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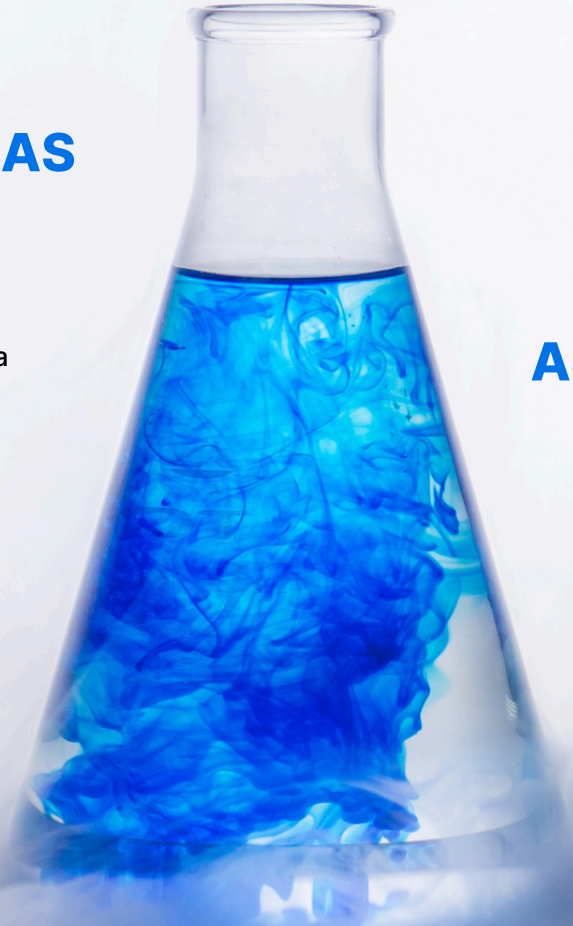

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Original article

Concurrent intravenous immunoglobulin and platelet transfusion for refractory alloimmune thrombocytopenia in patients undergoing allogeneic hematopoietic stem cell transplantation

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ABSTRACT

Background: Severe refractory alloimmune thrombocytopenia is a challenging and life-threatening complication in patients with hematologic disorders who are undergoing allogeneic hematopoietic stem cell transplantation. This study aimed to investigate the utility of continuous intravenous immunoglobulin and platelet transfusions as a therapeutic approach for alloimmune thrombocytopenia in patients undergoing allogeneic transplants.
Methods: A single-center retrospective analysis was conducted of ten adult allogeneic transplant patients hospitalized with transfusion-refractory alloimmune thrombocytopenia. Intravenous immunoglobulin (2 g/kg) was administered as a slow continuous infusion over 48 h along with a continuous apheresis platelet infusion (one apheresis unit over eight hours). Clinical response was defined as the resolution of bleeding or patients being able to undergo the required procedure without bleeding complications.
Results: The median time after the transplant was 27.5 (range: 7–299) days. Myeloablative and reduced-intensity conditioning were performed in 5 (50 %) and 5 (50 %) patients, respectively. The median platelet count at the time of infusion was 4.5×10^9/L. All patients were able to achieve clinical response with the median maximum platelet count within ten days of the infusion being 41.0×10^9/L. The median time to best response was three days with a median platelet count of 27.0×10^9/L.

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Conclusions: Continuous intravenous immunoglobulin and platelet infusions over 48 h may be able to overcome life-threatening refractory alloimmune thrombocytopenia in transplant patients and may provide a bridging measure until platelet engraftment or for life-threatening hemorrhage or invasive procedures with high bleeding risk.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established option for treating various malignant and non-malignant hematopoietic conditions [1]. Known complications of allo-HSCT include infections, graft failure or rejection, graft-versus-host disease (GvHD), and cytopenias [1,2]. Immune-mediated cytopenias following allo-HSCT probably result from immune dysregulation needing multimodal treatment approaches. Specifically, severe transplant-mediated immune thrombocytopenia (ITP), also known as alloimmune thrombocytopenia (AITP), presents as a frequent complication following allo-HSCT [3]. ITP is characterized by a persistent platelet count of less than 100×10^9/L and an increased risk of bleeding [4]. ITP is a general term that includes conditions where the underlying etiology may involve autoimmunity, such as in idiopathic ITP, wherein autoantibodies target platelets. However, AITP is caused explicitly by alloimmunization, where the immune system develops antibodies against transfused or transplanted donor platelets, leading to accelerated destruction or inefficient megakaryocyte production [3,5]. Allo-HSCT recipients with AITP often show no response to random donor platelet transfusions and limited response to HLA (human leukocyte antigen)-matched platelets [6]. Platelet refractoriness, a poor response to platelet transfusions resulting in lower post-transfusion count increments, is a concern in immune-mediated thrombocytopenia [7]. So, severe refractory thrombocytopenia due to alloimmunization remains a life-threatening complication of hematologic disorders requiring substantial platelet transfusions.

Management of ITP involves various options, including corticosteroids, splenectomy, platelet transfusions, thrombopoietin receptor agonists such as eltrombopag and romiplostim, or immunomodulatory therapies such as immunosuppressive agents (e.g., Rituximab), intravenous anti-D, and intravenous immunoglobulin (IVIG) [8–10]. Clinical management, particularly in severe thrombocytopenia cases refractory to platelet transfusion, often overlaps with idiopathic thrombocytopenia and AITP. Intravenous immunoglobulin (IVIG) is used in both contexts to modulate the immune response and temporarily increase platelet counts by mechanisms such as Fc receptor blockade and reduction of platelet destruction [7]. Studies have shown that IVIG can be administered when a more rapid increase in platelet count is necessary, especially during active bleeding or in preparation for an invasive or life-saving procedure [11–13]. When first-line corticosteroid therapy is insufficient or contraindicated, IVIG combined with concomitant platelet transfusion can effectively increase platelet counts in immune-mediated thrombocytopenia [13]. There are limited reports in the literature regarding the concomitant use of platelet transfusion and IVIG infusion investigated in alloimmunized HSCT patients [14]. In this study, the clinical outcomes of IVIG with concurrent platelet transfusion was assessed as a therapeutic approach for AITP for patients undergoing allo-HSCT.

Methods

A single-center retrospective analysis was conducted of all adult patients who had undergone allo-HSCT and had transfusion-refractory thrombocytopenia presumed to be secondary to AITP between April 2021 and August 2023. This study was approved by the University of Kansas Medical Center institutional review board, and all patients consented to receive blood products before receiving IVIG. Data were collected through a retrospective review of electronic medical records. Platelet refractoriness was defined as a corrected count increment (CCI) of ≤7500 one-hour after two consecutive platelet transfusions. Inclusion criteria included adult patients greater than 18 years old with presumed AITP and the need for urgent concurrent IVIG and continuous platelet transfusion for active bleeding or the requirement of a procedure. IVIG (2 g/kg) was given as a slow continuous infusion over 48 h with concomitant apheresis platelet infusion (one apheresis unit over eight hours). The platelet antibody screen was performed using Solid Phase Immune Adherence Assay. Platelet levels were checked one hour after the completion of each transfusion. Clinical response was identified as the resolution of bleeding or the ability of patients to undergo their procedure without bleeding complications. Data analysis was conducted using Microsoft Excel and SPSS. This study aimed to evaluate the effectiveness of IVIG with concurrent platelet transfusion as a treatment for AITP in patients undergoing allo-HSCT.

Results

This study included ten adult patients with transfusion-refractory AITP with a median age of 56 (range: 23.0–66.1) years. Five of the patients (50 %) were males. The median time after the transplant was 27.5 (range: 7–299) days. The primary hematologic malignancies were acute myeloid leukemia (n = 3; 30 %), myelodysplastic syndrome (n = 6; 60 %), and chronic neutrophilic leukemia (n = 1; 10 %). Myeloablative and reduced-intensity conditioning were used in five (50 %) patients each. The indication for an urgent increase in platelets included vaginal bleeding (n = 1; 10 %), epistaxis (n = 2;

20 %), intravitreal hemorrhage (*n* = 2; 20 %), melena (*n* = 1; 10 %), blood loss anemia of unknown origin (*n* = 1; 10 %), and the need of interventional radiology guided procedures (*n* = 3; 30 %). All patients in this analysis were hospitalized, and seven had sepsis requiring broad-spectrum antibiotics. Eight patients (80 %) had positive antiplatelet antibodies. Reasons for hospitalization included GvHD (*n* = 1; 14 %), neutropenic fever or infection (*n* = 5; 71 %), or active hemorrhage (*n* = 5; 71 %). The median platelet count at IVIG/platelet infusion was 4.5×10^9/L. The baseline characteristics and treatment response with IVIG are shown in Table 1. All patients had resolution of their hemorrhage or were able to achieve a platelet response high enough to receive their procedures without bleeding complications. No adverse events were observed from IVIG infusions in all ten patients. The median time to best response was three days with a median platelet count of 27.0×10^9/L (Figure 1). The individual responses to IVIG infusion are shown in Figure 2.

Discussion

Thrombocytopenia in the context of allo-HSCT is an effect of conditioning regimens and presents a multifaceted challenge with significant clinical implications. Its mechanism is usually multifactorial, underscoring the importance of ongoing research in elucidating and addressing its diverse contributors. Proposed causes of thrombocytopenia include prolonged isolated thrombocytopenia, secondary failure of platelet recovery, and poor graft function [15]. Prolonged isolated thrombocytopenia is characterized by persistently low platelet counts after allo-HSCT ($<20 \times 10^9$/L) with normal counts in other cell lineages for >90 days. It is seen in approximately 5 −20 % of patients who undergo allo-HSCT [15,16]. Its mechanisms remain elusive, potentially involving platelet destruction, ITP-related complications, and/or alterations in the bone marrow microenvironment [17]. Secondary failure of platelet recovery affects approximately 20 % of patients and carries an elevated risk of mortality influenced by factors such as GvHD prophylaxis, donor selection, organ dysfunction, and treatment strategies. Recent advances in transplantation and cytomegalovirus management have altered the incidence and risk factors for secondary failure of platelet recovery [18,19]. GvHD may contribute to reduced platelet production and increased consumption [18]. Poor graft function also presents as persistent cytopenia after allo-HSCT, with a complex etiology encompassing pre-transplant, peri-transplant, and post-transplant factors that create an inflammatory and immune microenvironment [15,19]. Patients with poor graft function experience poorer survival outcomes influenced by factors like the graft cell dose, ferritin levels, and splenomegaly. These conditions collectively underline the intricate nature of thrombocytopenia in stem cell transplantation, emphasizing the ongoing need for research to enhance patient care and outcomes.

This complexity is further highlighted by the persistence of thrombocytopenia even with routine platelet transfusions. In a comprehensive study involving 50 allo-HSCT patients with and without prolonged thrombocytopenia, 42 patients with idiopathic thrombocytopenic purpura, and 22 healthy individuals, the findings revealed that several factors, including the index for plasma glycocalin normalized for individual platelet count, plasma thrombopoietin levels, and circulating B cells producing anti-GPIIb-IIIa antibodies, were notably higher in patients after allo-HSCT [20]. Furthermore, the study by Leytin et al. in a murine model shows IVIG may increase the platelet count in animals that have antibodies for the GPIIb-IIIa receptor, which suggests that further studies should be carried out to investigate if this is a contributing factor in a human model [21].

While the concurrent use of IVIG and platelet administration was first documented in 1984 for leukemic patients resistant to standard platelet transfusions, Baumann et al. were the first to report on using this combination for ITP [22]. The study included six patients and established the effectiveness of concurrent IVIG and platelet administration. A recent retrospective review of 40 patients revealed that the simultaneous use of IVIG and platelets was safe and effective [13]. The combination therapy was observed to increase platelet count and effectively control bleeding symptoms rapidly. This result offers a promising insight that could aid in developing more effective treatment options for patients suffering from similar conditions. A case series by Ancevski et al. demonstrated that concurrent IVIG infusion and continuous platelet transfusion temporarily increased platelet counts in patients with AITP and bleeding complications [14]. Their findings closely align with our study, where a similar treatment stabilized platelet counts in refractory AITP patients. While earlier reports focused on using this approach in ITP, the study by Ancevski et al. provides direct insight into AITP management, highlighting its effectiveness in modulating immune responses and controlling bleeding. This supports the relevance of using IVIG and platelet transfusions in post-transplant patients with transfusion-refractory thrombocytopenia.

The few studies that researched the concurrent use of IVIG and platelet transfusion have variations in their definitions of a platelet response, patient populations, and comorbidities. These reports studied the use in ITP in adult and pediatric populations [13,22–24]. Bierling et al. reported platelet counts of $>50 \times 10^9$/L within a few days [23]. The findings of Spahr et al. demonstrate that average platelets increased to 55×10^9/L after 24 h and peaked at 69×10^9/L 48 h after treatment [13]. Most studies did not report clinical outcomes, such as controlling life-threatening bleeding or undergoing a procedure without bleeding complications. However, the study of Spahr et al. reported that all patients could achieve this clinical response in an ITP setting; the present study did not demonstrate such a high platelet count, likely due to a more significant challenge to achieve a response in the setting of AITP. Additionally, these studies do not report whether there is resolution of their life-threatening bleeding.

Of note, two patients in the current study (ID-06 and ID-08) did not demonstrate positive anti-platelet test results. However, they were still managed as refractory AITP as immune reconstitution following allo-HSCT is often delayed, and antibody testing is unreliable due to B-cell aplasia causing impaired antibody production [2]. The positive response after continuous platelet transfusion and concurrent IVIG infusion supports this assumption. We also did not have data on the degree of alloimmunization using methods like the Panel

Table 1 – Baseline characteristics and treatment response to intravenous immunoglobulin infusion.

Variable	Patient ID									
	ID-01	ID-02	ID-03	ID-04	ID-05	ID-06	ID-07	ID-08	ID-09	ID-10
Age (years)/Sex	28/F	59/M	52/M	64/F	52/F	23/M	47/F	66/M	64/M	61/M
Days between transplant and IVIG	8	11	299	12	7	149	129	17	40	38
Primary diagnosis	AML	MDS	AML	MDS	AML	AA + MDS	MDS	MDS	MDS	CNL
Type of Allogenic transplant	MUD	MSD	MSD	MUD	MSD	MUD	MUD	MUD	Haplo	Haplo
Conditioning type	MAC	MAC	MAC	RIC	MAC	RIC	MAC	RIC	RIC	RIC
Conditioning regimen	Cy/Bu	Cy/Bu	Cy/Bu	Bu/Flu	Cy/Bu	Cy/Flu/TBI	Cy/Bu	Cy/Flu/Mel	Flu/Mel/TBI	Bu/Flu/Cy
Graft source	BM	PSC	PSC	PSC	PSC	BM	BM	PSC	PSC	PSC
Indication of urgent need of platelet increase	Vaginal bleeding	Epistaxis	Ommaya reservoir placement	Trifusion catheter removal	Intravitreal hemorrhage	Intravitreal hemorrhage	Epistaxis	Melena	Blood loss anemia	Lumbar Puncture
Platelet antibody positive?	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes
Platelet count at start of IVIG infusion (x 10^9/L)	5	3	17	3	3	9	3	4	7	19
Days between IVIG and platelet >10×10^9/L	2	1	0	3	1	1	3	1	1	0
Days between IVIG and platelet 30×10^9/L	5	2	1	13	16	2	NA	6	2	3
Days between IVIG and platelet 50×10^9/L	6	2	2	15	20	112	NA	NA	NA	NA
Maximum platelet count within 10 days	52	55	116	19	16	42	27	40	30	43
Maximum platelet count within 21 days	87	55	116	194	54	42	27	40	30	43
HLA antibodies	Negative	Positive	NA	NA	NA	Negative	Positive	Positive	Negative	Positive

IVIG: Intravenous immunoglobulin infusion; F: Female; M: Male; AML: Acute myeloid leukemia; MDS: Myelodysplastic syndrome; AA: Aplastic anemia; CNL: Chronic neutrophilic leukemia; MUD: Matched unrelated donor; MSD: Matched sibling donor; Haplo: haploidentical transplant; Cy: Cyclophosphamide; Bu: Busulfan; Flu: Fludarabine; TBI: Total body irradiation; BM: Bone marrow; PSC: Peripheral stem cell; IVIG: Intravenous immunoglobulin; HLA: human leukocyte antigen; NA: Not available.

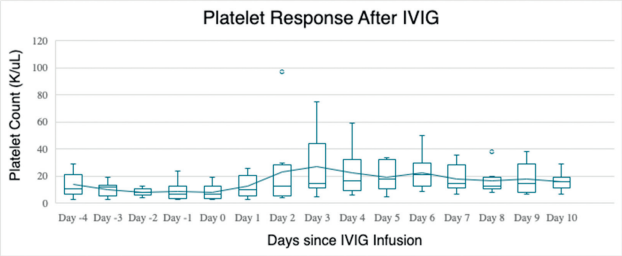

Figure 1 – Platelet count after concurrent IVIG infusion.

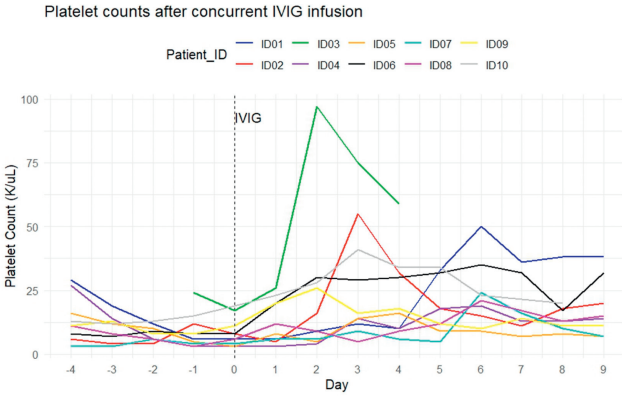

Figure 2 – Platelet count after concurrent IVIG infusion: individual patient data.

Reactive Antibody assay. This study is limited by its small sample size; however, AITP in the context of allogeneic HSCT is a rare condition, and this case series provides promising evidence to support the use of concurrent IVIG and platelet infusions.

Conclusions

The findings of this study demonstrate that the administration of continuous IVIG (2 g/kg) along with platelet infusion over 48-hour hours is a safe and effective method that could potentially overcome refractory AITP in allo-HSCT patients and serve as a temporary measure until platelet engraftment can occur. Furthermore, this combined treatment may also be helpful in cases of life-threatening hemorrhages or invasive procedures with a high risk of bleeding.

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This manuscript has not been previously published and has not been submitted for publication elsewhere while under consideration.

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Not applicable.

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Original article

Post-thaw dimethyl sulfoxide reduction in autologous peripheral blood progenitor cell suspensions

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Amyloidosis
Cell recovery

Background and objectives: Dimethyl sulfoxide has become the most common cryoprotectant used for cryopreservation of hematopoietic progenitor cells because of its efficiency, regardless of its potentially toxic side effects. Its application is considered safe, provided that the daily dose administered does not exceed 1 gram per kilogram of patient weight. Indications for its reduction after thawing are limited to patients with high risk of malignant arrhythmia and those with severely impaired renal function. However, dimethyl sulfoxide reduction can lead to the loss of viable progenitors.

Methods: A retrospective study of viable hematopoietic progenitor cell recovery after dimethyl sulfoxide reduction was performed with 13 patients (nine men, four women) with secondary amyloidosis in multiple myeloma (n = 9), primary amyloid light chain amyloidosis (n = 3), or severe adverse reaction at the beginning of the hematopoietic progenitor cell concentrate infusion (n = 1). The Wilcoxon signed-rank test was used.

Results: The results of the dimethyl sulfoxide reduction process showed a high recovery of viable nucleated cells (median: 120.85 %), and of viable mononuclear cells (median: 104.53 %). There was a significant decrease in total number of viable $CD34^+$ cells in comparison with data obtained after original collection (median: 51.49 %). No significant decrease in colony-forming unit capacity was observed after dimethyl sulfoxide reduction (median: 93.37 %).

Conclusion: The dimethyl sulfoxide removal process and total process recoveries revealed considerable individual variability. To minimize the risk of prolonged engraftment or non-engraftment, it is important to apply this process only to high-risk patients.

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Introduction

After a period of using glycerol for the cryopreservation of hematopoietic progenitor cells (HPCs) [1], dimethyl sulfoxide (DMSO) has become the most commonly used cryoprotectant for this purpose because of its high cryoprotective efficiency and rapid penetration across cell membranes [2–4]. The great advantage of its use is the possibility of infusing the thawed cell product without removing the cryoprotectant, which was not possible with glycerol. However, the potential toxicity of DMSO has given rise to debates on the safety of its use. Cases of adverse reactions such as increased heart or respiratory rate, facial flushing, increased or decreased blood pressure, dyspnea, nausea, and vomiting have been described after infusion of HPC concentrates containing DMSO [3–9]. DMSO toxicity is dose-dependent. Therefore, strategies are being developed to neutralize the toxicity, to reduce the concentration of the cryoprotectant, or to wash it off before clinical administration. Infusion of an HPC product containing DMSO is generally accepted as safe in concentrations below 10 % (v/v) [10] under the condition that the maximal daily dose of DMSO does not exceed 1 g per kg patient body weight [11,12]. While the above-mentioned symptoms usually cause only transient discomfort for the patient, serious adverse reactions have been described in patients with preexisting cardiovascular, respiratory, renal or central nervous system diseases, sometimes with fatal outcomes [6–8,13]. In the practice of University Hospital Hradec Králové, the most frequent medical indications for DMSO reduction are chronic renal failure caused by secondary amyloidosis in multiple myeloma and primary or secondary amyloidosis of the heart.

The most widely used technique in DMSO washing is gradual dilution of the cell suspension with its subsequent centrifugation and the addition of cryoprotectant-free solution [14,15]. The degree of dilution and the composition of the washing solution are usually adjusted to minimize osmotic changes. The components of the washing medium should be acceptable from a clinical point of view, i.e., it should not contain components of animal origin and should contain registered drugs, and use CE-certified medical devices, or products approved by the national competent authority (State Institute for Drug Control in the Czech Republic) [15]. In clinical practice, certified saline solutions/electrolytes, such as 0.9 % NaCl, Normosol-R® (Hospira, Inc., USA), Plasma-Lyte 148® (Baxter, USA), Ringer's solution (B. Braun, Germany) with dextran-40 (5–10 %), human serum albumin (1–5 %), hydroxyethyl starch (HES - 3–6 %), or acid citrate dextrose anticoagulant are acceptable. These media are often supplemented with dextran-40, HES, or human serum albumin at various concentrations [16–19].

Commercially available closed automatic systems developed for hematopoietic cell grafts, which are usually characterized by large-transplanted volumes, can also be used to wash out cryoprotectants. Examples of such systems are devices based on the principle of dilution and subsequent centrifugation, e.g., the COBE® 2991 Cell Processor (Terumo BCT, Inc.), Sepax S-100® (Sepax 2 S-100), and Biosafe SA® (GE HealthCare) [13,15,17,19,20], or on the principle of dilution and subsequent filtration, e.g., the Haemonetics ACP215 Automated Cell Processor® (Haemonetics Corp), CytoMate® (Baxter/Nexell), or Lovo® (Fresenius Kabi) [13,15,21,22].

Our clinical center has long experience with autologous HPC transplantation in multiple myeloma [23], and the infused DMSO dose is far below the limit [24] in most cases. A controlled study performed by Horacek et al. [25] did not report any differences in monitored vital functions between infusions of autologous and allogeneic HPC concentrates. Nevertheless, in a minority of cases, it was necessary to split the HPC dose over several days [24,26].

This retrospective study reviewed cases of primary or secondary amyloidosis as a complication of multiple myeloma treated by HPC autologous transplantation requiring DMSO reduction. Data regarding the influence of the freezing/thawing and DMSO reduction processes on the content of nucleated cells (NC), mononuclear cells (MNC), and $CD34^+$ cells were analyzed. This analysis included pre- and post-process viability, the recovery of viable cells, and repopulation potency, as measured by the colony-forming unit-granulocyte macrophage (CFU-GM) assay, for samples contained within one 100 mL cryobag.

Methods and materials

Patients and study design

A retrospective study of the influence of freezing/thawing and the DMSO removal process on HPC concentrate parameters was conducted in 2013–2022. Thirteen patients were included. The inclusion criteria this study were complete documentation, initial NC concentration not exceeding 400×10^9/L, and processing within 24 h after collection. The DMSO was reduced to approximately a quarter of the initial concentration and no more than two 100 mL bags were infused per day. For the purpose of this analysis, data from only one washing process of one 100 mL cryobag from the total of three or four cryobags of HPC concentrate obtained by one leukapheresis and stored for clinical application were compared. The process of HPC leukapheresis, transport, processing, and application is presented in Figure 1.

HPC stimulation and collection

The HPCs were collected by leukapheresis after mobilization by cyclophosphamide (2.5 g per m^2 of the patient body surface area) and granulocyte colony-stimulating factor (10 μg per kg of the patient weight) at a separator, namely Cobe Spectra or Spectra Optia (Terumo BCT, USA). Melphalan (140 or 200 mg per m^2 of the patient body surface area) was used for conditioning.

HPC cryopreservation

After transport to the Tissue Establishment, collected HPCs were processed in a laminar flow cabinet and under a laminary ceiling that adhered to Grade A purity with Class B background within 24 h of the harvest. A standard cryopreservation protocol for autologous HPCs using CE-certified DMSO (WAK Chemie GmbH, FRG) in a final concentration

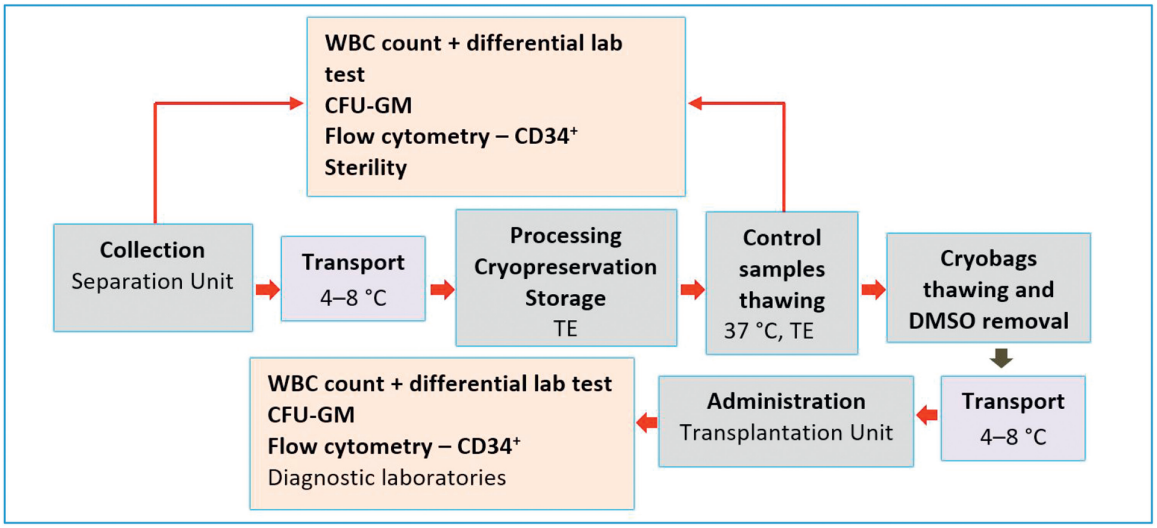

Figure 1 – Diagram of hematopoietic progenitor cell collection, processing, and application.WBC: white blood cell; CFU-GM: colony forming unit-granulocyte macrophage; DMSO: dimethyl sulfoxide

of 10 % (v/v), and HES (Voluven 10 %, Fresenius Kabi, GmbH, FRG) in the final concentration of 5 % supplemented with 20 % (v/v) human serum albumin (5 mL per 100 mL) were used. Three or four cryobags containing 70–100 mL of HPC suspension were created from the initial collection bag with a mean volume of 194 mL (154–205 mL) and were frozen in a programmable freezer (Planer Biomed, England) with a cooling rate of 1 °C/min to −90 °C and 5 °C/min to −150 °C. Cryopreserved HPCs were stored in the vapor phase of liquid nitrogen at a temperature below −160 °C in a biological container (Chart MVE, USA) with automatic filling and continuous temperature recording. The average time of storage was 103 days (range: 26–679 days).

DMSO reduction

According to the results of estimation of the dose $CD34^+$ cells from thawed control samples, the treating physician decided to use one or more bags. Metal cassettes containing bags with cryopreserved HPCs were removed from the storage container and thawed in a water bath at 37 °C. The thawing of each bag took approximately five minutes. The thawed bags were transferred to a clean room and further processed in the laminar flow cabinet (Grade A with Class B background). The total volume of each bag (mean 98 mL; range: 70–100 mL) was transferred to the washing bag, and was mixed with 258 mL of HES and 42 mL of ACD-A solution (Fresenius Kabi, GmbH, FRG). Then, the cell suspension was centrifuged for 20 min at 400 g at 4 °C in the adjacent room (Grade C). In all, 300 mL of the supernatant was removed again in the laminar flow cabinet after the centrifugation. The total time for the DMSO removal of each bag was approximately one hour. Bags containing washed HPCs were appropriately labeled and transferred to the clinical department in an insulated box at a temperature of 2–8 °C. The content was administered to the patient within two hours after thawing, without any complications. The infusion time of each bag was approximately ten minutes.

Sampling and quality control tests

The following quality parameters of collected, cryopreserved, and DMSO-depleted concentrates were determined: hematocrit and blood count including detailed white blood cell differential, total number of viable NC (TNC), MNC, and $CD34^+$, CFU-GM, and sterility.

Hematological parameters were determined by an automated hematological analyzer Sysmex XN3000 (Sysmex, Japan). $CD34^+$ phenotyping and viability determination was performed with the flow cytometer FACS (fluorescence-activated cell sorting) Navios (Beckman Coulter, USA) using SW Kaluza, Version 1.2 (Beckman Coulter, USA). Cell suspension was incubated with the anti-CD34-PE and anti-CD45-FITC monoclonal antibodies (Beckman Coulter, USA), and 7-AAD (Beckman Coulter, USA) as a vital dye.

Sterility testing was performed using a Bactalert (type BTA 3D 240, SW version B 50, BioMérieux, France) automatic microbial detection system situated in a clean room (Grade A with a Class B background) according to Czech Pharmacopoiea [27].

During CFU-GM assay in a biohazard safety cabinet certified for level II handling of biological materials, the defined amount of HPC suspension was diluted in Iscove's modified Dulbecco's medium (Sigma Aldrich, Czech Republic) and then cultured in a semi-solid matrix (Metho-Cult™, StemCell™ Technologies, USA) in Petri dishes. Cultivation took place in an incubator set at 37 °C with 5 % CO_2 in air and ≥95 % humidity (BBD Herasafe, USA). An inverted microscope (Olympus CK 40, Japan) was used for colony counting. To perform counting of CFU-GM, colonies were observed after 14 days in culture, using 10× objective (50–100× magnification) according to Czech Pharmacopoiea [28].

Table 1 – Data of patients included in the retrospective study.

Patient number	Sex	Age (year)	Weight (kg)	Diagnosis/reason for washing
1	male	55	81	Multiple myeloma - secondary amyloidosis - suspected amyloidosis of heart
2	female	61	52	Primary amyloidosis of bone marrow, liver, and kidneys
3	male	48	94	Multiple myeloma - allergic adverse reaction (DMSO)
4	male	56	91	Multiple myeloma - secondary amyloidosis - suspected amyloidosis of heart
5	female	55	80	Multiple myeloma - secondary amyloidosis of kidneys
6	female	57	61	Multiple myeloma - secondary amyloidosis - suspected amyloidosis of heart
7	male	69	93	Primary amyloidosis of liver and lungs
8	female	51	58	Multiple myeloma - secondary amyloidosis of gastrointestinal tract and kidneys
9	male	44	102	Multiple myeloma - secondary amyloidosis - suspected amyloidosis of heart
10	male	58	84	Multiple myeloma - secondary amyloidosis of duodenum and heart
11	male	66	77	Multiple myeloma - secondary amyloidosis of gastrointestinal tract
12	male	59	67	Primary amyloidosis of bone marrow and kidneys
13	male	70	103	Multiple myeloma - secondary amyloidosis - suspected amyloidosis of heart

Cell recovery calculation

Values of individual parameters were compared: total number of viable NC, MNC, and $CD34^+$ cells after collection, after cryopreservation, and after DMSO reduction. Recoveries of the individual phases of the DMSO reduction process such as: (1) freezing/thawing process, (2) DMSO removal, and (3) the total process (freezing/thawing + DMSO removal) were calculated according to the following equation:

$$Recovery = \frac{parameter\ x\ viability\ post - process}{parameter\ x\ viability\ pre - process}\ x\ 100$$

Statistical methods

The processes described above were evaluated using total numbers of viable NC, MNC, $CD34^+$ cells and CFU-GM. The data were statistically evaluated using MS Excel 2016 (Microsoft Corp., Redmond, WA, USA) and NCSS 10 statistical software (2015, NCSS, LLC., Kaysville, UT, USA, and available online: ncss.com/software/ncss [accessed on 21 April 2023]). Because the measured data did not show a normal distribution, the median and the first and third quartiles (1st Q, 3rd Q) were utilized as descriptive statistics. Bonferroni correction of the alpha significance level was used for multiple data comparisons. The data were compared using the Wilcoxon signed-rank test at the corrected alpha value $\alpha = 0.017$. Correlation analysis between the number of viable $CD34^+$ cells and CFU-GM after thawing and DMSO removal was conducted.

Results

Study inclusion criteria were met by 13 patients (nine men and four women) with an average age of 58 years (range: 44 −70 years) and weight of 80 kg (range: 52−103 kg). The retrospective study period was 2013−2020. The whole process of HPC collection, transport, processing, and administration is presented in Figure 1. Twelve patients had a diagnosis of primary amyloidosis or secondary amyloidosis as a complication of multiple myeloma, and one patient (No. 3) had an allergic adverse reaction to DMSO at the beginning of HPC infusion (Table 1).

Cell parameters of key processes

Descriptive statistics (median, 1st and 3rd quartile) of HPC key parameters characterizing individual processes, such as leukapheresis, freezing/thawing, and DMSO removal process are presented in Tables 2-4. These parameters were compared using the Wilcoxon signed-rank test with the aim of finding any significant differences between the values characterizing the pre- and post-processes. The freezing/thawing process significantly reduced the NC and MNC viability, TNC as well as the CFU-GM dose per kg of patient weight. The doses of MNC per kg and $CD34^+$ cells per kg were not affected, and the post-thaw MNC percentage increased significantly (Table 2).

Table 2 – Descriptive statistics of the data characterizing freezing/thawing process, and their comparison using the Wilcoxon signed-rank test. Data obtained from 13 patients.

Parameter	Pre-process (leukapheresis) median (Q1; Q3)	Post-process (freezing-thawing) median (Q1; Q3)	p-value
TNC/kg (x 10^8)	2.46 (1.96; 4.01)	1.79 (1.24; 3.04)	0.006*
NC viability (%)	100 (100; 100)	82 (74; 95)	0.002*
TMNC/kg (x 10^8)	1.51 (0.80; 1.78)	1.40 (1.01; 2.09)	0.364
MNC viability (%)	100 (100; 100)	87 (92; 99)	0.002*
MNC from TNC (%)	56 (33; 66)	70 (60; 83)	0.002*
$CD34^+$/kg (x 10^6)	3.71 (1.64; 6.15)	3.88 (1.42; 6.84)	0.529
$CD34^+$ from TNC (%)	1.33 (0.80; 2.00)	1.63 (0.87; 2.09)	0.014*
CFU-GM/kg (x 10^5)	2.97 (1.71; 4.05)	1.68 (1.42; 2.91)	0.002*

* Statistically significant differenceTNC: total number of viable nucleated cells; NC: nucleated cells; TMNC: total number of viable mononuclear cells; MNC mononuclear cells; CFU-GM: colony forming unit-granulocyte macrophage.

Table 3 – Descriptive statistics of the data characterizing dimethyl sulfoxide removal process, and their comparison using the Wilcoxon signed-rank test. Data obtained from 13 patients.

Parameter	Pre-process (freezing-thawing) median (Q1; Q3)	Post-process (DMSO removal) median (Q1; Q3)	P value
TNC/kg (x 10^8)	1.79 (1.24; 3.04)	1.79 (1.37; 2.20)	0.576
NC viability (%)	82 (74; 95)	78 (69; 85)	0.025
TMNC/kg (x 10^8)	1.40 (1.01; 2.09)	1.40 (0.87; 1.75)	0.402
MNC viability (%)	87 (92; 99)	87 (83; 92)	0.002*
MNC from TNC (%)	70 (60; 83)	63 (47; 83)	0.081
$CD34^+$/kg (x 10^6)	3.88 (1.42; 6.84)	1.76 (1.13; 3.65)	0.018
$CD34^+$ from TNC (%)	1.63 (0.87; 2.09)	0.90 0.51; 1.51)	0.002*
CFU-GM/kg (x 10^5)	1.68 (1.42; 2.91)	1.82 (1.31; 2.71)	0.133

* Statistically significant differenceTNC: total number of viable nucleated cells; NC: nucleated cells; TMNC: total number of viable mononuclear cells; MNC mononuclear cells; CFU-GM: colony forming unit-granulocyte macrophage.

Table 4 – Descriptive statistics of the data characterizing total dimethyl sulfoxide removal process, and their comparison using Wilcoxon signed-rank test. Data obtained from 13 patients.

Parameter	Pre-process (leukapheresis) median (Q1; Q3)	Post-process (DMSO removal) median (Q1; Q3)	P value
TNC/kg (10^8)	2.46 (1.96; 4.01)	1.79 (1.37; 2.20)	0.036
NC viability (%)	100 (100; 100)	78 (69; 85)	0.002*
TMNC/kg (10^8)	1.51 (0.80; 1.78)	1.40 (0.87; 1.75)	0.944
MNC viability (%)	100 (100; 100)	87 (83; 92)	0.002*
MNC from TNC (%)	56 (33; 66)	63 (47; 83)	0.036
$CD34^+$/kg (10^6)	3.71 (1.64; 6.15)	1.76 (1.13; 3.65)	0.010*
$CD34^+$ from TNC (%)	1.33 (0.80; 2.00)	0.90 0.51; 1.51)	0.003*
CFU-GM/kg (10^5)	2.97 (1.71; 4.05)	1.82 (1.31; 2.71)	0.006*

* Statistically significant differenceTNC: total number of viable nucleated cells; NC: nucleated cells; TMNC: total number of viable mononuclear cells; MNC mononuclear cells; CFU-GM: colony forming unit-granulocyte macrophage.

The DMSO removal process significantly reduced MNC viability and the percentage of $CD34^+$ cells from leukocytes. Other parameters were not significantly affected (Table 3).

Data from leukapheresis indicated that the DMSO removal process significantly reduced the number of viable NC and MNC, the percentage of $CD34^+$ cells from leukocytes, $CD34^+$ cells, and CFU-GM dose per patient body weight (Table 4).

Results of sterility determination

Sterility was verified in all samples after cryopreservation and after DMSO removal. All evaluated samples were sterile.

Post-thaw recovery of key hematopoietic progenitor cells parameters

Figures 2, 3, and 4 show HPC recoveries estimated from the thawed cryobags. Figure 2 shows that after cryopreservation and thawing, the number of all NC was reduced, and $CD34^+$ cell potency was also reduced.

Figure 3 shows greater inter-individual differences in cell recovery for TNC, TMNC, and $CD34^+$. Recoveries expressed as medians (Q1; Q3) were: TNC 120.85 % (61.16 %; 154.16 %), TMNC 104.53 % (38.98 %;139.27 %), $CD34^+$ 51.49 % (40.35 %; 91.82 %) and CFU-GM 93.37 % (90.86 %; 97.59 %) on comparing values obtained after freezing/thawing and after DMSO removal.

Figure 4 shows that DMSO removal decreased TNC, HPC potency and $CD34^+$ content. Recoveries, expressed as medians (Q1; Q3), were: TNC 83.98 % (42.62 %; 95.26 %), TMNC 98.71 % (57.00 %; 166.40 %), $CD34^+$ 50.69 % (35.90 %; 95.96 %), and CFU-GM 74.80 % (57.92 %; 85.07 %), on comparing values obtained after leukapheresis and after DMSO removal.

Results of correlation analysis between CFU-GM and $CD34^+$ cells

Table 4 shows considerable decreases in CFU-GM and $CD34^+$per kg after DMSO removal. Using Evans Handbook [29], the correlation between post-thaw values of viable $CD34^+$ content and CFU-GM content was found to be significant (r = 0.751; p-value = 0.003) (Figure 5). A comparable result was found after DMSO removal (r = 0.814; p-value = 0.001) (Figure 6).

Engraftment of neutrophils and platelets

In the group of patients with DMSO removal, the engraftment in neutrophils was on average 13.81 ± 2.58 days and of the platelets it was 13.77 ± 2.36 days, which was compliant with the European Society for Blood and Marrow Transplantation (EBMT) Handbook criteria [30]. Engraftment did not exceed the 21-day threshold for any patient.

