote the development of standard male characteristics. Desired characteristics include increased body and facial hair, deepening of the voice, increased muscle mass and reduced fat mass, as well as blocking the menstrual cycle and enlarging the clitoris.[1] In the laboratory setting, GAHT for trans men aims to elevate serum testosterone and reduce estradiol concentrations to achieve masculinizing levels.[2] Although these medications are considered relatively safe in the short and medium term,[3] GAHT is associated with adverse effects that may pose risks to the health of this population.[4]

The most common adverse effects attributable to testosterone use include erythrocytosis,[5] acne,[6] hair loss, increased sexual desire, temporary or permanent reduction in fertility,[2] and alterations in the lipid profile, with a decline in high-density lipoprotein cholesterol (HDL-c) and increases in both triglycerides (TG) and low-density lipoprotein cholesterol (LDL-c).[4] There is also a reduction in adiponectin levels associated with insulin resistance, with a greater predisposition to type II diabetes in this population.[7] Another concern is the increased risk of thromboembolism associated with testosterone use. However, research on this topic is limited in the literature, especially in the trans men population.[8,9]

The largest study available in the literature showed that the hazard ratio of venous thromboembolism (VTE) in trans men compared to cisgender (*cis*) men was 1.6 (95 % confidence interval [95 % CI]: 0.9–2.9) in the total group and 2.7 (95 % CI: 0.6–12.1) in the group starting GAHT with testosterone.[10] Although the observed increases in the risk of VTE from 60 % to 270 % were not statistically significant,[10] they may hold clinical relevance, especially in this population, which presents other risk factors for VTE, such as a higher prevalence of smoking and erythrocytosis.[11] It is important to highlight that the number of trans men that had VTE in these studies was considerably small, a fact that makes it difficult to draw an adequate conclusion regarding the risk of VTE in transgender men.[9]

In the absence of studies with a suitable sample size to reach a definitive conclusion about the risk of VTE in trans men, the effects of testosterone on blood clotting may be useful for generating hypotheses regarding the role of testosterone in hemostasis and in the risk of VTE. A recent study analyzed fibrinogen, specific blood clotting factors (FII, FIX, and FXI), natural anticoagulants (protein S and protein C), resistance to activated protein C, and hematocrit in trans men.[8] According to the authors, the use of testosterone, regardless of the route of administration (transdermal or intramuscular) for 12 months was not associated with relevant procoagulant alterations.[8] However, it is important to note that some clotting markers have not been evaluated in trans men using testosterone.

Given the lack of conclusive information regarding the risk of VTE and the paucity of studies on the effects of testosterone on hemostasis in this population, the aim of the present study was to assess the impact of intramuscular testosterone use on the hemostatic system of trans men 12 weeks after initiating GAHT through the quantification of blood-clotting markers.

Methods

Study design and settings

This study employed a prospective observational cohort design, enrolling trans men without previous use of testosterone, and was carried out in a trans people healthcare service at the Clinics Hospital and the "Saúde Escola" Center of Ribeirão Preto Medical School, University of São Paulo, Brazil. The study participants were selected by convenience sampling, enrolling all eligible patients who wished to start GAHT with testosterone. The studied period was from December 2021 to December 2022.

Compliance with ethical standards

The present research project was approved by the Research Ethics Committee of the Clinics Hospital of the Ribeirão Preto Medical School. The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants included in the study.

Participants

The participants were recruited by the main researcher (ETSR), who contacted the participants prior to the start of GAHT, explained the objectives of the study, and invited them to participate. Individuals aged from 18 to 40 years, with female genitalia and male gender identity, and who desired to initiate GAHT with testosterone were considered eligible for the study. The exclusion criteria were: smoking, history of current or previous arterial or venous thromboembolic disease, body mass index (BMI) $\geq$30 kg/m^2, contraindication to testosterone use, withdrawal from participating in the study or discontinuation of treatment.

Methodology and variables

After informed consent, the recruited patients attended an initial medical evaluation before starting GAHT. During this visit, the researchers collected sociodemographic and clinical data and measured the participants' weight and height to determine their body mass index (BMI).

A total of 20 mL of fasting venous blood was collected to measure the clotting times (prothrombin time - PT; International Normalized Ratio - INR; and activated partial thromboplastin time - APTT), clotting markers (activity of coagulation factors VII and VIII), the fibrin turnover marker (D-dimer), and the anticoagulation marker (antithrombin), as well as tests to monitor GAHT safety (lipid profile, hemogram, total testosterone, and estradiol).

After the initial evaluation, intramuscular testosterone cypionate (Depo-Testosterone) was prescribed every 21 days. To minimize and detect potential losses to follow-up, the participants were contacted biweekly by phone to ensure proper testosterone usage and to remind them about the tests scheduled for 12 weeks after starting GAHT.

The enzymatic method was adopted to measure total cholesterol (TC), HDL-c, LDL-c, and TG levels. The LDL-c was calculated using the Friedewald formula: LDL-c = TC - (HDL-c + TG/5), provided that the values were <400 mg/dL.[12] Testosterone and estradiol levels were measured using the radioimmunoassay method.[13]

Meanwhile, in order to assess the clotting factors, venous blood was collected in tubes containing sodium citrate and centrifuged for 15 min at room temperature for platelet removal. The plasma supernatant was stored at −35 °C in 0.2-mL aliquots for later analyses, at which time the frozen plasma samples were thawed directly in a water bath at 37 °C for at least 15 min and mixed by shaking before use.

The PT was determined using the photo-optical method with the STA-Neoplastine CI reagent.[14] The INR was calculated using two major PT 'correction factors', the mean normal PT and the international sensitivity index.[15] The APTT was also determined using the photo-optical method, but with the STA-PTT reagent,[16] which establishes the partial time of thromboplastin activation. This method is used to analyze clotting factors XII, XI, IX, X, V, II, and I.

Factor VIII was assessed by coagulometry using the STA-ImmunoDef VIII reagent.[14] Factor VII was also measured through coagulometry, using an automated coagulometer (Instrumentation Laboratory, United Kingdom).[14] Additionally, plasminogen activator inhibitor was evaluated using a chromogenic substrate assay.[14]

Antithrombin was measured using the chromogenic method by way of STA-Stchrom ATIII cleavage.[14] The determination of D-dimer levels was conducted via immunoturbidimetry using the Imubind Dimer Test Stripwell EIA Kit.[17]

Statistical analyses

Statistical analyses were conducted using the R software, version 4.2.2 (R Foundation for Statistical Computing), with the significance level set at p-value <0.05. Quantitative variables are summarized using measures of central tendency and dispersion. In order to detect possible statistical differences regarding the quantitative variables, the Wilcoxon non-parametric test for paired samples was used, as the variables did not present parametric distribution.

Results

Sixty-four patients were recruited from December 2021 to December 2022. In the initial evaluation before starting GAHT, 41 patients were excluded from the study for the following reasons: uncertainty in the diagnosis of gender incongruity (n = 2); indecision regarding starting testosterone treatment (n = 2); previous use of testosterone (n = 1); smoking (n = 35); and withdrawal from participating in the study (n = 1). Thus, 23 patients were considered eligible and consented to participate. After the beginning of GAHT, four patients were excluded based on the following criteria: initiation of smoking (n = 2), and discontinuation of testosterone on their own (n = 2). Finally, 19 patients were included in the study and had a second clinical evaluation and blood collection 12 weeks after starting testosterone cypionate (Depo-Testosterone) treatment (Figure 1).

The mean age (± standard deviation) of the participants was 23.7 ± 3.7 years. Of the participants included in the study,

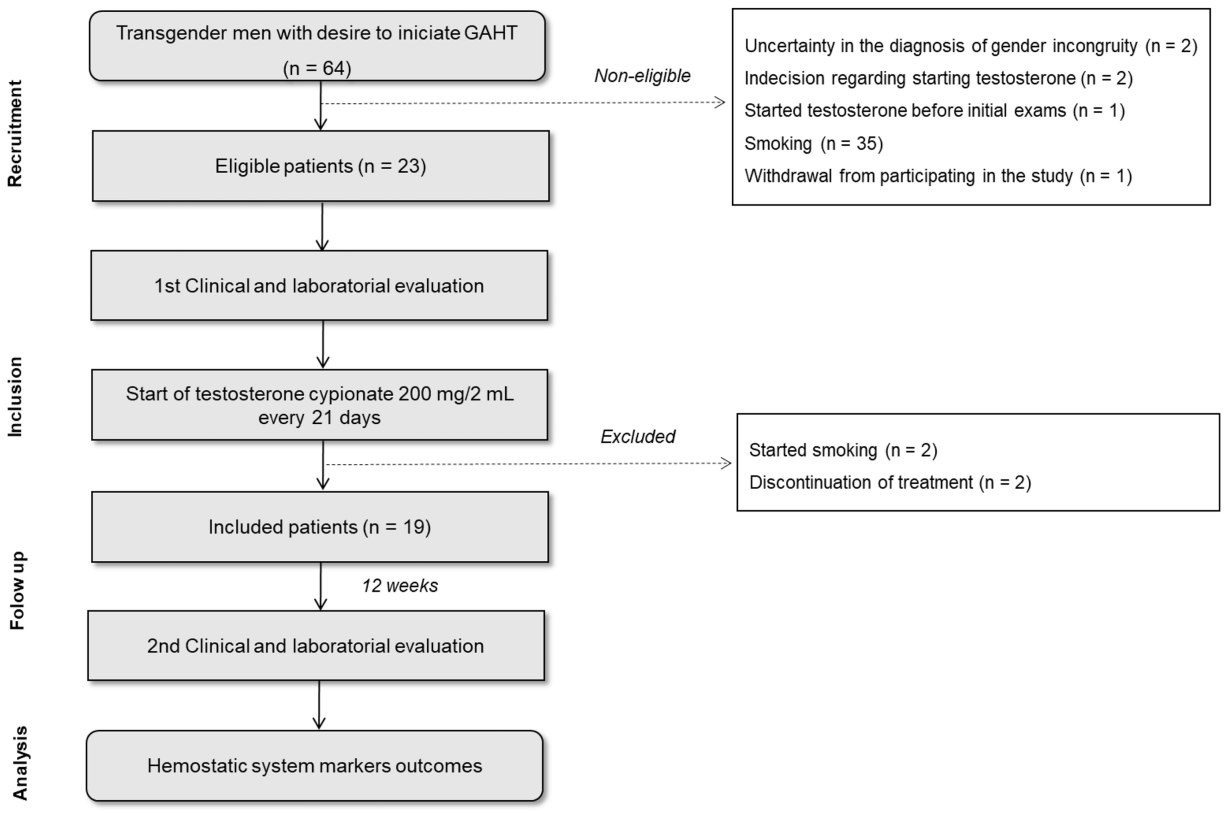

Figure 1 – Flowchart of the study.

Table 1 – Clinical and laboratory characteristics of transgender men before and 12 weeks after starting gender-affirming hormone therapy with testosterone.

Variable	Basal (Mean ± SD)	12 weeks (Mean ± SD)	*p*-value[a]
Age (years)	23.7 ± 3.74	n/a	
Weight (kg)	60.7 ± 9.16	63.62 ± 8.5	0.002
Height (m)	1.63 ± 0.05	n/a	
BMI (kg/m^2)	22.9 ± 3.7	23.94 ± 3.6	0.007
Total cholesterol (mg/dL)	166.1 ± 33.7	163.9 ± 29.3	0.30
HDL (mg/dL)	51.9 ± 14.0	46.3 ± 11.2	0.006
LDL (mg/dL)	97.3 ± 33.7	99.6 ± 28.5	0.71
Triglycerides (mg/dL)	84.9 ± 37.7	89.4 ± 49.5	0.86
Testosterone (ng/dL)	58.2 ± 28.7	483.6 ± 324.6	<0.001
Estradiol (pg/mL)	78.0 ± 61.9	57.3 ± 47.8	0.08

SD: standard deviation; HDL: high-density lipoproteins; LDL: low-density lipoproteins; n/a: not applicable.

[a] Wilcoxon non-parametric test.

five reported having comorbidities, including one case of type I diabetes mellitus and hypothyroidism (under use of insulin and levothyroxine (Levoxyl)), one case of dyslipidemia (without medication use), and two cases of depressive mood disorder taking psychoactive medications (sertraline, risperidone (Risperdal), and venlafaxine). In terms of contraceptive use, three participants were using intramuscular depot medroxyprogesterone acetate (Provera) every three months and one participant was using a copper intrauterine dispositive. None of the participants were using combined hormonal contraceptives.

The clinical and laboratory characteristics of the study participants are shown in Table 1. After 12 weeks of testosterone cypionate (Depo-Testosterone) use, there was a body weight gain of approximately 5 % (*p*-value = 0.002). An increase of 830 % was also observed in serum testosterone levels (p-value <0.001), as well as a 27 % reduction in estradiol (p-value = 0.08), and reduction in HDL-c, of around 10 % (*p*-value <0.001).

Table 2 shows the levels of blood-clotting markers before and after 12 weeks of GAHT with testosterone. Notably, there was a 7 % increase in hemoglobin (*p*-value <0.001), a 10 % rise in the hematocrit (p-value = 0.001), and a 5 % increase in INR (*p*-value = 0.046). A 15 % reduction in clotting factor VII was also observed (*p*-value <0.001), while the remaining variables showed no significant changes.

Discussion

After 12 weeks of GAHT, a statistically significant reduction in clotting factor VII activity was observed, as well as statistically significant increases in hemoglobin and hematocrit. Nonetheless, these alterations remained within normal values. Meanwhile, the remaining markers of the hemostatic system did not show significant changes.

The activation of blood coagulation initiates with the formation of a complex between the tissue factor (TF) and the activated factor VII (FVIIa) composed of a serin protease with procoagulant properties.[18] The 15 % reduction in factor VII activity, a deficiency which predisposes to the risk of hemorrhagic disorders,[18] may contribute to an anticoagulant effect. In line with this hypothesis, a recent study evaluating blood clotting in 100 trans men before and after 12 months of GAHT evidenced an increase in factor IX activity and in the hematocrit, suggesting procoagulant alterations.[8] On the other hand, in the same study, there were reductions in factor II and factor XI activity, in addition to increased levels of natural anticoagulants (protein S and activated protein C), which may have counterbalanced the procoagulant effect.[8] Prospective studies could be designed to evaluate whether there is a correlation between reduced coagulation factors and erythrocytosis in trans men.

The relationship between coagulation factor VII deficiency,[19] the activity of factors II, V, and X, and fibrinogen and the prolongation of the INR has already been documented in the literature.[15] The PT is a single-stage screening test used to assess the TF and overall coagulation that is influenced by the activity of coagulation factors (II, V, VII, X) and fibrinogen.[15] The prolongation of PT can be caused by deficiencies in one or more coagulation factors or may indicate the presence of coagulation factor inhibitors.[15] Corroborating a previous report[1] in which the use of testosterone in trans men was analyzed for over one year of follow-up, the present study observed an increase of 5 % in INR. However, no significant change in PT values was noted.

Among the possible procoagulant effects observed in this study, there was a 10 % increment in the hematocrit after 12 weeks of testosterone use. An elevated hematocrit indicates an increase in blood viscosity.[20] This alteration could potentially reduce the venous return and elevate the cardiovascular risk; however, due to the lack of specific studies in this population, the parameters used refer to cisgender individuals. In one study involving *cis* men, a 5 % increment in the hematocrit increased the risk of VTE by 33 % (odds ratio: 1.33; 95 % CI: 1.05–1.70),[20] while in *cis* women, this increase in hematocrit was not associated with increased risk when adjusted for age, BMI, and smoking status. It is noteworthy that trans men are at greater risk of elevated hematocrit due to the high prevalence of smoking.[21] On the other hand, the actual relationship between erythrocytosis and VTE in the general population is still debated in the scientific literature,[8] and there is still not enough data to determine whether or not erythrocytosis resulting from GAHT contributes to an increased risk of VTE in trans men.[22]

Table 2 – Laboratory evaluation of the hemogram and hemostatic system markers of transgender men before and after gender-affirming hormone therapy with testosterone.

Variable	Basal (Mean ± SD)	12 weeks (Mean ± SD)	*p*-value[a]
Hemoglobin (g/dL)	13.8 ± 1.3	14.9 ± 1.6	<0.001
Hematocrit (%)	42.0 ± 4.1	46.2 ± 4.9	0.001
Platelets (x10^3/μL)	289.4 ± 67.5	278.8 ± 69.8	0.55
D-dimer (μg/L)	0.3 ± 0.2	0.2 ± 0.1	0.31
Factor VII (%)	73.5 ± 23.5	62.4 ± 15.8	<0.001
Factor VIII (%)	127.7 ± 43.7	122.9 ± 36.3	0.65
Antithrombin (%)	106.7 ± 9.6	108.0 ± 7.8	0.43
APTT (seconds)	30.74 ± 3.24	32.37 ± 5.36	0.1183
PT (seconds)	12.06 ± 1.0	12.34 ± 1.09	0.1162
INR	1.00 ± 0.08	1.05 ± 0.13	0.046

SD: standard deviation; INR: International Normalized Ratio; APTT: activated partial thromboplastin time; PT: prothrombin time.
Reference values: D-Dimer: ≤0.5 μg/L; Factor VII: 50–129 %; Factor VIII: 50–150 %; Antithrombin: 80–120 %.

[a] Wilcoxon non-parametric test.

A systematic review conducted in 2021 showed that the incidence of VTE in trans men using testosterone was 10.8 in every 10,000 patients per year.[9] This frequency is comparable to the rates seen in cisgender men undergoing hormone replacement therapy with testosterone. According to the authors of that review, the majority of current findings do not support an association between GAHT and testosterone and an increased risk of VTE.[9]

In the present study, regarding the lipid profile, after 12 weeks of starting GAHT with testosterone, a nearly 10 % reduction in HDL-c was observed in relation to the mean of the participants (*p*-value = 0.006). Conversely, the other lipid profile parameters, such as total cholesterol, LDL-c, and TG, did not show marked changes. This decrease in HDL-c is in line with a study carried out in 2017[23] that investigated the effects of GAHT with testosterone on the lipid profile of trans men, in which the authors noted a reduction in HDL-c and an increase in LDL-c and in TG levels. However, the mechanism by which testosterone negatively impacts the lipid profile remains unknown.[24] During the 12-week period, there was also an increase in body weight, on average, of approximately 3 kg among the participants and, consequently, an increase in BMI. The reduction of 1 mg/dL of HDL-c is correlated with an increase of 2–3 % in cardiovascular incidents.[25,26]

Together with data from the literature, the findings of this study point to the hypothesis that the use of GAHT is not associated with procoagulant alterations. Therefore, it does not seem to be through altering hemostasis that GAHT increases the risk of VTE. Further research is necessary to determine whether increments greater than 5 % in the hematocrit of trans men undergoing GAHT would be capable of increasing the risk of VTE due to elevated blood viscosity. Regarding arterial thrombosis, one study reported a 3.7-fold increase in the risk of acute myocardial infarction in trans men compared to *cis* women.[27] The changes in the lipid profile caused by GAHT, with the reduction of HDL-c, may be implicated in this risk.

The present study should be interpreted in light of some limitations, namely the limited number of participants, the lack of an untreated control group, its observational design, and the short observation period. However, if even in small studies we have still not found a sign that GAHT can cause procoagulant alterations, the question arises as to what is the advantage of using more financial resources for randomized and controlled studies to evaluate this hypothesis. The exclusion of smokers in the present study represents a limitation, as it restricts the generalizability of the findings to the broader population of trans men undergoing GAHT. The high prevalence of smoking in the trans population has already been documented in the literature. When compared to their cisgender counterparts, transgender individuals are 2.7 times more likely to consume cigarettes throughout their lives.[28] Future clinical studies should consider including participants with diverse clinical profiles, including individuals who smoke, to better capture the variability of hemostatic responses. Additionally, expanding the sample to include trans men in different clinical conditions would provide a more comprehensive understanding of the potential cardiovascular and hemostatic effects of hormone therapy across heterogeneous populations.

Conclusion

GAHT with testosterone in 12 weeks of observation promoted an increase in hematocrit, hemoglobin, weight, and BMI, as well as a reduction in HDL-c and clotting factor VII; however, these alterations remained within the normal limits for the variables analyzed. Considering the increased hematocrit and the reduced HDL-c, it might be more pertinent to invest in studies that evaluate risk factors for arterial thrombosis in trans men. Finally, long-term follow-up studies of trans individuals are necessary to determine the actual risk of venous and arterial thromboembolism in this population and provide adequate risk management after the start of GAHT.

Conflicts of interest

The authors declare no conflicts of interest.

CRediT authorship contribution statement

Estella Thaisa Sontag dos Reis: Investigation, Formal analysis, Writing – original draft. **Carla Maria Franco Dias:** Writing – review & editing. **Carolina Sales Vieira:** Investigation, Writing – review & editing. **Mariane Nunes Nadai:** Conceptualization, Methodology, Supervision. **Sérgio Henrique Pires Okano:** Investigation, Writing – review & editing. **Silvio Antônio Franceschini:** Investigation, Writing – review & editing. **Lúcia Alves da Silva Lara:** Conceptualization, Methodology, Supervision.

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Original article

There is no transfer of mitochondria from donor hematopoietic cells to recipient mesenchymal stromal cells after allogeneic hematopoietic stem cells transplantation in humans

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ABSTRACT

Introduction: Multipotent mesenchymal stromal cells are progenitors of the bone marrow stromal microenvironment that support hematopoiesis. Mitochondria, which can be transferred between cells via nanotubes or extracellular vesicles, play a key role in the functions of mesenchymal stromal cells. In a murine model, donor hematopoietic stem and progenitor cells transfer functional mitochondria to bone marrow mesenchymal stromal cells of the recipient. The aim of this study was to find out whether such transfer occurs in humans after allogeneic hematopoietic stem cell transplantation.

Methods: This study included nine patients with acute leukemia who received a reduced intensity conditioning regimen. Donor hematopoietic stem and progenitor cells mobilized into peripheral blood were the source of transplanted stem cells. Total DNA was isolated from bone marrow mesenchymal stromal cells of each patient before and after transplantation and their respective donors' leukocytes. A fragment of mitochondrial DNA including the full-length control region was sequenced. The mitochondrial DNA sequence of each patient's mesenchymal stromal cells was compared before and after the procedure and with the respective donor leukocytes.

Results: Donor mitochondrial DNA was not detected in the mesenchymal stromal cells of any patient after transplantation even as trace amounts. Co-culturing donor leukocytes with intact and irradiated mesenchymal stromal cells *in vitro* did not lead to detection of donor mitochondrial DNA transfer.

Conclusion: The data show that there is no mitochondrial transfer from donor hematopoietic stem and progenitor cells to recipient mesenchymal stromal cells after

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transplantation. Thus, the results indicate that one cannot count on improved mesenchymal stromal cell metabolism due to mitochondrial transfer. It is necessary to look for other ways to restore the stromal microenvironment.

Introduction

Multipotent mesenchymal stromal cells (MSCs) support hematopoiesis and exhibit strong immunomodulatory properties due to the secretion of growth factors, cytokines, and extracellular vesicles. MSCs and their extracellular vesicles (MSC-EVs) are widely tested in clinical trials. For example, MSCs have been used to treat critically ill patients with sepsis,[1] COVID-19 pneumonia,[2] and for the prevention of graft-versus-host disease.[3] MSC-EVs can exert immunomodulatory effects in multiple sclerosis, rheumatoid arthritis, and type 1 diabetes, and are also effective in cardiac, hepatic, and renal regeneration.[4] MSC-EVs can deliver mRNA, miRNA, cytokines, and growth factors to target cells and some contain mitochondria that are transferred to recipient cells.[5]

The role of mitochondria is not limited to energy production: they are essential for redox balance maintenance, apoptosis regulation and metabolic programming and thus have a significant impact on cell fate and functionality. Mitochondria dysfunction disrupts tissue integrity causing a number of human diseases.[6] Their activity is important for many facets of stem cell fate, including quiescence, proliferation and differentiation.[7] In recent years, it has been found that mitochondria play a key role in regulating different functions of MSCs and are a putative target for various therapies.[8]

During cell division, mitochondria are transmitted from parent to daughter cells. Over the past two decades, an ever-growing body of evidence was accumulated that some cell types can export mitochondria via nanotubes[11] or extracellular vesicles[12] to unrelated cells in a process called horizontal mitochondrial transfer.[9,10] Mitochondrial exchange is believed to be an essential form of intercellular signaling involved in homeostasis, stress response and immune reactions.[13]

MSCs and their mitochondria are currently in the spotlight due to their clinical relevance and therapeutic potential. MSCs have been found to donate mitochondria to vascular smooth muscle cells, neurons, lymphocytes, macrophages and other cell types, including a variety of malignant cells.[14] In the bone marrow, stromal cells transfer mitochondria to hematopoietic progenitors as a response to acute infection, boosting their expansion and differentiation to leukocytes.[15]

On the other hand, MSCs-derived mitochondria support not only normal hematopoietic cells, but also leukemic cells, contributing to progression and therapy resistance.[16,17,18] MSCs primed by reactive oxygen species-inducing agents actively transferred mitochondria and were able to mitigate oxidative stress in co-cultured acute lymphoblastic leukemia (ALL) cell lines and in a murine ALL model.[19] The importance of mitochondria for this was highlighted by the fact that mitochondrial depletion or prevention of mitochondrial transfer abolished the rescue.[17] The therapeutic efficacy of MSCs appears to be closely tied to their ability to donate Mitochondria as well as to mitochondrial activity.[20]

Despite the fact that most studies focus on their ability to donate mitochondria, MSCs can be on the receiving end of the mitochondrial transfer as well. In a murine model, donor hematopoietic stem and progenitor cells (HSPCs) transfer functional mitochondria to the recipient bone marrow MSCs after allogeneic hematopoietic stem cell transplantation (allo-HSCT).[21] Notably, MSCs (but not other stromal or endothelial bone marrow cells) demonstrated a dramatic loss of mitochondrial mass after total body irradiation.[22] After mitochondrial transfer, both host stromal microenvironment recovery and donor HSPC engraftment were improved, resulting in better hematopoiesis reconstitution. These findings demonstrate that donor HSPCs not only restore the hematopoietic system after allo-HSCT, but also induce and support the recovery of the irradiated microenvironment via mitochondrial transfer.

HSPC-to-MSC transfer of mitochondria was also observed after *in vitro* co-cultures; however, the efficiency was much lower, possibly because of the shorter time or the absence of *in vivo* signals triggering the mitochondrial exchange.[21] *In vitro* co-culture of murine MSCs with both murine and human $CD34^+$ HSPCs led to similar rates of mitochondrial transfer.

Reciprocal mitochondrial transfer between host and recipient HSPCs was observed in a murine model using mitochondrial-nuclear exchange mice (hybrid mice with nuclear DNA from C57BL and mitochondrial DNA from C3H/HeN strains).[23]

Studying horizontal mitochondrial transfer presents certain challenges. Most studies use Mitotracker Red (or similar potential-based dyes) as an mitochondrial marker. However, recently this approach was proven unreliable by Chen et al.[14] The authors showed that tracking mitochondria with GFP-fused mitochondrial proteins results in the detection of mitochondrial transfer in significantly fewer cases than utilizing Mitotracker. Furthermore, staining of Mt-deficient cells convincingly proved that potential-based mitochondrial dyes are not specific enough and can lead to false-positive results.[14]

Objective

The aim of this work was to find out whether mitochondria are transferred from transplanted HSPCs to the recipient MSCs after allo-HSCT in humans. In order to avoid potential-based mitochondrial dyes, mtDNA Sanger sequencing was used as a highly specific method suitable for low copy detection of donor mtDNA in the recipient MSCs.

Method

Patients

This study was approved by the ethics committee of the Federal State Budgetary Institution National Medical Research Center for Hematology of the Ministry of Health of the Russian Federation (protocol No 171 dated April 27, 2023) and the donors and patients provided written informed consent before inclusion. The samples were obtained in accordance with the Declaration of Helsinki.

The study analyzed the data of nine patients with acute leukemia before and one month after allo-HSCT (Supplementary Table 1). In all cases, hematopoietic stem cells mobilized into peripheral blood were used (total $CD34^+$ cell dose $>6 \times 10^6$ cells/kg). The patients received a reduced intensity conditioning regimen (fludarabine, busulfan). The point of analysis after allo-HSCT was chosen as it matches the average time of donor hematopoiesis reconstitution (number of leukocytes in peripheral blood $>1 \times 10^3$ cells/μL).

Experimental design

To test the transfer of Mt, MSCs from patient bone marrow were studied before and one month after allo-HSCT. The patient bone marrow was obtained during a diagnostic puncture, and MSC culture was established (Figure 1A). In addition, patient or third-party donor confluent MSCs were co-cultured *in vitro* with donor $CD45^+$ cells - leukocytes (10^6- 5×10^6 cells per six-well plate: Figure 1B) for four days, and then the mitochondrial composition of the MSCs was analyzed. In some experiments, third-party donor MSCs were irradiated at a dose of 8 Gy using a gamma irradiator BioBeamGM 8000 (Gamma-Service Medical, Germany) at a dose rate of 1.4 Gy/min. The irradiation regimen was chosen in order to approximate the doses used *in vivo* to the *in vitro* model.

After co-culture MSCs were washed from leukocytes and passaged two more times to avoid the mtDNA of donor leukocytes being included in the analysis.

A. Analysis for the *in vivo* mitochondrial transfer from donor hematopoietic stem and progenitor cells (HSPCs) to patient mesenchymal stromal cells (MSCs)
   The bone marrow of patients was collected prior to the allogeneic hematopoietic stem cell transplantation (allo-HSCT) procedure, and the MSCs were isolated and screened for matching MSC criteria. The individual mitochondrial gene markers were analyzed at the second passage. The donor lymphocytes were also analyzed for individual mitochondrial gene markers. The bone marrow was collected during a diagnostic procedure one month after the allo-HSCT, and the MSCs were obtained for analysis, with the mitochondrial gene markers being analyzed at the second passage.
B. Analysis for the *in vitro* mitochondrial transfer from leukocytes to MSCs
   The patient MSCs at the second passage were cultured with donor lymphocytes for a period of four days. Subsequently, the lymphocytes were removed, MSCs were washed and passaged. The mitochondrial gene markers in the MSCs were examined. In certain experiments, the MSCs from a third-party donor were exposed to a dose of 8 Gy of radiation.

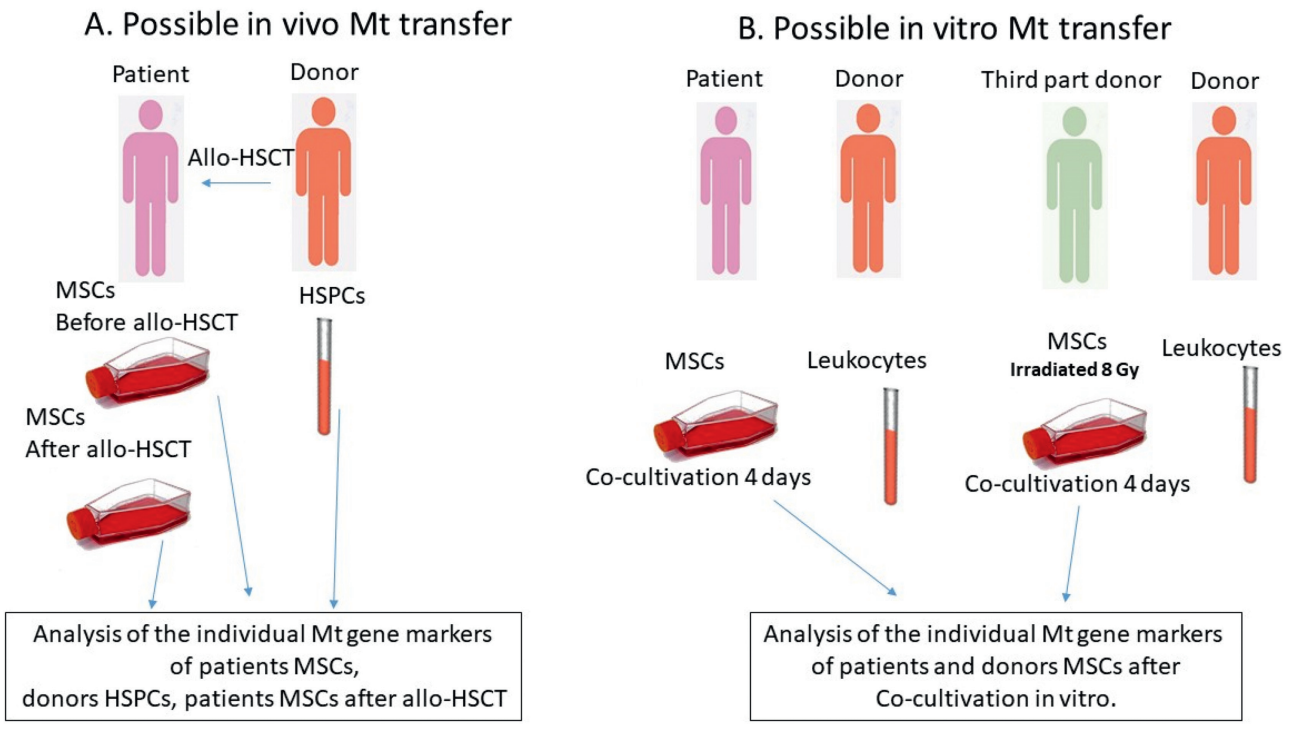

Figure 1 – Study design outline.

MSC isolation and culture

Bone marrow was obtained during diagnostic punctures of patients. Healthy bone marrow for MSC acquisition was obtained during exfusion for allo-HSCT. To prevent clotting, 2 −7 mL of bone marrow were placed in sterile tubes with 1 mL of heparin (50 U/mL). The bone marrow was diluted 2-fold with α-MEM (ICN, Canada) and 0.2 % methylcellulose (1500 cP, Sigma, USA) and left for 40 min at room temperature. The supernatant was collected and precipitated by centrifugation at 450 g for ten minutes. A total of 3×10^6 cells were seeded into a 25 cm^2 flask (Corning-Costar, USA) containing 5 mL of complete α-MEM nutrient medium (ICN, Canada) supplemented with 10 % fetal bovine serum (FBS; Hyclone, USA), 2 mM L-glutamine (ICN, Canada), 100 U/mL penicillin (Sintez, Russia), and 5 μg/mL streptomycin (BioPharmGarant, Russia). MSCs were cultured at 37°C and 5 % CO_2. After reaching confluency, the cells were passaged. During passage, 10^5 cells were seeded in a 25 cm^2 flask in 5 mL of the medium. Cultures were maintained for four passages.

The MSC population was characterized by flow cytometry. The mean fluorescence intensity (MFI) of fluorescently-labeled antibodies bound to the CD105, CD90, CD73, CD146, CD54 antigens was estimated. All studied MSCs matched the criteria of the International Society of Cellular Therapy.[24]

The time to reach the initial confluence (P0) was defined as the number of days from seeding the bone marrow to reaching confluence for the first time.

Cumulative cell production for three passages was calculated using the following formula:

$$Nsum = N0 + N1 * (N0 + N2)/2 \text{ x } 10^5 + N2 * (N1 + N3)/2 \text{ x } 10^5$$

where N0, N1, N2, and N3 are the number of cells obtained from 2 culture flasks at passages 0, 1, 2, and 3, respectively.

Mitochondrial membrane potential and mitochondrial mass assessment

In order to assess mitochondrial membrane potential, the MSCs were stained with tetramethylrhodamine methyl ester perchlorate (TMRM) (Abcam, UK), a potential-based dye that is sequestered by mitochondria and reflects their membrane potential. MitoView Green (Biotium, USA) staining was used to determine relative mitochondrial mass per cell.

The analysis was performed according to the manufacturers' recommendations using a CytoFLEX flow cytometer (Beckman Coulter, USA). Data were analyzed using the Kaluza Analysis 2.1 program (Beckman Coulter, USA).

In order to characterize mitochondrial activity, the ratio of MFI(TMRM)/MFI(MitoView Green) was calculated.

RNA and DNA isolation and real-time polymerase chain reaction

The relative gene expression levels (RELs) of MSCs were determined by reverse transcription followed by real-time polymerase chain reaction (PCR) (Taq-Man modification) using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). RNA was isolated using the Trizol reagent (Life Technologies, USA) according to the standard protocol with minor modifications for the MSCs after the first passage. M-MLV reverse transcriptase (Promega, USA) was used to build the first cDNA strands after RNA hybridization with a mixture of poly-T primers and random hexamers. The housekeeping genes *GAPDH* and *ACTB* were used for normalization. RELs were calculated using the ΔΔCt method.

To determine the ratio of mtDNA and nuclear DNA (nDNA), total DNA was isolated from all MSC samples. Cells were placed in 200 μL of lysing buffer (10 mM TrisHCl pH 7.5, 10 mM EDTA, 10 mM NaCl, 0.5 % w/v sodium lauryl sarcosine, proteinase K 1 mg/mL) for 12 h at 60 °C, DNA was precipitated in the presence of NaCl, ethanol and glycogen.

The NADH dehydrogenase-1 gene (*MT-ND1*) was used as an mtDNA marker, and a non-transcribed region of VISTA hs71 enhancer (LOC110120583) on chromosome 16 was used as a nDNA marker. ΔCt of these genes was obtained by multiplex PCR. The mtDNA/nDNA ratio was calculated using the formula $1.5^{\Delta Ct}$, where 1.5 is the primer efficiency in multiplex PCR.[25]

The sequences of primers and probes are presented in Supplementary Table 2. Samples were stored at −20 °C.

Determination of mtDNA control region sequence

Total DNA was extracted from patient MSCs before and after allo-HSCT and donor leukocytes as described above. The full-length control region (CR), including the most variable region of mtDNA 15967−605 (1208 base pairs), was amplified (Forward-1 primer: CCA TTA GCA CCC AAA GCT, reverse primer: GAT GTG AGC CCG TCT AAA CA). After purification by electrophoresis in agarose gel and the Cleanup S-Cap kit (Evrogen, Russia), the primary structure of the amplified fragment was determined using Sanger sequencing (NANOFOR 05, Syntol, Russia) with the forward-1 primer. In case the information obtained was insufficient to distinguish donor and patient mtDNA a forward-2 primer (GCC TAA ATA GCC CAC ACG TT) was used for sequencing. To determine polymorphisms, obtained primary structures were compared to the Cambridge sequence (NC_012920.1), as described by Murakami et al.[26] The mtDNA sequences of each patient after allo-HSCT were then compared with those of the same patient prior to allo-HSCT and to the respective donor.

Results

Sequence analysis of mtDNA from MSCs after allo-HSCT did not identify the presence of donor mtDNA in any of the cases even as trace amounts (Table 1 and Supplementary Figure 1). In the work by Golan et al.,[21] the horizontal mitochondrial transfer occurred both *in vivo* and *in vitro*, although the efficiency was much lower *in vitro*. Moreover, the authors detected horizontal mitochondrial transfer from human $CD34^+$ cells to murine MSCs *in vitro*. We attempted to replicate these *in vitro* co-culture experiments. After co-culture of MSCs with healthy donor leukocytes for four days, no donor mtDNA was detected in any of the MSC cultures studied.

In the work by Golan et al.,[21] mice were lethally irradiated as a pre-transplantation conditioning. Radiation is known to

Table 1 – Comparison of the mtDNA sequences from donors and patients before and after allogeneic hematopoietic stem cells transplantation.

Patient-donor group	Cambridge sequence position number	Patient mtDNA before allo-HSCT (MSCs)	Donor mtDNA (leukocytes)	Patient mtDNA after allo-HSCT (MSCs)
1.	16,069	C	T	C
	16,126	T	C	T
	73	A	G	A
	146	T	C	T
2.	16,126	C	T	C
	16,153	G	A	G
	93	A	G	A
3.	16,093	C	T	C
	16,356	T	C	T
	16,519	T	C	T
	73	A	G	A
4.	16,069	C	T	C
	16,163	G	A	G
	16,186	T	C	T
	16,189	C	T	C
	16,294	T	C	T
	16,519	T	C	T
5.	16,126	C	T	C
	16,163	G	A	G
	16,186	T	C	T
	16,189	C	T	C
	16,294	T	C	T
	16,355	T	C	T
	16,356	C	T	C
	16,519	C	T	C
6.	16,126	T	C	T
	73	A	G	A
	185	G	A	G
	188	A	G	A
	228	G	A	G
	295	C	T	C
	303	C_7	C_9	C_7
	462	C	T	C
	477	C	T	C
	489	T	C	T
7.	16,294	T	C	T
	16,296	T	C	T
	16,342	T	C	T
	16,519	C	T	C
8.	16,298	C	T	C
	72	C	T	C
	73	A	G	A
	152	T	C	T
	185	G	A	G
	228	G	A	G
9.	16,126	C	T	C
	16,129	G	A	G
	16,294	T	C	T
	16,296	T	C	T
	16,304	C	T	C
	16,316	A	G	A

gravely damage stromal cells. Since the patients studied in this work received a reduced intensity conditioning regimen instead, we set out to test whether the difference in conditioning method accounts for the lack of transfer. In order to do that, we co-cultured healthy donor $CD45^+$ cells with irradiated MSCs from a third-party healthy donor. However, no horizontal mitochondrial transfer from the leukocytes to the MSCs was detected. Thus, we concluded that the irradiation did not trigger mitochondrial exchange *in vitro*. It appears that human MSCs, despite being impaired by malignant cells, chemotherapy, and pre-transplantation conditioning, do not receive mitochondria from healthy donor HSPCs after allo-HSCT.

Mitochondrial activity affects MSC differentiation and even immunoregulatory properties. When comparing the growth characteristics of MSCs before and after allo-HSCT, no

Table 2 – Characteristics of mesenchymal stromal cells before and after allogeneic hematopoietic stem cell transplantation.

MSC characteristic	Patient number	1	2	3	4	5	6	7	8	Mean ± SE	*p*-value
MSCs for 3 passages	Before allo-HSCT	8.87	17.10	7.98	4.20	12.40	9.76	5.48	7.04	9.10 ± 1.45	**0.81**
	After allo-HSCT	19.20	8.80	6.70	5.95	1.36		4.70	22.80	9.93 ± 2.81	
Time to P0	Before allo-HSCT	10.00	12.00	13.00	12.00	18.00	12.00	10.00	11.00	12.25 ± 0.90	**0.007**
	After allo-HSCT	18.00	14.00	14.00	21.00	20.00	14.00	15.00	14.00	16.25 ± 1.05	
Cell proliferation rate	Before allo-HSCT	7.00	8.60	6.87	7.88	7.79	7.55	5.19	5.70	7.07 ± 0.41	**0.56**
	After allo-HSCT	9.30	7.10	6.80	4.58	1.24		4.93	9.43	6.20 ± 1.02	
REL of *PGC1A*	Before allo-HSCT	0.01	0.00	0.03	0.02	0.01	0.01	0.00	0.00	0.01 ± 0.00	**0.34**
	After allo-HSCT	0.28	0.04	0.01	0.00	0.02		0.02	0.01	0.05 ± 0.04	
REL of *NFE2L2*	Before allo-HSCT	1.59	1.37	2.02	3.21	2.80	0.93	1.57	2.08	1.94 ± 0.27	**0.37**
	After allo-HSCT	3.70	2.84	2.10	1.99	2.17	0.57	3.02	2.37	2.35 ± 0.33	
REL of *NQO1*	Before allo-HSCT	2.63	2.15	1.13	0.59	1.52	0.70	3.46	3.40	1.95 ± 0.40	**0.72**
	After allo-HSCT	1.08	1.32	1.64	1.75	3.93	0.56	1.86	1.88	1.75 ± 0.35	
REL of *HO1*	Before allo-HSCT	4.70	3.56	3.99	3.45	4.18	4.47	4.30	10.95	4.95 ± 0.87	**0.78**
	After allo-HSCT	9.09	6.61	4.59	3.64	10.13	0.61	3.38	4.88	5.37 ± 1.11	
REL of *GCLC*	Before allo-HSCT	1.87	1.61	4.06	4.23	2.77	1.60	2.94	2.77	2.73 ± 0.36	**0.59**
	After allo-HSCT	5.09	2.09	3.08	2.74	2.08		4.72	2.95	3.25 ± 0.42	
REL of *HIF1A*	Before allo-HSCT	1.16	0.82	0.27	1.78	0.73	0.62	0.70	1.51	0.95 ± 0.18	**0.21**
	After allo-HSCT	2.10	1.18	0.97	0.96	1.28		1.29	1.43	1.32 ± 0.14	
REL of *LDHA*	Before allo-HSCT	2.74	2.58	0.38	1.41	0.84	2.58	3.00	2.27	1.97 ± 0.34	**0.56**
	After allo-HSCT	1.24	1.27	1.49	2.94	2.28	0.07	1.85	1.98	1.64 ± 0.30	
mtDNA/nDNA	Before allo-HSCT	21.92	56.26	67.04	15.98	6.49	2.76	20.98	6.08	24.69 ± 8.50	**0.082**
	After allo-HSCT	6.97	6.26	15.20	13.84	3.20	3.78	6.90	11.79	8.49 ± 1.61	

SE: standard error; REL: relative gene expression level; MSC: mesenchymal stromal cell; allo-HSCT: allogeneic hematopoietic stem cell transplantation; P0: initial confluence.

significant differences were found in the cumulative cellular production over three passages nor the proliferation index (Table 2). On the other hand, the time to P0 was significantly longer for MSCs after allo-HSCT.

This study analyzed the RELs of genes regulating mitochondrial biogenesis and metabolic activity and the ratio of mtDNA/nDNA (Table 2).

Discussion

Treatment of patients with acute leukemia is associated with intensive chemotherapy courses that not only kill tumor and hematopoietic cells, but also damage the stromal microenvironment of the bone marrow.[27] After allo-HSCT, which involves conditioning the recipient, all recipient hematopoietic cells are eliminated, and stromal progenitor cells are damaged even more. Thus, the quality of hematopoiesis worsens. The murine model data that transplantation of healthy HSPCs can lead to the transfer of mitochondria from healthy cells to damaged MSCs were very attractive. This study attempted to determine whether such a transfer occurs after the restoration of hematopoiesis in patients. Contrary to data from murine models,[23,21] mitochondrial transfer was not detected from transplanted HSPCs to recipient MSCs in patients after allo-HSCT. In addition, the main properties of the patient MSCs both before and after allo-HSCT were studied. The only statistically significant difference was an increase in the time to P0 after allo-HSCT, indicating a decrease in the concentration of MSCs in the bone marrow. The mtDNA/nDNA ratio in MSCs of six out of eight patients studied decreased after allo-HSCT (Table 2), which confirms the data of other authors on mitochondrial damage after radiation and chemotherapy.[21,28] Additionally, in a larger cohort of patients, mitochondrial activity decreased after allo-HSCT, as evidenced by a decrease in the TMRM/MitoView ratio (Figure 2).