Discussion

Glycerol was used as a cryoprotectant in the first autologous hematopoietic cell transplantations performed before the start of the regular hematological transplantation program at University Hospital Hradec Králové, [31,32]. Later, the use of DMSO was introduced, and washing was performed as standard. Since the beginning of 1994, routine washing of DMSO was stopped, and the rule applied abroad was followed, namely that the daily dose of DMSO per kg of the recipient's weight should not exceed 1 g. Nevertheless, certain doubts persisted in the Czech Republic about the clinical use of DMSO, especially with regard to the quality of the product

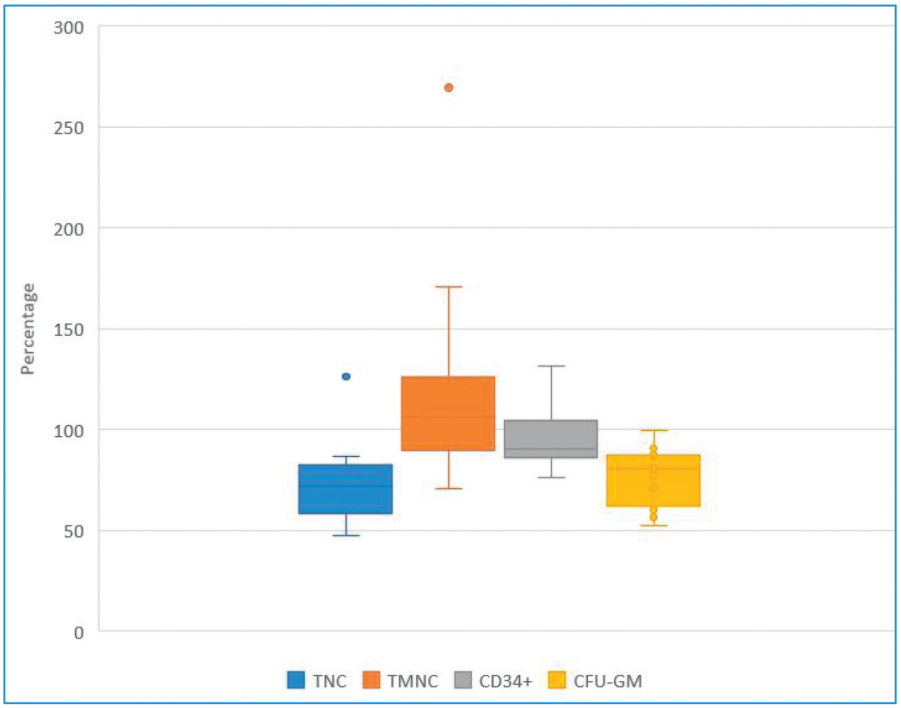

Figure 2 – Recovery (%) of key hematopoietic progenitor cell parameters after the freezing/thawing process, comparing data from collection and after freezing/thawing. Blue, orange, and yellow points denote outliers. TNC: total number of viable nucleated cells; TMNC: total number of viable mononuclear cells; CFU-GM: colony forming unit-granulocyte macrophage.

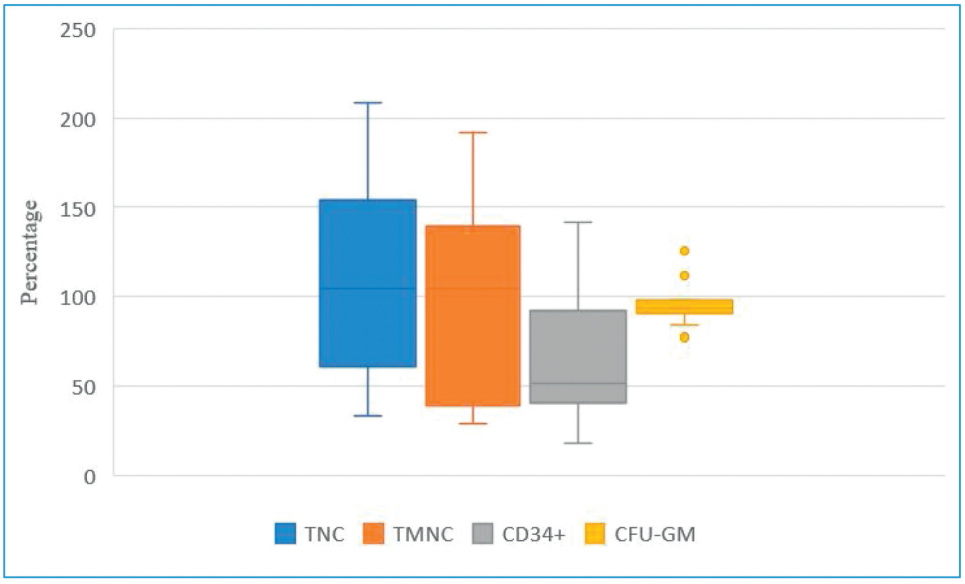

Figure 3 – Recovery (%) of key hematopoietic progenitor cell parameters after the dimethyl sulfoxide removal process, comparing data after freezing/thawing process and after dimethyl sulfoxide removal.TNC: total number of viable nucleated cells; TMNC: total number of viable mononuclear cells; CFU-GM: colony forming unit-granulocyte macrophage

[12]. These doubts were definitively resolved in 1996 when the State Institute for Drug Control fully accepted DMSO for use. This acceptance, however, mandated laboratory testing of DMSO products at individual transplantation centers until a clinically applicable CE-marked product became available in the Czech Republic. If the DMSO daily dose exceeded the recommended limit, the infusions were spread over several days [12].

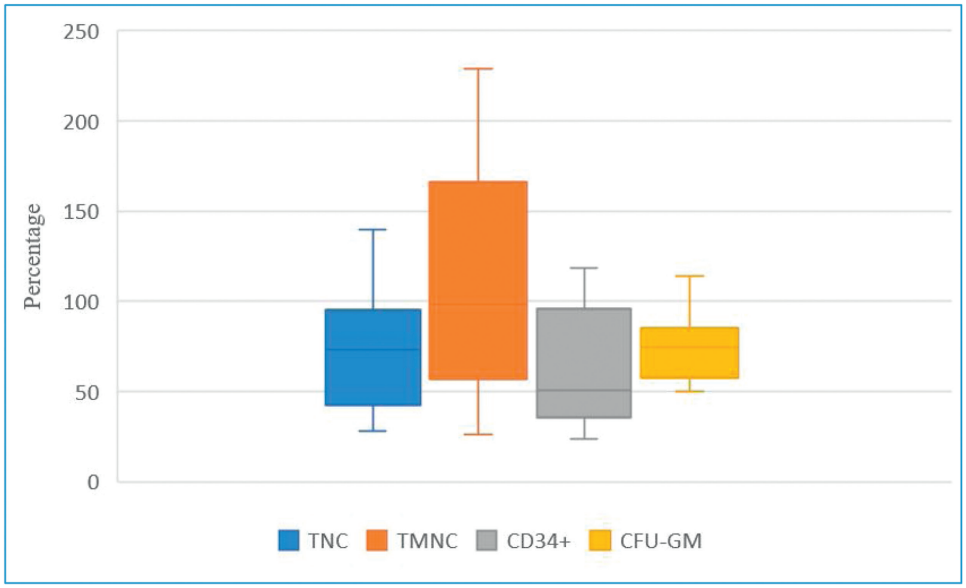

Figure 4 – Recovery (%) of key hematopoietic progenitor cell parameters after dimethyl sulfoxide removal, comparing data at collection and after dimethyl sulfoxide removal.TNC: total number of viable nucleated cells; TMNC: total number of viable mononuclear cells; CFU-GM: colony forming unit-granulocyte macrophage

Currently, there are new trends, the goal of which is either to find other equally effective cryoprotective solutions or to reduce the risk of adverse reactions [15]. In general, the rule of "three Rs" – Replace, Reduce, Remove [33] – is applied. This involves the search for suitable combinations with well-known, but individually less effective, cryoprotectants such as ethylene glycol, hydroxycellulose, sucrose, maltose, trehalose, and also some macromolecules (dextran, polyvinylpyrrolidone, etc.) [13]. Automatic washing systems working in a closed system are available. Compared with classic manual DMSO washing, their advantage is high viability of HPCs and minimal risk of microbial contamination. The disadvantage is the high price of the device [13,17,19,20].

Previous studies at this center with multiple myeloma patients who underwent autologous transplantation demonstrated that DMSO doses per kg administered at transplantation were, in the majority of cases, well below the maximum allowable daily dose [24]. The problem is posed by occasional poorly mobilized patients, with whom it was necessary to split the transplant dose into two after several days.

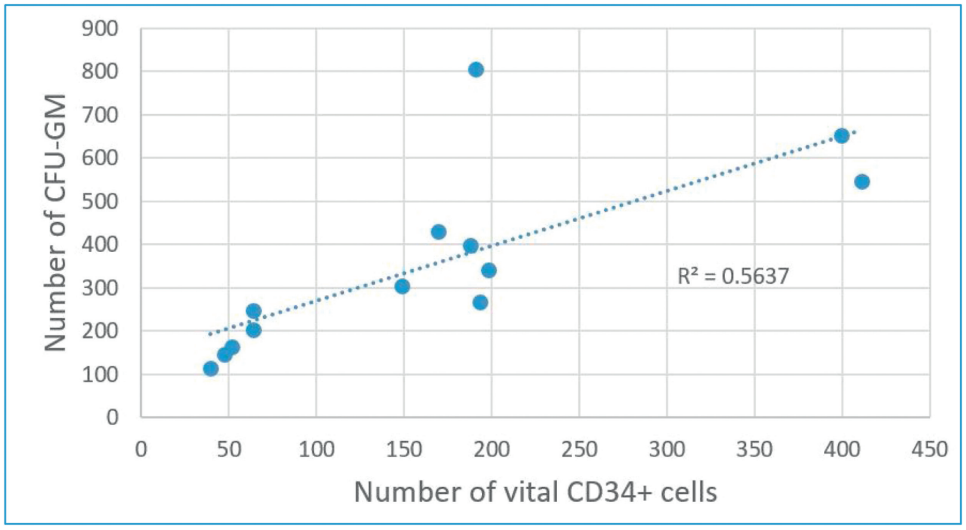

Figure 5 – Correlation between post-thaw values of CD34$^+$ cell content and colony forming unit-granulocyte macrophage (CFU-GM) content (r = 0.751; p-value = 0.003).

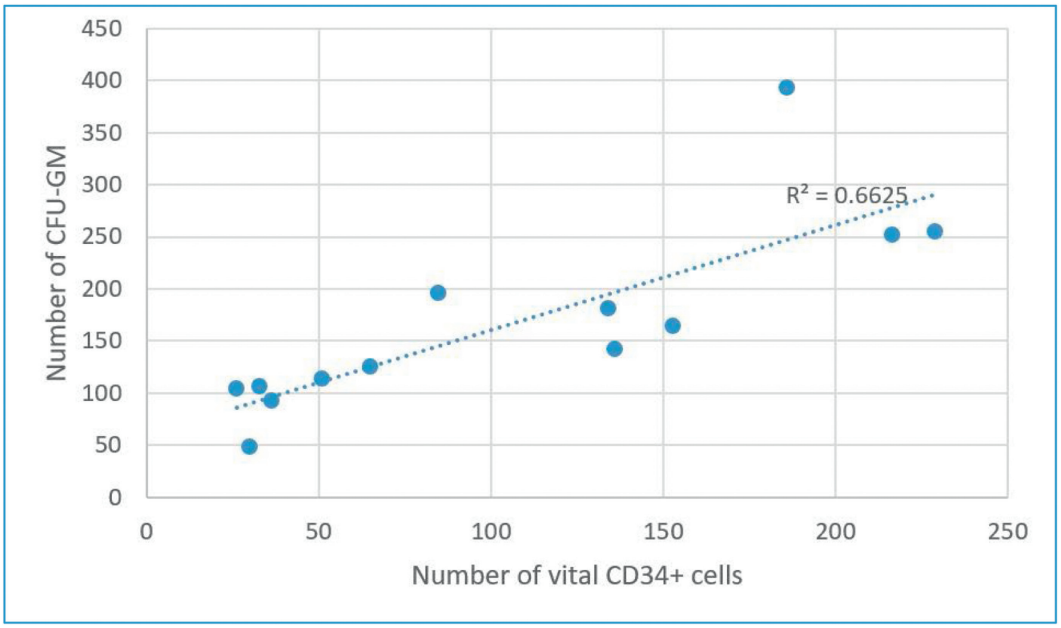

Figure 6 – Correlation between the CD34⁺ cell content and colony forming unit-granulocyte macrophage (CFU-GM) content after dimethyl sulfoxide removal (r = 0.814; p-value = 0.001).

In accordance with Yang et al. [34], this study demonstrated a correlation between the level of CD34⁺ cells and CFU-GM content. This correlation does not mean, however, that in individual cases such a correlation may not exist as demonstrated by Watts et al. [35] and Morgestern et al. [36]. The results of this study for the CD34⁺ cell freeze/thaw recovery process were higher that the results of Yang et al. [34], who established a median recovery of viable CD34⁺ cells in the freeze/thaw process of 66.4 % (versus 90.18 % in the present study), and CFU-GM of 63.0 % (versus 80.6 % in the present study). This difference can be explained by more efficient cryoprotection based on a combination of DMSO and HES. Mean CD34⁺ cell recovery after manual DMSO removal, as determined by Chen et al. [37] using Trypan blue solution, was 85.4 % which was higher than the present results determined by flow cytometry.

The results of this study were achieved in a relatively small group, as DMSO reduction after thawing was performed only for patients with a known higher risk of arrhythmia (amyloidosis of the heart) or who were at risk of impaired elimination of the DMSO (renal failure caused by amyloidosis of the kidney). In only one case, the removal of DMSO was not planned but was performed in an emergency situation, namely a severe adverse reaction after initiation of an infusion of thawed concentrate.

It was confirmed that the removal of DMSO by washing the cells leads to a significant decrease in the viability of MNCs and the dose of CD34⁺ cells per kg of recipient weight (Table 2) and that the results of the washing process and of the entire process show large individual differences. Decreased viability may be a manifestation of cryopreservation-induced delayed cell death [38]. Nevertheless, in all these patients, a sufficient dose was administered, and delayed engraftments of neutrophils or platelets were not reported. In our practice, we routinely estimate the dose of CD34⁺ cells and CFU-GM from thawed control samples, always comparing the resulting values with the doses determined before cryopreservation.

The results of this study confirm that DMSO washing should be limited to indicated cases only, which is in line with the European Directorate for the Quality of Medicines & HealthCare (EDQM) 2022 recommendation [11]. Another problem is that the result of determining the washed product sterility is known only after administration to the recipient. However, this risk is minimal if the thawed product is handled in purity Grade A clean rooms with Class B background.

We still regard the use of DMSO as safe if the daily dose of 1 g per kg is not exceeded [11].

Conclusion

DMSO removal should only be performed in indicated cases, as it leads to significant loss of progenitor cells. Despite the fact that data from only 13 patients were analyzed and that the resulting CD34⁺ dose was suboptimal, engraftments were achieved in all cases. The minimal CD34⁺ dose should be 1×10^6 per kg of patient body weight with optimum being 2×10^6 per kg of patient body weight as recommended by EBMT standards. Determination of the CD34⁺ level should be performed simultaneously with determination of CFU-GM to minimize the risk of prolonged engraftment or non-engraftment.

Financial disclosure statement

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Conflicts of interest

The author declares no conflicts of interest.

CRediT authorship contribution statement

Miroslava Jandová: Writing – original draft, Conceptualization, Data curation. **Pavel Měřička:** Methodology, Writing – review & editing. **Jiří Gregor:** Methodology. **Miriam Lánská:** Visualization. **Aleš Bezrouk:** Formal analysis. **Dana Čížková:** Writing – review & editing. **Jakub Radocha:** Writing – review & editing.

Data availability statement

The data will be available upon request to the corresponding author.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Updating the Brazilian clinical practice guidelines for sickle cell disease: Recommendations and development process

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ABSTRACT

Background: Sickle cell disease is a hereditary blood disorder that significantly impacts morbidity and mortality, requiring comprehensive care. In Brazil, its management in the National Health Service follows the Brazilian Clinical Practice Guidelines, based on evidence and expert consensus. Periodic updates ensure alignment with new scientific findings.

Objectives: This study describes the methodology for updating the clinical guidelines for sickle cell disease and provides an overview of recommendations for diagnosis, treatment and monitoring, emphasizing the evidence and health technology assessments for prioritized technologies.

Methods: The update followed the technical guide of the Brazilian Ministry of Health, and the Gradings of Recommendation, Assessment, Development and Evaluation (GRADE) approach. All the recommendations were assessed by the National Committee for Health Technology Incorporation (Conitec). The clinical guidelines panel included health technology assessment researchers, clinical experts, and policymakers. Systematic reviews assessed new evidence with stakeholder contributions being incorporated through public consultation. Cost-effectiveness analysis was applied to support new technology coverage or changes.

Results: The updated clinical guidelines provide structured recommendations for screening, diagnosis, prophylaxis, vaccination, and treatment, covering pharmacological and

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non-pharmacological approaches. It emphasizes patient and caregiver education to promote early recognition of complications. Expected benefits include fewer pain crises, fewer hospitalizations and transfusions, and improved fetal hemoglobin level, quality of life and survival rates. Key updates include listing epoetin alfa and 100 mg hydroxyurea tablets, expanding hydroxyurea eligibility criteria and revising monitoring protocols.

Conclusion: The updated clinical practice guidelines standardize sickle cell disease care in the Brazilian NHS aligned with current evidence. Dissemination and integration aim to enhance healthcare delivery, while future assessments should optimize real-world implementation.

Introduction

Sickle Cell Disease (SCD) is a genetic condition encompassing a group of hereditary disorders caused by alterations in the structure of hemoglobin (Hb) that may be associated with defects in its synthesis [1,2]. Sickle-shaped red blood cells (RBCs) are rigid and exhibit membrane alterations, making them prone to rupture, leading to intravascular hemolysis and chronic hemolytic anemia. Additionally, sickled RBCs express an increased quantity of adhesion molecules, promoting heightened interactions with endothelial cells, leukocytes, and platelets [1,2] These processes, coupled with endothelial damage, proliferative vasculopathy, and hypercoagulability, contribute to the primary clinical manifestations of SCD, including vaso-occlusive episodes, acute pain, inflammatory responses, and multi-organ damage [3–6].

SCD is among the most common genetic disorders worldwide with an estimated 5 % of the global population carrying the beta-S globin mutation [7]. Hemoglobinopathies resulting from structural hemoglobin defects are particularly prevalent in African populations, where approximately 15 % of infant mortality is attributed to SCD [8]. This elevated prevalence of the Hb S gene among populations of African descent is reflected globally [9].

In Brazil, approximately 4 % of the population carries the sickle cell trait, with a heterogeneous distribution across regions [10,11]. According to the annual data report of the National Neonatal Screening Program, 5428 newborns were diagnosed with SCD between 2014 and 2018 [12]. While recent data are lacking, estimates from 2007 suggest that 25,000 to 50,000 individuals in Brazil are affected by sickle cell anemia (Hb SS homozygotes), compound heterozygosity, or double heterozygosity for SCD [11,13].

Due to extensive racial mixing and population migrations, SCD is present throughout the Brazilian territory. Although the condition is not exclusive to any single group and can occur in individuals of all skin tones, it is most prevalent among populations with African, Mediterranean, Middle Eastern, and South Asian ancestry [11]. The estimated prevalence of the sickle cell trait ranges from 6-10 %. Historically, sickle cell trait was introduced to Brazil via the transatlantic slave trade, as enslaved individuals were forcibly brought from Africa [14]. Consequently, SCD remained neglected for a long time, rendered invisible by structural and institutional racism [15,16].

Despite being a prevalent genetic disorder, described over a century ago, with significant health and economic burden [17,18], patients with SCD are particularly vulnerable to health care disparities and treatment options for SCD remain limited [19,20]. Hydroxyurea (HU), blood transfusions, and stem cell transplantation are currently the primary therapeutic approaches, but access and utilization vary greatly [21]. This underscores the urgent need to improve the care of SCD patients by expanding access to existing treatments, promoting the adoption of new therapies.

In Brazil, the care of patients with SCD in the public National Health Service (*Sistema Único de Saúde* - SUS) is guided by the Brazilian Clinical Practice Guidelines (CPG). These guidelines outline comprehensive disease management and regulate the use of health technologies listed in the NHS, specifying eligibility criteria, monitoring requirements, and conditions for treatment discontinuation. The NHS is a publicly funded healthcare system that provides universal access to listed health services, including prevention, diagnosis, treatment, and rehabilitation. When a health technology is listed in the NHS, it must be provided free of charge to patients. Its distribution and availability may occur at the federal, state, or municipal level, depending on the management and procurement policies established.

This work aims to present the process of updating the CPG for SCD, including the health technology assessment process and final recommendations on diagnostic and therapeutic approaches.

Methods

The updated Brazilian CPG for the diagnosis, treatment, and monitoring of SCD patients was developed by the Ministry of Health (MoH) in conjunction with invited medical experts and methodological researchers from the Health Technology Assessment Unit at the Hospital Alemão Oswaldo Cruz. Although no clinical protocols aligned with our scope or recently updated CPG were identified to support the adoption (adolopment) process recommended by the GRADE-ADOLOPMENT methodology [22], in this update we reviewed previous SCD CPG recommendations (from 2018) [23] and considered

the addition of new recommendations when pertinent to the Brazilian context, and new scientific evidence available in the literature. The entire process of updating and drafting the guidelines followed the legislative and methodological guidelines established by the MoH [24], which are aligned with the GRADE guidelines [25].

The AGREE II Reporting Checklist was used to support reporting guideline recommendations (Supplementary Table S1).

Scope and purpose

The target population includes children and adults with suspicion or diagnosis of SCD, whether with the sickle cell trait (Hb AS), the homozygous Hb S mutation (Hb SS), or compound heterozygosity (e.g., Hb Sβ^0, Hb Sβ^+, Hb SC, Hb SD, Hb Sβ^{thal}, according to the International Classification of Diseases-10 (ICD-10) codes D57.0, D57.1, D57.2.

Guidelines development group

The CPG Development Group was composed of a multidisciplinary team consisting of researchers, clinical experts, and policymakers to ensure a comprehensive and evidence-based approach. The process was coordinated by the General Coordination of Clinical Protocols and Therapeutic Guidelines (CGPCDT), a division of the Brazilian MoH responsible for the development and revision of CPGs (management committee) [26].

The researchers were from the Health Technology Assessment Unit (UATs) of the Hospital Alemão Oswaldo Cruz, a recognized Center with expertise in health technology assessment (HTA) and pharmacoeconomics (drafting group). Researchers were responsible for mapping relevant technologies for reimbursement, conducting systematic literature reviews and full HTA, including cost-effectiveness and budget impact analyses.

The clinical specialists (panelist group) were selected based on their expertise in hematology and SCD care. They contributed to the definition of the scope of the guidelines, formulation of PICO (Population, Intervention, Comparator, Outcome) questions, and drafting of clinical recommendations.

The government representatives included technical advisors of the MoH and representatives from the Secretariat of Science, Technology, and Innovation. The Health Economic-Industrial Complex (SECTICS), Department of Specialized and Thematic Care (DAET), and Department of Pharmaceutical Assistance and Strategic Supplies (DAF) provided strategic advice and ensured alignment with public health priorities. The final version of the CPG was reviewed by CGPCDT to ensure consistency and alignment with MoH regulations.

Meetings and updating process

The selection of CPG for periodic updates follows predefined criteria established by the Brazilian MoH. These include the time since the last update, availability of new evidence, listing of new health technologies in the NHS, horizon scanning studies and recommendations from the MoH technical areas [26].

The CPG development process began with a broad literature mapping and identification of international protocols. Initially, three virtual meetings were held between June and August 2022 involving the CPG Development Group.

The first preliminary meeting focused on defining the scope of the CPG and identified priority topics for revision. During this phase, researchers presented a synthesized overview of the literature mapped, along with a comparative analysis of key recommendations from international CPGs and the current version of the national CPG.

The second meeting delved deeper into clinical aspects, including the discussion of eligibility criteria and key recommendations for diagnosis, treatment, and monitoring. During this meeting, medical specialists identified specific sections of the CPG for revision or rewriting, ensuring that the document reflects current clinical standards and practices [27].

The final meeting was dedicated to prioritizing PICO questions related to the technologies listed in the NHS that required evaluation in order to be included in the CPG. These technologies, either diagnostic tools or treatments, were typically identified through the initial literature search. This step ensured that the guidelines incorporate relevant, evidence-based technologies that enhance disease management and align with national healthcare priorities.

Subsequently, two parallel processes were initiated. While clinical specialists began revising the existing text and drafting the updated CPG, researchers undertook the HTA process for the prioritized technologies to be evaluated for reimbursement. This dual approach ensured that the CPG text and the economic and clinical assessments were developed concurrently, allowing a seamless integration of evidence-based recommendations and updated technological evaluations.

Health technology assessment

The National Committee for Health Technology Incorporation (Conitec) plays a critical role in the evaluation and recommendation of health technologies for inclusion in the NHS. Conitec is responsible for assessing the efficacy, safety, cost-effectiveness, and budgetary impact of medicines, devices, and other health technologies proposed for use within the NHS, as well as to approve the publication of the CPGs.

For this reason, researchers conducted the entire HTA process following the methodological guidelines of the MoH [28–30], including systematic reviews, cost-effectiveness and budgetary impact analyses of technologies prioritized during the third meeting. These findings were then presented and discussed in plenary meetings of Conitec, which included representatives from various governmental and technical bodies. The commission deliberated on the evidence and formulated recommendations considering the benefits and harm, values and preferences, resource use, acceptability, cost and feasibility with all these aspects being comprehensively described in the published reports [31–33]. Although the assessment of Conitec and recommendation process was not conducted by an expert panel, the evaluation of health technologies considered key domains aligned with the GRADE Evidence to

Decision (EtD) framework. Therefore, the results are presented in accordance with this structure in the present article.

All aspects related to evidence retrieval, economic evaluations, and the criteria considered by Conitec during plenary discussions for recommending listed health technologies are detailed in the respective recommendation reports, which are publicly available on the website of the agency [34]. These recommendation reports comprehensively document key methodological aspects, including search methods, evidence selection criteria, strengths and limitations of the evidence and the consideration of benefits and harm. These aspects have been summarized in Supplementary Table S2.

In parallel, clinical specialists drafted the CPG based on the discussions and agreements from the preliminary meetings. If Conitec recommended a technology for inclusion in the NHS, the CPG draft was updated to reflect the technologies listed. This included specifying criteria for patient inclusion and exclusion, usage recommendations, and monitoring requirements, ensuring that the CPG remained aligned with national healthcare priorities.

Public consultation and final recommendations

After the initial presentation of HTA in the plenary meetings of Conitec, preliminary recommendations underwent a public consultation phase to gather input from stakeholders and to capture the views and preferences of patients and the public, further ensuring that the final decision aligns with societal needs and values. During this phase, the public, including patients, caregivers, healthcare professionals, and other stakeholders, were invited to provide feedback. Contributions could be submitted via an online platform, and all the input was systematically reviewed by the drafting group and presented to Conitec [35].

Conitec was also responsible for preliminary and final recommendations of the CPG, which also went through the process of evaluation, recommendation, and public consultations. The updated CPG was subsequently subjected to a Public Consultation (No. 25/2024) held from May 24 to June 12, 2024. A total of 184 contributions were received and reviewed, influencing the final recommendations of the CPG [36].

Recommendations

The target users of the CPG are the healthcare professionals involved in the care of SCD patients, particularly physicians and nurses working in primary and specialized care, including outpatient settings within the NHS.

SCD treatment requires coordinated actions by a multidisciplinary team for the prevention of crises, infection and complications, and for this reason, these CPG cover key aspects of screening, diagnosis, and disease identification, as well as prophylaxis and vaccination strategies. They also outline recommended actions and clinical approaches for healthcare professionals, patients, and families, addressing both pharmacological and non-pharmacological dimensions of comprehensive SCD care (Figure 1). Educating patients on self-care and family members on recognizing emergencies is of

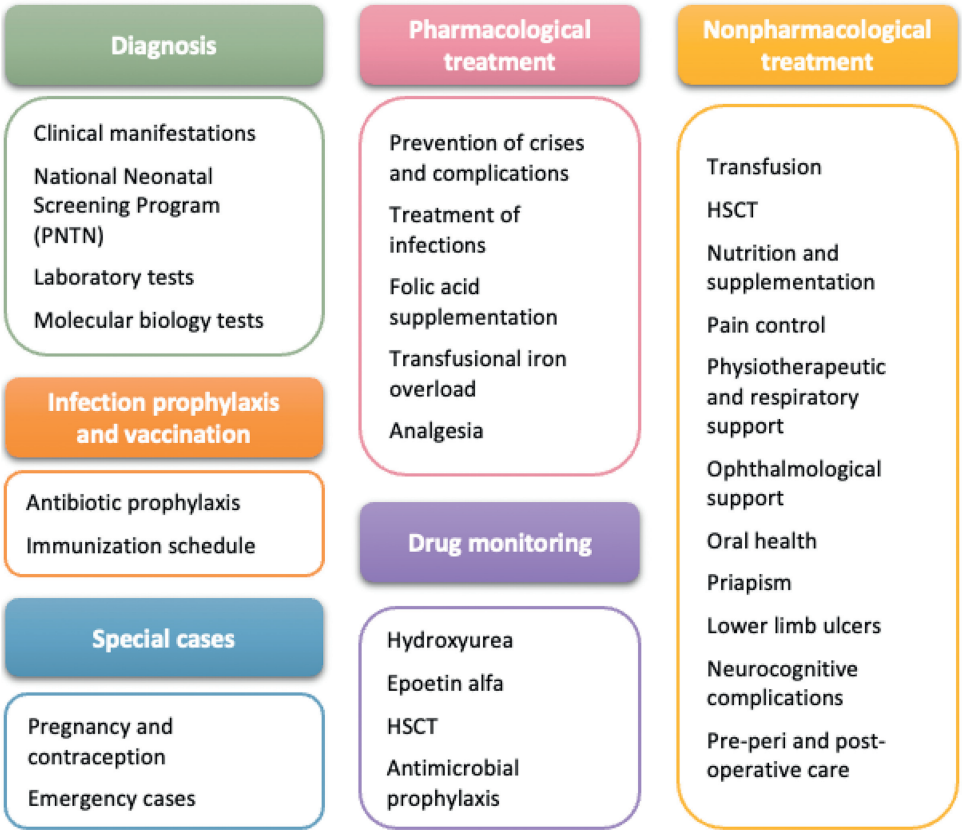

Figure 1 – Overview of major topics for recommendations in the updated national guidelines.

utmost importance to ensure early care, particularly for acute conditions such as fever and autosplenectomy.

Expected benefits from the treatments and monitoring outlined in these CPG include: (i) elimination or reduction of pain episodes; (ii) increased fetal hemoglobin (Hb F) production; (iii) slight increase in total Hb concentration; (iv) reduced acute chest syndrome (ACS) episodes; (v) reduced hospitalizations; (vi) reduced transfusions; (vii) regression or stabilization of organ or tissue damage; (viii) lower infection risk; and (ix) improved well-being, quality of life, and survival rates.

Diagnosis

Clinical presentation

SCD presents diverse clinical manifestations that aid in diagnosis, including signs of anemia (pallor, jaundice, fatigue, tachycardia, and heart murmurs), vaso-occlusive episodes, and renal complications that may progress to chronic kidney failure, with consequences such as glomerular hyperfiltration, hyposthenuria, and the need for hemodialysis [3–6]. Frequent infections, especially in young children, are the leading cause of mortality due to functional asplenia, which increases susceptibility to pathogens like *Streptococcus pneumoniae*. Severe crises, such as splenic sequestration, can result in hypovolemic shock and risk of death [37–39].

National neonatal screening program

SCD can be diagnosed at birth through the National Neonatal Screening Program (national policy for the newborn blood spot test) [40] or later in children and adults who did not have access to neonatal screening. It is recommended that detection and initiation of treatment occur before four months of age to ensure proper prevention of infections and other potentially fatal complications.

Laboratory tests

Hematological abnormalities can be observed in the complete blood count of SCD individuals, including reduced mean corpuscular volume and mean corpuscular hemoglobin concentration, as well as increased leukocyte counts and reticulocyte counts (ranging from 4-10 %). In optical microscopy of blood smears, additional features may include sickle-shaped RBCs, codocytes, hypochromia, microcytosis, polychromasia, Howell-Jolly bodies, and Heinz bodies. While these findings may assist in clinical suspicion for late diagnosis, they are not confirmatory of the disease.

Hemoglobin electrophoresis using isoelectric focusing or high-performance liquid chromatography is recommended for confirming the diagnosis, as both are accurate and usually do not require repeat testing. Conventional hemoglobin electrophoresis on agarose gel or cellulose acetate can also be used in adults. Inconclusive results from any method may be clarified with a complementary technique. For neonatal screening, repeat testing, if required, should use direct patient samples to prevent sample mix-ups.

Molecular biology tests

Molecular biology tests, such as polymerase chain reaction (PCR), should be incorporated into the diagnostic process to elucidate the genotype and identify polymorphisms, which are important for understanding the prognosis and severity of the disease [41].

The quantification of Hb A_2 and Hb F is recommended, as well as familial analysis of the parents and siblings, and the identification of alpha-thalassemia variants, haplotypes associated with the Hb S gene, and other Hb variants [14].

Primary healthcare services, upon receiving the newborn blood spot test result or the hemoglobin electrophoresis test result, must initiate care for SCD individuals. The diagnostic and referral workflow are represented in Supplementary Figure S1.

Infection prophylaxis and vaccination

Daily antibiotic prophylaxis is recommended for children with asplenia or hyposplenism until age five or at least one-year post-splenectomy. High-risk groups, including children up to 16 years, adults over 50, immunocompromised patients, and those with prior sepsis, should prioritize prophylaxis. For over five-year-old patients without high-risk features, 1-2 years of prophylaxis post-splenectomy may suffice [42,43].

Oral penicillin V is the preferred agent, with injectable penicillin G as an alternative when oral administration is not possible. For penicillin-allergic children, erythromycin is recommended. Amoxicillin and penicillin are first-line choices, while cephalosporins, fluoroquinolones, and macrolides are alternatives (Table 1).

Vaccination should align with the MoH schedule, including 23-valent pneumococcal polysaccharide vaccine (PPSV23) and 13-valent pneumococcal conjugate vaccine (PCV13) after age two, with a booster after 3-5 years [44]. An annual

Table 1 – Recommended prophylactic treatment for children aged three months to five years.

Drug	Criteria	Dosage regimen
Oral penicillin V (phenoxymethylpenicillin)	Children under three years old or weighing up to 15 kg	125 mg (equivalent to 200,000 IU or 2.5 mL) every 12 h (250 mg/day)
	Children over three years old or weighing between 15 and 25 kg	250 mg (equivalent to 400,000 IU or 5 mL) every 12 h (500 mg/day)
Intramuscular benzathine penicillin G (benzylpenicillin) *	Patients weighing up to 10 kg	300,000 IU every 4 weeks
	Patients weighing between 10 and 20 kg	600,000 IU every four weeks
	Patients weighing over 20 kg	1,200,000 IU every four weeks
Oral erythromycin estolate	For penicillin allergy	20 mg/kg twice daily (40 mg/kg/day)

* IM: Intramuscular; IU: international units

influenza vaccination is recommended for all ages, and COVID-19 vaccination is advised for children and adults with SCD [44].

Non-pharmacological treatment

Transfusion support

Transfusion support is essential for acute and chronic disease management, as well as during pregnancy and in perioperative periods [45,46]. Transfusion strategies include simple transfusion, which restores volume and oxygen capacity but risks volume and iron overload, and exchange transfusion, which removes sickled RBCs and replaces them with normal RBCs through erythrocytapheresis or partial manual exchange. Indications for each approach depend on the patient's clinical condition, including age, comorbidities, transfusion history, and treatment alternatives (Table 2) [45–47]. It is recommended to avoid unnecessary transfusions to reduce risks like alloimmunization and iron overload, and monitor serum ferritin levels, especially in irregular transfusions.

Phenotyping for ABO, Rh, and Kell antigens is required at diagnosis, with transfused RBCs matched to prevent sensitization. If antibodies develop, extended phenotyping is needed. Immunohematology tests are available through public blood centers, and confirmatory testing is advised after six months of age and before the first transfusion [47,48].

Leukoreduced, phenotyped, and sickle cell-free RBCs are recommended for all transfusions. In rare cases, RBCs with sickle cell trait may be used for unique antigen profiles. Exchange transfusions are preferred over simple transfusions when feasible to minimize iron overload and alloimmunization. For patients eligible for hematopoietic stem cell transplantation (HSCT), irradiated blood components should be used, following MoH guidelines [49].

Hematopoietic stem cell transplantation

Myeloablative allogeneic HSCT from an HLA-matched sibling or haploidentical donor, or using cord blood, peripheral blood, or bone marrow is recommended for treating SCD in patients with the Hb SS or Hb Sβ^0 genotypes experiencing severe vaso-occlusive complications despite HU treatment. HLA testing should begin at age five or upon the onset of severe complications to identify potential candidates. Transplantation age criteria are not explicitly defined, and the choice of stem cell source should balance donor and recipient risks [50,51].

Strategies for pain management

Families and individuals with SCD should prioritize hydration and avoid vaso-occlusive crisis triggers, such as temperature changes, infections, menstruation, pregnancy, and stress [52]. Non-pharmacological pain management includes psychosocial support, cognitive-behavioral therapy, relaxation techniques, and addressing contributing factors like stress, insomnia, and malnutrition. Individualized care plans are crucial for managing chronic and acute pain [53,54].

Physical activity is beneficial but requires medical guidance. Light exercises with gradual progression, stretching, and breaks every 20 min are recommended to avoid fatigue and pain. Activities like walking, swimming, and cycling at 50-90 % maximum heart rate for 20 min are ideal. Hydration, proper nutrition, and avoiding prolonged or intense workouts are essential [52,55,56].

Nutrition and supplementation

A balanced diet rich in vitamins A, C, and E, as well as minerals such as zinc and copper are recommended. Bone health should be evaluated regularly, including assessments of calcium intake and annual or semiannual vitamin D levels [57]. Bone density measurements should begin at age 12, with vitamin D assessments repeated annually and bone density testing every three years. Supplementation with vitamin D and calcium can support the bone metabolism, helping to prevent growth and maturation delays in children and adolescents with SCD [58,59].

Physiotherapeutic and respiratory support

Aerobic kinesiotherapy, analgesic electrotherapy, and electroacupuncture may benefit SCD patients. Continuous incentive spirometry is recommended during hospital stays or when ACS develops [60]. For patients with increasing oxygen needs or reduced respiratory effort, non-invasive ventilation techniques like Continuous/Bilevel Positive Airway Pressure (CPAP/BiPAP) can be beneficial [61,62].

Oral health

Periodic dental evaluations are essential for SCD patients. Preventive care, monitoring, and treatment of maxillofacial alterations, such as malocclusion, help reduce disease-associated complications [63,64].

Table 2 – Indications for each type of transfusion.

Simple transfusion	Exchange transfusion
• Aplastic crisis and pancytopenia; • Acute and progressive infections with a hemoglobin drop of 1.5 g/dL from baseline or Hb below 7 g/dL; • Acute hepatic or splenic sequestration; • Pregnancy; • Acute stroke when exchange transfusion is unavailable; • Acute chest syndrome (ACS) with increasing oxygen requirement to maintain saturation above 95 %.	• Drop in baseline Hb with symptomatic anemia or acute Hb drop without reticulocytosis; • ACS, with transfusion continuing for at least six months post-episode; • Frequent and severe pain crises; • Acute stroke or cerebral infarction; • Transient ischemic attack (TIA); • Multi-organ failure; • Priapism unresponsive to urological procedures; • Increased velocity on transcranial Doppler; • Preparation for major surgeries or procedures involving vital organs.

Ophthalmological support
Patients with the Hb SS, Hb Sβ^0, and Hb SC genotypes are most significantly affected, particularly within the 20-39 year age group; therefore, annual ophthalmological monitoring should begin at age ten and continue throughout adulthood [65].

Priapism
Prophylactic measures for priapism include increased fluid intake and frequent bladder emptying. For episodes under two hours, hydration, bathing, walking, warm compresses, and analgesia are recommended. Priapism lasting more than 2-4 h is a medical emergency requiring immediate evaluation, hydration, analgesia, and a urological consultation [66,67].

Leg ulcers
Wound care involves keeping the skin hydrated and using appropriate footwear. Ulcers should be cleaned with 4 % sodium chloride solution using specific techniques based on wound type (e.g., jet irrigation for clean wounds, mechanical cleaning with dry gauze for infected wounds or wounds with debris). Dexamethasone cream and mineral oil are recommended for perilesional skin, with mineral oil use continuing post-healing. The tetanus vaccination should be updated [68].

Neurocognitive complications
Stroke prevention includes annual transcranial Doppler ultrasound for patients aged 2-16 years to assess risk [69]. Increased cerebral artery velocity indicates a higher stroke risk, requiring chronic transfusion therapy to lower Hb S levels. Selective screening with cerebral magnetic resonance angiography may also be needed.

Silent strokes, defined as lesions visible on at least two planes by T2-weighted magnetic resonance imaging (MRI), measuring at least 3 mm in the largest linear dimension [70], without associated neurological findings, can be detected early by cognitive tests conducted by qualified psychology professionals, and should be included in care for children and adolescents with SCD, enabling measures to mitigate these consequences [71–74].

Surgical procedures in sickle cell disease
SCD patients require special care before, during, and after surgical procedures [75], as presented in Table 3.

Pharmacological treatment

Four research questions for listing technologies were prioritized for the updated CPG, for which the working group developed recommendation reports (Table 4). As a result of the HTA process and Conitec deliberation, epoetin alfa [33] and presentation of HU in 100 mg tablets [32] were listed in the NHS. Additionally, the criteria for the use of HU were expanded [31], allowing broader access to treatment for SCD patients (Supplementary Table S2). Crizanlizumab was also prioritized for assessment; however, its evaluation was suspended, and the assessment report did not proceed to the plenary deliberation of Conitec following the cancellation of its marketing authorization by National Health Surveillance Agency (Anvisa) on October 30, 2023. The eligibility criteria for using listed treatments were established in accordance with the listing recommendations of Conitec, and the original text was revised to prevent access barriers. (Supplementary Table S3).

Prevention of crises and complications

HU is the cornerstone of SCD management and the most effective pharmacological therapy, requiring regular monitoring due to a risk of hematologic toxicity albeit low [76,77]. While HU has shown in-vitro carcinogenic and teratogenic potential, this has not been confirmed in vivo [78]. Treatment should continue indefinitely if clinical and laboratory responses are achieved [79]. For about 25 % of patients with an unsatisfactory response, discontinuation may be considered after two years. Treatment adherence should be reassessed to optimize outcomes, with healthcare teams emphasizing the benefits of HU to the patient.

The recommended starting dose of HU is 15 mg/kg/day, taken orally as a single daily dose. The dose can be increased by 5 mg/kg/day every four weeks, up to a maximum of 35 mg/kg/day or until hematological toxicity or serious adverse effects occur. For children weighing up to 25 kg, the 100 mg coated tablet is advised.

Recombinant human epoetin alfa is recommended for Hb SS and Hb Sβ^0 patients on HU who experience declining hemoglobin levels or frequent transfusion needs. The suggested dose is 12,000 IU per week, divided into three

Table 3 – Recommended care for patients with sickle cell disease undergoing surgical procedures.

Preoperative care	Perioperative care	Postoperative care
• Clinical evaluation; • Complete blood count and coagulation tests; • Glucose, urea, creatinine, liver function tests, and urinalysis; • Electrocardiogram (ECG) for surgical cardiac risk assessment; • Chest X-ray; • Hb A and Hb S quantification; • Pulse oximetry and respiratory physiotherapy evaluation; • Transfusion preparation and immuno-hematological testing with red blood cell phenotyping; • Full hydration for 12 hours preoperatively	• Maintaining a moderate temperature in the operating room; • 50 % oxygenation combined with the anesthetic agent; • Clinical monitoring (ECG, blood pressure, pulse, temperature, urinary output); • Laboratory monitoring (serum electrolytes, inspired O^2 concentration, pulse oximetry, or arterial blood gas analysis).	• Immediate postoperative oxygenation; • Pulse oximetry monitoring; • Parenteral hydration; • Respiratory physiotherapy.

Table 4 – Summary of health technology prioritized and assessed for coverage decisions.

Technology and research question	Clinical evidence*	Economic assessments	Coverage decision
Epoetin alfa for reduced kidney function	Eight observational studies analyzed before/after clinical and hematological outcomes. Epoetin alfa use significantly increased: • Hb levels (4-32.8 % improvement, p<0.05) (certainty of evidence: **VERY LOW**) • Hb F levels (5.2-17.1 % increase, p<0.05), and reduced transfusion requirements (quantitative data not reported) (certainty of evidence: **VERY LOW**) No increase in vaso-occlusive crises (VOC) or venous thromboembolism (VTE) was observed, suggesting safety (quantitative data not reported) (certainty of evidence: **VERY LOW**) For a renal impairment subgroup (n = 4), Hb concentration improved by 29 % (certainty of evidence: **VERY LOW**)	A CEA using a one-year decision tree model evaluated its impact on reducing transfusion needs. Epoetin alfa showed modest clinical benefit (incremental 0.033 QALY) and cost savings of BRL 11,564. Sensitivity analyses confirmed epoetin alpha as a dominant option. The eligible population for the BIA was estimated at 5,274 patients annually. With a 10-50 % market share, direct acquisition costs was BRL 806,129 in year one to BRL 4.8 million in year five. Over five years, a cumulative savings of BRL 96.5 million, due to reduced transfusion-related costs, including iron chelation therapy.	After public consultation, Conitec members unanimously recommended incorporating epoetin alfa into the NHS for treating SCD patients with renal impairment and worsening hemoglobin levels, following the Ministry of Health's clinical protocol. The decision was formalized under Resolution No. 871/2024.
Hydroxyurea to nine-month-old children	A randomized clinical trial (BABY HUG - NCT00006400) and three non-comparative observational studies were included as clinical evidence. Hydroxyurea showed a significant impact on the following efficacy outcomes compared to placebo: • Hb levels (difference of 0.9; 95 % CI: 0.5-1.3; p<0.001) (certainty of evidence: **MODERATE**) • Hb F levels (difference of 6.7 %; 95 % CI: 4.8-8.7 %; p<0.001) (certainty of evidence: **MODERATE**) • Pain (HR 0.59; 95 % CI: 0.42-0.83; p = 0.002) (certainty of evidence: **MODERATE**) • Acute chest syndrome (HR 0.36; 95 % CI: 0.15-0.87; p = 0.02) (certainty of evidence: **MODERATE**) • Transfusion requirements (HR: 0.55; 95 % CI: 0.32-0.96; p = 0.03) (certainty of evidence: **MODERATE**) No significant difference was found for hospitalizations (HR: 0.73; 95 % CI: 0.53-1.00; p = 0.05) (certainty of evidence: **MODERATE**)	In the CEA, hydroxyurea was cost-effective when considering QALY and may be cost-effective (ICER – BRL 12,258 per QALY gained). In BIA, it was estimated that the 5-year cumulative impact for the adoption of hydroxyurea for patients aged 9-24 months could range from BRL 105,556 to BRL 484,805.	After a public consultation, the Conitec members unanimously recommended incorporating hydroxyurea in the treatment of patients with sickle cell SCD (Hb SS, Hb Sβ0, severe Hb Sβ+, and Hb SD Punjab), aged 9-24 months, without symptoms or complications, considering the clinical benefits and favorable budget impact.
Hydroxyurea 100 mg tablet and 1000 mg tablet	It was considered that the new formulations (100 mg and 1000 mg) are bioequivalent or similar to the 500 mg formulation, and, for this reason, only economic only viability assessment was made. Certainty of the evidence was not assessed since the studies were based on bioequivalence analyses.	In the BIA, two populations were considered: individuals >9 months (17,400 per year) and 9 months to 12 years (5,000 per year). With a 10-50 % market share, the 5-year impact would be approximately BRL 396.7 million and BRL 43 million, respectively.	After public consultation, the members of Conitec unanimously recommended the incorporation of 100 mg hydroxyurea and not to incorporate 1000 mg hydroxyurea for the treatment of patients with SCD aged at least 9 months. The Committee maintained this decision, considering that the potential clinical benefits of using the 1000 mg form would not justify its costs.

BIA: budget impact analysis; BRL: Brazilian Reais; CEA: cost-effectiveness analysis Hb: Hemoglobin; Hb F: Fetal hemoglobin; ICER: incremental cost-effectiveness ratio; 95 % CI: 95 % confidence interval; HR: Hazard ratio.

* Further aspects of the GRADE Evidence-to-Decision (EtD) framework, considered in the recommendations reports and discussed during the meetings of Conitec are presented in the Supplementary Material.

administrations of 4,000 IU each, delivered via subcutaneous or intravenous injection [80,81].

Treatment of Infections

Prompt recognition and treatment of infections are critical due to the high risk of septic shock. Fever is an emergency, especially in under three-year-old children with temperatures above 38.2°C. Signs of infection require immediate transport to an emergency facility and a Reference Center notification. Diagnostic tests include blood count, reticulocytes, urinalysis, c-reactive protein, cultures, chest X-ray, and lumbar puncture if suspicion of central nervous system infection. Empirical antibiotics for encapsulated organisms should start immediately for undiagnosed febrile cases. Antibiotic selection varies by infection type:

- ACS: Add macrolides (e.g., clarithromycin, azithromycin).
- Meningitis: Use CNS-penetrating antibiotics (e.g., ceftriaxone).
- *Mycoplasma pneumoniae*: Add erythromycin, clarithromycin, or azithromycin.
- Osteomyelitis: Provide 4-6 weeks of antibiotics covering *S. aureus* and *Salmonella spp*. along with hydration and analgesia.

SCD patients with COVID-19 require close monitoring, hospitalization at the first complication sign, and careful management even with mild symptoms [82].

Folic acid supplementation

Folic acid is recommended for low serum folate levels or specific conditions such as pregnancy, with a dosage of 1 mg/day. Serum folic acid levels should be maintained below 17 ng/mL. If therapeutic monitoring is unavailable, dietary guidance is advised alongside supplementation. For children up to 1 year or 10 kg, a half tablet (2.5 mg) three times per week is recommended. For patients above this age or weight, one tablet (5 mg) three times per week is advised.

Transfusional iron overload

Treatment should follow the current iron overload CPG of the MoH [83].

Analgesia

Pain management during vaso-occlusive crises requires fixed-dose analgesics based on intensity, guided by pain scales which can direct treatment, monitor responses, and predict hospitalization duration [84,85]. Treatment must be individualized to avoid opioid toxicity when alternative therapies are more appropriate, following the current National CPG for Chronic Pain [86]. Chronic pain from avascular necrosis, compression fractures, or arthropathies may require surgical interventions. Menstrual pain may benefit from hormonal therapies or NSAIDs, while priapism requires adequate analgesia pending a urological evaluation [87].

Iron supplementation

Iron supplementation is not routinely recommended and should only address specific deficiencies identified by ferritin, iron, and transferrin levels, with treatment limited to correcting the deficiency [88].

Special cases

Pregnancy and contraception

Pregnant women with SCD face higher risks of placental dysfunction, infections, preterm birth, and perinatal mortality. Untreated bacteriuria can cause intrauterine growth restriction (IUGR) and preterm labor, while anemia may worsen due to increased blood demand, hemodilution, or deficiencies. Women should be informed about risks such as IUGR, preeclampsia, eclampsia, infections, thromboembolism, gestational diabetes, and preterm delivery. Miscarriages are more common, especially in Hb SS patients, and delayed puberty may postpone pregnancy.

High-risk obstetric care, including ultrasound, fetal biophysical profile, umbilical Doppler, and cesarean delivery, when necessary, is essential to reduce perinatal mortality. Regular prenatal visits, hydration, and timely management of fever or pain crises are critical. Pregnant women must interrupt unsafe medications such as iron chelators and discontinue HU three months before conception. Folic acid (5 mg/day) is recommended during pregnancy.

Pain crises, more frequent late in pregnancy, are treated similarly to non-pregnant patients. NSAIDs may be used between 18-30 weeks but should be avoided after 30 weeks to prevent ductal closure. Routine monthly transfusions are reserved for severe cases, while intermittent transfusions may address significant anemia (<7 g/dL).

Initial care includes clinical and obstetric history, paternal Hb electrophoresis, and comprehensive lab tests. Monitoring at each trimester and updating maternal immunizations are recommended. Maternal assessments should cover nutrition, hydration, blood pressure, weight gain, and cervical evaluation.

Progestogen-only contraceptives, like medroxyprogesterone acetate, are preferred due to lower thrombosis risk. Copper intrauterine devices are an option but require monitoring for increased menstrual flow. Bone density should be evaluated before starting progestogen-based contraceptives and reassessed every two years.

Emergency cases

ACS is identified by new pulmonary infiltrates on chest X-ray and symptoms like chest pain, cough, fever, hypoxemia, or respiratory distress. Urgent hospitalization is required, with intensive care unit transfer if oxygen saturation falls below 93 %. Management includes oxygen therapy to maintain saturation ≥93 %, monitoring blood counts, reticulocytes, blood cultures, and chest X-rays. Bronchodilators and respiratory physiotherapy may be used. Fluid management should avoid overhydration to prevent pulmonary edema. Simple RBC transfusion is indicated if Hb drops by ≥1.5 g/dL below baseline. For severe or rapidly progressing cases, exchange transfusion or urgent simple transfusion is recommended. Post-recovery, exchange transfusion should continue for at least six months [89].

Splenic Sequestration progresses rapidly and requires immediate hospitalization and notification of a reference center. Families should monitor spleen size to detect recurrence, which occurs in up to 50 % of cases [90]. Treatment involves IV hydration (10-15 mL/kg plasma expanders or 40-100 mL/kg

saline over two hours), bed rest, oxygen therapy, and leg elevation. RBC transfusion (10 mL/kg) may be needed to achieve Hb levels of 6-7 g/dL, with careful volume monitoring post-crisis to prevent hyperviscosity. Splenectomy is often performed after the first acute event in over five-year-old children to prevent recurrence.

Hyper-Hemolysis Syndrome (HHS) is a severe hemolysis exacerbation following transfusion, marked by a rapid Hb drop below pre-transfusion levels. Immediate treatment includes hydration and immunosuppressive therapy with glucocorticoids (1-4 mg/kg/day prednisone or equivalent methylprednisolone), IV immunoglobulin (0.4-1 g/kg/day for 3-5 days), or both. Therapy should be individualized, with prompt intervention essential to prevent fatal outcomes [91,92].

Monitoring

Hydroxyurea

Monitoring HU in SCD aims to ensure clinical effectiveness and safety. Initial tests include Hb electrophoresis, Hb F levels, complete blood count (CBC), liver and kidney function tests, and pregnancy testing. During treatment, Hb F, CBC, and liver and kidney function should be monitored periodically to guide dose adjustments (Supplementary Table S4).

If toxicity occurs, HU should be discontinued until recovery and resumed at a dose 5 mg/kg/day lower than the previous dose. Recurrent toxicity at the same dose defines the maximum tolerated dose. HU dose must be adjusted for renal impairment based on creatinine clearance (if 10-50 mL/min, administer 50 % of the dose; if less than 10 mL/min, administer 20 % of the dose) [93]. Myelosuppression is the primary dose-limiting toxicity. Toxicity levels and frequency of recommended tests are described in Supplementary Table S5 [94–97]. Severe mucocutaneous reactions require discontinuation, while long-term use does not affect growth or development in children with SCD.

Lactating patients should decide between discontinuing breastfeeding or treatment, considering the benefits for the mother. However, pharmacokinetic studies have reported low HU transfer to breast milk, suggesting its use may be acceptable if breastfeeding occurs at least three hours after the daily dose [98]. Therefore, the risks and benefits should be carefully assessed in these cases. Women of childbearing age should use contraception. HIV or hepatitis patients require close monitoring for potential complications.

Epoetin alfa

Hemoglobin levels should be monitored weekly until stabilization, then monthly for six months, and every three months thereafter. Epoetin alfa should be discontinued if no reduction in transfusion needs or failure to achieve target Hb levels (≥8.5 g/dL or an increase ≥1.5 g/dL from baseline) occurs within six months [33]. Otherwise, treatment may continue indefinitely to reduce transfusion dependency.

Hematopoietic stem cell transplantation

HU should be discontinued four weeks before starting conditioning and reintroduced only in cases of engraftment failure.

Antimicrobial prophylaxis

Prophylactic phenoxymethylpenicillin (penicillin V) should be continued from diagnosis until age 5. Asplenia is not a contraindication for vaccinations, including live vaccines. No evidence suggests vaccine-related complications in SCD patients on HU, and the benefits of reducing infections outweigh potential risks [99].

Transition process

Adolescence is a period of considerable vulnerability, as patients face challenges inherent to their age, considering social, physical, and psychological development. For people with SCD, this stage of life is often marked by a shift in disease behavior, with increased severity observed in some individuals. This progression is largely attributed to the underlying biology and pathophysiology of SCD, which tends to worsen due to the cumulative burden of organ damage over time. The absence of a structured transition process can result in disruptions in care, with serious consequences such as increased use of emergency services, deterioration of organ function, and early death. Therefore, preparation, transfer, and integration are essential in the transition process, which should occur in an organized and planned manner [100,101].

A planned transition is considered a quality standard in services for patients with chronic conditions. It involves not just the transfer of care - understood as the act of moving the patient from one institution or care team to another - but a broader, dynamic, and complex process. This includes evaluating the acquisition of self-management skills and implementing educational strategies and appropriate healthcare resources [101].

The main goals of a transition clinic are: (1) to improve the capacity of adolescents and young adults, with or without special healthcare needs, to manage their own care and use health services effectively; and (2) to ensure an organized process within pediatric and adult care teams for transition preparation, transfer, and integration into adult-centered healthcare. The stages of this process, clearly outlined in the updated CPG (Supplementary Figure S2), should guide services aiming to address this important gap.

Conclusion

The updated national Guidelines for SCD is publicly available on the MoH website and has been officially published through a Joint Ordinance (SAES/SECTICS No. 16, of November 1, 2024), ensuring accessibility of healthcare professionals, patients and all the population [102]. In addition to these dissemination efforts, the present article aims to contribute to the international discussion on SCD management, describing the Brazilian approach for evidence-based guideline development.

Regarding evaluation of implementation, no formal assessment of facilitators and barriers was planned within this update. However, it is important to emphasize that the guidelines should not be regarded as a standalone document. Given the decentralized structure of the NHS, states and municipalities have the autonomy to develop complementary

tools and local protocols, aligning with the national guidelines while addressing regional healthcare needs and operational constraints. Future studies assessing the real-world application of these recommendations could provide valuable insights to optimize implementation strategies and improve patient outcomes.

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2) Acquisition of data, or analysis and interpretation of data: LPG, MMF, LAO, CL, KNTM, SS, RCL, MRKR, DFG
3) Drafting the article: LPG, MMF, RCL, LAO
4) Revising it critically for important intellectual content: RCL, LAO, HAOJ, MCLSM
5) Final approval of the version to be submitted: LPG, MMF, RCL, LAO, HAOJ, CL, KNTM, SS, MRKR, DFG, MCLSM

Declaration of generative ai and ai-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve language and readability. After using this tool/service, all authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Conflicts of interest

The authors declare that they have no conflicts of interest to disclose.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2025.103964.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Overall survival in multiple myeloma in Brazil: A cohort of 16 years

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ABSTRACT

Multiple myeloma constitutes approximately 1 % of all malignancies, with a higher incidence observed in over 65-year-old individuals. New technologies have shown promising results with an increased overall survival. The objective of this cohort study was to evaluate the survival analysis of patients with multiple myeloma treated by the Brazilian Unified Health Service over 16 years and compare the effectiveness of bortezomib (Bortezomib)-based treatment with other regimens used. A retrospective national cohort study was conducted utilizing real-world evidence derived from the Brazilian Unified Health System big data. This study focused on 25,370 patients with multiple myeloma who underwent chemotherapy between 2000 and 2015. Of these patients, 50.71 % were male, and the median age was 62 years. The median overall survival was 37 months. Hematopoietic stem cell transplantation (HSCT) was the best prognostic factor with overall survival of 87 months. The bortezomib (Bortezomib)-based chemotherapy provided the best results of the different chemotherapy regimens in terms of overall survival (67 months), followed by thalidomide-based schemes with an overall survival of 54 months. Despite the significant progress made in the Brazilian health system, the National Committee for Technology

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Incorporation (CONITEC) needs to make quicker decisions to improve access to new oncology drugs for patients, while maintaining rigorous evaluation criteria. Earlier adoption and adequate funding for oncology services could have saved more lives compared to the treatments made available by the Unified Health Service at that time.

Introduction

Multiple Myeloma (MM) is a hematological disease characterized by the multiplication of malignant plasma cells in the bone marrow. As the second most common malignant hematological disease after lymphoma, MM represents approximately 10 % of such cases and accounts for 1 % of all types of cancer [1,2].

Demographically, MM predominantly affects elderly individuals, with a mean age at diagnosis of 66 years, and a majority of patients (56 %) being male [3]. The actual incidence of MM in Brazil is unknown according to information available in reports of the National Cancer Institute [4,5]. Data provided by the Institute for Health Metrics and Evaluation show that 1.83 deaths per 100,000 inhabitants occurred in Brazil in 2019 due to MM, whereas data from the United States report 5.47 deaths per 100,000 inhabitants [6].

The diagnosis of MM is characterized by bone marrow clonal plasma cells ≥10 % or bone or extramedullary plasmacytoma proven by biopsy, in addition to one or more of the following: evidence of target organ damage that may be attributed to an underlying plasma cell proliferative disorder, specifically: [C] Hypercalcemia: serum calcium >11 mg/dL or >1 mg/dL above the upper limit of normal; [R] Renal failure: creatinine clearance <40 mL in one minute or serum creatinine >177 mmol/L (>2 mg/dL); [A] Anemia: hemoglobin value <10 g/dL or 2 g/dL below the lower limit of normal; [B] Bone lesions: one or more osteolytic lesions on skeletal radiography, computed tomography (CT) or Positron emission tomography–computed tomography (PET-CT). And one or more of the following biomarkers of malignancy: percentage of plasma cells in the bone marrow biopsy ≥60 %; Ratio of Serum Free Light Chains ≥100; >1 focal lesion in magnetic resonance studies [5,7].

The treatment of symptomatic MM is with drugs, such as chemotherapeutics, immunomodulatory agents, proteasome inhibitors, monoclonal antibodies, and more recently, bispecific antibodies and advanced cell therapy combined or not with radiotherapy. HSCT is an important therapeutic option and may be performed in eligible patients. The goal of treatment is to reach an objective overall response rate (symptom and biochemical control), since it is an incurable disease. Patients experience multiple recurrences until becoming refractory to the treatment [8], leading to death.

In the Brazilian Unified Health System (SUS), the available drugs (bortezomib, cyclophosphamide, cisplatin, dexamethasone, doxorubicin, liposomal doxorubicin, etoposide, melphalan, vincristine and thalidomide) may be used in different combinations [9–11].

Limited research has been published regarding MM in Brazil, a nation comprising approximately 210 million inhabitants. Most of the reports cover single institution experiences or small numbers of patients compared to this nationwide sixteen years cohort [12,13]. The purpose of this study is to perform a broad evaluation and description of the epidemiological profile, access to treatments and the main clinical outcome of the MM patients treated by SUS.

Methods

Study design and setting

This study employed a nationwide, non-concurrent, open cohort design, with patient follow-ups conducted from 2000 to 2015. Data were developed through deterministic-probabilistic linkage of the patient-centered registry within the Hospital Information System, Ambulatory Information System and Mortality Information System [14]. The Hospital Information System contains data on hospitalization from both public and private hospitals contracted by SUS. The High-Complexity Procedure Authorization subsystem of the Ambulatory Information System database contains all information about chemotherapy including records about the medical diagnoses for which treatment was prescribed using the International Classification of Diseases, Tenth Revision (ICD-10) codes.

The chemotherapy dispensations recorded in the database were decoded, listed, and cleaned to extract information regarding the protocols utilized. Treatment effectiveness was assessed by comparing outcomes of patients exposed to bortezomib-based regimens compared to those treated with other chemotherapeutic regimens.

Patients were categorized into therapeutic groups based on exposure to specific agents at any time during their treatment, regardless of treatment line. For instance, the 'bortezomib-based' group comprised all patients who received bortezomib at any point during the study period. This inclusive approach aimed to evaluate the overall impact of drug exposure across the disease trajectory.

The study entry period was between January 2000 to December 2014, and patients were followed up from January 2000 to December 2015 (16 years). This strategy assured a minimum follow-up of 12 months. The inclusion criteria for this study were as follows: patients who received one or more treatments for MM (ICD C90.0), individuals over 18 years of age, and those initially treated between 01/01/2000 and 31/12/2014. Patients were censored if they abandoned or interrupted their treatment or at the end of follow-up (right censoring). Treatment failure events were characterized by death (Figure 1).

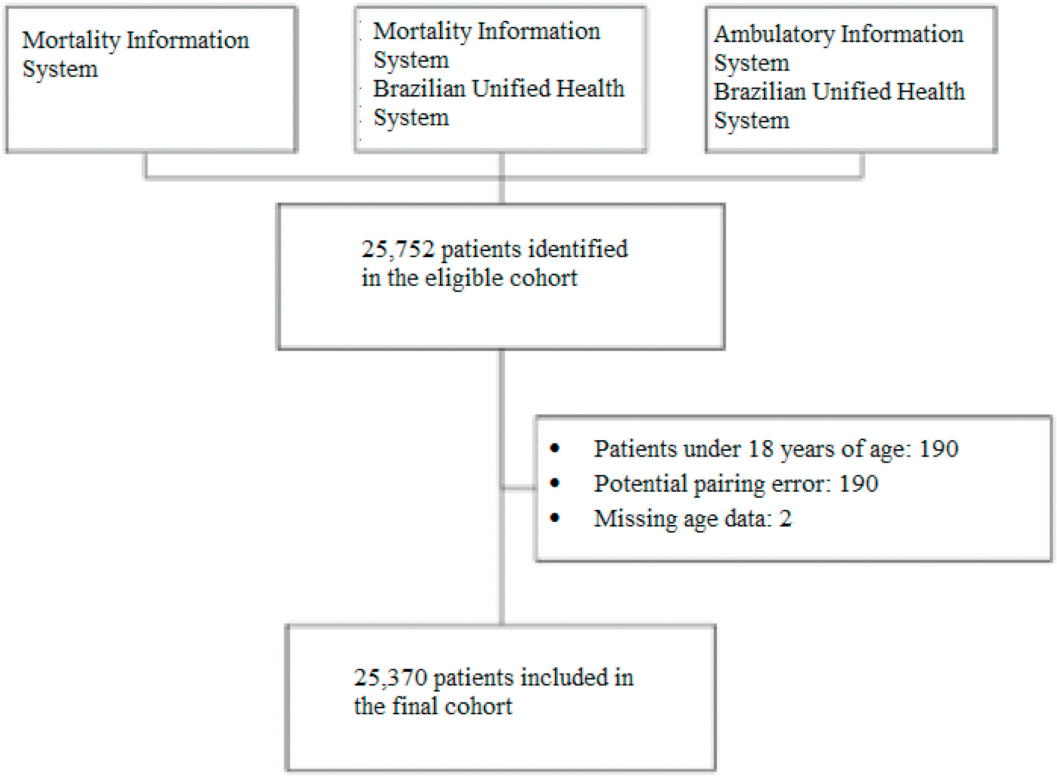

Figure 1 – Cohort selection flow.

Ethical aspects

The use of the National Database was evaluated and approved by the Research Ethics Committee of Federal University of Minas Gerais (CAAE - 16334413.9.0000.5149).

Statistical analysis

The frequency distribution was analyzed for categorical variables. Measures of central tendency and variability were determined for numerical and quantitative variables (sociodemographic and clinical characteristics). Among other available variables, the chemotherapy regimen was used to stratify survival based on drug treatment.

The baseline was used as the first event (first chemotherapy or hospitalization for chemotherapy) to calculate the overall survival (OS). The Kaplan-Meier technique was used to determine the cumulative probability of survival of patients included in the study and according to the explanatory variables of the study. The Log-Rank test was used for subgroup analyses and test the hypothesis of equality between survival curves.

The proportional hazards model – Cox Model – was used to calculate hazard ratios (HR) and 95 % confidence intervals (95 % CI) of covariables that were statistically significant (p-value <0.05) in the Log-Rank test. The software "R" version 4.1.3, of R Foundation for Statistical Computing, Microsoft Excel® business 2019 was used for statistical analysis.

Results

The characteristics of the cohort are shown in Table 1. The final cohort consisted of 25,370 patients with 50.71 % being males. The median age was 62 years, with 70 % of patients over 56 years of age (Table 1). The distribution according to region identified a higher concentration of patients in the southeastern region (49.9 %), followed by the northeast and the south of the country (21.7 % and 19.3 %, respectively) (Table 1). The OS of the total study population was 37 months (95 % CI: 36–38 months) (Figure 2).

The assessment by sex found an OS of 40 months (95 % CI: 39–42 months) for women versus 36 months (95 % CI: 34–37 months) for men (Figure 3).

Table 2 presents the relative risk estimates (HRs) and corresponding 95 % CIs for the main sociodemographic and clinical characteristics analyzed in the cohort based on a multivariable Cox proportional hazards model. This table allows for the identification of groups with higher or lower risk of death within the cohort, contributing to the understanding of disparities in survival outcomes. In the univariate analysis, male sex was associated with an increased risk of death (HR: 1.12; 95 % CI: 1.08–1.16).

Over 65-year-old patients had an OS of 29 months versus 44 months for the other age groups (Figure 4).

The risk of death for patients from the south of Brazil was the highest in the country (HR: 1.11; 95 % CI: 1.03–1.20) and the lowest risk of death was identified in patients from the northeastern region (HR: 0.84; 95 % CI: 0.78–0.91). The relative

Table 1 – Characteristics of the patients included in the cohort.

Variable	*n* = 25,370
Sex – *n* (%)	
Female	12,505 (49)
Male	12,865 (51)
Age at baseline - Median (IQR)	62 (54 to 71)
Age range at baseline - *n* (%)	
>65 years	10,122 (40)
18 - 25 years	103 (0.4)
26 - 35 years	408 (1.6)
36 - 45 years	1850 (7.3)
46 - 55 years	5089 (20)
56 - 65 years	7798 (31)
Self-declared skin color - *n* (%)	
Asian	258 (1)
White	8032 (32)
Indigenous	3 (<0.1)
Unknown	12,299 (48)
Brown	3879 (15)
Black	899 (3.5)
Residence region at baseline - *n* (%)	
Central-West	1703 (6.7)
North	635 (2.5)
Northeast	5596 (22)
South	4394 (17)
Southeast	13,042 (51)
ICD10 Description at baseline - *n* (%)	
Extramedullary plasmacytoma	604 (2.4)
Gammopathy	480 (1.9)
Multiple myeloma	23,833 (94)
Multiple myeloma and malignant plasma cell neoplasms	71 (0.3)
Plasma cell leukemia	382 (1.5)
Cohort entry period - *n* (%)	
2000 - 2003	6185 (24)
2004 - 2007	5306 (21)
2008 - 2011	7293 (29)
2012 - 2015	6586 (26)
Medication at baseline - *n* (%)	
bortezomib (Bortezomib) Based	445 (1.8)
Thalidomide Based	2633 (10)
Others	22,292 (88)
Hematopoietic stem cell transplantation - *n* (%)	
No	22,644 (89)
Yes	2726 (11)
Comorbidity Charlson Index at baseline - Median (IQR)	2.00 (2.00 to 3.00)
Frailty Index at baseline - Median (IQR)	0 (0 to 11)
Mean time of illness before baseline - Median (IQR)	0 (−1 to 0)
Mean time in the cohort - Median (IQR)	18 (6 to 40)
Event type - *n* (%)	
Censure	12,328 (49)
Death	13,042 (51)

risks of the main characteristics evaluated in the study are shown in Table 2.

Although bortezomib had not been formally incorporated into the SUS at the time of the study, patients receiving therapeutic regimens containing bortezomib were nevertheless identified (*n* = 445 patients). In terms of OS, bortezomib-based chemotherapy showed the best results, achieving a median time of 67 months (95 % CI: 55-NA]). This corresponds to a Hazard Ratio of 0.60 (95 % CI: 0.50–0.73), indicating a significantly reduced hazard of death compared to other treatment regimens (Figure 5).

The second most common scheme was thalidomide-based with a median OS of 54 months (95 % CI: 50–62) and HR 1.30 times better when compared to all other options (HR: 0.77; 95 % CI 0.72–0.82) (Figure 6).

The comparison of the OS for all the therapeutic regimens is shown in Figure 7.

In this study, 2726 patients were identified as having undergone HSCT. This subgroup achieved a median survival time of 87 months (95 % CI: 81–95), and HR 1.51 times better (HR: 0.36; 95 % CI: 0.34–0.39) versus 33 months (95 % CI: 32 –34) for patients who did not undergo HSCT (Figure 8).

Discussions

MM is an onco-hematological neoplasm with a low incidence. The median age at start of treatment in this study was 62 years, which is consistent with the Brazilian literature. In a study conducted in Reginal Hospital in Mato Grosso do Sul of patients treated from January 2013 to December 2017, the median age of patients was 63 years, corroborating the findings of this study [15]. The same median (63 years; range: 37 –82 years) was found in a study conducted by Silva et al. [16] in Clinical Hospital of Minas Gerais [16]. The variation found in this cohort was 18 to 98 years, with 70 % of patients being over 56 years old. The median age in the present study is comparable to the 60.5 years reported by Hungria et al. [5]. Given that SUS provides care for most of the population [17], with no significant access restrictions compared to the private healthcare system, our results are likely representative of the overall national profile of MM patients.

The age of patients at the beginning of treatment for MM is lower in Brazil than in other countries. In a study using data from the French health care system, the median age was 74 years (range: 63–81 years). In the United States, the median age at diagnosis was 69 years, with 60 % of patients over 65 [18]. In that report, there was no significant difference in incidence between sexes, but mortality was higher in men (HR: 1.12; 95 % CI: 1.08–1.16) [18]. In the United States, the incidence of MM was 1.5 times higher in men (2.1/100,000) than in women (1.4/100,000) and the mortality in 2018 was 59,000 deaths in men versus 47,000 in women, in the same period.

The life expectancy in Brazil in 2010 was assessed at 73.9 years, which can be considered lower than countries such as the USA, which had an approximate life expectancy of 80 years in 2010 [19,20]. Regarding the epidemiological profile of MM, the age at diagnosis found in this cohort is also lower when compared to patients in the USA (66–70 years), with 37 % of patients being younger than 65 years, as reported by Kazandjian [21].

The OS found in the current cohort reached a median time around three years (37 months), a result consistent with the literature, considering the same period [21]. Different factors affect the OS of MM patients in Brazil. Notably are the lack of access to or availability of newer medicines throughout the country and the low rates of autologous HSCT despite financing by SUS. The low rates of HSCT can be attributed to a

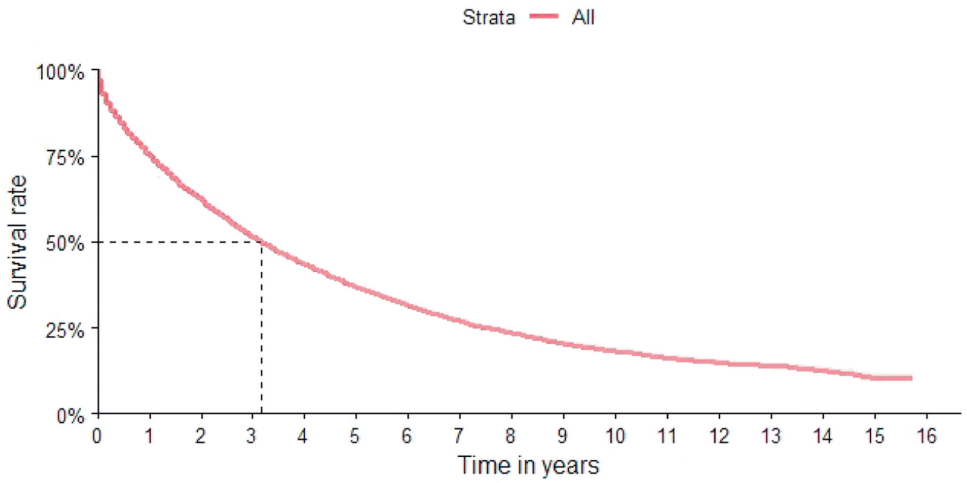

Figure 2 – Overall survival.

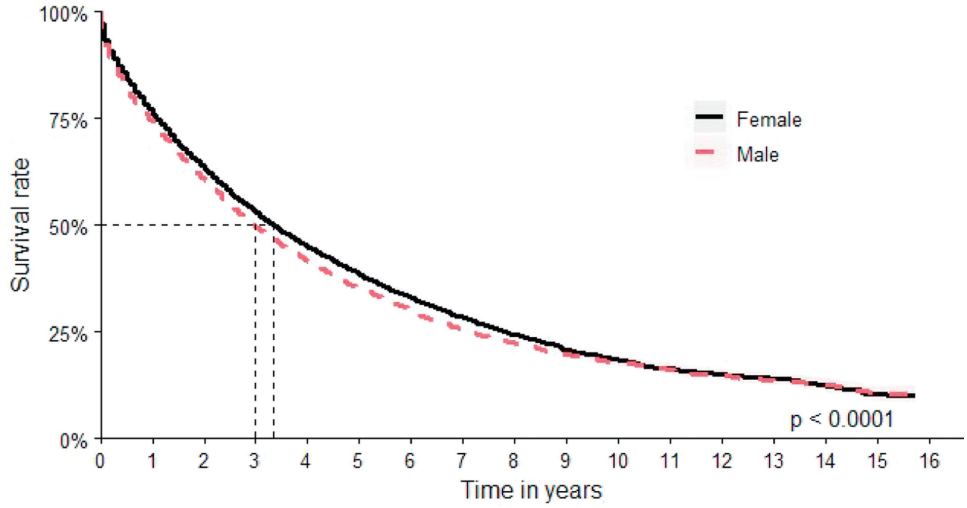

Figure 3 – Overall survival by sex.

combination of factors, including insufficient specialized medical centers, geographic disparities in healthcare access, long waiting times, and socioeconomic barriers that limit patient access to the treatment. The current study observed that transplant-eligible patients exhibited a longer OS when compared to their non-transplanted counterparts, aligning with findings from other published studies [22,23].

Eligibility to HSCT is the best prognostic factor in MM; data obtained in this study are compatible to data from the International Myeloma Working Group in five countries in Latin America, where OS of HSCT-eligible patients was 73.6 months versus 43.0 months for ineligible patients [24].

According to Moore et al. [25] the incidence of MM is on the rise in Nordic countries and other Western nations. Despite this demographic change, the inclusion of individuals from the older age group in clinical trials can be a challenge as evidenced in studies such as VISTA [26], FIRST [27], ALCYONE [28] and MAIA [29]. Over 65-year-old patients often present clinical conditions that hinder their participation in clinical trials, particularly due to frailty and the complexities involved in testing new therapies. In this context, real world evidence becomes relevant, as it reflects outcomes in the actual MM population, taking into account the age, sex, and other factors [25].

The improvement in OS following the incorporation of novel agents has been well described in the literature. In this study, an OS of 54 months was observed among patients who received thalidomide in the therapeutic regimen. Two studies evaluated the regimen of melphalan and prednisone with or without thalidomide in previously untreated patients and elderly patients. The study by Hulin et al. [30] in over 75-year-old patients with early MM, reported an OS of 45.3 months versus 27.7 months. The study conducted by Facon et al. [31] of over 65-year-old patients showed an OS of 51.6 months versus 33.2 months in the group without thalidomide. These studies reinforce the finding of the benefits of associating thalidomide to the therapeutic regimen and the difference in OS regarding age at diagnosis [30,31].

Table 2 – Estimated risk rate according to the COX proportional analysis model for the total cohort (*n* = 25,370; deaths: 13,042).

Characteristic	HR	95 % CI	*p*-value
Sex			
Female	—	—	
Male	1.09	1.05–1.13	**<0.001**
Age at baseline	1.02	1.02–1.02	**<0.001**
Age range at baseline			
>65 years	—	—	
18–25 years	0.47	0.34–0.66	**<0.001**
26–35 years	0.50	0.43–0.59	**<0.001**
36–45 years	0.48	0.44–0.52	**<0.001**
46–55 years	0.66	0.63–0.70	**<0.001**
56–65 years	0.76	0.73–0.79	**<0.001**
Self-declared skin color			
Asian	—	—	
White	1.36	1.09–1.70	**0.007**
Indigenous	2.38	0.58–9.67	0.23
Unknown	2.26	1.81–2.83	**<0.001**
Brown	1.23	0.98–1.54	**0.080**
Black	1.15	0.90–1.47	0.25
Residence region at baseline			
Central-West	—	—	
North	0.94	0.82–1.08	0.38
Northeast	0.86	0.80–0.93	**<0.001**
South	1.12	1.04–1.21	**0.003**
Southeast	0.93	0.87–1.00	**0.052**
ICD 10 Description at baseline			
Extramedullary plasmacytoma	—	—	
Gammopathy	1.14	0.95–1.35	**0.15**
Multiple myeloma	1.25	1.12–1.41	**<0.001**
Multiple myeloma and malignant plasma cell neoplasms	1.26	0.92–1.72	**0.15**
Plasma cell leukemia	1.49	1.25–1.79	**<0.001**
Cohort entry period			
2000 - 2003	—	—	
2004 - 2007	1.22	1.16–1.28	**<0.001**
2008 - 2011	0.99	0.94–1.03	0.53
2012 - 2015	0.85	0.81–0.90	**<0.001**
Medication at baseline			
Bortezomib Based*	—	—	
Thalidomide Based	1.31	1.07–1.60	**0.009**
Others	1.71	1.41–2.06	**<0.001**
HSCT			
No	—	—	
Yes	0.36	0.34–0.39	**<0.001**
Comorbidity Charlson at baseline	1.06	1.05–1.07	**<0.001**
Frailty Index at baseline	1.00	1.00–1.00	**<0.001**
Mean time of illness before baseline	1.00	1.00–1.01	**<0.001**
Mean time in the cohort	0.95	0.95–0.95	**<0.001**

Thalidomide was market approved in Brazil for MM treatment in 2000 and was integrated into the SUS during the study period [32]. The low percentage of patients using this drug may be due to the need for patient monitoring and guidance, particularly in a vast country like Brazil, where access to Thalidomide and HSCT is more limited outside major urban centers.

In a study conducted by Hungria et al. in five Latin American countries, HSCT was performed in 58.6 % of the patients for whom it was initially planned, and in only 26.9 % of the total patient population [24]. Despite the observed benefits in treatments involving thalidomide or HSCT, and their availability in the SUS, physicians and medical institutions have the possibility to choose which treatments to prescribe for MM patients. The guideline that enumerates the available procedures is not obligatory, leading to variations in therapy access based on the clinical judgment of the medical team.

The median OS for patients using bortezomib was 67 months versus 37 months in the total study population. In the Phase 3 ENDEAVOR study of relapsed or refractory over 18-year-old patients using bortezomib and dexamethasone (Vd), the OS was 40 months [33]. In the VISTA study,

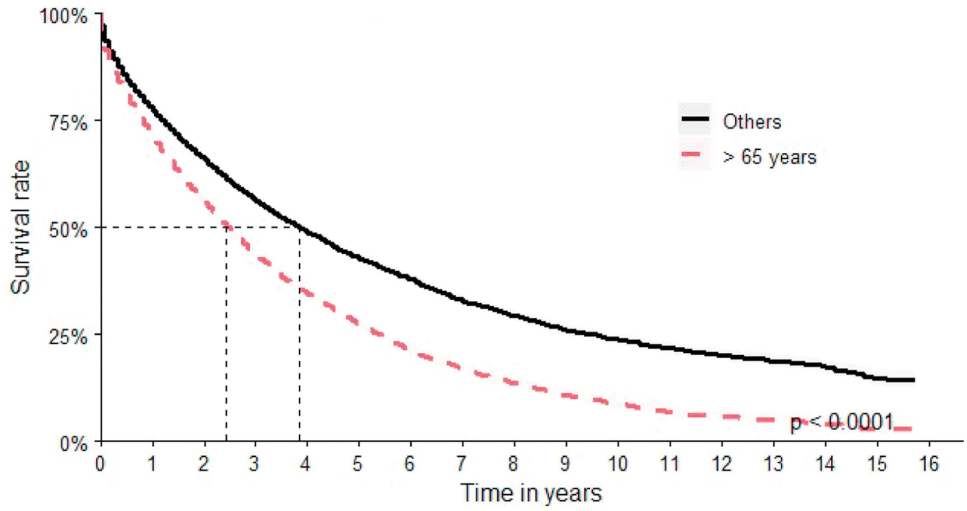

Figure 4 – Overall survival by age.