In their quiescent state, MSCs seem to favor glycolysis, whereas during proliferation their metabolism shifts to rely more on mitochondrial activity.[29] RELs of genes regulating mitochondrial biogenesis (*PGC1A, NFE2L2* and *HIF1A*),[30] associated with oxidative stress (*NQO1, HO1, GCLC*),[31] and regulating anaerobic glycolysis (*LDHA*) did not differ significantly in MSCs before and after allo-HSCT (Table 2). Thus, the data of this study demonstrate that HSPC transplantation from a healthy donor does not promote MSC metabolism.

The absence of donor mitochondrial markers in recipient MSCs after allo-HSCT and changes in the expression of genes responsible for mitochondrial metabolism indicate the absence of mitochondrial transfer from transplanted healthy HSPCs to recipient mesenchymal cells. In this work, no methods were used to label potentially transferred Mt, except the natural mitochondrial DNA markers. As has been described, dye markers may distort the results.[14] In studies on mice, sorted cells were used and mitochondrial markers were examined in the total bone marrow cell population.[23] In this study, recipient MSCs were studied after allo-HSCT without any additional processing. It has been shown previously that the proteome of MSCs from patients at the onset and remission of acute leukemia lacks many proteins associated with mitochondrial biogenesis, which are present in MSCs from donors.[32] One could expect an improvement in the metabolic status of MSC mitochondria after allo-HSCT, but this did not happen.

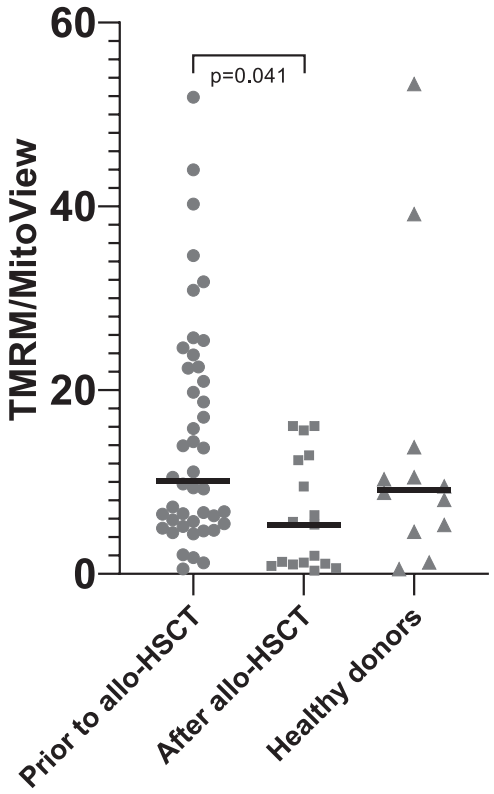

Figure 2 – The mean fluorescence intensity (MFI) tetramethylrhodamine methyl ester perchlorate (TMRM) to MFI MitoView green ratio. To assess mitochondrial membrane potential, MSCs were stained with TMRM. MitoView green staining was used to determine relative mitochondrial mass per cell. Mitochondrial activity, characterized by the MFI (TMRM)/MFI(MitoView Green) ratio, was found to be significantly decreased after allo-HSCT.

Conclusion

Based on the data obtained, one cannot count on an improvement in the stromal microenvironment due to horizontal mitochondrial transfer from healthy donor HSPCs to patient MSCs after transplantation. It is obvious that the stromal microenvironment after allo-HSCT is severely damaged and the development of new therapies is required to improve its condition.

Conflicts of interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2025.103859.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Prevalence of malaria parasites among blood donors in two hospitals in Enugu metropolis, Nigeria

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ABSTRACT

Introduction: Screening of blood donors for malaria parasites as recommended by the World Health Organization (WHO) is currently not included in the protocols and procedures for pre-screening blood donors of many private and public health facilities in Nigeria.

Methods: A cross-sectional study was conducted of voluntary, family, and remunerated blood donors in two hospitals in the Enugu metropolis. A well-structured questionnaire was used to collect demographics and blood donation history data. Five milliliters of blood were collected from each blood donor, of which 2 mL were used to screen for malaria parasites.

Results: Three hundred and seventy-seven blood donors participated in the study with 148 (39.3 %) being malaria-positive. Most of the blood donors were in the age groups 16–25 and 26–35 years old with prevalences of 40.0 % and 44.1 %, respectively. The prevalence of malaria in both age groups was high compared to the 36–45 years age group (26.7 %). Still, the overall difference in malaria prevalence across the four age groups was not statistically significant (χ^2 = 5.437; *p*-value = 0.142). The majority (*n* = 290; 76.9 %) of the donors were male, while 87 (23.1 %) were female. Although female blood donors had a higher prevalence of malaria (47.1 %) compared to male donors (36.9 %), the difference was not statistically significant (*p*-value = 0.057).

Conclusion: The high prevalence of malaria in the studied area, suggests the need for careful screening of blood samples of blood donors for malaria parasites.

Introduction

Malaria has become one of the most widespread and most important single disease entities of the tropics with unprecedented high morbidity and mortality. In 2022, there were an estimated 249 million malaria cases reported across 85

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malaria-endemic countries and regions. This marked an increase of 5 million cases compared to 2021. The primary contributors to this rise were Pakistan (+2.1 million), Ethiopia (+1.3 million), Nigeria (+1.3 million), Uganda (+597,000), and Papua New Guinea (+ 423,000). In comparison, in 2015, the baseline year for the Global Technical Strategy for Malaria 2016–2030, there were approximately 231 million malaria cases. Malaria case incidence decreased from 81 per 1,000 people at risk in 2,000 to 57 per 1,000 in 2019. Despite a slight 3 % increase in 2020, the incidence rates have remained stable over the past three years, with the incidence rate in 2022 recorded at 58 per 1,000 people at risk.[1] Studies have shown that there may be about 350–500 million cases of malaria each year. [2,3] About 41 % of the world population is said to be at risk of infection causing up to 2.7 million deaths annually.[3]

There are high levels of blood-demanding health conditions in Nigeria such as severe anemia in pregnancy and some hemorrhagic complications that occur particularly during childbearing, and after road traffic accidents, hence the need for blood transfusions. These amplify the possibility of the transmission of blood-borne diseases.[4] The spread of multidrug-resistant *Plasmodium falciparum* can be acquired equally by transfusion and the infection can be life-threatening in a patient population that is impoverished and vulnerable.[5,6] The frequent occurrence of blood-transfused malaria has raised concerns about potential infection worries in the fight to eradicate malaria. Also, children under the age of 5 years old, pregnant women, accident victims, immune-suppressed patients, and others requiring emergency blood transfusions are constantly at risk of its attendant infections.[7]

In Sub-Saharan Africa, where malaria is endemic, the screening of blood donors for malaria parasites is often not prioritized. This occurs despite international policies mandating that donated blood be quality-assured and free from transfusion-transmissible infections, including malaria.[8] As most of the people living in malaria-endemic regions are semi-immune, the test used to screen blood must be sensitive. This research sought to assess the prevalence of malaria parasites among blood donors in two hospitals in Enugu Metropolis, Nigeria.

Methods

Population and design

This was a cross-sectional study. The sample for this study included 377 individuals (remunerated, volunteer, and family members) who donated blood at two hospitals, the blood donor units of the University of Nigeria Teaching Hospital (UNTH) and the Enugu State University Teaching Hospital (ESUTH) in the Enugu metropolis, Nigeria. The sample size was determined using the standard formula of Cochran for calculating the minimum sample size.[9] A semi-structured interviewer-administered questionnaire was administered to all consenting participants to obtain information on their sociodemographic status. Ethical clearance for this study was obtained from the Enugu State Ministry of Health and Enugu State University Teaching Hospital, Parklane Research Ethics Committee.

Laboratory procedure for blood sample collection and malaria screening

From each donor, 5 mL of whole venous blood was collected for preliminary screening to assess donor suitability. Of this volume, 2 mL was subsequently aliquoted into ethylenediaminetetraacetic acid (EDTA) bottles. Malaria was diagnosed microscopically by staining thick and thin blood films on grease-free glass slides to visualize malaria parasites using Giemsa staining. Thick and thin blood films were prepared according to the technique outlined by the World Health Organization.[3]

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corporation, Armonk, USA). The prevalence of malaria was estimated by the chi-square test. The questionnaire responses are summarized as frequency distributions. Statistical significance was determined at an alpha level of 0.05 (p-value <0.05), corresponding to a 95 % confidence level.

Results

Blood donor demographic characteristics

The demography of blood donors who partook in the study is outlined in Table 1. All 377 individuals tested for malaria answered the study questionnaire. The blood donors were aged between 16 and 65 years. Most of the blood donors were in the 16- to 25- and 26- to 35-year-old age groups with prevalences of malaria of 40.0 % and 44.1 %, respectively. There were more than three times more male blood donors than female blood donors (76.9 % versus 23.1 %) yet female blood donors had a higher prevalence of malaria. Moreover, there were over three times more unmarried blood donors than their married counterparts (76.7 % versus 23.3 %). Although the prevalences of malaria in both the younger age groups were higher than the 36- to 45-year-old age group (26.7 %), the overall difference in prevalence across the four age groups was not statistically significant ($\chi^2 = 5.437$; p-value = 0.142).

Blood donation history

The blood donors fell into three categories: those who had to donate because family members needed blood (n = 277; 73.5 %), those making voluntary donations (n = 81; 21.5 %) and those donating for remuneration (n = 19; 5.0 %). Most (n = 241; 63.9 %) of the blood donors are repeated donors compared to 136 (36.1 %) doing so for the first time. The majority of the repeat donors had donated blood about six months previously (n = 138; 57.3 % - Table 2).

Prevalence of malaria parasites among blood donors

Blood group *O+* was the dominant blood type registered in this study followed by *A+*, *B+*, O-, and B-. No blood group A- or AB individuals were registered in this study. Of the 188 (O+),

Table 1 – Demographic characteristics and prevalence of malaria infection among blood donors in two hospitals in Enugu metropolis, Nigeria.

Variable		*n*	Frequency (%)	Infected (%)	
Age (years)	16–25	200	53.1	80 (40.0)	$\chi^2 = 5.437$; df = 3
	26–35	111	29.4	49 (44.1)	*p*-value = 0.142
	36–45	60	15.9	16 (26.7)	
	46–65	6	1.6	3 (50.0)	
Gender	Male	290	76.9	107 (36.9)	$\chi^2 = 2.937$; df = 1
	Female	87	23.1	41 (47.1)	*p*-value = 0.057
Marital status	Single	289	76.7	108 (37.4)	$\chi^2 = 1.849$; df = 1
	Married	88	23.3	40 (45.5)	*p*-value = 0.109
Level of education	Primary	15	4.0	8 (53.3)	$\chi^2 = 3.371$; df = 2
	Secondary	162	43.0	56 (34.6)	*p*-value = 0.185
	tertiary	200	53.1	84 (42.0)	
Occupation	Civil servant	53	14.1	32 (60.4)	$\chi^2 = 20.104$; df = 5
	Business	134	35.5	49 (36.6)	*p*-value = 0.001
	Professionals	20	5.3	8 (40.0)	
	Skilled artisans	27	7.2	7 (25.9)	
	Students	131	34.7	52 (39.7)	
	Unemployed	12	3.2	0 (0)	

df: degree of freedom.

Table 2 – Blood donation history.

Blood donor attribute	*n* (%)
When was the last time you donated blood? (*n* = 241)	
1–4 weeks ago	57 (23.7)
6 months ago	138 (57.3)
I cannot remember	46 (19.1)
Were you tested for malaria before donating blood?	
Yes	3 (0.8)
No	244 (64.7)
No response	130 (34.5)
Where was the test taken? (*n* = 3)	
Hospital	2 (66.7)
Laboratory	1 (33.3)
Source of knowledge about blood donation	
Television	20 (5.3)
Internet	28 (7.4)
Newspaper	2 (0.5)
People	297 (78.8)
Books	18 (4.8)
Others	12 (3.2)

Table 3 – Prevalence of malaria parasites grouped by blood group and donation history.

Variable	*n*	Infected *n* (%)	
Frequency of blood donation			
First-time donors	136	59 (43.4)	$\chi^2 = 1.116$; df = 1
Repeat donors	241	49 (20.3)	*p*-value = 0.172
Type of blood donor			
Voluntary	81	37 (45.7)	$\chi^2 = 1.788$; df = 2
Family	277	104 (37.5)	*p*-value = 0.409
Remunerated	19	7 (36.8)	
Blood group			
A+	88	22 (25.0)	$\chi^2 = 20.020$; df = 4
B+	53	31 (58.5)	*p*-value = 0.000
B-	16	3 (18.8)	
O+	188	81 (43.1)	
O-	32	11 (34.4)	

df: degree of freedom.

32 (O-), 88 (A+), 53 (B+) and 16 (B-) blood group subjects examined, the malaria infection rates were 43.1 %, 34.4 %, 25.0 %, 58.5 % and 18.8 %, respectively. The chi-square analysis ($\chi^2 = 20.020$; *p*-value = 0.000) revealed a significant association between the blood group and infection (Table 3).

Discussion

The prevalence of malaria parasites among blood donors in two hospitals in Enugu Metropolis was studied. The result of this study revealed that of 377 blood donors screened for malaria parasites, 148 (39.3 %) were malaria-positive. This high prevalence of malaria parasites was similar to prevalences reported in different studies of blood donors in Southeastern Nigeria (40.9 %, 30.2 %, and 51.5 %).[4,7,10] The implication of this for blood transfusions is enormous; blood transfusions carry the risk of transmitting malaria to hospitalized patients who are in dire need of blood. The risk of the recipient developing transfusion-transmitted malaria (TTM) is however dependent on other factors including the immune status and parasite density. Malaria prevalence rates in blood donors across Africa vary widely and depend on the local endemicity and transmission season. This study is contrary to a study conducted among blood donors in a hospital in Kumasi, Ghana where a low prevalence of malaria was recorded[11] and a study in Sudan also reported a figure lower than the current study (13 %).[12]

The recorded parasite density in blood films ("+" and "++") was not considered indicative of severe infection. This observation was cited by a laboratory scientist at one of the study hospitals as a rationale for excluding malaria testing from the

routine pre-screening protocol for assessing blood donor eligibility. However, preservation of blood at 4 °C does not destroy the parasites, and even one to two parasites per mL of blood, which may be undetected on thick or thin blood films, are sufficient to transmit the illness. [13]

The majority of blood donors in this study were males (n = 290; 76.9 %). The reason for the low number of female blood donors in this study (n = 87; 23.1 %) could be because of physiological issues such as menstruation, pregnancy, lactation, and iron deficiency anemia, which hinder them from donating blood regularly. Furthermore, the percentage of infections in this study was significantly higher in females than in males (47.1 % versus 36.9 %). This finding concurs with previous studies that reported a higher infection rate in females than in males.[14–18] Another reason for the higher prevalence among females may be the contemplation of differences in disease susceptibility or reporting. However, there appears to be no scientific evidence linking malaria to gender.[18] Generally, males donate blood more often than females, particularly in developing countries.[19] The reason has been attributed to socio-cultural influences and beliefs.[20]

This study shows that the 46- to 65-year-old age group had the highest percentage of malaria infection. This finding did not agree with the observations in some previous studies where higher prevalences among younger age groups were recorded.[4,13,21] The different observations in this study may be a result of the level of exposure, number of patients examined, low immunity, and lifestyle. Prevalence of malaria among 16- to 25- and 26- to 35-year age groups was also high compared to the 26.7 % in the 36- to 45-year age group, nonetheless the overall difference in prevalence across the four age groups was not statistically significant (χ^2 = 5.437; p-value = 0.142).

From this present study, those identified as being married had the highest number of positive cases for malaria (n = 40; 45.5 %) compared to unmarried blood donors (n = 108; 37.4 %). This conforms with a previous study conducted in a general hospital in Minna, Niger State, Nigeria.[22] The high malaria prevalence among married women was attributed to the existence of stagnant water around the homes which provides an environment for mosquitoes to breed, while another study with a high malaria prevalence among blood donors in Kaduna, Nigeria could not attribute the high rate to any specific reason.[23]

All blood groups were found to be parasitized with the malaria parasite in this present study indicating that no blood group can be exempted. Therefore, malaria can be transmitted through the transfusion of any of the different blood groups. Blood group *B+* donors had the highest prevalence (58.5 %). The finding in this study is similar to a previous study in Southeastern Nigeria,[24] but does not agree with other studies that recorded a higher prevalence of malaria among blood group *O+* individuals.[25,26]

First-time donors had a higher prevalence (n = 59; 43.4 %) compared to repeat donors (n = 49; 20.3 %). The high percentage of malaria among new blood donors as seen in this study could be that previous donors might have had antimalaria treatment or were not exposed to malaria parasites before donating blood.

Those who identified themselves as voluntary blood donors had the highest prevalence of infection (n = 37; 45.7 %) while family or replacement blood donors and remunerated donors had prevalences of 37.5 % and 36.8 %, respectively. This present study contradicts previous findings where all studies recorded higher malaria infection rates in family blood donors.[23,27]

The highest infection rate was observed in donors with primary education (53.3 %), followed by those with tertiary education (42.0 %) while the lowest rate was in donors with secondary education (34.6 %). In contrast to a previous study in the Abagana community in Eastern Nigeria, the lowest prevalence of malaria was observed in individuals with tertiary education.[28] One observation argued that those with tertiary education have better knowledge about the mode of transmission of malaria and ways to prevent and control the disease, thus they protect themselves better.[29] The highest prevalence observed in the primary education group is also in agreement with the suggestion that those with little or no education may not have good knowledge about transmission and control of the disease.

Conclusion

The prevalence of infection by the malaria parasite in donors, as seen in this present study, is significantly high. In as much as blood transfusion is required in severe anemia due to malaria and other illnesses, safe blood transfusion should be guaranteed. As the world searches for ways to curtail the spread and mortality due to malaria, there is an urgent need to screen blood donors for malaria parasites before donation. This is a neglected but important public health issue in Nigeria where malaria is endemic.

Author contributions

ST: Conceptualization, data curation, original draft; POU: Supervision, review and editing; KIO: Review; GAO: Review; COU: Review; FR: Review.

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Conflicts of interest

The authors declare no conflicts of interest.

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Original article

Epidemiological characterization of chronic myeloid leukaemia patients at an oncologic centre: A retrospective observational study

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ABSTRACT

Background: The chronic myeloid leukaemia population, treatment patterns and responses in Portugal are unknown. The aim of this study is to describe these features in a Portuguese reference centre.
Methods: A retrospective cohort study included patients with chronic myeloid leukaemia, treated between 2012 and 2022 at the Instituto Português de Oncologia of Porto. Data were obtained from the Cancer Registry of the institution and clinical records. Variables included demographic data, treatments administered, responses (hematologic, cytogenetic, major and deep molecular responses), adverse events, and survival. Patients without available data, those treated in a clinical trial context, and those admitted only for hematopoietic transplantation were excluded.
Results: Ninety-nine patients were included in this study, with a median age of 52 years (range: 7–84 years) at diagnosis. The first-line treatment was imatinib in 96 patients however 33 required second-line with dasatinib, and 17 discontinued treatment while maintaining response. Regarding responses, 95 (96 %) patients achieved cytogenetic response, 90 (94 %) achieved major molecular response, and 71 (72 %) achieved deep molecular

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response. At three months, the early molecular response rate was 77 %. At 12 months of treatment, of the 67 patients with response evaluation, 93 % achieved complete cytogenetic response and 49 % major molecular response. Both imatinib and dasatinib were well tolerated. The median follow-up was eight years. The five-year overall survival was 96 %.

Conclusion: This study is the first to characterize chronic myeloid leukaemia patients at a Portuguese centre. The patient characteristics, responses, and overall survival were within the expected range according to the literature. This study confirms the good prognosis of chronic myeloid leukaemia and the good responses using imatinib as first-line treatment.

Introduction

Chronic myeloid leukaemia (CML) is a myeloproliferative neoplasm that accounts for 15 % of adult leukaemias and whose prevalence has been increasing.[1] This pathology is defined by the presence of a *BCR::ABL1* fusion gene, which encodes a protein with the same name. The expression of this fusion protein leads to the activation of signaling pathways. This activation contributes to increased cell division, reduced apoptosis, and altered adhesion to stromal cells/extracellular matrix, leading to the leukaemic phenotype.[2,3]

CML is more common in older individuals.[1] The natural history of the disease includes three phases: a chronic phase, an accelerated phase, and a blastic phase.[4] Until 2000, only 10 % of patients achieved a complete cytogenetic response.[5] However, with the study of the disease's pathophysiology and the subsequent development of tyrosine kinase inhibitors (TKIs), the paradigm and course of this disease have changed substantially.[6]

Currently, CML has become a chronic disease, requiring regular molecular monitoring, adherence to TKI therapy, and proper management of toxicities.[7] Patients adequately treated with these agents, who have a good response to TKI, have a survival rate similar to the general population.[8–12] Moreover, some patients may discontinue TKI therapy and maintain remission. Since the approval of imatinib in 2001, the therapeutic arsenal for CML has expanded with the introduction of other TKIs such as nilotinib, dasatinib, bosutinib, ponatinib, and asciminib.[8–11,13,14] However, clinical trial candidates are not the same as real-world patients, and several authors argue for the need to understand these outcomes.[15,16] Although many countries have epidemiological databases and published literature on real-world outcomes for their CML patients,[17–21] to our knowledge, there are no published studies on these outcomes in Portugal.

The aim of this article is to describe the population of CML patients treated at Instituto Português de Oncologia do Porto (IPO-Porto), including the characterization of the patients, treatment patterns, therapeutic efficacy, documented adverse effects of TKIs, number of candidates for TKI discontinuation, and whether there was a need to resume TKI therapy.

Materials and methods

This retrospective cohort, single-centre study was conducted at the Haematology and Bone Marrow Transplantation Department of IPO-Porto. Patients were selected from the Cancer Registry of the institution. The inclusion criteria were: patients with CML (International Classification of Diseases for Oncology, 3rd Edition, ICD-O-3: 9863/3; 9875/3; 9876/3) treated at IPO-Porto between 1/11/2012 and 31/10/2022 and aged 18 years or older during the study period. Patients whose data were unavailable, incorrect diagnoses, patients admitted solely for hematopoietic progenitor cell transplantation, or those treated in a clinical trial context were excluded. The follow-up of patients continued until death, loss to follow-up, or administrative closure of this project in May 2024.

Patient data were collected through electronic medical records and cancer registry. The extracted variables included:

1. At the time of diagnosis: gender, age, Charlson comorbidity index,[22] clinical presentation (number of leukocytes, percentage of peripheral blasts, splenomegaly, constitutional symptoms, disease phase), presence of additional cytogenetic abnormalities, type and quantity of *BCR::ABL1* transcript, and prognostic scores: Sokal Index for CML (SOKAL),[23] and EUTOS long-term survival (ELTS) score.[24]
2. During treatment: the treatments administered, hematological, cytogenetic, major and deep molecular responses, adverse events, the need for TKI switch and reasons for the switch, progression, death, cause and date of death were collected.

Response evaluation was conducted according to the European Leukaemia Net 2020 recommendations.[6] Progression was defined as death from any cause, loss of cytogenetic response, or progression to accelerated or blastic phase.

For event-free survival (EFS), an event was defined as the first occurrence of any of the following: death from any cause during treatment, progression to accelerated-phase CML (characterized by ≥15 % blasts in the blood or bone marrow, ≥30 % blasts plus promyelocytes in the blood or bone marrow, ≥20 % peripheral basophils, or thrombocytopenia $<100 \times 10^3/\mu$L unrelated to treatment), or progression to blast-phase CML (defined by ≥30 % blasts in the blood or bone marrow or extramedullary involvement, excluding hepatosplenomegaly). Loss of complete hematologic response (CHR) was defined by the occurrence of any of the following in two blood samples obtained at least one month apart: a white blood cell count $>20 \times 10^3/\mu$L, a platelet count $\geq 600 \times 10^3/\mu$L, the appearance of extramedullary disease, ≥5 % myelocytes and metamyelocytes in the peripheral blood, or the presence of blasts or

promyelocytes in the peripheral blood. Loss of major cytogenetic response (MCyR) was defined as an increase in Philadelphia chromosome-positive (pH^+) cells in metaphase by at least 30 percentage points on two cytogenetic analyses performed at least one month apart. An increasing white blood cell count was defined as a doubling to $>20 \times 10^3/\mu L$ measured on two occasions at least one month apart in a patient who had never achieved a strict CHR despite receiving maximally tolerated doses of therapy. Overall survival (OS) was calculated from time to death from any cause. The criteria for discontinuing TKI therapy was based on the 2020 European Leukaemia Net guidelines.[6]

Statistical analysis

For descriptive analysis, categorical variables were presented as frequencies and percentages, and continuous variables as medians and ranges. Survival analysis was performed using the Kaplan-Meier estimator. Statistical analysis was conducted using the software R.

Ethical consideration

This project was submitted to the Ethics Committee of IPO-Porto (Ref. CES. 79/023). All data were processed in accordance with European and Portuguese data protection laws.

Results

Patient characteristics

Data from 157 patients were extracted from the oncologic registry of the IPO Porto. Of these, 58 cases were excluded for the following reasons: a diagnosis other than CML, lack of follow-up at the institution, referral solely for hematopoietic stem cell transplantation, inclusion in clinical trials, or unavailable data.

The median age at diagnosis of the 99 patients included was 52 years (range: 7–84 years), with a slight predominance of males (57 %). Two of the included patients were under 18 at the time of diagnosis, but were followed up only after becoming 18 years of age. The median Charlson comorbidity index was 0 (range: 0–9), and hypertension was the most common comorbidity (40 %). The patient characteristics are detailed in Table 1. Most patients were in the chronic phase at diagnosis ($n = 84$); however, for 15 patients, the disease phase could not be determined. The total of high-risk patients was 14 (14 %) and 5 (5 %) according to the SOKAL and the ELTS scores, respectively. The transcript was classic (e13a2 or e14a2) in 74.8 % of patients. The disease characteristics are described in Table 2.

Table 1 – Characteristics of the study population.

	Overall ($n = 99$)
Gender – *n* (%)	
Female	43 (43.4)
Male	56 (56.6)
Age at diagnosis[a]	
Median (range)	52.0 (7.00–84.0)
ECOG – *n* (%)	
0	84 (84.8)
1	10 (10.1)
2	3 (3.0)
3	1 (1.0)
4	1 (1.0)
Charlson comorbidity index (excluding the presence of CML +2)	
Median (range)	1.00 (0–9.00)
Smoker - *n* (%)	
No	87 (87.9)
Yes	12 (12.1)
Hypertension - *n* (%)	
No	59 (59.6)
Yes	40 (40.4)
Dyslipidaemia - *n* (%)	
No	70 (70.7)
Yes	29 (29.3)
Diabetes - *n* (%)	
No	84 (84.8)
Yes	15 (15.2)
Previous cardiovascular event - *n* (%)	
No	93 (93.9)
Yes	6 (6.1)

[a] Although two patients were under 18 years of age at the time of diagnosis, their follow-up in this study only began once they reached 18.

Treatment

First-line treatment was imatinib 400 mg (Glivec®) in 96 patients (97 %). Other treatments included nilotinib ($n = 1$), interferon ($n = 1$) and interferon with hydroxyurea ($n = 1$). The median time from diagnosis to initiation of treatment was 13 days (range: 0–45 days).

Among patients treated with imatinib, 32 required dose adjustments. Three patients escalated to imatinib 600 mg due to insufficient response. An insufficient response was defined as failure to achieve *BCR::ABL1* <1 % (International Scale) at 12 months of TKI therapy. Of the 29 patients who reduced their dose, 13 did so due to toxicity, initially decreasing to 300 mg and later to 200 mg. Sixteen patients reduced their dose due to sustained deep molecular responses (≥4 years) before attempting TKI discontinuation. Additionally, 34 (35 %) patients switched TKIs: 30 due to insufficient response and four due to intolerance. Criteria for discontinuing imatinib according the ELN guidelines of 2020[6] was met in 22 patients: 11 maintained molecular responses with TKI suspension and 11 needed to resume treatment, responding quickly to the reintroduction.

Second-line treatment was dasatinib in 33 patients. Other treatments included azacitidine associated with interferon and imatinib. Of these, 11 changed the dose: two increased due to insufficient response, and the remaining nine reduced the dose. Criteria for discontinuing dasatinib was met in one patient, who maintained sustained molecular response.

There was no preferential treatment for 3rd and 4th lines. The treatments administered and their sequence are illustrated in Figure 1. Only two patients were treated with allogenic stem cell transplantation.

Table 2 – Baseline chronic myeloid leukaemia characteristics.

	Overall (*n* = 99)
Splenomegaly - *n* (%)	
No	57 (57.6)
Yes	22 (22.2)
Unknown	20 (20.2)
Spleen size (mm below the costal margin)	
Median (range)	0 (0–100)
Missing - *n* (%)	21 (21.2)
Constitutional symptoms - *n* (%)	
No	60 (60.6)
Yes	23 (23.2)
Unknown	16 (16.2)
Disease stage - *n* (%)	
Chronic	84 (84.8)
Unknown	15 (15.2)
SOKAL Score - *n* (%)	
High	14 (14.1)
Intermediate	32 (32.3)
Low	33 (33.3)
Unknown[b]	21 (21.2)
ELTS Score - *n* (%)	
High	5 (5.1)
Intermediate	26 (26.3)
Low	47 (47.5)
Unknown	21 (21.2)
Additional cytogenetic abnormalities at diagnosis - *n* (%)	
No	66 (64.6)
Yes[a]	4 (4.0)
Unknown[b]	29 (29.3)
Transcript type - *n* (%)	
e13a2	36 (36.4)
e14a2	33 (33.3)
e13a2 and/or e14a2	5 (5.1)
e1a2	3 (3.0)
e19a2	1 (1.0)
Not found	1 (1.0)
Missing	20 (20.2)

[a] 3 patients had a second pH, and the other clonal evolution −1.
[b] Unavailable data.

TKI domain mutation testing

All the patients who had treatment failure (*n* = 31) underwent TKI domain mutation testing, confirming mutations in eight patients: one mutation conferred partial resistance to imatinib (p.(Cys475*)); five conferred complete resistance (Q252R, M388L, M244V, F311L, G250R); one patient had two mutations conferring resistance to imatinib and nilotinib simultaneously (E255V and Y253H), as is described in Table 3. The remaining mutations had indetermined significance, including c.708G>T, p.(Glu236Asp): and N322S. The majority of these mutations are not mentioned in reports as they are not significant.

Adverse effects

During the median follow-up of 8.25 years (Interquartile Range [IQR]: 7.14 years), the most common adverse events of imatinib including all lines of treatment (*n* = 101 – two patients who restarted imatinib at a later point were counted as having an additional line of therapy) were: gastrointestinal (39 %), myalgias (23 %), oedema (19 %), hematologic changes (13 %), fatigue (13 %), arthralgias (11 %), and rash (6 %). For dasatinib (*n* = 36), the most notable effects were: pleural effusion (25 %) and hematologic changes (8 %). For bosutinib (*n* = 3), one patient experienced cardiac toxicity and another gastrointestinal toxicity. For nilotinib (*n* = 2), one patient experienced critical limb ischemia. Only one patient was treated with asciminib, experiencing hypertension.

The adverse events reported prompted a change of TKI due to intolerance in 4 % of patients treated with imatinib, 11 % with dasatinib (pleural effusion), and 50 % of patients treated with nilotinib. Grade 3 and 4 adverse events by drug are shown in Table 4.

Responses and survival

During the follow-up, 98 patients (99 %) achieved a complete hematologic response, 95 (96 %) achieved a complete cytogenetic response, 90 (94 %) achieved a major molecular response, and 71 (72 %) reached a deep molecular response. At three months, 77 % of patients had achieved an early molecular response. By 12 months, 93 % achieved a complete cytogenetic response, 49 % achieved a major molecular response, and 21 % reached a deep molecular response. Among patients treated exclusively with imatinib, 95.7 % (45/47) achieved a cytogenetic response by 12 months, and 96.7 % (58/60) achieved it during the study follow-up. Among patients with a high risk according to the ELTS score, 75 % achieved a complete cytogenetic response within the first year.

During the study period, seven patients lost hematologic or cytogenetic response, though none progressed to the accelerated or blast phase. The median progression free survival was 268 months. The one-year event-free survival rate was 84 %, and at five years, it was 53 %. No patients were excluded due to loss to follow-up. Of under 45-year-old patients (*n* = 19), 47 % achieved a deep molecular response. Overall survival was 88 %, with no deaths due to CML-related causes (Figure 2). The five-year survival rate was 96 %.

Discussion

This study is, to our knowledge, the first to describe patients with CML in Portugal. The patient population exhibited characteristics similar to those seen in clinical trials, with a similar age (52 years), and small proportion of high-risk patients according to the ELTS score.[8–11] Imatinib was the main first-line therapy, with good tolerance. A third of patients needed a second line and a fifth successfully discontinued TKI. The rate of Grade 3 and 4 complications was higher in patients with TKI of higher generations. The responses achieved were comparable to expectations, with a complete cytogenetic response of 93 % and major molecular response at 12 months of 49 %, as was the overall survival.

This study reinforces the good outcomes currently achieved in CML. All TKIs are highly effective in newly diagnosed chronic-phase CML, with long-term overall survival

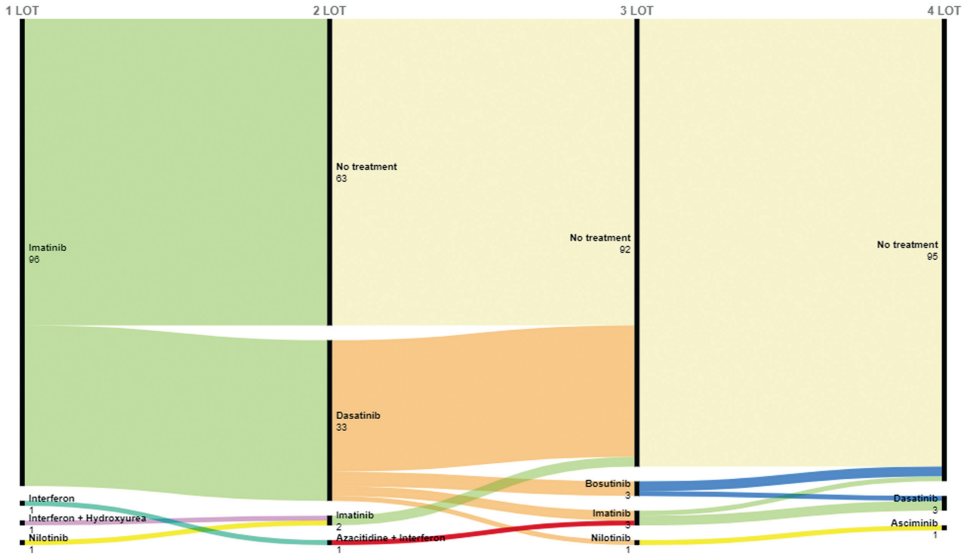

Figure 1 – Treatment patterns during the study period.

Table 3 – Identified mutations which configured resistance to tyrosine kinase inhibitors.

Mutation	Resistance
Q252R	Resistance to Imatinib
Y253H	Resistance to Imatinib and Nilotinib
G250R	Insensitivity to Imatinib
p.(Cys475Tyrfs*11) in ABL1	Resistance to Imatinib
F311L	Insensitivity to Imatinib
p.(Cys475*)	Of controversial significance, reported as conferring partial resistance to Imatinib
M244V	Resistance to Imatinib
M388L	Insensitivity to Imatinib

rates comparable to age-matched controls.[25] This study underlines the results obtained in clinical trials, showing no significant differences in overall survival between patients starting treatment with imatinib versus second-generation TKIs, as described in Table 5.[8–11] In this study, the rate of disease progression was low, achieving outcomes as good as those obtained with second-generation TKIs in clinical trials.[9–11] However, the study corroborates the faster achievement of molecular responses and deeper responses with second-generation TKIs, which may facilitate TKI discontinuation in selected patients. At 12 months, 49 % achieved major molecular response, while 94 % achieved molecular response. The good tolerability of TKIs was also confirmed. In this sample, as in the DASISION trial, patients treated with imatinib had more myalgias and peripheral oedema, and those treated with dasatinib had more pleural effusion. Although rare, one patient treated with dasatinib developed pulmonary hypertension.[9] As demonstrated in the ENESTnd trial, the risk of cardiovascular events was higher in patients treated with nilotinib, even in a small sample ($n = 4$).[11]

Several real-world studies have evaluated the efficacy of first-line imatinib treatment in countries such as Italy[17] and Spain.[18] These studies showed responses comparable to the present study, with major molecular responses at one year of around 50 %. Other studies evaluated not only first-line imatinib but also second-generation TKIs, including countries like Switzerland,[19] The Netherlands[20] and Italy[21] with heterogeneous treatment patterns and response evaluations. Therefore, comparing study results is difficult.

The selection of first-line treatment remains controversial. Several authors advocate starting treatment with a second-generation TKI[26]; one meta-analysis even recommended the use of second- and third-generation TKIs for younger individuals without comorbidities.[27] However, the choice of first-line TKI involves considerations not only of age and comorbidities, but also of treatment intent (survival versus TKI discontinuation), risk scores, costs, and availability.[25,28,29]

Table 4 – Grade 3 and 4 toxicities documented during the study.

	Imatinib ($n = 103$)	Dasatinib ($n = 33$)	Bosutinib ($n = 3$)	Nilotinib ($n = 4$)	Asciminib ($n = 1$)
Myalgias	1 (1 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Pancreatitis	1 (1 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Rash	2 (2 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Hematologic toxicity	1 (1 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Pleural effusion	0 (0 %)	4 (12 %)	0 (0 %)	0 (0 %)	0 (0 %)
Cardiovascular event	0 (0 %)	0 (0 %)	0 (0 %)	1 (25 %)	0 (0 %)
Hepatic toxicity	0 (0 %)	0 (0 %)	0 (0 %)	1 (25 %)	0 (0 %)

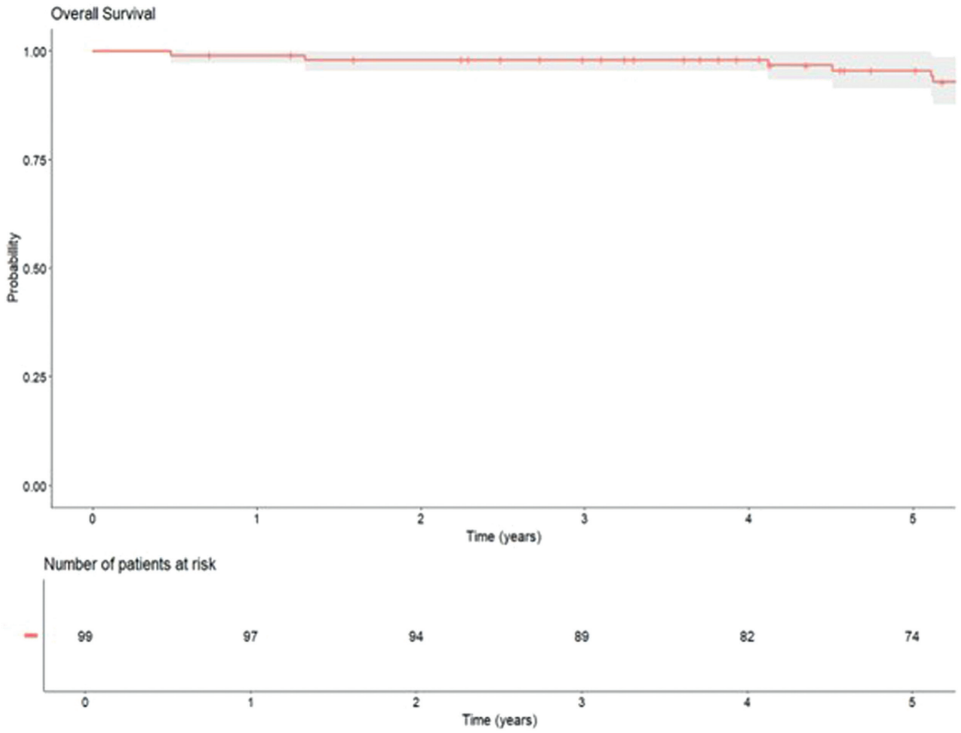

Figure 2 – Overall survival of patients with chronic myeloid leukaemia over ten years of follow-up.

This study shows that first-line imatinib, even for higher-risk and younger patients, continues to be a good option, allowing for excellent responses, good tolerability, and lower financial burden on the National Health Service.

As already mentioned, this study analyses the Portuguese population with CML, including treatment patterns, responses, and adverse effects. Characterizing this population is relevant because it may have different characteristics from other populations and because of the unique organization of the Portuguese healthcare system. The data from this study could potentially inform future therapeutic decisions regarding CML at a national level and improve care for these patients.

This study has limitations, including the small sample size, especially concerning the number of patients treated with second-generation TKIs and inhibitors other than imatinib, which limits the conclusions regarding these drugs. Additionally, due to its retrospective nature, this study is subject to information bias, given that some data were not available.

Conclusion

This is first study to characterize the Portuguese CML patient. The features, responses, survival, and adverse effects of the population are similar to those described in the literature. Furthermore, this study reinforces the good efficacy-tolerability profile of imatinib as a first-line treatment. A more detailed understanding of the population, treatment patterns, and outcomes in Portugal could improve the clinical practice in the country.

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Table 5 – Comparison of the main findings of the present study with other studies in literature.

Variable	Present cohort	Italian cohort[1]	Australian cohort[2]	Brazilian cohort[3]	Spanish cohort[4]
n	99	226	86	227	62
Age (median)	53	60	55	50	40
First line treatment	Imatinib 400 mg (97 %)	Imatinib 400 mg (86 %)	Imatinib 400 mg	Imatinib 400 mg (80 %)	Imatinib 400 mg (89 %)
MMR at 12 months (%)	49	55	44	56	91 in all follow-up
Follow up	2012–2022	2008–2012 (followed until 2015)	2001–2018	2007–2017	–
5-year EFS (%)	53 %	93	76	–	–
5-year survival (%)	98	85	94	91	100

CCyR, complete cytogenetic response; MMR, major molecular response; EFS, event free survival.

Authorship contributions

Ana Maria Meireles contributed to data collection and manuscript writing. Rita Calisto performed the statistical analysis. All other authors—Maria José Bento, Pedro Martinho Gouveia, Susana Bizarro, Manuel Teixeira, Cláudia Moreira, Ana Espírito Santo, and Mário Mariz—contributed to the critical revision of the manuscript and approved the final version for submission.

Conflicts of interest

The authors declare no conflicts of interest.

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Risk factors associated with HIV infection in four large Brazilian blood centers: A multicentric case-control study (2009–2017)

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ABSTRACT

Background: Strategies to reduce contamination by transfusion-transmissible infections are constantly evolving. Over the years, HIV residual risk has decreased in several countries. However, in Brazil a recent study showed that the residual risk remains substantially higher than in other countries. Continuous surveillance of risk behaviors for infection in donors can help in pre-donation screening to reduce the risk of HIV in blood transfusions.
Methods: This analysis evaluated risk factors related to HIV infection among blood donors from four large Brazilian blood centers located in São Paulo, Rio de Janeiro, Belo Horizonte and Recife, from 2009–2017. A binary logistic model was used to evaluate any association between risk characteristics and behaviors and the occurrence of HIV. The significant variables were included in a saturated model, to which the backward strategy was applied to arrive at the final model. The analyses were carried out using the R program version 4.1.2 and p-value <0.05 was considered significant.
Results: A total of 1507 blood donors were included in the study, 716 were HIV positive and 791 were uninfected controls. Demographics significantly associated with infection were: Male sex, incomplete secondary education, separated/divorced/widowed, and bisexual/homosexual orientation. Behaviors most strongly associated with infection were: workplace exposure, intravenous drugs and men who had sex with other men.

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Conclusion: The risk factors identified suggest that the blood donor screening process in Brazilian blood centers does not adequately identify donors at increased risk for HIV and further studies should be carried out to support changes to improve the process.

Introduction

Blood transfusions are critical interventions widely used to treat a range of clinical indications.[1] Strategies to reduce contamination by transfusion-transmissible infections (TTIs), such as clinical and epidemiological screening of blood donors, serological screening tests and microbiological inactivation are constantly evolving.[2] Currently, the improvement in process controls of blood banks and the use of techniques, such as the nucleic acid amplification test (NAT) to detect the genetic material of HIV, have reduced the risks of TTIs.[3]

The risk of HIV transmission through transfusion following the adoption of safety interventions is called residual risk (RR). RR has decreased in countries that have adopted measures to control blood quality from the emergence of HIV to the present day. However, a recent study showed that in Brazil there is no evidence that there has been a decrease in RR over the last ten years. The RR of HIV transmission in Brazil is 5.46 per million transfused red blood cell units,[4] remaining substantially higher than in other countries such as Germany (0.52 per million transfusions),[5] France (0.40)[6] and Canada (0.04).[7] HIV contamination through transfusion is still possible even with current procedures due to factors such as the coincidence that the donation period is within the HIV immunological window.[4] Furthermore, risks change over time and a study from Brazil demonstrated that donors using antiretroviral prophylaxis have different periods of the immunological window, and that the use of antiviral prophylaxis by blood donors in the acute phase of HIV infection delays seroconversion.[8]

In Brazil, the RR of TTI transmission decreased significantly after the implementation of NAT; however, it remained stable after this initial reduction and remains a major concern,[9–12] especially with the introduction of antiretroviral therapies, which prolong the immunological window. Cases of blood donors using antiretrovirals have already been reported in Brazilian studies, highlighting the need for greater surveillance in this context.[13] Thus, transfusion safety measures need to be improved to reduce the RR of HIV transmission. In addition to the laboratory measures applied for quality control, the steps prior to blood collection must be assessed, which involves the process and evaluation of the donor during screening prior to donation.[14] The identification of risk factors related to HIV infection, such as age, gender, sociodemographic factors, medical history and sexual behavior in pre-donation interviews can inform changes in the donor selection process during the eligibility interview.