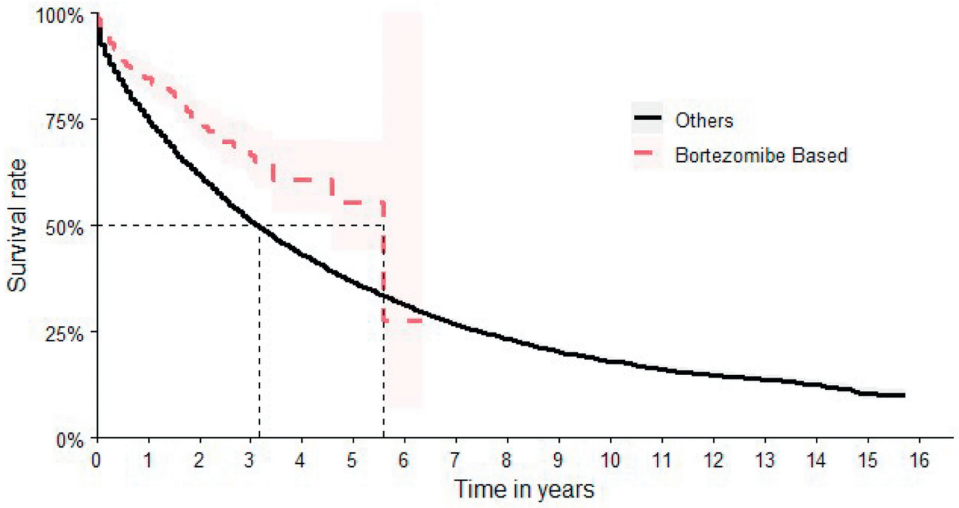

Figure 5 – Overall survival comparing bortezomib-based chemotherapy with other regimens.

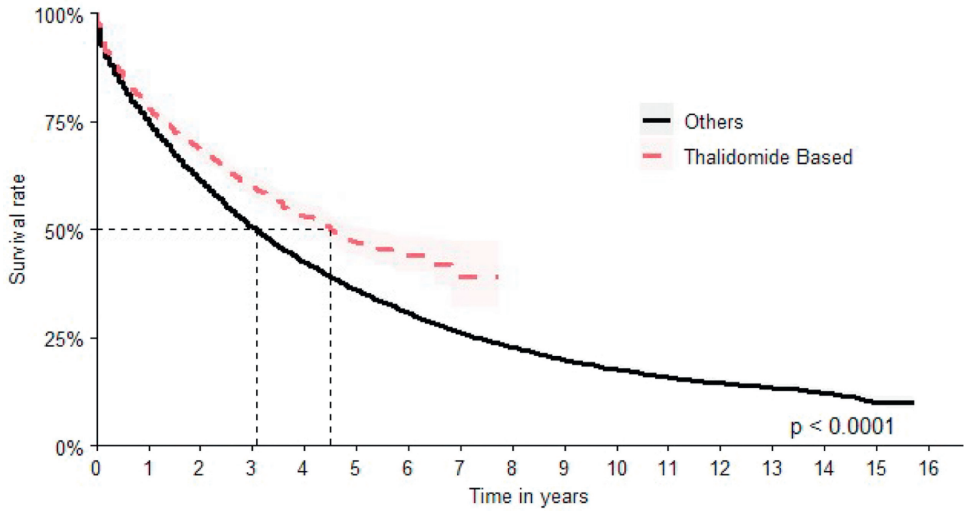

Figure 6 – Overall survival for thalidomide-based chemotherapy.

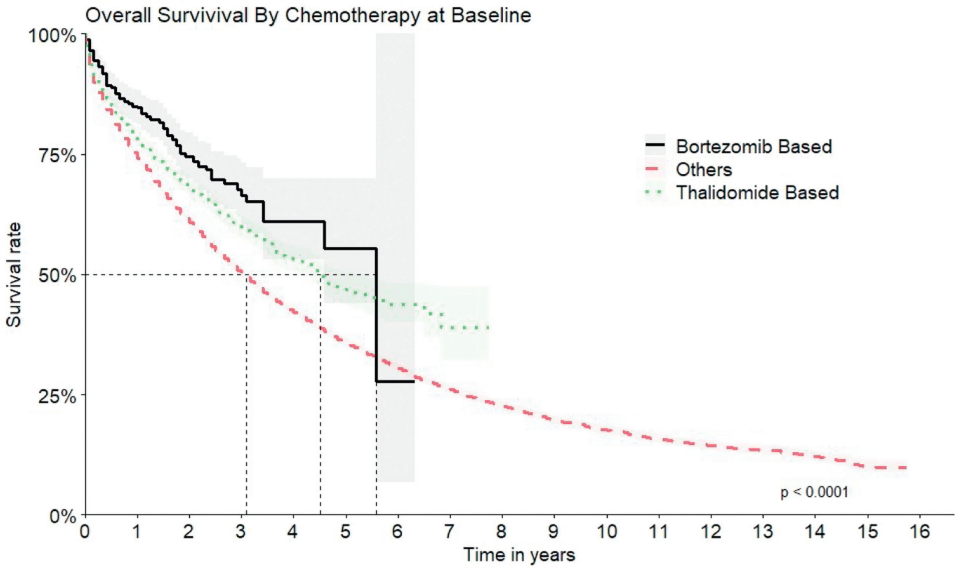

Figure 7 – Overall survival by therapeutic regimen.

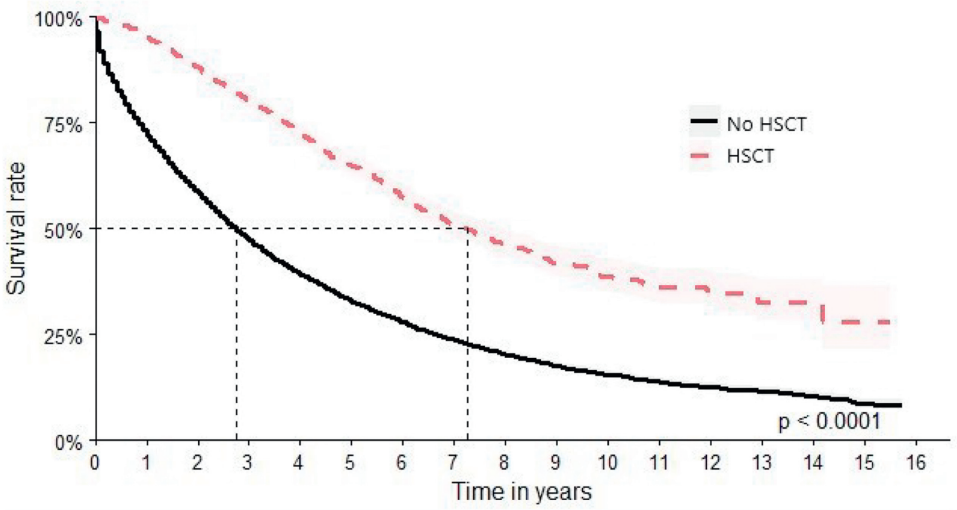

Figure 8 – Overall survival after hematopoietic stem cell transplantation.

previously untreated patients using an association of bortezomib, melphalan and prednisone (VMP), OS was 56.4 months over a five-year follow-up [34], supporting what has already been discussed regarding the increased OS related to the early use of technology.

A key methodological consideration is that patients were classified according to exposure to specific therapeutic agents at any point during the treatment course, rather than being limited to first-line therapy. This methodological choice aimed to assess the overall impact of drug exposure on patient survival across the entire disease trajectory. Although this approach does not allow for the isolation of the effects of bortezomib when used exclusively as first-line treatment, it better reflects the real-world complexity of therapeutic regimens and captures the cumulative benefit associated with access to effective agents. The improved OS observed among bortezomib-exposed patients may partially reflect treatment selection bias and the advantage of longer survival allowing access to subsequent lines of therapy. However, the findings suggest that bortezomib exposure, regardless of treatment line, is associated with favorable survival outcomes. Future studies designed to evaluate line-specific treatment effects are warranted to further elucidate the role of bortezomib in different therapeutic stages.

The Brazilian National Committee for Technology Incorporation (CONITEC) carefully carries out and deliberates on the continuous assessment of new technologies, costs, and equity in access to healthcare. This process considers several factors, such as effectiveness, safety, cost-effectiveness, and epidemiological needs. However, Bortezomib was only

formally incorporated by the CONITEC into the SUS in 2020 thereby explaining the low number of patients treated with this drug in this cohort [9,10].

However, prior to this formal incorporation some factors such as approval for market entry by the national regulatory agency (National Health Surveillance Agency - ANVISA) with its scientific evidence of efficacy, encouraged its use by physicians. Another reason is the model of finance of the oncology service providers in Brazil where certain flexibility is allowed for when prescribing chemotherapy. SUS makes a fixed payment for patient treatment and oncology services providers are free to choose among therapeutic options between approved medicines. Despite the significant progress made by SUS in expanding access to a broad range of therapeutic options, there is still a need for more timely decisions by CONITEC [35] regarding the incorporation of new oncology drugs. Accelerating this process, while maintaining rigorous evaluation criteria, could improve access and reduce delays in the availability of innovative treatments. Litigation about oncology treatments is a major issue in Latin America, especially in Brazil and a faster assessment would reduce the conflict. In the case of Bortezomib, an earlier incorporation into SUS, coupled with adequate funding for oncology services, could have potentially saved lives, given the observed impact on OS in the current study compared to the treatments available at that time within SUS.

Conflicts of interest

The author declares no conflicts of interest.

CRediT authorship contribution statement

Deborah Marta do Santos Oliveira: Formal analysis, Investigation, Methodology, Software, Writing – original draft. **Isabela Cristina Menezes de Freitas:** Formal analysis, Methodology, Visualization, Writing – review & editing. **Wallace Mateus Prata:** Formal analysis, Methodology, Visualization, Writing – review & editing. **Isabella Zuppo Laper:** Formal analysis, Investigation, Methodology, Software. **Pâmela Santos Azevedo:** Formal analysis, Investigation, Methodology, Software. **Adriano de Paula Sabino:** Conceptualization. **Marisa Yurico Itonaga:** Conceptualization. **Carmino Antonio de Souza:** Conceptualization. **Mariângela Leal Cherchiglia:** Conceptualization. **Juliana Alvares Teodoro:** Conceptualization, Supervision. **Francisco de Assis Acurcio:** Conceptualization, Supervision. **Augusto Afonso Guerra Junior:** Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Visualization, Writing – review & editing.

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Original article

Risk factors associated with the use of red blood cells in elective cardiac surgeries: A patient blood management (PBM) view

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ABSTRACT

Background: Blood is a biological, irreplaceable, and perishable resource, provided through voluntary, altruistic, and free donation in Brazil. Although blood components are widely used in hospital settings, several challenges persist, including the limited availability of these resources, the high costs associated with their procurement, storage, and transfusion, as well as the risks inherent in the allogeneic transfusion process due to potential transfusion reactions. Therefore, there is a need to focus on Patient Blood Management (PBM) within the Brazilian transfusion system to reduce the need for transfusions, particularly of packed red blood cells, during elective cardiac surgeries.

Objective: To evaluate the risk factors associated with the use of packed red blood cells in elective cardiac surgeries performed at the Hospital das Clínicas, Faculty of Medicine, Botucatu.

Methods: This retrospective study involving 741 individuals was conducted between 2018 and 2021 with the approval of an ethics research committee. Data were analyzed using descriptive statistics, the Chi-square test, and stepwise logistic regression, with the level of significance set at 5 %.

Results and conclusion: Preoperative factors such as female sex (Odds ratio: 9.074; p-value <0.0001), low hematocrit levels (Odds ratio: 7.498; p-value = 0.0034), and the presence of diabetes mellitus (Odds ratio: 1.779; p-value = 0.0318), as well as intraoperative factors such as extracorporeal circulation time greater than 90 min (Odds ratio: 1.68; p-value = 0.0442), were identified as risk factors for excessive bleeding and the need for packed red blood cells during surgery.

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Introduction

Knowing that transfusion is possible exclusively due to blood donation [1], the rational use of this resource must be evaluated and implemented in blood therapy centers. While transfusions are an undeniable need, several challenges arise. The limited availability of blood components, combined with high costs of procurement, storage, and transfusion, as well as the risks associated with the allogeneic transfusion process – such as transfusion reactions [2–4] - contrasts sharply with the high demand for blood products. This demand is further exacerbated by the constant use of blood components as therapeutic resources, population aging, increasingly restrictive screening criteria [5,6], difficulties in recruiting new donors [3], and festive, climatic and epidemiological events, such as COVID-19 pandemic [7].

Moreover, the irrational use of blood and the failure to minimize unnecessary exposure to blood products [2,8,9] which directly affects already declining stocks, continues to be problems. This issue seems to stem from clinical practices where professionals recommend and approve blood transfusion when other interventions, mainly during the perioperative period, have been shown to be more effective and safer in the treatment of patients [8].

While transfusion of packed red blood cells can be life-saving, it also presents risks to the patient and entails significant costs [2]. These factors disrupt the cost-benefit balance of this type of therapy, underscoring the need for safer and more effective alternatives. In this context, Patient Blood Management (PBM) is particularly relevant given the realities of blood transfusions in Brazil and as recommended by the World Health Organization (WHO) [2].

This program consists of an interdisciplinary, individualized, and multimodal approach based on evidence and medical interventions, when necessary and timely, to maintain the blood mass, minimize the need for allogeneic transfusions, and promote safe and rational use of available blood therapy resources [5,8,10].

To this end, different studies have identified three main strategies or pillars for implementing PBM in healthcare settings: (1) management of the patient's anemia, which includes addressing preoperative anemia through the administration of intravenous or oral iron, for example; (2) minimization of iatrogenic or unnecessary blood loss through blood-sparing surgical techniques, perioperative autologous blood collection and re-transfusion, and the use of hemostatic agents such as antifibrinolytics; and (3) optimization of the patient's specific physiological tolerance to anemia by adhering to transfusion triggers [5,8,9,11].

Regarding the first pillar, literature has shown that the majority of blood transfusions (around 94 %) are due to low preoperative hemoglobin levels (diagnosed in between 20 and 60 % of cases), excessive blood loss during surgery, and iatrogenic transfusion practices [9,12]. These findings are consistent with other studies, which report a prevalence of 22–30 % of preoperative anemia in patients undergoing cardiac surgery. Anemic patients experience worst postoperative outcomes compared to non-anemic patients, including longer hospital stays, higher risk of infectious complications, increased morbidity, and even mortality [6,12].

One the other hand, the implementation of PBM has been associated with improved perioperative outcomes across various types of surgery, including significant reductions in complications and mortality, particularly among cardiac and orthopedic surgery patients [5,13]. In fact, PBM has contributed to enhanced patient safety, cost savings, and a reduction in hospital readmissions [10].

Recent and important studies emphasize the need for perioperative interventions in cardiac surgeries, as these practices substantially reduce transfusion requirements and are associated with better patient health outcomes [8]. Scientific evidence indicates that the need for transfusions increases the risk of morbidity and mortality in these surgeries. As a result, guidelines from the Society of Cardiovascular Anesthesia recommend promoting interventions that increase red blood cell volume to reduce the risk of transfusions of packed red blood cells [13].

In addition to the preexistence of anemia, several other factors may contribute to an increased risk of excessive bleeding as observed in both literature and unpubliched data [14]. These factors include gender [15,16], age [15–17], systemic arterial hypertension, diabetes mellitus, platelet count, prothrombin activation time, activated partial thromboplastin activation time [17] and the use of medications such as anticoagulants, antiplatelet agents, and non-steroidal anti-inflammatory drugs. The potential suspension of these medications should be carefully considered for each surgery candidate due to individual predisposition to perioperative bleeding [17,18].

Similarly, intraoperative factors – those present during the surgical procedure - can also pose a risk for the transfusion of packed red blood cells. For example, the use of extracorporeal circulation and its duration are already known to be high-risk factors for bleeding, directly influencing the number of allogeneic blood units transfused [16,17,19]. Among the various intraoperative measures identified in the literature, the use of topical or systemic agents such as tranexamic-acid and epsilon-aminocaproic acid stands out. When used appropriately, these agents help prevent excessive blood loss during cardiac surgery [5,20,21].

Therefore, this study aimed to evaluate the risk factors associated with the use of packed red blood cells in elective cardiac surgeries from a PBM perspective. It sought to evaluate the preoperative and intraoperative variables related to increased bleeding during these procedures, considering their significant implications for in-hospital practices and medical protocols in relation to patient care and the use of blood components.

Methods

All activities carried out were approved by the Hospital das Clínicas da Faculdade de Medicina de Botucatu (HCFMB) Research Ethics Committee (opinion 5627,357) recognized by the National Research Ethics Commission, and in accordance with the guidelines outlined in Resolution 466/12 of the

Table 1 – Preoperative and intraoperative variables and criteria associated with excessive bleeding risk.

Variables	Operational definitions and criteria for excessive bleeding risk as described in the literature and reference values
Age	Obtained through registration in electronic medical records in preliminary evaluation to surgery. Bleeding risk: elderly[15–17]
Sex	Obtained through registration in electronic medical records. Bleeding risk: male[15,16] and female
Systemic arterial hypertension (SAH)	Verified as a clinical diagnosis prior to surgery and previous pathological history recorded in the electronic medical records. Bleeding risk: occurrence[17]
Diabetes Mellitus (DM)	Verified as a clinical diagnosis prior to surgery and previous pathological history recorded in the electronic medical record. Bleeding risk: not characterized[17]
Hemoglobin (Hb) Levels	Obtained from the last laboratory test collected prior to surgery (up to 10 days) and recorded in the electronic medical record. Risk of bleeding: anemia present[6]. Reference values: Birth (18.0 ± 4.0 g/dL), 3 days (18.0 ± 3.0 g/dL), 1 month (14.0 ± 2.5 g/dL), 2 months (11.2 ± 1.8 g/dL), 3 to 6 months (12.6 ± 1.5 g/dL), 1 year (12.6 ± 1.5 g/dL), 2 to 6 years (12.6 ± 1.5 g/dL), 6 to 12 years (12.5 ± 1.5 g/dL)[22] adult women (14.0 ± 2.0) and adult men (15.5 ± 2.0 g/dL). All adopted by HCFMB
Hematocrit (Ht) Levels	Obtained from the last laboratory test collected prior to surgery (up to 10 days) and recorded in the electronic medical record. Bleeding risk: below 30 %[15,17]. Reference values: Birth (60 ± 15 %), 3 days (56 ± 11 %), 1 month (43 ± 10 %), 2 months (35 ± 7 %), 3 to 6 months (35 ± 5 %), 1 year (34 ± 4 %), 2 to 6 years (37 ± 3 %), 6 to 12 years (40 ± 5 %), adult women (41 ± 5 %)[22] and adult men (47 ± 6 %). All adopted by HCFMB
Platelet Count (PLT)	Obtained from the last laboratory test collected prior to surgery (up to 10 days) and recorded in the electronic medical record. Bleeding risk: below 150,000 cells/mm^3[17]. Reference value adopted by HCFMB: 140,000 to 440,000 10^3/mm^3
Prothrombin activation time	Obtained from the last laboratory test collected prior to surgery (up to 10 days) and recorded in the electronic medical record. Bleeding risk: above 14 s[17]. Reference value adopted by HCFMB: above 14 s, approximately International Normalized Ratio > 1.2
Activated partial thromboplastin time (APTT)	Obtained from the last laboratory test collected prior to surgery (up to 10 days) and recorded in the electronic medical record. Bleeding risk: above 40 s[17]. Reference value adopted by HCFMB: above 40 s, with a normal ratio of ≥ 0.70 to 1.25; above > 1.25
Antiplatelet therapy	Verified in medication prescriptions prior to surgery (up to 5 days) recorded in the electronic medical record. Examples of medications: non-steroidal anti-inflammatory drugs (NSAIDs), such as acetylsalicylic acid (AAS), dipyrone and paracetamol; blood thinners, such as warfarin, heparin, and enoxaparin; and antiplatelet agents, such as ketoprofen, clopidogrel and cilostazol. Bleeding risk: non-interruption or interruption for <5 days before surgery[11,16–18]
History of Covid-19	Obtained in the past history in the years 2020 and 2021 and prior to surgery. Registered in electronic medical records
Type of surgery performed	Recorded in surgical map and electronic medical record. The surgeries analyzed were: Communication closure (intraventricular or interatrial); pacemaker implantation; valve prosthesis implantation; and myocardial revascularization. Risk of bleeding: when ECC is used[16,17]
Classification as reoperation	Record in surgical map and history prior to surgery recorded in electronic medical records. Risk of bleeding: when present[16]
Need for Extracorporeal Circulation	Registered in electronic medical record. Risk of bleeding: when ECC is used[16,17]
Extracorporeal Circulation Time	Obtained through electronic medical records after surgery. Bleeding risk: prolonged ECC, around 90–110 min[15]. Reference Value used by HCFMB: normal up to 90 min[19]
Activated Clotting Time (ACT)	Obtained through electronic medical records after surgery. Reference value used by HCFMB: normal between 80–120 s[19]
Therapy with systemic hemostatic agents	Recorded in electronic medical records with prescription from 5 days before surgery until 1 day after surgery. Medications: tranexamic-acid, epsilon-aminocaproic acid[4,5,18,20]

Source: Adapted from Braga & Brandão, 2018, and Souza, 2022 (unpublished data).

National Health Council. This study was conducted at the Transfusion Agency of the Blood Center at the HCFMB.

This retrospective study is based on data collected from surgical and electronic medical records focusing on preoperative and intraoperative factors associated with the use of packed red blood cells in elective cardiac surgeries performed between 2018 and 2021 (Table 1). It is important to note that the reference values used for statistical analysis are those adopted by the hospital.

The main exclusion criteria were unsatisfactory or incomplete data, meaning patients who did not have all the necessary information for the required parameters; patients with test results that were older than ten days prior to surgery, based on the clinical validity determined by the Laboratory of Clinical Analysis and the Blood Center of the HCFMB, patients who underwent two elective cardiac surgeries simultaneously; and patients who died during surgery, as this event negatively impacted the complete documentation of surgical information and its relevance to the study.

The collected data were subjected to a descriptive analysis, which included the calculation of mean, standard deviation, minimum, maximum, and median values for quantitative

variables, as well as frequencies and percentages for categorized variables. The chi-square test was used to assess associations between the use or non-use of packed red blood cells and the other study variables.

Subsequently, a Logistic Regression (stepwise analysis) was conducted, with the use or non-use of packed red blood cells as the dependent variable and the other variables as explanatory, in order to identify risk factors for the event. A 5 % significance level was applied to all tests, using the SAS 9.4 computer software.

Results

The present study analyzed data from all patients undergoing elective cardiac surgery and who met the inclusion criteria, with a total number of 741 patient records reviewed. The patient's ages ranged from 1–95 years, with a mean age of 61.79 years, a standard deviation of 15.19, and a median age of 63.00 years. These results indicate a higher prevalence of advanced age. Regarding intraoperative parameters, myocardial revascularization surgery was the most common, accounting for 50.88 % of all procedures.

Additionally, more than half of the surgeries (53.31 %) involved the use of extracorporeal circulation, a known major risk factor for bleeding, as reported in the literature. Descriptive statistics for the remaining parameters are presented in Table 2. It is important to note that all patients met the inclusion criteria, and their reference values were based on the hospital's practice for surgeries.

By analyzing the parameters studied and the number of blood bags used in elective cardiac surgeries, an association was found between these factors during the observed period, as shown in Table 2. It is important to note that, only for this statistical analysis, blood bags used to fill the heart-lung system during extracorporeal circulation (when applicable) were excluded from the total count. This was done to ensure that the results solely reflect the transfusion of red blood cell concentrates required to meet the patient's needs in the event of transfusion during the surgical procedure.

Logistic regression was performed using stepwise analysis, with the odds ratio (OR) estimated based on the use or not of packed red blood cells as the response variable and the other factors as explanatory variables. The goals were to identify risk factors for the event. Only four of the evaluated parameters were classified as risk factors, despite other factors being identified as significant by the chi-square test.

The factors associated with a higher risk of requiring transfusions during elective cardiac surgery included female sex (OR: 9.074; 95 % Confidence interval [95 % CI]: 5.218; p-value <0.0001), low hematocrit (OR 7.498; 95 %CI 4.362; p-value = 0.0034), presence of diabetes mellitus (OR 1.779; 95 % CI 1.052; p-value = 0.0318), and cardiopulmonary bypass time exceeding 90 min (OR 1.68; 95 % CI 1.013; p-value = 0.0442).

Discussion

As the prescription for packed red blood cells has become more prevalent in recent years [5,6], the need for a more attentive and careful approach is urgent. This is not only due to the significant financial burden it places on the hospital system and, in many cases, on the government, but also because of the progressive decline in blood donations [3].

Therefore, there is an urgent need to evaluate the factors associated with the use of packed red blood cells during elective cardiac surgeries, in order to identify risk of excessive intraoperative bleeding and the need for transfusions related to these variables. This highlights the importance of understanding PBM Programs [4–6,9], which are crucial for promoting a significant reduction in transfusions. Such programs can help to avoid unnecessary costs, mitigate risk to patients undergoing transfusion therapy, and ensure better control of blood stocks in the blood bank. It is also important to note the data obtained in this study may aid medical practice by providing insights for surgery teams to more carefully consider patients who present one or more of the risk factors for needing transfusions.

The current research yielded preliminary results indicating a significant relationship between the use or not of packed red blood cells and the following parameters: sex, diabetes mellitus, hemoglobin, hematocrit, non-steroidal anti-inflammatory drugs (acetylsalicylic-acid), type of surgery, reoperation, extracorporeal circulation, duration of extracorporeal circulation, and the use of systemic hemostatic agents (Table 2).

Although all these parameters showed some correlation with the use or not of packed red blood cells during the surgical procedure, a secondary logistic regression analysis revealed that only four were associated with significant risk related to the studied outcome. These variables were female sex, diabetes mellitus, hematocrit below the reference value for age adopted by the study hospital (Table 2) and extracorporeal circulation time exceeding 90 min. This factor may have arisen due to the small sample sizes of certain variables (presence or absence, above, normal or below) which were too limited to establish a reliable correlation when compared to the total number of participants in the study.

Nevertheless, although the current study showed a male predominance (64.1 %) of advanced age patients, with a median age of 63 years, which aligns with similar studies [15,17] that identify these as risk factors for excessive perioperative bleeding, an OR 9.074 (p-value <0.0001) was observed for females. While many studies do not support this finding, it may be closely linked to the higher prevalence of anemia in women [23], which could be associated with sex-specific factors such as menstruation, pregnancy, and lactation.

Despite this, the present study observed an OR 7.498 (p-value = 0.0034) for hematocrit levels below the reference values adopted by the study hospital, strongly suggesting the presence of preoperative anemia. This finding is further supported by the significant data observed for hemoglobin (p-value = 0.0001) and by the comparison of the number of individuals with hemoglobin and hematocrit levels below the reference values who received packed red blood cells during surgery versus those who did not. Specifically, 134 (57.8 %) and 132 (56.9 %) individuals with low hemoglobin and low hematocrit, respectively received blood transfusions, compared to 184 (36.3 %) and 188 (37.2 %) who did not.

This situation is further supported by discussions surrounding PBM Programs, which focus on treating anemia, particularly iron deficiency, given that anemia is widely

Table 2 – Descriptive values of preoperative and intraoperative variables and their association with the use or not of packed red blood cells during elective cardiac surgery.

Variable	Subvariable	n (%)	Did not receive packed red blood cells	Received packed red blood cells	p-value
Sex	Female	266 (35.9 %)	149 (29.3 %)	117 (50.4 %)	<0.0001**
	Male	475 (64.1 %)	360 (70.7 %)	115 (49.6 %)	
Systemic arterial hypertension	Present	479 (64.64 %)	323 (63.5 %)	156 (67.2 %)	0.3178
	Absent	262 (35.36 %)	186 (36.5 %)	76 (32.8 %)	
Diabetes mellitus	Present	241 (32.52 %)	153 (30.1 %)	88 (37.9 %)	0.0339*
	Absent	500 (67.48 %)	356 (69.9 %)	144 (62.1 %)	
Hemoglobin	Below	318 (43.03 %)	184 (36.3 %)	134 (57.8 %)	<0.0001**
	Above	13 (1.76 %)	9 (1.8 %)	4 (1.7 %)	
	Normal	408 (55.21 %)	314 (61.9 %)	94 (40.5 %)	
Hematocrit	Below	320 (43.36 %)	188 (37.2 %)	132 (56.9 %)	<0.0001**
	Above	14 (1.90 %)	6 (1.2 %)	8 (3.4 %)	
	Normal	404 (54.74 %)	312 (61.7 %)	92 (39.7 %)	
Platelets	Below	39 (5.28 %)	30 (5.9 %)	9 (3.9 %)	0.2774
	Above	8 (1.08 %)	4 (0.80 %)	4 (1.7 %)	
	Normal	691 (93.63 %)	472 (93.3 %)	219 (94.4 %)	
Prothrombin activation time	Above	66 (8.91 %)	49 (9.6 %)	17 (7.3 %)	0.3082
	Normal	675 (91.09 %)	460 (90.4 %)	215 (92.7)	
Activated partial thromboplastin time	Below	246 (33.20 %)	176 (34.6 %)	70 (30.2 %)	0.2298
	Above	8 (1.08 %)	7 (1.4 %)	1 (0.4 %)	
	Normal	487 (65.72 %)	326 (64.0 %)	161 (69.4 %)	
Non-steroidal anti-inflammatory drugs - total	Present	397 (53.58 %)	254 (49.9 %)	143 (61.6 %)	0.003*
	Absent	344 (46.42 %)	255 (50.1 %)	89 (38.4 %)	
Non-steroidal anti-inflammatory drugs - acetylsalicylic acid	Present	352 (47.50 %)	224 (44.0 %)	128 (55.2 %)	0.0048*
	Absent	389 (52.50 %)	285 (56.0 %)	104 (44.8 %)	
Non-steroidal anti-inflammatory drugs - dipyrone	Present	51 (6.88 %)	35 (6.9 %)	16 (6.9 %)	0.9919
	Absent	690 (93.12 %)	474 (93.1 %)	216 (93.1 %)	
Non-steroidal anti-inflammatory drugs - paracetamol	Present	22 (2.97 %)	16 (3.1 %)	6 (2.6 %)	0.6786
	Absent	719 (97.03 %)	493 (96.9 %)	226 (97.4 %)	
Anticoagulant - total	Present	68 (9.18 %)	49 (9.6 %)	19 (8.2 %)	0.5298
	Absent	673 (90.82 %)	460 (90.4 %)	213 (91.8 %)	
Anticoagulants - Warfarin	Present	3 (0.40 %)	1 (0.2 %)	2 (0.9 %)	0.1858
	Absent	738 (99.60 %)	508 (99.8 %)	230 (99.1 %)	
Anticoagulants - enoxaparin	Present	58 (7.83 %)	44 (8.6 %)	14 (6.0 %)	0.22
	Absent	683 (92.17 %)	465 (91.4 %)	218 (94.0 %)	
Anticoagulants - heparin	Present	8 (1.08 %)	5 (1.0 %)	3 (1.3 %)	0.7042
	Absent	733 (98.92 %)	504 (99.0 %)	229 (98.7 %)	
Antiaggregant - total	Present	14 (1.89 %)	9 (1.8 %)	5 (2.2 %)	0.7197
	Absent	727 (98.11 %)	500 (98.2 %)	227 (97.8 %)	
Antiplatelet agent - ketoprofen	Present	1 (0.13 %)	1 (0.20 %)	0	0.4993
	Absent	740 (98.87 %)	508 (99.8 %)	232 (100 %)	
Antiplatelet agent - Cilostazol	Present	3 (0.40 %)	3 (0.6 %)	0	0.2413
	Absent	738 (99.60 %)	506 (99.4 %)	232 (100 %)	
Antiplatelet agent - clopidogrel	Present	12 (1.62 %)	7 (1.4 %)	5 (2.2 %)	0.4354
	Absent	729 (98.38 %)	502 (98.6 %)	227 (97.8 %)	
COVID-19	Present	7 (0.94 %)	7 (1.4 %)	0	0.0727
	Absent	734 (99.06 %)	502 (98.6 %)	232 (100 %)	
Type of surgery	Communication closure	30 (4.05 %)	12 (2.4 %)	18 (7.8 %)	<0.0001*
	Pacemaker implant	229 (30.9 %)	228 (44.8 %)	1 (0.4 %)	
	Valve prosthesis implantation	105 (14.17 %)	40 (7.9 %)	65 (28.0 %)	
	Revascularization of the myocardium	377 (50.88 %)	229 (45.0 %)	148 (63.8 %)	
Reoperation	Present	115 (15.52 %)	101 (19.8 %)	14 (6.0 %)	<0.0001*
	Absent	626 (84.48 %)	408 (80.2 %)	218 (94.0 %)	
Extracorporeal circulation	Present	395 (53.31 %)	191 (37.5 %)	204 (87.9 %)	<0.0001*
	Absent	346 (46.69 %)	318 (62.5 %)	28 (12.1 %)	
Extracorporeal circulation time	Above	245 (63.97 %)	108 (58.1 %)	137 (69.5 %)	0.0194
	Normal	138 (36.03 %)	78 (41.9 %)	60 (30.5 %)	
Activated clotting time	Below	5 (1.29 %)	2 (1.0 %)	3 (1.5 %)	0.5723
	Above	249 (64.01 %)	118 (61.8 %)	131 (66.2 %)	
	Normal	135 (34.70 %)	71 (37.2 %)	64 (32.3 %)	
Systemic hemostatic agents - total	Present	2 (0.27 %)	0	2 (0.9 %)	0.0359*
	Absent	739 (99.73 %)	509 (100 %)	230 (99.1 %)	

Table 2 (*continued*)

Variable	Subvariable	n (%)	Did not receive packed red blood cells	Received packed red blood cells	p-value
Systemic hemostatic agents - Tranexamic acid	Present	1 (0.13 %)	0	1 (0.4 %)	0.1383
	Absent	740 (99.87 %)	509 (100 %)	231 (99.6 %)	
Systemic hemostatic agents - Epsilon-aminocaproic acid	Present	1 (0.13 %)	0	1 (0.4 %)	0.1383
	Absent	740 (99.87 %)	509 (100 %)	231 (99.6 %)	

The categories 'below', 'above' and 'normal' are related to reference values as adopted by the HCFMB.

* Statistically significant values, *p*-value <0,05.
** Statistically significant values, *p*-value <0,0001.

recognized as an important factor contributing to the need for transfusions [5,6,11,15,17]. An additional key factor in this context is the association between preoperative anemia and comorbidities, advanced age, and female gender - all of which are identified as risk factors for adverse outcomes following cardiac surgery [24].

Regarding the duration of extracorporeal circulation exceeding the reference values adopted by the hospital (over 90 min), with an OR 1.68 (p-value = 0.0442), a relationship is observed with findings from other studies [15,16]. This is consistent with the preliminary results of the present study, which also showed significant associations with the use of extracorporeal circulation (p-value <0.001) and the type of surgery performed (p-value <0.001). These findings may be particularly influenced by myocardial revascularization surgery followed by valve prosthesis implantation, which are associated with a prolonged use of extracorporeal circulation.

Cardiopulmonary bypass is potentially one of the main causes of bleeding during the perioperative period due to coagulation disorders and the activation of fibrinolysis, factors that are further exacerbated by prolonged usage. This is also considered one of the primary contributors to bleeding in cardiac surgeries involving extracorporeal circulation [14,21]. Additionally, it is known that patients with a hematocrit below 40 % who require extracorporeal circulation have a higher likelihood of needing blood transfusions during myocardial revascularization surgery, which is a concern regarding the use of blood components [4].

Finally, although the presence of diabetes mellitus was classified for the present study with OR 1.779 (p-value = 0.0318), a finding that contrasts with what is generally reported in the literature [17], this comorbidity is considered prevalent in complicated patients undergoing cardiac surgery [25]. Furthermore, its prevalence is significantly associated with the group of patients who receive transfusions, as seen in various studies, whether related to other diseases or not [26,27].

Thus, it is evident that hyperglycemia is closely linked to cardiovascular events due to chronic inflammation [28], as well as to the occurrence of systemic events, particularly those related to atherosclerosis, which results from the severity and duration of insulin resistance (IR) in diabetic patients [29,30]. Pre-existing anemia [27,31] and altered hemostatic events further contribute to this relationship.

Although the literature presents an extensive range of issues regarding diabetes mellitus, it is still not possible to prove the relationship between the presence of this comorbidity and the need for transfusions, thus raising speculations on the subject as presented below:

(1) Anemia: The first proposal highlights the need for transfusions due to pre-existing anemia in diabetic patients. Type 2 diabetes mellitus, due to the hemodilution caused by high glucose levels in the body [31], contributes to reduced hemoglobin and hematocrit values, indicating anemia [27,31]. Two additional sub-proposals included in this item focus on the role of erythropoietin, which may be insufficient in the presence of this comorbidity, especially when considering the hypoxia induced by the disease [31] and the reduction in hemoglobin levels. Furthermore, diabetic patients are more likely to have iron deficiency due to impaired intestinal absorption, which further contributes to anemia, along with an impaired ability to produce erythropoietin in response to decreased hemoglobin [27].
(2) Vascular complications: It is well established that insulin resistance is strongly associated with endothelial dysfunction, especially when proinsulin levels are elevated, which stimulates the production of plasminogen activator inhibitors and impairs fibrinolysis [29,30]. A study conducted on patients undergoing transcatheter aortic valve implantation found that clinically significant bleeding complications were linked to the presence of diabetes mellitus and associated with vascular diameters [32]. Additionally, the prevalence of hyperfibrinogenemia in patients with type 2 diabetes mellitus, where fibrinogen levels contribute to an increasing cardiovascular risk [33], was observed. This may indirectly relate to the need for transfusions, although this connection has not been extensively discussed in the literature.
(3) Women: Carotid atherosclerosis caused by diabetes mellitus was found to be more prevalent in women than in men, along with increased oxidative stress and endothelin-1 levels, which contribute to vasoconstriction and platelet aggregation. This leads to a more pro-thrombotic fibrin profile, promoting the formation of denser fibrin clots and prolonged fibrinolysis [34], as well as a higher risk of vascular complications, which are more pronounced in women [35]. These factors are associated with the gender-related findings on the use of packed red blood cells in the present study. Therefore, an important aspect to highlight about women is their concurrent incidence of diabetes mellitus. The explanation for this finding lies in the fact that diabetes mellitus diminishes the development of heart disease and

nephropathies, predisposing them to higher cardiovascular risks compared to men. This is related to genetic factors, endocrine disorders, psychosocial stress, and differing sociocultural behavior between women and men [34].

Although all of the factors mentioned above are clinically significant, it is important to note that, despite the patients in the present study being under medical care shortly before surgery, given that the procedures were classified as elective, many of the individuals were likely not admitted to the hospital immediately prior to the procedure. This may have led to a lack of control over their daily care, particularly concerning the proper use of medication and adherence to the recommended diet for diabetes mellitus.

In addition, body mass index calculations, blood glucose measurements before surgery, and classification between type 1 and 2 diabetes mellitus were not provided, highlighting the absence of important data to better understand the relationship between diabetes mellitus and the need for transfusions.

Finally, it is important to emphasize that, although the literature describes the connection between diabetes mellitus and complications in cardiovascular diseases, there are still no pathophysiological explanations that establish a causal link between the presence of this comorbidity and the need of transfusions, even though this relationship has been observed. Therefore, experimental studies are necessary to offer a clearer explanation of the data observed in the present study.

Conclusion

Preoperative risk factors, such as female sex, decreased hematocrit, and presence of diabetes mellitus, along with intraoperative risk factors, such as the use of extracorporeal circulation for >90 min, have been shown to be associated with the use of packed red blood cells in elective cardiac surgeries conducted between 2018 and 2021.

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Conflicts of interest

During the preparation of this work, the author(s) used ChatGPT to improve the translation into English. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Haploidentical hematopoietic stem cell transplantation with post-transplant cyclophosphamide in the public Chilean national health system: A single center study

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ABSTRACT

Introduction: Haploidentical peripheral stem cell transplantation with post-transplant cyclophosphamide is the most common modality in low-and-middle-income countries. This article reports the consecutive adult patients who received this modality of transplant in a single center in Chile between 2016-2021.

Methods: The primary outcome was overall survival. Secondary outcomes were event-free survival, II-IV acute graft-versus-host disease at Day +100, chronic graft-versus-host disease at two years and cumulative incidence of relapse.

Results: The median age was 25 years (Range: 15-51), and 65 % of patients were male. Ninety-four percent had a neoplastic disease (77/82), with the most common diagnosis being acute lymphoblastic leukemia (57 %). Forty-seven percent proceeded to transplant in the first complete response. Conditioning was mostly myeloablative (96 %). Primary graft failure and poor graft function were observed in 1.2 % and 13 %, respectively with five patients (6.1 %) dying before engraftment. Grade II-III acute graft-versus-host disease was seen in 29 % and chronic graft-versus-host disease was 41 % of the patients. With a median follow-up of 33 months (Range: 1-84), the estimated three-year overall survival and event-free survival were 68.3 % (95 % CI: 59–79 %) and 64.6 % (95 % CI: 55–76 %), respectively. The three-year cumulative incidence of relapse was 23 % (95 % CI: 15–33 %).

Conclusion: These results demonstrate encouraging survival outcomes and acceptable rates of graft-versus-host disease following haploidentical peripheral stem cell transplantation with post-transplant cyclophosphamide, suggesting its potential as a feasible option in low-resource settings.

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) offers a therapeutic option for varied neoplastic and non-neoplastic hematologic diseases. In patients without a matched sibling donor (MSD), alternative sources like HLA-matched unrelated donors (MUD) or umbilical cord, have extended the access to this therapy, but usually associated with a longer latency, and higher complexity and cost. In this context, the option of haploidentical (Haplo) related donors identifies suitable donors for almost 95 % of patients. This alternative source has become a valid option for ethnic minorities and for low- and middle-income countries (LMIC) with limited access to MUD.

Haplo-HSCT was introduced in Chile for the first time at the Luis Calvo Mackenna Children Hospital, with technological support of the St. Jude Children's Research Hospital providing encouraging results. However, the complexity and cost of ex-vivo T-lymphocyte depletion prevented its widespread use [1,2]. In 2001, investigators from Johns Hopkins Hospital published a Phase I/II trial of Haplo-HSCT with in-vivo T-lymphocyte depletion with post-transplant cyclophosphamide (Haplo-PTCy) [3]. Using this platform, meta-analysis and non-randomized studies have shown similar or even, better results, when Haplo donors are compared with MSD and MUD [4–7]. These results explain why this modality of hematopoietic stem cell transplantation (HSCT) has increased worldwide [8,9]. In 2020, Sarmiento et al. reported the first local series including 49 cases in a private institution with a three-year overall survival (OS) of 48 % [10]. At the public level, a national adult HSCT program for public health insurance patients, was implemented in 2010. Limited transplantation teams and budgets forced to impose important access limitations in age and donor type. At the beginning only allo-HSCT recipients up to 40 years old with an MSD were included. MUD transplantation was not considered given its high cost and the low chance of successful searches for ethnic minorities, like those of Chile. In 2016, Haplo-PTCy was incorporated for patients up to 40 years of age, and expanded up to 60 years in 2019. The aim of this study is to describe the outcomes of the first 82 consecutive adult Haplo-PTCy transplants between 2016-2021 at the main public center of the Chilean national HSCT program.

Materials and methods

Patients

All patients that received an Haplo-PTCy at the Intensive Hematological Unit, Hospital del Salvador, from 2016 to 2021 were included. As mentioned, all cases belonged to the national HSCT program, and were approved by a national adult HSCT committee following a specific Haplo-HSCT protocol established in 2016. Inclusion criteria were age ≥15 and ≤40 from 2016-2018 and ≤60 years old since 2019, Eastern Cooperative Oncology Group (ECOG) performance status <3, no concomitant active cancer, and adequate organ function. For patients with acute leukemias, complete response before transplantation was mandatory. This study was performed according to the Helsinki declaration and was approved by the institutional ethics committee.

Endpoint and definitions

The main endpoints were event-free survival (EFS), OS, cumulative incidence of relapse (CIR), non-relapse mortality (NRM) at two years and graft-versus-host disease (GvHD)-free, relapse-free survival (GRFS) at one year after transplantation. Secondary endpoints were incidence of Grade II-IV acute graft-versus-host disease (aGvHD) at Day +100 and chronic GvHD (cGvHD) at two years. OS was calculated from the day of infusion until the last visit or death by any cause. Glucksberg criteria were used for aGvHD [11,12]. Chronic GvHD was graded using the National Institutes of Health (NIH) criteria [13]. NRM was defined as death from any cause other than relapse. EFS was calculated from the day of infusion until the day of relapse, graft failure or death. GRFS was defined as one year post-transplant survival without Grade III-IV aGvHD, systemic therapy required for cGvHD, relapse, or death.

The haploidentical donor was defined based on molecular techniques for HLA-A, HLA-B and HLA-DRB1 loci. Disease stage at the time of transplantation was classified by the Disease Risk Index (DRI) [14]. The hematopoietic cell transplantation-specific comorbidity index (HCT-CI) was used to stratify patients according to pre-transplant comorbidities [15].

Myeloablative conditioning (MAC) was defined as a regimen containing either total body irradiation with a dose greater than 6 Gy, a total dose of oral busulfan greater than 8 mg/kg bodyweight, or a total dose of intravenous busulfan >6.4 mg/kg bodyweight [16].

Cytokine release syndrome (CRS) was defined as post-infusion fever up to Day +6, with no clinical focus nor microbiologic agent identified and classified according to Lee (Supplementary Table 1) [17]. Neutrophil engraftment was defined as the first day of an absolute neutrophil count $\geq 0.5 \times 10^9$/L lasting for three or more consecutive days and platelet engraftment as $\geq 20.0 \times 10^9$/L for five consecutive days without transfusional support. Graft failure (GF) was defined as either lack of initial engraftment of donor cells (primary graft failure) or loss of donor cells after initial engraftment (secondary graft failure) with donor chimerism ≤5 %. Engraftment syndrome (ES) was defined as the presence of fever, weight gain, skin rash, and/or respiratory distress according to Spitzer classification [18]. Poor graft function (PGF), as frequent dependence on blood and/or platelet transfusions and/or growth factor support with donor chimerism >5 % in the absence of relapse, drugs, or infections [19]. Quality of life was evaluated based on Karnofsky performance scale.

Treatment

Hematopoietic progenitors were obtained from unmanipulated peripheral blood mobilization. The best donor was selected, prioritizing negative specific anti-HLA antibodies (DSA) and crossmatch: age <40 years, male gender, ABO compatibility and lower parity of female donor. The conditioning protocols are shown in Table 1 [20–23].

Table 1 – Patient and transplantation characteristics (n = 82).

	n, (%)
Age, median (years) - n (range)	25 (15-51)
Male sex - n (%)	56 (68)
Disease - n (%)	
ALL	47 (57)
AML/MDS	25 (31)
SAA	3 (4)
PNH	2 (2)
HL	2 (2)
CML	2 (2)
BPDCN	1 (1)
DRI - n (%)	
Low	9 (11)
Intermediate	46 (56)
High	25 (30)
Not assessed	2 (2)
HCT-CI - n (%)	
Low	60 (75)
Intermediate	17 (21)
High	4 (5)
Type of conditioning - n (%)	
Flu (120 mg/m^2) TBI (6×2Gy)	34 (41)
Bu (16 mg/kg/PO) Flu (120 mg/m^2)	26 (32)
Cy (120 mg/kg) TBI (2×6Gy)	10 (12)
Bu (16 mg/kg/PO) Cy (120 mg/kg)	5 (6)
Flu (150 mg/m^2) Cy (29 mg/kg) ATG (7.5 mg/m2) TBI (2 Gy)*	4 (5)
Bu (8 mg/kg/oral) Flu (150 mg/m^2) Cy (29 mg/kg)*	3 (4)
Donor relationship - n (%)	
Sibling	49 (60)
Parent	17 (21)
Other	16 (19)
Donor-Specific Antibodies - n (%)	
Negative	71 (87)
Positive	1 (1)
Not assessed	10 (12)
ABO Incompatibility - n (%)	
No/Minor	69 (84)
Major/bidirectional	13 (16)
Donor/Receptor CMV status - n (%)	
D+/R+	70 (85)
D+/R-	8 (10)
D-/R+	3 (4)
D-/R-	1 (1)

ALL: Acute lymphoblastic Leukemia; AML/MDN: Acute Myeloid Leukemia/myelodysplastic neoplasms; SAA: Severe Aplastic Anemia; CML: Chronic Myeloid leukemia; PNH: Paroxysmal Nocturnal Hemoglobinuria; HL: Hodgkin Lymphoma; BPDCN: Blastic Plasmacytoid Dendritic Cell Neoplasm; PBSC Peripheral Blood Stem Cell; *: Reduced Intensity Conditioning; PO: orally; FLU Fludarabine; TBI Total Body Irradiation; Bu Busulfan; ATG Thymoglobulin; Cy Cyclophosphamide; GVHD Graft versus Host Disease; CNI Calcineurins; MMF Mycophenolate Mofetil; PT-Cy Post Transplant Cyclophosphamide; D donor; R recipient.

Patients were hospitalized in individual isolation units with positive pressure and 4th generation high efficiency particulate air filters and were assisted by a multidisciplinary Intensive Hematology team. According to the institutional protocol, filgrastim 300 mcg/d sc was universally used from Day +5 until absolute neutrophil count (ANC) >1000. Additional filgrastim, erythropoietin and eltrombopag were allowed if sustained or progressive cytopenias due to infections, drug toxicity, PGF or GF were observed.

Acute GvHD prophylaxis was performed with cyclosporine aiming at levels between 250 and 350 ng/mL from Day +5, mycophenolate 1 g every 8 h orally from Day +5 to Day +35 and in vivo T-cell depletion with cyclophosphamide 50 mg/kg bodyweight iv, on Days +3 and +4 [24].

Antibacterial prophylaxis with ciprofloxacin, acyclovir and fluconazole was used. Febrile neutropenia was managed according to the institutional protocol. Acute GvHD was treated according to its severity. Briefly, global Stage I cases were observed or treated with topical or low-dose systemic steroids (equivalent to prednisone 0.5 mg/kg bodyweight/day). Stage II were treated with standard dose steroids (prednisone 1 mg/kg bodyweight/dose). For Stage III-IV aGvHD, high-dose steroids (prednisone 2 mg/kg bodyweight/dose) were used as first line and calcineurin inhibitors were optimized or restarted. If no response was achieved, mycophenolate or methotrexate was used as second-line treatments. Ruxolitinib was not available during the study period.

Discharge was indicated when engraftment, full oral medication and fluid intake >2.5 L/24 h were achieved. Cyclosporine levels were monitored weekly and cytomegalovirus (CMV) by real time polymerase chain reaction (RT-PCR) biweekly until Day +100. CMV reactivation was defined as a viral load exceeding 1000 copies/mL. CMV disease was diagnosed in cases of clinical signs and symptoms. Preemptive treatment with valganciclovir was used as the first choice in cases of CMV reactivation. Ganciclovir was used in CMV disease. Foscarnet was used in case of severe cytopenias or ganciclovir refractoriness.

Statistical analysis

All epidemiological data and clinical characteristics of the patients were expressed as frequencies with percentages for categorical variables and the mean with range for numeric variables. OS and EFS were estimated using the Kaplan-Meier method. The cumulative incidence of relapse was calculated using relapse as the primary event and death without relapse as a competing event. R software was used for statistical analysis.

Results

The characteristics of patients, donors and conditioning regimens are shown in Table 1. Eighty-five haploidentical transplants were performed during the period of analysis. Three second Haplo-PTCy were excluded. Mean age was 25 years (Range: 15-51 years), 94 % (77/82) were for neoplastic diseases with the most common diagnosis (57 %) being acute lymphoblastic leukemia. Sixty-one percent of patients underwent transplants less than one year after diagnosis and 47 % proceeded to transplant in the first complete response. Most patients (76 %) had low-risk HCT-CI scores. Conditioning was mostly myeloablative (96 %). The median number of CD34$^+$ cells infused was 8.02 × 10^6/kg bodyweight (Range: 2.42-10.02×10^6/kg bodyweight). No patient needed a

desensitization regimen. All patients received aGvHD prophylaxis with the planned protocol. PHSP were used in all patients, including those with non-malignant diseases, to reduce the risk of graft failure and to avoid the risk of SARS-CoV2 transmission during the pandemic [25].

Early post-transplantation events

Grade 1-2 CRS was seen in 83 % however no Grade 3-4 CRS was observed. Almost all (98 %) patients had mucositis and 61 % needed parenteral nutrition, with a median duration of 11 days (Range: 2-29 days). All patients had at least one FN episode, 56 % with a gastrointestinal focus. Bacteremia was observed in 23 % of the patients, with *Staphylococcus epidermidis* being the most common agent. Eighteen percent of patients had a probable invasive aspergillosis.

Neutrophil engraftment was achieved in 96 % of patients, (Median: Day +17; Range: 11-25). Platelet engraftment (>20 $\times$ 10^9/L) was achieved in 95 %, (Median: Day +19; Range: 9-84) and platelets >50$\times$$10^9$/L (Median: Day +19; Range: 10-175). PGF was observed in 11 (13 %) patients. Eltrombopag was used in 37 %.

Criteria for ES were identified in 21 (26 %) patients; two patients required low-dose vasopressor drugs or non-invasive ventilatory support, both for less than 48 hours. All patients had a good response with low dose steroids. No mortality was observed.

The incidence of Grade II-IV aGvHD and Grade III-IV at 100 days were 29 % and 5 %, respectively. Grade IV was not observed (Table 2). The median onset was on Day +31 (Range: 13-83): most (89 %) had a good response to oral prednisone (0.5-1 mg/kg bodyweight) with only three (4 %) receiving 2 mg/kg bodyweight. Mycophenolate was used as the second line in four patients and methotrexate in one.

Table 2 – Acute Graft-Versus-Host staging (n = 82).

	n (%)
Skin aGVHD	**40 (49)**
I	17 (21)
II	12 (15)
III	11 (13)
IV	0 (0)
Liver aGVHD	**2 (2)**
I	0 (0)
II	2 (2)
III	0 (0)
IV	0 (0)
Upper gastrointestinal aGVHD	**30 (37)**
I	27 (33)
II	3 (4)
III	0 (0)
IV	0 (0)
Lower gastrointestinal aGVHD	**4 (5)**
I	1 (1)
II	2 (2)
III	1 (1)
IV	0 (0)

Data is presented in simple rates at Day +100, and using the Glucksberg criteria (see text).
aGVHD: Acute Graft-versus-Host Disease.

The present cohort showed a two-year incidence of cGvHD of 41 % (31/75) with a median time of presentation on Day +187 (Range: 112-562). Nine (12 %) patients developed moderate-to-severe cGvHD requiring systemic treatment. Overall, complete response was observed in 25 (33.3 %), and partial response in 5 (6.7 %) patients with one patient presenting progressive disease. There were two cGvHD -related deaths, one due to refractory pulmonary cGvHD and one with systemic progression due to poor treatment compliance.

Cytomegalovirus (CMV) reactivation was observed in 63 % (50/82) of patients at a median of Day +37 (Range: 15-77). Of these patients, four developed CMV disease. The median viral load for these reactivations was 1595 copies/mL (Range: 39-87,000). Treatment with valganciclovir was effective in 66 % of cases. Four patients required treatment with ganciclovir, and one patient received foscarnet. Three patients (4 %) developed post-transplant lymphoproliferative disorder, with one requiring chemotherapy.

Main endpoints

With a median follow-up of 33 months (Range: 1-84), the estimated three-year EFS and OS of the whole cohort were 64.6 % (95 % confidence interval [95 % CI]: 55-76 %) and 68.3 % (95 % CI: 59-79 %), respectively. Patients with neoplastic disease (n = 77), had a three-year CIR of 23 % (95 % CI: 15-33 %). The two-year NRM cumulative incidence was 13.4 % (95 % CI: 12.2-14.8 %). GRFS at one year was 40.2 % (Figure 1).

Cause of death

As a whole, 26 patients died, 16 due to relapse at a median Day +250 (Range: 81-510), eight due to transplant related mortality (three to sepsis before engraftment from Klebsiella pneumoniae carbapenemase-producing bacteria when specific antibiotic therapy was not available, two due to secondary GF, one on Day +53 due to aGvHD and two on Days +273 and +276 due to cGvHD). Two patients died due COVID-19 pneumoniae on Days +97 and +228.

Discussion

This is the first account of Haplo-PTCy in the public health system in Chile. It reports the experience in 82 consecutive patients, showing that it is a feasible and safe procedure to be considered in the absence of an MSD.

These results are encouraging, similar to other adult cohorts using peripheral blood stem cells [7,25–30]. The two-year OS in those studies range between 57- 68 %, while our cohort shows a 3-year OS of 68 %. Regarding aGvHD, the incidence of Grade II-IV aGvHD was also consistent with the results previously reported by other groups, ranging from 18–42 % globally, and 8–14 % for Grade III-IV, compared with 29 % and 5 % in the preset study, respectively. The two-year cumulative NRM incidence of 0.134 is also similar to that reported in the same studies (16–28 %), as well the two-year CIR of 0.22 (Range: 17–36 %). Finally, our study also shows a good quality of life after Haplo-PTCy with a one-year GRFS of 40 %, compared with 23–43 % in the literature.

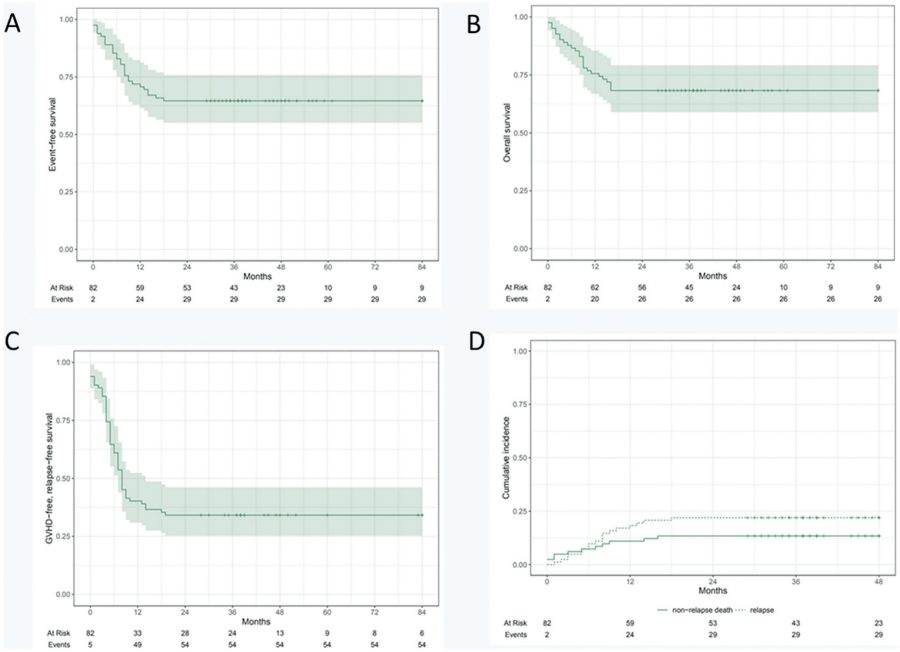

Fig. 1 – Overall survival (A), event-free survival (B), GVHD-free, relapse-free survival (C) and non-relapse mortality (D).

When comparing the OS, EFS, CIR and GRFS of the current study to the aforementioned reports, one must consider that this was a carefully selected cohort of young patients. The median age was 25 years (compared to 41-60 years in other studies), and all patients met strict response criteria before transplantation (every patient with acute leukemia was in CR). These variables have been consistently identified as significant good risk factors for OS and EFS after HSCT [15,31,32]. Furthermore, studies of Haplo-PTCy in children with acute lymphoblastic leukemia, with a median age of 10-12 years, from Spain and China, have shown OS of 59–82 % resembling the OS of our young cohort [33,34]. Another factor could be the young age of most of the donors, with a median of 29 years of age (Range: 15-63). Younger donors have also been associated with better outcomes in the Haplo-PTCy setting [35].

This study has several limitations. As mentioned, it is a single-center cohort with a relatively small number of highly selected patients, and it is not possible to compare directly with prior studies. Additionally, we could not compare it with other types of donors, namely MSD (low number of cases) or MUD (not available).

Conclusions

In conclusion, the experience of this center adds to the evidence that Haplo-PTCy is a safe and effective allogeneic transplant option, when following strict inclusion criteria. The results are comparable to the literature and stand out as the center is a public institution in a LMIC characterized by less investment and facilities in public health.

Author contribution

BP: Concept/design, Data collection, Data analysis/interpretation, Drafting article, Critical revision of article, Approval of article, Statistics; FB: Data collection, Critical revision of article, Approval of article; JM: Concept/design, Drafting article, Critical revision of article, Approval of article; RB: Data analysis/interpretation, Drafting article, Critical revision of article, Approval of article, Statistics; AA: Concept/design, Critical revision of article, Approval of article; AM Concept/design, Critical revision of article, Approval of article; MEC: Data analysis/interpretation, Drafting article, Critical revision of article, Approval of article; MK: Concept/design, Data analysis/interpretation, Drafting article, Critical revision of article, Approval of article

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.2025.103982.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Experience with second allogeneic hematopoietic stem cell transplantation in Chilean patients: A single-center study

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ABSTRACT

Introduction: Allogeneic hematopoietic stem cell transplantation is potentially a curative treatment for several hematological diseases. However, post-transplant relapse remains a significant challenge. For patients who achieve a second complete remission, a second allogeneic transplantation may be a promising therapeutic option. The aim of this study was to analyze clinical outcomes including graft-versus-host disease, non-relapse mortality, and relapse rates, as well as graft sources in patients who underwent a second allogeneic transplantation in a university-based transplant program.

Patients and Methods: A retrospective analysis of 21 adult patients who underwent a second allogeneic transplantation between 2001 and 2023 was performed. Data on demographics, underlying disease, graft source, conditioning, graft-versus-host disease, relapse, and survival were collected. Survival estimates were calculated using the Kaplan–Meier method.

Results: The graft source was bone marrow in 60 % and peripheral blood in 40 % of cases. Grade III–IV acute graft-versus-host disease occurred in 5 % and extensive chronic graft-versus-host disease in 17 %. The non-relapse mortality was 69.2 %, and disease relapse occurred in 23.1 %. The one-year progression-free survival was 26.5 %, and overall survival was 42.3 %. Compared to those transplanted before 2010, patients who underwent transplantation after 2010 showed improved two-year PFS and OS, reaching 55 % and 45.4 %, respectively.

Conclusion: A second allogeneic transplantation may offer a survival benefit in selected patients with relapsed hematologic malignancies or bone marrow failure syndromes. Despite high non-relapse mortality, outcomes have improved in recent years with better salvage strategies.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is administered with curative intent in multiple hematologic disorders. However, a substantial proportion of patients relapse following this treatment. A second allogeneic transplantation (ALO2) is an option for a subgroup of patients who achieve remission after relapse, as demonstrated by leading transplant centers, predominantly in developed countries [1]. In Chile, the National Public Health Transplantation Program does not offer an ALO2 to patients who experience relapse after HSCT. Nevertheless, university and private centers provide ALO2 to fit patients who achieve a second remission.

Patients who relapse after an initial HSCT have a dismal prognosis and poor long-term survival [2,3]. Recent developments in salvage therapies have made it possible for selected patients to achieve remission and proceed to ALO2. In this context, the objective of this study was to analyze the outcomes of patients undergoing ALO2 in a Chilean university hospital, focusing on survival, complications, and treatment feasibility.

Methods

This was a retrospective, descriptive study conducted in patients aged 18 years or older who underwent a ALO2 between 2001 and 2023 at the Hematology Department of Red de Salud UC Christus, Pontificia Universidad Católica de Chile.

The primary outcomes were overall survival (OS) and progression-free survival (PFS), estimated using Kaplan–Meier methodology and the secondary outcomes were incidence of acute and chronic graft-versus-host disease (GvHD), non-relapse mortality (NRM), and relapse. GvHD was defined and graded per standard criteria.

Data on demographics, underlying hematologic disease, conditioning regimens, donor source, $CD34^+$ cell dose, engraftment, complications, and cause of death were obtained from clinical records and the transplant program database.

The study protocol was approved by the institutional review board of the Pontificia Universidad Católica de Chile.

Results

A total of 1203 patients underwent HSCT at the center between 2001 and 2023. Of these, 602 (51 %) received an allogeneic transplant. Of this group, 21 patients underwent a ALO2 due to relapse or graft failure. The median age was 33.4 years (Range: 18–59 years), and 42 % were female.

The graft source was bone marrow in 60 % and peripheral blood in 40 % of cases. Human leukocyte antigen (HLA)-identical family donors were used in 55 %, haploidentical in 20 %, and unrelated donors in 25 %. The characteristics of the patients are summarized in Table 1.

Table 1 – Patient characteristics.

Characteristic	
Gender -%	
Male	57.9
Female	42.1
Median Age (years) – median (range)	30
Hematologic Disorder - n (%)	
Acute Myeloid Leukemia	8 (38)
Acute Lymphoblastic Leukemia	5 (23)
Chronic Myeloid Leukemia	2 (8)
Hodgkin Lymphoma	3 (13)
Non-Hodgkin Lymphoma	1 (5)
Severe Aplastic Anemia	3 (13)
Donor Type - n	
HLA-Identical Family	11
Haploidentical	4
Unrelated	5
Stem Cell Source -%	
Bone Marrow	60
Peripheral Blood	40

Conditioning regimens varied by period. Between 2001 and 2010, regimens included busulfan/cyclophosphamide, total body irradiation (TBI)/etoposide (Etoposide)/cyclophosphamide, and fludarabine/cyclophosphamide. From 2011 to 2023, regimens included fludarabine/cyclophosphamide/TBI, fludarabine/busulfan, fludarabine/treosulfan, and cyclophosphamide/ anti-thymocyte globulin (ATG).

GvHD prophylaxis used calcineurin inhibitors in all patients, methotrexate (Methotrexate) in 71 %, and post-transplant cyclophosphamide in 29 %. The mean $CD34^+$ cell dose was 6.62×10^6/kg bodyweight (95 % CI: 2.9–11.0). Median neutrophil and platelet engraftment times were 16 days (range: 8–35 days) and 17 days (range: 9–75 days), respectively.

Grade III–IV acute GvHD occurred in 5 %, and extensive chronic GvHD in 17 % of the cases. NRM was 69.2 %, while disease relapse accounted for 23.1 % of deaths. The median follow-up time was 13.5 months. Transplantation data are summarized in Table 2.

The one-year PFS and OS were 26.5 % and 42.3 %, respectively. When stratified by transplant period, patients transplanted between 2010 and 2023 had improved outcomes: one-year PFS was 55 %, and OS was 45.4 %. In contrast, for patients treated between 2001 and 2010, both one-year PFS and OS were 12.5 %, with median survival times of 51 and 52 days, respectively. Figs. 1-4 show the Kaplan Meier survival curves for PFS, OS, PFS by transplant decade and OS by transplant decade.

Discussion

This retrospective study contributes to the limited data available from Latin America on the outcomes of an ALO2. While ALO2 is a well-established salvage option in developed countries, its role in resource-constrained settings is less defined.

There is currently no standard of care for patients who relapse after an initial allogeneic transplant. Treatment strategies are often individualized, depending on multiple variables including patient comorbidities, disease characteristics, access to salvage therapies, donor availability, and functional

Table 2 – Transplantation Characteristics.

Characteristic	
Conditioning Regimen (2001–2010) - n	
Busulfan, Cyclophosphamide	4
TBI 100 Gy, Etoposide Cyclophosphamide	3
Fludarabine, Cyclophosphamide	1
Conditioning Regimen (2011–2023) - n	
Flu-Cy-TBI (200–400 Gy)	4
Fludarabine, Busulfan	3
Flu-Treosulfan	2
Cyclophosphamide, ATG	3
GvHD Prophylaxis - n	
Calcineurin Inhibitors	21
Methotrexate	15
Post-Transplant Cyclophosphamide	6
$CD34^+$ Cell Dose ($\times 10^6$/kg bodyweight) - median (range)	6.62 (2.9–11.6)
Granulocyte Engraftment (days) - median (range)	16 (8–35)
Platelet Engraftment (days) - median (range)	17 (9–75)
Acute GvHD Grade 3–4 -%	5
Extensive Chronic GvHD -%	17

Flu: fludarabine; ATG: anti-thymocyte globulin; Gy: grays, Cy: cyclophosphamide; TBI: total body irradiation; GvHD: graft-versus-host diseases.

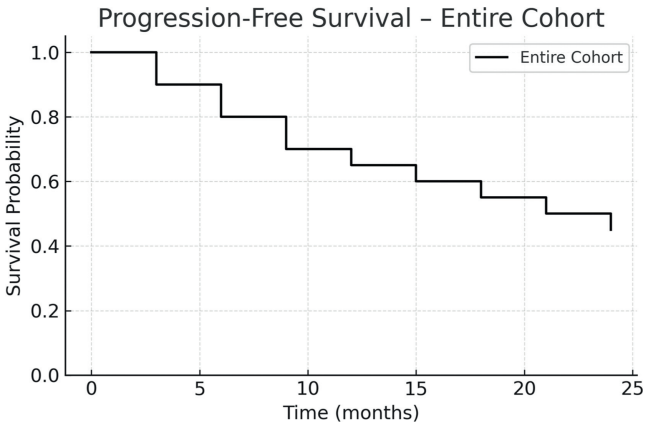

Figure 1 – Progression-free survival in patients undergoing second allogeneic hematopoietic stem cell transplantation.

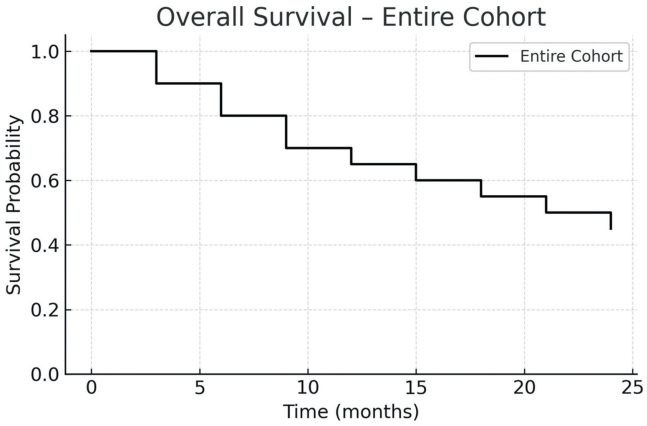

Figure 2 – Overall Survival in patients undergoing second allogeneic hematopoietic stem cell transplantation.

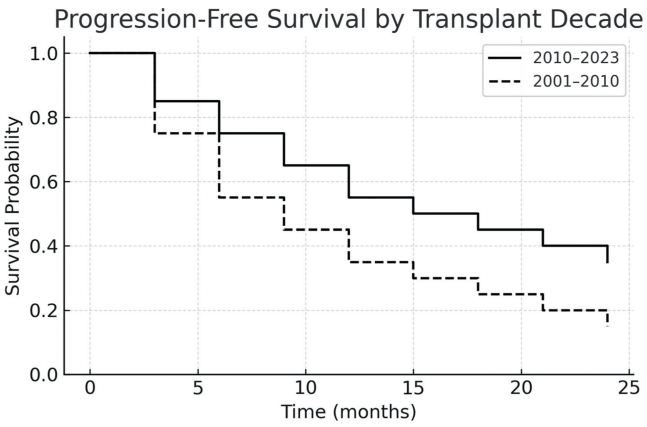

Figure 3 – Progression-free survival in patients undergoing second allogeneic hematopoietic stem cell transplantation according to the transplant decade.

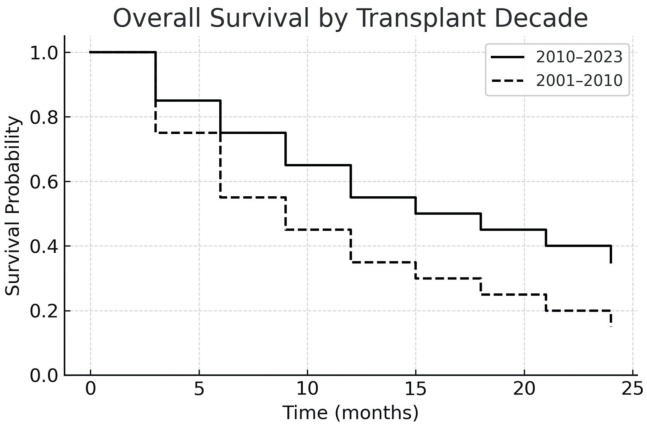

Figure 4 – Overall Survival (OS) in patients undergoing second allogeneic hematopoietic stem cell transplantation according to the transplant decade.

status at relapse [4,5]. Options include donor lymphocyte infusions, immunosuppression withdrawal, targeted agents, chimeric antigen receptor (CAR)-T therapies, and repeat transplantation. A ALO2 remains one of the few potentially curative strategies in selected patients, particularly those who achieve a second remission.

Multiple retrospective studies have shown that ALO2 can lead to long-term survival in a minority of patients. The Société Française de Greffe de Moelle (SFGM) [6] reported a two-year disease-free survival (DFS) and OS of 35 % and 41 %, respectively in 150 patients, 61 % of whom had acute myeloid leukemia (AML). Similarly, a Center for International Blood & Marrow Transplant (CIBMTR) Research report [7] found a three-year OS of 27 % in AML patients who underwent ALO2. In a European Society for Blood and Marrow Transplantation (EBMT) analysis [8], the NRM rates at two and five years were 24 % and 26 %, respectively. More recently, ALO2 has been used in combination with CAR-T cell therapy [9,10] to consolidate remission after relapse, with promising results, especially in relapsed/refractory B-cell malignancies. However, such strategies remain largely inaccessible in Latin American countries due to financial and logistical constraints.

The findings of this study demonstrate that survival after ALO2 is possible even in resource-limited settings. The one-year OS in the present cohort was 42.3 %, and PFS was 26.5 %, which aligns with international data. Notably, patients treated after 2010 had significantly better outcomes, suggesting improvements in patient selection, salvage therapy efficacy, and transplant protocols. However, non-relapse mortality was high (69.2 %) with infections and GvHD being major contributors. Another Latin-American experience [11] reported a 66 % mortality rate in 12 patients who underwent ALO2, highlighting similar challenges in the region. These findings emphasize the need for real-world data and local treatment strategies tailored to regional limitations.

Despite the inherent limitations of retrospective design and small sample size, this study provides valuable insights into the feasibility and outcomes of ALO2 in Latin America. Future prospective studies and international collaborations are needed to better define best practices and improve accessibility to potentially curative treatments in developing countries.

Conflicts of interest

The author declares no conflicts of interest.

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Original article

Coagulation factor stability and sterility of thawed fresh frozen plasma stored at 2-6 ° C for five days: Towards optimizing utilization

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ABSTRACT

Background: Fresh frozen plasma plays a crucial role in managing trauma and bleeding patients. The concern about a decline in labile coagulation factors limits its usage beyond 24 hours. This study aimed to analyze coagulation factor levels and microbial contamination of thawed fresh frozen plasma stored at 2-6 °C for five days.

Material and methods: A prospective observational study was conducted on 40 male donors with blood groups A and O selected through purposive sampling. Blood was collected in 450 mL bags and freshly prepared plasma was aliquoted and frozen at -80 °C. Aliquots were thawed at 37 °C and tested on Days 0, 1, and 5 after storage at 2-6 °C. Coagulation screening assays and activity of coagulation factors V, VIII, IX, fibrinogen, and von Willebrand factor were performed. Samples were tested for sterility on Day 5.

Results: One-way ANOVA revealed a significant increase in mean prothrombin time, activated partial thromboplastin time, and international normalized ratio during storage (p-value < 0.001). The activity of factors V and VIII showed a significant decrease over five days (factor V - 20.0 % and factor VIII - 42.2 %; p-value < 0.001), with factor VIII activity declining by 30.8 % within the first 24 hours and remaining relatively stable thereafter. Mean von Willebrand factor activity was lower in fresh frozen plasma from O blood group donors (p-value < 0.05) on Days 1 and 5 of storage using an unpaired t-test. Cultures were sterile on Day 5.

Conclusion: Key coagulation factors were well preserved in thawed plasma till five days of storage at 2-6 °C without compromising product sterility suggesting potential for extended shelf life.

Introduction

Fresh frozen plasma (FFP) is a rich source of coagulation factors. It is currently indicated for transfusion in conditions of impaired hemostasis resulting from multiple

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coagulation factor deficiencies such as liver diseases, disseminated intravascular coagulation, major trauma, and as a replacement fluid during therapeutic plasma exchange [1,2]. FFP should be thawed at 30-37 °C in a plasma thawing bath before transfusion. As per the Indian National Regulatory Authority, FFP should be used within 24 hours of thawing [3]. If not utilized within 24 hours, FFP is discarded due to concerns about a decline in coagulation factor activity and microbial contamination. This leads to the wastage of this precious resource.

The shelf life of thawed FFP is a topic of interest. The recommendations pertaining to the maximum permitted storage time post-thawing vary in different countries. The Association for the Advancement of Blood and Biotherapies (AABB) introduced the concept of thawed plasma (TP) to maintain inventory and reduce plasma wastage. TP is plasma stored at 1-6 °C for an additional four days after the 24 hours period [4]. The British Society of Hematology states that once thawed, FFP may be stored at 2-6 °C for up to 24 hours [5]. In Australia, TP is known as Extended Life Plasma (ELP) and has a shelf life of up to five days at 2-6 °C from the day of thawing. TP and ELP are similar to FFP but have lower levels of labile coagulation factors [6–9]. In developing countries like Malaysia, FFP is discarded if unused within six hours of thawing [10].

In severe trauma-induced bleeding, precious lives can be saved by rapid replacement of coagulation factors with the administration of plasma. Availability of pre-thawed FFP in trauma centers will be a great asset to clinicians for immediate resuscitation of these patients. Therefore, the present study aimed to determine the levels of labile and stable coagulation factors and to check for bacterial contamination of thawed plasma during storage at 2-6 °C for five days. Utilization of FFP beyond 24 hours of thawing may help in timely availability and prevent potential wastage of unused thawed FFP.

Materials and methods

Study design

This prospective cross-sectional study was conducted in the Department of Transfusion Medicine of a tertiary care hospital. This study was conducted on 40 healthy male donors over 12 months after approval from the Institutional Ethics Committee and informed written consent from blood donors. All whole blood donors were screened as per the Indian National Regulatory Authority [3]. Blood donors meeting the eligibility criteria and weighing more than 55 kg were included in the study using a purposive sampling method. Only male donors were included as female donors usually do not meet the minimum weight eligibility criteria of 55 kg for the collection of 450 mL blood. Exclusion criteria were donors where blood was not collected using a single clean venipuncture, collection time of more than eight minutes, technical errors, under or over-collected bags, and donors reactive for any transfusion-transmitted infections. The donors were further divided into one of two groups:

Group 1 (n = 20): donors of the O Rh D positive blood group and Group 2 (n = 20) donors of the A Rh D positive blood group.

Blood collection and processing

After obtaining informed consent, whole blood was collected in 450 mL triple blood bags with Citrate Phosphate Dextrose Adenine Solution (CPDA-1) as an anticoagulant (Terumo Penpol, Thiruvananthapuram, India). Whole blood was collected within eight minutes using a single clean venipuncture. Packed red blood cells (PRBCs), FFP, and platelet concentrates (PCs) were prepared within six hours of blood collection according to the departmental standard operating procedure using the same cryofuge by a dedicated trained laboratory technologist. Plasma was rapidly frozen at -80 °C in a mechanical deep freezer (Terumo Penpol, Thiruvananthapuram, India) immediately after preparation. Once frozen, FFP was subsequently stored below −30 °C in a deep freezer (Terumo Penpol, Thiruvananthapuram, India).

FFP units were thawed in a plasma thawing bath (Helmer Scientific, Indiana, USA) at 37 °C for 30 minutes within three months of freezing. After thawing, 50 mL aliquots were prepared in a transfer bag using a sterile connecting device (TSCD-II, Terumo Penpol, Thiruvananthapuram, India), and a baseline 10 mL sample was taken in a plain tube after proper mixing of contents. The aliquot was stored at 2-6 °C and sampled again at 24 hours and five days for coagulation factor analysis. The rest of the unit was stored at 2-6 °C for issue to patients within 24 hours.

Laboratory analysis

Coagulation screening assays, including Prothrombin time (PT), Activated Partial thromboplastin time (APTT), Prothrombin index (PTI), and International normalized ratio (INR), were analyzed on Days 0, 1, and 5 using a fully automated coagulation analyzer (ACL Top TM 500 CTS, Instrumentation Laboratory, Bedford, MA, USA) as per the manufacturer's instructions. Factor V (FV), factor VIII (FVIII), factor IX (FIX), fibrinogen and von Willebrand factor (vWF) levels were also measured using the same analyzer as per the manufacturer's instructions. The thawed FFP samples were also sent for sterility testing on Day 5.

Statistical analysis

The sample size was estimated based on the mean difference of FVIII activity in thawed FFP based on a previous study [10]. For possible attrition, it was decided to include at least 30 subjects. The following formula was used for sample size calculation n = (Zα/2+Zβ)2 *2*σ2 / d2 where Zα/2 is the critical value of the normal distribution at α/2 (e.g. for a 95 % confidence level, α is 0.05 and the critical value is 1.96), Zβ is the critical value of the normal distribution at β (e.g. for a power of 95 %, β is 0.05 and the critical value is 1.64), σ2 is the population variance, and d is the difference between means.

All data was compiled using Google Sheets and data analysis was done using IBM SPSS statistics software. For quantitative variables, data were analyzed either as the mean and

standard deviation or as the median and interquartile range. The distribution of the variables was tested with the chi-square test. Student t-test was applied to compare two groups and the ANOVA test was applied to compare three groups for normally distributed data. Categorical variables were reported as counts and percentages. Discrete (categorical) groups were compared by the chi-square (χ^2) test. The ANOVA test was applied for time-related variables of scores/skewed data; the paired t-test was carried out for normally distributed data. Comparisons of categorical data were made using the Pearson chi-square test. All the statistical tests were two-sided and were performed at a significance level of $\alpha = 0.05$, thus a p-value < 0.05 was considered significant.

Results

The mean age of the study population was 34.3 ± 9.3 years with a range of 19-60 years. The mean time taken to collect 450 mL of whole blood from all the study subjects was 6.2 ± 0.83 minutes (range: 5-8 minutes). The mean time between whole blood collection and component preparation was 3.7 ± 0.88 hours (range: 2-5 hours). The mean time between the day of collection and thawing was 73.3 ± 19.6 days (range: 35-103 days).