Some studies have shown that Audio Computer-Assisted Structured-Interviews (ACASI) allow donors to report stigmatizing behaviors (such as sexual behaviors), and may help to close a gap in the pre-donation screening process.[15,16] In addition, studies show that changing the approach to assessing infection risks can help in pre-donation screening to reduce the RR of HIV transmission.[15–18]

This analysis evaluates risk factors related to HIV infection in blood donors from four large Brazilian blood centers from 2009–2017. This period of time precedes the federally mandated removal of sexual orientation as part of the donor selection process. Before 2020, Brazilian regulations did not allow donations from men who have sex with men (MSM) in the 12 months before donation. In May 2020, the Brazilian Supreme Court prohibited asking questions about sexual orientation to qualify blood donation candidates based on equal treatment of all potential blood donors.[19]

Study design and methods

This study was part of the NHLBI REDS-II and III International program (Brazil). The study included Fundação Pró-Sangue in São Paulo, Fundação Hemominas in Belo Horizonte and Fundação Hemorio in Rio de Janeiro, all in the southeastern and most populous region of Brazil, and Fundação Hemope in Recife in Northeastern Brazil. Blood samples from each donation were screened at each center with two different enzyme immunoassay assays (EIAs) (third and fourth generation) performed in parallel. By standard procedure, if the EIAs were reactive or discordant, a Western blot was performed. After 2012, with the gradual implementation of NAT in Brazilian blood banks, a serological test was performed, carried out in duplicate. Risk factor interviews for HIV infection of donors were conducted from April 2009 to March 2017. In the first phase of the study, from April 2009 to March 2011, HIV positive (cases) and HIV negative (controls) donors were interviewed. In the second phase, from April 2011 to March 2017, only cases were interviewed. The two datasets were combined for this analysis. All subjects included in the study completed the risk factor questionnaire at the time of return to the blood center for notification and counseling. Potential controls were recruited into this study after donation. Controls tested negative for all infections for which donations are screened in Brazil and were randomly selected based on scheduled donation visits during the first phase.

The ACASI risk factor questionnaire was based on HIV risk interviews developed by the US Centers for Disease Control (CDC)[20] but was modified to reflect potential risk behaviors in Brazil. The questionnaire included sociodemographic factors, incentives and motivations to donate, sexual history, and risks of sexual partners. A social matrix was used to obtain detailed information about sexual behaviors and other risks of the participant's last five sexual partners before blood

donation. Other factors, including alcohol and drug use, medical history, other potential risk factors (like tattooing, piercing, acupuncture, grooming at barbershops or beauty salons) and workplace exposure, were also assessed. In the analysis, the sexual orientation of the donor was included, in addition to the self-reported behaviors reported during the interview. For example, donors who reported sex exclusively with same sex partners were classified as homosexual, if they also reported sex with opposite sex partners, they were classified as bisexual, and if they reported only opposite sex partners, as heterosexual.

Statistical analysis

Summary descriptive statistics comparing cases and controls were generated and are presented as frequencies. Bivariable logistic regression was used to assess the unadjusted association between donor characteristics or behaviors and the occurrence of HIV. Significant variables from the bivariable models were included in a saturated model, to which a backward elimination strategy was applied to arrive at the final multivariable model. Results are presented as odds ratios (OR) and 95 % confidence intervals (95 % CI). The goodness of fit was evaluated by the Hosmer-Lemeshow test, and multicollinearity was evaluated by the variance inflation factor (VIF) statistic. The analyses were performed using R version 4.1.2 and a p-value <0.05 was considered significant.

Ethical considerations

The original ACASI interview studies were reviewed and approved by Ethics Committees in Brazil and Institutional Review Boards (IRBs) in the United States. The study protocols were approved by the Federal Committee on Human Subjects (CONEP) of the Ministry of Health in Brazil as part of the REDS-II/III International Program, ethics committees at all participant blood centers, and also IRBs in the United States. This analysis of anonymized previously collected data was not reviewed by Ethics Committees or IRBs.

Results

A total of 1507 blood donors participated in the study, of which 716 were cases with HIV infections and 791 were controls without infection. The ACASI were conducted from April 2009 to March 2017.

Table 1 presents the sociodemographic characteristics of all study participants. Female donors compared to males were less likely to be HIV positive (OR: 0.62; 95 % CI: 0.49 −0.78). Donors from Fundação Pró-Sangue (OR: 0.58; 95 % CI: 0.43−0.76), Hemominas (OR: 0.38; 95 % CI: 0.28−0.51) and Hemorio (OR: 0.71; 95 % CI: 0.54−0.92) showed lower associations with HIV infection when compared to donors from Hemope. According to the educational level, people with incomplete high school educations were more likely to be HIV positive when compared to individuals with higher levels of education (OR: 1.30; 95 % CI: 1.04−1.63). Donors who reported being separated, divorced or widowed (OR: 4.28; 95 % CI: 2.81 −6.59), followed by the single and never married group (OR: 3.55; 95 % CI: 2.58−4.91) and people who live together without being married (OR: 3.62; 95 % CI: 2.80−4.72) were more likely to be HIV positive. Donors defined as bisexual (OR: 27.14; 95 % CI: 4.02−60.90) and homosexual (OR: 8.33; 95 % CI: 5.63−12.69) presented higher ORs when compared to heterosexuals.

Table 2 shows the association between risk behaviors according to HIV status, such as workplace exposure, piercing, tattooing, blood transfusion, intravenous drug use (IVDU), acupuncture, hair removal, among others. Individuals who had already used intravenous drugs at least once in their lives or had IVDU sex partners had a greater association with HIV infection (OR: 6.77; 95 % CI: 3.58−14.17). inmate populations (OR: 3.19; 95 % CI: 1.32−8.90) or individuals who have had sexual partners who were inmates at least once in their lives (OR: 4.35; 95 % CI: 2.42−8.42) were more likely to be HIV positive. Being MSM (OR: 23.54; 95 % CI: 15.90−36.12), having MSM sex without condom use (OR: 21.64; 95 % CI: 13.95−35.23) and sexual partners of MSM (OR: 20.02; 95 % CI: 13.92−29.72) were associated with HIV infection. Likewise, individuals who had sex with an HIV positive partner (OR: 23.38; 95 % CI: 11.67 −55.58) were more likely to be HIV positive. However, many of the univariate data presented in Table 2 lost significance in Table 3, which presents multivariate data.

Adjusted ORs are presented for the risk factors associated with HIV infection from the multivariable analysis in Table 3. Characteristics that were independently associated with HIV infection were lower level of education (OR 2.93; 95 % CI: 1.93 −4.42), donors who reported being separated, divorced or widowed, (OR: 3.97; 95 % CI: 2.26−8.47), followed by the single and never married group (OR: 3.47; 95 % CI: 2.23−5.48) and people who live together without being married (OR: 3.20; 95 % CI: 1.90−5.40). Behavioral factors such as workplace exposure (OR: 3.14; 95 % CI: 1.69−5.92), tattooing (OR: 1.86; 95 % CI: 1.18 −2.93), manicuring or shaving in beauty salons or barber shops (OR: 1.71; 95 % CI: 1.17−2.50), medical procedures within the last 12 months (OR: 2.60; 95 % CI: 1.81−3.76), IVDU or IVDU partner (OR: 5.75; 95 % CI: 1.88−21.89), MSM sexual partner (OR: 11.78; 95 % CI: 6.17−23.53), sex with an HIV positive partner (OR: 15,57; 95 % CI: 5.78−54.62), having had two or more heterosexual partners within the last 12 months (OR: 2.64; 95 % CI: 1.74−4.05) remained associated with greater odds of being HIV positive. Table 3 also shows the multivariate analysis stratified by gender. Male blood donors who were classified as bisexual (OR: 8.10; 95 % CI: 2.47−36.66) or homosexual (OR: 5.71; 95 % CI: 2.13−17.13) were more likely to be HIV positive. The analyzed behaviors, such as exposure at work (OR: 2.81; 95 % CI: 1.44−5.60), tattooing (OR: 2.03; 95 % CI: 1.18 −3.52), manicuring or shaving in beauty salons or barber shops (OR: 1.65; 95 % CI: 1.10−2.49), medical procedures within the last 12 months (OR: 1.78; 95 % CI: 1.18−2.70), IVDU or partner IVDU (OR: 8.31; 95 % CI: 2.19−54.71), being MSM (OR: 8.30; 95 % CI: 4.18−17.37), sex with an HIV positive partner (OR: 5.75; 95 % CI: 1.69−26.73), having had two or more heterosexual partners within the last 12 months (OR: 2.33; 95 % CI: 1.48−3.70) when reported by male donors were more associated with being HIV positive. On the other hand, female blood donors were more likely to be HIV positive when classified in the bisexual group (OR: 14.35; 95 % CI: 1.89−297.81), and when they reported behaviors such as tattooing (OR: 2.72; 95 % CI: 1.32−5.73)

Table 1 – Sociodemographic characteristics of donors according to HIV status among Brazilian blood donors

Sociodemographic characteristic	Control (*n* = 791)	Case (*n* = 716)	OR (95 % CI)	P-value
Gender				
Male	556 (70.3)	568 (79.3)	1.00	–
Female	235 (29.7)	148 (20.7)	0.62 (0.49; 0.78)	**<0.001**
Age (*n* = 1506)				
18–25	189 (23.9)	176 (24.6)	1.00	–
26–30	130 (16.4)	150 (21.0)	1.24 (0.91; 1.69)	0.178
31–39	235 (29.7)	205 (28.7)	0.94 (0.71; 1.24)	0.645
40+	237 (30.0)	184 (25.7)	0.83 (0.63; 1.10)	0.205
Blood Donation Center				
Hemope	194 (24.5)	264 (36.9)	1.00	–
Fundação Pró-Sangue	194 (24.5)	152 (21.2)	0.58 (0.43; 0.76)	**<0.001**
Hemominas	195 (24.7)	100 (14.0)	0.38 (0.28; 0.51)	**<0.001**
Hemorio	208 (26.3)	200 (27.9)	0.71 (0.54; 0.92)	**0.011**
Donation type				
Community	362 (58.6)	371 (61.9)	1.00	–
Replacement	256 (41.4)	228 (38.1)	0.87 (0.69; 1.09)	0.231
Donor type (*n* = 1261)				
First time	311 (48.7)	285 (45.8)	1.00	–
Repeat	328 (51.3)	337 (54.2)	1.12 (0.90; 1.40)	0.311
Education (*n* = 1502)				
< High School	206 (26.1)	225 (31.5)	1.30 (1.04; 1.63)	**0.022**
≥ High School	582 (73.9)	489 (68.5)	1.00	–
Marital status (*n* = 1506)				
Married	326 (41.3)	115 (16.1)	1.00	
Single, never married	298 (37.7)	381 (53.2)	3.62 (2.80; 4.72)	**<0.001**
Living together, never married	119 (15.1)	149 (20.8)	3.55 (2.58; 4.91)	**<0.001**
Separated/Divorced	47 (5.9)	72 (9.9)	4.28 (2.81; 6.59)	**<0.001**
Widowed				
Sexual orientation (*n* = 1447)				
Heterosexual	713 (94.8)	417 (60.0)	1.00	–
Bisexual	8 (1.1)	127 (18.3)	27.14 (14.02; 60.90)	**<0.001**
Homosexual	31 (4.1)	151 (21.7)	8.33 (5.63; 12.69)	**<0.001**

OR, odds ratio; 95 % CI, 95 % confidence interval.

medical procedures within the last 12 months (OR: 4.11; 95 % CI: 2.15–8.12), a MSM sexual partner (OR: 5.06; 95 % CI: 1.17 –27.31), sex with an HIV positive partner (OR: 88.6; 95 % CI: 15.58–1924.91) and having had two or more heterosexual partners within the last 12 months (OR: 6.38; 95 % CI: 2.66–16.71).

Discussion

This study shows that male donors are still more likely to be HIV positive, as demonstrated in other researches published in the same period,[21] probably because the epidemiology of the states in the present study is predominantly clade B, which means a greater prevalence of sexual infection. The greater chance of being HIV positive found among blood donors from Recife probably is also related to the number of partners and other regional issues, such as disparity in the availability of resources, such as reduced access to prevention and health promotion. At the time of this study the number of sexual partners allowed for donor candidates was different at study sites: Hemominas, in Minas Gerais, allowed up to two partners, while Fundação Pró-Sangue in Sao Paulo and Hemope in Pernambuco allowed up to three partners. Previous studies have shown that there were differences in the deferral criteria between blood centers and this can have had a direct impact on the chances of having an HIV positive donor.[17,22]

Low education was also a factor associated with greater chances of being HIV positive. Access to information and mass education are fundamental components in strategies to reduce the incidence and prevalence of HIV in populations. However, the interaction between sociocultural, political and economic issues and its relation with social and sexual behavior cannot be underestimated. The diversity of socio-epidemiological risk exposure interferes in this process, differentiating individuals in terms of their knowledge about HIV and their behavior.[23–25] In the current sample, marital status was shown to be a protective factor when compared to unmarried donors, as in other studies.[18,26] Theoretical epidemiological models on HIV/AIDS transmission ignore marital status as a risk factor.[27] Marriage is considered an aggravating or protective risk of infection between people, with contradictory results in different studies.[28,29] According to a social psychology study, there is a higher prevalence of HIV in MSM associated with a context of exclusion and discrimination of these individuals, which impacts access to health care.[30] When experiencing prejudice in public health services, for example, MSM and transwomen may begin to avoid such services, due to the expectation of discrimination.[31] The CDC performed a study[32] emphasizing the

Table 2 – Risk behaviors according to HIV status among Brazilian blood donors

Characteristic	Control (*n* = 791)	Case (*n* = 716)	OR (95 % CI)	P-value
Potential job exposure, ever (*n* = 1503)	33 (4.2)	104 (14.6)	3.92 (2.65–5.98)	**<0.001**
Piercing, ever (*n* = 1504)	96 (12.1)	159 (22.3)	2.08 (1.58–2.75)	**<0.001**
Tattoo, ever (*n* = 1504)	106 (13.4)	178 (25.0)	2.15 (1.65–2.81)	**<0.001**
Acupuncture, ever (*n* = 1503)	46 (5.8)	47 (6.6)	1.14 (0.75–1.74)	0.537
Manicure or Shave in salon/barber shop, ever (*n* = 1504)	371 (46.9)	436 (61.2)	1.78 (1.45–2.19)	**<0.001**
Medical procedures in the last 12 months (*n* = 966)	152 (31.9)	251 (51.2)	2.24 (1.73–2.91)	**<0.001**
Surgery in the last 12 months (*n* = 941)	126 (27.3)	221 (46.1)	2.28 (1.74–3.00)	**<0.001**
Endoscopy in the last 12 months (*n* = 296)	40 (27.4)	68 (45.3)	2.20 (1.36–3.59)	**0.001**
IVDU or sexual partner of IVDU, ever (*n* = 1506)	10 (1.3)	57 (8.0)	6.77 (3.58–14.17)	**<0.001**
Blood Transfusion/Sex partner with blood transfusion, ever (*n* = 1506)	42 (5.3)	62 (8.7)	1.69 (1.13–2.56)	**0.011**
Inmate, ever (*n* = 1504)	6 (0.8)	17 (2.4)	3.19 (1.32–8.90)	**0.015**
Sex with inmate, ever (*n* = 1474)	13 (1.7)	50 (7.0)	4.35 (2.42–8.42)	**<0.001**
MSM (*n* = 1123)	29 (5.2)	320 (56.4)	23.54 (15.90–36.12)	**<0.001**
MSM, unprotected sex last 12 months (*n* = 1051)	22 (4.0)	239 (47.5)	21.64 (13.95–35.23)	**<0.001**
Sexual partner of MSM, ever (*n* = 1489)	33 (4.3)	337 (47.1)	20.02 (13.92–29.72)	**<0.001**
Sex with person with potential job exposure, ever (*n* = 1474)	41 (5.4)	80 (11.2)	2.23 (1.51–3.32)	**<0.001**
Sex with HIV+ person, ever (*n* = 1475)	7 (0.9)	127 (17.8)	23.38 (11.67–55.58)	**<0.001**
2 or more heterosexual partners, protected sex, last 12 months (*n* = 1452)	27 (3.6)	16 (2.3)	0.62 (0.32–1.15)	0.136
2 or more heterosexual partners, last 12 months (*n* = 1440)	127 (17.0)	153 (22.1)	1.39 (1.07–1.81)	**0.013**
Sex with sex worker, protected sex, last 12 months (*n* = 1388)	1 (0.1)	3 (0.4)	3.14 (0.40–63.61)	0.322
Sex with unknown person, protected sex, last 12 months (*n* = 1369)	7 (1.0)	11 (1.6)	1.65 (0.65–4.51)	0.303
Sex with sex worker/unknown or ≥2 heterosexual partners, unprotected sex, last 12 months (*n* = 1469)	31 (4.1)	27 (3.8)	3.14 (0.40–63.61)	0.322

OR, odds ratio; 95 % CI, 95 % confidence interval; IVDU, intravenous drug use; MSM, men who have sex with men.

importance of annual testing for the MSM population and the importance of prevention programs to improve their strategies to reach the population at greatest risk, aiming to facilitate this population's access to testing and treatment services and, consequently, to provide more information and care thereby reducing the spread of HIV. When analyzing risk behaviors, the rate of infection through IVDU is probably related to the frequency of use, the sharing of needles and poor access to effective HIV control and prevention therapies.[33]

An unexpected finding was the greater chance of being HIV positive in donors with the habit of going for manicures or shaves in beauty salons or barber shops. There are several studies that support the risk of other blood-borne infections, such as hepatitis and syphilis, through unsafe tattooing practices,[34–36] although the evidence is less clear when it comes to HIV transmission.[36,37] Although this transmission pattern of HIV is not common or widely reported, a few studies report this pattern of increased risk for HIV transmission.[38] It is likely that there are confounding factors involved in these results, probably behavioral factors. More studies are needed to highlight the lack of knowledge on the factors involved in this pattern of HIV infection in people who perform these practices. The association of HIV in people who undergo aesthetic procedures may be associated with the popularity of these procedures, especially for young people. The risk in the prison population is also associated with HIV infection in the present study. These factors deserve more attention.[34]

As to increased risk sexual behaviors, some widely reported in the literature are heterosexual sex with two or more partners within the last 12 months, being MSM, unprotected sex with an MSM partner, as well as sex with an HIV-positive partner. The latter three have the highest ORs found in the univariate and multivariate analysis for both males and females in the present study.[15–18,39,40] When the behavioral factors associated with HIV infection between men and women are compared, being bisexual or having MSM sexual partners are increased risk factors for both, with the magnitude of the ORs being greater in women.[41]

Almeida-Neto et al.[8] reported on HIV risks in blood donors in Brazil with similar data, but their donors were recruited between 2009 and 2011. The present study evaluated data from donors recruited from 2009 to 2017. The current results do not differ substantially from previous data, showing a tendency towards the maintenance of increased risk behaviors already identified, but this study shows a significant relationship between HIV and women who reported being bisexual, which was not reported in the previous publication.

This study has limitations. One limitation is that only factors in the ACASI interview were evaluated. Therefore, it is possible that other risk factors could be attributed to greater exposure to HIV and were not identified in the present study, such as questions about the use of pre- and post-exposure prophylaxis, having had some sexually transmitted diseases, such as chlamydia or gonorrhea, or Early age at first intercourse. Another limitation is that the study was conducted from 2009 to 2017, and the profile of donors may have changed since then. Furthermore, it was a period prior to the recent change in the screening criteria of Brazilian blood centers that occurred in 2020, which censored questions about the sexual orientation of donors in clinical screening.[42] These changes may have an impact on the profile of donors and further studies are needed to understand the new profile of donors after this change.

Table 3 – Multivariate analysis of factors associated with HIV infection among Brazilian blood donors

Characteristic	OR	95 % CI	P-value
BOTH GENDERS			
Education			
≥ High School	1.00	–	–
< High School	2.91	(1.93–4.42)	**<0.001**
Marital status			
Married	1.00	–	–
Living together, never married	3.20	(1.90–5.40)	**<0.001**
Single, never married	3.47	(2.23–5.48)	**<0.001**
Separated/Divorced	3.97	(2.26–8.47)	**<0.001**
Widowed			
Sexual orientation			
Heterosexual	1.00	–	–
Homosexual	2.45	(1.07–5.73)	0.035
Bisexual	7.55	(2.60–27.60)	**<0.001**
Behavioral factors (ref. No)			
Manicure or Shave in salon/barber shop, ever	1.71	(1.17–2.50)	**0.005**
Tattoo, ever	1.86	(1.18–2.93)	**0.008**
Medical procedures in the last 12 months	2.60	(1.81–3.76)	**<0.001**
2 or more heterosexual partners, last 12 months	2.64	(1.74–4.05)	**<0.001**
Potential job exposure, ever	3.14	(1.69–5.92)	**<0.001**
IVDU or sexual partner of IVDU, ever	5.75	(1.88–21.89)	**0.004**
Sexual partner of MSM, ever	11.78	(6.17–23.53)	**<0.001**
Sex with HIV+ person, ever	15.57	(5.78–54.62)	**<0.001**
MALE			
Sexual orientation			
Heterosexual	1.00	–	–
Homosexual	5.71	(2.13–17.13)	**<0.001**
Bisexual	8.10	(2.47–36.66)	**0.002**
Behavioral factors (ref. No)			
Manicure or Shave in salon/barber shop, ever	1.65	(1.10–2.49)	**0.016**
Medical procedures in the last 12 months	1.78	(1.18–2.70)	**0.006**
Tattoo, ever	2.03	(1.18–3.52)	**0.011**
2 or more heterosexual partners, last 12 months	2.33	(1.48–3.70)	**<0.001**
Potential job exposure, ever	2.81	(1.44–5.60)	**0.003**
MSM	8.30	(4.18–17.37)	**<0.001**
IVDU or sexual partner of IVDU, ever	8.31	(2.19–54.71)	**0.007**
Sex with HIV+ person, ever	5.75	(1.69–26.73)	**0.010**
FEMALE			
Sexual orientation			
Heterosexual	1.00	–	–
Homosexual	0.07	(0.01–1.01)	0.094
Bisexual	14.35	(1.89–297.81)	**0.023**
Behavioral factors (ref. No)			
Tattoo, ever	2.72	(1.32–5.73)	**0.007**
Medical procedures in the last 12 months	4.11	(2.15–8.12)	**<0.001**
Sexual partner of MSM, ever	5.06	(1.17–27.31)	**0.039**
2 or more heterosexual partners, last 12 months	6.38	(2.66–16.71)	**<0.001**
Sex with HIV+ person, ever	88.60	(15.58–1924.91)	**<0.001**

OR, odds ratio; 95 % CI, 95 % confidence interval; IVDU, intravenous drug use; MSM, men who have sex with men.

Conclusion

After analyzing the findings of the present study, there are factors that lead to an increased risk of acquiring HIV. Many have already been widely reported in the literature, but persist over time, such as IVDU, behaviors related to sexual activity, such as being bisexual (for men and women) and multiple sexual partners. More studies are needed to better understand these increased risk factors in order to develop policies to approach the most vulnerable populations.

This study shows that the donor profile has not changed over the years, there is little variability related to risk factors. In 2019 there were specific changes in the screening criteria in Brazilian blood centers, such as the removal of restrictions on blood donations by MSM. This fact, associated with the use of pre- and post-exposure antiretroviral medications, can generate significant changes. Therefore, this pattern must be

constantly monitored by new studies, especially due to the high transmissibility rate of the virus.

Conflicts of interest

None.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Oral post-surgical complications in patients with hemophilia and von Willebrand disease

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ABSTRACT

Objective: To determine the prevalence of post-surgical complications in patients with hemophilia and von Willebrand disease.
Methods: A prospective, cross-sectional study with descriptive and exploratory data analysis was conducted at the outpatient clinic of the Arthur de Siqueira Cavalcanti State Institute of Hematology (Hemorio). The sample included 26 patients who underwent tooth extraction following the protocols of the Brazilian Ministry of Health.
Results: The prevalence of post-surgical complications identified in the study was 26.07 %, with 15.38 % of cases presenting bleeding after extraction.
Conclusion: The prevalence of postoperative complications found in this study was notably higher in patients with von Willebrand disease, followed by those with severe hemophilia.

Introduction

Coagulopathies are hemorrhagic disorders caused by deficiencies in one or more coagulation factors, which may be either quantitative or qualitative.[1] Patients with coagulopathies can clinically present with bleeding of varying severity, either spontaneous or post-traumatic, predominantly in the oral cavity.[2]

Hemophilia is an X-linked hereditary bleeding disorder, characterized by the deficiency of coagulation factors VIII (hemophilia A) or IX (hemophilia B).[1,3,4] Its prevalence is approximately 1 per 5000–10,000 live male births for hemophilia A, and 1 per 30,000–40,000 live male births for hemophilia B.[1,4,5] According to the consensus of the International Society of Thrombosis and Haemostasis, this dyscrasia is classified based on plasma levels of factor VIII (FVIII) or factor IX (FIX): severe (<1 % of normal), moderate (1–5 % of normal), and mild (5–40 % of normal).[1,4]

Von Willebrand disease (VWD) is a hereditary coagulopathy caused by a genetic deficiency in von Willebrand factor (VWF), a plasma glycoprotein. It is the most prevalent coagulopathy among hemorrhagic disorders, affecting approximately 1 in every 100–1000 individuals. VWD is divided into three types: Types 1 and 3 involve quantitative deficiencies of VWF, while Type 2 is associated with qualitative defects.[1,6]

Patients with coagulopathies often neglect their oral health due to a fear of bleeding during toothbrushing and flossing.[7] Among dental procedures, oral surgeries carry the highest risk of bleeding and complications, whether

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intraoperative or postoperative.[1] Dental professionals must follow proper care protocols, including conducting a thorough medical history, consulting with the patient's hematologist to discuss the severity of the condition, and evaluating the risks of proposed procedures. Familiarity with local hemostatic measures, such as the use of fibrin sealants, proper anesthetic techniques, tranexamic acid, trichloroacetic acid, and other antifibrinolytics, is essential to minimize complications during treatment.[2,8,9]

In this context, the present study aims to identify the prevalence of post-surgical complications in patients with hemophilia and von Willebrand disease.

Methods

This research was submitted and approved by the Research Ethics Committee of Hemorio under number CAAE 57792122.3.0000.5267, in accordance with Resolution 466/2012 of the National Health Council.

This prospective, cross-sectional research using descriptive and exploratory data analysis was conducted at the dental outpatient clinic of Hemorio from July to December 2022. The sample size of 1493 patients was calculated, taking into account the number of patients diagnosed with Hemophilia A, Hemophilia B, and Von Willebrand Disease enrolled at Hemorio in the last two years. From the sample calculation, a maximum sample size of 306 was obtained, with a 5 % margin of error and a 5 % significance level. Since patients voluntarily seek dental care, the minimum sample size was not reached. The inclusion criteria were: being over 18 years old, requiring tooth extraction, having a panoramic radiograph, and voluntarily consenting to participate by signing the informed consent form. Twenty-six participants who met the inclusion and exclusion criteria after clinical examination were enrolled.

Data collection took place in a dental office under artificial lighting, using a flat mirror. Panoramic radiographs were taken, and participants completed a questionnaire developed by the researcher, which included questions on oral hygiene habits and dental bleeding history.

On the day of extraction, participants completed a health history questionnaire, and their vital signs were measured. After the extraction, an intraoperative form was filled out to document technical details of the procedure, such as tooth impaction, odontosection, osteotomy, and crown fracture. Participants returned for a follow-up visit 7–10 days later for suture removal and clinical evaluation, at which time a postoperative assessment form was completed.

Surgical procedures followed protocols of the Brazilian Ministry of Health.[1,10] Transfusion strategies were implemented to elevate FVIII or FIX levels to 80 % with a single preoperative dose for patients with hemophilia. Additional doses or oral tranexamic acid (25 mg/kg every eight hours) were administered in the days following the procedure, depending on the surgical details and the patient's bleeding history.

For patients with von Willebrand disease, the goal was to increase plasma levels of the deficient protein. Treatment options included desmopressin (DDAVP) or FVIII/VWF concentrates, often supplemented with oral tranexamic acid (25 mg/kg every eight hours), as recommended by a hematologist.

Local hemostatic measures included the application of a lyophilized hydrolyzed collagen hemostatic sponge (Hemospon, Maquira, Maringá, Paraná, Brazil), 3–0 silk sutures, and a hemostatic paste made by combining macerated tranexamic acid tablets with 0.2 % chlorhexidine digluconate gel.

Postoperative recommendations included maintaining regular oral hygiene, refraining from smoking and consuming alcoholic beverages, and eating soft, room temperature or cold foods for the first 48 h. Patients were advised to apply extraoral ice packs and take relative rest during the first 24 h, and rinse with a 0.12 % chlorhexidine digluconate mouthwash for one week. Moreover, they were warned to avoid exposure to sun, and refrain from vigorous rinsing, using a straw, or spitting during the first 72 h. In case of bleeding, patients were instructed to bite on a gauze pad for 15 min and return to the clinic if the bleeding persisted.

Data were tabulated using Microsoft Excel, followed by quantitative and descriptive statistical analysis.

Results

In the studied sample, von Willebrand disease was the most prevalent coagulopathy, accounting for 46.2 % of participants, followed by Hemophilia A (38.5 %) and Hemophilia B (15.4 %). Male patients were more prevalent, representing 57.7 % of the sample. The mean age was 39.9 years (standard deviation: 15.1 years), with the most represented age groups being 21–30 years and 31–40 years, each comprising 30.8 % of the participants (Table 1).

Regarding oral hygiene habits, all participants reported brushing their teeth daily; 46.2 % brushed three times a day, 42.3 % twice a day, and 11.5 % four or more times daily. Additionally, 84.6 % reported cleaning their tongue during routine brushing. When asked about flossing, 69.2 % of participants reported flossing, with 33.3 % doing so sporadically, 33.3 % at least once a day, 11.1 % twice daily, 11.1 % three times daily, and 11.1 % four or more times daily (Table 2).

When questioned about receiving hygiene guidance, 18 participants (69.2 %) reported having received some type of orientation, primarily from a dentist (83.3 %), followed by family members (11.1 %) and others (5.6 %). All patients reported having visited a dentist at least once before.

Table 1 – Profile of research participants.

Variable		n (%)
Gender	Female	11 (42.3)
	Male	15 (57.7)
Type of coagulopathy	von Willebrand disease	12 (46.2)
	Hemophilia A	10 (38.5)
	Hemophilia B	4 (15.4)
Age (years)	21–30	8 (30.8)
	31–40	8 (30.8)
	41–50	3 (11.5)
	51–60	3 (11.5)
	61–70	3 (11.5)
	71–80	1 (3.8)

Table 2 – Oral hygiene habits.

	Yes n (%)	No n (%)	Frequency per day n (%)				
			1	2	3	4 or more	Sporadic use
Brushing	26 (100)	0 (0)	0 (0)	11 (42.3)	12 (46.2)	3 (11.5)	0 (0)
Tongue brushing	22 (84.6)	4 (15.4)	4 (18.2)	10 (45.5)	7 (31.8)	1 (4.5)	0 (0)
Flossing	18 (69.2)	8 (30.8)	6 (33.3)	2 (11.1)	2 (11.1)	2 (11.1)	6 (33.3)

Twenty-two participants (84.6 %) reported experiencing bleeding episodes in their lifetime, with 12 (54.5 %) requiring urgent care for these episodes. Regarding tooth extractions, 21 participants (80.8 %) had previously undergone dental extractions, with 15 (71.4 %) reporting bleeding after the procedure. None of the patients had a history of inhibitors.

The absence rate for follow-up consultations after surgery was five (19.2 %) participants. Among those who attended follow-ups, complications related to the surgical procedure were observed in 23.07 % of the cases. Bleeding was reported in 47.6 % of these cases, with 40 % (15.38 % of the total sample) requiring emergency care to manage the bleeding.

Hematomas, hospitalizations, and other postoperative complications were evaluated. None of the participants required hospitalization. One patient developed a hematoma at the extraction site, and two patients experienced complications unrelated to bleeding. All teeth associated with bleeding complications were posterior and located in the maxilla. These included three third molars (one impacted) and one premolar with extensive caries and a crown fracture, leaving only a root remnant. Additionally, two molars developed alveolitis: one in the maxilla and one in the mandible. Table 3 summarizes the postoperative complications and their details.

Table 4 presents data on the reassessments conducted for patients with postoperative complications. The first reassessment (Reassessment 1) was performed within seven days of the procedure to address urgent needs. The second reassessment (Reassessment 2) occurred within 14 days after the extraction and included urgent care as necessary. Only one of these patients did not return for the second reassessment.

Discussion

In the present study, a postoperative complication rate of 23.07 % was identified, with 15.38 % attributed to bleeding episodes. In comparison, Franchini et al.[11] reported a 3.1 % bleeding rate in 288 procedures using fibrin glue for local hemostasis. Similarly, Hsieh et al.[12] observed a bleeding incidence of 18.9 % (10 out of 53 extractions) with hemostatic measures like gelatin sponges and oxidized cellulose. Bajkin and Dougall[13] found general bleeding rates of 11.9 % and 11.4 % for pre- and post-procedure factor concentrate use. Yagyuu et al.[14] reported post-extraction bleeding in 16.3 % of cases (9 out of 55), while Cesconetto et al.[15] found five cases of bleeding among 73 patients. The higher complication and bleeding rates in this study may be due to the small sample size and the inclusion of all patients undergoing tooth extractions.

Effective local hemostasis is essential to reduce bleeding risk. Commonly used materials, such as collagen sponges, fibrin sealants, and oxidized cellulose, stabilize clots, and non-absorbable sutures should be applied.[1] Epsilon

Table 3 – Postoperative complications.

	Event	Diagnosis	Gender	Age	Tooth	Indication for extraction	Postoperative recommendations	Details
1	Hemorrhage	Hemophilia B - Severe	Male	31	28	Caries	Not followed	- The patient reported not having taken the recommended dose of tranexamic acid as prescribed by the physician.
2	Hemorrhage	von Willebrand disease*	Female	31	28	Orthodontic	Followed	- With a history of bleeding during previous extractions.
3	Hemorrhage	von Willebrand disease*	Male	36	28	Impaction	Followed	- Osteotomy
4	Hemorrhage	Hemophilia A - Severe	Male	56	24	Caries/Radicular remnant	Not followed	- Osteotomy/The patient reported not having taken the recommended dose of tranexamic acid as prescribed by the physician.
5	Alveolitis	von Willebrand disease*	Female	48	26	Caries/Radicular remnant	Followed	- Poor hygiene in the extraction region
6	Alveolitis	Hemophilia A - Severe	Male	34	38	Impaction	Not followed	- Osteotomy/The patient reported smoking during the postoperative period.

* Without specification of type.

Table 4 – Postoperative reassessments.

	Reassessment 1			Reassessment 2		
	Suture	Presence of malformed clot	Healing	Suture	Healing	Bleeding
1	Intact	Yes	Unsatisfactory	Intact	Satisfactory	No
2	Intact	Yes	Satisfactory	–	–	–
3	Intact	Yes	Unsatisfactory	Intact	Satisfactory	No
4	Intact	Yes	Satisfactory	Intact	Satisfactory	No
5	Intact	Yes	Unsatisfactory	Intact	Satisfactory	No
6	Intact	No	Satisfactory	Intact	Satisfactory	No

aminocaproic acid and 10 % trichloroacetic acid are also used for minor gingival bleeding.[1,16] In this study, Hemospon filled the socket, and 3–0 silk sutures were applied. A paste of macerated tranexamic acid tablets mixed with 0.2 % chlorhexidine gel was used, chosen for its consistency and ability to concentrate the material at the extraction site. Fibrin glue and other local hemostatics were unavailable, but the protocol remained effective. The literature suggests using tranexamic acid as a mouthwash, intravenously, or mixed with local anesthetics for direct application.[1,9,16] Due to the risk of dislodging the clot, mouthwash was not recommended after extraction, and tranexamic acid was used only in paste form.

When appropriately indicated, systemic tranexamic acid offers significant benefits, particularly in controlling bleeding and promoting healing.[9] The study results support the effectiveness of the protocol of the Brazilian Ministry of Health with tranexamic acid, as only four patients experienced bleeding, two of which were due to underdosing.

In cases of post-surgical hemorrhage, the hematologist must be contacted for transfusion replacement, followed by local anesthesia, suture removal, socket curettage, clot removal, and management of granulation tissue. Local hemostasis should be achieved using collagen sponges, fibrin sealants, or other hemostatic materials, along with firm sutures and antifibrinolytic paste mixed with 0.2 % chlorhexidine gel.[1] No patient in this study had recurrent bleeding after this procedure.

This study also observed two cases of alveolitis: one dry socket and one purulent socket. The dry socket patient presented bone pain, halitosis, and smoking during the postoperative period, consistent with the report by Kuśnierek et al.,[17] who found smoking increases the risk of alveolitis. The purulent socket patient had poor oral hygiene in addition to the symptoms above. While the exact causes of alveolitis are unclear, poor hygiene is believed to be a contributing factor.[18]

Despite clear verbal and written postoperative instructions, three patients experienced complications due to neglecting these guidelines. Silva[19] emphasized the need for healthcare providers to tailor instructions to the understanding of patients to minimize complications and improve postoperative quality of life. Thus, it is the healthcare professional's responsibility to communicate care guidelines effectively to reduce the risk of forgetfulness and associated complications.

Good oral hygiene is crucial for patients with blood disorders, as healthy gums do not bleed spontaneously.[1] In this study, 100 % of patients reported daily tooth brushing, 84.6 % brushed their tongues, and 69.2 % used dental floss. Czajkowska et al.[20] found worse interdental hygiene in patients with blood disorders compared to healthy individuals, often due to a fear of gingival bleeding. Regular dental visits, at least every six months, are recommended for monitoring and prevention.

The limitations of this study include the small sample size and the unavailability of 'gold standard' medications like fibrin glue. However, the prospective design minimizes exposure determination bias, offering an advantage over retrospective studies. Further prospective studies are needed to optimize the safety of oral surgeries for patients with blood dyscrasias and minimize associated risks.

In conclusion, the prevalence of postoperative complications in this study was notably higher in patients with von Willebrand disease, followed by those with severe hemophilia. With this knowledge, dental surgeons can feel more confident about performing surgery on patients with blood dyscrasias.

Conflicts of interest

The author declares no conflicts of interest.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Investigation of haematological, inflammatory parameters and the incidence of alloimmunization in multi-transfused sickle cell diseased patients

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ABSTRACT

Introduction: Sickle cell disease is a haemoglobinopathy caused by an aberrant mutation of the beta chain with the amino acid valine replacing glutamic acid at the 6th position. Patients with sickle cell disease suffer from complications including chronic inflammation and the development of allogeneic antibodies due to multiple blood transfusions. This study investigated the association between haematological, inflammatory markers and alloimmunization in multi-transfused patients with sickle cell disease.

Methods: This was a cross-sectional study, that enrolled 100 participants; 50 young adults (18–48 years) with homozygous sickle cell disease (Sickle cell Group) from the Obafemi University Health Centre in Nigeria, and 50 age and sex matched individuals who did not have the disease (Control group) but who had also received blood transfusions. Complete blood counts and differentials were processed on an auto-analyser (SFRI H18 Light, France). Red cell antigen identification used the saline and anti-human globin method while the abnormal haemoglobinopathy was evaluated using electrophoresis. ABO and Rhesus blood groups were analysed using a direct method on tile, and the determination of inflammatory markers including C-reactive protein, tumour necrosis factor-alpha, interleukin-6, and interleukin-1β was by the enzyme-linked immunosorbent assay technique. The data were statistically analysed using SPSS version 24.0 and GraphPad Prism. Additionally, the student *t*-test and Chi-square test were employed as appropriate. Data were presented as mean ± standard deviation, with a p-value <0.05 considered statistically significant.

Result: As expected, the Sickle Cell group had an increased rate of alloimmunisation and significantly reduced haemoglobin and red cell parameters except for the mean cell volume. Although both groups had platelet counts within the reference range the Sickle Cell group had significantly higher counts than the Control group. The Sickle Cell group displayed evidence of inflammation with significantly increased levels (p-value = 0.001) of

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C-reactive protein and tumour necrosis factor-alpha. This was supported by higher white cell counts and neutrophilia. The majority of the antibodies detected in sickle cell disease were anti-Kell, Jka and Fya while the controls showed a higher prevalence of anti-M and Kell antibodies. Despite the elevated inflammatory markers, no significant correlation was observed between these and the rate of alloimmunization.

Conclusion: In this study, the Sickle Cell group had an elevated rate of alloimmunization with higher levels of anti-kell, Jka and Fya as well as inflammatory markers. However, despite these findings, no significant correlation between inflammatory markers and alloimmunization could be detected. This suggests that elevated alloimmunization rates are multifactorial and involve other processes which require further investigation.

Introduction

Sickle cell disease (SCD) is a genetic disorder characterized by haemoglobin S (Hb S) resulting from the inheritance of an abnormal beta-globin chain gene from one or both parents. Globally, 50 million people are affected, with Africa experiencing from 50–90 % of childhood mortality.[1] In Ghana, screening of newborns between 1995 and 2004 recruited 177,283 newborns with 3346 having SCD. Annually, SCD makes up about 2 % of 5 million total births per year.[2] Screening is to ensure that SCD in children is captured early enough for medical treatment. Meanwhile, in Nigeria, it has been estimated that 24 % of the population has the sickle cell trait (SCT), with an estimated 150,000 babies born with SCD annually. SCD makes up 3 % of the total births while the annual infant mortality rate is approximately 100,000.[3]

In Africa, blood transfusion remains the most common form of therapy for SCD effectively reducing complications including vaso-occlusive crises, acute pain, and chest pain syndrome by increasing the oxygen-carrying capacity of blood.[4] However, there are risks associated with blood transfusions such as blood-borne diseases, and allergic and haemolytic reactions. Chronically transfused patients suffer from iron overload and alloimmunization which is particularly prominent in those suffering from SCD. The production of alloantibodies can affect up to one-third of the SCD population potentially resulting in delayed haemolytic transfusion reactions (DHTR).[5] Identifying suitable and compatible blood for a patient with multiple alloantibodies has therefore become a challenge.[6]

The presence of delayed reactions and the development of antibodies in multi-transfused patients has been implicated in various pathological conditions. It has been proposed that one of the causes of the increased alloimmunization rate in individuals with SCD is chronic inflammation.[7]

Inflammation arises from an abnormal activation of innate immune responses which can be initiated by the process of haemolysis. This commences chronically in SCD when red blood cells are damaged, releasing various molecules into the peripheral blood, including Hb S and heme (iron compound). It was recently discovered that free heme increases in both SCD and beta thalassaemia, however, the inflammation in SCD is triggered by circulating abnormal haemoglobin.[8] The abnormal Hb S binds to Toll-like receptor 4 (TLR4: also known as CD284) expressed on monocytes, a key activator of the innate immune response.[9,10] The resulting inflammation has been correlated with mortality and therefore it has been hypothesized that elevated levels of pro-inflammatory markers could predict the buildup of allogeneic antibodies.

It has been observed in a murine model that inflammation plays an important role in RBC alloimmunization.[11] This work supported the theory that SCD is characterized by chronic inflammation[12] and has led to the hypothesis that inflammation plays a role in the increased rate of alloimmunisation observed in these patients despite little published data. This hypothesis appears reasonable because inflammatory signals activate the immune response and advance the recognition of foreign antigens. The release of cytokines, such as interleukin-6 (IL-6), interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α) as well as the activation of antigen-presenting cells and tissue damage may lead to initiation of alloimmunization when foreign antigens are introduced during transplantation and transfusion.

Alloimmunization poses a complex challenge with the risk increasing after every additional blood transfusion.[13] A study carried out in the United States reported that 50 % of all immunized subjects had multiple antibodies.[14–16] Over time many of these became undetectable, potentially challenging future transfusion and putting the patient at risk of a DHTR.[17] The most common red cell antigens involved are the Rh, Kell, Kid, Duffy, Lewis, and MNS blood group systems.[18,19] Other factors include the recipient's age and sex, number and frequency of transfusions, history of pregnancy, recipient clinical diagnosis and treatment, ethnic differences between recipient and donors and genetic factors related to antigenic responses.[16]

The overall incidence of post-transfusion alloimmunization in Nigeria varies from 18.7 %[20] to lower rates of 8.8 %[21] depending on the region where the study took place.

With this background, it has been hypothesized that those with elevated levels of inflammatory markers have a higher risk of alloimmunization and delayed transfusion reactions. Therefore, this study aimed to investigate the association between the increased incidence of alloimmunization in multi-transfused individuals with SCD and chronic inflammatory markers. This study focused on a cohort of individuals

with SCD diagnosed at the haematology department of an academic hospital in Nigeria.

Research design and methodology

This was a cross-sectional study conducted at the Department of Haematology and Immunology, Obafemi Awolowo University Teaching Hospital, Nigeria. The complete blood count (CBC), differential count, inflammatory markers, and blood group antigen profiles of patients with SCD (SCD group) who received two or more doses of blood were compared to a group of individuals without the disease (Control group) but who also received blood transfusions.

Inclusion and exclusion criteria

All patients who were diagnosed with SCD who had received at least two blood transfusions were included in the SCD group. The Control group was made up of age and sex-matched individuals who did not have SCD who had received at least two blood transfusions. Individuals who were experiencing a sickle cell crisis were excluded.

Ethical consideration

The study received ethical clearance from both the Human Research Ethics Committee, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, (CPUT/HWS-REC2021 renewal) Bellville, South Africa and the Research and Ethics Committee of Obafemi Awolowo University Health Centre, Ref: (D.MHS/2023) Ile-Ife. Informed written consent was obtained from all participants involved in the research.

Diagnosis of sickle cell disease

All patients with SCD were diagnosed according to established criteria using traditional haematological parameters including blood smear morphology. Thereafter the diagnosis was confirmed using haemoglobin electrophoresis which was performed on a Helena manual electrophoresis instrument (Helena Biosciences, UK) according to a previously described method.[22]

Analysis of samples

Six millilitres of blood were collected into ethylenediaminetetraacetic acid (EDTA) and serum separator tubes. A CBC, haemoglobin electrophoresis, ABO and Rhesus blood group typing and red cell antibody typing of Rh (D, C, E, c, e), Kell (K, k), Duffy (Fya, Fyb) and Kidd (Jka, Jkb) M, N, S, s, PI, Lu^a, Kp^a, Le^a, Le^b were investigated in both the SCD and Control groups. The red cell antibodies were interpreted using the ID panel profile. TNF-α, C-reactive protein (CRP), IL-6, and Interleukin-1 beta (IL-1β) were also analysed using enzyme-linked immunosorbent assays (ELISA).

Complete blood count and blood smear analysis

CBCs were performed using an H18Light auto analyser (SFRI, France) which uses the impedance technique to enumerate blood cells and spectrophotometry to determine haemoglobin levels. Red cell indices were calculated using the red cell count and haemoglobin values. Before analysis, three levels of control were used to ensure the accuracy of the autoanalyzer.

A routine blood smear was stained with Leishman stain for 3–5 min and after washing the slides they were allowed to dry before examining under an x100 objective. A manual differential was performed, and the red cells were examined for the presence of sickling and other red cell abnormalities.

Haemoglobin electrophoresis

Blood samples were haemolysed using hemolysate and an appropriate volume of Tris buffer (pH 8.4) was added to the electrophoresis chamber. Cellulose acetate paper was soaked for 20–30 min in the buffer, after which the excess was blotted and 0.5–0.6 mL of the specimen was applied. The cellulose acetate paper was placed in the electrophoresis chamber and covered to run at 450 V for 20 min. The cellulose paper was then removed and stained with Ponceau S for three minutes. The results of the abnormal haemoglobins were compared with the relative mobility of control samples.[23]

Determination of ABO and rhesus blood groups

Biotech's blood grouping reagents for ABO and Rhesus were used to determine the blood groups. This was achieved by tile grouping and confirmed by tube grouping methods utilizing anti-sera A, B and D for Rhesus, while tube grouping employed pooled A, B and O cells. Equal volumes of each type of cell and antisera were added and mixed and agglutination was observed and interpreted appropriately. Red cell antibody analysis was carried out to determine the presence of clinically significant red cell antibodies such as Kell, Kidd, and Lewis which could cause alloimmunization. This was achieved using the ID panel cells for red cell antibodies (ID Panel cells, product code PR144, NHSBT Reagents) which were processed according to the manufacturer's instructions (NHS Blood Transplant PR 1444–2020).

Inflammatory markers

ELISA KITS for TNF-α, CRP, IL-6, and IL-1β (Elabscience Biotechnology, USA) were used for the measurement of inflammatory cytokines. The micro-Elisa plates were pre-coated with antibodies specific for Human TNF-α, CRP, IL-6, and IL-1β. Samples (or standards) were added to the micro-ELISA plate wells and incubated with each specific antibody. Thereafter, a biotinylated detection antibody and avidin-horseradish peroxidase (avidin-HRP) conjugate was added to all the microplate wells. Free components were washed away, and a substrate solution was added. Only those wells that contained human TNF-α, CRP, IL-6, and IL-1β, biotinylated detection antibody and Avidin-HRP conjugate appeared blue and the reaction was terminated by the addition of a stop solution

resulting in the colour turning yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 ± 2 mm with the OD values being proportional to the concentrations of the relevant analyte. The concentrations of each analyte were calculated by comparing the OD of all the respective samples to their standard curves.

Statistical analysis

Data were subjected to statistical analysis using SPSS version 24.0 and the GraphPad Prism version 8 statistical package and relevant statistical values were obtained. Student *t*-test was used and data were presented as means ± standard deviation (SD). The Chi-square test was also employed where appropriate. *P*-values <0.05 were considered statistically significant.

Results

Demographics and clinical characteristics

All participants were of African descent and between the ages of 18 and 48 with no significant difference between the mean age of those with (30.66 ± 9.25 years) and without (31.46 ± 9.87 years) SCD. Thirty of the SCD group were females compared to 26 in the Control group. As expected, those suffering from SCD received significantly more transfusions (165 versus 108) compared to the controls (*p*-value <0.001). On average each person in the SCD group received 3.4 transfusions compared to 2.2 in the Control group (*p*-value <0.0001). Nineteen (38 %) of the SCD group experienced a transfusion reaction compared to ten (20 %) of the Control group. Although the number of reactions was higher in those with SCD, there was no significantly difference between groups (*p*-value = 0.2038 - Table 1). The types of transfusion reactions observed were mostly haemolytic reactions and included allergy, coldness, shivering, rash, and itching.

Complete blood count and haematology

As expected, the red cells, haematocrit (Hct), haemoglobin and all red cell indices apart from the mean cell volume were significantly different between the SCD and Control groups. In addition, those with SCD had significantly higher neutrophil counts (*p*-value <0.0001) and a lower lymphocyte count (*p*-value <0.0001). Although both groups had platelet counts within the reference range, those with SCD had a significantly higher platelet count (*p*-value <0.0001 - Table 2).

Development of alloantibodies

Those with SCD had an increase of alloantibodies with a mean of 0.6 alloantibody reactions compared to 0.4 in the controls (p-value = 0.2038). This was however not statistically significant. The majority of the antibodies in those with SCD were anti-Kell, Jka and Fya whereas the Control group had a higher prevalence of anti-M, as well as anti-Kell antibodies (Table 3).

Inflammation markers

Participants with SCD had significantly elevated levels of CRP and the pro-inflammatory cytokine TNF-α. Table 4 demonstrates the independent-samples *t*-test for the inflammatory parameters of participants by group. The result showed that there was a significant difference in the means between the Control and SCD groups for CRP (t[97] = −3.099; *p*-value = 0.003) and TNF-α (t[97] = −2.449; *p*-value = 0.016). The result also showed that there was no significant difference in the means between the Control and SCD groups for IL-6 (t[97] = 1.380; *p*-value = 0.171) and IL-1β (t[97] = −1.710; *p*-value = 0.090).

Correlation between inflammation markers and alloimmunization

Correlation analysis showed weak positive correlations between alloimmunization and two of the pro-inflammatory

Table 1 – General characteristics of participants.

	Control *n* = 50 (%)	SCD *n* = 50 (%)	Total *n* = 100 (%)	*P*-value
Age (years)	31.46 ± 9.877	30.66 ± 9.255		0.6769
Gender				
Female	26 (52.00)	30 (60.00)	56 (56.00)	χ^2 = 0.6494,
Male	24 (48.00)	20 (40.00)	44 (44.00)	df =1
				P-value = 0.4203
Haemoglobin electrophoresis				
Hb AA	36 (72.00)		36 (36.00)	χ^2 = 100.00,
Hb AC	5 (10.00)		5 (5.00)	df =3
Hb AS	9 (18.00)		9 (9.00)	*P*-value <0.0001*†
Hb SS		50 (100.00)	50 (50.00)	
The mean number of blood units transfused	2.160 ± 0.5095	3.360 ± 1.045		<0.0001
Mean number of blood transfusion reactions	0.400 ± 0.8571	0.6327 ± 0.9507		0.2038

SCD: sickle cell disease.

Table 2 – Comparing the haematological parameters in sickle cell disease and non-sickle cell anaemia patients.

Parameter	Control group		SCD group		*p*-value
	Mean ± SD	Median	Mean ± SD	Median	
WBC (x10^9/L)	15.702 ± 40.587	5.550	14.803 ± 6.453	13.450	0.0001[a]
Platelets (x10^9/L)	182.300 ± 87.196	177.500	289.063 ± 128.976	267.500	0.0001[a]
Hct (%)	31.20 ± 23.13	32.50	23.13 ± 4.741	23.00	0.0001[a]
Hb (g/dL)	10.41 ± 2.852	11.45	7.642 ± 1.482	7.650	0.0001[a]
MCV (FL)	79.72 ± 4.468	80.00	80.75 ± 4.468	80.00	0.3603
MCH (pg)	31.70 ± 11.84	30.00	27.62 ± 6.469	26.00	0.0001[a]
MCHC (g/dL)	31.76 ± 1.364	32.00	31.45 ± 0.9237	31.10	0.0763
Neutrophil (%)	57.74 ± 12.58	59.00	67.60 ± 9.498	70.00	0.0001[a]
Neutrophil (x10^9/L)	9.883 ± 25.636	3.051	10.206 ± 5.029	9.694	0.0001[a]
Lymphocyte (%)	39.74 ± 12.62	40.00	30.00 ± 9.042	29.00	0.0001[a]
Lymphocyte (x10^9/L)	4.640 ± 10.137	2.388	4.275 ± 2.025	3.585	0.0001[a]
Monocyte (%)	0.4800 ± 0.8142	0.0000	0.5000 ± 0.8864	0.0000	0.9225
Monocyte (x10^9/L)	0.628 ± 0.114	0.0000	0.141 ± 0.711	0.0000	0.5315
Eosinophil (%)	1.600 ± 1.917	0.0000	1.600 ± 1.953	1.0000	0.0065[a]
Eosinophil (x10^9/L)	0.7371 ± 3.494	0.0000	0.2174 ± 0.2651	0.1485	0.0002[a]
Basophil (%)	0.5000 ± 1.111	0.2500	0.4200 ± 0.7025	1.0000	0.6397
Basophil (x10^9/L)	0.2455 ± 1.135	0.0000	0.0583 ± 0.1015	0.0000	0.3778

SD: standard deviation; WBC: white blood cell; Hb: haemoglobin; Hct: haematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration.

[a] Mann-Whitney test: significant difference between the Test and Control groups (*p*-value <0.05).

Table 3 – Distribution of alloantibodies in the sickle cell and control groups.

Alloantibody	Control *n* (%)	SCD *n* (%)	Total *n* (%)
C	2 (4)	2 (4)	4 (4)
E	5 (5)	2 (4)	7 (7)
Fya	3 (6)	6 (12)	9 (9)
Fyb	1 (2)	2 (4)	3 (3)
Jka	0 (0)	8 (16)	8 (8)
K	9 (18)	9 (18)	18 (18)
Kpa	3 ((6)	4 (8)	7 (7)
Lea	5 (10)	2 (4)	7 (7)
M	12 (24)	5 (10)	17 (17)
N	1 (2)	2 (4)	3 (3)
S	3 (6)	2 (4)	5 (5)
C	5 (5)	2 (4)	7 (7)
Cw	1 (2)	2 (4)	3 (3)
E	0 (0)	2 (4)	2 (2)

SCD: sickle cell disease.

Table 4 – Inflammatory markers in the SCD and control groups.

	Group Control		SCD				
	M	SD	M	SD	t	df	*p*-value
CRP	1.2	2.78	3.9	5.62	−3.099	97	0.003[a]
IL-6	84.3	101.96	58.1	86.10	1.380	97	0.171
TNF-α	2.8	5.76	8.1	14.31	−2.449	97	0.016[a]
IL-1β	2.5	.38	4.1	6.73	−1.710	97	0.090

M: mean; SD: standard deviation; df: degree of freedom; CRP: C-reactive protein; IL-6: interleukin-6; TNF-α: tumour necrosis factor-alpha; IL-1: interleukin-1.

[a] Significant differences between the groups.

markers, CRP, and TNF-α. In contrast, very weak negative correlations between alloimmunization and two other inflammatory markers, IL-1β and IL6, were observed. These however were not statistically significant (Table 5).

Discussion

This cross-sectional study aimed to explain the complex relationship between inflammation and alloimmunization in individuals with SCD. It highlights the increased transfusion frequency and transfusion reactions in these patients. The participants with SCD developed allogeneic antibodies which were mostly anti-Kell, Jka and Fya while the non-SCD group developed anti-Kell and anti-M antibodies. Despite the individuals with SCD having significantly elevated levels of pro-inflammatory markers (TNF-α and CRP), no significant correlation was detected with the

Table 5 – Correlation between inflammatory markers and alloimmunization.

Inflammatory marker	Pearson's correlation	*p*-value
CRP	0.1127	0.2665
IL6	−0.08318	0.4130
IL-1β	−0.02402	0.8135
TNF-α	0.1179	0.2452
Neutrophil	−0.02975	0.7700
WBC	−0.03534	0.7284

CRP: C-reactive protein; IL-6: interleukin- 6; IL-1 β: interleukin 1beta; TNF-α: tumour necrosis factor-alpha; WBC: white blood cell.

rate of alloimmunization. This suggests that while inflammation is prevalent in patients with SCD, it does not directly predict alloimmunization.[24]

These findings align with previous research by Kangiwa et al.[20] which reported similar alloimmunization patterns but at higher rates. Differences in sample size, subject age, regional practices, and transfusion protocols likely contribute to the variance in alloimmunization rates. This study focused on young adults in the Western region of Nigeria, while Kangiwa et al.[20] included both adults and children in the Eastern region, indicating possible regional differences in antigen prevalence and healthcare practices.

Other studies[25,26–28] corroborate these findings, highlighting the clinically significant increases in Rhesus and Kell antibodies. The alloimmunization rate in Nigeria is higher compared to other African countries like Ghana[29] Uganda[30] and Tanzania.[17]

These differences might be explained by variations in the genetic backgrounds between populations which can influence immune responses. For instance, the prevalence of certain antigens like Kell, Jka, and Fya might vary across different ethnic groups, affecting the likelihood of developing corresponding antibodies. Likewise, variations in blood transfusion protocols and donor screening processes between different regions and hospitals can impact alloimmunization rates and the types of antibodies formed.

At our institution, blood units are prophylactically phenotype-matched between the recipient and the donor using ABO and Rhesus grouping and when compatible given to the recipient without necessarily carrying out antigen testing. However, in this current study, an extended antigen testing panel was performed.

The immunogenicity of antigens may also significantly influence the likelihood of antibody development. Antigens like Kell, Jka, and Fya, which were elevated in the SCD group, are known to be highly immunogenic, leading to a higher prevalence of corresponding antibodies in transfused patients. The Duffy antigen, specifically Fya, is particularly immunogenic and prevalent in African populations, making it a common target for alloimmunization in individuals with SCD.[31,32]

In this current study, individuals with SCD had significantly higher levels of the proinflammatory proteins TNF-α and CRP while IL-6 and IL1 levels were similar to the Control group. The increase in inflammatory proteins has been reported by previous studies[33] and it has been proposed that these proteins could be used as clinical biomarkers. For example, CRP has been associated with acute chest pain (ACP) and vaso-occlusive crises.[34] This has been confirmed by others whose findings were consistent with this study.[35,36]

TNF-α is a cytokine with several properties including the activation of endothelial cells and leucocytes. The action of macrophages and the chemotaxis of inflammatory cells has been implicated in the pathogenesis of various acute and chronic states such as sepsis, chronic infections, and inflammatory conditions. TNF-α plays an essential role in the synthesis of protein and the expression of adhesion molecules in vascular endothelial cells.[37,38] In SCD, this cytokine has been proposed as a risk factor for the occurrence of painful crises, as well as being involved in the occlusion of the microcirculation.[39,40]

High white blood cell counts and neutrophils are also associated with inflammation and during infections, neutrophils are the first cells to respond. Activated neutrophils release enzymes, such as reactive oxygen species, proteases and myeloperoxidase, which combat foreign organisms at the infection site.[41] These enzymes are also involved in several inflammatory processes.[41] When adhesion takes place, chemokines and cytokines are produced which go on to stimulate dendritic cells resulting in the presentation of antigens to memory CD4-positive T cells as well as to naïve CD8-positive T cells which consequently leads to the activation of the adaptive immune response.[42]

Several other studies have reported elevated platelet counts in individuals with SCD.[43–45] Increased platelets in this study could contribute to the chronic and acute complications of SCD by promoting molecular and diverse cellular events within the microcirculation that eventually lead to vaso-occlusive and vascular injury.

Despite the increase in inflammatory markers, no significant correlation between the rate of alloimmunization and any of the inflammatory markers could be detected, which was similar to a previous study by Tatari-Calderone et al.[24] Their study, conducted in Washington, DC, USA, involved 83 children with SCD who received multiple red blood cell transfusions for both the prevention and treatment of disease-related complications. The levels of cytokines were correlated with the development of anti-RBC antibodies within the seven-year period post-recruitment and demonstrated that twelve subjects had significantly elevated levels of all cytokines, both pro-inflammatory and anti-inflammatory. Interestingly, higher levels of cytokines were also found in the patients without anti-RBC allo- or autoantibodies. Therefore, it was concluded that high cytokine levels were not indicative of alloantibody development and that the increased concentration of multiple cytokines is not a biomarker of either the presence of or susceptibility to the development of RBC alloimmunization.

Several other studies have reported increased inflammation and innate immune activation in individuals with SCD[46–48] however, the pattern of cytokine expression varies.[49,50] High plasma levels of TNF-α have been reported while others have suggested that reduced production of IFN-γ was the first evidence of the onset of DHTR in individuals with SCD.[51]

Researchers have shown that various factors, aside from inflammation can influence the development of alloimmunization in SCD. These factors include iron overload, haemolysis, delayed haemolytic transfusion reactions, pregnancy, haemolytic disease of the newborn, infection, genetic factors, the antigenic immunogenicity of RBCs, recipient exposure to foreign donor antigens, the immunological status of the recipient, age at first transfusion, and the duration of transfusions. A further factor which could play a role is differences in the human leukocyte antigen alleles. These findings all warrant future research.[52,53]

Limitations

This study was conducted in only one region of Nigeria and therefore the results cannot be applied to the general Nigerian population or other countries within Africa. Regional genetic

variations, environmental factors, and healthcare practices can influence the results, thereby limiting the broader applicability of the conclusions. A further limitation was the relatively low number of participants. A larger sample size would have provided more robust data and enhanced the statistical power of the study, allowing for more definitive conclusions. Moreover, only four inflammatory markers were analysed which may have overlooked other relevant biomarkers which could provide additional insights into the conditions being studied.

Conclusion

Despite these limitations, the results indicate that SCD individuals in this region of Nigeria have high rates of alloimmunization and elevated levels of anti-Kell, Jka, and Fya antibodies, along with inflammatory markers, TNF-α and CRP, compared to those without SCD. However, no significant correlation was found between the inflammatory markers and alloimmunization. This study underscores the multifactorial nature of alloimmunization in SCD and the importance of considering genetic, regional, and procedural factors to optimize transfusion practices. Further research is needed to explore these differences and develop strategies to reduce alloimmunization risks in individuals with SCD.

Conflicts of interest

The author declares no conflicts of interest

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Translation, cross-cultural adaptation, and validation of the HCT frailty scale for hematopoietic stem cell transplant candidates: an observational study

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ABSTRACT

Introduction: Hematopoietic stem cell transplantation (HSCT) is a treatment option for patients with hematologic malignancies. The aim of this study is to validate the Hematopoietic Cell Transplantation Frailty Scale in a Chilean population.

Methods: This was a cross-sectional scale validation study. The sample consisted of patients with various hematologic malignancies who were transplantation candidates. The study had two stages: (1) translation (forward and backward) and (2) psychometric analysis, including face validity, test-retest reliability, and content validity. Descriptive analyses included mean, standard deviation, and the 95 % confidence interval. Reliability was assessed with Spearman's correlation, and content validity used Kendall's W test.

Results: Fifty-four patients (53.7 % women) were included, with multiple myeloma being the most frequent diagnosis (33.3 %). Positive and strong correlations were identified (Spearman's Rho [ρ]: 1.0; p-value <0.001) for all items on the scale. Regarding content validity, there was agreement among evaluators for the categories of relevance and coherence (p-value <0.01; Kendall's W range: 0.13–0.17) but not for "clarity" (p-value = 0.11; Kendall's W: 0.07). Some terms in the content were adjusted without affecting the overall structure of the scale. In the retest analysis, descriptive values were similar to the initial test.

Conclusion: The Spanish version of the Hematopoietic Cell Transplantation Frailty Scale for Chile is conceptually and linguistically equivalent to the original instrument. Additionally, it demonstrated adequate psychometric properties in terms of validity and reliability.

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Introduction

Hematopoietic stem cell transplantation (HSCT), is a treatment for hematological pathologies.[1] The selection of HSCT candidates involves assessing the patient's tolerability to determine the risk of treatment-related complications, including comorbidity burden, functional status, and chronological age.[2] Traditional pre-transplant assessment parameters, such as chronological age, comorbidity indices, and Karnofsky performance status, may fail to specifically detect the presence of frailty and functional conditions.[3] Therefore, incorporating variables related to frailty and functionality may enhance the predictive capacity of these existing tools across all age groups, particularly in older adults.

Frailty and functionality are predictors of mortality in patients diagnosed with hematological disorders in general, and particularly in HSCT candidates.[4] Functionality is a relevant parameter that has been correlated with survival in the older adult population in both oncological and non-oncological settings.[5] Similarly, poor functionality has also been correlated with worse outcomes in cancer patients, particularly in HSCT recipients with poor exercise tolerance and reduced physical function.[6,7]

Likewise, frailty is common in patients undergoing HSCT and, when present, it has been associated with an increased risk of post-transplant morbidity and mortality.[8] In this context, frailty can be present in adults of all ages and has been shown to have a negative impact on transplant outcomes,[9] is associated with greater HSCT complexity, an increased risk of non-relapse mortality, and reduced survival.[10]

With the aim of classifying HSCT candidates, professionals at the Princess Margaret Cancer Center, Toronto, Canada, developed the Hematopoietic Cell Transplantation (HCT) Frailty Scale, a prognostic tool that is quick and easy to apply. The HCT Frailty Scale consists of eight variables, including functional assessments and laboratory tests, that allow for the categorization of HSCT candidates into three groups: "fit," "pre-frail," and "frail," regardless of age.[8,10]

Currently, there are no validated scales to assess frailty and functionality in HSCT candidates in the Chilean population. Therefore, the objective of this study is to validate the HCT Frailty Scale for this population.

Methods

Design

An observational study with a cross-sectional design, translation, and adaptation, aimed at validating a measurement instrument that follows the guidelines of the COSMIN (COnsensus-based Standards for the selection of health Measurement INstruments) framework.[11] This study was approved by the Scientific Ethics Committee of the Metropolitan Eastern Health Service (December 5, 2023).

Participants

Fifty-four HSCT candidates aged ≥17 years with a diagnosis of onco-hematological diseases participated in this psychometric study in a public hospital in Santiago, Chile. Individuals with observed functional or cognitive deficits, or significant disabilities that prevented them from understanding the study, performing simple functional tests, or giving their written informed consent, were excluded. Additionally, individuals with insufficient understanding of Spanish, which hindered comprehension of instructions and evaluator directions, were also excluded.

Procedures

Patients attending their first consultation with the hematologist in the HSCT program were recruited from the HSCT unit. Those who met the eligibility criteria were invited to participate in the study, with a detailed explanation of the objectives and procedures involved. Those who voluntarily agreed to participate signed an informed consent form prior to enrollment.

The evaluations were conducted between December 2023 and June 2024 by two physical therapists at a physical medicine and rehabilitation clinic.

The original authors authorized the use of the scale, and the study was conducted in two stages:

Forward and backward translation

This process was carried out in the following order:

Forward translation

The scale was first translated into Spanish by two native Chilean speakers who are bilingual in English. They worked independently during the translation process.

Comparison and merging

The resulting translations were compared and merged into a single version by a test coordinator. Any discrepancies between the versions were analyzed and resolved by the translators and the coordinator.

Backward translation

The scale was then translated back into English by a native English speaker (language teacher) who is bilingual in Spanish and did not participate in the translation stage.

Comparison and evaluation

The back-translated version was compared and evaluated in terms of similarities and conceptual equivalence with the version obtained in phase 1.2 and, in parallel, with the original scale.

Final consensus meeting

In a consensus meeting of the researchers and translators, a second unified version was obtained that was consistent with the original version, with minor adjustments made for the Spanish scale tailored for Chile. Finally, through consensus, a

final version derived from the previous process was sent to the original authors for review. After some corrections, they approved the final version to be applied in a second pilot testing phase (Figure 1).

Psychometric properties analysis

Apparent validity

Since the version obtained in the first stage could not be limited to a simple translation, conceptual and semantic equivalence must be ensured between the original version and the adapted version, as well as the understanding of the obtained version by the target population. In this stage, the degree to which the content of the scale adequately reflects the construct to be measured was assessed. For this purpose, a pilot test was conducted with 54 patients, with some guiding questions being applied. The scale was first administered to 27 patients, observations were compiled, and necessary changes were made. Subsequently, the remaining 27 patients were evaluated, and new observations were gathered.

Reproducibility (test-retest reliability)

In this stage, the stability of the scale over time was evaluated by administering it at two different timepoints. The scale was applied twice by two physical therapists to a group of 30 patients, with a 24-h interval between assessments. To improve data reliability and facilitate interpretation, the two assessments were conducted within a maximum interval of 24 h, as recommended by the reviewers.

This interval was chosen to ensure a sufficient period of time to minimize the risk of progressive physical changes in the patients.

Content validity (face validity)

Content validity assessed whether the scale made sense to the professionals who care for HSCT candidates. Twenty-one professionals from different HSCT care centers nationwide (hematologists, physiotherapists, and nurses) with at least 5 years of experience in hematology and HSCT patient care were consulted. For content validity, an individual method was used, involving a written survey that each participant answered without having contact with the others. The scale was evaluated in terms of "coherence," "clarity," and "relevance" for each of the eight items composing the scale. A Likert-type survey with five response alternatives was used: "Strongly agree," "Agree," "Neither agree nor disagree," "Disagree," and "Strongly disagree" for each statement. An observation section was also included for additional information.

Instruments used

Hematopoietic Cell Transplantation Frailty Scale

This scale was developed by professionals at the Princess Margaret Cancer Centre and is designed to classify patients who are HSCT candidates. It consists of eight items, which include various subjective and objective tests and scales. These items were carefully selected and appropriately modified based on previous studies conducted in older populations and transplant centers.[8,10]

The items are: Clinical Frailty Scale (CFS) score[12]; Instrumental Activities of Daily Living (IADL) [13]; Self-Rated Health Questionnaire (SRH-Q)[14]; Fall Risk Assessment (Falls-test); Grip Strength (Dynamometry)[15]; Timed Up and Go test (TUGT)[16]; and Laboratory Tests such as serum albumin[17] and C-reactive protein (CRP).[18] Each variable is scored as either "normal" (0 points) or "abnormal" (1, 1.5, or 2 points, depending on the specific variable and its defined cut-off value). The total score is derived from the total of individual item scores, yielding a possible range of from 0 to 10.5 points. This allows for the classification of HSCT candidates in three categories: "fit," "pre-frail," and "frail," regardless of age and underlying diagnosis.

In the present study, a hydraulic dynamometer was used for the grip strength test (Jamar®, J A Preston Corporation, New York, USA).

Performance status

This variable was assessed using the Karnofsky Performance Status (KPS) scale, a numerical scale from 0 to 100. A lower score indicates a worse performance status.[19] In this study, the ranges used were: 50–60, 70–80, and 90–100.

Sociodemographic and clinical background

Data was collected on sociodemographic and clinical factors such as age, sex, education level, marital status, employment status, smoking and alcohol drinking habits, weight, height, diagnosis, type of HSCT, treatments received, Disease Risk Index (DRI), and the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI).[20]

Statistical analysis

The data were tabulated and analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0. Descriptive analyses were conducted, considering the mean, standard deviation, and the 95 % confidence interval. Spearman's correlation test was used for reliability analysis between the two assessments considering the items of the scale in the test-retest. The following values were considered for interpretation: between 0.00 and 0.10, insignificant correlation; between 0.10 and 0.39, weak correlation; between 0.40 and 0.60, moderate correlation; between 0.70 and 0.89, strong correlation; and between 0.90 and 1.00, very strong correlation.[21]

Content validity was determined using Kendall's W test, considering the dimensions of "clarity,", "coherence," and "relevance" for each item of the scale based on data from expert evaluators. The following interpretation was applied: 0: No agreement; 0.10: Weak agreement; 0.30: Moderate agreement; 0.60: Strong agreement; and 1.0: Perfect agreement.[22]

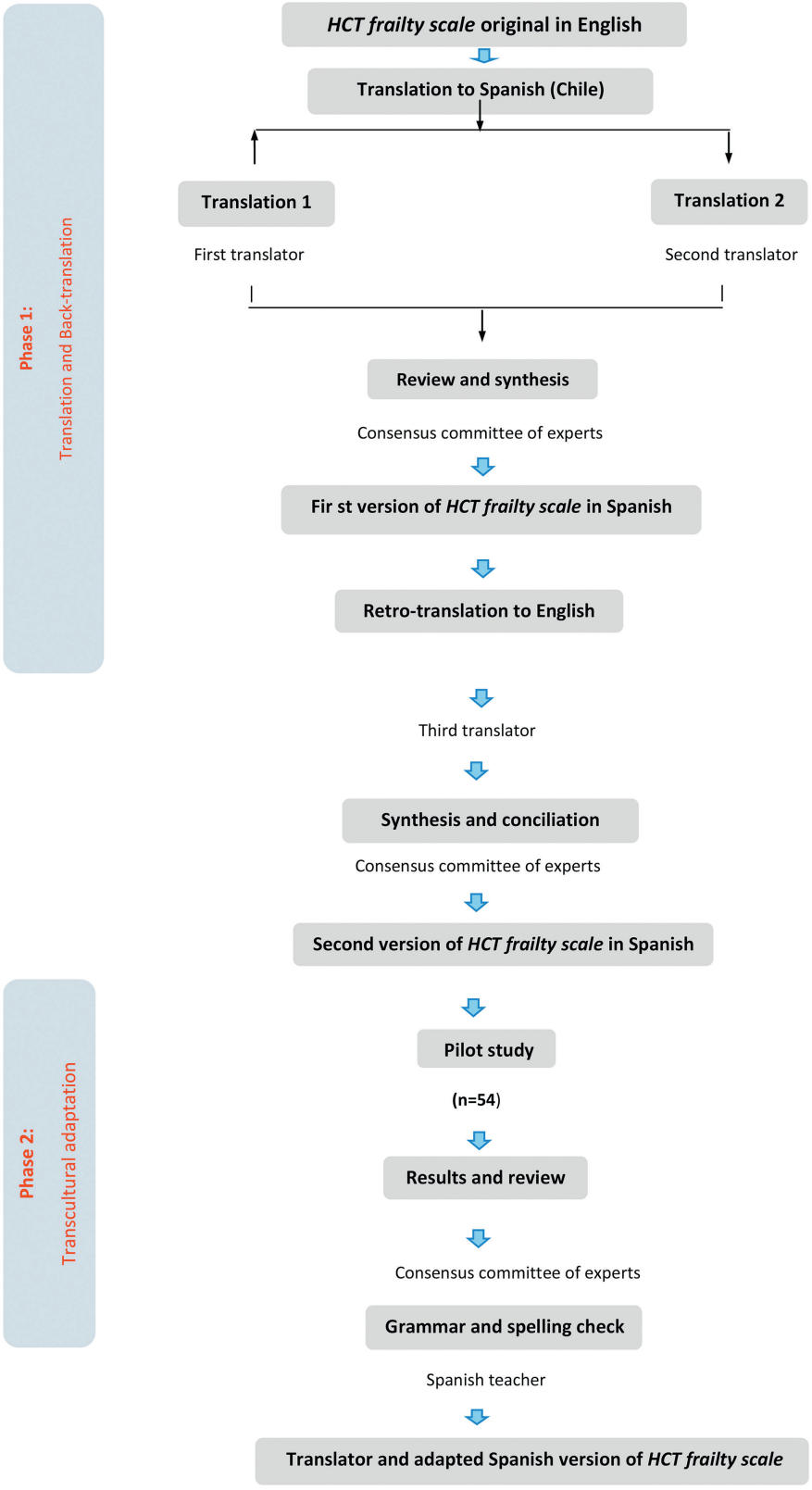


Figure 1 – Flowchart of the translation and cross-cultural adaptation phases.

Results

Translation and cross-cultural adaptation

After the forward translation process carried out by two independent translators, the versions were compared and deliberations took place to determine which words should be adjusted for better understanding, resulting in a single version (Table 1), which then proceeded to the back-translation process. Subsequently, the back-translated version was compared with the original version, and no significant differences were found, confirming that the translations were similar.

In the section of instructions for applying the scale, there were differences in the translation of the word "test," where the first translator translated it as "prueba," and the second translator kept it as "test," with the final consensus being "prueba." Similarly, in the application instructions, the word "fit" in the original version was translated into Spanish as "apto." In the back-translation, it was rendered as "suitable," but it was consensually accepted as "fit."

Psychometric properties

A total of 54 HSCT candidates participated in the psychometric evaluation of the scale. The median age was 36.9 ± 14.6 years, with the majority being women (53.7 %) and multiple myeloma being the most prevalent diagnosis (33.3 %). Twelve patients (12.2 %) were categorized as "fit," 26 (48.1 %) as "pre-frail," and 16 (29.6 %) as "frail." The sociodemographic and clinical background of the participants are shown in Table 2.

Apparent validity

The first 27 patients evaluated stated that the scale was easy to understand, except for Item 6, "self-reported health question" which required further explanation for 12 patients. Regarding the functional tests, they mentioned that they were not difficult to perform. They also reported that the instructions were clear and the items were relevant and appropriate for assessing their frailty and functionality before the HSCT. No modifications were made during this stage.

Table 1 – Forward translation results.

Item	Original version HCT-Frailty Scale	First Spanish translation	Second Spanish translation	Final agreed version
1	Clinical frailty score (CFS): ≥ 3 (frail) [vs 1–2 (no frail)]	Puntaje de fragilidad clínico (PCF): ≥ 3 (frágil) [vs 1–2 (no frágil)]	Puntuación clínica de fragilidad (PCF): ≥ 3 (frágil) [vs 1–2 (no frágil)]	Puntaje clínico de fragilidad (PCF): ≥ 3 (frágil) [vs 1–2 (no frágil)]
2	Instrumental Activities of daily living (IADL) score: ≥1 limitation [vs no limitation]	Actividades instrumentales de la vida diaria (AIVD)puntaje: ≥ 1 Limitación [vs sin limitación]	Puntaje Actividades instrumentales de la vida diaria (AIVD): ≥ 1 Limitación [vs sin limitación]	Puntaje en Actividades instrumentales de la vida diaria (AIVD): ≥ 1 Limitación [vs sin limitación]
3	Time and go test (TUGT): Abnormal> 10 seg. [vs normal]	Prueba de levantarse y caminar cronometrada: Anormal > 10 seg. [vs normal]	Test de tiempo de levantarse y caminar: Anormal > 10 seg. [vs normal]	Prueba de levantarse y caminar cronometrada: Anormal> 10 seg. [vs normal]
4	Grip Strength (GS): Abnormal [vs normal] If female <16 kg. If male <26 kg.	Fuerza de agarre (FA): Anormal [vs normal] Si es mujer menos de 16 kg. Si es hombre menos de 26 kg.	Fuerza de prensión manual (FPM): Anormal [vs normal] Si es mujer menos de 16 kg. Si es hombre menos de 26 kg.	Fuerza de prensión manual (FPM:) Anormal [vs normal]. Si es mujer menos de 16 kg. Si es hombre menos de 26 kg.
5	Self-rated Health question (SRH-Q): Fair, poor (vs excellent, very good, good)	Pregunta sobre autopercepción de salud (PAS): Regular,mala (vs excelente, muy buena,buena)	Pregunta auto informada de salud (PAS): Regular,mala (vs excelente, muy buena,buena)	Pregunta auto informada de salud (PAS): *Se le pide al paciente que califique su salud actual en comparación con otras personas de su edad entre*: Regular, mala (vs excelente, muy buena,buena)
6	Falls in last 6 months Yes (vs no)	Caídas en los últimos 6 meses Sí (v no)	Caídas los últimos 6 meses Sí (vs no)	Caídas los últimos 6 meses Sí (vs No)
7	Albumin serum level (Alb): Abnormal (<38 g/L) [vs normal]	Nivel de albumina sérica (Alb): Anormal (<38 g/L) [vs normal]	Nivel de albumina sérica (Alb): Anormal (<38 g/L) [vs normal]	Nivel de albumina sérica (Alb): Anormal (<38 g/L) [vs normal]
8	C-reactive protein (CRP): Abnormal(≥11 mg/L) [vs normal]	Proteína C reactiva (PCR): Anormal (≥11 mg/L) [vs normal]	Proteína C reactiva (PCR): Anormal (≥11 mg/L) [vs normal]	Proteína C reactiva (PCR): Anormal (≥11 mg/L) [vs normal]
	Total score	***Puntuación total***	***Puntaje total***	***Puntaje total***
	Patient risk classifications	***Clasificación de riesgo del paciente***	***Categorización de riesgo del paciente***	***Categorización del paciente***

Table 2 – Participant characterization for face validity (*n* = 54).

Variable	
Sex - *n* (%)	
Female	29 (53.7)
Male	25 (46.3)
Age – years	36.9 ± 14.6 (32.9–40.9)[a]
Height - m	1.64 ± 0.10 (1.62–1.67) [a]
Weight – kg	73.8 ± 16.7 (69.2–78.4) [a]
Body Mass Index - kg/m^2	27.1 ± 5.0 (25.7–28.4) [a]
Educational level - *n* (%)	
Primary	7 (13.0)
Secondary	22 (40.7)
Technical	14 (25.9)
University	11 (20.4)
Marital status - *n* (%)	
Single	32 (59.3)
Married	16 (29.6)
Cohabiting	2 (3.7)
Widowed	1 (1.9)
Divorced	3 (5.6)
Employment status - *n* (%)	
Employed	6 (11.1)
On medical leave	22 (40.7)
Unemployed	7 (13.0)
Other (student or homemaker)	19 (35.2)
Drinking habit - *n* (%)	
No	17 (31.5)
Occasionally	37 (68.5)
Smoking habit - *n* (%)	
No	27 (50.0)
Yes	8 (14.8)
Former smoker	19 (35.2)
Diagnosis - *n* (%)	
Multiple myeloma	18 (33.3)
Hodgkin lymphoma	9 (16.7)
Non-Hodgkin lymphoma	5 (9.3)
Acute lymphoblastic leukemia	13 (24.1)
Acute myeloid leukemia	4 (7.4)
Myelodysplastic aplasia	4 (7.4)
Hypoplastic myelodysplastic syndrome	1 (1.9)
Type of treatment - *n* (%)	
Chemotherapy/Radiotherapy/Immunotherapy	1 (1.9)
Chemotherapy/Immunotherapy	5 (9.3)
Chemotherapy/Radiotherapy	5 (9.3)
Chemotherapy	39 (72.2)
Immunotherapy	3 (5.5)
Not declared	1 (1.8)
Type of HPCT - *n* (%)	
Autologous	31 (57.4)
Allogeneic-MRD (matched related donor)	5 (9.3)
Allogeneic-Haploidentical	18 (33.3)
DRI - *n* (%)	
Low	6 (11.0)
Intermediate	35 (64.8)
High	11 (20.4)
Very high	1 (1.9)
Not evaluable	1 (1.9)
Karnofsky - *n* (%)	
50–60	6 (11.1)
70–80	34 (73.0)
90–100	14 (25.9)
HSCT-CI - *n* (%)	
0	32 (59.3)
1–2	14 (25.9)
≥ 3	5 (9.3)
Not evaluable	3 (5.6)
Categorization of patients according to the HCT Frailty Scale - *n* (%)	
Frail	12 (22.2)
Pre-frail	26 (48.1)
Fit	16 (29.6)

MRD: matched related donor; DRI: disease risk index. HPCT: Hematopoietic Progenitor Cell Transplantation; HSCT-CI: Hematopoietic Cell Transplantation-Specific Comorbidity Index.

[a] Mean ± standard deviation (95 % confidence interval).

Later, in the second round, the scale was applied to another 27 patients, of whom two also had some difficulty answering Item 6. Four patients experienced some difficulty executing the TUGT. All 54 evaluated patients emphasized the importance of being assessed on their "functional status" as a critical aspect prior to the transplant.

Reliability (test-retest)

Regarding the reliability analysis between the two assessors (test-retest), positive and strong correlations were identified (Spearman's Rho [ρ]: 1; p-value <0.001) for all items of the scale (Table 3).

Content validity

In general, all the variables of the scale were evaluated as consistent and relevant (Kendall's W range: 0.13–0.17; p-value <0.05). However, there were discrepancies regarding the clarity of some items (Kendall's W: 0.07; p-value = 0.11; Table 4).

Based on the analysis and observations made by the experts, improvements were incorporated to enhance the clarity and understanding of the scale, and some changes were made to the version from the first stage.

For Item 3, it was agreed to use the TUGT without translation, as this test is widely recognized and accepted, and has been integrated by professionals in the national clinical context.

For Item 4, which evaluates handgrip strength, some experts noted that while the test is coherent and relevant for

Table 3 – Test-retest reliability analysis of the Hematopoietic Cell Transplantation Frailty Scale (*n* = 30).

Dimension	Item	Spearman's Rho
Clinical Frailty Scale	1	1.00[a]
Instrumental activities of daily living (IADL)	2	0.93[a]
Timed and go test (TUGT)	3	0.97[a]
Handgrip strength	4	0.92[a]
Self-reported health question	5	1.00[a]
Falls in the last 6 months	6	1.00[a]
Albumin level	7	1.00[a]
C-reactive protein (CRP)	8	1.00[a]

[a] p-value <0.01.

Table 4 – Content validity and inter-rater agreement on the "clarity," "consistency," and "relevance" of the items in the Hematopoietic Cell Transplantation Frailty Scale (*n* = 21).

		Mean	Standard deviation	Minimum	Maximum	W Kendall	
	Item					Range	Kendall's W (*p*-value)
Clarity	1	4.9	0.21	4.0	5.0	4.5	0.07 (0.11)
	2	4.9	0.21	4.0	5.0	4.5	
	3	4.9	0.21	4.0	5.0	4.5	
	4	4.9	0.21	4.0	5.0	4.5	
	5	4.9	0.30	4.0	5.0	4.3	
	6	4.8	0.35	4.0	5.0	4.1	
	7	5.0	0.00	5.0	5.0	4.7	
	8	5.0	0.00	5.0	5.0	4.7	
Consistency	1	4.9	0.21	4.0	5.0	4.6	0.13 (<0.01)
	2	4.9	0.30	4.0	5.0	4.4	
	3	4.9	0.21	4.0	5.0	4.6	
	4	4.9	0.21	4.0	5.0	4.6	
	5	4.9	0.30	4.0	5.0	4.4	
	6	4.7	0.43	4.0	5.0	3.8	
	7	5.0	0.00	5.0	5.0	4.7	
	8	5.0	0.00	5.0	5.0	4.7	
Relevance	1	4.9	0.21	4.0	5.0	4.5	0.17 (<0.01)
	2	4.9	0.21	4.0	5.0	4.5	
	3	4.9	0.21	4.0	5.0	4.5	
	4	4.9	0.21	4.0	5.0	4.5	
	5	4.9	0.21	4.0	5.0	4.5	
	6	4.7	0.43	4.0	5.0	3.8	
	7	5.0	0.00	5.0	5.0	4.7	
	8	5.0	0.00	5.0	5.0	4.7	

this population, they inquired about the appropriateness of using the scale with values adjusted for the Chilean population. The original authors argued that the cutoff points (16 kg for women and 26 kg for men) used in the scale's design methodology, supported by previous studies, were specifically chosen to make the scale applicable to other institutions. Therefore, the original values were retained.

Additionally, to improve comprehension, the phrase "patient classification" was changed to "patient categorization." Furthermore, for the CFS scoring, the original version mentioned that it should be performed by "a physician," which was changed to "healthcare professional" to adjust to the national clinical context, providing the option for these assessments to be conducted by other professionals.

A final version of the HCT-Frailty Scale adapted for use in Chile is provided in Supplementary Material 1.

There were also differences regarding the time required for application. The original authors mentioned 5–6 min, which was insufficient, as professionals took between 20–25 min to complete the scale. Additionally, it was consensually deliberated that the most suitable professionals for administering the scale are physiotherapists, as they frequently conduct all the tests that make up the scale in various clinical settings.

Regarding the results of each item on the Frailty-Functionality Scale reported by participants in the test and retest evaluations (Figure 2).

Discussion

This study resulted in a Spanish (Chile) version of the HCT Frailty Scale, which was culturally adapted for the Chilean population after a process of translation, back-translation, and evaluation of apparent validity in patients undergoing HSCT. The translation and cultural adaptation process aimed to produce a version of the HCT Frailty Scale that maintains equivalent semantic, conceptual, and technical levels as the original instrument, ensuring that it can be understood by individuals when evaluating their functional status and frailty in their local context.[23]

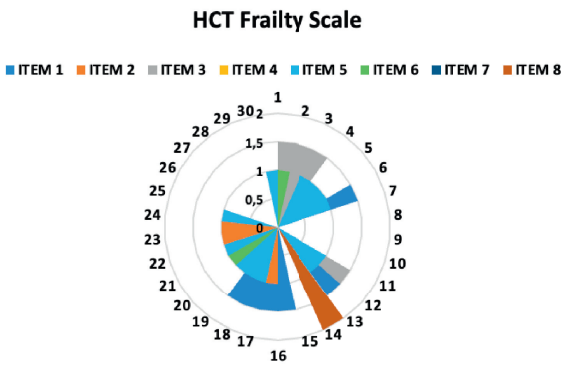
To our knowledge, this is the first validated version for Spanish-speaking individuals in Latin America and could serve as a reference for its use in these countries. However, it is recommended that before using this version of the scale, the authors conduct a thorough review for cultural adaptation and linguistic validation.[24,25] Although the main mission of the Royal Spanish Academy (Real Academia Española) is to ensure that changes in the Spanish language do not break its essential unity, there are certain nuances and terminology preferences in each Spanish-speaking country.

Regarding the apparent validity of the HCT Frailty Scale, it was found to be appropriate for assessing the construct in HSCT candidates. Patients reported that the version was clear and easy to understand. They also highlighted the relevance of being evaluated on their "frailty and functionality" condition as a critical aspect prior to transplantation.

Similarly, for clinical use, scales require valid, reproducible, and reliable evaluation methods. In this study, the reliability analysis through test-retest showed that the Spanish (Chile) version of the HCT Frailty Scale has adequate reliability in terms of information stability.

Regarding content validity according to the consulted experts, the results of this study indicate that the eight items of the scale are relevant and consistent for evaluating the

A



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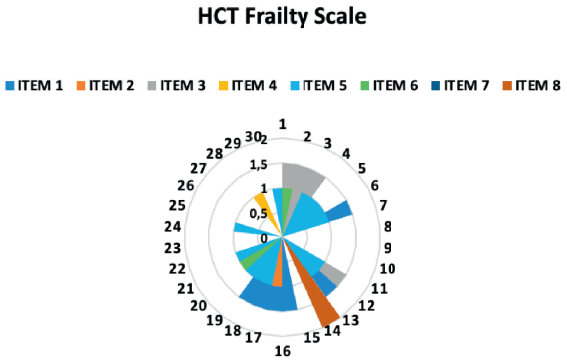

Figure 2 – Results of the test with the Hematopoietic Cell Transplantation Frailty Scale - A: Test; B: Retest.

construct "frailty and functionality" in HSCT candidates, but not for the dimension of clarity. Considering these results and the qualitative input provided by the experts in the observations section, some changes were made to the Spanish version of the scale to improve aspects related to clarity. These changes were made because the benefits derived from these suggestions aim to enhance the validity of the scale, as they directly impact the content of the items and certain aspects related to its structure, thereby avoiding potential content biases and/or errors during subsequent application, as mentioned by some authors.[26,27]

In general, the professionals reported that the scale was easy to apply and they were confident that they had understood the instructions correctly. However, they noted that more time was needed than the 5–10 min stipulated by the original authors of the scale, as it involves several items that require precision, along with functional tests that necessitate additional "learning" time for patients who are performing the tests for the first time. Additionally, a significant number of these patients experience substantial functional deterioration prior to HSCT, a condition that may limit their performance in functional tests.[2,28,29]

Moreover, it is suggested that for better understanding and to facilitate the application of the scale, training and the development of a support manual for healthcare professionals who will assess these patients should be provided.