Conventional coagulation tests of thawed fresh frozen plasma

Table 1 shows the changes over five days of storage at 2-6 °C in the values of conventional coagulation tests of thawed FFP. The baseline PT ranged between 10.2 s and 14.1 s while the APTT ranged between 26.1 s and 37.2 s. According to one-way ANOVA, the mean increases in PT and APTT values were statistically significant over five days of storage (p-value < 0.001). The increases in mean PT of thawed FFP stored at 2-6 °C were statistically significant from Day 0 to Day 1 (p-value = 0.004), Day 0 to Day 5 (p-value < 0.001), and Day 1 to Day 5 (p-value < 0.001) using an unpaired t-test. The changes in mean APTT from Day 0 to Day 1 (p-value < 0.001), Day 0 to Day 5 (p-value < 0.001), and Day 1 to Day 5 (p-value < 0.05) were also statistically significant using an unpaired t-test.

Coagulation factors in thawed fresh frozen plasma

The activities of FV, FVIII, FIX, and vWF, and fibrinogen levels in thawed FFP over five days of storage at 2-6 °C are shown in Table 2. The baseline FV activity ranged between 73.5 % and 137.5 %, and FVIII activity ranged between 43 % and 184.8 %. The coagulation FV and FVIII activities in thawed FFP showed significant declines during storage at 2-6 °C over five days using the one-way ANOVA test (p-value < 0.001; Table 2). The decline in FVIII activity from Day 0 to Day 5 was 42.2 % with a major decline (30.8 %) in activity being observed in the first 24 hours. Out of 40 donors, 55 % (n = 22) had FVIII activity greater than 50 % while in 20 % (n = 8) of donors the activity was more than 70 % on Day 5. The FIX activity, vWF activity and fibrinogen levels remained stable over the five-day storage period of thawed FFP at 2-6 °C. The mean activities of all the factors studied were well within the reference ranges on Day 5 of storage at 2-6 °C.

Donor blood group and coagulation parameters in thawed fresh frozen plasma

The mean FVIII activity was lower in FFP from donors of the O blood group as compared to A blood group donors on all three days; however, the difference was statistically insignificant. Mean FIX activity was significantly lower in FFP from O blood group donors on Day 5 of storage (p-value: 0.029). Mean vWF activity was also lower in FFP from O blood group donors with the difference being significant on Days 1 (p-value = 0.014) and 5 of storage (p-value = 0.019). The mean fibrinogen levels were significantly higher in FFP prepared from O blood group donors on Day 1 of storage (p-value: < 0.001; Table 3).

Culture of thawed fresh frozen plasma

All thawed FFP samples were sent for culture on Day 5 of storage. The cultures were sterile after 72 hours of incubation.

Discussion

Trauma accounts for nearly 20 % of mortality in India, with a high impact on the younger population [11]. FFP is crucial for managing trauma-related coagulopathy, and pre-thawed liquid plasma could aid early resuscitation. However, guidelines for using thawed FFP beyond 24 hours at 2-6 °C are lacking in developing nations. Coagulation factors and sterility in thawed FFP from O and A Rh D-positive donors stored at 2-6 °C for five days were compared due to potential ABO influence on vWF and FVIII [8,12,13]. To ensure uniformity, FFPs were thawed within three months of storage.

In this study, the mean PT, PTI, APTT, and INR of thawed FFP increased significantly during storage likely due to coagulation factor deterioration at 2-6 °C. Nevertheless, all values

Table 1 – Conventional coagulation tests of thawed fresh frozen plasma over five days of storage at 2-6 °C.

Parameter	Normal Range	Day 0 (Mean ± SD)	Day 1 (Mean ± SD)	Day 5 (Mean ± SD)	p-value ANOVA
Prothrombin Time (s)	9.4-12.5	11.3 ± 0.9	11.9 ± 0.9	13.0 ± 1.2	**<0.001**
PTI (%)	96-100	107.0 ± 11.0	98.7 ± 10.1	87.3 ± 10.6	**<0.001**
APTT (s)	25.1-36.5	30.9 ± 2.7	33.1 ± 3.1	34.6 ± 3.2	**<0.001**
International Normalized Ratio	0.9-1.1	0.94 ± 0.1	0.99 ± 0.1	1.1 ± 0.1	**<0.001**

PTI: prothrombin time index; APTT: activated partial thromboplastin time; SD: Standard Deviation.
Note: Bold p-values indicate statistically significant results.

Table 2 – Coagulation factors in thawed fresh frozen plasma over five days of storage at 2-6 °C.

Parameter	Normal range	Day 0 (Mean ± SD)	Day 1 (Mean ± SD)	Day 5 (Mean ± SD)	p-value*	Reduction percentage on Day 1	Reduction percentage on Day 5
Factor V activity (%)	62-139	98.7 ± 16.6	93.1 ± 23.3	78.7 ± 17.4	**<0.001**	5.6	20.0
Factor VIII activity (%)	50-150	97.7 ± 30.4	66.9 ± 24.1	55.5 ± 19.1	**<0.001**	30.8	42.2
Factor IX activity (%)	65-150	117.1 ± 18.6	109.6 ± 22.5	111.5 ± 20.2	0.248	7.5	5.6
Fibrinogen (mg/dl)	238-498	257.0 ± 54.8	272.3 ± 79.3	317.4 ± 53.4	**<0.001**	-	-
von Willebrand Factor (%)	Blood group O: 41.1-125.9 Blood group A: 61.3-157.8	112.5 ± 40.5	111.2 ± 37.2	102.6 ± 35.5	0.456	1.3	9.9

*Using one-way ANOVA test
SD: Standard Deviation
Note: Bold p-values indicate statistically significant results.

remained within the normal reference range over five days. A study observed a significant increase in mean PT from 11.3 ± 0.83 s on Day 0 to 13.3 ± 1.10 s on Day 5 in thawed FFP stored at 2-6 °C [14]. Another study also showed 23 % increase in mean PT and APTT values from Day 0 to Day 5 of storage [15]. Authors have reported an increase in mean INR in thawed FFP from 0.95 ± 0.05 on Day 0 to 1.20 ± 0.21 on Day 5 of storage [8].

FV, a labile pro-coagulant factor with 40 % homology to FVIII, showed a 20 % activity loss during storage at 2-6 °C. However, the mean FV activity (78.7 ± 17.4 %) on Day 5 remained within the reference range and met the quality control criteria of 70 %, making it suitable for use in isolated FV deficiency. One study reported a decline (114.1 ± 38.7 % on Day 0 to 70.6 ± 18.9 % on Day 5) in mean FV activity in thawed FFP stored over five days at 1-6 °C [8].

FVIII activity declines during prolonged plasma storage at 2-6 °C and its measurement is a quality control requirement. In the present study, FVIII activity decreased by 43 % over five days, with a major drop (30.8 %) in the first 24 hours, with stabilization thereafter. This suggests that extending thawed FFP storage to five days does not adversely affect FVIII levels. Studies report a 36-60 % decline in mean FVIII activity by Day 5 in thawed FFP stored at 2-6 °C [15]. In this study, FVIII

Table 3 – Donor blood group and coagulation parameters of thawed fresh frozen plasma.

Days of storage	Blood group 'O'	Blood group 'A'	p-value**
Factor V (%)			
Day 0	100.4 ± 18.2	96.9 ± 15.1	0.518
Day 1	96.1 ± 26.5	89.9 ± 19.5	0.413
Day 5	77.6 ± 17.9	79.9 ± 17.3	0.685
p-value*	**0.003**	**0.014**	
Factor VIII (%)			
Day 0	90.3 ± 32.2	105.4 ± 27.1	0.123
Day 1	61.7 ± 25.3	72.5 ± 22.1	0.165
Day 5	50.6 ± 16.3	60.7 ± 20.6	0.097
p-value*	**<0.001**	**<0.001**	
Factor IX (%)			
Day 0	112.5 ± 17.2	122.1 ± 19.3	0.109
Day 1	106.5 ± 22.3	112.8 ± 22.8	0.389
Day 5	104.7 ± 19.5	118.6 ± 18.9	**0.029**
p-value*	0.432	0.372	
Fibrinogen (mg/dL)			
Day 0	259.1 ± 47.2	254.9 ± 63.1	0.815
Day 1	375.3 ± 73.9	269.1 ± 86.4	**<0.001**
Day 5	321.3 ± 43.9	313.4 ± 62.8	0.650
p-value*	**<0.001**	**0.006**	
von Willebrand factor (%)			
Day 0	100.7 ± 35.6	125.1 ± 42.4	0.058
Day 1	97.3 ± 34.8	125.9 ± 34.7	**0.014**
Day 5	89.9 ± 34.5	116.0 ± 32.2	**0.019**
p-value*	0.610	0.655	

* Using one-way ANOVA test; ** Using unpaired t-test; Note: Bold p-values indicate statistically significant results.

activity remained within the normal reference range up to five days of refrigerated storage, sufficient for achieving clinical hemostasis. Mean baseline FVIII activity was lower in group O donors, consistent with existing literature [12].

FIX showed a non-significant decrease in activity in thawed FFP throughout the storage period as seen in previous reports [8,14]. A significantly lower mean FIX activity was observed in FFP from group O donors on Day 5 of storage. A recent study from India also reported significantly lower FIX levels in O group donors as compared to non-O group donors [16].

Fibrinogen is a key protein involved in clot formation [17]. The current study found no significant decline in fibrinogen levels in thawed FFP over five days of refrigerated storage, consistent with previous reports [8,10,14,18]. This supports its use in hypofibrinogenemia, particularly in resource-limited settings. Fibrinogen levels in cryoprecipitate were higher in O group donors than A group donors on Day 1, challenging the notion that O group donors are unsuitable for cryoprecipitate due to lower FVIII and vWF levels. Given the high prevalence of the O group in India, further research is needed to explore its potential in cryoprecipitate production. vWF is a multifunctional protein and an acute-phase reactant [17]. In this study, vWF activity in thawed FFP remained stable during storage, whereas another study reported a decline from 110.8 ± 39.01 % at baseline to 67.0 ± 35.5 % on Day 5 [14]. A significantly lower vWF activity was found in O group donors on both Day 1 and Day 5, consistent with reports that vWF levels are 25 % lower in O group plasma due to the effect of ABO(H) carbohydrate structures on its glycans [19].

In the normal population, clotting factor levels range from 50-150 %, with hemostasis requiring much lower levels [7]. In this study, baseline coagulation factor activity remained above 70 %. Plasma is rarely contaminated due to frozen storage, but thawing in a water bath poses a risk. All samples remained sterile after 72 hours of incubation, supporting the safe storage of FFP for five days at 2-6 °C. Similarly, another study found no bacterial growth in culture samples collected on Day 5 and tested on Day 7 [20].

The present study had a few limitations. Firstly, it was a single-center study with a small sample size, which may limit the generalizability of the findings. The exclusion of female donors, primarily due to weight requirements, could have introduced gender-related bias by overlooking physiological differences. Larger, multi-center studies are needed to validate these results and assess clinical implications. Despite these limitations, the findings provide valuable insights for managing coagulopathies with FFP transfusions. Future research should explore the impact of extended storage on additional factors and assess potential cost-effectiveness.

Conclusion

This study demonstrates that extending the storage duration of thawed FFP to five days at 2-6 °C is feasible while maintaining clinically effective levels of key coagulation factors and product sterility. This signifies the potential of pre-thawed FFP in improving patient care by facilitating faster resuscitation in trauma situations and reducing plasma wastage.

Ethics approval statement

The study was approved by the institutional ethics committee and informed written consent was obtained from all participants.

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None.

Author contributions

RB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft; **PK:** Conceptualization, Formal analysis, Methodology, Resources, Supervision, Validation, Writing – review & editing; **KM:** Formal analysis, Methodology, Supervision, Validation, Writing – review & editing; **AT:** Formal analysis, Methodology, Supervision, Validation; **VG:** Methodology, Supervision, Validation; **RK:** Methodology, Resources, Supervision, Writing – review & editing; **GK:** Methodology, Visualization, Writing – review & editing.

Data availability

The data pertaining to the study is available from the first author and corresponding author upon reasonable request.

Conflicts of interest

The manuscript has been seen and approved by all authors, it is not under active consideration for publication, has not been accepted for publication, nor has it been published, in full or in part.

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Original article

Correlation of serum interleukin-8 with disease severity in aplastic anemia

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ABSTRACT

Background: Aplastic anemia is a heterogeneous group of hematological disorders identified by the presence of one or more cytopenias in the bone marrow or the peripheral blood or both, affecting either one or multiple blood cell lineages. The pathophysiology of this disorder is unclear; however, the prevailing hypothesis posits that an aberrant immune response is responsible for the death of hematopoietic precursor stem cells due to autoreactive cytotoxic T lymphocytes in individuals with a genetic predisposition. Interleukin-8 acts as a pleiotropic prototype chemokine and serves as a powerful inhibitor of myelopoiesis and assumes an essential role in both the initiation and progression of acute inflammation and tissue damage. Consequently, it is postulated that the sustained elevation in interleukin-8 production could potentially lead to immune-mediated bone marrow failure in aplastic anemia. The aim of this study was to evaluate the serum interleukin-8 concentrations in individuals with aplastic anemia as well as investigate its potential connection with disease severity.

Methods: This study was performed at the National Center of Hematology and included 28 aplastic anemia patients and 30 healthy individuals matched by age and gender as a control group. The serum interleukin-8 levels were measured by the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) method.

Results: Considerrable elevation in serum interleukin-8 levels were observed between the two groups (p-value = 0.405). Of the aplastic anemia patients, severe cases had significantly higher levels of interleukin-8 compared to non-severe patients (p-value = 0.0495), with a cutoff serum level of 7.71 pg/mL.

Conclusion: Interleukin-8 may have a role in the immune-mediated pathophysiology of aplastic anemia as well as have a significant correlation with disease severity.

Introduction

Idiopathic acquired aplastic anemia (AA), an uncommon and potentially fatal bone marrow failure, is identified by hypocellular bone marrow and peripheral blood cytopenias [1]. Its

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effects may range in severity from mild to severe cytopenia [2]. AA is classified into three clinical and hematological categories, which are determined by evaluating the cellularity of the bone marrow (BM), platelet count, absolute neutrophil count (ANC), and reticulocyte count [3]. AA is a disease based on an immune meditated pathway in which the dysfunctional effector cells recognize and destroy the marrow progenitor cells, resulting in pancytopenia [4,5]. The immunopathology theory of AA is supported by the positive response to immunosuppressive therapy with the administration of anti-thymocyte globulin and cyclosporine [5]. A variety of growth factors and cytokines, specifically chemokines, synthesized by stem cells, regulatory cells, and marrow stromal cells, play vital roles in the BM microenvironment. These components exert regulatory control over hematopoiesis through autocrine and paracrine mechanisms [6,7]. Interleukin-8 (IL-8), a pleiotropic prototype CXC chemokine, is produced by different cell types, primarily by macrophages and fibroblasts in the BM, and neutrophils and T-cells in the peripheral blood [8,9]. Via a receptor-ligand mediated mechanism, IL-8 may contribute to the suppression of hematopoiesis, a process by which the blood cells are originated [8]. It serves as a powerful inhibitor of myelopoiesis and assumes an essential role in both the initiation and progression of acute inflammation and tissue damage [10,11]. Research conducted on the pathophysiology of the disease has predominantly centered on stem cell abnormalities and immunological pathways, while, limited data exists regarding the involvement of the BM microenvironment in this condition [1]. Consequently, it is postulated that the sustained elevation in IL-8 production could potentially play a role in the onset of immune-mediated BM failure in individuals with AA [11]. Furthermore, evidence shows that IL-8 correlates with disease severity and progression [12]. This study evaluates the serum IL-8 concentrations in individuals with AA as well as investigates potential connections with disease severity.

Patients and methods

The present study was carried out from December 2020 to December 2022 at the National Center of Hematology, and included 28 patients with newly diagnosed idiopathic AA. Their initial diagnostic data was established from their medical records at the time of sampling. Another 30 apparently healthy individuals, with similar ages and genders, were chosen as a control group. The researchers received ethics approval and only subjects who gave written consent were included in the study. Patient data comprised an analysis of blood components, including a complete blood count, assessment of peripheral blood smears, and testing of BM samples. Other criteria for the inclusion of patients were: no organomegaly, normal renal function, normal liver function, normal Erythrocyte Sedimentation Rate (ESR), a negative serological connective tissue screen, no history of acute febrile illness, and no evidence of organ dysfunction. AA patients were categorized into mild, moderate, severe and very severe. Severe AA was characterized by a reduction in blood counts affecting two or more hematopoietic lineages (i.e., absolute reticulocyte count $<60 \times 10^9$/L, an ANC $<0.5 \times 10^9$/L, or platelet count $<20 \times 10^9$/L) that is accompanied by bone marrow hypocellularity, defined as less than 25 % of the normal cellularity. Very severe AA was identified by an ANC $<0.2 \times 10^9$/L. While, the decrease in blood counts that did not meet the criteria for severe disease was classified as moderate AA [13]. Both patients and controls were free from chronic illness like diabetes, hypertension and recent febrile illness, as well as serological evidence of acute or chronic viral hepatitis.

Under aseptic circumstances, 3 mL of peripheral blood were drawn from both patients and controls, then dispensed in sterile plain tubes, and centrifuged for ten minutes at 4000 rpm. The serum samples were collected and stored at −20 °C until the IL-8 level was analyzed. As per the instructions provided by the manufacturer, the levels of IL-8 for all participants were measured using a commercial quantitative sandwich ELISA assay kit (R&D Systems, USA). The concentration in pg/mL was used to express the results.

Statistical analysis

The Statistical Analysis System (SAS 2012) program was employed to detect the effect of differences between the groups. The Mann-Whitney test was employed for non-normally distributed continuous variables and a *t*-test was employed for normally distributed continuous variables. Correlation coefficients between variables were estimated in this study. A p-value ≤0.05 was considered statistically significant, whereas a p-value ≤0.01 was considered highly significant [14].

Results

Twenty-eight AA patients were included in this study with a median age of 36.5 ranging from 8–68 years. Of these patients, 15 (53.6 %) were male and 13 (46.4 %) were female with a M:F ratio of 1:1. Thirty samples of apparently healthy volunteers were included as a control group in the analysis with median age of 36.5 ranging between 17 and 67 years and a M:F ratio of 1:1 (Table 1).

Regarding the IL-8 mean serum level, there was an increase in the serum level of IL-8 in AA patients in comparison to the control group (p-value = 0.405). However, data

Table 1 – General characteristics of aplastic anemia patients and the control group.

	Patient (n = 28)	Control (n = 30)	p-value
Age in years - median (Range)	36.5 (8–68)	36.5 (17–67)	0.8260*
Sex - n (%)			0.7993**
Male	15 (53.6 %)	15 (50 %)	
Female	13 (46.4 %)	15 (50 %)	
Hemoglobin (g/dL) Mean ± SE (Range)	7.225 ± 0.2803 (5.1–10)	–	
Platelet x $10^3/\mu$L Mean ± SE (Range)	20.79 ± 1.816 (7-44)	–	
ANC x $10^3/\mu$L Mean ± SE (Range)	583.9 ± 53.68 (110–1100)	–	

SE: Standard error; ANC: absolute neutrophil count.

* *t*-test.

** Fisher's exact test.

Table 2 – Comparison of mean interleukin-8 (IL-8) serum level between aplastic anemia patients and controls.

Group	Mean ± SE IL-8 (pg\mL)
Aplastic Anemia (*n* = 28)	15.02 ± 2.81
Control (*n* = 30)	10.19 ± 1.01
p-value	0.405

Mann-Whitney test was used for statistical analysis. Test is considered significant if p-value ≤0.05.

Table 3 – Relationship between severity of disease and mean serum level of interleukin-8 (IL-8) in aplastic anemia patients.

Severity of disease	Mean ± SE IL-8 (pg\mL)
Severe (No = 21)	17.54 ± 3.59
Non-severe (No = 7)	7.43 ± 0.52
p-value	0.0149

Mann-Whitney test was used for statistical analysis. Test is considered significant if p-value ≤0.05.

Table 4 – Relationship between gender and mean serum level of interleukin-8 (IL-8) of aplastic anemia patients.

Sex	Mean ± SE IL-8 (pg\mL)
Male	15.76 ± 3.93
Female	14.14 ± 4.16
p-value	0.383

T-test was used for statistical analysis. Test is considered significant if P-VALUE ≤0.05.

indicate a significant difference in mean IL-8 (p-value = 0.0149) between severe and non-severe AA patients (Tables 2 & Table 3).

Moreover, a significant difference in mean serum level of IL-8 was observed between different age groups of patients with AA; higher mean serum levels were found in older patients (p-value = 0.0406). While there was no statistically difference observed in the mean serum level of IL-8 between sexes in AA patients (Tables 4 & 5).

A non-statistically significant (p-value >0.05) correlation coefficient was observed between IL-8 levels and other patient parameters, such as hemoglobin, platelet count, and ANC (Table 6).

Table 5 – Relationship between age groups and mean serum level of interleukin-8 (IL-8) of aplastic anemia patients.

Age (Year)	Mean ± SE IL-8 (pg/mL)
≤40	8.31 ± 0.4492
>40	22.25 ± 5.922
p-value	0.0406

T-test was used for statistical analysis. Test is considered significant if p-value ≤0.05.

Table 6 – Correlation coefficient between interleukin-8 (IL-8) and different hematological parameters among aplastic anemia patients.

Parameters	Correlation coefficient (r) with IL-8	p-value
Hemoglobin	−0.18	>0.05
platelet count	−0.04	>0.05
ANC	−0.03	>0.05

Receiver operating characteristic (ROC) analysis showed that IL-8 with a cutoff value of 7.71 pg/mL was highly significant with good sensitivity and specificity to discriminate between severe and non-severe AA patients (Table 7).

Discussion

IL-8 has the ability to suppress the production of myeloid progenitor cells by impeding their proliferation through a mechanism involving a receptor-ligand interaction (transmitting an inhibitory signal), specifically, IL-8 (CXCR-2), which is expressed on progenitor cells [15]. Since IL-8 has strong stimulatory effects on neutrophils [16], elevated concentrations of this cytokine in the blood stream have the potential to promote activation-induced apoptosis of fully developed neutrophils in the peripheral system [17].

This study shows that AA patients have elevated, albeit non-significant, mean serum IL-8 concentrations in comparison to the control group (p-value >0.05). Previous studies conducted in pediatric and adult patients by Gupta et al., Bhargawa et al., and Singh et al. revealed significant incre-

Table 7 – Receiver operating characteristic (ROC) analysis evaluating the diagnostic accuracy of interleukin-8 levels in aplastic anemia patients.

Interleukin-8	AUC	Explanation	AUC at 95 % CI	p-value	Optimum cut-off value	SP (%)	SN (%)
Aplastic Anemia versus Control	0.564	Poor	0.415–0.714	0.401	8.33 pg/mL	50	64.3
Severe versus non-severe	0.806	Good	0.637–0.975	0.017	7.71 pg/mL	71.4	81

AUC: Area under curve; 95 % CI: 95 %confidence interval; SN: sensitivity; SP: specificity.

ments (p-value <0.001) in the IL-8 level of AA patients in comparison to controls (15.58 ± 18.0 versus 1.85 ± 0.95, 122.56 ± 97.79 versus 3.42 ± 1.73, and 120.28 ± 94.98 versus 1.79 ± 0.78, respectively). A recent study confirms these results showing that the median IL-8 level is significantly higher in AA patients than in a control group (320 versus 97; p-value <0.0001) [2,12,18,19]. These findings may give us an idea about the pathology of the AA and the role of cytokines like IL-8 in disease activity and marrow suppression.

Additionally, in the current study, patients diagnosed with AA showed significantly increased mean serum levels of IL-8 in severe AA patients, signifying that IL-8 levels may be playing a role in exacerbating disease progression and severity. This finding agrees with many other studies such as those by Gupta et al., Bhargawa et al., Singh et al., and Khalifa et al., that revealed a significant correlation between an elevated level of IL-8 and the severity of the disease [2,12,18,19]. A study conducted by Tripathy et al. examining bone marrow and peripheral blood samples obtained from AA patients, demonstrated an increase of IL-8 in both samples that correlated with disease severity [8]. Tang et al. also demonstrated the same result using peripheral blood samples [20].

There are several studies that revealed a substantial association between elevated levels of IL-8 in peripheral blood and the severity of the disease. Therefore, IL-8 could have a significant role in the pathogenesis of a severe type of idiopathic AA [18,21]. The present study confirmed other studies suggesting that patients with AA may have an inflammatory background mediated by IL-8 acting as a negative regulator of hematopoiesis [19]. Further studies are encouraged to explore a targeted therapy that inhibits IL-8 in the context of hematological and immunological settings.

Higher levels of IL-8 were detected in the older age group in comparison to patients aged between 20–40 years. This finding is inconsistent with the data on serum IL-8 levels detected in children diagnosed with AA, as elevated concentrations were associated with younger severe AA patients [18].

There was no statistically significant relationship between the mean IL-8 serum level and the mean levels of hemoglobin, platelets, and ANC in the AA patient group. The relationship between IL-8 and peripheral blood indices might not be consistent across patients due to heterogeneity in disease pathophysiology or the severity of AA.

When combining other investigations with our findings, it is evident that patients diagnosed with severe and very severe AA exhibit considerably higher peripheral blood IL-8 levels compared to those with non-severe AA. Moreover, these elevated IL-8 levels demonstrate a positive correlation with disease severity, suggesting a crucial role in the pathogenesis of AA [9,12,19]. Thus, IL-8 might be useful as a discrimination marker with the current study suggesting that the cutoff value of 7.71 pg/mL may separate severe from non-severe AA.

One of the primary limitations of this study is the limited sample size only from a single institution. Consequently, further research involving a large sample size from many institutions is imperative to enhance the generalizability of these findings. Additionally, the lack of patient follow-up after therapy in this study represents a constraint that should be acknowledged.

Conclusion

IL-8 may have a role in the clinical presentation and severity of the AA; the immune-mediated action of IL-8 in patients diagnosed with AA may be associated with an increment in BM hypocellularity and subsequently more profound peripheral blood cytopenias.

Data availability

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

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Consent

Informed consent was obtained from each participant prior to enrollment in this study

Final approval of manuscript

All the authors.

Ethics approval

Ethics approval is sought from the scientific ethical committee in the National Center of Hematology/ Mustansiriyah University (Reference: nch-erc-20-27), and it conformed to the ethics guidelines of the Declaration of Helsinki.

Conflicts of interest

The authors declare no competing interests.

CRediT authorship contribution statement

Shahla'a Fadhil Sabir: Methodology, Data curation, Project administration, Writing – review & editing. **Huda Ibraheem Abd AL-Lateef:** Project administration, Writing – review & editing. **Naam Ali Hamza:** Project administration, Writing – original draft.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Original article

Immunological consequences of past transfusions in kidney transplant candidates: A focus on anti-HLA antibody formation

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ABSTRACT

Introduction: Blood transfusions are crucial for saving lives but can affect the recipient's immune system. A significant concern is the development of anti-human leukocyte antigen (HLA) antibodies, which can influence organ transplantation outcomes. The presence of these antibodies increases the risk of transplant rejection. The aim of this study was to evaluate how blood component characteristics (leukodepletion, type, number, volume) and timing from the last transfusion to anti-HLA antibody detection affect sensitization in kidney transplant candidates.

Materials and Methods: This retrospective study analyzed 115 candidates on the cadaveric kidney transplant list from South Bačka and Novi Sad, Serbia. Among them, 69 received blood transfusions, classified as either leukodepleted or containing leukocytes (WBCs), for sensitization control. Anti-HLA antibodies were detected using Complement-Dependent Cytotoxicity, Enzyme-Linked Immunosorbent Assay, and Luminex technology. This study evaluated demographic data, transfusion history, and sensitization. Statistical analysis focused on the relationship between sensitization and blood component variables.

Results: In this study, 53.7 % were sensitized. The number of blood components received (p-value = 0.437), blood unit (p-value = 0.6809), and blood volume (p-value = 0.5857) were not significantly associated with sensitization rates. The use of leukodepleted blood components (p-value = 0.0057), as well as blood components containing WBCs (p-value = 0.030) is associated with a higher sensitization. Sensitization was detected in 67.57 % of cases more than 12 months after transfusion (p-value = 0.046). A significant difference in sensitization was shown when packed red blood cells were used (89.19 % versus 68.75 %; p-value = 0.006).

Conclusions: Sensitization was higher with blood components containing WBCs and packed RBCs. The longer time after transfusion, the more often sensitization is detected.

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Introduction

According to a 2023 report,[1] the overall kidney transplant rate across 40 European countries increased by 1.9 % from 2010 to 2018. Notably, this rate remains higher in Western Europe compared to Eastern Europe. Serbia, however, experienced a 10.8 % decrease in the kidney transplant rate during this period and a significant 46.0 % reduction in live donor kidney transplant rate from 2016 to 2018, positioning it among the top six and top nine countries in these respective categories. Kidney transplantation has a long history in Serbia, with the first procedures conducted in 1970 in Ljubljana and then in 1973 in Belgrade. Over the past 50 years, fewer than 1500 transplants have been performed, with over 70 % being related transplants.[2] From 2010 to 2021, Serbia carried out 460 kidney transplants, while 725 additional patients were added to the waiting list in 2022.

Kidney transplant is widely recognized as the preferred treatment for end-stage chronic kidney disease (CKD), offering enhanced survival and quality of life. Despite its advantages, kidney transplant is not without significant risks and challenges, including anemia, a common comorbidity in terminal-stage CKD patients. Over the last two decades, management of CKD-related anemia has advanced significantly. Historically, blood transfusions were the primary treatment, but they come with complications such as infections, iron overload, fluid imbalance, and adverse reactions to transfusions.[3] Additionally, repeated transfusions increase the risk of alloimmunization, complicating outcomes for patients awaiting renal transplantation.[4] The introduction of recombinant erythropoietin in the late 1980s, followed by erythropoiesis-stimulating agents (ESAs), revolutionized anemia treatment.[5] These therapies not only reduced the need for transfusions but also improved survival rates, quality of life, cardiac function, and decreased hospital admissions. Importantly, they helped lower the percentage of transplant candidates with high panel reactive antibodies (PRA) levels on the waiting list.[6]

Sensitization refers to the presence of antibodies in a potential transplant recipient's serum, typically against HLA class I or class II antigens, and occasionally against non-HLA antigens. A key tool used to quantify immune sensitization is the PRA test, which estimates the percentage of the general population to which a patient has preformed anti-HLA antibodies. Higher PRA values indicate a greater degree of sensitization and are associated with longer waiting times, increased risk of graft rejection, and poorer transplant outcomes.[7,8] Sensitization can occur due to a variety of causes, including blood transfusions, prior transplants, pregnancy, and ventricular assist devices, or sensitization may occasionally arise spontaneously.[9] Among these factors, blood transfusions are a major contributor, responsible for approximately 20–33 % of sensitization events, especially in patients who did not receive leukoreduced blood components.[10]

Despite red blood cells (RBCs) expressing low levels of HLA class I molecules, their sheer number results in a comparable HLA load to residual WBCs in leukocyte-depleted blood.[11] Previous research highlights the link between RBC transfusions and HLA sensitization, leading to prolonged waiting times for transplantation and reduced graft survival.[12] Studies, such as one by Aston et al. in 2014,[13] found no significant benefit from additional washing of red cells in reducing HLA sensitization risk. Prevention strategies focus on minimizing transfusions by optimizing iron stores, judicious use of erythropoietin, and selecting transplants with the best possible HLA match.

PRA levels are not only diagnostic but also prognostic: high PRA values prior to transplantation correlate with an increased risk of graft rejection and delayed graft function.[13,8] For instance, each 1 % increase in PRA above 20 % has been linked to a 5 % rise in rejection risk.[14]

Preformed anti-HLA antibodies pose a significant risk to transplant success, increasing the likelihood of antibody-mediated rejection and graft loss. Allosensitization from blood transfusions exacerbates these risks, limiting graft availability, prolonging waiting times, and shortening graft survival.[15] Minimizing blood transfusions in CKD patients awaiting transplant is therefore critical to mitigate sensitization and improve transplant outcomes.[12] In kidney recipients, preformed anti-HLA antibodies elevate the risk of antibody-mediated rejection, leading to damage to the transplanted organ. These antibodies adhere to the endothelium, causing hyperacute and acute graft rejection, which results in poor survival outcomes.[16]

Reducing the formation of new HLA antibodies and minimizing existing ones in kidney patients can enhance transplant success. Research indicates that HLA class I antibodies correlate with acute rejection,[17] while HLA class II antibodies are linked to chronic rejection.[18]

Objective

The aim of this study was to evaluate the influence of the number, volume and type of blood components received on the appearance of anti-HLA antibodies in patients on the waiting list for kidney transplantation, as well as to evaluate the effect of time from the blood transfusion to the detection of the appearance of HLA antibodies.

Materials and methods

Study population

A one-year (2016) retrospective study involved 115 cadaveric kidney transplant candidates from South Bačka and Novi Sad, Serbia. Of these, 69 received blood component transfusions for sensitization control, while 46 did not. The study adhered to the Declaration of Helsinki, ensuring respondent anonymity, with data presented in aggregated form. This study was approved by the Ethics Committee of the Faculty of Medicine in Novi Sad and Clinical Center of Vojvodina (Novi Sad) - No. 01-14/17-3.

Demographic data and transfusion history were retrospectively collected from the database of the Vojvodina Blood Transfusion Institute. Sensitization was defined as the presence of positive HLA antibodies or a positive Complement Dependent Cytotoxicity (CDC) test result on at least one occasion. The inclusion criteria were patients on the cadaveric

kidney transplant waiting list, undergoing four anti-HLA antibody tests annually (in accordance with the guidelines of the European Federation of Immunogenetics),[19] and receiving blood component transfusions before sensitization testing.

The blood components that patients received included: packed RBCs, leukoreduced RBCs, resuspended RBCs depleted of WBCs and platelets, resuspended RBCs, fresh frozen plasma, double-leukoreduced RBCs, leukoreduced and washed RBCs, fresh frozen plasma without cryoprecipitate, leukocyte-depleted platelets, and preserved whole blood. For the purposes of this study, we categorized the blood components into two groups: leukodepleted (without WBCs) and those containing WBCs. This classification helps to better understand the impact of the WBC content on sensitization.

Detection of anti-HLA antibodies

Detection of anti-HLA antibodies was performed in the tissue typing laboratory of the Vojvodina Blood Transfusion Institute. The results of detection of anti-HLA antibodies used in the analysis are based on the application of three methods: CDC test, Enzyme-Linked Immunosorbent Assay (ELISA) and Luminex bead-based technology (LMX).

Serum samples were screened for preformed anti-HLA class I antibodies using the CDC test following Terasaki's method as per National Institutes of Health (NIH) guidelines. Samples (1 μL) were dispensed onto Terasaki trays with a 20-cell panel sourced from diverse HLA donors, covering the Vojvodina population alleles. Controls comprised negative sera from male AB donors and positive pooled sera (>80 % PRA). Fresh donor cells (1 μL of a 2 $\times$ 10^6/mL suspension) were added and incubated at room temperature for 30 min. Subsequently, 5 μL of rabbit complement was added to each well, followed by a 60-minute incubation at 22 °C. Lysed and viable lymphocytes were assessed using 5 % eosin dye and 37 % formaldehyde under an inverse phase contrast microscope. Reactivity against 10 % or more panel members indicated significant presensitization. The performance of the ELISA and LMX tests for detecting target analytes was assessed. Reference samples with known concentrations were prepared and tested using both assays. In the ELISA procedure, microplate wells were initially coated with specific capture molecules, followed by incubation with the reference samples. Subsequent steps involved detection of antibody binding, washing, and the addition of a substrate solution for colorimetric detection. The LMX was conducted using the Luminex 100 System with xMap technologies and xPONENT Software, designed for protocol-based data acquisition and robust data regression analysis. The reagents for the LMX were supplied by Immucor (Norcross, GA, USA). Specifically, the LIFECODES LifeScreen Deluxe (LMX) is a Luminex® Screening Assay designed for the detection of IgG antibodies against HLA Class I and Class II molecules of human origin. The test involved the preparation of microspheres coated with capture molecules, incubating them with reference samples, adding fluorescently labeled detection molecules, and quantifying the resulting fluorescence intensity using a flow cytometer. Data were analyzed using the MATCH IT! Antibody software.

Analyzed parameters included demographic (gender, age), ABO type, RhD type, anti-HLA class I and II antibody tests, PRA percentage, blood component details (number, volume, type), and time since blood transfusion to HLA antibody detection.

Statistical analysis

Data were processed in the IBM Statistical Package for Social Sciences, version 23 (SPSS Inc., Chicago, IL) and MedCalc for PC (Medcalc Software, Mariakerke, Belgium). Data analysis methods used descriptive and inferential statistics.

Numeric variables were presented as mean (±SD), while discrete outcomes were shown as absolute and relative (%) frequencies. Two groups were formed based on sensitized patient values, with group comparability assessed through demographic data and follow-up duration. Normality and heteroskedasticity of continuous data were examined using Shapiro-Wilk and Levene's tests, respectively. Continuous outcomes were compared using unpaired Student *t*-test, Welch *t*-test, or Mann-Whitney U test based on data distribution. Discrete outcomes were compared using chi-squared or Fisher's exact test accordingly.

Receiver Operating Characteristic (ROC) curves were utilized to predict sensitization based on the number and volume of blood units received. The area under the curve and 95 % confidence intervals were calculated. Multivariate logistic regression was conducted to assess the relationship between sensitization and explanatory variables, including the number of blood components received, number of blood units received, volume of received blood in milliliters, and types of received blood components (with or without WBCs reduction). Data were checked for multicollinearity, heteroskedasticity and normality. Statistical significance was set at a p-value <0.05. Sampling weights were applied during statistical analysis, and results are presented in tables, charts, and diagrams.

Results

Of 115 patients on the kidney transplant waiting list, 46 (40%) had no history of receiving blood units. Consequently, the studied group included the 69 patients (60%) with a history of receiving blood units before transplantation. Table 1 shows data on the gender and ABO type of the examined patients. A total of 69 patients participated in the study, of which 53.7% were sensitized and 46.3% were not sensitized. Among the sensitized patients, almost 60% were female, with the A, RhD positive blood type predominating. Median age was 56.0 (interquartile range [IQR]: 19.0) in patients who were sensitized and 52.0 (IQR 19.25) in patients who were non-sensitized (Median [Yes - No] = 4.0; p-value = 0.032).

A statistically significant difference in the detection of class I and class II HLA antibodies was demonstrated using different techniques (ELISA and LMX) between patients who were sensitized and those who were not. Of the sensitized patients, HLA class I antibodies were detected more often, and in a higher percentage by the LMX technique (81.08%) than by the ELISA technique (45.95%) (Table 2).

No statistically significant difference in the number of received blood units was proven between the examined

Table 1 – Demographic and clinical characteristics.

	Sensitized % (53.7 %)	Non-sensitized % (46.3 %)	p-value
Gender			0.015
Male	40.5	71.9	
Female	59.5	28.1	
Age	56.0 (IQR: 19.0)	52.0 (IQR: 19.25)	0.032
ABO type			0.318
A	43.2	50	
B	21.6	12.5	
AB	16.2	6.3	
0	18.9	31.3	
RhD type			0.496
positive	59.2	81.2	
negative	10.8	18.8	

IQR: Interquartile range

Table 2 – HLA antibodies detection between groups.

Variable	Sensitized % (53.7)	Non-sensitized % (46.3)	p-value
ELISA I			<0.001
not valid	8.11	12.5	
detected	45.95	0.0	
not detected	45.95	87.5	
ELISA II			0.002
not valid	8.11	12.5	
detected	27.03	0.0	
not detected	64.86	87.5	
LMX I	0.0	3.12	<0.001
not valid	81.08	0.0	
detected	18.92	96.88	
not detected			
LMX II	0.0	3.12	<0.001
not valid	59.46	0.0	
detected	40.54	96.88	
not detected			

ELISA I: Investigation of anti-HLA class I antibodies using the ELISA method; ELISA II: Investigation of anti-HLA class II antibodies using the ELISA method; LMX I: Investigation of anti-HLA class I antibodies using the LMX method; LMX II: Investigation of anti-HLA class II antibodies using the LMX method.

groups (33.27 versus 16.56; p-value = 0.366) (Table 4). An ROC curve (Figure 1) was built to assess the prediction of sensitization in relation to the number of blood units received, with the area under the curve being 0.564 (95% CI: 0.467-0.66) with a cut-off value of 36 units, indicating a weak predictive value of sensitization. Based on the cut off value, we divided the respondents into two groups; a significantly higher percentage of non-sensitized patients received more than 36 units of blood (93.75% versus 70.27%; p-value <0.001) (Table 4). No statistically significant difference was found for the number of blood components received between the two groups (3.19 versus 2.97; p-value = 0.787) (Table 4). No statistically significant difference was identified for the volume of blood received (in milliliters) between the two groups (9.636 L versus 4.673 L; p-value = 0.396) (Table 4). An ROC curve (Figure 2) was performed to assess the prediction of sensitization in relation to volume of blood received with the area under the curve being 0.56 (95% CI: 0.463-0.657) and a cut-off value of 10.898 mL, which shows a weak predictive value of sensitization. Based on the cut off value, we divided the respondents into two groups; a significantly higher percentage of non-sensitized patients received over 11 L of blood (93.75% versus 72.97%; p-value = 0.003) (Table 4). In multivariate analysis, the number of blood components received (OR = 0.88; 95% CI: 0.65-1.21; p-value = 0.437), number of blood unit received (OR = 0.96; 95% CI: 0.77-1.18; p-value = 0.6809), received blood volume in milliliters (OR = 1.0; 95% CI: 1.0-1.0; p-value = 0.5857) were not associated with the rate of sensitization (Table 6).