One limitation of this study was the lack of published psychometric studies for other countries using the HCT Frailty Scale, which prevented the possibility of making broader comparisons with these results.

Furthermore, this study had a small sample size, which is inherent to the type and objective of the study. However, as this study represents an initial step in the evaluation and application of the scale, ongoing research is focused on analyzing other psychometric properties of the Spanish version of the HCT Frailty Scale in a larger patient sample.

The use of the validated HCT Frailty Scale is important for assessing the true extent of frailty and functionality in this population, which could enable the proposal of pre-transplant interventions, such as pre-habilitation, for patients who are not "fit."[1,29]

A key strength of the study is that it proposes a scale the application of which does not require additional costs and can be implemented using existing resources. Additionally, this study recruited a nationally representative sample, as patients from across the country participated, considering that the Hospital del Salvador is a national referral center for HSCT.

Conclusions

The Spanish version of the HCT Frailty Scale for Chile is conceptually and linguistically equivalent to the original instrument. Furthermore, it demonstrated adequate psychometric properties in terms of validity and reliability. Therefore, it is recommended for clinical use to categorize patients who are HSCT candidates.

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Ethical approval

Approved by the Scientific ethics committee of the Metropolitan East Health Service on December 5, 2023.

Data availability

Data supporting the results can be accessed by previous requires to the corresponding author.

Conflicts of interest

The authors declare they have no financial interests.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2025.103933.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Incidence of hepatocellular carcinoma in beta thalassemia: a systematic review and meta-analysis

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ABSTRACT

Background: Current evidence indicates that iron overload increases the risk of hepatocellular carcinoma. However, the incidence of hepatocellular carcinoma in thalassemia is still unclear. This review aims to summarize the current evidence regarding the incidence of hepatocellular carcinoma in thalassemia patients.

Methods: Detailed searches were conducted in several databases, including PubMed, Europe PMC, EBSCOHost, and ProQuest. Keywords such as "thalassemia" and "hepatocellular carcinoma," along with other relevant synonyms, were used. Articles investigating the incidence of hepatocellular carcinoma in thalassemia patients were included. Pooled estimates were calculated using the DerSimonian Laird inverse-variance random effect model and presented as incidence (%) along with their 95 % confidence intervals and 95 % prediction intervals.

Results: From a total of 318 articles, five studies encompassing a total of 9592 thalassemia patients were included in this study. The cumulative incidence of hepatocellular carcinoma in thalassemia patients was 1.96 % (95 % confidence interval: 0.88 %–4.27 %; prediction interval: 0.12 %–24.74 %; $I^2 = 86.8$ %). Of the 139 hepatocellular carcinoma patients, 121 were reported positive for anti-HCV, 78 for HCV RNA, three for HbsAg, and 50 positive for anti-HBV or had past infections. The liver iron concentration and ferritin level ranges in all studies were 2.95–10.5 mg/g and 3.1–2950 μg/L, respectively.

Conclusions: The present meta-analysis demonstrates that the incidence of hepatocellular carcinoma in thalassemia patients was high (1.96 %). It might be caused by liver infection, iron overload, or something else.

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Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer with more than one million people affected each year.[1] It is also the third cause of cancer-related death worldwide.[2] There are various risk factors related to HCC such as viral hepatitis, alcoholic liver diseases, and metabolic diseases.[3] Nowadays iron overload has been linked to the development of HCC. Iron overload induces hepatocyte proliferation, ferroptosis, impaired p53 expression, and mitochondrial iron accumulation that could promote HCC.[4] There are several causes of iron overload including transfusion dependent thalassemia.[5]

Thalassemia is a condition where there is inadequate production of globin protein leading to ineffective oxygen transport[6] with 35 % of thalassemia patients being dependent on routine transfusions: this can lead to the development of iron overload if not monitored regularly.[7] Excessive deposits of iron in various organs can lead to chronic liver disease and thalassemia patients are more exposed to blood-transmitted diseases such as chronic viral hepatitis.[8]

Thalassemia patients are potentially at higher risk for developing HCC compared to the normal population. However, the incidence of HCC in thalassemia is still unclear. This review aims to summarize the current evidence regarding the incidence of HCC in thalassemia patients.

Methods

Three independent investigators performed detailed searches for relevant studies in several databases including PubMed, Cochrane Controlled Register of Trials (CENTRAL), Europe PMC (medRxiv and bioRxiv), EBSCOHost (Medline), and ProQuest (Gray Literatures) from inception to 30 July, 2023 using keywords such as "thalassemia" and "hepatocellular carcinoma," along with other relevant synonyms. Articles investigating the incidence of HCC in thalassemia patients were included in this study. There were no restrictions on time or settings. Studies were excluded if they met any of the following criteria: 1) case reports, letters to editors, reviews; 2) non-English articles; or 3) irretrievable full-text articles.

The study selection was done by three authors independently, and disagreement was resolved by the fourth author. Duplicates and irrelevant articles were excluded. The authors screened the titles and abstracts obtained through the search before excluding any work that did not meet the inclusion criteria. Selected studies at this stage were screened further using the full text of the records to determine their eligibility. Any disagreements at each stage of the selection process were resolved by discussion. Data extraction, including author's name, year of publication, study characteristics, patient characteristics, and outcomes, was input into a web-based word processor.

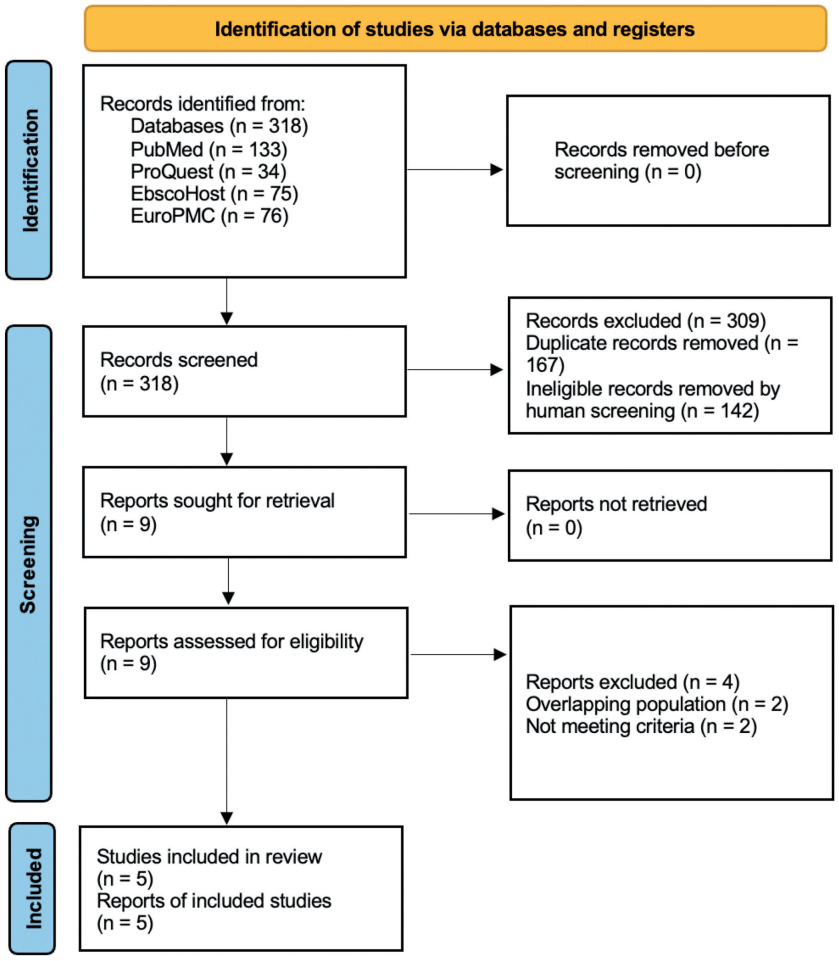

Figure 1 – Literature search process and results.

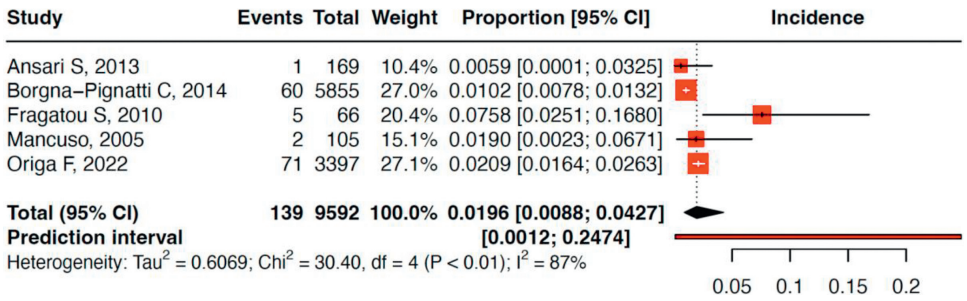

Figure 2 – Pooled overall incidence of hepatocellular carcinoma in thalassemia patients.

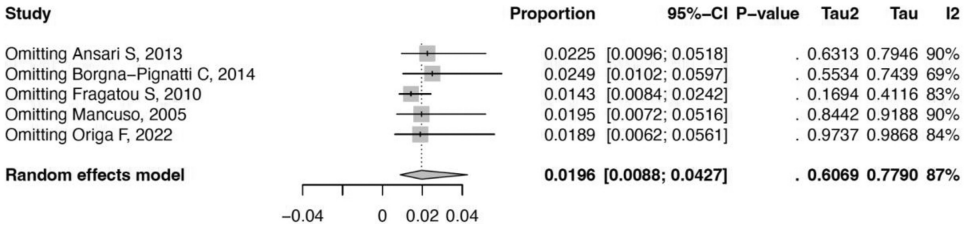

Figure 3 – Leave-one-out sensitivity analysis.

To assess the risk of bias, all authors independently assessed methodological quality of the studies using the Quality in Prognosis Studies tool.[9] I-squared statistics were employed to analyze the heterogeneity of the studies. Pooled estimates were calculated using the DerSimonian Laird inverse-variance random effect model and presented as incidence (%) along with the 95 % confidence intervals and 95 % prediction intervals. Sensitivity analysis was done by leave-one-out analysis.

Results

From a total of 318 articles, 167 duplicates and 142 ineligible records were removed. Nine studies were assessed for eligibility resulting in four studies excluded because of overlapping populations and not meeting the study criteria. Five studies, encompassing a total of 9592 thalassemia patients, were included in this study (Figure 1).[10–14]

Of all the patients, 73.8 % (n = 7083) had thalassemia major, and 26.1 % (n = 2509) had thalassemia intermedia. Three studies from Italy, one study from Iran, and one study from Greece reported HCC incidence rates of from 1.02 % and 2.09 %, 0.6 %, and 7.57 %, respectively. Of the 139 HCC patients, 121 were reported positive for anti-HCV, 78 for HCV RNA, three for HbsAg, and 50 were positive for anti-HBV or had infections. The liver iron concentration (LIC) and ferritin level ranges in all studies were 2.95–10.5 mg/g and 3.1–2950 μg/L, respectively.

The cumulative incidence of HCC in thalassemia patients was 1.96 % (95 % confidence interval: 0.88 %–4.27 %) with a prediction interval of 0.12 %–24.74 % and I^2 of 86.8 %. Sensitivity analysis revealed similar estimates when each study was sequentially removed. This indicates that the results are robust and without inter-studies heterogeneity. Risk of bias assessment using Joanna Briggs Institute Critical Appraisal Tools found that all studies had a low risk of bias (Figures 2-4, Table 1).

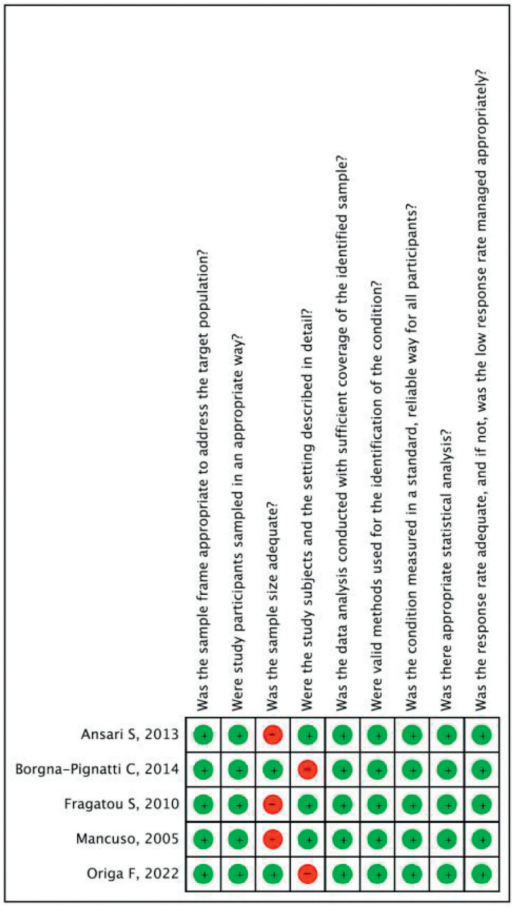

Figure 4 – Risk of bias summary for included studies assessed using Joanna Briggs Institute Critical Appraisal Tools.

Table 1 – Characteristics of included studies.

Ref	Author	Year	Setting	No of patients	Other hemoglobinopathies	Total HCC incidence (Thal Only)	Total HCC incidence (All hemoglobinopathies)	HBV/HCV status in Thal patients with HCC	Iron status in Thal patients with HCC	Thal with HBV	Thal with HCV
[10]	Ansari S	2013	Iran	170 (TM = 164; TI = 5; SCD = 1)	SCD = 1	1/169 (0.6 %)	1/170 (0.6 %)	Anti-HCV and HCV RNA (+) = 1/1	NR	–	TM = 164; TI = 5
[13]	Borgna-Pignatti C	2014	Italy	5857 (TM = 4248; TI = 1607 SCD = 2)	SCD = 2	60/5855 (1.02 %)	NR	HBsAg (+) = 3/60; Anti-HBc (+) = 36/60; HBV vaccination = 22/60; Anti-HCV (+) = 52/60; HCV RNA (+) = 43/60; HBV/HCV (-) = 4/60; Unknown serology status = 1/60	TM LIC (median) = 2.95 mg/g; TI LIC (median) = 9 mg/g; TM ferritin (median and peak) = 937 μg/l and 2001 μg/l; TI ferritin (median and peak) = 1181 μg/l and 2950 μg/l	NR	NR
[11]	Fragatou S	2010	Greece	66 (TM = 57; TI = 9)	–	5/66 (7.57 %)	–	Anti-HCV (+) = 2/5; HCV RNA (+) = 2/5 Anti-HBc = 1/5; HBV/HCV (-) = 3/5	TM LIC = 4.9 and 0.215 mg/g; TI LIC = 4.8, 5.2, and 6.9 mg/g; TM ferritin = 18.9 and 3.1 μg/l (1890 and 310 ng/dL); TI ferritin = 6, 13.5, and 14.5 μg/l (600, 1350, and 1450 ng/dL)	–	TM = 23
[14]	Mancuso A	2005	Italy	105 (TM = 35; TI = 70)	–	2/105 (1.90 %)	–	Anti-HCV (+) = 2/2; HCV RNA (+) = 2/2	Iron overload (+) = 2/2; LIC = NR	TI = 2	TM = 28; TI = 18
[12]	Origa F	2022	Italy	4631 (TM = 2579; TI = 818; Others = 1234)	SCD = 815; HbH = 384; Others = 35	71/3397 (2.09 %)	78/4631 (1.68 %)	Anti-HBV = 14/67; HBV DNA = 1/25; Anti-HCV (+) = 64/78; HCV RNA (+) = 30/68 (Serology status including other hemoglobinopathies)	LIC at diagnosis = 5.2 mg/g; LIC peak before diagnosis = 10.5 mg/g; Ferritin at diagnosis (median) = 786 ng/ml (786 μg/l); Ferritin peak before diagnosis = 2704 ng/ml (2704 μg/l)	NR	NR

Thal: thalassemia; TM: thalassemia major; TI: thalassemia intermedia; SCD: sickle cell disease; HBV: hepatitis B virus; HCV: hepatitis C virus; HCC: hepatocellular carcinoma; LIC: liver iron concentration; NR: not reported.

Discussions

HCC can increase morbidity and mortality in thalassemia population especially when they are 41–50 years old for thalassemia major, and 61–65 years old for thalassemia intermedia. HCC was one of the most frequent solid malignancies in thalassemia patients.[15] HCC is because of iron overload and/or transfusion-transmitted viral infections, hepatitis B or hepatitis C, immunology abnormality, hydrea use, bone marrow stimulation due to chronic anemia. According to the subject characteristics in this study, most were hepatitis B or hepatitis C positive. Only seven subjects did not have hepatitis B or C. One study did not give the details.[12]

The mean age at diagnosis of HCC was younger than for the non-thalassemia population. This might be due to hemosiderosis as an additional factor for HCC.[16,17] Iron overload can happen primarily due to the suppression of hepcidin synthesis in the liver, it increases recycled iron released from the reticuloendothelial system and also increases intestinal absorption. It also occurs secondary to regular transfusions especially in thalassemia major patients.[18] Iron induces toxicity damage which results in genotoxicity, immunological aberrancies, and attenuating cancer immune surveillance.[19]

According to this analysis LIC and ferritin level ranges in all studies were 2.95–10.5 mg/g and 3.1–2950 μg/L. Borna-Pignatti et al. found that three out of four patients without hepatitis B or hepatitis C had high levels of ferritin.[13] Another study by Maakaron et al. also mentioned two cases of HCC in hepatitis negative patients with thalassemia intermedia with both having high levels of ferritin and liver iron.[20] These conditions had been studied in other populations such as hereditary hemochromatosis (HH) and iron overload. The researchers found a significant relationship, stating that patients with HH had a 23-fold higher risk of developing HCC compared to healthy individuals.[20] The annual incidence rate of HCC related liver cirrhosis was 3 %–4 %.[21]

In general, it was believed that HCC was more common in patients with transfusion dependent thalassemia than non-transfusion dependent thalassemia with the milder progression of iron overloading and a lower incidence of chronic viral liver infections being possible explanations.[17] But there was also another theory related to the difference of iron overload impact between thalassemia major (TM) and intermedia (TI). In TI, similar to genetic haemochromatosis, the iron is absorbed directly from the intestinal tract and loads to hepatocytes. A different process happens in TM. The transfused iron initially goes to Kupffer cells. This different pathway makes the liver iron level in TI higher than in TM which might increase the prevalence of HCC in TI than in TM.[13]

This high iron level, if it happens above the ferritin synthesizing capacity of the cells, may generate reactive oxygen species (ROS) and mutations. Imbalance of immune regulation as another result of iron overload decreases the CD4/CD8 ratio and modulates cytokine activity. Both are responsible for self-defense against viruses and malignant cells. These changes may lead to cancer development.[19] Iron overload also activates stellate cells and profibrogenic effects of lipid peroxidation, thus accelerating fibrosis to cirrhosis and HCC.[17]

The role of iron in the development of HCC can be prevented by using iron chelation. Some guidelines recommend initiation of chelation therapy in non-transfusion dependent patients with ferritin levels >800 ng/L or LIC >5 mg/g dry weight.[22] An experimental study by Qian Ba et al. proved that a potent iron chelator can suppress tumor growth of HCC. It reduced available iron, triggering cell-cycle arrest, and apoptosis. An experimental study by Qian Ba et al. proved that iron chelators can suppress tumor growth in HCC. It reduced available iron, triggering cell-cycle arrest, and apoptosis.[23] The most widely iron chelators used in clinical settings are desferrioxamine (DFO), deferasirox (DFX), and deferiprone.[24] DFX-DFO combination or DFX as monotherapy have been proven to reduce LIC effectively.[25]

Conclusions

The present meta-analysis demonstrates that the incidence of HCC in thalassemia patients was high (1.96 %). It might be caused by liver infection, iron overload, or something else. More studies are needed to further estimate the incidence of HCC in thalassemia patients and its pathogenesis.

Conflicts of interest

The authors declare no conflicts of interest.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Review article

Blood storage effect of G6PD on RBC quality

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ABSTRACT

Background: The most prevalent metabolic condition of red blood cells, glucose-6-phosphate dehydrogenase (G6PD) deficiency, affects around 35 million people globally. The highest prevalence is seen in tropical and subtropical areas of the eastern hemisphere, where it can affect up to 35 % of the population. G6PD deficiency, the most prevalent enzyme deficit, is not currently tested for in blood products. G6PD deficiency is a genetic factor that influences the quality of stored red blood cells impacting their ability to respond to oxidative stress. This hospital-based cross-sectional study aimed at assessing the prevalence of G6PD deficiency in donor blood and the impact of the enzyme deficiency on red cell indices during storage.

Method: A total of 57 blood bags were screened for G6PD deficiency. Red cell indices and blood film comments were investigated on Day 0, Day 7 and Day 14 of storage.

Results: Eight out of 57 (14 %) had the G6PD full defect and 86 % (49/57) had no defect. Over the course of 14 days storage, the hemoglobin and red blood cell count significantly decreased in G6PD-deficient blood units with a corresponding significant increase in mean corpuscular volume and red cell distribution width-standard deviation compared to baseline and normal G6PD activity. The blood film comment showed 85.7 % normocytic normochromic, 2.0 % microcytic hypochromic and 12.2 % macrocytic hyperchromic from G6PD-non-deficient donors whereas G6PD-deficient donors had 75 % normocytic normochromic with 12.5 % microcytic hypochromic and 12.5 % macrocytic hypochromic after 2 wk in storage.

Conclusion: Red blood cell count and hemoglobin reduce significantly in G6PD-deficient donor units during storage with an associated increased mean corpuscular volume indicating progressive loss of the cellular membrane homeostatic mechanism that could potentially result in further hemolysis during long term storage.

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Introduction

One of the most common therapies for anemic hospitalized patients is red blood cell (RBC) transfusions.[1] Patients with sickle cell disease and thalassemia, in particular, require chronic transfusions because of inherent RBC abnormalities linked to increased hemolysis and inefficient erythropoiesis. Accelerated clearance of transfused RBCs results in several side effects related to continuous RBC transfusion therapy, including iron overload, alloimmunization, and perhaps increased susceptibility to infection.[2] As a consequence, numerous initiatives are made to supply the highest quality RBC products. The Food and Drug Administration (FDA) establishes acceptance criteria for RBC units at the end of their maximum permitted storage period (42 days), which are primarily based on an average 24-hour post-transfusion recovery (PTR) rate of at least 75 % (i.e., 75 % of the transfused RBCs should still be circulating 24 h after transfusion) and a <1 % rate of in vitro hemolysis.[3] Additionally, the proportion of successful PTRs must have a one-sided, lower limit of the 95 % confidence interval of at least 70 %; in other words, there can be no more than two unsuccessful PTRs of 75 % in a cohort of 20 healthy volunteer blood donors.

PTRs are remarkably different between blood donors,[4] with these variations being distinct and recurrence-free for each donor, indicating that some donors are strong iron storers and others are poor iron storers.[1] Inter-donor metabolic heterogeneity was discovered by in vitro tests of preserved RBCs; this heterogeneity can affect the metabolic age of stored RBC units at least as much as their chronological age.[5] Furthermore, as RBC storage quality is heriTable,[6] genetic factors might be to blame for at least some of these variances.

The most prevalent human enzymopathy, glucose-6-phosphate dehydrogenase (G6PD) deficiency, is an X-linked illness that affects around 400 million people worldwide.[7] The pentose phosphate pathway (PPP), which produces reduced nicotinamide adenine dinucleotide phosphate (NADPH), a cofactor that powers a number of antioxidant pathways in RBCs, also depends on G6PD as its rate-limiting enzyme.[8] In fact, NADPH is necessary for glutathione reductase to recycle oxidized glutathione into its reduced form. The thioredoxin reductase system, biliverdin reductase B, and the ascorbate-tocopherol axis are just a few examples of the numerous NADPH-dependent antioxidant enzymes it supports.[9] It also enhances catalase, glutathione peroxidase, peroxiredoxins, glutaredoxins, and the thioredoxin reductase system. The reduced ability of G6PD-deficient RBCs to produce NADPH,[1] which can be brought on by drugs, infections, and nutrition, makes them more vulnerable to oxidative stress.[10]

In refrigerated storage, oxidative stress indicators increase,[11,12] indicating that storage itself may contribute to oxidative stress. PTR also increases noticeably in mice and humans when RBCs are maintained under hypoxic conditions[13] or in the presence of the antioxidant ascorbic acid,[14] which reduces oxidative stress. RBCs do not appear to have evolved to withstand the oxidative damage brought on by cold storage however, they evolved defenses against oxidative stress as they age in vivo with some of these defenses being triggered during typical blood bank storage. Studies using stable isotope-labeled tracers, for instance, indicate that storage-induced oxidation of Cys152 of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) results in a shift in the glucose metabolism toward the oxidative phase of the PPP; this phenomenon is attenuated or exacerbated by hypoxic or hyperoxic storage, respectively.[15] G6PD-deficiency reduces NADPH generation in RBCs, which reduces their capacity to replenish the reduced form of glutathione and prevent the buildup of peroxidation/inflammatory products.[16] G6PD is the most important enzyme in the oxidative phase of the PPP. In fact, blood units obtained from G6PD-deficient donors have altered glutathione homeostasis and antioxidant defenses.[17]

Method

Study design

This was a cross-sectional study to assess the prevalence of G6PD deficiency among blood donors. It also has a comparative study design to assess the impact of G6PD deficiency on stored RBCs as compared to non-G6PD-deficient stored RBCs.

Ethical considerations

Ethical clearance was obtained from the Committee on Human Research Publications and Ethics (CHRPE) of the School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology before the inception of the study. The management of Living Waters Hospital also gave their approval for their facility to be used for this study. Moreover, consent was sought from blood donors who were assured of the highly confidential nature of this study.

Sample collection

About 5 mL of blood was collected from each blood unit donated in the blood bank from patients who had passed the donor screening tests. These samples were used for the initial analysis. Subsequently after 7 and 14 days, additional samples were collected from the same blood bags that had been kept in a storage fridge.

The first set of samples were screened for G6PD deficiency using the methemoglobin reductase technique. Thin films were prepared, stained with Leishman stain and observed for general film comment on the red cell morphology. Furthermore, a complete blood count was performed on the samples to determine red cell hematological indices.

Laboratory investigations

The procedure of the G6PD screening test

The methemoglobin technique of G6PD testing was done by arranging three test tubes in a test tube rack with the labels 'Positive', 'Test' and 'Negative'. One mL each of a well-mixed blood sample from a CPD-A1 anti-coagulant blood storage bag was introduced into the three test tubes. Fifty μL of a mixture of sodium nitrite and glucose was dispensed into the tubes

labelled 'positive' and 'test' and mixed and 50 μL of methylene blue was added to the tubes labelled 'test' and 'negative' and mixed.

The test tube setups were then corked and incubated in a water bath at 37 °C for 3 h. At the end of this time, the contents of the tubes were diluted with physiological saline solution and observed against a white background. The result was read as either full defect, partial defect or no defect.

Complete blood count

The blood sample collected from the blood bags into a plain test tube was swirled to evenly distribute blood cells.

Following standard protocols, the complete blood count of all samples was analyzed using a MINDRY BC-3000Plus 3PARTS Automated Hematology Analyser from the Kumasi Technical University Clinic laboratory.

The parameters of interest of the complete blood count analysis were the hemoglobin (Hb) concentration, RBC count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH) and mean corpuscular Hb concentration (MCHC) since the study focuses on RBC indices.

Blood film comment

Thin blood films of each sample were prepared and stained with Leishman stain using the standard staining protocol, with Leishman stain being flooded on the smear for 1–2 mins and then diluted with buffered water at about twice the volume of the stain and allowed to stand for 15 mins. The slides were then washed and blotted for observation.

The stained slides were observed by a student and the blood picture was confirmed by an independent experienced hematologist at the facility. The observed morphological characteristics of the cells were then used to categorize the cells.

Results

Socio-demographic characteristics of study participants

A total of 57 male blood donors were recruited for this study. The mean age of the blood donors was 26.47 ± 3.723 years (range: 19–38 years). The majority of the blood donors were in the 21–25 (46.6 %) age group followed by 26–30 (36.2 %), whilst the smallest age group was that of 36–40 (1.8 %) years old. Of the various blood groups, 45.6 % were of the O^+ blood group, followed by 24.6 %, 17.5 %, 5.3 %, 3.5 %, 1.8 % and 1.8 % of the A^+, B^+, AB^+, B^-, A^- and O^- blood groups, respectively.

From the total of 57 blood donors recruited, 8 (14 %) had the full defect for G6PD enzyme activity whilst 49 (86 %) had no defect for G6PD activity. This gives a 14 % (8/57) prevalence of G6PD deficiency among blood donors of this study (Table 1 and Figure 1).

General effect of storage on RBC indices of donor blood

At baseline, the mean Hb, RBC count, MCV, MCH, MCHC and red cell distribution width-standard deviation (RDW-SD) of the donor units were 13.00 ± 1.99 g/dL, 4.55 ± 0.62 × 10^{12}/L, 82.57 ± 9.71 fL, 27.48 ± 4.36 pg, 33.10 ± 2.22 g/dL and 48.16 ± 3.5 fL, respectively (Table 2).

Comparing the hematological indices of the donor samples from the baseline to Day 7 in storage, the mean Hb decreased significantly (p-value = 0.023) from 13.00 ± 1.99 g/dL to 12.78 ± 2.26 g/dL while the RDW increased significantly (p-value = 0.00) from 48.16 ± 3.5 fL to 50.24 ± 4.1 fL. However, the RBC count (p-value = 0.368), MCV (p-value = 0.220), MCH (p-value = 0.336) and MCHC (p-value = 0.080) showed no significant changes (Table 2).

Comparing the data again from the baseline to day 14 in storage, the mean Hb and MCHC decreased significantly from 13.00 ± 1.99 g/dL to 12.87 ± 2.57 g/dL (p-value = 0.009) and

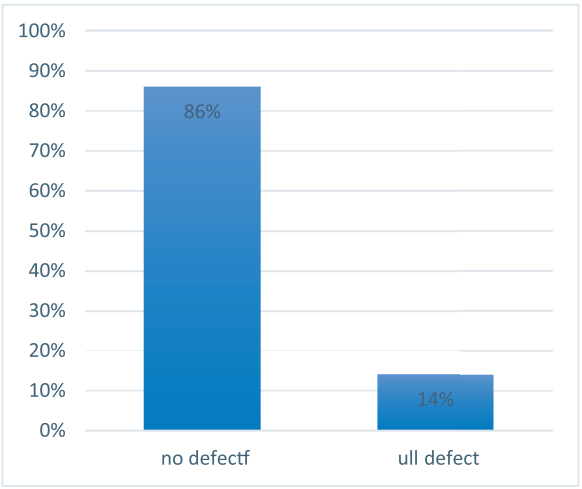


Figure 1 – Prevalence of G6PD status among blood donors.

Table 1 – Shows descriptive statistics of the blood donors in the study.

Variable	Frequency	Percentage (%)
Gender		
Male	57	100
Female	0	0
Total	57	100
Age group-years		
16–20	2	3.5
21–25	27	47.4
26–30	21	36.8
31–35	6	10.5
36–40	1	1.8
Total	57	100
Blood group		
A-	1	1.8
A+	14	24.6
AB+	3	5.3
B-	2	3.5
B+	10	17.5
O-	1	1.8
O+	26	45.6
Total	57	100
G6PD Status		
No defect	49	86
Full defect	8	14
Total	57	100

Table 2 – Changes in red blood cell parameters of donor blood over a 7-day storage period.

Variable	Baseline	7 days of storage	*p*-value
Hb (g/dL)	13.00 ± 1.99	12.78 ± 2.26	0.023
RBC ($\times 10^{12}$/L)	4.55 ± 0.62	4.50 ± 0.66	0.368
MCV (fL)	82.57 ± 9.71	83.43 ± 10.91	0.22
MCH (pg)	27.48 ± 4.36	27.21 ± 4.41	0.336
MCHC (g/dL)	33.10 ± 2.22	32.60 ± 1.61	0.08
RDW-SD (fL)	48.16 ± 3.5	50.24 ± 4.1	0.00

Hb: hemoglobin; RBC: Red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: red cell distribution width-standard deviation.

33.10 ± 2.22 g/dL to 30.90 ± 2.08 g/dL (*p*-value = 0.002), respectively, whereas the mean MCV and RDW increased significantly from 82.57 ± 9.71 fL to 87.96 ± 14.32 fL (*p*-value = 0.001) and 48.16 ± 3.5 fL to 51.28 ± 4.0 fL (*p*-value = 0.00), respectively. However, the RBC count (*p*-value = 0.300) and MCH (*p*-value = 0.284) showed no significant changes (Table 3).

Impact of G6PD deficiency on RBC indices of stored donor blood units

The mean values of the RBC indices (Hb, MCV, MCH and MCHC) of G6PD-deficient and G6PD-non-deficient blood during baseline analysis were slightly lower in full-defect blood compared to non-defect blood. However, the mean RBC count remained the same and the RDW was slightly higher in full-defect blood compared to non-defect blood.

G6PD-deficient samples showed significant decreases in Hb concentration (*p*-value = 0.015) and RBC count (*p*-value = 0.025) and a significant increase in RDW (*p*-value = 0.00) by the 7th day of storage whilst donor blood with normal G6PD enzyme activity maintained stable for Hb concentration (*p*-value = 0.161) and RBC count (*p*-value = 0.997) over this period. Additionally, a significant reduction in MCHC (*p*-value = 0.053) and an increase in RDW (*p*-value = 0.000) occurred in donor blood with normal G6PD activity (Table 4).

Again, G6PD-deficient samples showed significant decreases in Hb (*p*-value = 0.03) by the 14th day of storage whilst donor blood with normal G6PD enzyme activity maintained a stable Hb concentration over this period (*p*-value = 0.079). Additionally, a significant reduction in the RBC count (*p*-value = 0.03) occurred in G6PD-deficient blood but not in donor blood with normal G6PD activity. There was a general increase in MCV (*p*-value = 0.034) and RDW (*p*-value = 0.05) which occurred in both G6PD-deficient and G6PD-non-deficient blood by the 14th day of storage (Table 5).

Table 4 – Comparison of red blood cell indices between G6PD-deficient (*n* = 8) and non-deficient donor blood (*n* = 49) after 7 days storage.

Variable	Baseline	7 days of storage	*p*-value
Hb (g/dL)			
G6PD defect	12.61 ± 1.64	11.92 ± 1.88	0.015
G6PD no defect	13.06 ± 2.04	12.92 ± 2.31	0.161
RBC ($\times 10^{12}$/L)			
G6PD defect	4.56 ± 0.44	4.21 ± 0.44	0.025
G6PD no defect	4.55 ± 0.65	4.55 ± 0.68	0.997
MCV (fL)			
G6PD defect	79.23 ± 12.85	79.80 ± 15.13	0.761
G6PD no defect	83.12 ± 9.15	84.02 ± 10.14	0.238
MCH (pg)			
G6PD defect	25.65 ± 5.71	25.59 ± 5.48	0.923
G6PD no defect	27.78 ± 4.10	27.48 ± 4.22	0.333
MCHC (g/dL)			
G6PD defect	31.88 ± 2.45	32.08 ± 1.44	0.746
G6PD no defect	33.29 ± 2.14	32.69 ± 1.64	0.053
RDW-SD (fL)			
G6PD defect	48.90 ± 50	50.99 ± 5.2	0.00
G6PD no defect	48.03 ± 3.3	50.12 ± 3.9	0.00

Hb: hemoglobin; RBC: Red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: red cell distribution width-standard deviation.

Microscopic morphological assessment of G6PD-deficient and non-deficient donor blood after storage

Analysis of blood film comments of 57 donor samples presented with 89.8 % of RBC samples with normocytic normochromic and 10.2 % samples with microcytic hypochromic blood pictures from G6PD-non-deficient donors whereas G6PD-deficient donor samples showed 75 % of samples with normocytic normochromic blood picture, 12.5 % with microcytic hypochromic picture and 12.5 % with anisopoikilocytosis during baseline analysis (Table 6).

After seven days of storage, 93.9 % of samples from G6PD-non-deficient donors presented with normocytic normochromic and 6.1 % with microcytic hypochromic blood pictures whereas 75 % of samples from G6PD-deficient donors were

Table 3 – Changes in red cell parameters of donor blood over a 14-day storage period.

Variable	Baseline	7 days of storage	14 days of storage	*p*-value
Hb (g/dL)	13.00 ± 1.99	12.78 ± 2.26	12.87 ± 2.57	0.009
RBC ($\times 10^{12}$/L)	4.55 ± 0.62	4.50 ± 0.66	4.58 ± 0.74	0.300
MCV (fL)	82.57 ± 9.71	83.43 ± 10.91	87.96 ± 14.32	0.001
MCH (pg)	27.48 ± 4.36	27.21 ± 4.41	27.28 ± 4.42	0.284
MCHC (g/dL)	33.10 ± 2.22	32.60 ± 1.61	30.90 ± 2.08	0.002
RDW-SD (fL)	48.16 ± 3.5	50.24 ± 4.1	51.28 ± 4.0	0.000

Hb: hemoglobin; RBC: Red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: red cell distribution width-standard deviation.

Table 5 – Comparison of red blood cell indices between G6PD-deficient and non-deficient donor blood after 14 days storage.

Variable	Baseline	7 days of storage	14 days of storage	*p*-value
Hb (g/dL)				
G6PD defect	12.61 ± 1.64	11.92 ± 1.88	11.8 ± 2.12	0.03
G6PD no defect	13.06 ± 2.04	12.92 ± 2.31	13.05 ± 2.62	0.079
RBC ($\times 10^{12}$/L)				
G6PD defect	4.56 ± 0.44	4.21 ± 0.44	4.24 ± 0.46	0.03
G6PD no defect	4.55 ± 0.65	4.55 ± 0.68	4.63 ± 0.76	0.778
MCV (fL)				
G6PD defect	79.23 ± 12.85	79.80 ± 15.13	83.40 ± 17.70	0.034
G6PD no defect	83.12 ± 9.15	84.02 ± 10.14	88.70 ± 13.77	0.00
MCH (pg)				
G6PD defect	25.65 ± 5.71	25.59 ± 5.48	25.71 ± 5.48	0.968
G6PD no defect	27.78 ± 4.10	27.48 ± 4.22	27.53 ± 4.24	0.195
MCHC (g/dL)				
G6PD defect	31.88 ± 2.45	32.08 ± 1.44	30.68 ± 1.81	0.197
G6PD no defect	33.29 ± 2.14	32.69 ± 1.64	30.94 ± 2.13	0.00
RDW-SD (fL)				
G6PD defect	48.90 ± 5.0	50.99 ± 5.2	52.23 ± 5.5	0.05
G6PD no defect	48.03 ± 3.3	50.12 ± 3.9	51.12 ± 3.8	0.00

Hb: hemoglobin; RBC: Red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: red cell distribution width-standard deviation.

Table 6 – Microscopic morphological variations between G6PD-deficient and G6PD-non-deficient donor units.

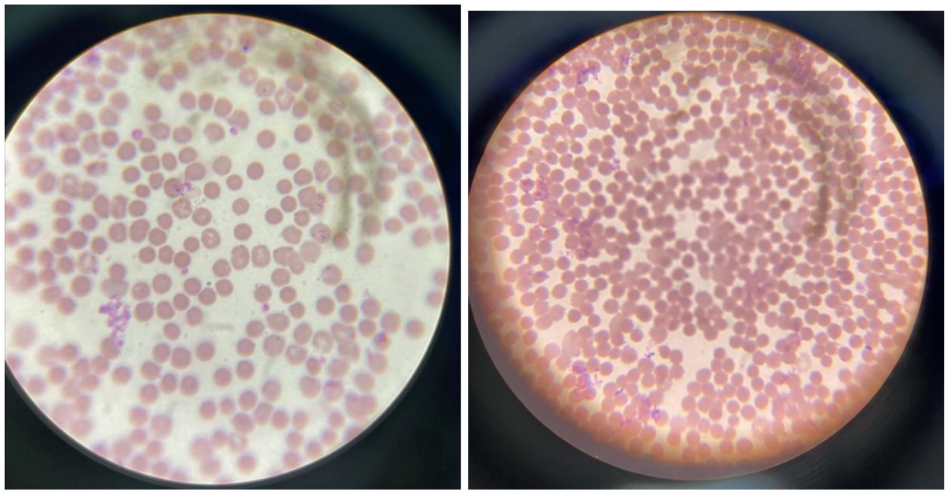
Variable	Baseline	7 days of storage	14 days of storage
Film comment	n (%)	n (%)	n (%)
G6PD defect			
normocytic normochromic	6 (75)	6 (75)	6 (75)
microcytic hypochromic	1 (12.5)	1 (12.5)	0 (0)
macrocytic hypochromic	0 (0)	0 (0)	1 (12.5)
anisopoikilocytosis	1 (12.5)	1 (12.5)	1 (12.5)
Total	8 (100)	8 (100)	8 (100)
G6PD no defect			
normocytic normochromic	44 (89.8)	46 (93.9)	42 (85.7)
microcytic hypochromic	5 (10.2)	3 (6.1)	1 (2)
macrocytic hypochromic	0 (0)	0 (0)	6 (12.2)
anisopoikilocytosis	0 (0)	0 (0)	0 (0)
Total	49 (100)	49 (100)	49 (100)

normocytic normochromic, 12.5 % were microcytic hypochromic and 12.5 % had anisopoikilocytosis (Table 6).

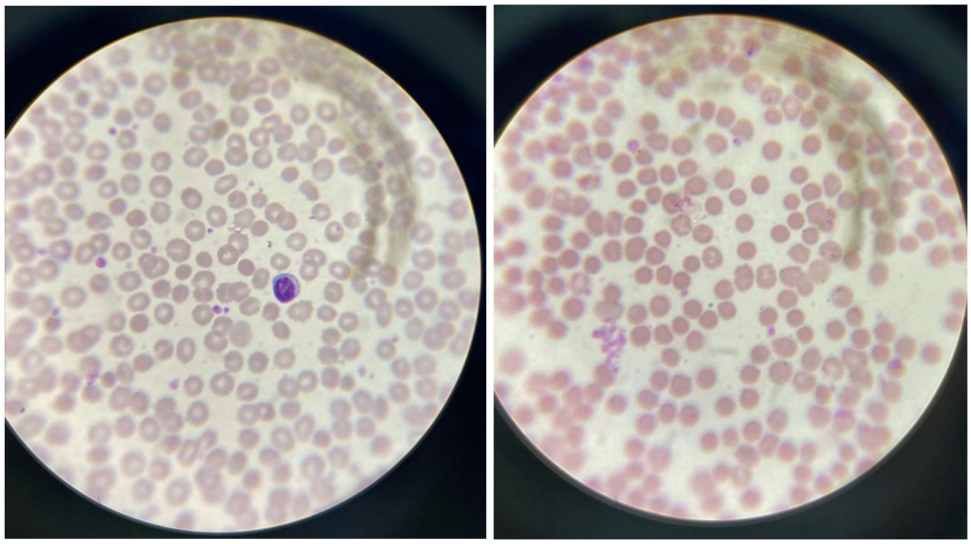
Moreover, after 14 days of storage, the blood film comments of G6PD-non-deficient donors identified 85.7 % normocytic normochromic, 2 % microcytic hypochromic and 12.2 % macrocytic hypochromic samples and from G6PD-deficient donor blood 75 % samples were normocytic normochromic, 12.5 % were macrocytic hypochromic and 12.5 % had anisopoikilocytosis (Table 6 and Figure 2).

Discussion

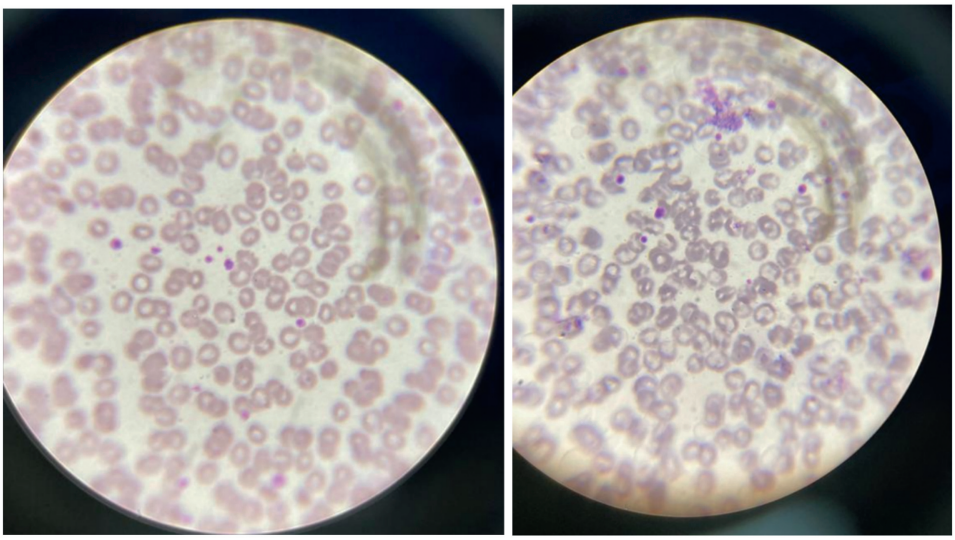
This study was geared towards establishing the prevalence of G6PD deficiency among blood donors at the Living waters Hospital in the Ashanti region and any potential effect of G6PD enzyme deficiency on RBC indices during storage in the blood bank. The study recruited 57 blood donors all of whom were male with the majority being between 21 and 25 (46.6 %) and 26–30 (36.2 %) years old. The finding on males is that men are the dominant gender in blood donations in line with a study conducted at Sokoto in North Western Nigeria where of a total of blood 14,965 donors from January 2010 to July 2013, 14,871 (99.4 %) were males and only 94 (0.64 %) were female.[18] Most studies in Africa reported a male dominance in blood donation programs: 61 % in Togo,[19] 71.2 % in Burkina Faso[20] and 90 % in Ghana.[21] In a recent survey in Central, Western, and Eastern Franco-phone African regions, all seven countries surveyed reported <30 % females in their donor populations.[22] One contributing factor might be that women do not meet donation cut-off values for hemoglobin due to normal menses, menorrhagia, prenatal iron deficiency anemia and postnatal blood loss. From a cultural perspective also, in various African countries it is more likely for males to donate blood given long-standing beliefs that women are not as physically strong as men.[23] In Western regions, such as Europe, women were found to have higher rates of adverse reactions, primarily vasovagal events, and were also not as likely to meet hemoglobin cut-off requirements for donation.[24]



Sample 20: Normocytic normochromic on Day 7 and normocytic normochromic on Day 14



Sample 15: Macrocytic hypochromic on Day 14 and normocytic normochromic on Day 7



Sample 24: Anisopoikilocytosis on Day7 and anisopoikilocytosis on Day 14

Figure 2 – Examples of the film comment results.

The age distribution observed in the present study was very similar to those reported by studies in Kenya, East Africa, where 59 % of voluntary donors were <25 years old,[25] in Burkina Faso, with a reported mean age of 28.9 ± 7.9 years,[26] and in Rwanda, where >75 % were <30 years old,[23] highlighting the fact that young people form the backbone of blood donation in these countries.

ABO distribution in this study showed that blood group O Rh positive (45.6 %) was the most predominant among the donors followed by A Rh positive (24.6 %) and B Rh positive (17.5 %). The rarest blood groups were A Rh negative (1.8 %) and O Rh negative (1.8 %). This finding is similar to a study conducted in Cape Coast, Ghana by Patrick Adu et.al., where O-positive was found predominant in 36.59 % and AB-positive was the least common in 6.33 % of the donations. Another study, also in line with this result, reported that the O-positive group was predominant and AB-positive was the least common.[27] But other studies have reported different results with A-positive being the predominant group followed by O-positive however AB-positive was still the least frequent.[28]

It was observed that, the prevalence of G6PD deficiency among blood donors was 14 % (8/57) which is higher than the 7.9 % reported by Stephen et al. in Cameroon, Central Africa.,[29] and slightly lower than the 19.5 % reported by Patrick et.al. at Berekum in the Brong Ahafo region of Ghana.[30] However, Soheir et.al. reported a prevalence of G6PD deficiency of 4.3 % in Egypt, East Africa.[31] The differences in prevalence between this study and other studies may be attributed to the variations in population studied including genetic factors, screening methods used and the sample size of the population studied.

Storage of whole blood and components is necessary in order to provide support in many accident emergencies, and for obstetric bleeding and post-partum hemorrhage. Provision and storage of blood and blood components is therefore important in the hospital setting.[32]

This study showed a general significant decrease in the Hb concentration and MCHC levels during storage throughout the study period whereas MCV levels had significantly increased by Day 14 suggesting that osmosis of fluid into the RBC increases during storage as the RBC membrane is impaired; this may ultimately lead to RBC hemolysis. This observation confirms the report of Christian Eze et al. that, as storage time increases, hemolysis increases in stored blood.[33] In line with this assertion, L'Acqua et al. demonstrated that, transfusion of RBCs stored for longer than 4 wk, considerably increased plasma free Hb.[34] Additionally, a study by Houxiang et al.,[35] also showed that free Hb and percentage of free to total Hb in storage medium also significantly increased after storage as adenosine triphosphate and 2,3-difosfoglicerato levels were significantly decreased compared to fresh RBCs.

This study also showed that, despite both G6PD-deficient and non-deficient blood donors fulfilled the minimum Hb concentrations for blood donation, G6PD-deficient donors had lower mean Hb concentrations compared to those of donors with normal G6PD enzyme activity. Additionally, over the course of 14 days storage, the Hb concentration and RBC count significantly decreased in G6PD-deficient blood units with a corresponding significant increase in MCV compared to the baseline which differed from insignificant variations observed in Hb, RBC and MCV of donor units with normal G6PD activity. D'Almeida et al. reported decreases in RBC deformability of 34 % following 4 wk of storage,[36] while Tsai et al. also demonstrated that prolonged storage causes increases in intracellular potassium and free Hb concentrations in the suspending fluid plasma, resulting in a drop in pH leading to decreased fraction of RBCs that survive after being returned to circulation through transfusions.[37]

The significant drop in RBC count and concentration could be due to increased hemolysis as demonstrated by Mattew et al.,[38] the impact of G6PD status on RBC storage and transfusion outcomes. This could be the result of increased glycolysis, impaired glutathione homeostasis, and increased purine oxidation.

Studies in which RBCs were exclusively stored in a mannitol-containing additive solution (i.e., SAGM, AS-1, or AS-5) showed a significant decrease in G6PD activity during storage.[39] In contrast, studies of RBCs in other storage solutions, in general, did not suffer this effect.[10] Consistent with the finding of decreased G6PD activity in some studies, the trend of declining PPP activity upon stimulation is seen during RBC storage.[15] Therefore, these varied results may be explained by differences in storage conditions or the methods used to assess G6PD function.

Very few studies have been carried out on the effect of G6PD deficiency on peripheral blood film comment. One study conducted by Sutasir et al. on G6PD deficiency shows that routine staining of peripheral smears reveals polychromasia, representing increased RBC production. So-called bite cells caused by the splenic removal of denatured Hb may be seen as can Heinz bodies (denatured Hb) on the peripheral smear in cases of G6PD deficiency.[40]

Contrary to our findings, there were no significant presentations on peripheral blood film of G6PD-deficient donor blood as compared to normal G6PD donor blood throughout the study period. This difference in findings can be attributed to the small sample size of the present study because of the short period given for the study and the short duration of storage of only 14 days. Significant changes were seen by other researchers from 3 wk.

Limitations

Because this study was conducted in the era of the COVID-19 pandemic, the rates of blood donation at various health centers were drastically reduced hence the small sample size.

Again because of limited resources, extension of unit monitoring beyond 14 days and inclusion of additional parameters such a cellular oxidative stress indices were not possible.

Recommendations

Based on the findings, the authors recommend;

The need for a multifacility study with a larger sample size to assess a holistic information on the burden of G6PD deficiency, especially in sub-Saharan Africa. This will enhance donor blood quality during transfusions.

A policy should be formulated for G6PD deficiency screening to be included in the screening list for blood donors. This should be observed in all facilities involved in blood donation.

Conclusion

The most prevalent enzyme deficiency worldwide is G6PD-deficiency. Overall, despite the strong recommendations of the World Health Organization, screening blood donors for G6PD deficiency is not a common practice, and so blood banks and transfusion services have G6PD-deficient RBCs in their inventories. The RBC count and Hb concentration reduce significantly in G6PD-deficient donor blood units in storage with an associated increase in MCV indicating progressive loss of the cellular membrane homeostatic mechanism that could potentially result in further hemolysis during long term storage.

Transfusion of G6PD-deficient blood units may thus not yield optimum transfusion outcomes. This may show up in individuals with higher underlying oxidative stress, such as newborns, people with sickle cell disease, and those using oxidative drugs, as well as lower post-transfusion reactivity of stored G6PD-deficient RBCs and decreased transfusion efficacy in patients.

Declaration

I hereby declare that this submission is my own work towards the BSc. Degree in Medical Laboratory Technology and that to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the university, except for references to other people's work, which have been duly acknowledged.

Ethics approval and consent to participate

Ethical clearance was obtained from the Committee on Human Research Publications and Ethics (CHRPE) of School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology before the inception of the study. Management of Living Waters Hospital also gave approval for their facility to be used for this study. Consent was sought from blood donors who were assured of the highly confidential nature of this study.

Consent for publication

Consent for publication was sought from the different authors involved in the development of this work.

Availability of data and material

Data of this research is available only on request since is a clinical data.

Funding

The research work was financed solely by the corresponding author.

Authors contribution

BS is the principal investigator and carried out the model design and the computational framework. AEC designed the model, the computational framework and the analysis of the data and the writing of the article. SO was involved in reagent preparation, laboratory investigations and data analysis. HD helped in the reagent preparation and laboratory investigations. DFA helped in sample collection and storage monitoring. MO assisted in the manuscript development and editing.

Abbreviations

G6PD: Glucose 6-phosphate dehydrogenase, NADPH: Nicotinamide adenine dinucleotide phosphate, Hb: Hemoglobin, MCV: Mean cell volume, MCH: Mean cell hemoglobin, RBC: Red blood cell, MCHC: Mean cell hemoglobin concentration, PPP: Pentose phosphate pathway

Conflicts of interest

Not applicable.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Review article

CD36 as a marker of acute myeloid leukemia prognosis: A systematic review

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ABSTRACT

CD36 is a glycoprotein associated with resistance to chemotherapy and the recurrence of acute myeloid leukemia. This systematic review aims to evaluate the impact of CD36 on the prognosis of acute myeloid leukemia, a complex heterogeneous malignant hematopoietic disease. The Embase, Scopus, Web of Science, Cochrane Library and SciELO databases were searched until September 2023. Only studies that analyzed CD36 expression in humans were included. Of 905 articles identified from the databases, 600 were screened and nine were included. The Newcastle-Ottawa Scale was used to evaluate the methodological quality of the studies. According to this systematic review, CD36 is associated with different prognostic factors in acute myeloid leukemia, including remission and relapse of the disease, overall survival, and chemoresistance.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of fatal and aggressive diseases affecting hematopoietic stem cells. Mutations in myeloid stem cells occur during AML progression, leading to an immature cell population.[1] Currently, the treatments for AML are chemotherapy and bone marrow transplantation; however, there is a need for new therapeutic approaches.[2,3]

Among leukemia subtypes, AML accounts for the highest percentage of leukemia-related deaths, representing 62 % of cases.[4] AML is the second most common subtype in children,[5] resulting in about 15–20 % of leukemia cases in this population.[6] In developed countries, survival rates in pediatric patients are high (65 %) compared to low- and middle-income countries, where rates are lower than 40 %.[7] AML relapse rates vary from 30–35 % in younger patients with favorable risk factors and can reach 80 % in older patients with adverse risk factors.[8] The prognosis of AML patients is based on the absence or presence of cytogenetic and/or molecular biology abnormalities and is divided into favorable, intermediate, and unfavorable subgroups.[9] Prognostic markers in AML include mutations in the *NPM1, FLT3, MLL*, and *CEBPα* genes, as well as alterations in the expression levels of *BAALC, MN1, ERG*, and *AF1q*,[10] and chromosomal abnormalities such as t (8;21) and t(15;17).[11] Yet, accurately predicting prognosis in

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AML remains challenging due to factors such as disease heterogeneity, clonal evolution, and the influence of microenvironmental factors. There is still debate about the influence of blast immunophenotyping on the prognosis in AML.[12] Recent studies have shown worse prognoses for AML associated with increased CD34 and CD318 expressions.[13,14] Also, CD36 is positively associated with the dissemination of leukemic blasts and is highly expressed in tumors at an advanced stage.[15] Furthermore, leukemia cells resistant to cytarabine (Cytarabine) exhibit a high expression of CD36.[16]

CD36 is a glycoprotein of the scavenger receptor class B superfamily, the gene of which is located on the long arm of chromosome 7 (7q11.2).[17] This glycoprotein has different physiological functions such as cell adhesion, establishment of connections with collagen, thrombospondin, phospholipids, and low-density lipoprotein, and can serve as a regulatory glycoprotein for fatty acid transport.[18] CD36 has been described as contributing to tumor formation and the development of various types of cancer, including breast cancer, gastric cancer, and AML.[19] CD36 expression is correlated with low survival in patients with lung carcinoma, bladder cancer, and luminal breast cancer.[20,21] This study aims to review evidence of the association of CD36 with worse prognosis in AML.

Material and methods

Protocol

The protocol of this systematic review was registered *a priori* in the International Prospective Register of Systematic Reviews (PROSPERO) with registration number CRD42023481493.[22]

Search

The search for articles was carried out by two reviewers, on September 16th, 2023, in six bibliographic databases: PubMed, Embase, Scopus, Web of Science, the Cochrane Library, and SciELO. The terms used were “acute myeloid leukemia” and all of its synonyms in MeSH Terms (or Emtree, in Embase) AND ‘CD36’ and all of its synonyms in MeSH Terms (or Emtree, in Embase). The complete search strategy can be accessed in the PROSPERO protocol.[22] Additional research was also carried out using Google Scholar before extracting data, looking for new studies not yet peer-reviewed.

Eligibility criteria

Studies that analyzed the expression of CD36 in human patients, including clinical trials, case-control studies, and case reports were included. The articles should evaluate the impact of CD36 on factors related to the prognosis of AML, including, for example, survival and response to treatment. Reviews, editorials, letters, abstracts from conference annals, and studies that only used animals or lineage cells for the analyses of CD36 were excluded. Studies that addressed only one specific subtype of AML and not the disease spectrum were also excluded.

Study selection

Two independent reviewers selected studies based on eligibility criteria. Disagreements between individual judgments were solved by a third reviewer. The software used for blinding and recording decisions was Rayyan.[23]

Data extraction

From each eligible study, data were input in duplicate in an Excel worksheet, following a standardized template created for this review. The following data were extracted from articles: first author, publication year, research location, study design, sample size, age of participants, percentage of males, eligibility criteria, stem cell source, method of CD36 quantification, comparators, statistical analysis, method of AML classification, evaluated prognostic factors and results related to the impact of CD36 on factors influencing the prognosis of AML.

Quality assessment

The methodological quality of nonrandomized studies was evaluated using the Newcastle-Ottawa Scale. Studies rated 3−4 stars in the selection domain, 1−2 stars in the comparability domain and 2−3 stars in the outcome/exposure domain were classified as good quality. Studies rated 2 stars in the selection domain, 1−2 stars in the comparability domain and 2−3 stars in the outcome/exposure domain were considered as fair quality. Those rated 0−1 star in the selection domain or 0 stars in the comparability domain or 0−1 star in the outcome/exposure domain were classified as poor quality.[24] Two independent reviewers evaluated the quality of the studies, and disagreements between individual judgements were solved by a third reviewer to reduce the risk of bias.

Results

Search results

The search strategy resulted in 905 articles, and one more article was added after a manual review to identify eligible articles that might not have been captured by the search strategy. After excluding 305 duplicates, the titles and abstracts of 600 articles remained were assessed and another 583 articles were excluded based on the eligibility criteria, leaving 17 articles and one added manually giving a total of 18 to be read in full. Of these, nine articles[25−33] were included in this review with the other nine being excluded for not complying with the inclusion criteria (six did not report the prognoses, two were conference abstracts and one was exclusively an animal study). The agreement between evaluators during the full-text phase was 93.75 % (Cohen’s κ: 0.875). Figure 1 represents the flowchart detailing the excluded articles at each stage, along with the reasons for their exclusion.

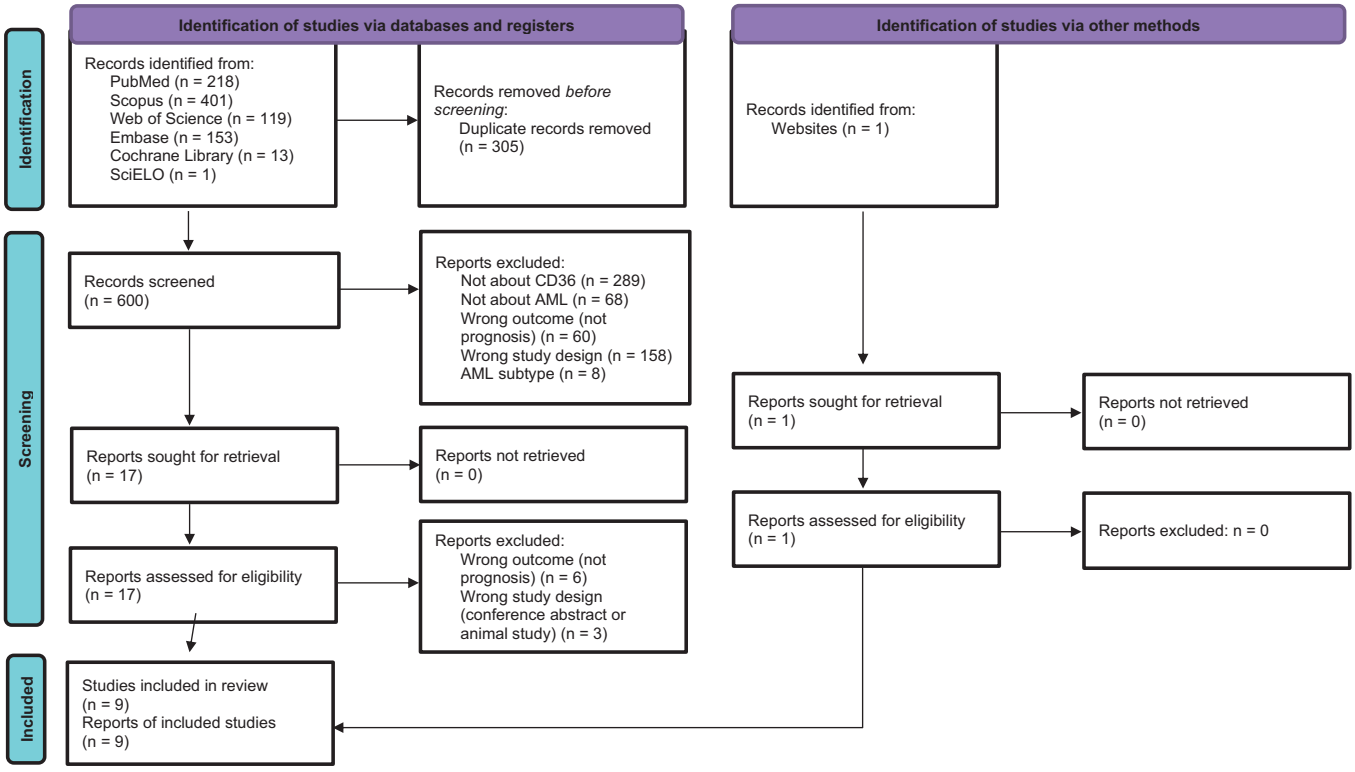

Figure 1 – Flow diagram of study selection process.

Study characteristics

Table 1 summarizes the characteristics of the studies included in the main analysis. Articles were published between 1992 and 2023. Some of the articles do not provide all the collected data, such as the mean age and gender of the study participants. From the studies that reported the mean age of the participants, the mean age ranged from more than one month to approximately 44 years. The stem cell source in most articles was the bone marrow. Two studies used peripheral blood in addition to bone marrow, and one of the articles used databases and did not clarify the stem cell source but mentioned the use of tumor tissue. The most commonly used method for CD36 quantification was flow cytometry, in addition to RNA sequencing and immunohistochemistry. The comparison was mainly conducted between patients with complete remission of AML versus patients who relapsed, patients with positive or increased CD36 expression versus negative or decreased CD36 expression, but there were also comparisons with healthy individuals and people with other types of cancer. The articles primarily classified AML based on the French-American-British (FAB) classification and Cytogenetic and Molecular Risk Groups. The studies demonstrate the impact of CD36 on the prognosis of AML across different categories, such as survival, remission, chemotherapy resistance, and tumor cell proliferation. All results linking CD36 expression to any of these characteristics were collected.

Quality assessment

The methodological quality of the studies analyzed in this review ranged from low to good, with most of the articles being classified as fair quality, primarily due to significant gaps in crucial information reported by the articles. Scores on the Newcastle-Ottawa Scale for the studies ranged from 5–7 stars.

Synthesis of results

In a prospective study investigating the prognostic significance of cell surface antigens associated with myeloid differentiation, such as CD36, the authors found that the presence of these myeloid-associated cell surface antigens did not have prognostic significance for children.[25] However, a higher frequency of CD36-positive cells at diagnosis was identified in cases of children who experienced disease recurrence.[26] In contrast, in adults, CD36 expression was significantly higher in AML when compared to other types of cancer,[27] and the increase or even the expression of CD36 is associated with several factors that appear to be associated with a poor prognosis. Bone marrow mononuclear cells from AML patients expressing CD36 are less susceptible to chemotherapeutic agents such as cytosine arabinoside (Ara-C) compared to cells that do not express CD36,[28] demonstrating that CD36 expression interferes with chemotherapy. Another study also

Table 1 – Characteristics of studies included in the analysis.

First author	Country	Study quality	n	Stem cell source	Method of CD36 quantification	Classification of AML	Results
Smith, FO[25]	USA	Fair	176	BM	Flow cytometry	FAB	No prognostic significance for the cell surface expression of CD36 in myeloid cells.
Valet, G[31]	Germany	Fair	724	BM and PB	Flow cytometry	C + M Risk	5-year non-survivors had a higher percentage of CD36-positive AML blasts than survivors.
Perea, G[30]	Spain	Good	266	BM	Flow cytometry	FAB and C + M Risk	The 2-year LFS rate was lower in CD36+ patients (32 %) compared to CD36− patients (56 %; p-value = 0.01). The risk of relapse was higher in CD36+ patients (63 %) than in CD36− patients (38 %). CD36+ patients with trisomy 8 had poorer LFS (0 %) compared to CD36+ patients without trisomy 8 (37 %; *p*-value = 0.0001). Trisomy 8 was significantly associated with CD36 expression.
El-Aziz, A[29]	Egypt	Good	97	BM and PB	Flow cytometry, FISH	FAB	Remission was achieved in 23 of 45 (51.1 %) evaluable CD36+ patients and in 32 of 46 (69.5 %) CD36− patients. Although a higher number of responders was observed among CD36− patients compared to CD36+ patients, the difference was not statistically significant (*p*-value = 0.073). The 2-year OS rates were 37.5 % for CD36+ patients and 44.9 % for CD36− patients (p-value = 0.001). The 2-year LFS rates were 33.3 % for CD36+ patients and 40.6 % for CD36− patients (*p*-value = 0.03). CD36 expression, along with WBC count and adverse cytogenetics, were significant factors influencing OS and LFS. Multivariate analysis confirmed that CD36 expression retained its significance as an independent negative prognostic factor for both OS and LFS. CD36 expression was more commonly observed in the unfavorable cytogenetic group.
Zhang, T[33]	USA	Fair	196*	BM and PB	Flow cytometry	FAB and C + M Risk	Using the GSE30377 dataset (*n* = 23), patients were dichotomized based on the median CD36 expression. Patients with high CD36 expression exhibited shorter OS, although the difference was not statistically significant (*p*-value = 0.114). Analysis of the TCGA dataset (*n* = 173) showed that when patients were dichotomized by APOC2 and/or CD36 mRNA expression Z-scores into high (Z-score ≥ 2) and low (Z-score <2) groups, those with high APOC2 and/or CD36 expression had significantly shorter OS compared to patients with low APOC2 and low CD36 expression (median OS 9.2 vs. 21.5 months, *p*-value = 0.017).
Chen, YJ[27]	China	Low	70*	Database, undetermined (possibly tumor tissue)	RNA sequencing and immunohistochemistry	–	CD36 expression was significantly elevated in AML based on RNA-seq data. CD36 expression showed a strong positive correlation with infiltrating stromal scores in AML (*r* = 0.618, *p*-value <0.001). CD36 expression was positively correlated with immune scores (*r* = 0.609, *p*-value <0.001) and with the ESTIMATE score (*r* = 0.669, *p*-value <0.001), indicating a robust association between CD36 expression and the tumor immune microenvironment. CD36 expression was negatively correlated with the expression of four methyltransferases in AML
Hoch, REE[26]	Brazil	Good	51	BM	Flow cytometry	FAB and C + M Risk	Higher frequency of CD36+ cells at diagnosis was observed in cases with disease recurrence.
Zhang, Y[28]	China	Fair	5*	BM	Flow cytometry	–	CD36+ cells exhibited lower sensitivity to chemotherapeutics than CD36− cells.

Table 1 (*continued*)

First author	Country	Study quality	n	Stem cell source	Method of CD36 quantification	Classification of AML	Results
Farge, T[32]	France	Fair	1273	BM and PB	Flow cytometry	C + M Risk	CD36 expression in blasts at diagnosis is associated with human AML progression and relapse. CD36 was significantly associated with a worse EFS (HR: 1.55; 95 % CI: 1.17−2.05, *p*-value = 0.002), and a worse OS (HR: 1.69; 95 % CI: 1.18−2.41, *p*-value = 0.005). Median EFS of CD36-high patients was half that of CD36-low patients (252 days vs. 538 days, respectively; HR:1.65, *p*-value <0.0001). Median OS, which was 462 days in CD36-high patients, was not reached after 3 years in CD36-low patients (HR: 1.88, *p*-value <0.0001). A high CD36 protein expression at diagnosis was associated with an increased CIR after intensive chemotherapy, and a high expression of CD36 was associated with a shorter CIR (SHR: 1.53; 95 % CI: 1.07−2.19, *p*-value = 0.02). Intermediate and unfavorable karyotypes had a higher percentage of CD36-expressing blasts. A comparison of the genomic landscape in 224 AML did not reveal a pattern related to CD36 expression, except for FLT3 abnormalities. there's an enrichment of AML with KMT2A (11q23) or t(9;22) abnormalities in CD36-high group and an increase in t(15;17) and t(8;21) AML in CD36-low group.

BM: bone marrow; PB: peripheral blood; LFS: leukemia-free survival; OS: overall survival; WBC: white blood cell; EFS: even-free survival; 95 % CI: 95 % confidence interval; CIR: cumulative incidence of relapse; FAB: French-American-British; C + M Risk: cytogenetic and molecular risk groups.

* Sample size used to evaluate the outcome of interest.

demonstrated a tendency for a lower response to chemotherapy in the group of patients with positive CD36 expression compared to the group with negative CD36 expression.[29]

Furthermore, CD36 expression is associated with relapse and recurrence of the disease. The higher frequency of CD36-positive cells at diagnosis was identified in cases that presented recurrence of the disease,[26] as well as a higher risk of relapse for $CD36^{+}$ patients, thereby presenting a shorter leukemia-free survival rate.[30]

In addition to a higher risk of relapse, CD36 expression is also associated with shorter overall survival. Five-year non-survivors showed increased levels of CD36-positive AML blasts,[31] while complete remission was achieved in a higher percentage of CD36-nagative patients, as well as overall survival rates and leukemia-free survival rates.[29]

CD36 expression in blasts at diagnosis is also associated with human AML progression and relapse. CD36 was significantly associated with worse survival. The survival of CD36-high patients was half that of CD36-low patients. Furthermore, a high CD36 protein expression at diagnosis was associated with an increased cumulative incidence of relapse after intensive chemotherapy, and a multivariate analysis showed that a high expression of CD36 was associated with a shorter cumulative incidence of relapse.[32]

Some proteins may be associated with this CD36 effect, such as apolipoprotein C-II (APOC2), which cooperates with CD36 to promote leukemia growth, as described by Zhang et al.[33] Using a dataset of 23 patients, high CD36 expression had shorter, but not statistically significant, overall survival. Nevertheless, analyzing a dataset of 173 patients, those with high APOC2 or CD36 levels had significantly shorter overall survival than patients with low APOC2 and low CD36 expressions.[33]

A connection between CD36 expression and the tumor immune microenvironment is suggested. CD36 expression is significantly positively correlated with infiltrating stromal scores in AML, and there are positive correlations between CD36 expression and infiltrating levels of immune score in AML, as well as infiltrating levels of the Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data (ESTIMATE) score in AML.[27] Additionally, negative correlations were observed between CD36 expression and four methyltransferases in AML.[27]

CD36 expression was more frequently observed in an unfavorable cytogenetic group,[29] suggesting a link between CD36 and high-risk cytogenetic profiles. Cytogenetic and molecular abnormalities appear to contribute to the severity driven by CD36 expression. CD36-positive patients with trisomy 8 had significantly poorer leukemia-free survival compared to CD36-positive patients without trisomy 8,[30] highlighting the interplay between CD36 and specific cytogenetic alterations. Supporting this association, intermediate and unfavorable karyotypes exhibited a higher proportion of CD36-expressing blasts.[32] CD36 expression was characterized as an independent marker for AML progression, but has a higher association in patients with *FLT3* abnormalities, reinforcing its role in poor prognosis.[32] Moreover, AML cases in the CD36-high group were enriched with *KMT2A* (11q23) or t(9;22) abnormalities, while cases in the CD36-low group showed a higher frequency of favorable cytogenetic abnormalities, such as t

(15;17) and t(8;21).[32] This suggests that CD36 expression correlates with more aggressive disease phenotypes driven by adverse genetic features.

Discussion

The results of this systematic review indicate that CD36 is associated with different prognosis factors in AML. For example, remission and relapse of the disease, leukemic cell metabolism and growth, overall survival, and chemoresistance.

There are some narrative reviews about the impact of CD36 on different types of cancer, which include AML, such as those reported by Wang et al.,[34] Guerrero-Rodríguez et al.,[15] and Feng et al.[35] Some authors described studies that did not comply to the inclusion criteria, for instance, the study by Ye et al.[36] demonstrated that leukemia stem cells with a higher CD36 expression seemed to be resistant to different chemotherapy drugs, such as cytarabine, doxorubicin, etoposide, SN38, and irinotecan. Since those were not systematic reviews, the authors used less strict criteria for inclusion, while this study aimed at demonstrating the correlation between CD36 and the prognosis of AML by evaluating clinical and not pre-clinical studies.

Some of the studies evaluated in this systematic review focus on children, associating a higher frequency of CD36-positive cells at diagnosis in children who experienced recurrence of AML[26]; therefore, studies comparing the role of CD36 in AML in different age ranges have not been done. Moreover, results from this research demonstrated that cells from AML patients expressing CD36 were less susceptible to chemotherapeutic agents such as Ara-C compared to cells that did not express CD36[28]; nevertheless, there are other drugs that must be investigated in further studies to support CD36 as a treatment target. Additionally, even though some studies focused on cytogenetic and molecular abnormalities in AML,[29,30,32] this area is expanding and might be explored in further research to correlate the CD36 expression with different genetic features in AML patients.

The present study has some limitations. Many studies did not report or adjust the prognostic factor evaluated for other confounding factors and interventions that could affect the outcome. Also, some of the studies did not describe the characteristics of the population from which they obtained the bone marrow or peripheral blood samples. Moreover, the lack of data from the studies did not allow a meta-analysis. Therefore, further studies are necessary to empower evidence-building.

Conclusions

In conclusion, this systematic review suggests that CD36 is associated with the prognosis of AML. The role of CD36 in the pathogenesis of AML remains to be evaluated to support CD36 as a treatment target.

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Conflicts of interest

The authors declare no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2025.103861.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Review article

Advanced molecular approaches to thalassemia disorder and the selection of molecular-level diagnostic testing in resource-limited settings

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ABSTRACT

Beta-thalassemia is a genetic disorder that significantly burdens healthcare systems globally. This inherited blood disorder, categorized into beta-thalassemia and alpha-thalassemia, results in insufficient globin production, leading to anemia and iron overload from frequent transfusions. Severe cases, known as thalassemia major, require regular blood transfusions. Beyond clinical suspicion and biochemical tests, molecular techniques are essential for confirming the diagnosis and guiding treatment. Advanced molecular profiling methods such as Polymerase Chain Reaction (PCR), Multiplex Ligation-dependent Probe Amplification (MLPA), Next-Generation Sequencing (NGS), Third-Generation Sequencing (TGS), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are effective in detecting mutations. Epigenetic factors also play a crucial role, driving the development of epidrugs for targeted therapy. This review covers various molecular techniques, established gene-editing methods, epigenetic mechanisms, and the impact of artificial intelligence on thalassemia management. It highlights the importance of selecting precise and sensitive molecular tools for detecting thalassemia gene mutations and stresses the need to make these testing methods accessible in resource-limited clinical settings.

Introduction

Thalassemia, a monogenic disorder among hemoglobinopathies, has a universally recessive inheritance affecting both children and adults worldwide. In India, beta-thalassemia accounts for 25 % of the global burden.[1] Beta-thalassemia is particularly prevalent in Mediterranean countries, Middle East, and South Asia, regions historically affected by malaria. In India, the highest prevalence is found in the northern states of Punjab, Haryana, and Delhi, and the western states of Maharashtra and Gujarat, with the lowest prevalence in the southern states of Tamil Nadu and Karnataka.[2,3] The challenges faced by the patients and their families due to this disease are substantial. Affected individuals require lifelong regular blood transfusions and chelation therapy, leading to complications such as heart disease, liver damage, and endocrine disorders.[4] In rural areas, the cost of the treatment, medical care and testing services pose a significant

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constraint. In urban areas, too, the disease burden significantly impacts the health system and resources.

Thalassemias are clinically categorized as thalassemia major (TM), thalassemia intermedia (TI) and thalassemia minor or trait, based on the severity. TM is the most serious form, requiring regular treatment. Thalassemia is classified into two based on the globin gene defect: alpha-thalassemia (Hemoglobin Subunit Alpha 1: *HBA1* and Hemoglobin Subunit Alpha 2: *HBA2* genes) and beta-thalassemia (Hemoglobin Subunit Beta: *HBB* gene). In beta-thalassemia, substitutions of bases occur in the introns, exons and promotor regions of the beta-globin genes, whereas in alpha-thalassemia, base deletions lead to the removal of alpha genes.[5] The alpha gene is located on chromosome 16p13.3, and the beta gene is clustered among other hemoglobin genes on chromosome 11p15.15.

The diagnosis and detection of thalassemia involve several laboratory examinations such as complete blood count, blood smear, iron studies, hemoglobinopathy studies, DNA analysis by genetic testing, and prenatal genetic testing.[6] Based on the clinical, hematological and molecular features, beta-thalassemia is categorized into two distinct types based on blood transfusion: non-transfusion-dependent β-thalassemia (NTDT), which is TI, and transfusion-dependent β-thalassemia (TDT), which is TM. Preliminary screening methodologies are economical and feasible for mass coverage of the disease-causing genes in the society, helpful in triaging patients who require a DNA analysis through superior and high-throughput technology. However, in routine clinical practice, mutation testing for these genes is not commonly practiced. Instead, driven by market forces, patients are often directly referred for NGS testing assuring one-stop solutions. Therefore, it is advocated that discussions between clinical genetic departments and diagnosticians should prioritize less expensive methodologies with superior specificity and reliability in terms of test quality to triage patients and effectively utilize NGS technology.[7] Hence this review aims to study different molecular methodologies and high-throughput tests affecting the detection level of thalassemia, a hematological disorder of high societal impact, and its future implications in clinical practice.

Molecular profiling of thalassemia

Various molecular profiling methods exist for diagnosis, each with its limitations. The available molecular genetic testing for thalassemia is single gene testing.[8] For beta-thalassemia, *HBB* gene sequencing analysis is offered to detect mutations. However, due to the identical length of the *HBA1* and *HBA2* genes, sequencing analysis for alpha-thalassemia has been challenging.[9] Protein-based detection methods such as electrophoresis and chromatography are commonly used in routine practice. To prevent adverse outcomes of globin genetic disorders, along with genetic confirmation in a given patient, genetic testing is important for potential carriers in prenatal and premarital contexts.[10] The list of molecular techniques used for detecting thalassemia is summarized in Table 1 and Figure 1.

Recent molecular approaches

Advancements in techniques for detecting thalassemia include NGS, which can accurately distinguish rare mutations and reduce misdiagnoses. Extensive work has been conducted on alpha- and beta-thalassemia mutation screening using NGS technology over the past few years. While whole genome sequencing, exome sequencing, RNA sequencing, and methylation sequencing are widely used NGS applications, targeted sequencing is the most effective and economical approach for thalassemia, covering indels and point mutations in the *HBA, HBB,* Hemoglobin Subunit Delta (*HBD*) and Hemoglobin Subunit Gamma (*HBG*) genes.[11] Conventional methods only detect specific mutations targeted by primer sets, but NGS provides a more extensive and thorough analysis of the individual's genetic make-up in a single test and

Table 1 – List of molecular techniques, their application and disadvantages.

Technique	Application	Disadvantages
Sanger sequencing	Detects all possible mutations in an individual.	Not useful for detecting large deletions
Allele-specific methodologies (allele-specific polymerase chain reaction)	Useful in genetically homogeneous populations, high throughput and economical	Less useful in ethnically diverse populations
Gap-Polymerase Chain Reaction	Rapid and multiplexed	Cannot detect point mutations, requires specific primers
Multiplex ligase-dependent probe amplification (MLPA)	Can cover large chromosomal regions for deletion analysis	Low resolution, cannot detect point mutations or small deletions
Next-generation sequencing (NGS)	Has potential to characterize mutations and deletions throughout all globin genes in parallel	Needs to create awareness about the technique
Comparative genomic hybridization (CGH)	covers large chromosomal regions for deletion analysis, high resolution and has exact breakpoints	Not reliable due to cross-hybridization
Mass spectrometry	Hemoglobin variants can be characterized rapidly	Yet to be approved in routine diagnostics
Artificial Intelligence (AI)	Differentiates between thalassemia and other microcytic anemia by using different algorithms and web-based prediction tools.	Not yet approved in routine diagnostics

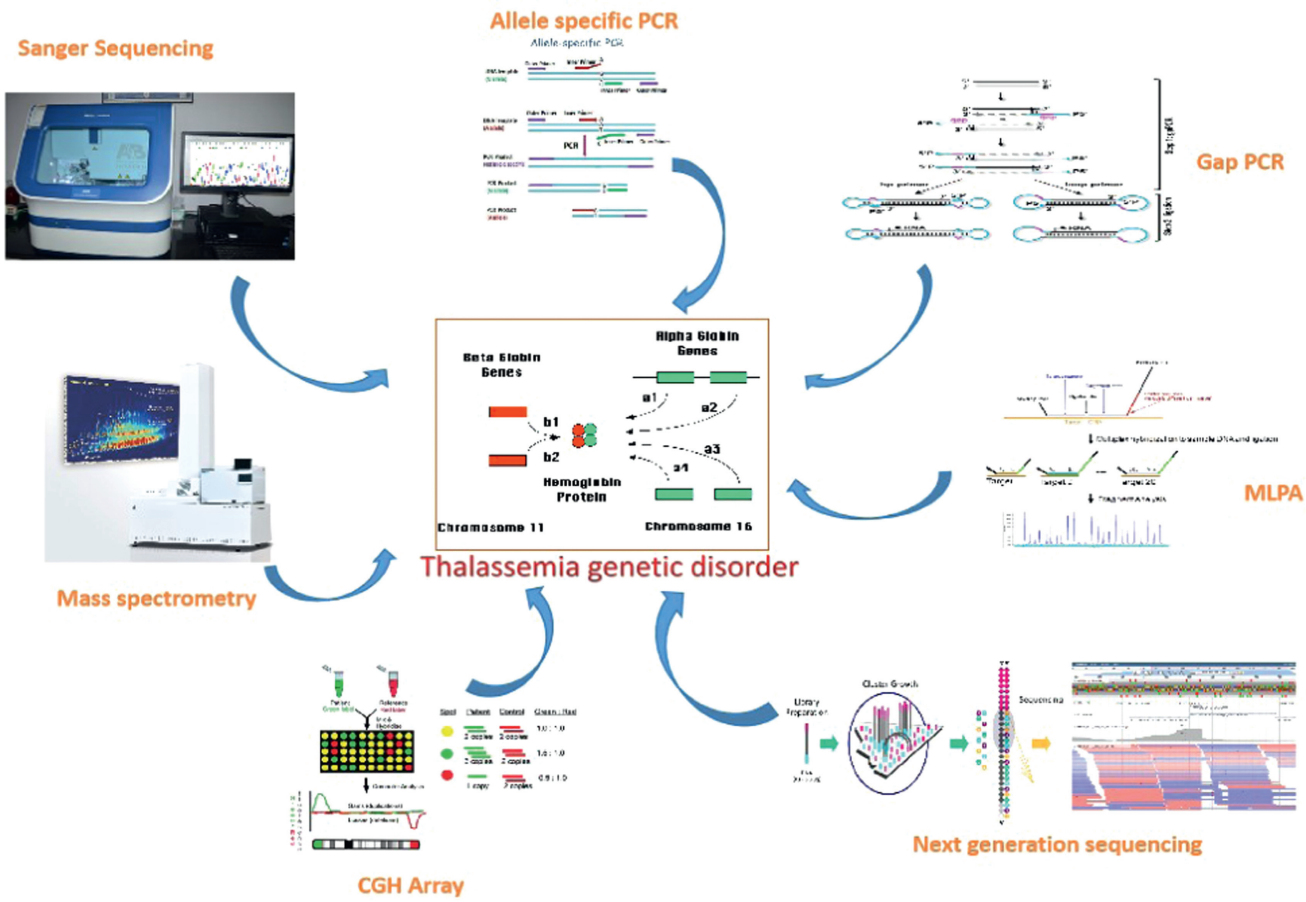

Figure 1 – Illustration of the different molecular tools for diagnosis of thalassemia.

can detect multiple mutations from a single gene or multiple genes. NGS is more reliable in characterizing the disease genotype, and its deep sequencing is used to identify mutations in diagnosing many human genetic disorders.[12]

Recently, Gupta et al. developed a scalable non-invasive amplicon-based precision sequencing (SNAPseq) assay system, a unique strategy-based NGS approach to detect virtually all *HBB* mutations. The SNAPseq assay utilizes a simple, extraction-free non-invasive buccal swab crude lysate or finger prick blood sample directly for detecting allele-specific beta-thalassemia and sickle cell disorder genotypes. Their study showed a simplified sampling procedure combined with an NGS approach to develop and optimize pipelines to prioritize pathogenic mutations with allele-specific sensitivity. They concluded that their assay could serve as a gold standard technique applicable for precise diagnosis of beta-hemoglobinopathies with high sensitivity.[13]

Third-Generation Sequencing (TGS) - the next era of DNA sequencing technology, has gained prominence in molecular biology, studying genomes, transcriptomes, and metagenomes without the need for clonal amplification. Oxford Nanopore Technology (OCT) and the Pac-Bio Single Molecule Real-Time Sequencing (SMRT) are the two TGS technologies currently available. The major challenge in TGS is the accurate identification of the nucleotide bases due to the instability of the molecular machinery involved, resulting in higher error rates than NGS. Several studies have clinically utilized the TGS approach to identify both alpha- and beta-thalassemia genetic carrier statuses, with results showing complete concordance with conventional molecular techniques.[14,15] A study conducted by Zhen-min et al.[17] reported rare mutations in *HBA*, *HBB*, *HBD*, and Hemoglobin H genes in children with mild anemia. They identified rare mutations in children with suspected transfusion-dependent thalassemia (TDT), necessitating long-term blood transfusions using the TSG approach. Zhuang et al.[16] also reported identifying rare variants in the *HBA* gene by TGS technology. Hence, TSG can serve as a diagnostic tool to effectively screen thalassemia carrier trait in at-risk individuals or couples.[16-19]

The innovation of the CRISPR-associated protein 9 (CRISPR-Cas9) system, a genome editing technology, revolutionized biomedical research. This system is widely used for DNA base editing, RNA targeting, gene expression regulation and epigenetic editing for preventing and managing various genetic diseases. Though the technology has many challenges, due to its ease of use, higher efficiency, specificity and cost-effectiveness, it is more extensively used than other genome editing techniques.[20] Current curative stem cell or bone marrow transplantation for thalassemia has the limitation of obtaining an HLA matched donor within the family or an unrelated individual. Graft-versus-host disease and the high cost compared to gene editing make gene editing a

potential curative option.[21] CRISPR-Cas9 gene editing technology is applied to correct the alpha- or beta-globin chain imbalance in thalassemia hematopoietic stem/progenitor cells by down regulating the alpha-globin locus to control *HBB* gene expression.[22] An editorial by Parums discusses the first regulatory approval for CRISPR-Cas9 gene editing therapy, Casgevy (exagamglogene autotemcel) and Lyfgenia (lovotibeglogene autotemcel), for treating patients with transfusion-dependent beta-thalassemia and sickle cell disease. He discusses the therapeutic challenges and outcomes of patients treated with CRISPR-Cas9 therapy.[23] The end-point of several clinical trial studies will warrant the treatment management of thalassemia and sickle cell anemia through gene editing therapy. Still, gene editing technology has limitations, which will be overcome by the new prime editing technology.[24] Advancements in gene editing technology, such as CRISPR, may soon surpass allogeneic transplants as the preferred treatment for patients with sickle cell disease or thalassemia. These cutting-edge techniques offer the potential for more precise and personalized treatments, potentially reducing the risks and complications associated with traditional transplant methods.

Epigenetic aspect of thalassemia

Developments in the field of medical genetics focus more on the regulatory machinery of gene expression through epigenetics, thereby providing a new entity of therapeutic targets for treating various genetic disorders. The alteration of gene activity without the change in DNA sequence by histone modification and DNA methylation is an epigenetic concept. Epigenetic modifiers play a significant role in alpha- and beta-thalassemia disorders. In alpha-thalassemia, the common mutation types are often deletions affecting one or more of the alpha-globin genes (HBA1 and HBA2) or one pseudogene with a homozygous configuration of the allele, which results in the hydrops fetalis form. The DNA methylation level in association with this mutation results in a differential methylation pattern between placenta and leukocytes.[25] In beta-thalassemia, the epigenetic modification changes fetal hemoglobin (Hb F) to adult hemoglobin (Hb A). The delay in conversion of Hb F to Hb A is due to the regulatory single nucleotide polymorphism (r-SNP), which leads to clinical complexity of the disease by keeping Hb F levels high. The DNA methylation in beta-thalassemia down regulates the beta-globin gene and up regulates the production of the gamma-globin gene with co-inheritance of alpha-thalassemia, which improves beta-thalassemia severity. The enhancement of gamma-globin gene expression in beta-thalassemia is due to the demethylation of the promotor Cytosine-phosphate-Guanine (CpG) sites in erythroid progenitor cells[26]. In a study by Yassim et al.[27] it was found that the Immunoglobin superfamily 4 (IGSF4) has an important role in the synthesis of the globin chain. Due to the methylation of *IGFS4,* the synthesis of the globin chain is affected by its interaction with other genes in the regulation network of globin expression.

This disease-causing epigenetic change can be revisited by the use of epigenome editing to control the regulation of gene expression by writing and erasing the epigenetic modifiers. Some of the epigenetic modifiers include DNA modifiers, mRNA modifiers and histone protein modifiers. The IGSF4 and La ribonucleo protein 2 (LARP2) modifiers were hypermethylated in beta-thalassemia major patients.[26] The development of epigenetic drugs called epidrugs was utilized initially to reverse the nature of epigenetic alterations. Epidrugs target different epigenetic marks and inhibit disease-causing alterations. Their effect is not sequence-specific and can lead to cell death due to a broad alteration of gene expression.[28] To assuage this effect, epigenome editing technology has upsurged in the medical field as a solution for treatment of rare genetic disorders. In beta-thalassemia, zinc finger protein (ZF)-based epigenome editors were fused to epigenetic modifiers to achieve activation of specific endogenous genes and modulate the gene expression. The limitation of using the zinc finger-based editors is their low specificity and binding to off-target sites.[29] Hence, other epigenome editing platforms with higher DNA recognition capacities play a crucial role in the stable regulation of gene expression, namely Transcription activator-like effectors (TALE) and CRISPR-Cas9. However, they still have limitations. Comparatively, CRISPR-Cas9-based epigenome editors present several advantages over TALE and ZFs. With the use of only one Cas9 enzyme, the CRISPR-Cas9 system facilitates simultaneous epigenome editing of multiple regions.[30] Several studies were conducted to investigate the beta-globin gene regulation mechanism using artificial transcription factors and epigenome editors to reactivate human gamma- or beta-globin gene expression.[31–34] To step into therapeutics, several paces are needed to be taken care of for the usage of epigenome editing, and it is necessary to develop protocols for the delivery system.