A significantly higher percentage of sensitization was found in patients who received blood components with WBCs (89.19% versus 68,75%; p-value = 0.006) compared to non-sensitized patients (Table 4). Among patients who received blood components without WBCs, 91.89% were not sensitized and 96.88% were sensitized; however, this difference was not statistically significant (p-value = 0.618) (Table 4). There was a significant difference in the frequency of using blood components containing white blood cells (WBCs) between sensitized and non-sensitized patients (p-value = 0.011); of the sensitized patients, 78.38% received both leukocyte-containing and leukocyte-depleted blood components, whereas 10.81% received only one or the other; in the non-sensitized group, 62.5% received both types of blood components (with and without WBCs), while 31.25% received components without WBCs, and 6.25% received components with WBCs (Table 4). Multivariate analysis showed that the receipt of blood components with and without white blood cells (WBCs) was associated with a higher rate of sensitized patients (OR = 3.62; 95% CI: 1.45−9.04; p-value = 0.0057). Additionally, the receipt of blood components containing WBCs alone was also associated with a higher rate of sensitization (OR = 5.0; 95% CI: 1.17 −21.39; p-value = 0.03).

Within six months after transfusion, 21.62% of the patients developed sensitization. An additional 10.81% became sensitized between 6 and 12 months, while in the majority (67.57%) sensitization was detected more than 12 months after the transfusion (Table 4). When analysed according to transfusion-related parameters, the highest sensitization rate was observed in patients who developed HLA antibodies 6-12 months after transfusion (80.0%), compared with 56.82% in those with antibody emergence after more than 12 months, and 40.0% in those with antibodies detected within the first six months (Table 5). Patients who developed HLA antibodies within the first year after transfusion had received a significantly higher number of blood units (p-value <0.001), greater volume of transfused blood (p-value <0.001), and more blood components compared to those with later antibody development (p-value = 0.019). Moreover, the type of transfused blood components differed significantly among groups: patients in the >12-month group predominantly received both leukocyte-containing and leukocyte-reduced components, whereas in the <12-month groups, only leukocyte-reduced components were most frequently administered (p-value <0.001).

According to the Eurotransplant Kidney Allocation System (ETKAS) classification, 51.35% of the group of sensitized

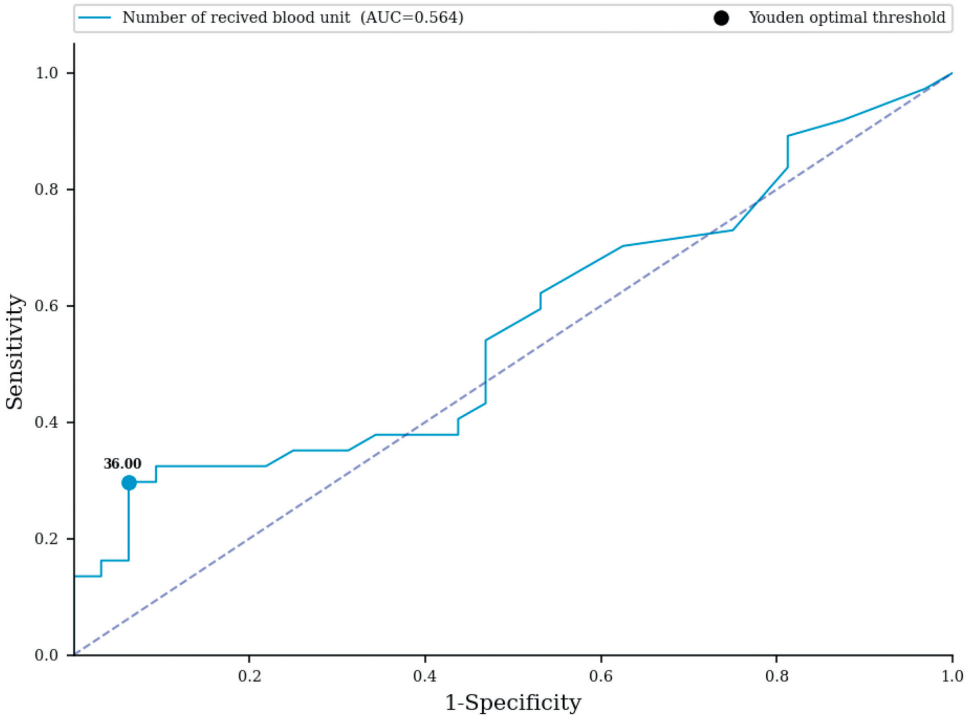

Fig. 1 – Receiver Operating Characteristic curve - prediction of sensitization in relation to the number of blood units received.

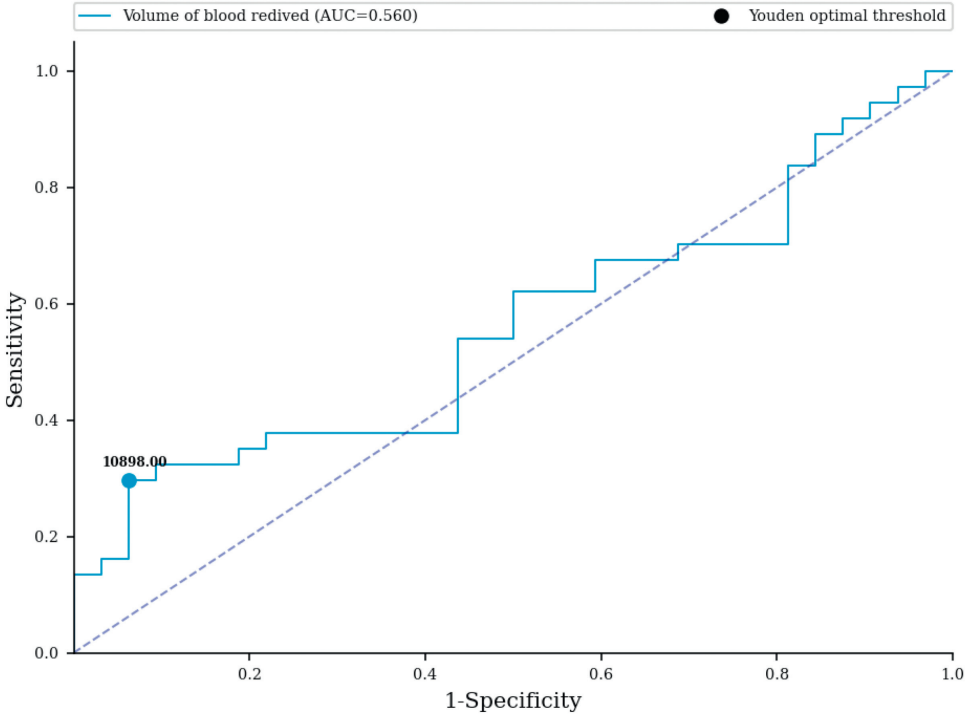

Fig. 2 – Receiver Operating Characteristic curve - prediction of sensitization in relation to the volume (in milliliters) of blood received.

patients were transplantable (Group I), 48.65% were immunized (Group II), and there were no highly immunized patients (Group III). No significant difference was demonstrated in the sensitized patients according to the ETKAS classification (p-value = 0.869). In this study, significant differences in HLA antibody detection were found between PRA-positive and PRA-negative groups (Table 3; p-value <0.001). Among PRA-positive patients, 61.11% had antibodies for both

Table 3 – Human leukocyte antigen antibody detection between panel reactive antibody groups.

	HLA I	HLA I and II	HLA II	negative	Total	p-value
PRA positive	5	11	0	2	18	<0.001
	27.78 %	61.11 %	0.0 %	11.11 %	26.09 %	
	41.67 %	57.89 %	0.0 %	5.88 %		
	7.25 %	15.94 %	0.0 %	2.9 %		
PRA negative	7	8	4	32	51	
	13.73 %	15.69 %	7.84 %	62.75 %	73.91 %	
	58.33 %	42.11 %	100.0 %	94.12 %		
	10.14 %	11.59 %	5.8 %	46.38 %		
Total	12	19	4	34	69	
	17.39 %	27.54 %	5.8 %	49.28 %	100 %	

PRA: panel reactive antibodies; HLA: human leukocyte antigen.

Table 4 – Characteristics of blood component in relation to sensitization.

Variable	Sensitized (53.7 %)	Non-sensitized (46.3 %)	p-value
Number of blood unit received	12.0 (IQR: 36.0)	10.5 (IQR: 16.5)	0.198
Number of blood units received (based on cut off) - %			<0.001
0–36	29.73	6.25	
>36	70.27	93.75	
Number of blood components received – mean (± SD)	3.19 (± 1.79) Range: (1.0–8.0)	2.97 (± 1.47) Range: (1.0–6.0)	0.696
Number of blood components received (based on cut off)			0.106
0–6	10.81	3.12	
>6	89.19	96.88	
Volume of blood received (mL)	3520 (IQR: 9859)	2992.5(IQR: 3916.75)	0.226
Volume of blood received in liters (based on cut off) - %			0.003
0–11	27.03	6.25	
>11	72.97	93.75	
Received blood component with WBCs - %			0.006
Yes	89.19	68.75	
No	10.81	31.25	
Received blood component without WBCs - %			0.618
Yes	91.89	96.88	
No	8.11	3.12	
Received blood component - %			0.011
with WBCs	10.81	6.25	
without WBCs	10.81	31.25	
both	78.38	62.5	

WBC: White blood cell; HLA: human leukocyte antigen; IQR: Interquartile range.

HLA class I and II, while 27.78% tested positive for HLA class I antibodies alone, and none had isolated HLA class II antibodies. In contrast, PRA-negative patients had a lower overall rate of sensitization, with 15.69% showing dual sensitization and 7.84% testing positive for isolated HLA class II antibodies. Additionally, PRA-negative patients had a higher percentage of negative HLA results (62.75%) compared to the PRA-positive group (26.09%).

In relation to the type of blood components received, the only significant difference between sensitized and non-sensitized patients was demonstrated when using packed RBCs (89.19% versus 68.75%; p-value = 0.006) (Table 7). The use of other types of blood components is shown in Table 8. In multivariate analysis, blood transfusion (OR = 3.75; 95% CI:1.52-9.26; p-value = 0.0042) were associated with higher rates of sensitization (Table 6).

Discussion

Compared to the findings of this study, where 53.7 % of the subjects were sensitized, other studies reported slightly lower rates. The study of Loupy et al. in 2012[20] found pre-transplant sensitization in up to 30 % of kidney transplant candidates. Susal et al.[21] reported nearly 25 % of patients on kidney waiting lists had pretransplant anti-HLA antibodies. Of transfused patients, females showed higher rates of sensitization (33-

Table 5 – Time from blood transfusion to HLA antibody detection.

	<6 month % (29.0)	6–12 month % (7.2)	>12 month % *n* = (63.8)	p-value
Gender – %				
male	55.0	80.0	52.27	0.248
female	45.0	20.0	47.73	
Age – years	51.85 (± 10.89) Range: (31.0–71.0)	53.8 (± 14.2) Range: (28.0–64.0)	53.5 (± 11.55) Range: (35.0–74.0)	0.642
Sensitization - %				0.046
Yes	40.0	80.0	56.82	
No	60.0	20.0	43.18	
Number of blood units received	38.05 (± 43.11) Range: (2.0–167.0)	65.4 (± 74.56) Range: (9.0–188.0)	15.3 (± 19.46) Range: (1.0–107.0)	<0.001
Number of blood components received	3.6 (± 1.91) Range: (1.0–8.0)	3.8 (± 1.4) Range: (2.0–6.0)	2.77 (± 1.45) Range: (1.0–6.0)	0.019
Volume of blood received (mL)	11,054.75 (± 12,543.96) Range: (490.0–47,142.0)	18,263.4 (± 20,259.7) Range: (2765.0–50,097.0)	4401.14 (± 6070.36) Range: (165.0–35,083.0)	<0.001
Received blood component - %				<0.001
with WBCs	0.0	0.0	13.64	
without WBCs	40.0	0.0	13.64	
both	60.0	100.0	72.73	

WBC: Leukocyte; RBC: Red blood cell.

60 %) compared to males (17-34 %), consistent with the findings of this study with 59.5 % of sensitized women.[22]

Several studies have investigated the relationship between blood group antigens and HLA antibodies with varying results. Rouger et al.[23] suggested that blood group antigens could influence the detection and formation of HLA antibodies, which is a crucial factor for transplant compatibility and outcomes. Conversely, Erikoglu et al.[24] found that blood type does not directly impact the distribution of HLA antigens. Supporting this, Cruz-Tapias et al.[25] reported that while blood group antigens might have indirect effects on HLA antibody formation and detection - potentially complicating test interpretation due to cross-reactivity -they are not directly linked to HLA antibody detection but can influence the overall immune response. In contrast, this study found that ABO blood type did not influence the detection of HLA antibodies, aligning with the observations of Erikoglu et al.[24] and suggesting that, within this cohort, the blood group antigens do not significantly affect HLA antibody detection.

Table 6 – Multivariate analysis.

Characteristic of blood components in relation to sensitization		
	Odds Ratio	**p-value**
Intercept	1.2 (0.546–2.64)	0.649
Number of received blood units		
Risk for each 1-unit increase	0.957 (0.775–1.18)	0.681
Number of received blood components		
Risk for each 1-unit increase	0.884 (0.649–1.21)	0.437
Received blood volume in milliliters		
Risk for each 1-unit increase	1 (0.999–1)	0.586
Type of blood components in relation to sensitization		
Intercept	1.45 (0.969–2.17)	0.0706
Reference: blood components with and without WBCs		
blood components containing WBCs	1.38 (0.389–4.89)	0.619
leukodepleted blood components	0.276 (0.111–0.688)	0.005
The effect of the use of packed RBCs on sensitization		
Intercept	0.4 (0.176–0.908)	0.028
packed RBCs	3.75 (1.52–9.26)	0.004

WBC: Leukocyte; RBC: Red blood cell.

In the study by Pandey et al. in 2022,[26] transfused blood showed a high rate of alloimmunization for HLA class I antigens. Picascia et al.[27] observed a higher frequency of anti-HLA antibodies for class I compared to class II, although not statistically significant. In this study, of the sensitized patients receiving blood transfusions, HLA class I antibodies were more frequently detected by LMX (81.08 %) than ELISA (45.95 %). Of the PRA-positive patients, HLA class I antibodies were detected in the highest percentage (88.89 %), and HLA class II antibodies in 61.11 %. Vasic et al.[28] found that nearly half of the patients received less than ten units of blood, with an average sensitization level of 13.61 %. Handa et al.[29] reported no significant association between the number of transfused units and alloimmunization. Similarly, Vasic et al.[28] noted higher sensitization levels in patients receiving more than 3000 mL of transfused blood. In the present study, patients receiving less than 11 L of blood components had an average sensitization level (PRA) of 4.82 %, compared to 25 % in those receiving more.

Bilgin et al.[30] found that transfusion of leukocyte-depleted platelets significantly reduces the formation of anti-HLA antibodies. In the current study, blood components with depleted WBCs showed a higher rate of sensitized patients (12.5 %)

Table 7 – Transfusion of different blood components between groups.

Variable		Sensitized %(53.7)	Non-sensitized %(46.3)	p-value
Packed RBCs				0.006
Yes	89.19	68.75		
No	10.81	31.25		
Leukoreduced RBCs				>0.999
Yes	40.54	40.62		
No	59.46	59.38		
Resuspended RBCs depleted of WBCs and platelets				0.368
Yes	62.16	53.12		
No	37.84	46.88		
Resuspended RBCs				>0.999
Yes	62.16	62.5		
No	37.84	37.5		
Fresh Frozen Plasma				0.246
Yes	29.73	40.62		
No	70.27	59.38		
Double Leukoreduced RBCs				0.686
Yes	5.41	3.12		
No	94.59	96.88		
Leukoreduced and washed RBCs				0.686
Yes	5.41	3.12		
No	94.59	96.88		
Fresh Frozen Plasma without cryoprecipitate				0.545
Yes	13.51	18.75		
No	86.49	81.25		
leukocyte-depleted platelets				>0.999
Yes	2.7	3.12		
No	97.3	96.88		
preserved whole blood				0.285
Yes	8.11	3.12		
No	91.89	96.88		

WBC: Leukocyte; RBC: Red blood cell.

compared to those without (5 %), but the difference was not statistically significant (p-value = 0.03). Vasic et al.[28] similarly found no significant difference in sensitization levels between patients receiving leukoreduced RBC units and those who did not. Previous studies by Karpinski et al.[31] also showed no difference in allosensitization rates between standard and leukoreduced RBC transfusions. Despite WBC reduction, alloimmunization rates vary widely, ranging from 7–44 % among recipients of leukocyte-reduced blood transfusions and from 20–50 % among control recipients of non-leukoreduced blood components.[32] As explained in one study,[33] sensitization levels are similar between recipients of leukoreduced and non-leukoreduced blood components. The authors suggest that residual WBCs and RBCs carrying HLA antigens may contribute to the reduced effectiveness of leukoreduced units in preventing sensitization. Vasić et al.[28] observed lower sensitization levels with longer periods since the last transfusion, suggesting that the longer the time elapsed, the less likely it is for the patient to develop alloimmunization. This contrasts with the findings of the present study, where patients who developed HLA antibodies more than 12 months post-transfusion still showed a relatively high rate of sensitization (56.82 %). However, a closer examination of these data reveals that those who developed antibodies earlier (within 6–12 months) exhibited the highest sensitization rates (80 %), indicating that the intensity and frequency of transfusions within the first year play a critical role in sensitization. While the findings of Vasić et al. highlight the potential decline in sensitization over time, the results of this study emphasize the significance of transfusion-related parameters, such as the number of transfusions and blood components, in the development of HLA antibodies, which could suggest that the type and extent of exposure to transfused blood have a more substantial impact on the sensitization process than the mere passage of time.

In the study of Vasic et al. in 2013,[28] the mean PRA value in patients receiving blood components was 16.04 %, consistent with the average PRA value of the current study with 15.54 %, both falling into the ETKAS II group (PRA 6–85 %). Karahan et al.[34] reported that among patients with positive PRA, 47.6 % had positive HLA class I antibodies, 16.7 % had positive HLA class II antibodies, and 35.7 % had positive HLA antibodies for both classes. In this study, 27.78 % of the PRA-positive patients had HLA class I, 61.11 % had HLA class I and II, and none had only class II. Additionally, 11.11 % of PRA-positive patients were negative for HLA antibodies. The findings of this study show a higher prevalence of dual sensitization (both class I and II) compared to Karahan et al.[34] Marfo K. et al.[16] reported 35 % of patients on the waiting list had PRA values >0 %, with 15 % highly sensitized (PRA levels >80). In the present study, according to the ETKAS classification, 51.35 % of the sensitized patients were transplantable (Group I, PRA<6 %), 48.65 % were immunized (Group II, PRA 6–85 %), and none were highly immunized (Group III, PRA >85 %). No significant difference was found in sensitized patients based on the ETKAS classification. In this study, a significant difference in sensitization rates was found between patients who received packed RBCs and those who did not, with a 3.75 times higher likelihood of sensitization in the former group. Recent analysis using LMX technology from the US Renal Data System revealed that RBC transfusions can strengthen and broaden HLA antibodies. Laffell et al.[10] reported a 20 % antibody response rate in patients receiving RBCs transfusions, leading to a tenfold increased relative risk of broad sensitization and a 32-point mean increase in PRA. These findings suggest a causal link between RBCs transfusions and clinically relevant HLA antibody development, resulting in a significant decrease in available donor organs. Therefore, minimizing transfusions whenever possible for patients on the transplant waiting list is crucial.

Conclusion

The study findings suggest that while the total number, volume, and units of blood components received do not significantly contribute to an increase in anti-HLA antibodies or

sensitization, the kind of blood component plays a crucial role. Specifically, transfusions involving blood components containing leukocytes are more likely to lead to sensitization. Among blood cell components, the transfusion of packed RBCs is associated with a higher incidence of sensitization compared to other blood components. Additionally, the time elapsed since transfusion is a significant factor, with a longer interval post-transfusion being correlated with a higher likelihood of detecting sensitization.

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Conflicts of interest

None.

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Review article

COVID-19 Microangiopathy: Insights into plasma exchange as a therapeutic strategy

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ABSTRACT

COVID-19-associated thrombotic microangiopathy has emerged as a severe complication that exacerbates morbidity and mortality in critical cases. Thrombotic microangiopathy, characterized by microvascular thrombosis and endothelial injury, includes conditions like thrombotic thrombocytopenic purpura and atypical hemolytic uremic syndrome. This review investigates therapeutic plasma exchange as a potential strategy to mitigate COVID-19-induced thrombotic microangiopathy, examining its role in removing pro-inflammatory cytokines, immune complexes, and pro-thrombotic factors. Additionally, it highlights the synergistic effects when therapeutic plasma exchange is combined with treatments such as complement inhibitors and immunosuppressants. Preliminary evidence, drawn from case reports and early trials, supports the efficacy of therapeutic plasma exchange in improving outcomes for COVID-19-associated thrombotic microangiopathy. However, larger randomized controlled trials are necessary to definitively establish its place in COVID-19 management, particularly for high-risk and transplant patients with underlying immunological vulnerabilities.

Introduction

COVID-19, caused by the SARS-CoV-2 virus, has demonstrated a wide range of complications, with thrombotic microangiopathy (TMA) being among the most severe [1]. TMA encompasses conditions such as thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic uremic syndrome (aHUS), both of which involve microvascular thrombosis, organ dysfunction, and poor outcomes [2]. COVID-19-induced endothelial injury plays a central role in the development of these conditions [3]. Endothelial cells, when damaged by the virus, become dysfunctional and initiate coagulation pathways, contributing to TMA development. This endothelial dysfunction is also compounded by the 'cytokine storm', where excessive inflammatory responses lead to widespread tissue damage and thrombosis [4].

Understanding the pathophysiology of these conditions in the context of COVID-19 is critical for optimizing treatment. COVID-19 has been shown to trigger complement activation, a vital part of innate immunity, which unfortunately also leads to exaggerated inflammatory and coagulation

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responses [5]. Studies have highlighted that excessive complement activation leads to microthrombus formation in several organs, such as the lungs and kidneys, contributing to multi-organ failure in critically ill patients [6].

Additionally, imbalances in von Willebrand factor (VWF) and ADAMTS13, a metalloprotease that cleaves VWF, are implicated in COVID-19-related microangiopathy [7]. An increase in circulating VWF, coupled with a deficiency of ADAMTS13, leads to unchecked thrombosis and is particularly evident in TTP and aHUS [7,8]. This imbalance exacerbates thrombus formation, especially in microvascular beds, which contributes to organ damage, particularly in the lungs and kidneys [8]. Thus, addressing these pathophysiological changes is crucial for effective management of COVID-19-associated TMA.

Therapeutic plasma exchange (TPE) has emerged as a potential treatment modality for managing COVID-19-associated TMA. TPE works by removing large volumes of plasma containing pro-inflammatory cytokines, immune complexes, and pro-thrombotic factors, thus addressing the hypercoagulable state seen in these patients [9]. Several case reports and small trials have reported promising results with TPE, especially when used in conjunction with other therapies such as complement inhibitors and immunosuppressants [10,11].

Pathophysiology of microangiopathy in COVID-19

SARS-CoV-2 primarily affects the endothelium, leading to widespread endothelial dysfunction and microvascular thrombosis. The virus directly infects endothelial cells via the angiotensin-converting enzyme 2 receptor, leading to cellular damage, inflammation, and apoptosis [12]. The resulting endothelial injury activates both immune and coagulation systems, creating a pro-thrombotic state and driving the development of TMA [13]. This process is particularly problematic in critically ill patients, where endothelial injury plays a central role in organ dysfunction and multi-organ failure. Key contributors to this pathophysiology include:

- Cytokine storm: The hyperinflammatory state induced by COVID-19, often referred to as a 'cytokine storm', triggers widespread endothelial injury. This storm is characterized by the excessive release of pro-inflammatory cytokines such as interleukin-6, tumor necrosis factor-alpha, and interleukin-1 [14]. These cytokines amplify immune responses and lead to direct endothelial cell activation and damage. This hyperinflammatory state contributes to an uncontrolled immune response that results in microvascular damage and thrombus formation.
- Complement dysregulation: Excessive activation of the complement system is a hallmark of severe COVID-19, exacerbating microthrombus formation [15]. Normally, the complement system functions as part of the innate immune defense, but in COVID-19, overactivation leads to endothelial cell injury and the formation of microthrombi. Specifically, complement factors such as C5b-9, a membrane attack complex, have been found to deposit on endothelial surfaces, leading to cell lysis and inflammation [16]. This dysregulation has been strongly implicated in both thrombotic TTP and aHUS, both of which are forms of TMA.
- VWF and ADAMTS13 imbalance: One of the most critical aspects of COVID-19-associated microangiopathy is the imbalance between VWF and ADAMTS13, a metalloprotease responsible for cleaving VWF to prevent excessive clot formation [7]. COVID-19 induces a significant release of VWF from endothelial cells due to the extensive endothelial activation and damage. Simultaneously, levels of ADAMTS13 decrease, possibly due to consumption or direct inhibition by inflammatory mediators [17]. The resultant accumulation of high-molecular-weight VWF multimers leads to unchecked platelet aggregation and microthrombus formation, particularly in the lungs and kidneys. This imbalance is a driving force behind TTP and aHUS, contributing to severe complications such as acute kidney injury and respiratory failure.

Additionally, other coagulation abnormalities in COVID-19, such as elevated D-dimer levels and fibrin degradation products, further support the role of endothelial dysfunction and the pro-thrombotic state in the development of microangiopathies [18]. This constellation of endothelial injury, immune dysregulation, and coagulation imbalance highlights the complexity of managing COVID-19-induced microangiopathies and underscores the need for targeted therapeutic interventions.

Clinical manifestations of COVID-19-Associated thrombotic microangiopathy

TMA in COVID-19 patients presents with a wide range of clinical manifestations, affecting multiple organs. The endothelial damage, hyperinflammation, and coagulation abnormalities that characterize COVID-19 contribute to the widespread nature of TMA, impacting the lungs, kidneys, and other organ systems [19]. The severity of these manifestations often correlates with poor clinical outcomes, including multiorgan failure and death.

- Lung involvement: One of the most severe clinical manifestations of COVID-19-associated TMA is lung involvement, which can lead to acute respiratory distress syndrome (ARDS) [20]. SARS-CoV-2-induced endothelial injury in the pulmonary vasculature results in microvascular thrombosis, impaired gas exchange, and widespread inflammation [21]. Studies have shown that microthrombi in the pulmonary capillaries contribute significantly to the severe hypoxia seen in ARDS [22]. Autopsy findings in patients who succumbed to COVID-19 have revealed extensive pulmonary microthrombi, reinforcing the link between microangiopathy and respiratory failure [23]. Furthermore, elevated levels of VWF in the lungs exacerbate clot formation, contributing to the development of TMA-related lung complications such as ARDS.
- Renal involvement: The kidneys are also highly susceptible to damage in COVID-19-associated TMA, with acute kidney injury being a frequent and severe complication [24]. Endothelial injury, along with microvascular thrombosis in the

renal vasculature, leads to reduced perfusion, ischemia, and tissue damage. In severe cases, COVID-19 patients may develop aHUS, characterized by hemolysis, thrombocytopenia, and kidney failure [25]. The activation of the complement system and its deposition in the renal microvasculature are critical drivers of kidney injury in COVID-19 patients with TMA [26]. Complement-mediated endothelial injury further exacerbates kidney damage, leading to a rapid decline in renal function and, in many cases, the need for renal replacement therapy [26].

- Systemic microvascular dysfunction: Systemic microvascular dysfunction is a hallmark of severe COVID-19-associated TMA and is often indicative of a poor prognosis [27]. As microthrombi form in various organs, multiorgan failure ensues. The heart, liver, gastrointestinal system, and central nervous system are among the organs affected by systemic microthrombi [28]. For instance, myocardial injury in COVID-19 patients has been attributed to microthrombosis within the coronary vasculature, contributing to heart failure and arrhythmias [29]. Hepatic involvement, manifested by elevated liver enzymes and, in severe cases, liver failure, is also observed due to microvascular injury [30]. Neurological symptoms, such as confusion, delirium, and stroke, have been associated with microthrombi and endothelial dysfunction in the cerebral vasculature [31].

As systemic microvascular thrombosis progresses, patients often develop multi-organ failure, a leading cause of death in critically ill COVID-19 patients [32]. The widespread endothelial dysfunction and uncontrolled microthrombosis place immense strain on multiple organ systems, ultimately leading to irreversible damage. Elevated markers of endothelial damage, such as VWF and fibrinogen, are frequently observed in these patients and serve as indicators of severe disease progression [33].

Mechanism of therapeutic plasma exchange (TPE)

TPE is a blood filtration method used to treat a variety of immune-mediated and inflammatory conditions by removing large volumes of plasma, which contains pathological components such as pro-inflammatory cytokines, immune complexes, and coagulation factors [34]. In the context of COVID-19-associated TMA, TPE has gained attention due to its ability to address the underlying mechanisms driving the severe inflammatory and thrombotic responses observed in patients [10]. By clearing the responsible agents from the bloodstream, TPE helps restore balance to the immune and coagulation systems, thus mitigating the progression of TMA and improving clinical outcomes [35]. The benefits of TPE in COVID-19-associated TMA stem from its ability to target several key pathological processes:

- Reduction in inflammatory mediators: By removing large volumes of plasma, TPE significantly reduces the circulating levels of these cytokines, thereby helping to control the cytokine storm and reduce the risk of multi-organ failure [36]. Studies have shown that the reduction of cytokines through TPE can lead to clinical improvement in critically ill COVID-19 patients, reducing inflammation and its associated complications [37].
- Restoration of coagulation balance: By clearing excess VWF and replenishing ADAMTS13, TPE helps to restore normal clotting function, reducing the risk of thrombus formation in the lungs, kidneys, and other organs. Furthermore, the removal of immune complexes, which can also contribute to microvascular damage, helps to decrease the pro-thrombotic state [38].
- Mitigation of complement activation: TPE is effective in removing circulating complement proteins, including C3a and C5a, which play a crucial role in the inflammatory and thrombotic processes associated with TMA. By reducing complement activation, TPE can mitigate endothelial damage and reduce the likelihood of microvascular thrombosis.

In addition to these mechanisms, TPE also provides benefits by removing other circulating pathological substances, such as autoantibodies, that may contribute to the progression of TMA [39]. The process typically involves exchanging large volumes of plasma, which is then replaced with donor plasma or albumin. This replenishment helps to restore the levels of essential plasma components, including clotting factors and regulatory proteins, further supporting the patient's recovery [40].

Evidence supporting therapeutic plasma exchange in COVID-19 microangiopathy

There are several case reports, case series, and studies supporting the benefit of TPE in the treatment of COVID-19-associated TMA (Table 1). Elkayam et al. described a patient who developed TMA in the context of severe COVID-19 infection [41]. The patient received TPE in addition to dexamethasone as standard of care and showed clinical improvement including renal function and declining vasopressor requirement as well as increase in platelets and normalization of hemolysis laboratory results. They attributed to this improvement to cytokine removal of inflammatory mediators which is also suggested by Zhang et al. in their case series of three patients with severe COVID-19 patients with ARDS [42]. Ten days after the treatment, all patients showed resolved respiratory symptoms, improvement of lesions on computed tomography scan, two negative nucleic acid tests and normal body temperature for three days. They claimed that TPE had an acute effect on the treatment of cytokine storm and henceforth led to improvement of the symptoms and radiological/laboratory findings. Similarly, Shi et al. presented a patient with COVID-19 infection who developed respiratory failure, shock and diarrhea and failed to respond to standard therapies [43]. The patient received TPE followed by Intravenous immunoglobulin (IVIG) and showed an immediate clinical response to therapy, supporting the hypothesis that TPE might help signs and symptoms of COVID-19 by removing the cytokines from circulation.

In a study by Arulkumaran et al., five patients who received TPE for severe COVID-19 complicated by ARDS showed improvements in oxygenation and reversal of the thrombo-inflammatory markers [44]. They identified a high

Table 1 – Evidence supporting therapeutic plasma exchange in COVID-19 microangiopathy.

Study/Authors	Patients and Condition	Treatment Provided	Clinical Results
Elkayam et al. [41]	1 patient with severe COVID-19 and TMA	TPE + dexamethasone	Improved renal function, decreased vasopressor requirement, increased platelet counts, normalization of hemolysis parameters
Zhang et al. [42]	3 patients with severe COVID-19 and ARDS	TPE	Resolution of respiratory symptoms, improved CT lesions, negative nucleic acid tests, normalized temperature
Shi et al. [43]	1 patient with COVID-19, respiratory failure, shock, diarrhea	TPE followed by IVIG	Immediate clinical improvement
Arulkumaran et al. [44]	5 patients with severe COVID-19 complicated by ARDS	TPE	Improved oxygenation and reversal of thrombo-inflammatory markers; high VWf:ADAMTS13 ratio observed
Eriko et al. [45]	1 HIV-positive patient with COVID-19-associated TMA	TPE, glucocorticoids, hemodialysis, antihypertensives	Improved renal function and thrombocytopenia
Vrecko et al. [46]	8 patients: 1 with TTP, 7 with aHUS and COVID-19	TTP patient: TPE, steroids, caplacizumab aHUS patients: TPE ± steroids, C5 complement inhibitor, IVIG	Complete hematologic recovery in TTP patient. Rapid hematologic recovery in all aHUS patients; full renal recovery in only one aHUS patient
Cohen et al. [47]	1 patient with COVID-19-associated TTP	TPE, steroids, caplacizumab	Improved abdominal pain, normalization of platelets, hemoglobin, and ADAMTS13
Tehrani et al. [48]	4 patients with COVID-19-associated TTP (2 treated with TPE)	TPE ± Rituximab	Patient 1: Normalization of hemoglobin and platelets Patient 2: No initial response; later response with Rituximab but eventual death due to hemorrhagic stroke

VWf Ag:ADAMTS13 ratio in the patients which is comparable to the parameters seen in TMA. Eriko et al. reported a patient with a past medical history of human immunodeficiency virus, who was diagnosed with TMA secondary to COVID-19 [45]. The patient received TPE, glucocorticoids, hemodialysis, and antihypertensives which contributed to improvements in renal function and thrombocytopenia. Vrecko et al. reported the results of their case series of eight patients with COVID-19-associated TMA including one patient with TTP and seven patients with aHUS [46]. The patient with TTP was treated with TPE, steroids and caplacizumab resulting in complete hematologic recovery. Six patients with aHUS were treated with TPE with or without steroids, C5 complement inhibitor and IVIG while the other patient with aHUS was only treated with steroid and C5 complement inhibitor. While all patients with aHUS showed rapid hematologic recovery, only one of them had full renal function recovery.

Cohen et al. presented a case of TTP in the setting of COVID-19 who was treated with TPE, steroids, and caplacizumab. This patient responded to therapy with improvement of the abdominal pain and normalization of platelets, hemoglobin, and ADAMTS13 [47]. Tehrani et al. reported another case series of four COVID-19-associated TTP patients two of whom were treated with TPE [48]. The first patient responded well to the therapy with normalization of hemoglobin and platelet levels. Even though the second patient did not respond to TPE initially, with the addition of rituximab, hemoglobin and platelet levels became normal although she succumbed to death due to hemorrhagic stroke in her frontal cortex.

Discussion

The emergence of TMA as a complication of severe COVID-19 has brought renewed attention to the complex interplay between endothelial injury, inflammation, coagulation dysregulation, and immune activation. This review consolidates the current understanding of COVID-19-associated TMA and explores TPE as a targeted intervention.

The pathogenesis of COVID-19-associated TMA appears multifactorial. SARS-CoV-2 infects endothelial cells through the angiotensin-converting enzyme 2 receptor, triggering endothelial dysfunction and subsequent activation of coagulation and complement cascades. The cytokine storm further amplifies these processes, while the imbalance between VWF and ADAMTS13 promotes unchecked microthrombus formation. This cascade results in systemic microvascular injury affecting critical organs such as the lungs, kidneys, heart, and brain.

TPE has shown potential in addressing multiple pathological mechanisms underpinning COVID-19-induced TMA. By removing pro-inflammatory cytokines, immune complexes, complement components, and excess VWF, TPE helps attenuate the hyperinflammatory and prothrombotic state. In addition, the replacement plasma replenishes regulatory proteins such as ADAMTS13 and mitigates ongoing vascular injury.

The clinical evidence supporting the use of TPE in COVID-19-associated TMA remains limited but encouraging. Case reports and small case series have documented favorable

outcomes, including rapid hematologic improvement, reversal of organ dysfunction, and improved survival in patients with both TTP and aHUS phenotypes. Notably, these outcomes were observed when TPE was employed in combination with immunosuppressants, steroids, and complement inhibitors, highlighting the importance of multimodal therapy.

Nevertheless, the use of TPE in COVID-19 remains controversial due to a lack of large-scale randomized controlled trials. The heterogeneity of TMA presentations, variability in TPE protocols, and differences in co-interventions make it challenging to draw definitive conclusions. Furthermore, TPE is resource-intensive, limiting its widespread application in resource-limited settings. Careful patient selection and early recognition of TMA features are crucial for optimizing outcomes.

Conclusion

COVID-19-associated TMA represents a severe manifestation of endothelial and immune dysregulation with significant morbidity and mortality. TPE offers a promising strategy to mitigate the cascade of inflammation, complement activation, and thrombosis characteristic of this condition. Early evidence supports its use, especially in cases mimicking TTP or aHUS, and when integrated with other immunomodulatory therapies. However, robust clinical trials are urgently needed to clarify patient selection criteria, treatment timing, and optimal adjunctive therapies. As our understanding of COVID-19-associated microangiopathy evolves, TPE may emerge as a cornerstone of therapy for selected patients, offering a lifeline in an otherwise devastating disease trajectory.

Author contributions statement

Dr. Yigit Baykara, Dr. Yamac Akgun and Kaan Sevgi contributed to all aspects of this review, including conceptualization, conducting the literature review, and manuscript preparation.

Conflicts of interest

The authors declare no conflict of interest.

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Ethical approval statement

Ethical approval is not applicable to this study as it does not involve human or animal subjects requiring institutional review board approval.

Patient consent statement

Patient consent is not applicable as no individual patient data is included in this manuscript.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Case Report

An enigmatic tale of macrophages in bone marrow causing inflammation of the brain: A case report on CNS HLH

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ABSTRACT

Background: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening immune disorder characterized by excessive inflammation and multiorgan involvement. Rarely, HLH can manifest with signs and symptoms isolated to the central nervous system (CNS). This case report highlights the unique clinical course of CNS-isolated HLH in a 19-year-old female who, despite a nine-year delay in diagnosis, achieved disease remission following a hematopoietic stem cell transplant (HSCT).

Case: The patient initially presented at 9 years old with seizures, ataxia, and progressive cognitive decline. Over the next nine years, extensive diagnostic evaluations were performed, including neuroimaging, cerebrospinal fluid analysis, and genetic testing. Genetic testing identified a compound heterozygous mutation in the PRF1 gene, confirming a diagnosis of familial HLH (FHL). The patient underwent hematopoietic stem cell transplant (HSCT) from an HLA-matched unrelated donor. Despite significant complications, including multiple infections and renal failure, she achieved remission. Six years post-transplant, the patient exhibited stabilization of neurological function, cessation of seizures, and absence of active HLH.

Conclusion: This case underscores the importance of considering genetic testing in patients with unexplained CNS symptoms and atypical radiological findings. Timely HSCT, even in cases with delayed diagnosis, can lead to remission and improved quality of life.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disorder of immune dysregulation, manifesting as either familial or secondary to infection, malignancy, or autoimmune

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disease. Familial HLH has been associated with mutations in four genes (PRF1, STX11, STXBP2, and UNC13D), which account for over 90 % of familial cases [1].

Diagnosis of HLH is currently based on the HLH-2004 criteria, which emphasizes systemic disease markers such as fever, splenomegaly and cytopenias [2,3]. Another frequent finding is central nervous system (CNS) involvement due to infiltration of activated lymphocytes and macrophages into the meninges and brain, which can present as seizures, encephalopathy, and cerebellar involvement on MRI [4]. From 30–73 % of patients with systemic HLH have CNS-HLH, but rarely patients will present with isolated CNS involvement [2,4].

Hematopoietic stem cell transplant (HSCT) is now recognized as the only curative option for HLH [3,5]. Numerous studies have demonstrated the effectiveness of HSCT in achieving long-term remission, however, delays in diagnosis, especially with CNS involvement, are associated with increased risk of relapse and poorer outcomes due to the cumulative irreversible CNS damage [3,4,6]. Few cases exist where HSCT has successfully cured CNS-isolated HLH years after initial presentation, with the longest documented period being seven years [7]. Here, we report a unique case of a 19-year-old girl with CNS-isolated HLH, successfully cured with HSCT nine years after symptom onset.

Case presentation

In March 2009, a previously healthy nine-year-old girl presented with progressive neurological symptoms, including headaches, vomiting, and dizziness, and was subsequently admitted to the hospital with sudden-onset confusion, blindness, and incoherent speech. She had an unremarkable perinatal history and no significant personal or familial medical history.

Initial investigations included serological and cerebral spinal fluid (CSF) testing for opportunistic and routine viral infections, all of which were negative. Magnetic resonance imaging (MRI) showed diffuse white matter abnormalities and T2 hyperintensity around the optic nerves. Based on the clinical and radiological findings, a diagnosis of optic neuritis secondary to acute demyelinating encephalomyelitis (ADEM) was made, and the patient was treated with intravenous corticosteroids. A lumbar puncture revealed an opening pressure of 28 mmHg, necessitating the placement of an external ventricular drain, which was successfully removed after one week. The patient regained full vision and was clinically stable until May 2009, when she began to exhibit focal seizures and was started on clobazam. Over the next three months, her condition worsened, with gait disturbances, memory deterioration, and persistent focal seizures despite increasing doses of clobazam.