Non-coding RNA in thalassemia

The non-coding RNA (ncRNA) is a functional RNA molecule and constitutes a heterogeneous group of transcripts not translated into proteins. The two major types of ncRNA are small RNA (sRNA) and long non-coding RNA (lncRNA). sRNA are important regulatory molecules in the control of gene expression at both transcription and post-transcriptional level by gene silencing or RNA silencing.[35] The types of sRNA include microRNA (miRNA), small interfering RNA (siRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and piwi-interacting RNA (piRNA) which are majorly involved in regulating various biological processes. lncRNA, including intergenic, intronic, sense and antisense lncRNAs, are reported to be the most prevalent and functionally diverse members of ncRNA.[36] The role of lncRNA can be perceived in gene expression, genomic imprinting, nuclear organization, gene dosage compensation, chromatin structure modulation, RNA translation, splicing and epigenetic regulation.[37]

The role of epigenetic regulators and modifiers, including lncRNA in hemoglobin synthesis and the role of dysregulated lncRNA have been studied extensively, but their role in changing the expression of the human globin gene has not been studied in depth.[38] In normal cells, lncRNA prevents the binding of miRNA to maintain the Hb F levels, whereas in disease conditions like beta-thalassemia, due to dysregulated lncRNAs the level of Hb F is elevated.[39] The possible mechanism for high levels of Hb F is the activation of Hemoglobin

Subunit Epsilon 1 (HbE1) and haemopoietic cell lineage-inducible molecule by lncRNA. Several studies have reported various mechanisms of regulation of lncRNA in the expression of the gamma-globin gene.[40–43] The lncRNAs, like Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1), Myocardial Infarction Associated Transcript (MIAT), Antisense Non-coding RNA in the Inhibitors of cyclin-dependent kinase 4 Locus (H19 and ANRIL), are differentially expressed in beta-thalassemia, thereby acting in a putative role in beta-thalassemia pathophysiology.[43,44]

Apart from lncRNA, miRNA plays a major role in hemoglobinopathies, such as regulating gene expression, erythroid cell mechanism, iron hemostasis, and oxidative cell damage. *miR15a/16–1, miR-486–3p, miR-26b, miR-199b-5p, miR-210, miR-34a, miR-138, miR-326, let-7, and miR-17/92* cluster elevate gamma-globin expression, whereas *miR-451* induces alpha-, beta- and gamma-globin expression.[45] Down regulation of the circulating miRNAs *miR-let-7d, miR-200b* and up-regulation of *miR-122* in TDT can serve as biomarkers for cellular damage under excessive iron conditions in tissue.[46] In a recent study, Penglong et al.[47] showed a biphasic expression of *miR-214* in beta- and alpha-thalassemia and the molecular mechanism of miRNA and transcription factors in the regulation of oxidative status in erythroid cells in thalassemia.

Role of artificial intelligence in thalassemia

The simulation of human intelligence using machines to generate, classify and perform cognitive functions through technology is called artificial intelligence (AI). The use of AI has increased in the field of healthcare for accurate and swift diagnosis of disease.[48] Several machine learning algorithms play a key role in diagnosing and differentiating thalassemia from iron deficiency anemia.[49] AI-based tools are required to predict the prevalence of genetic mutations in thalassemia much earlier, before expending more on diagnosis and treatment.[50] It is essential to have collaboration between engineers and healthcare practitioners to decide on the development of algorithms and models to solve problems using specific knowledge and approaches to improve the quality of life for patients.

Molecular diagnostics demand for thalassemia

Though different molecular approaches exist for the detection, screening and diagnosis of thalassemia their utilization in routine clinical practice is limited by socioeconomic conditions and by the awareness of the patients and their relatives. At the same time, in-depth knowledge about the currently available techniques, along with the advantages and limitations of the same, is important to choose the correct testing methodology applicable to concerned tertiary healthcare facilities and the patients attending them. NGS is widely available in the market, and because of the numerous publications, all departments are aware of its existence and usage. Broader panels including multiple genes or shorter panels with the targeted genes, which have clinical implications prevalent in our population, can be studied using the same technique. Quantitative polymerase chain reaction (qPCR) is another more sensitive methodology when compared to NGS, as the results obtained from NGS during a research protocol are always validated using qPCR. qPCR is a cost-effective methodology that can be used in diagnostics and hence can be used with increased sensitivity for thalassemia mutation testing, both in patients and also in instances of prenatal screening. Testing of the *HBB* gene using the qPCR technique is capable of detecting the most common mutations known to occur in the Indian population. Using the NGS technique, additional mutations (both prevalent and non-prevalent) in the *HBB* gene can be covered with less sensitivity. This pitfall of reduced sensitivity is associated with the errors and false-positive data that can occur as part of data analysis. Expertise is needed for pre-analytical, analytical and post-analytical procedures, since error-free performance of the technique is not widely available everywhere, especially in resource-poor settings.

Conclusion

Although various molecular approaches exist to detect and treat thalassemia, the burden of the disease is increasing worldwide. It is necessary to create widespread awareness and adopt diagnostic and prenatal screening programs to provide appropriate supportive care and treatment for affected patients and, at same time, to prevent the birth of affected babies. The testing methodology adopted for this should be cost-effective, sensitive, specific and easy to perform in resource-poor settings. Based on the extensive literature review available, it is suggested that qPCR may be considered a viable option for thalassemia mutation testing of the Indian population. NGS may be reserved for a clinically suspected thalassemia patient who does not harbor a detectable mutation by qPCR. Moreover, qPCR is sufficient to detect mutations in prenatal screening. In summary, this review aimed to discuss multiple molecular-level approaches to detect mutations in thalassemia and therapeutic knowledge about the application of gene editing technologies in the treatment of thalassemia. It also discusses the epigenetic mechanisms and the role of non-coding RNA which may serve as a biomarker for disease diagnosis. The need for molecular testing diagnostics is warranted, and it should, at the same time, be made affordable.

Conflicts of interest

The authors declares no conflict of interest.

Authors contribution

B.M. designed the scope and objective of the review by conducting comprehensive literature search and drafted the manuscript; C.K. edited and revised the manuscript to improve clarity and accuracy; S.D. created tables and reviewed the final version of the manuscript.

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Special article

A dream or reality: Consideration of 'bloodless' hematopoietic stem cell transplants for Jehovah's witness patients

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ABSTRACT

Background: Hematopoietic stem cell transplantation (HSCT) is an important part of treatment for many hematologic conditions. The high-dose chemotherapy used in HSCTs puts patients at risk of significant cytopenias which often necessitate blood product transfusions. Certain populations, including Jehovah's Witnesses, are unable to receive blood product transfusions during their transplant and thus, in the past, they have been seen as unsuitable candidates for transplantations. However, there has been growing evidence of the safety and efficacy of so-called "bloodless" HSCT protocols.
Methods: The most recent and relevant literature on "bloodless" transplants were identified through Embase, MEDLINE, and PubMed, and analyzed to construct a "bloodless" HSCT protocol at a Canadian centre. Since 2021, the regimen was utilized for four autologous transplantations in three different Jehovah's Witness patients.
Results: None of the patients had a significant bleeding event nor a hemoglobin nadir below 8.0 g/dL. Minor bleeding events, predominantly mucositis, resolved with site-specific management. No patient had significant thrombocytopenia, and all the cell lines of patients had normalized without transfusions by the time of discharge. All patients were hospitalized for <30 days, similar to the experience of the centre with "regular" autologous transplants.
Conclusion: Careful planning and tailored regimens support the achievability of "bloodless" HSCTs in patients, such as Jehovah's Witnesses, allowing practitioners to provide care to a previously excluded group and minimize the use of blood products in all HSCT patients.

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Introduction

Hematopoietic stem cell transplantation (HSCT), both autologous and allogeneic, is a key pillar in the treatment of many non-malignant and malignant hematologic conditions.[1] Protocols for HSCT rely on high-dose chemotherapy which often leads to the complication of severe pancytopenia. In these instances, typical hematopoietic support for HSCT patients utilizes transfusion of blood products to manage possible life-threatening cytopenias. For example, most institutions initiate red blood cell transfusions at hemoglobin concentrations of <7.0 g/dL and platelet transfusions for platelet counts $<10 \times 10^9$ cells/L in non-bleeding patients.[2] These are the values at which patients are at increased risk of end organ dysfunction and spontaneous intracranial hemorrhage, respectively.[3] The transfusion requirements for HSCTs can often be quite high. For example, on average, it has been reported that allogeneic HSCT recipients necessitate a mean of 6.8 units of packed red blood cells within the first 60 days alone.[4] Given this frequent necessity of transfusions in HSCTs, patients who refuse blood product transfusions have previously not been candidates for HSCT. This has left certain groups, particularly the approximately 8.5 million Jehovah's Witnesses worldwide,[5] marginalized, and unable to access standard-of-care treatment for conditions with high morbidity and mortality.[6]

While there has been an increase in recent years in attempts to minimize transfusion products and optimize "bloodless" HSCTs,[7] there remains a scarcity of descriptions of the implementation of "bloodless" protocols, particularly in Canada. This study sought to assess the feasibility and safety of a "bloodless" HSCT protocol at a Canadian centre for Jehovah Witness patients based on the most relevant literature.

Methods

Relevant literature describing "bloodless" HSCT protocols were identified through Embase, MEDLINE and PubMed databases. The first reported "bloodless" HSCT was in 1996.[8] In total, ten relevant articles were identified which described "bloodless" protocols. While there remains no consensus guidelines for "bloodless" transplantations, general principles were identified. These specialized protocols have also been shown to be quite effective with good overall patient outcomes.[7–16] Assessing these recent and relevant articles, London Health Sciences Centre's (LHSC) Hematology Division was able to construct a "bloodless" HSCT protocol based on their core principles and features (Table 1).

Management strategies for HSCT-related cytopenias were divided into two general categories: pre-transplant optimization and post-transplant management. Pre-transplant optimization involves targeting safe cell count levels prior to the conditioning therapy with these levels being achieved by erythropoietin agonists, intravenous (IV) iron and limiting blood loss.[8–10] We utilized pre-transplant cell count targets for hemoglobin of >11.0 g/dL and for platelets $>150 \times 10^9$ cells/L (Table 1) with similar thresholds generally used in other studies.[8–10] These thresholds were primarily based on retrospective assessments of pre-transplant values which led to more severe outcomes,[9] while still maintaining the principles of a "transfusion-restrictive" approach and limiting any sequelae of increased viscosity at higher hematocrits.[2] To achieve these cell counts, patients received regular erythropoietin stimulating agent (ESAs) injections, a strategy also commonly used for Jehovah's Witnesses perioperatively or when critically ill.[17] Specifically, our protocol used 40,000 units subcutaneously (SC) weekly of Eprex® (Epoetin alfa). In conjunction, erythropoiesis was further supported with IV iron supplementation,[8–10] with our regimen using IV iron sucrose 300 mg every week. Additionally, to further prevent unnecessary menstrual blood loss, hormonal contraceptives have been used in various protocols,[8–10] therefore, for women of childbearing age, we provided the option between an oral hormonal contraceptive or an intramuscular medroxyprogesterone injection to control menstruation. ESAs and intravenous iron were discontinued when hemoglobin reached >12.0 g/dL (Table 1).

Management is more complex post-transplant with prophylactic hematopoietic support, bleeding prevention, minimizing unnecessary blood loss, as well as reactive management for severe cytopenias and significant bleeding (Table 1). Prophylactic nutrient supplements have been utilized in a variety of "bloodless" regimens to support hematopoiesis.[8–10,12,13] At LHSC, patients were given folic acid 5 mg oral (PO) once daily (q.d.) and vitamin B12 1,000 mg PO q.d., in addition to vitamin K 10 mg PO q.d. on Mondays, Wednesdays, and Fridays to prevent coagulopathies. Further hematopoietic support was given to improve platelet and neutrophil counts as these cell lines are often profoundly depleted during HSCT conditioning. It has become common to provide scheduled thrombopoietin receptor agonists (TPO-RAs) and granulocyte colony-stimulating factor (G-CSF) post-transplant when patients are expected to reach their cell count nadir.[8–10,12,13] We administered filgrastim 300 mg, or 480 mg if patient weight was greater than 90 kg, SC q.d. starting on Day +5 after the transplant until the absolute neutrophil count reached 1.0×10^9 cells/L and romiplostim 4 mg/kg SC weekly starting on Day +5 until the platelet count reached $>100 \times 10^9$ cells/L.

To prevent unnecessary blood loss, other regimens sought to prevent upper and lower gastrointestinal bleeding, and epistaxis,[8–10,12,13] so our patients were given lansoprazole 30 mg PO twice daily (b.i.d.), PEG3350 17 g PO q.d., and saline nasal spray one spray per nostril q.d. A major source of blood loss in HSCT patients is the regular, at least daily, blood draws. Therefore, based on similar protocols in other studies,[8–16]

Table 1 – London health sciences centre 'bloodless' hematopoietic stem cell transplant regimen.

Prerequisites & informed consent	
	• Ideal parameters: Hb >11.0 g/dL, Plt >150 × 10^9 cells/L
	• Acceptable parameters: Hb >9.0 g/dL, Plt >100 × 10^9 cells/L
	• 10 % risk of overall mortality from bleeding/anemia/infections
	• CNS involvement increases risk of fatal intracranial hemorrhage (10 % risk)
	• Informed documented consent of refusal of blood product
Pre-transplant optimization	
	• Eprex 40,000 units SC q1wk. Target Hb ≥11.0 g/dL. Discontinue if Hb ≥12.0 g/dL
	• Iron sucrose 300 mg IV over two hours q1wk. Target Hb ≥10.0 g/dL. Discontinue if Hb ≥12.0 g/dL
	• If female patient, consider: oral contraceptive, or Medroxyprogesterone IM injection
During transplant hematopoietic support	
Medication support	• No aspirin/NSAIDS
	• Vitamin K 10 mg PO q.d. Monday, Wednesday, and Friday
	• Folic acid 5 mg PO q.d.
	• Vitamin B12 1,000 mg PO q.d.
	• Polyethylene glycol 3350 17 g PO q.d. (can increase if constipation develops)
	• Lansoprazole 30 mg PO b.i.d.
	• Saline nasal spray 1 spray each nostril t.i.d.
	• Filgrastim 5 mg/kg (300/480 mg) SC q.d., start Day +5
	• Eltrombopag 50–150 mg q.d. or romiplostim 4 mg/kg SC q1wk starting Day +5 until Plt >100 × 10^9 cells/L
	• fluconazole (Diflucan) 400 mg PO or IV q.d. start Day 0
	• Acyclovir 400 mg PO or 250 mg IV every 12 h, start Day 0
	• Levofloxacin 500 mg PO q.d., start Day 0; when febrile, stop levofloxacin and start imipenem 500 mg IV q6h
	• Acetaminophen 650 mg PO q4h if febrile
	• IV fluids to ensure euvolemic status
	• If female patient: continue oral contraceptive if started pretransplant
	• Only twice weekly blood (Monday and Thursday)
Blood conservation	
	• CBC, LFTs, creatinine, electrolytes (+ PT, INR, fibrinogen once weekly)
	• Use pediatric vacutainers-MICROTAINERS for sampling (total volume = 0.25+ 0.4 + 0.4 + 0.4 = 1.5 mL each draw [1.8 mL for coagulation studies once weekly])
	• Do not use central line for blood draw
	• If fever, blood culture x2 – one central venous catheter, peripheral venipuncture only once/week (Use Bactec Adult/F bottles-10 mL x2)
	• If clinical unstable or other collections indicated, then above collection rule exempted
Anemia	• If Hb <11.0 g/dL, iron sucrose 300 mg IV q1wk; discontinue when Hb >12.0 g/dL
	• If Hb <10.0 g/dL, Eprex® 40,000 units SC q1wk; discontinue when Hb >12.0 g/dL
	• If Hb < 8.0 g/dL, then supplemental O_2 2 L/min by nasal prongs
Thrombocytopenia	• If Plt <30 × 10^9 cells/L, tranexamic acid 1 g PO q4h
	• If Plt <10 × 10^9 cells/L, tranexamic acid 4 g IV q4h
Active bleed	• Local hemostasis/pressure dressing
	• Change PO lansoprazole to pantoprazole 40 mg IV q12h if GI bleed
	• Tranexamic acid 1 g mouth rinse swish and spit QID for mucosal bleed
	• If systemic bleed, tranexamic acid 1 g IV q4h (avoid if hematuria)
	• If minor bleed, desmopressin IV 0.3 mg/kg IV q12H for 3 days
	• If continued bleeding after desmopressin, can consider Factor VIIa 90 mg/kg IV q2h until bleeding subsides
	• For epistaxis, xylometazoline (Otrivin®)/saline nasal spray two sprays in each nostril q15 min until bleed resolves

Hb: hemoglobin; Plt: platelets; PO: oral; SC: subcutaneous; IV: intravenous; q.d.: once daily; b.i.d.: twice daily; t.i.d.: three times daily; q: every; wk: week; h: hour; min: minute; CNS: Central nervous system; NSAIDS: non-steroidal anti-inflammatory drugs; CBC: complete blood count; LFTs: liver function tests; PT: prothrombin time; INR: international normalized ratio.

bloodwork was reduced from daily to twice per week and pediatric-sized vacutainers were used. Additionally, in the event of a fever, given post-transplant infections are another common risk of HSCTs, blood cultures were limited to only once per week. These blood conservation techniques have been estimated to reduce the average blood drawn from 40 mL per day to approximately 3 mL.[16]

If severe anemia or thrombocytopenia were to arise, various protocols increased hematopoietic support in addition to antifibrinolytic agents, achieving significant success.[7–12] For our protocol, when the patient's hemoglobin was <11.0 g/dL, we initiated IV iron sucrose (300 mg weekly) and when their hemoglobin was <10.0 g/dL they were started on Eprex® (40,000 units SC weekly). If their platelets were less than 30 × 10^9 cells/L, they were started on tranexamic acid (TXA - 1 g PO every four hours), which was increased to 4 g IV every four hours if their platelets dropped below 10 × 10^9 cells/L.

Finally, in the event of bleeding, previous protocols implemented various systemic and local therapies to gain hemostasis.[7–16] For minor hemorrhages, defined as localized bleeding without significant hemoglobin reduction, hemodynamic instability, or life-threatening implications, TXA 1 g was administered orally four times daily and desmopressin IV 0.3 mg/kg every 12 h for three doses. In addition,

Table 2 – Jehovah's witness patients who underwent London health sciences centre's "bloodless" hematopoietic stem cell transplant (HSCT) protocol

Patient	1	2	3	
Age at treatment (years)	50	64	60	
Sex	Male	Female	Female	
Diagnosis	Relapsed primary CNS DLBCL with multifocal lesions	Recurrent primary CNS DLBCL	High risk MM	
Prior treatment	2019 MVPC with radiation and procarbazine maintenance 2020 Ibrutinib 2021 CHOP-R	2020 MPVC with radiation and procarbazine maintenance 2022 Ibrutinib	2022 CyBorD	
HSCT regimen (date, conditioning regimen, cell dose)	Autologous HSCT September 2021 Conditioning: Carmustine & Thiotepa Cell dose: 7.2×10^6 CD34 cells/kg	Autologous HSCT November 2022 Conditioning: Carmustine & Thiotepa Cell dose: 3.8×10^6 CD34 cells/kg	Autologous HSCT May 2023 Conditioning: Melphalan Cell dose: 6.2×10^6 CD34 cells/kg	Autologous HSCT August 2023 Conditioning Melphalan Cell dose: 3.2×10^6 CD34 cells/kg
Pretransplant Hb (g/dL)	15.7	13.3	9.0	12.7
Days to Hb Nadir	+13	+11	+13	+17
Hb Nadir (g/dL)	9.4	8.4	8.1	10.4
Days Hb <7.0 g/dL	0	0	0	0
Hb at discharge (g/dL)	12.0	10.4	9.9	13.2
Pretransplant Plt count (x 10^9cells/L)	168	145	223	119
Days to Plt nadir	+9	+7	+10	+10
Plt nadir (x10^9cells/L)	<5	<5	8	<5
Days Plt count <10 (x 10^9 cells/L)	4	4	7	7
Plt count at discharge (x 10^9 cells/L)	235	47	157	76
Pretransplant ANC (x 10^9cells/L)	1.0	5.6	11.7	4.2
Days to ANC nadir	+2	+15	+6	+10
ANC nadir (x 10^9cells/L)	0.0	2.5	0.0	0.2
Days ANC <0.5 $\times$ 10^9cells/L	14	0	7	3
ANC at discharge (x 10^9cells/L)	1.4	2.5	4.3	5.9
Days to neutrophil engraftment	+13	+11	+13	+13
Days to Plt engraftment	+13	+11	+21	+13
Filgrastim days administered	+5 to +12	+5 to +10	+5 to +16	+5 to +8
Days of grade 3–4 anemia	0	0	0	0
Days of grade 3 thrombocytopenia	7	5	0	4
Days of Grade 4 thrombocytopenia	1	4	7	11
Bleeding complications & interventions	No significant bleeding TXA given for low plt	No significant bleeding TXA given for low plt	No significant bleeding TXA given for low plt	No significant bleeding TXA given for low plt
Infection complications and interventions	Culture negative febrile neutropenia – imipenem until recovered ANC	None	Culture-negative febrile neutropenia – imipenem and vancomycin until recovered ANC	BCx neg febrile neutropenia – IV imipenem *C. difficile* colitis versus neutropenic colitis versus diverticulitis – PO vancomycin + metronidazole, bowel rest + parenteral nutrition Day +7 to +11

Other complications	Grade 2 mucositis with mild dysphagia and nausea – treated with koolstat, olanzapine (Zyprexa) and ondansetron Constipation – treated with desmopressin	Grade 2 mucositis with mild dysphagia – treated with koolstat, olanzapine (Zyprexa) and ondansetron	Grade 1 mucositis	None
Follow-up post-HSCT and clinical status	Complete metabolic response	Complete metabolic response	Pending follow-up	

Day 0: day of transplant; Hb: hemoglobin; Plt: platelet count; ANC: absolute neutrophil count; CNS: central nervous system; DLBCL: diffuse large B cell lymphoma; MM: multiple myeloma; MVPC: methotrexate (Methotrexate), vincristine, procarbazine, cyclophosphamide; CHOP-R: cyclophosphamide, hydroxydaunorubicin (doxorubicin), oncovin (vincristine), prednisone, rituximab; CyBorD: cyclophosphamide, bortezomib (Bortezomib), dexamethasone.
Epidemiologic information, diagnosis, prior treatments, HSCT regimen, cell count information, engraftment time, complications, and their interventions.

alterations could be made based on the location of the bleeding. For example, basic pressure dressings, local topical TXA for mucosal bleeds, IV proton pump inhibitors for gastrointestinal bleeding, or nasal spray and packing for epistaxis. If the bleeding were to be more severe or systemic, IV TXA as well as IV Factor VIIa could be considered.

Results

This regimen has been utilized for three different inpatients at LHSC since 2021 (Table 2); one patient had two transplants as part of the scheduled plan of care. All three were practicing Jehovah's Witnesses who did not consent to most blood-derived products, including red blood cells and platelets. Two of the patients underwent salvage autologous HSCTs in the setting of relapsed/recurrent primary central nervous system diffuse large B cell lymphoma while the other patient received tandem HSCTs for high-risk multiple myeloma. None of the patients had a significant bleeding event, while all three patients did experience mild oral mucositis which was controlled with topical TXA. Furthermore, none had a hemoglobin nadir below 8.0 g/dL. All patients did have significant thrombocytopenias and neutropenias, including in both tandem HSCT procedures, but these resolved with TPO-RAs and G-CSF administration giving engraftment times comparable to "regular" autologous HSCTs. For any additional bleeding risk associated with thrombocytopenia, all patients received both oral and IV TXA. Two of the three patients also experienced febrile neutropenias and were treated with an appropriate course of antibiotics. All the cell lines of the patients had normalized by the time of discharge with all patients being hospitalized for <30 days, similar to the experience of our centre with "regular" autologous HSCTs. All the patients are doing clinically well with the two lymphoma patients having an ongoing complete metabolic response.

Discussion

In summary, based on existing literature,[7–16] we developed a "bloodless" HSCT protocol (Table 1) with three Jehovah's Witness patients undergoing treatment according to this protocol (Table 2). The protocol entailed pre-transplant targets for hemoglobin concentration >11.0 g/dL and platelet count $>150 \times 10^9$ cells/L achieved through ESAs, IV iron, and, when indicated, hormonal contraceptives. Our post-transplant regimen supported hematopoiesis with supplements including iron, vitamin B12, and folic acid as well as vitamin K for coagulopathy prevention. Hematopoiesis was additionally supported by injections of TPO-RAs and G-CSF. Unnecessary blood loss was prevented by decreasing the frequency and volume of blood draws to twice a week bloodwork using pediatric test tubes. Bleeding prevention was achieved via oral proton pump inhibitors, laxatives, and nasal spray. Response measures to severe anemias, thrombocytopenias, and bleeding were in place and entailed ESAs, TXA, iron, desmopressin, and tailored hemostasis measures. Of the three patients who underwent autologous HSCT with our "bloodless" protocol, none developed any significant bleeding event nor any severe anemia that would have necessitated blood product transfusion in regular HSCT protocols. While all the patients did develop severe thrombocytopenias, these were managed conservatively as outlined above and the patients did not develop any adverse outcomes (Table 2). These findings of safety of "bloodless" HSCTs are in line with previous experiences in other centres.[7–16] Therefore, both our review of the literature and own experiences demonstrate the feasibility of implementing "bloodless" HSCTs with specialized and carefully planned protocols at Canadian centres.

The major limitation of this study is the small sample size of patients, an inherent issue given the niche population group being assessed; most of the previous literature represents small case series or retrospective analyses. However, in conjunction, there is a clear body of evidence showing that "bloodless" HSCTs can be done safely and with similar efficacy to "regular" HSCTs. This allows certain populations, especially Jehovah's Witnesses, access to a previously unavailable treatment option. It is also important to note that negative findings in this study population are rarely published.

Moving forward, conducting further prospective trials would be beneficial to better elucidate the risks or

disadvantages associated with "bloodless" protocols. Arranging such trials would be difficult given the small number of Jehovah's Witnesses requiring HSCTs, especially in Canada, and would likely necessitate enrolling multiple centres to have a large enough population. As blood products are both a time-intensive and scarce resource, implementation of aspects of the "bloodless" protocol for all patients may reduce the number of blood product transfusions required in HSCTs overall. A notable feature of this protocol is its applicability to other patients undergoing HSCT, with the objective of conserving blood products at our center. However, patients must be informed regarding the implications of declining transfusions, which include the possibility of clinical deterioration and the inability to provide support in critical situations such as life-threatening anemias and hemorrhages.

In conclusion, with careful planning and tailored regimens there is evidence supporting the achievability of "bloodless" HSCTs in patients, such as Jehovah's Witnesses, who are not able to receive blood products through the course of their treatment. These advancements not only allow for practitioners to provide care to a previously excluded group but could minimize the use of resource scarce blood products in all HSCT patients.

Author contributions

MM collected the data, framed the ideas, drafted, and critically revised the manuscript. AF and DC were responsible for data analysis, design of protocol, and manuscript review. UD designed the protocol, critically revised the manuscript, and supervised the project.

Conflicts of interest

The authors declare no conflicts of interest.

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Case Report

Influenza A-triggered Bickerstaff brainstem encephalitis successfully treated with therapeutic plasma exchange: A case report

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Introduction

Bickerstaff brainstem encephalitis (BBE) is an extremely rare autoimmune disease with the first reports being published in the 1950s [1]. The most characteristic neurological symptoms of BBE include the following: ophthalmoplegia, ataxia, impaired consciousness, limb weakness, facial paralysis, positive Babinski's sign and impaired superficial sensation [2]. Impaired consciousness was shown to be most likely caused by dysfunction of the ascending reticular activating system [3]. Diagnosis of the disease is complex due to similarity of symptoms to other neurologic diseases (e.g. meningitis, encephalitis). Initially, the diagnosis in our case was infective meningitis or encephalitis, but it was revised to BBE after ruling out neuro-infection. Laboratory confirmation of BBE is often delayed as in most institutions anti-ganglioside antibody (anti-GQ1b) tests are performed externally, leading to prolonged turn-around time. There are three diseases in which anti-ganglioside antibodies are present – BBE, Miller Fisher syndrome (MFS), Guillaine-Barre syndrome (GBS) – and overlapping of diseases may occur. Characteristic for MFS are ophthalmoplegia, ataxia, areflexia, and obtunded consciousness. Some authors view BBE and MFS as a continuous spectrum of one disease, the so-called Fisher-Bickerstaff syndrome [4,5]. Anti-ganglioside antibodies, produced in response to infective agents (mostly bacteria) sharing ganglioside structures, can damage myelin sheaths through molecular mimicry [6].

Case presentation

A 32-year-old patient was admitted to the local intensive care unit (ICU) due to deteriorating consciousness with a preliminary diagnosis of neuro-infection or autoimmune encephalitis. Past medical history was negative. The current presentation was preceded by contact with an influenza A-positive child. Three days before hospital admission, the patient became feverish (up to 40 °C), complained of nasal congestion and dry cough. Notably, the patient reported

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numbness of hands and feet that started one day before admission. On the day of hospital admission, the patient reported spinning dizziness intensifying on standing and sitting and with head movements, and nausea. On admission to the neurology department the patient was conscious, with logical verbal contact, oriented, with no meningeal symptoms, the only abnormality in the neurologic examination was first degree fine-wave nystagmus when looking to the right. An antigen test for respiratory viruses was negative, C-reactive protein was 22.3 mg/L, cerebrospinal fluid (CSF) was colorless and clear, with elevated cytosis (17 cells/μL; norm <5 cells/μL) and slightly elevated glucose (78.3 mg/dL with 76 % of serum concentration; norm 40–60 mg/dL with 60 % of serum concentration) and immunoglobulin G concentration (4.1; norm 1–3 mg/dL). A non-contrast computed tomography (CT) of the head showed only paranasal sinusitis. A fast deterioration of the neurological status was noted – the patient became periodically uncooperative with dysarthric speech, positive Kernig's sign, ophthalmoplegia with predominant leftwards gaze, ataxia, vivid deep reflexes, and positive Babinski sign on the left side. First-line treatment included empirical broad-spectrum antibiotic therapy, anti-viral agents, and corticosteroids. Initial treatment was unsuccessful: the patient became more obtunded and bradypnoe was noticed, therefore the patient was transferred to the ICU where he was intubated and mechanical ventilation was commenced (<24 h after hospital admission). Appropriate samples were obtained for additional laboratory and microbiological tests. Further diagnostic imaging included: contrast-enhanced CT of the head, CT angiogram of the head, contrast-enhanced magnetic resonance imaging (MRI) and contrast-enhanced CT of the chest, abdomen and pelvis however, no abnormalities apart from paranasal sinusitis were detected. The electroencephalogram revealed disturbed spatial organization and generalized retardation of basic activity. A nerve conduction study (NCS) showed mostly axonal motor-sensory polyneuropathy with predilection to lower extremities. Following exclusion of neuro-infection, the constellation of the symptoms of external ophthalmoplegia, ataxia, and deterioration of consciousness, as well as the result of NCS, made the diagnosis of BBE most probable. The patient was scheduled for emergency therapeutic plasma exchange (TPE). A dialysis cannula was inserted through the right internal jugular vein and a series of five procedures scheduled every other day was carried out. A 5000 mL volume of 4 % human albumin solution was utilized as the substitution fluid. The procedure was performed using a standard continuous renal replacement therapy apparatus (multiFiltratePRO, Fresenius Medical Care, Germany) and a special filter (Plasma Flux P2 dry, Fresenius Medical Care, Germany). Standard therapeutic doses of heparin sodium were used for anticoagulation during the procedure. Following the initiation of TPE, a blood sample for anti-ganglioside antibodies (anti-GM1, anti-GD1b, anti-GQ1b) was sent for analysis but the results came back negative. During the course of TPE the patient showed multiple episodes of vegetative excitation with tachycardia, hypertension, sweating, and muscle tension. As the duration of mechanical ventilation was prolonged (>7 days), percutaneous dilatational tracheotomy (Griggs technique) was performed [7]. Due to inability to feed the patient orally, a percutaneous gastrostomy was inserted. Three days after the last TPE procedure the patient regained consciousness and non-verbal logical contact. The only laboratory test that came back positive was anti-glutamic acid decarboxylase antibodies (a high titer >2000 IU/mL). The patient was then transferred to the neurology department and later to the neurological rehabilitation department where he made full neurological recovery.

Discussion

Clinical features of our patient were characteristic of anti-GQ1b-positive BBE: prior upper respiratory tract infection, mildly elevated cell count and protein concentration in the CSF, normal brain MRI (performed twice: during diagnosis and after regaining consciousness), and relatively fast return of consciousness [8]. The negative anti-GQ1b test in this patient could be due to the fact that a blood sample was collected only after starting the patient on TPE, by which time pathologic antibodies could have been removed or their concentration significantly decreased. Nevertheless, initiation of appropriate therapy should not wait until these test results come back. As soon as neuro-infection was excluded by negative CSF cultures, TPE was started. In our institution standard CSF cultures (final result after approximately five days) are used to establish the etiologic agent in neuro-infections: this could be achieved in a matter of one hour if polymerase chain reaction was used. The examination of CSF in BBE may show increased or normal cytosis and elevated protein [9]. In our case both were elevated. A nerve conduction study in BBE may reveal axonal demyelination [10]. In this case, NCS also revealed axonal involvement. This test is particularly useful if there is a suspicion of BBE with overlapping GBS. In the case of severe or rapidly progressing BBE, the initiation of TPE seems to be the most beneficial therapeutic option, as intravenous immunoglobulins and corticosteroids may not be effective. Physicians performing TPE should be aware of this disease that can present in both children and adults, in order to commence treatment at an early stage of BBE.

BBE is a rare disease with partly unspecific neurological symptoms. Following exclusion of an infective cause of neurological symptoms, patients should optimally be started on TPE as alternative therapies may not be effective. The turnaround time for confirmatory laboratory tests is prolonged and should not delay the start of appropriate treatment.

Contribution statement

PFC: Conceptualization. **PFC, MP, TJ, PL:** Manuscript Writing & Editing.

Conflicts of interest

None.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Case Report

CAR T cell therapy-related lumbosacral polyradiculopathy with myelitis and stiff person syndrome with response to intravenous immunoglobulin and corticosteroids in a patient with acute lymphoblastic leukemia

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Introduction

Chimeric antigen receptor (CAR) T cell therapy has emerged as a promising treatment for relapsed/refractory acute lymphoblastic leukemia (ALL) in children and adults. However, major toxicities can occur after CAR T cell therapy, most notably cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). The pathophysiology and long-lasting effects of these adverse reactions are still unknown. We report a case of lumbosacral polyradiculopathy with myelitis and stiff person syndrome (SPS) with glycine receptor antibodies after treatment with CAR T cells in a patient with ALL. To our knowledge, this is the first such report in the literature of this manifestation in a patient receiving CAR T cell therapy.

Case

A 55-year-old female diagnosed with CD20^{+} Philadelphia chromosome-positive B-cell ALL underwent induction chemotherapy following the European Working Group on Adult ALL (EWALL) protocol using a combination of dasatinib and

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rituximab. The patient received a reduced intensity conditioning regimen of fludarabine and melphalan and underwent a peripheral blood stem cell transplant from a matched related donor while in first complete remission with measurable residual disease (MRD) *BCR-ABL1* by polymerase chain reaction (PCR). The patient experienced T315I mutated relapsed disease with a detectable *BCR-ABL1* PCR of 18.1521 on Day +144. There was evidence of bone marrow involvement on Day +183 and she was started on blinatumomab and ponatinib therapy. The patient received five cycles of blinatumomab followed by a donor lymphocyte infusion on Day +307.

The patient was scheduled for a second donor lymphocyte infusion but developed facial pain and swelling, prompting teeth extractions. She continued to experience facial pain and underwent a computer tomography scan of the sinuses on post-transplant Day +482, which showed ethmoid and maxillary sinus mucosal thickening and soft tissue inflammatory changes. On Day +489, she underwent maxillary antrostomy, total ethmoidectomy, and sphenoidotomy. Pathology of the resected tissue was positive for $CD20^+$ B-ALL, consistent with extramedullary involvement of the sinuses.

Blinatumomab and ponatinib were administered in preparation for leukapheresis, which occurred on Day +496. The patient received bridging therapy with attenuated FLAG-Ida (fludarabine, cytarabine, granulocyte colony stimulating factor, and idarubicin) resulting in MRD negative remission with a positron emission tomography (PET) scan consistent with resolution of extramedullary disease (Figure 1). The patient received brexucabtagene autoleucel (Tecartus), which was complicated by Grade 1 CRS on Day +10 and resolved with a dose of tocilizumab.

Six days after receiving CAR T cell therapy, the patient reported body aches in the upper extremities, back, and hips, which were initially thought to be related to the use of granulocyte colony stimulating factor. The pain moved to the lower back and lower extremities and was described as “electrical” and “burning.” Hydromorphone and gabapentin were given with minimal improvement in symptoms. Over the course of the ensuing three weeks the pain severely limited her ability to walk, necessitating the use of a cane. Neurologic examination showed diffuse hyperreflexia, bilateral lower extremity weakness, distal sensory loss, and wide-based unsteady gait.

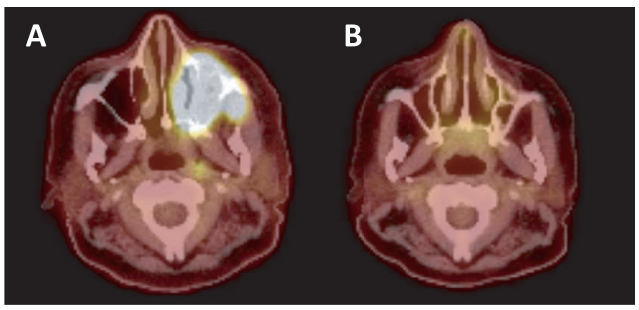

Figure 1 – A) Positron emission tomography – computed tomography (PET-CT) scan demonstrating extramedullary disease prior to bridging and CAR T cell therapy B) PET-CT scan demonstrating response to bridging and CAR T cell therapy.

A total spine magnetic resonance imaging exam (MRI) performed on Day +39 was significant for a circumferential disc bulge at L5-S1 and an annular fissure, both impinging on the bilateral descending S1 nerve roots; however, no spinal cord compression, signal abnormalities, or enhancement were observed. Electromyography (EMG) on Day +52 demonstrated active denervation throughout both lower extremities with slight myopathic dysfunction in both hip flexors and right biceps. A brain MRI on Day +53 was normal. A lumbar puncture was performed on Day +55 with cerebrospinal fluid (CSF) analysis showing lymphocytic pleocytosis (white blood count: 9/μL with 86 % lymphocytes) with normal protein and glucose. CSF cytology and flow cytometry did not demonstrate any malignant cells. The presentation was thought to be most consistent with CAR T cell therapy-associated polyradiculomyelitis. Hence, on Day +64, the patient was treated with dexamethasone (20 mg) and continued at 10 mg daily for six additional days with no immediate significant improvement in the symptoms. On Day +76, treatment was switched to intravenous immunoglobulin (IVIG) at 1 g/kg for two doses every two weeks for three cycles, resulting in significant improvement in the paresthesia, weakness, and gait. She no longer required a cane to ambulate, and IVIG was decreased to a monthly schedule.

Four weeks later, on Day +156, the patient developed severe acute pain affecting her trunk and proximal extremities requiring hospital admission for pain management. She described stiffness, muscle spasms, involuntary extremity movements, and transient inability to move after rapid standing. A neurological exam at this time showed normal leg strength bilaterally and resolution of hyperreflexia. A repeat total spine MRI was unchanged from before. Serum analysis revealed the presence of glycine receptor alpha1 subunit (GlyR) and glutamic acid decarboxylase 65 (GAD65) antibodies, at a concentration of 0.14 nmol/L (reference range $\leq$0.02 nmol/L). Repeat EMG showed denervation of lumbosacral muscles with resolution of active denervation in the lower extremities. She started a pain management regimen which included duloxetine, gabapentin, baclofen, and controlled-release morphine sulfate. This was around Day +165 after CAR T cell therapy and a *BCR-ABL1* gene fusion was detected at this time prompting the resumption of ponatinib. Given the initial improvement she had with IVIG, the patient was restarted on IVIG at 1 g/kg every two weeks with the addition of weekly rituximab at 375 mg/m^2. The rituximab was discontinued after three doses because of severe thrombocytopenia, and IVIG was also discontinued after four doses because of persistent pain and thrombocytopenia. Throughout this period, the patient had evidence of B-cell aplasia as demonstrated by the absence of B cells by flow cytometry.

At Day +204, after only a brief period of symptom improvement, the patient redeveloped pain and stiffness in the neck, shoulders, bilateral upper and lower extremities interfering with her daily activities and ability to sleep. It was decided not to do plasma exchange given the possibility of removing persistent CAR T cells. Therefore, she was treated with two doses of obinutuzumab on Days +302 and +316 with significant improvement in symptoms. Although the plan was to dose obinutuzumab every six months, the patient had a flare of symptoms on Day +388 and received an extra dose earlier

than expected. The patient continues to require analgesics and has mild upper body stiffness, but has been able to resume activities of daily living. Ponatinib was switched to asciminib because of recurrent thrombocytopenia, and her disease remains in MRD negative remission.

Discussion

CAR T cell therapy has arisen as a highly effective therapy in hematologic malignancies with high response rates. More than 60 % of patients receiving CAR T cell therapy may experience neurologic toxicity with variable degrees of severity.[1] Patients with ALL are described as having a high rate of ICANS, with a reported incidence of 40–62 %.[2–4] ICANS can manifest as delirium, headache, language disturbance, tremor, transient focal weakness, behavioral disturbances, ataxia, peripheral neuropathy, visual changes, generalized weakness, seizures, and acute cerebral edema.[5–7] More severe cases of ICANS have been seen with severe CRS, suggesting a possible overlap between these two syndromes.[3,4,8] Proposed mechanisms of ICANS include endothelial activation and the upregulation of proinflammatory cytokines in the central nervous system.[9–11] Ideal management of these patients and long-term effects of ICANS are areas of active investigation.

Our patient's course has several unique aspects. On Day +6 after receiving CAR T cell therapy, she developed progressive severe bilateral lower extremity burning pain followed by paraparesis, symptoms not typically reported with this therapy. She was ultimately treated for CAR T cell therapy-related lumbosacral polyradiculopathy treated with corticosteroids and IVIG to which she had a positive response. However, after initial improvement, her symptoms recurred with severe back pain, and stiffness of her neck, shoulders and bilateral upper and lower extremities. Additional testing demonstrated GlyR antibodies within the patient's serum. Low titer GAD65 antibodies were also present, which have been described in patients with GlyR antibody syndromes.

GlyR autoantibodies have been described in patients with SPS, particularly the subtype progressive encephalomyelitis with rigidity and myoclonus (PERM). SPS (formerly stiff man syndrome) is a rare and disabling disorder characterized by truncal stiffness, muscle spasms, and impaired gait. Additional features of active denervation on EMG and CSF pleocytosis are atypical for SPS, and suggest possible PERM. However, the patient did not have other specific symptoms of PERM such as encephalopathy, brainstem features, or autonomic dysfunction.[12] Carvajal-González et al.[13] found that nine out of 52 cases of GlyR antibody syndrome had a diagnosis of malignancy at some point in their lives. The literature suggests a paraneoplastic incidence rate of 20 %, including in patients with thymoma, Hodgkin's lymphoma and cancers of the lung, kidney and breast. GlyR autoantibodies have been reported in patients with underlying malignancies including thymoma, B-cell lymphoma, Hodgkin's lymphoma, breast cancer, and small cell lung cancer.[14–16] IVIGs, plasma exchange and B-cell depletion using rituximab have been used to suppress the presumed causative antibody-mediated process.

The temporal relationship of our patient's symptoms with CAR T cell therapy point strongly towards a CAR T cell-mediated etiology, although the mechanism remains uncertain. The positive serological findings for GAD65 and glycine receptor antibodies could support a paraneoplastic etiology, such *As SPS*, however persistent remission and the timing of the emergence of symptoms shortly after CAR T cell therapy suggest an association with the therapy. A complication of CAR T cell therapy is B-cell aplasia and hypogammaglobulinemia and therefore the therapy has been under investigation for the treatment of autoimmune diseases. Hence, it is counter-intuitive that an autoimmune phenomenon develops post-treatment. It is possible that CAR T cells were exposed to certain antigens that led to antibody formation prior to development of B-cell aplasia, or that the threshold for the development of symptoms in the presence of pre-existing antibodies is lower. The etiology may also be cell-mediated secondary to immune dysregulation, cytokine release syndrome, and CAR T cell proliferation and persistence. In these scenarios, T cells are responsible for the immune response with subsequent activation of phagocytes, cytotoxic T cells and cytokine production.

We believe that CAR T cell-related autoimmune/inflammatory phenomena are a worthwhile consideration for future patients who may have unexplained neurological symptoms, especially as the long-term side effects remain uncertain. Importantly, treatment with corticosteroids and IVIG can be effective in such cases.

Conflicts of interest

The authors declare no conflicts of interest

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Case Report

Onset of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis triggered by sudden reactivation of asymptomatic latent Epstein-Barr virus infection

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory disease characterized by hypercytokinemia and immune-mediated injury of multiple organ systems. HLH is categorized into two subgroups. Primary HLH is an inherited immune disorder, whereas secondary HLH develops in various settings, such as infection, autoimmune disorder, and malignancy.[1] Epstein-Barr virus (EBV) is the most frequent cause of HLH associated with infection. EBV-associated HLH (EBV-HLH), like HLH due to other causes, is clinically characterized by fever, splenomegaly, and cytopenia with histologic evidence of hemophagocytosis, along with extremely high levels of serum ferritin and soluble interleukin-2 receptor (sIL-2R). EBV-HLH has a high rate of mortality, often resulting from multiple organ failure.[1,2] Immunomodulatory treatment, including etoposide, has demonstrated effectiveness in controlling EBV-HLH, although refractory cases require multiagent chemotherapy or hematopoietic stem cell transplantation.[1]

EBV-HLH can occur following primary EBV infection or as a result of EBV reactivation due to EBV-associated diseases, such as chronic active EBV disease (CAEBV) or certain malignancies.[2] However, it is not clear whether EBV-HLH can be caused by reactivation of asymptomatic latent EBV infections. Here, we report such a case involving a patient who

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developed EBV-HLH as a result of sudden reactivation of latent EBV infection.

Case report

A 36-year-old Japanese man who had been healthy all of his life was admitted to our hospital with a one-month history of fever and anorexia. Review of personal and family history revealed no signs of immunodeficiency. A peripheral blood count showed a white blood cell count of 2.0×10^9/L, a hemoglobin level of 13.6 g/dL, and a platelet count of 148×10^9/L. Morphologic analysis of peripheral blood showed 6.5 % atypical lymphocytes. Biochemical studies revealed hepatic dysfunction and significantly elevated levels of serum ferritin and sIL-2R (Table 1).

A contrast-enhanced computed tomography (CT) scan revealed enlarged mediastinal and abdominal lymph nodes, splenomegaly, and a space-occupying lesion in segment 6 of the liver. A positron emission tomography-CT (PET-CT) scan showed pathologic uptake of ^{18}F-fluorodeoxyglucose (FDG) in mediastinal and abdominal lymph nodes, spleen, liver lesion, and systemic bone (Figure 1). Examination of the bone marrow aspirate by May-Grünwald-Giemsa staining showed mild hemophagocytosis but no abnormal cells. Flow cytometry analysis of bone marrow cells revealed no neoplastic cell populations, and immunoglobulin heavy chain and T-cell receptor beta chain rearrangement studies detected no rearrangement bands. After admission to our hospital, the patient had a persistent high fever and showed signs of exacerbated pancytopenia and liver dysfunction (Table 1). He met all eight clinical and laboratory diagnostic criteria outlined in the HLH-2004 diagnostic and therapeutic guidelines for HLH and was diagnosed with secondary HLH.

A variety of conditions can cause secondary HLH, including infection, autoimmune disorder, and malignancy. There were no clinical or laboratory findings to suggest the presence of autoimmune disease in this patient. We conducted further evaluations based on EBV-related assays. Antibody titers for EBV viral capsid antigen (VCA) IgG, VCA IgM, early antigen (EA) IgG, and nuclear antigen were 1:320, <1:10, 1:10, and 1:80, respectively. Levels of EBV DNA in plasma (4.85 log IU/mL) and in whole blood (5.48 log IU/mL) were significantly elevated. EBV clonality was not detected by terminal repeat analysis of the EBV genome. Percutaneous biopsy of the segment 6 liver mass revealed a proliferation of CD68-positive epithelial histiocytes (i.e., activated macrophages) accompanied by small lymphocytes. Most of the lymphocytes that clustered with these histiocytes were T cells, with a predominance of CD8-positive T cells; EBV-encoded early RNA (EBER)-positive cells were also present (Figure 2). Bone marrow biopsy confirmed nearly the same histology. These pathologic findings were consistent with HLH. On the basis of these results, together with the findings of EBV activation, the patient was diagnosed with EBV-HLH.

His general condition deteriorated rapidly, including impaired consciousness. Levels of serum ferritin (20,064.4 ng/mL) and sIL-2R (28,114.0 U/mL) were markedly elevated (Table 1). Serum interferon-gamma levels, which are considered to play a particularly key role in the cytokine storm in HLH, also were strikingly high at 50 IU/mL (reference range, ≤ 0.1 IU/mL).[1] We started pulse steroid therapy on admission Day 10, but the treatment was unsuccessful. A reduced dose of SMILE (steroid dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide) chemotherapy was administered as salvage therapy. One week after the start of SMILE therapy, the patient's overall condition began to improve. After completion of the first course of SMILE therapy, his liver function, sIL-2R level, and ferritin level improved significantly, and his general condition demonstrated a complete recovery. A CT scan of the chest and abdomen revealed significantly decreased sizes of the previously enlarged lymph nodes and liver mass.

Because the reduction of the EBV DNA load in the blood after two cycles of SMILE therapy was insufficient (from 5.48 log IU/mL to 3.48 log IU/mL), the patient underwent allogeneic hematopoietic stem cell transplantation from a human leukocyte antigen–matched unrelated donor. The patient has since maintained a stable condition and achieved an undetectable level of EBV DNA.

Table 1 – Laboratory findings of the patient.

	Days after hospitalization				Reference range
	1	6	8	10	
WBCs ($\times 10^9$/L)	2.00	1.06	1.31	2.41	3.30–8.60
Neutrophils ($\times 10^9$/L)	1.290	0.763	0.983	2.085	
Lymphocytes ($\times 10^9$/L)	0.650	0.170	0.183	0.133	
Hb (g/dL)	13.6	11.2	10.8	7.7	13.7–16.8
Platelets ($\times 10^9$/L)	148	85	59	50	158–348
T-bil (mg/dL)	0.70	0.91	1.33	0.99	0.40–1.50
AST (U/L)	83	199	506	451	13–30
ALT (U/L)	57	158	554	449	10–42
LDH (U/L)	735	786	908	1,308	124–222
CRP (mg/dL)	1.65	3.34	3.94	7.79	0.00–1.14
Ferritin (ng/mL)	1,583.7	N/A	11,162.5	20,064.4	14.4–303.7
sIL-2R (U/mL)	8,847.0	N/A	N/A	28,114.0	122.0–496.0

WBC: white blood cell; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CRP: C-reactive protein; Hb: hemoglobin; LDH: lactate dehydrogenase; N/A: not available; sIL-2R: soluble interleuikin-2 receptor; T-bil: total bilirubin.

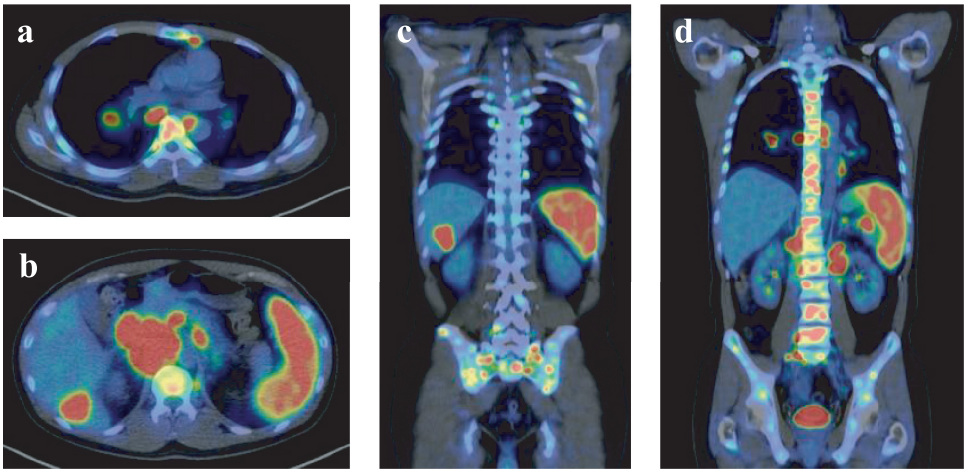

Figure 1 – Positron emission tomography-computed tomography demonstrated pathological ^{18}F-fluorodeoxyglucose uptake in mediastinal lymph nodes (a, d), abdominal lymph nodes (b, d), spleen (b, c, d), liver mass (b, c), and systemic bone (a, b, c, d).

Discussion

We report here a case involving sudden onset of EBV-HLH triggered by reactivation of latent EBV infection. The clinical course of this patient included no features suggesting the presence of any EBV-related disease, such as CAEBV or an associated malignancy, including malignant lymphoma. Serologic testing did not reveal high antibody titers for EBV VCA IgG or EA IgG, which are seen in most cases of CAEBV. Antibody titers for EBV VCA IgG, VCA IgM, and nuclear antigen were 1:320, <1:10, and 1:80, respectively, suggesting latent EBV infection.[3] Additionally, the positive finding of EA IgG antibodies with a low titer (1:10) suggested reactivation of latent EBV infection.

EBV-HLH is known to occur following primary EBV infection or as a result of reactivation due to EBV-related diseases, such as CAEBV or certain malignancies, and is most common among children and young adults.[2] It is not clear whether EBV-HLH can result from asymptomatic latent EBV infection in the absence of immunodeficiency. However, the present case is considered to represent EBV-HLH due to a reactivation of latent EBV infection. Some reported cases of adult-onset EBV-HLH include previously healthy individuals whose condition is unlikely to have resulted from primary EBV infection, CAEBV, or EBV-related malignancies.[4,5] There is a report of nine cases of previously healthy adults with prior EBV infection who experienced a rapid clinical course with fever, pancytopenia, and hepatic dysfunction; it was proposed that the condition seen in these cases be named progressive adult-onset EBV lymphoproliferative disorder, given their poor prognoses, although no clonal lymphocytes were identified.[6] We hypothesize that these prior cases may involve EBV-HLH due to a sudden reactivation of latent EBV infection. While the precise mechanism underlying the sudden onset of HLH from latent infection remains unclear, it has been suggested that EBV latent membrane protein 1 (LMP-1) specifically inhibits the expression of signaling lymphocytic activation molecule-associated protein (SAP).[7] This inhibition could potentially create an immunological deficiency similar to that observed in XLP1-associated primary HLH. These findings suggest that certain triggers in patients with latent EBV infection, even without underlying immunodeficiency, might induce an immunodeficient state reminiscent of that seen in primary HLH.[8]

The histology of the liver biopsy in the present case showed no evidence of malignant lymphoma but rather a proliferation of activated macrophages (epithelial histiocytes) accompanied by EBER-positive EBV-infected small lymphocytes. This histologic finding was consistent with the pathologic finding of EBV-HLH, in which EBV-infected lymphocytes activate macrophages and epithelial histiocytes replace normal tissue.[9] It is not clear which lymphocyte subpopulation was infected with EBV; however, it is likely that EBV-infected lymphocytes were CD8-positive T cells, as the lymphocytes seen around the histiocytes were mostly T cells (specifically, predominantly CD8-positive T cells).

In adult-onset EBV-HLH, it is important to distinguish between non-neoplastic EBV-HLH and HLH secondary to EBV-related malignancy (primarily malignant lymphoma), as the prognosis and treatment strategies differ between these conditions.[2] PET-CT scans are used to evaluate for the presence of malignancy, including malignant lymphoma. In non-neoplastic HLH, however, activated macrophages show high FDG uptake on PET-CT scans, similar to that seen in malignant lymphoma. Therefore, histologic examination is required for the accurate differential diagnosis of HLH. However, the rapid progression of EBV-HLH often results in insufficient time and/or medical conditions for obtaining a biopsy, and treatment must often be initiated on the basis of a clinical diagnosis. In the context of this challenging clinical diagnosis, it is important to recognize that EBV-HLH can suddenly develop from latent EBV infection; in the absence of this understanding, the diagnosis may be missed in some cases of EBV-HLH.

To date, there is no standard treatment for EBV-HLH in adults. The HLH-94/2004 treatment regimens, based on the protocols of international collaborative studies in HLH, consist of dexamethasone, cyclosporine, and etoposide and are most commonly used to suppress hyperinflammation, but relapse is often observed. For relapsed or refractory EBV-HLH,

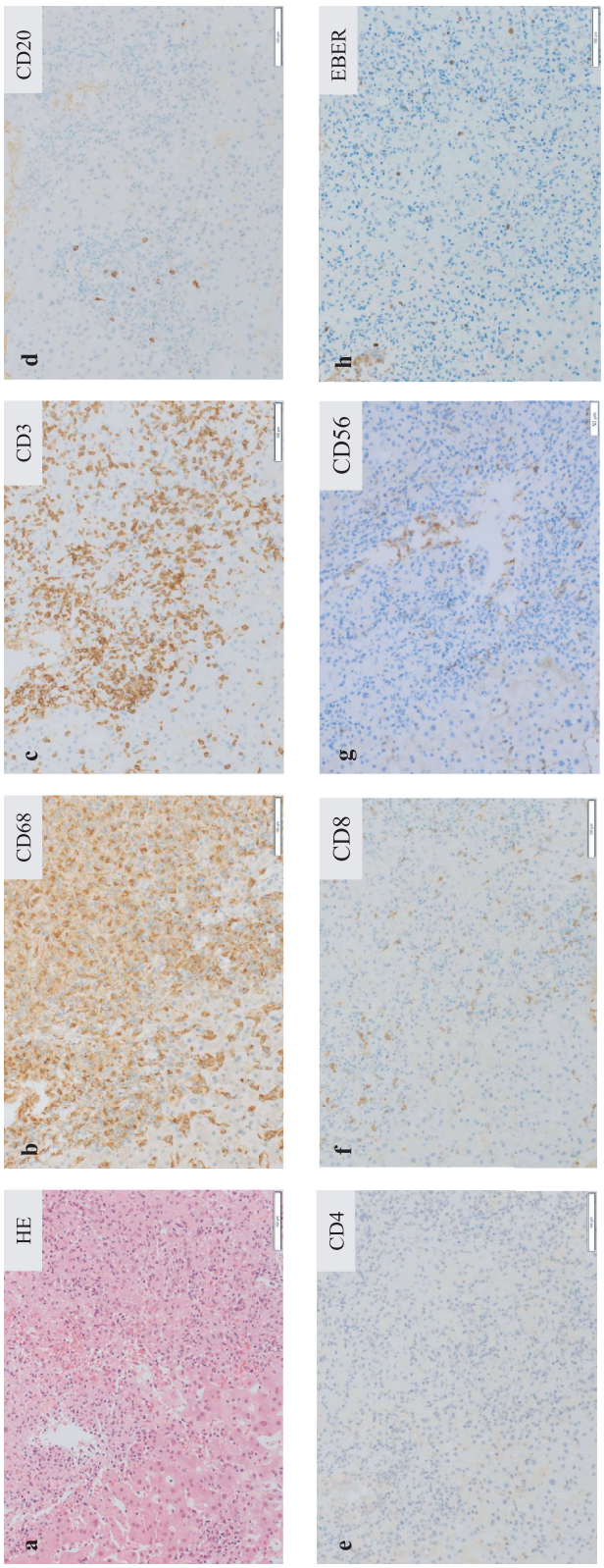

Figure 2 – Histopathological findings of liver mass biopsy were consistent with hemophagocytic lymphohistiocytosis. Hematoxylin and eosin stain (a), immunohistochemical stain for CD68 (b), CD3 (c), CD20 (d), CD4 (e), CD8 (f), CD56 (g), and in-situ hybridization for Epstein-Barr virus-encoded early RNA (h). HE: hematoxylin and eosin; EBER: Epstein-Barr virus-encoded early RNA.

combination chemotherapy and hematopoietic stem cell transplantation are required.[1,2] In the present case, the patient's general condition rapidly deteriorated, with impaired consciousness two weeks after the onset of the disease; combination chemotherapy was selected after implementing pulse steroid therapy. As EBV-HLH is known to be caused by EBV-infected T cells or natural killer cells, we administered a reduced dose of SMILE therapy, which has demonstrated its effectiveness in natural killer/T-cell lymphoma, and achieved a successful outcome. Other cases involving successful SMILE therapy in EBV-HLH have also been reported, and this therapy can thus be considered a treatment option for EBV-HLH.[10]

Conclusion

This case provides a significant clinical insight into EBV-HLH in adults. We demonstrated that EBV-HLH can develop from latent EBV infection even in immunocompetent adults without a history of CAEBV or EBV-associated malignancy. This finding expands our understanding of the pathogenesis of EBV-HLH and suggests the need for careful consideration of this diagnosis in adults presenting with HLH symptoms, regardless of their EBV-related medical history. This observation contributes to our understanding of the clinical spectrum of EBV-HLH and may help improve the early recognition of this life-threatening condition in adult patients.

Informed consent

Informed consent was obtained from the patient for publication of this case report.

Author contributions

MM and SU drafted the manuscript. All authors were involved in the diagnosis, treatment, and follow-up of the patient, critically revised the manuscript, read, and approved the current version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Letter to the Editor

Challenges in diagnosing thrombotic thrombocytopenic purpura

Thrombotic thrombocytopenic purpura (TTP) is a medical emergency necessitating rapid therapeutic intervention to prevent mortality. This rare hematologic condition is characterized by a deficiency in the ADAMTS13 enzyme (a disintegrin and metalloprotease with thrombospondin type motifs, member 13) [1]. ADAMTS13 is a metalloprotease which functions to cleave ultra-large von Willebrand factor (UL-VWF) multimers. Without ADAMTS13, these UL-VWF multimers bind to circulating platelets, resulting in the formation of microthrombi in arterioles and capillaries, leading to end-organ ischemia and hemolysis due to shearing of red blood cells (RBCs) as they transverse these microthrombi [2].

A deficiency in ADAMTS13 is most commonly due to autoantibodies which result in functional inhibition and/or accelerate the clearance of the ADAMTS13 protein from plasma (immune-mediated TTP - iTTP). However, in a minority of cases (<10 %), mutations in the *ADAMTS13* gene, cytogenetically located on chromosome 9q34.2, result in the inability to produce the ADAMTS13 protein or lead to the production of a dysfunctional enzyme [3]. In the case of abnormal or absent ADAMTS13 production secondary to genetic mutations, the condition is known as congenital TTP (cTTP) or Upshaw-Schulman syndrome.

In contemporary assays, ADAMTS13 activity is significantly reduced in patients with cTTP and iTTP. As such, it is not possible to differentiate these conditions by ADAMTS13 activity alone. However, this distinction is important, as the therapeutic, prophylactic, and prognostic characteristics differ. Moreover, there are instances where ADAMTS13 activity may be falsely low, or even undetectable, due to interferences in laboratory assays. Therefore, a nuanced discussion of the differences in cTTP and iTTP, and the assays used to diagnose the specific condition are warranted. We believe the importance of these nuances are particularly highlighted by a recent case of purported iTTP presented by Martins de Oliveira Filho et al. [4] In this report, the authors described a 50-year-old male with systemic lupus erythematous who was initially treated for immune thrombocytopenic purpura (antibody-mediated platelet destruction). The patient did not respond to corticosteroids, and further evaluation demonstrated evidence of thrombocytopenia and microangiopathic hemolytic anemia. The authors stated that "ADAMTS13 activity was undetectable, confirming a diagnosis of acquired thrombotic thrombocytopenic purpura"; while not incongruent with a diagnosis of iTTP, we believe that the information provided to the readers is insufficient to 'confirm' a diagnosis of iTTP. Further, we believe that this case illustrates the difficulties in confirming a diagnosis of iTTP, contributes to discrepancies in the literature, and precludes the ability to perform accurate epidemiological studies.

As mentioned above, both cTTP and iTTP are associated with low to undetectable (generally <10 %) ADAMTS13 activity. However, to definitively diagnose iTTP, an assessment for the presence of an autoantibody against ADAMTS13 should be performed. While not all patients with iTTP will have a detectable autoantibody, especially at initial presentation [5], the absence of such should evoke suspicion of cTTP wherein antibodies are absent [6]. As such, without the identification of an autoantibody, genetic testing should be performed to exclude mutations in the *ADAMTS13* gene. Given that the authors did not report an antibody, nor did they assess for genetic mutations, this case cannot be considered a 'confirmed' case of iTTP. While both iTTP and cTTP can present similarly with thrombocytopenia, hemolytic anemia, and end-organ ischemia, iTTP requires immunosuppression (corticosteroids, rituximab) to eliminate the inhibitor, plasma exchange to further reduce the acute effects of the inhibitor and UL-VWF and replenish the ADAMTS13 enzyme, and in many cases, an adjunct agent, caplacizumab, is used to further reduce microthrombi formation by inhibiting VWF-platelet binding [7] Conversely, cTTP usually only requires plasma infusion to replete the deficient ADAMTS13. Notably, recombinant ADAMTS13 has been approved in both Europe and the US, the use of which eliminates the risks inherent to exposure to donor plasma [8].

Secondly, it is important to mention the assays currently in use to evaluate ADAMTS13 activity, and how these assays may give a decreased result that is not due to an autoantibody

or genetic mutation. At present, the most commonly used assays are based on either fluorescence resonance energy transfer (FRET) or enzyme linked immunosorbent assay (ELISA) technologies utilizing recombinant VWF substrates [9]. A variety of factors can interfere with these assays; for example, hyperlipemia, elevated plasma hemoglobin, or hyperbilirubinemia may interfere with fluorescence-based assays [10]. Free hemoglobin and bilirubin may also directly inhibit the ADAMTS13 enzyme, while other plasma proteases may interfere with VWF cleavage or degrade ADAMTS13 [10].

While iTTP has a greater prevalence than cTTP, if comprehensive evaluation is not undertaken (or reported), it is difficult to draw conclusions from the presented findings. Many patients present with low or inconclusive inhibitor results on initial presentation and must be followed to determine antibody status. Further, although many patients with cTTP present early in life, a subset do not develop overt disease until advanced age [11]. Given these considerations, in patients with suspected iTTP, it is important to thoroughly assess for not only ADAMTS13 activity but also autoantibodies; if the latter are not detected, further testing for cTTP is warranted. It is also important to understand the assay methodology for ADAMTMS13 activity, and the potential limitations and interferences, to ensure an accurate diagnosis.

Contribution statement

JWJ: Reviewed the literature, drafted the manuscript, approved the final version. **GSB:** Revised the manuscript, approved the final version. **BDA:** Revised the literature, revised the manuscript, approved the final version.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Letter to the Editor

Cutaneous T-cell lymphomas may require an exception to the ABHH consensus regarding empiric vancomycin use in febrile neutropenia

Dear Editor,

We read with great interest the recently published guidelines "Management of febrile neutropenia: consensus of the Brazilian Association of Hematology, Hemotherapy and Cell Therapy - ABHH" by Nucci et al. [1]. We fully support the overall recommendations, particularly the more conservative approach to the use of vancomycin as part of the empiric antibiotic regimen, which is well justified by recent epidemiological evidence [2–5]. However, we would like to highlight a specific subgroup of patients who, in our view, should be considered an exception to the general recommendation against routine empirical anti-MRSA coverage: patients with advanced-stage cutaneous T-cell lymphomas (CTCL), particularly those with Sézary syndrome or extensive mycosis fungoides.

As noted in several studies, these patients have a significantly higher risk of skin and bloodstream infections caused by Staphylococcus aureus, including methicillin-resistant strains (MRSA) with this being one of the main causes of death [6–8]. The combination of profound immune dysregulation, extensive skin barrier disruption, and frequent colonization with S. aureus places these patients at a distinctively high risk of infections, which may progress rapidly to sepsis and death [9,10]. Additionally, the epidemiological studies cited in the guidelines to support the recommendation against empirical anti-MRSA coverage do not include a sufficient representation of patients with CTCL [2–5], making it difficult to extrapolate findings for this population.

Given these considerations, we suggest that advanced-stage mycosis fungoides and Sézary syndrome patients should be explicitly recognized as a subgroup that may warrant empirical anti-MRSA coverage in cases of febrile neutropenia until further studies focusing on this specific population bring additional valuable information to optimize their management.

Author Contribution

Yung Gonzaga conceived and wrote the paper. **Jose A. Sanches** wrote the paper.

Conflicts of interest

Yung Gonzaga received honoraria and speakers' fees from Takeda. Jose A. Sanches received honoraria and speakers' fees from Takeda.

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Letter to the Editor

Closing the gaps: Tackling myeloma inequities in Latin America

Dear Editor,

Multiple myeloma (MM), a malignancy of plasma cells, poses a significant global health challenge, characterized by disparities in incidence, treatment access, and outcomes. Advances in MM therapies have significantly improved survival rates globally, but inequities in care and treatment outcomes remain particularly pronounced in Latin America.[1–3]

The global burden of MM is shaped by genetic, environmental, and demographic factors. Latin America faces higher rates of late-stage diagnoses compared to wealthier regions due to limited awareness and inadequate screening initiatives. These disparities are especially evident in Indigenous and underserved populations, reflecting systemic health inequities.[2–4] The projected rise in MM cases highlights the urgent need for interventions to address these gaps.[4]

Social determinants of health

Social determinants of health (SDOH) play a critical role in shaping MM outcomes. Socioeconomic status, education, and geographic location influence access to timely diagnosis and effective treatment. Patients from lower socioeconomic backgrounds are more likely to experience delayed diagnoses and poorer survival outcomes.[2,3] Rural populations, in particular, face challenges in accessing specialists and advanced diagnostic tools, exacerbating these disparities[3,5,6]

Healthcare systems in Latin America are fragmented, leading to significant disparities between public and private sectors. Many facilities lack access to critical diagnostic tools, including next-generation sequencing and cytogenetics, which are essential for precise risk stratification. Advanced treatment options, such as autologous stem cell transplantation (ASCT) and proteasome inhibitors, remain inaccessible to many patients due to resource constraints.[3,4,5]

Financial barriers are a significant challenge for MM patients in Latin America. High costs of novel therapies combined with limited insurance coverage force many patients to opt for suboptimal care or forgo treatment altogether. Policies aimed at expanding insurance coverage and subsidizing treatment are crucial to alleviating financial toxicity.[2,6,7]

Timely initiation of treatment is a key determinant of MM prognosis. However, logistical challenges such as referral bottlenecks, lack of infrastructure, and delays in diagnosis contribute to poorer outcomes for patients in the region.[3,5,6]

MM outcomes in Latin America are poorer than in high-income countries due to delayed diagnoses, limited treatment availability, and systemic socio-economic inequities. Closing these gaps through targeted interventions, improved healthcare access, and addressing SDOH is critical to improving patient survival and quality of life.[1,2,4]

Strategies to address disparities

Investing in healthcare infrastructure and fostering equitable access to MM therapies should be priorities for policymakers in Latin America. Collaborative efforts among governments, non-governmental organizations, and international stakeholders are essential for reducing resource gaps and promoting capacity building.[3,4]

Community engagement is vital for reducing health disparities. Awareness campaigns tailored to cultural and regional contexts can improve early detection and encourage treatment adherence. The integration of community health workers into healthcare teams has proven effective in expanding access to underserved populations.[5–7]

Expanding research initiatives in Latin America is essential for understanding region-specific challenges and developing evidence-based interventions. Establishing regional MM registries can provide valuable data on disease patterns, treatment efficacy, and healthcare disparities, enabling targeted policy decisions.[4,6]

Conclusion

Health disparities in MM care across Latin America highlight systemic inequities rooted in socio-economic and healthcare

barriers. Addressing these challenges requires a multifaceted approach involving policy reforms, community-based strategies, and enhanced research efforts. By prioritizing health equity, stakeholders can ensure that advancements in MM care benefit all patients, irrespective of their geographic or socio-economic circumstances.

Conflicts of interest

The author declares no conflicts of interest.

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Letter to the Editor

Multi-cohort gene expression model enhances prognostic stratification in diffuse large B-cell lymphoma

Dear Editor,

Diffuse large B-cell lymphoma (DLBCL), the most common type of lymphoma, in most cases is marked by significant heterogeneity and aggressive clinical behavior. While standard chemotherapy often achieves initial responses, these are short-lived, and resistance and relapse are frequent challenges.[1] Traditionally, risk stratification has relied on clinical tools, including the International Prognostic Index (IPI) and its variation.[2] However, molecular stratification is promising to predict outcomes with greater accuracy, though gene-based approaches are still preliminary.[3] Progress in this field is hindered by limited sample sizes and the substantial intra- and inter-regional variability of DLBCL.[4,5] Consequently, large-scale studies are essential to refine risk stratification and optimize patient outcomes.

This study aimed to establish a prognostic gene expression signature for patients with DLBCL based on tumor transcriptome patterns. To achieve this, we analyzed transcriptome and survival data from 11 diverse cohorts worldwide. Given the variability in RNA sequencing or microarray platforms across the 11 datasets, we focused on the genes common to all datasets, resulting in a panel of 11,425 genes. Detailed information regarding the datasets can be found in Supplementary Table 1. Due to platform-specific differences in scale, the gene expression values were transformed into z-scores. Datasets with fewer than 100 patients were combined into a cohort referred to as the Merged Cohort. In total, six cohorts were used in this study: the National Cancer Institute Cohort (GSE10846), University of York Cohort (GSE181063), University of York II Cohort (GSE32918), Universitätsmedizin Berlin Cohort (GSE4475), University of Leeds Cohort (GSE69053), and the Merged Cohort (GSE69053, E_TABM_346, GSE11318, GSE21846, GSE23501, GSE57611, and TCGA-DLBC).

For each cohort, a univariate Cox regression was performed employing all genes in the panel, identifying those with a p-value <0.05 as prognostic. Genes were defined as core prognostic genes (CPGs) if they consistently predicted either favorable prognosis in at least 5 out of 6 cohorts or unfavorable prognosis in at least 5 out of 6 cohorts, with no conflicting outcomes.

This process led to the identification of 50 CPGs. To mitigate the risk of overfitting, a penalized Cox regression was applied using the Least Absolute Shrinkage and Selection Operator (Lasso-Cox), thereby allowing for the selection of only the most significant CPGs. The University of York cohort had the largest number of patients and was therefore used to train the Lasso-Cox model, while the other cohorts were used for validation. The final risk score was developed based on the expression levels of 22 CPGs selected through the Lasso-Cox regression (Figure 1A). The formula for calculating the risk score is as follows:

$$\text{Risk Score} = (\beta 1 \times \text{Gene1}) + (\beta 2 \times \text{Gene2}) + \ldots + (\beta 22 \times \textit{Gene22})$$

where 'βX' represents the coefficients derived from the Lasso-Cox regression, and 'GeneX' refers to the z-score of the expression of each gene for a given sample. The list of selected genes and their corresponding coefficients can be found in Supplementary Table 2.

Patients were then divided into High Risk (> median) and Low Risk (≤ median) Groups based on the risk score. Survival analysis using Kaplan-Meier curves was conducted, revealing that the developed risk groups were significant predictors of overall survival in all cohorts (Figure 1B). Additionally, the risk score demonstrated high predictive accuracy, achieving great (≥0.69) areas under the receiver operating characteristic curve (AUC) across all cohorts (Figure 1C). By pooling the hazard ratios (HR) from the cohorts using a random effects model, the HR for death of being in the High Risk Group was 2.73 (range: 2.43–3.05; Figure 1D), further validating the risk score as a strong predictor of survival.

To ensure the prognostic value of the risk groups, even when assessed alongside clinical data, we conducted multivariable Cox regressions for each cohort. The results demonstrated that the risk groups remained strong predictors of survival. Figure 2A presents the clinical characteristics of the cohorts analyzed in this study, along with the results of the multivariate Cox regression analysis.

To integrate the established risk groups with other clinical variables, we developed a nomogram (Figure 2B) using a meta-cohort of patients who provided complete information on sex,

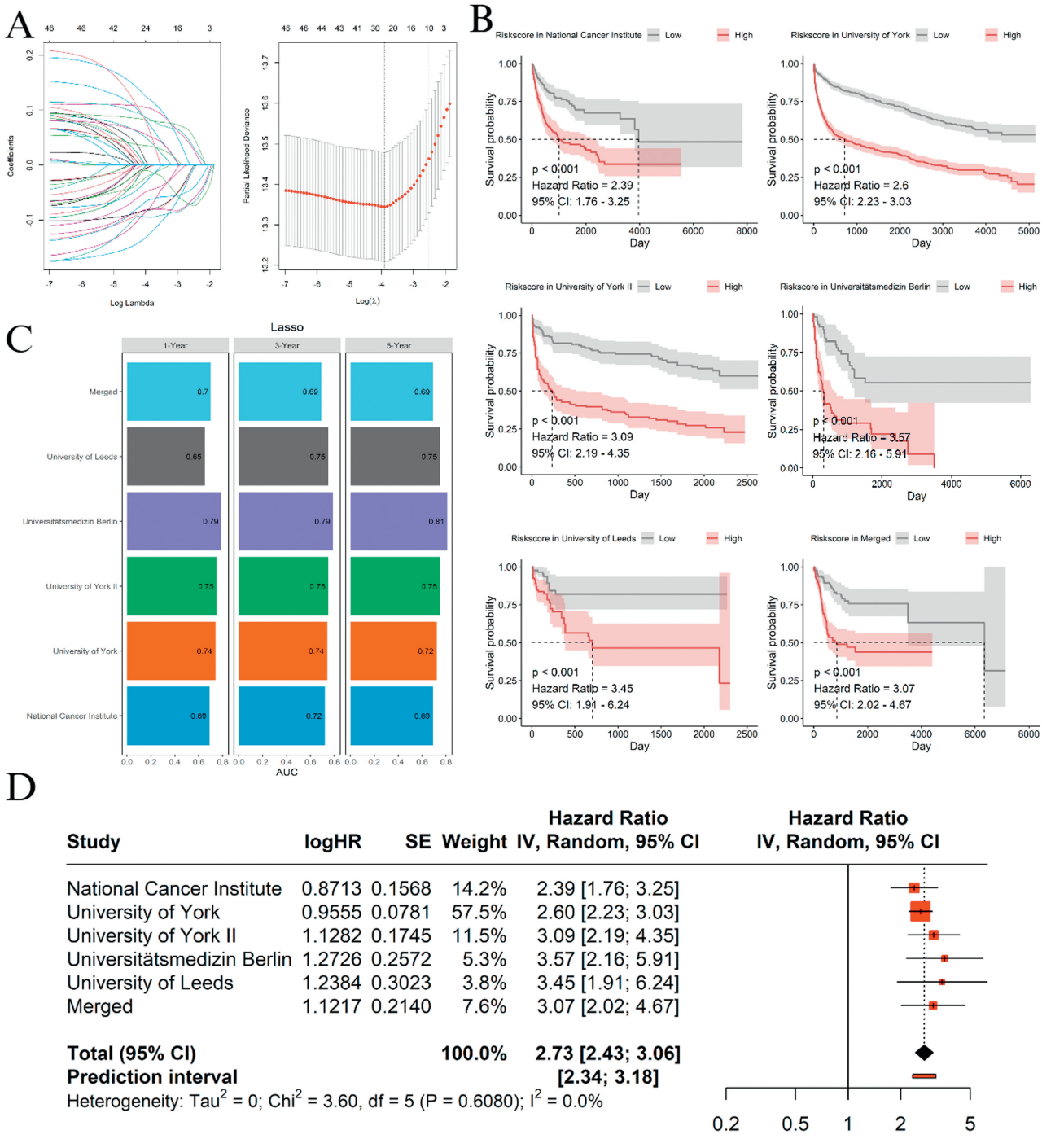

Figure 1 – A: least absolute shrinkage and selection operator penalized cox regression feature selection. B: Kaplan-Meier analysis of the different cohorts used in this study comparing Low Risk to High Risk Groups. C: Area under the receiver operating characteristic curve (AUC) for the model in different cohort and time-point evaluations. D: Pooled analysis of the hazard ratio of being in the High Risk Group.
Lasso: least absolute shrinkage and selection operator.

age (over 65 years or 65 years and younger), and DLBCL subtype (germinal center B-cell-like, activated B-cell-like, molecular high-grade B-cell lymphoma, and unclassified), comprising a total of 2102 patients. The nomogram showed an excellent AUC for survival prediction at 1, 3, and 5 years (Figure 2C) and generated survival predictions that closely matched observed outcomes as determined by the calibration plot (Figure 2D). Moreover, the nomogram attained the highest c-index for survival prediction when compared to risk groups and clinical variables alone (Figure 2E). A free online platform has been developed and made accessible at https://costafilhoetal.shinyapps.io/CoreProgDLBCL/ to enhance the applicability of the nomogram.

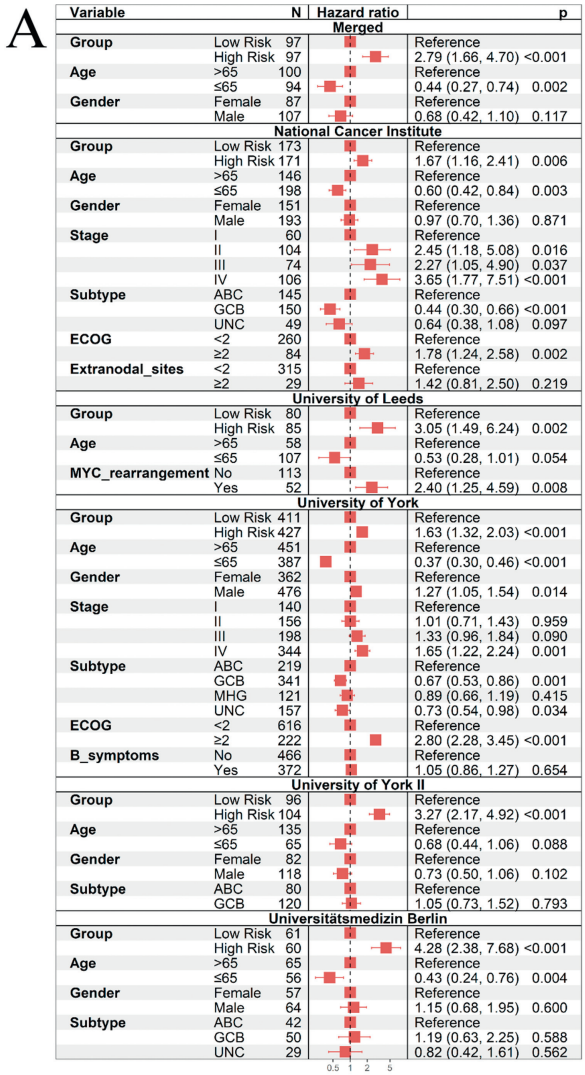

Figure 2 – A: Multivariate cox-regression analysis of the risk groups and other available clinical information. B: Nomogram integrating our risk groups with clinical information. E: Comparison of the concordance index of our model and other variables. F: Gene set enrichment analysis plot comparing high-risk and low-risk groups in the University of York cohort. GCB: Germinal center B-cell-like; ABC: Activated B-cell-like; MHG: Molecular high-grade B-Cell lymphoma; UNC: Unclassified.

We performed a Gene Set Enrichment Analysis (GSEA) using raw data from the University of York cohort and the Hallmark of Cancer gene sets from the Molecular Signatures Database (MSigDB) to better understand the biological processes distinguishing the risk groups. Notably, the GSEA (Supplementary Table 3) results revealed that the High Risk Group was predominantly enriched for E2F targets, MYC targets, and G2M checkpoint pathways, while showing downregulation of inflammatory response, interferon-gamma response, and epithelial-mesenchymal transition pathways (Figure 2F).

This study introduces a promising approach to prognostic stratification in DLBCL, utilizing gene expression data to identify CPGs and develop a validated risk score. While the IPI and its variations remain widely used for stratification in DLBCL, their discriminative power is often limited, with various studies reporting suboptimal overall survival prediction when used alone.[6,7] Nonetheless, the European Society for Medical Oncology (ESMO) currently endorses age-adjusted IPI, which has a reported c-index of 0.613, for stratifying under 60-year-old patients who may benefit from involved-field radiotherapy or autologous stem-cell transplantation.[7]

Furthermore, neither the ESMO nor the National Comprehensive Cancer Network guidelines have incorporated transcriptomic and exome stratification in patient management.[8,9] By outperforming traditional approaches focused on histopathology, our model was able to refine risk stratification by integrating precision oncology and shows promise in aiding treatment decisions, addressing the urgent need for improved stratification in a context where 30–50 % of DLBCL patients are not cured by standard chemotherapy.[10] In conclusion, our global multi-cohort study represents a significant advancement in the prognostic stratification of DLBCL. The integration of this model with clinical variables enabled the development of an accurate nomogram for survival prediction. Future studies should aim to validate this model in large prospective cohorts and explore its integration into clinical practice to enhance patient outcomes.

Conflicts of interest

The author declares no conflicts of interest

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.htct.2025.103847.

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Letter to the Editor

Digital polymerase chain reaction enhances analysis of the cKIT D816V mutation in systemic mastocytosis patients: Clinical insights

Systemic mastocytosis (SM) is a rare disease with heterogeneous presentation and the clonal proliferation of mastocytes.[1–3] Over 90 % of cases are well-marked with a molecular signature of the KIT D816V gene mutation.[4–6] However, detecting this mutation depends on the methodology applied, which, to date, lacks standardization, particularly for diagnostic purposes.[6] Digital polymerase chain reaction (dPCR) is a promising molecular instrument for analyzing low allele burden disease due to its high sensitivity and the absence of a requirement for calibration to achieve quantification. Therefore, many authors advocate its use in SM research and the clinical practice.

The objective of this study was to investigate the correlation between dPCR of the KIT D816V gene mutation and serum tryptase levels, as well as the clinical involvement of disease assessed using the Mastocytosis Activity Score (MAS) and the clinical subtype of SM.

This study involved a prospective analysis of patients diagnosed with SM who were followed-up as outpatients at Hospital das Clínicas, Medicine Faculty of the University of São Paulo between January 2019 and December 2021.

Peripheral blood samples were collected to measure serum tryptase levels using the immunofluorometric method (reference value: up to 14 ug/L), and synchronously genomic DNA was extracted using the QIAamp DNA Blood Midi Kit for the evaluation of the KIT D816V gene mutation via dPCR. The analysis was conducted using the Taqman LiqBiopsy Digital Hs0000000039_rm assay on the QuantStudio3D platform. Each sample was tested twice, with variant allele frequency levels near 0.1 % tested in triplicate. A clinical evaluation and adapted MAS through a one-day interview were conducted by one assistant. SM subtype classification followed 2022 World Health Organization guidelines.[2]

Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki and the protocol was approved by the institutional review board (CAAE number, 29975120.7.0000.0068).

The statistical analysis considered the KIT D816V mutation as the main variable, with serum tryptase levels, MAS and SM subtype as explanatory variables. Fisher's exact test compared categorical variables by SM subtype, while Pearson's correlation analyzed gene mutation and continuous variables. Normal distribution was assessed by the Shapiro-Wilk test, and a significance of 5 % was applied to discriminate significant results.

Out of nineteen patients assessed, 31.6 % were classified as having the aggressive subtype, while 68.4 % had the indolent subtype. Additionally, in the aggressive subtype, four out of six were in cytoreductive treatment. Serum tryptase levels were available for 16 patients, with a median of 61.3 μg/L (range: 16.4–200 μg/L). The KIT D816V mutation was detected in 15 out of 19 patients (median: 1.80; range: 0.14–6.79). Additionally, the median of MAS was 12 (range: 0–27).

A significant association was observed between the clinical subtype of SM and serum tryptase levels (p-value = 0.034 - Table 1). Higher values were noted in the aggressive subtype (median: 108.1 μg/L; quintile = 53.8 μg/L) compared to the indolent subtype (median: 51.2 μg/L; quintile = 42.3 μg/L). Furthermore, a modest correlation was found between the KIT D816V mutation and serum tryptase levels (correlation coefficient = 0.52; 95 % confidence interval: 0.03 to 0.08; *p*-value = 0.038 - Table 2).

A significant association was observed between the KIT D816V mutation and the clinical subtype of SM (*p*-value = 0.02) with higher values noted for the aggressive subtype (median: 3.52; quintile = 2.46) compared to the indolent subtype (median: 1.20; quintile = 1.00). Additionally, a weak correlation was found between the KIT D816V mutation and MAS (correlation coefficient = 0.45; 95 % confidence interval: −0.004 to −0.751; *p*-value = 0.052).

Table 1 – . Association between serum tryptase, the KIT D816V mutation and mastocytosis activity score (MAS) stratified by systemic mastocytosis subtype.

	Aggressive subtype	Indolent subtype	*p*-value
	n = 6	*n* = 13	
Serum tryptase (μg/L) median (quintile)	108.1 (53.9)	51.3 (42.3)	0.034[a]
KIT D816V mutation median (quintile)[b]	3.52 (2.46)	1.20 (1.00)	0.020[a]
Adapted MAS median (quintile)	11.5 (4.4)	9.2 (8.6)	0.555

[a] Statistical significance (Fisher exact test <0.05).
[b] Only those with measurable mutation (*n* = 15) were included.

Table 2 – Correlation between the KIT D816V mutation with mastocytosis activity score (MAS) and serum tryptase.

	KIT D816V mutation correlation	95 % confidence interval	*p*-value
Adapted MAS	0.45	−0.004 to −0.751	0.052
Serum tryptase	0.52	0.351 to 0.808	0.038[a]

[a] Statistical significance (Pearson's correlation test <0.05).

This study revealed a mutation positivity rate of 78.9 % (15 out of 19 patients) using dPCR specific for the D816V locus of the *KIT* gene. The remaining four patients may harbor other mutations (such as V560G, D815K, D816Y, D816F, D816H and D820G) within the same gene, which are found in nearly 5 % of SM cases.

As highlighted before, conventional methods for diagnosing the KIT D816V mutation lack sensitivity. Given its ability to analyze low tumor burden diseases, dPCR has emerged as a promising technique due to this challenge. In a comparison with quantitative PCR, Greiner et al. found a concordance rate of 96 %, indicating non-inferior performance between these two methods for KIT D816V analysis.[7]

The literature currently lacks consensus regarding the optimal sample for detecting the D816V mutation gene. Notably, Greiner et al.[7] observed a slight advantage for bone marrow samples compared to peripheral blood samples, although this difference was not statistically significant. The present study opted for peripheral blood samples due to their feasibility and the scarcity of literature addressing this issue.

Tumoral burden correlates with serum tryptase levels, which are higher in aggressive subtypes.[1] This study reaffirms these conclusions and adds the insight of a modest correlation between the KIT D816V mutation and serum tryptase levels, potentially enhancing the assessment of tumor burden.

Hoermann et al.[6] reported higher values for the KIT D816V mutation in aggressive SM subtypes. However, their study utilized quantitative PCR for this purpose. In this regard, the present study presents pioneering results by employing dPCR to achieve the same goal.

Evaluating and monitoring signs and symptoms in SM is complex. To address this challenge, the MAS has been proposed to standardize medical practice reports and clinical trial assessments.[7] While a mild correlation was observed between the KIT D816V mutation and MAS in this study, the potential clinical relevance of KIT D816V values exploring symptomatic load underscores the need for a broader cohort study.

It is well known that KIT D816V correlates with multilineage involvement, aggressive subtype, and outcomes.[6] The current study yielded similar results, with higher variant allele frequency values consistently associated with a more severe clinical burden disease. In conclusion, medical literature currently lacks data on the use of dPCR in SM. We emphasize its value in these patients as it correlates with symptomatic and tumoral burden, measured by adapted MAS/SM subtype and serum tryptase levels, respectively.

Conflicts of interest

The authors declare no conflicts of interest.

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