In early July, she was readmitted with encephalopathy, including a three-week history of increased lethargy, worsening memory, confusion, ataxia, and vomiting. MRI revealed new leptomeningeal enhancement with increased nodularity in the brain parenchyma. Two lumbar punctures performed during this hospitalization revealed only elevated protein (predominantly albumin) with a mild pleocytosis. All other investigations, including antinuclear antibody, oligoclonal banding, cryptococcal antigen testing, culture and cytology, testing were unremarkable. A repeat brain MRI just before discharge demonstrated significant improvement with the resolution of cortical lesions, although the patient continued to experience cognitive dysfunction and a wide-based gait.

In August 2009, a brain biopsy revealed T-cell lymphocytosis surrounding small vessels, leading to a diagnosis of small vessel vasculitis. The patient was treated with prednisone, mycophenolate mofetil (CellCept), cyclophosphamide, and acyclovir. Figure 1 shows the timeline of the patient's course over the next nine years, including multiple treatment modalities and relapses.

In March 2018, the patient underwent genetic testing which revealed a compound heterozygous mutation in the PRF1 gene, consistent with familial HLH. Further testing of her parents revealed the PRF1.886T>C (p.Tyr296His) mutation in her father, and the PRF1.481A>G (p.Lys161Glu) mutation in her mother. Given that the PRF.886T>C (p.Tyr296His) mutation was known to be pathogenic for HLH, she was referred for a potential curative HSCT.

In October 2018, the patient underwent an allogeneic HSCT from a 10/10 HLA-matched unrelated donor. A pre-transplant MRI was performed one month before transplant that found no enhancements to suggest any active disease (Figure 2). She underwent pre-transplant conditioning with fludarabine, treosulfan, and cytarabine (Cytarabine). Neutrophil and platelet engraftment took place 15 days post-transplant. Tacrolimus, rabbit anti-thymocyte globulin, methylprednisolone,

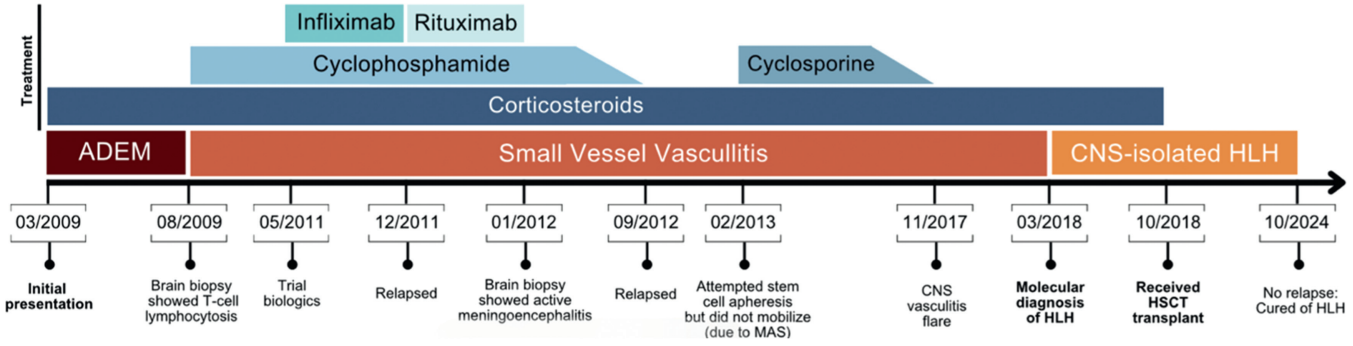

Figure 1 – Timeline of patient course (not to scale). Running diagnosis indicated in shades of orange, medications administered to patient indicated in shades of blue. Corticosteroids were administered PO or IV sporadically throughout the disease course. Notable events during disease progression are dated and detailed below the arrow.

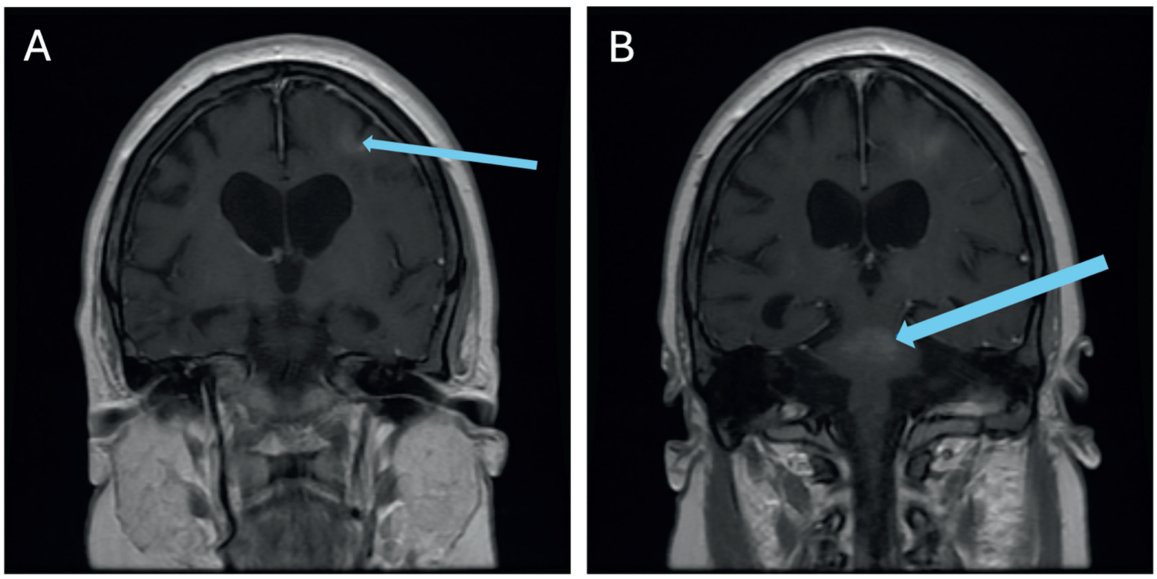

Figure 2 – Pre-transplant magnetic resonance images demonstrating low grade enhancement in the precentral gyrus of the left frontal lobe extending in deep aspects of cortex asymmetrically (A) and stippled enhancement in dorsal aspect of mid pons (B) on post-gadolinium images.

and methotrexate (Methotrexate) were given for graft-versus-host disease (GvHD) prophylaxis. She developed acute GvHD (overall Grade I) of the skin and gut, which resolved with topical and systemic steroids. Chimerism 60 days post-transplant showed >95 % donor cells, and at 280 days post-transplant the patient demonstrated stable chimerism with CD3 donor cells at 97.4 %, CD19 at 98.9 %, and myeloid cells at 98 %, and remained at these levels thereafter.

Unfortunately, her post-transplant course was complicated by multiple infections including Epstein–Barr virus (EBV) viremia, varicella infection, and adenoviremia one-month post-transplant. She also experienced acute kidney injury secondary to tacrolimus and cidofovir toxicity that progressed to acute-on-chronic renal failure due to sepsis requiring hemodialysis in March 2019, and eventually a related living donor kidney transplant in January 2023.

Despite these complications, her neurological symptoms significantly improved. She experienced two seizures within the first-year post-transplant but has since been seizure free. Despite ongoing dystonic movements (which predated her transplant) for which she is on trihexyphenidyl, her cognitive function has returned to baseline. MRI findings one-year post-transplant showed no active disease, and she has been deemed cured of HLH. She is now six years post-transplant and is doing well, with no recurrence of disease or neurological symptoms.

Discussion

Isolated CNS symptoms in the absence of systemic manifestations makes differentiating CNS-HLH from other CNS inflammatory diseases, such as ADEM, extremely challenging, as seen in this case. A study conducted by Deiva et al. compared radiological presentations of CNS-isolated HLH and ADEM and found that while both may present with white matter hyperintensity on MRI, CNS-HLH often features symmetrical, periventricular lesions, whereas ADEM typically involves the brainstem [8]. Our patient had asymmetrical white matter lesions and brainstem involvement, demonstrating that additional studies are necessary to fully differentiate these conditions (Figure 2).

The challenges of diagnosing CNS-isolated HLH are further highlighted by Benson et al., who describe three cases of CNS-isolated HLH initially misdiagnosed as ADEM or small vessel vasculitis [7]. Similar to our case, the diagnosis was ultimately confirmed through genetic testing. This case, in addition to those of Benson et al., underscores the importance of prompt genetic testing in patients with unexplained neurological symptoms and abnormal neuroimaging which would allow for earlier initiation of curative interventions like HSCT.

Although HSCT is widely known to be curative for systemic HLH, its role in CNS-isolated HLH is less established [3]. Previous cases have shown that early HSCT can halt disease progression and offer a cure when performed soon after symptom onset [7,9,10]. Our patient was unique in that she underwent HSCT nine years after her initial symptom onset. Despite the delay, her post-transplant MRI findings showed no further disease activity, although existing lesions and some neurological symptoms remain unchanged. This aligns with reports emphasizing the irreversible nature of CNS damage when treatment is delayed. Nevertheless, at six years post-transplant, our patient exhibits no further disease progression and is now considered cured of her CNS-HLH. This suggests that HSCT can still offer significant benefits, even after prolonged disease progression.

In conclusion, genetic testing for HLH-associated mutations should be pursued in patients with refractory inflammatory CNS disease and neurological impairment, as timely HSCT can halt disease progression, improve quality of life

and provide curative outcomes, even after years of ongoing symptoms

Ethics approval and consent to participate

Ethics approval is not required at our institution for individual cases or case series.

Consent for publication

Verbal, informed and written consent was obtained from the patient through a legally authorized representative for anonymized patient information.

Availability of data and materials

The authors confirm that the data generated and analyzed in this study are included in this published article.

Clinical trial number

Not applicable

Authors' contributions

IEV collected the raw patient data and wrote the manuscript. JL also collected patient data, designed the study and contributed to writing and editing the manuscript. AF provided guidance and obtained patient consent. UD designed the study and contributed to writing and editing of the manuscript with overall supervision. All authors read and approved the final manuscript.

MAS: macrophage activation syndrome; ADEM: acute demyelinating encephalomyelitis; HSCT: Hematopoietic stem cell transplant

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Conflicts of interest

The authors do not have any competing interests to declare.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Case Report

Daratumumab-EPOCH for transformed anaplastic multiple myeloma

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Introduction

Anaplastic multiple myeloma is not an established diagnostic entity but is described many times and has distinct histopathological features. It is associated with poor prognosis, high Ki67, rapid growth, and extramedullary myelomas. It can debut as the first symptom of multiple myeloma (primary anaplastic myeloma) or transform from an existing myeloma. Anaplastic transformation of multiple myeloma is typically associated with a survival of only a few months [1,2]. Myeloma treatments are mainly based on dexamethasone, proteasome inhibitors, immunomodulatory drugs, and CD 38 antibodies [3]. Traditional cytotoxic drugs are nowadays rarely used, except melphalan, mostly together with autologous stem-cell transplantation (ASCT). There have been reported cases where primary anaplastic multiple myeloma has responded well to the EPOCH regimen used for lymphoma [4]. There are, however, no reports of successful treatment of transformed anaplastic multiple myeloma. Here we present two heavily treated myeloma patients who had an anaplastic transformation of their disease. They received a combination of modern myeloma treatment with traditional lymphoma treatment with surprisingly good results.

Case report

Case 1 involved a 64-year-old woman diagnosed in 2015 with multiple myeloma, characterized by an IgG monoclonal protein (Table 1). She had been diagnosed with a monoclonal gammopathy of undetermined significance four years earlier. Her past medical history included rheumatoid arthritis, hypertension, ovarian cancer, herpes zoster, and sclerotic aortic and mitral valves. First line treatment was induction therapy followed by high-dose melphalan with ASCT. Three years later she had a biochemical recurrence. Second-line treatment was a second induction and high-dose melphalan with ASCT followed by maintenance therapy with lenalidomide and dexamethasone (Rd - Table 1). In December 2021 she was diagnosed with transformed anaplastic multiple myeloma (Table 2). She experienced persistent high fever with deteriorating general condition. She received four cycles of daratumumab, carfilzomib, and dexamethasone with a reduction of plasma cells in the bone marrow from 70 % to 40 %, but the fever remained. Four months later, a biopsy

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Table 1 – Baseline patient and disease features at multiple myeloma diagnosis.

	Case 1	Case 2
Sex	Female	Female
Age at multiple myeloma diagnosis	64 years	61 years
Characteristics of the myeloma	Monoclonal IgG protein 41 g/L, 30 % plasma cells in the bone marrow. Anaemia, hypercalcaemia, and skeletal involvement.	Non-secretory myeloma, 90 % plasma cells, hypercalcaemia, renal failure and osteolytic skeletal lesions.
ISS	III	II
Cytogenetics	Small extra marker chromosome	t(11;14)(q13;q32)
1st line treatment	VCd x 4, HDM with ASCT	VCd x 2, VTd x 2, VRd x 2, HDM with ASCT
2nd line treatment	VRd x 4 + HDM with ASCT + 27 x Rd maintenance therapy.	RT, Vd x 1, PomVD x 5, HDM with ASCT, 7 x DaraVd maintenance therapy

VCd: bortezomib, cyclophosphamide, dexamethasone; VRd: bortezomib, lenalidomide, dexamethasone; VTd: bortezomib, thalidomide, dexamethasone; HDM: high-dose melphalan; RT: radiotherapy; PomVd: pomalidomide, bortezomib, dexamethasone; DaraVd: daratumumab, bortezomib, dexamethasone.

Table 2 – Treatment given after anaplastic transformation and survival.

	Case 1	Case 2
Characteristics of anaplastic transformation	70 % plasma cells in the bone marrow, monoclonal protein IgG of 19 g/L, Ki67 of 100 %, the plasma cells positive for c-MYC protein.	Extramedullary disease, Ki67>60 %, C-Myc protein positive and cyclin D1 positive. Normal bone marrow biopsy.
Age at anaplastic transformation	70 years	66 years
Cytogenetics	Loss of 17p13	Normal
1st line treatment after anaplastic transformation	Dara-Kd	Dara-EPOCH
2nd line treatment after anaplastic transformation	Dara-EPOCH	Daratumumab maintenance therapy
3rd line treatment after anaplastic transformation	Daratumumab maintenance therapy	Dara-modified POMP
4th line treatment after anaplastic transformation	Dara-modified POMP	Belantamab mafodotin
5th line treatment after anaplastic transformation	Belantamab mafodotin	Talquetamab
6th line treatment after anaplastic transformation	Carfilzomib EPOCH	Isa-Kd-IME
Survival after anaplastic transformation	19 months	26 months
Overall survival after multiple myeloma diagnosis	7 years 9 months	8 years

Dara-Kd: daratumumab, carfilzomib, dexamethasone; Dara-EPOCH: daratumumab, etoposide, prednisolone, vincristine, cyclophosphamide, doxorubicin; Dara-modified POMP: daratumumab, dexamethasone, vinblastine, mercaptopurine, methotrexate; Isa-Kd-IME: isatuximab, carfilzomib, dexamethasone, ifosfamide, methotrexate, etoposide.

from an axillary lymph node confirmed anaplastic multiple myeloma. The EPOCH chemotherapy regimen traditionally used for aggressive lymphomas had been tried previously for anaplastic myeloma [4,5]. We therefore decided to give her daratumumab together with EPOCH, i.e., continuous infusion of etoposide 100 mg/m^2 Day 1–4, prednisolone 60 mg/m^2 Day 1–5, vincristine 0.8 mg/m^2 on Day 1–4, doxorubicin 20 mg/m^2 Day 1–5, and cyclophosphamide 750 mg/m^2 Day 5 (Dara-EPOCH). Daratumumab was administered weekly, and EPOCH was administered every third week. She received five cycles of Dara-EPOCH, the last cycle in July 2022. In November 2022 there were 0.5 % plasma cells in the bone marrow and the monoclonal component was zero. A positron emission tomography-computed tomography (PET-CT) scan identified residual lesions in the left iliac bone and a left rib. Radiotherapy for these lesions was initiated in November 2022. At the same time, maintenance treatment was started with daratumumab weekly. That winter she sent us a picture where she went cross-country skiing.

In January 2023 the monoclonal protein increased and we started treatment based on the POMP regimen [6] with vinblastine every fourth week, dexamethasone Day 1–5 of each 28 day cycle, together with daily oral mercaptopurine and weekly oral methotrexate in addition to the weekly daratumumab with dexamethasone she was already receiving (Dara-modified POMP). In May 2023 she had increased skeletal lesions and was administered two doses of the antibody-drug conjugate belantamab mafodotin. A lesion at the right orbit was treated with radiotherapy in July 2023. The disease continued to progress evidenced by extramedullary lesions and

following discussion with the patient we decided to give a new EPOCH cycle with carfilzomib. She died due to febrile neutropenia in late July 2023.

Case 2 was a 61-year-old woman diagnosed with non-secretory multiple myeloma in 2016. She received induction treatment and high-dose melphalan with ASCT (Table 1). She started Rd as maintenance treatment, but developed a rash and the treatment was discontinued. Five years later she was admitted to hospital with malignant spinal cord compression. She was treated with radiotherapy followed by subsequent induction and a second high-dose melphalan with ASCT. A response assessment PET-CT showed a lesion in the left femur. She then received seven cycles of daratumumab-bortezomib-dexamethasone (Table 1). Six months later, in March 2022, the disease recurred with tumours in the left pleura, behind the aorta, and a skeletal lesion in L1. A biopsy showed anaplastic multiple myeloma (Table 2). She received two cycles of EPOCH, and four cycles of Dara-EPOCH. A PET-CT in October 2022, six months after the first EPOCH cycle, demonstrated almost complete remission with a residual lesion in the left femur. She then started weekly daratumumab and the residual lesion in the left femur was treated with radiotherapy.

In January 2023 the treatment was shifted to Dara-modified POMPIn May 2023, a PET-CT revealed progressive disease with bilateral rib involvement, multifocal lesions in all four extremities, and paravertebral tumors. Treatment was changed to belantamab mafodotin which was discontinued after two doses due to keratopathy. In August, the treatment was changed to talquetamab, a bispecific antibody that induces apoptosis of myeloma cells by means of T-cell recruitment and activation [7]. She received 40 mg subcutaneous every 14th day, and the disease stabilized until November 2023 when she was readmitted to the hospital with increasing pain. A PET-CT showed increased size of the myeloma lesions. Treatment was shifted to a 28-day cycle of isatuximab Day 1 and 15, carfilzomib Day 1, 8 and 15, ifosfamide 1000 mg/m^2 Day 1–5, etoposide (Etoposide) 100 mg/m^2 Day 1–3, methotrexate (Methotrexate) 30 mg/m^2 Day 3 with dexamethasone and mesna (Mesna) resulting in effective pain control. A PET-CT in February 2024 showed regression of the myeloma lesions. In April 2024 the chemotherapy no longer provided pain relief. A subsequent PET-CT showed recurrence of the lesions from December 2023. She was transitioned to palliative care and died in May 2024.

Discussion

These two case reports illustrate that conventional chemotherapy used for aggressive lymphomas combined with anti-myeloma treatment is efficient in the treatment of transformed anaplastic multiple myeloma. The expected survival of patients with transformed anaplastic myeloma is only a few months. In contrast, these two patients lived for 19 and 26 months with a reasonably good quality of life. Most published cases of anaplastic myeloma are from patients who debut with anaplastic myeloma [1,4,8]. Although transformed anaplastic myeloma is a well-known aggressive end stage of myeloma,[2] we only found one publication reporting on the treatment of two such cases [1]. One of the two cases received thalidomide, vincristine, doxorubicin, dexamethasone (Thal-VAD) and lived for three months. The other received one cycle of bortezomib, cisplatin, cyclophosphamide, etoposide, dexamethasone followed by five cycles of Thal-VAD with at least nine months survival. Previous studies have shown good outcomes with EPOCH (without daratumumab) in patients with anaplastic myeloma at diagnosis, thus guiding its application in our two cases [4,9]. Others have tried more standard myeloma treatment with and without effect [8,10].

A possible reason for the effect of the EPOCH regimen might be that anaplastic myeloma, in contrast to regular myeloma, is a rapidly growing malignancy. Hence, more myeloma cells are in a cell cycle state where they are vulnerable to conventional chemotherapy. We do not know if the addition of daratumumab/isatuximab or carfilzomib to the lymphoma regimens was beneficial or not, but it certainly was tolerable. In both patients, we continued treatment combining traditional lymphoma treatments with myeloma treatment to mitigate the recurrence of the myeloma.

We suggest that EPOCH combined with other less cytotoxic myeloma drugs, such as anti-CD38 antibodies or proteasome inhibitors, is a possible option for anaplastic myeloma, and perhaps also for other rapid growing variants of multiple myeloma, e.g., plasma cell leukaemia. A possible way forward for aggressive myelomas could be to use Dara-EPOCH to bring the patient in remission, followed by bi-specific antibodies such as talquetamab as maintenance therapy.

Author contribution

ES treated the patients, collected data, and Writing - review & editing. AD treated the patients, collected data and wrote the first draft of the manuscript.

Data availability

Not applicable.

Conflicts of interest

Stormorken: No conflicts of interest.

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Letter to the Editor

The battle against pediatric retinoblastoma: insights from 2024 medical literature

This literature review, based on 2024 peer-reviewed publications from the National Library of Medicine, provides a concise overview of retinoblastoma, a pediatric cancer that necessitates multidisciplinary care. The primary objectives of treatment are to preserve life and vision [1]. Extraocular retinoblastoma can likely be cured with systemic chemotherapy and radiation therapy. In contrast, extra-orbital disease demands intensive chemotherapy, potentially consolidated with high-dose chemotherapy and autologous hematopoietic stem cell rescue, with or without radiation therapy [1]. Retinoblastoma, although relatively uncommon and accounting for about 3% of cancers in children under 15 years, is a significant concern [1]. It originates from the retina and may grow under the retina or towards the vitreous cavity. Most patients present with leukocoria, often first noticed after a flash photograph is taken. Screening guidelines have been established for children at risk of developing retinoblastoma [1].

As the most common pediatric ocular malignancy, retinoblastoma is typically caused by a mutation in the *RB1* gene or *MYCN* oncogene amplification [2]. The disease can be unilateral (60%–70%) or bilateral (30%–40%), with hereditary cases being bilateral and appearing earlier than unilateral ones, especially in India. Outcomes in developing countries are worse due to high prevalence, delayed presentation, and healthcare inaccessibility. Treatment has evolved from enucleation and radiotherapy to less aggressive eye salvage modalities. Multimodality treatment, including chemotherapy and focal consolidation therapies, is now standard. Genetic testing, counseling, and screening are essential, with advances in genetics paving the way for potential targeted therapy [2].

Addressing *RB1*-deficient cancers presents a challenge. Dysfunctional or deleted *RB1* can enhance cancer proliferation and spread [3]. Scientists are striving to understand the role of *RB1* in various biological processes and molecular pathways to develop innovative therapies for *RB1*-deficient cancers. Recent advancements have shown promising strategies for combating these cancers, leading to more precise and effective treatments. They emphasized the crucial role of *RB1* in cancer research and its potential for personalized therapies. *RB1*, a critical tumor suppressor gene, plays a significant role in cell cycle control and DNA repair mechanisms, restraining abnormal cell growth and maintaining genomic stability. Understanding the complex interactions of *RB1* with cellular pathways is key to developing targeted therapies [3].

Retinoblastoma, a prevalent pediatric vision-threatening condition, has traditionally been treated with conventional therapies like systemic chemotherapy and focal therapy [4]. However, the quest for tumor eradication with minimal impact on surrounding tissues continues. Researchers explored the genetic origin, classification, and treatment modalities with their combination with nano-scale delivery systems for tumor targeting. They also discussed ongoing clinical trials, patents, and emerging therapies like gene therapy and immunotherapy. Understanding genetics in retinoblastoma development has refined treatment strategies. The novel approaches discussed here could overcome limitations of conventional therapy, thereby improving retinoblastoma treatment outcomes [4].

Balaji et al. reported that retinoblastoma is a pediatric eye cancer occurring in 1/15000 live births [5]. Initiated by *RB1* gene inactivation, its progression relies on transcriptional changes. This study used RT2 Profiler™ PCR array to analyze 84 cancer-specific genes, identifying 68 dysregulated genes. Key alterations were validated by real time quantitative reverse transcription polymerase chain reaction (RT-qPCR) and Western blot, revealing potential therapeutic targets. Polymerase chain reaction (PCR) arrays are suggested for rapid, cost-effective gene expression analysis in retinoblastoma [5].

Cobrinik et al. reported that retinoblastoma is a childhood retinal cancer affecting six out of 100,000 live births, with 250–300 new cases annually in the U.S. and 8000 worldwide [6]. Early detection often leads to a cure, with advanced cases posing clinical challenges. Initiated by *RB1* gene inactivation, recent studies have identified the cell of origin and genomic changes linked to treatment resistance. This understanding may improve therapies and prevention for genetically predisposed children [6].

Zhou et al. states that retinoblastoma, a primary ocular malignancy in children that without prompt treatment poses

a significant mortality risk [7]. Survival and vision preservation depend on disease severity at diagnosis. Initiated by *RB1* gene mutations, tumorigenesis involves genetic and epigenetic changes. The *RB1* gene encodes a protein crucial for cell replication control. Diagnosis and treatment consider disease stage, germline mutation status, psychosocial factors, and institutional resources, enhancing care quality for this pediatric cancer [7].

Singh et al. concluded that retinoblastoma is the most common childhood intraocular tumor, caused by *RB1* gene mutations on chromosome 13 [8]. Annually, 8000 children are diagnosed globally, with 1500 cases in India. Survival rates exceed 90% in developed countries. Leukocoria and proptosis are common in Asian Indian populations. Diagnosis involves fundus examination and ultrasound. Prenatal and genetic testing benefit high-risk families. Histopathologic risk factors predict metastasis, requiring aggressive treatment for advanced cases [8].

Rabelo et al. reported that retinoblastoma, the most common childhood intraocular tumor, faces diagnostic and treatment challenges, especially in low- and middle-income countries [9]. Improving time to diagnosis and treatment access is crucial. A systematic review identified 21 studies with potential interventions, categorized into surveillance, genetic counseling, education, public assistance, and international partnership. These initiatives aim to enhance diagnosis and treatment access, proposing a comprehensive chain of initiatives to improve clinical outcomes for retinoblastoma patients [9].

The literature review provides a comprehensive overview of retinoblastoma, a significant pediatric cancer requiring multidisciplinary care. It emphasizes the primary treatment objectives of preserving life and vision, discussing various modalities such as systemic chemotherapy, radiation therapy, high-dose chemotherapy, and autologous hematopoietic stem cell rescue. The review delves into the genetic causes, particularly *RB1* gene mutations and *MYCN* oncogene amplification, and highlights the challenges faced in developing countries due to high prevalence, delayed presentation, and healthcare inaccessibility. It notes the evolution of treatment from enucleation and radiotherapy to less aggressive eye salvage modalities and multimodality treatment, stressing the importance of genetic testing, counseling, and screening. The review also addresses the challenges of *RB1*-deficient cancers and ongoing research to develop innovative therapies, including gene therapy, immunotherapy, and nano-scale delivery systems. Additionally, it mentions ongoing clinical trials and patents, diagnostic and treatment challenges, especially in low- and middle-income countries, and suggests potential interventions to improve clinical outcomes. Overall, the review provides an up-to-date understanding of retinoblastoma, its genetic basis, treatment options, and research efforts to enhance patient outcomes.

Author contribution

Yoshiyasu Takefuji completed this research and wrote this article.

Conflicts of interest

The author declares no conflicts of interest.

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Letter to the Editor

Electroacupuncture as a tool to stimulate bone marrow megakaryocytes in mice: A pilot study

Dear Editor,

We report the first evidence that electroacupuncture stimulates megakaryocyte production in the bone marrow (BM) of mice, offering a novel approach to support megakaryopoiesis and improve animal research.

Platelets, derived from megakaryocytes, are critical not only for hemostasis but also for vascular integrity, inflammation, pathogen defense, and tissue repair. They orchestrate innate and adaptive immune responses by expressing Toll-like receptors, activating leukocytes, releasing defensins, and engaging the complement system [1,2]. Enhancing platelet production is particularly important in thrombocytopenia and BM suppression [2,3].

In mouse models, BM sampling typically involves euthanasia and femur dissection, limiting longitudinal evaluations and conflicting with the 3Rs principles (replacement, reduction and refinement) [4–6]. We aimed to test whether electroacupuncture, a modern adaptation of Traditional Chinese Medicine, stimulates megakaryocyte production and to validate a minimally invasive iliac crest aspiration technique that permits repeated sampling in the same animal. We studied 30 six-month-old male BALB/c mice (15–22 g), housed individually under controlled conditions (20 °C, 12/12-h light-dark cycle, 70 % humidity, ad libitum food and water). Mice were randomized into three groups (n = 10 each): control group, electroacupuncture-treated (Electroacupuncture group), and sham electroacupuncture-treated (Sham group). Electroacupuncture was applied at large intestine (LI)-4 and LI-11, bladder (BL)-12 and BL-13, governing vessel (GV)-14 and GV-20 acupoints using sterile stainless-steel needles (0.16 mm × 9 mm, 0.18 mm × 8 mm; DUX®, Brazil). Treatments were performed under general anesthesia, using alternating currents (2 Hz/50 Hz) with 10s/30 s stimulation cycles and 5 s breaks at 2.0 mA, for 45 min, repeated over two weeks. Animals of the Sham group received identical stimulation at non-meridian points. Treatments were conducted by a veterinary acupuncture specialist (Figure 1). BM aspiration was performed under anesthesia on Days 0 and 44 by exposing the iliac crest through a 0.5 cm skin incision and aspirating up to 0.5 % of body weight. Postoperative care included monitoring and analgesia. BM smears were stained with May-Grünwald-Giemsa, and megakaryocyte counts were analyzed histologically. Statistical comparisons were conducted using the Mann-Whitney U and Wilcoxon tests (GraphPad Prism 9.4.1; p-value <0.05). Ethics approval was granted by the Pontifícia Universidade Católica do Paraná Animal Ethics Committee (registration 1247).

We completed 60 BM collections (two per animal). Mean weights were 22.0 ± 3.5 g at first and 26.0 ± 2.7 g at second collection point (p-value = 0.164). Anesthesia induction averaged 4.0 ± 0.89 min, aspiration 12.0 ± 3.82 min, and recovery 75.0 ± 24.0 min. No adverse events were noted; three animals discontinued electroacupuncture two minutes early. No postoperative medications beyond standard care were needed, and sutures were removed after seven days without infection or self-injury. Histological evaluation confirmed the presence of myeloid and erythroid precursors and megakaryocytes (Figure 2). Megakaryocyte counts significantly increased in the electroacupuncture group between first and second aspirations (p-value = 0.040) and were significantly higher than controls at the second timepoint (p-value = 0.040). The Sham group showed no significant changes (Figure 2).

These initial findings indicate that electroacupuncture stimulates megakaryocyte production in vivo. The experimental design reduced animal use by allowing paired comparisons over time, supporting the 3Rs principles. The iliac crest aspiration method was effective, minimally invasive, and allowed full recovery, making it suitable for longitudinal studies.

Megakaryopoiesis is regulated by thrombopoietin (TPO), a liver-derived cytokine that promotes megakaryocyte differentiation from hematopoietic stem cells and drives platelet production [2]. While we did not measure TPO levels, it is plausible that electroacupuncture may enhance megakaryocyte production by modulating the TPO pathway, which should be evaluated in future studies.

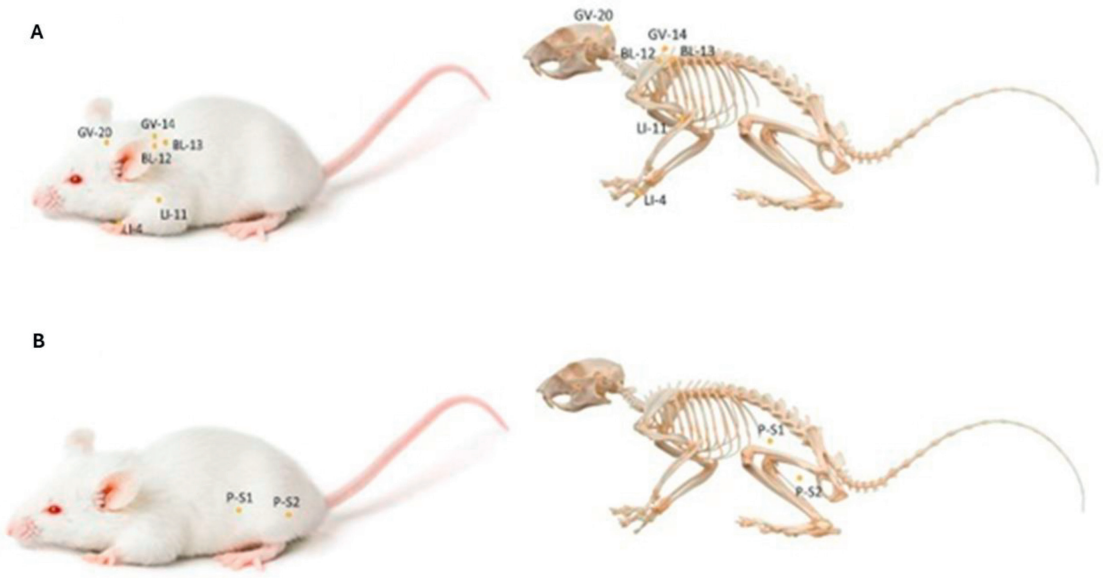

Figure 1 – Locations of electroacupuncture and sham electroacupuncture points in BALB/c mice. A) Electroacupuncture points: LI-4 (between the first and second metacarpal bones), LI-11 (lateral to the elbow crease), BL-12 and BL-13 (paraspinal region, at thoracic vertebral levels), GV-14 (between the spinous processes of C7 and T1), and GV-20 (at the midline of the skull). B) Sham electroacupuncture points: P-S1 (lateral abdomen, on the transverse abdominal muscle at the level of the 11th rib) and P-S2 (lateral hindlimb, on the gastrocnemius muscle, proximal to the lateral saphenous vein).

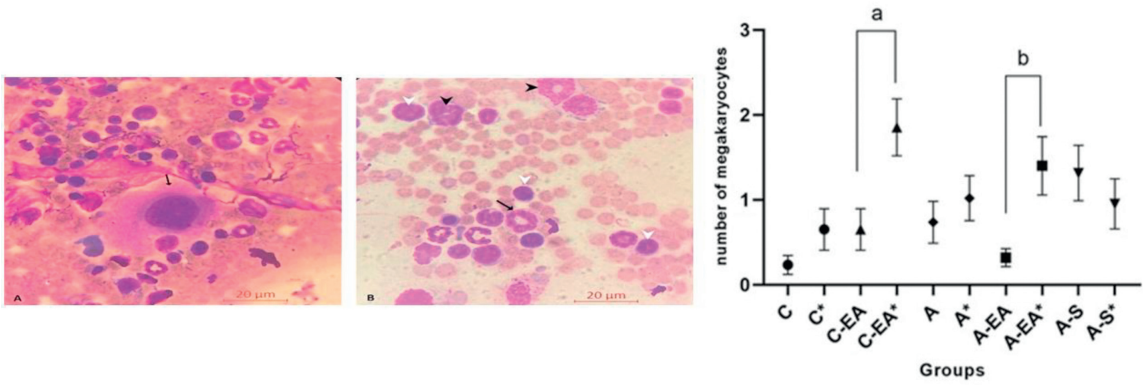

Figure 2 – Bone marrow samples from BALB/c mice stained with May-Grünwald-Giemsa. (A) Bone marrow smear showing myeloid and erythroid precursors; a megakaryocyte is indicated by the arrow (× 1000). (B) Bone marrow aspirate highlighting multiple megakaryocytes indicated by arrows (× 100). The graph shows the effect of electroacupuncture on megakaryocyte counts in bone marrow at baseline and after treatment. *Second bone marrow aspiration. A significant increase in megakaryocyte numbers was observed in the electroacupuncture group at the second aspiration. (C) Control group; EA: Electroacupuncture group; SEA: Sham electroacupuncture group.

We believe electroacupuncture could emerge as a novel supportive strategy in clinical contexts such as chemotherapy-induced thrombocytopenia or BM failure. Prior studies show that acupuncture influences neuroendocrine and inflammatory pathways [7,8] and promotes stem cell mobilization [9,10], providing mechanistic plausibility to our findings. Importantly, the inclusion of a sham group confirmed that megakaryocyte stimulation was specific to true acupuncture points.

We recognize the preliminary nature of this work and the small sample size. Future investigations should assess TPO modulation, platelet counts, and functional outcomes to elucidate the full hematopoietic impact of electroacupuncture and its translational potential.

In conclusion, our pilot study demonstrates that electroacupuncture stimulates megakaryocyte production in mice and provides a minimally invasive model for repeated BM sampling. We thank the editor for considering this letter and

welcome feedback from the Hematology, Transfusion and Cell Therapy readership.

Conflicts of interest

The authors declare no conflicts of interest.

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Letter to the Editor

The tower of babel in hematology: The World Health Organization and International Consensus Classification systems

Let us begin with a thought experiment. Imagine, dear reader, that you and we – the authors – are touring a large zoo. As we pass by the reptile section, Dr. Rego, who to my knowledge knows nothing about reptiles, suddenly exclaims: "Look at this beautiful specimen of an alligator!" Observing the animal, I concur: "Indeed." However, you, the reader, upon seeing the same animal, object: "This is not an alligator, but a crocodile." Dr. Rego reexamines the animal and insists: "I am certain this is an alligator. I have seen many alligators in other zoos, and I am confident this is one." Yet you persist: "It is easy to see that this is not an alligator, but a crocodile; one only needs to examine its features carefully." The discussion begins to escalate but is abruptly interrupted when our guide announces it is time to move on to the bird section. Later, I find myself reflecting on the nature of classification and what it means to define entities in the world. Was that reptile an alligator or a crocodile? As someone who is not an expert in herpetology, I cannot say with certainty. However, I am sure of one thing: the animal was either an alligator or a crocodile – it could not be both. In philosophical terms, this presents a genuine dichotomy: the reptile in question must be one or the other. These two categories are mutually exclusive. Given that the creature was a single, individual animal, it was either an alligator or, alternatively, a crocodile, but never both.

But what do alligators and crocodiles have to do with issues in hematology? Believe it or not, dear reader, the fact is that nowadays hematologists not only fail to distinguish between alligators and crocodiles (which in itself is not a serious concern, since such distinctions are strictly the domain of zoologists), but also, and this is a real problem, they have come to believe that "the same animal can be both an alligator and a crocodile!" Or, what amounts to the same thing, that a single hematologic disease can be simultaneously classified as disease X and disease Y. Allow us to explain this in a more appropriate way.

The current problems began in 2020, when preparations for the final version of the 5th Edition of the World Health Organization (WHO) Classification – commonly referred to as the "blue book" – were initiated. However, a brief historical overview is necessary to understand the present situation. Up until the 4th Edition of the WHO blue book, a collaborative group of hematologists, pathologists, oncologists, and geneticists formed the Clinical Advisory Committee (CAC), which operated under the auspices of the International Agency for Research on Cancer (IARC), the Society for Hematopathology (SH), and the European Association for Haematopathology (EAHP). Following each CAC meeting, the principal pathologists worked to resolve the difficult issues related to the committee's recommendations and published their conclusions in scientific articles prior to the formal release of the WHO blue book [1]. However, as reported by Daniel Arber and colleagues [1], "In 2020, Ian Cree, Head of the Evidence Synthesis and Classification Branch of the IARC in charge of the publication of the WHO blue books, notified SH and EAHP that IARC was ending the successful partnership with SH and EAHP for the 5th edition WHO classification of hematopoietic tumors and that they would no longer follow the process described above for the three prior books." Next, "the Executive Committees of the SH and EAHP organized different multidisciplinary working groups that culminated in the CAC meeting held in Chicago in September 2021." Finally, the CAC members published the International Consensus Classification (ICC) of Myeloid and Lymphoid Neoplasms in four articles [2–5]. For its part, the WHO published its independent and final classification in the traditional blue book format in 2024 [6].

At first glance, this dispute between two professional groups may appear to be a remote concern for hematologists who lead demanding clinical lives, often far removed from academic debates and focused on treating patients with life-threatening diseases. And indeed, this may largely be the case. In fact, a recent study comparing the WHO and ICC classifications found that only 1.3 % of acute myeloid leukemia (AML) cases showed "major diagnostic

discrepancies" – defined as differences in diagnosis with significant and clear therapeutic implications [7]. However, consider the following scenario: a patient presents with anemia, megakaryocytic dysplasia, 7 % bone marrow blasts, and mutations in *DNMT3A*, *NRAS*, and *NPM1*. According to the WHO blue book, an "increase in blasts in the peripheral blood and/or bone marrow" is essential for an AML diagnosis. Traditionally, >5 % in bone marrow or >2 % in the blood have been considered abnormal. Thus, according to the WHO, the diagnosis for this case is AML with mutated *NPM1*. In contrast, according to the ICC, because the blast percentage is below 10 %, this patient would be diagnosed with myelodysplastic syndrome, not otherwise specified (MDS-NOS). This discordance between the WHO and ICC regarding the minimum blast percentage for the diagnosis of AML is a very strange situation because, in effect, the patient in question clearly has a single disease, which cannot simultaneously be classified as both AML with mutated *NPM1* and MDS-NOS.

The crucial point here is that the existence of two divergent systems for the classification of AML creates problems that extend beyond the classification of individual cases. For instance, due to the coexistence of the WHO and ICC proposals, the 2024 Brazilian consensus on acute promyelocytic leukemia (APL) does not clearly specify the minimum blast percentage required for an APL diagnosis [8].

There are two potential solutions to this problem. The first is to accept both classifications. The second is to pursue rapid reconciliation between the WHO and ICC. We believe the latter is the best option. Indeed, we recently adopted an unorthodox approach to this issue and concluded that the existing literature already supports a single diagnostic criterion for at least five clinical entities within the group of "AML subcategories with defining genetic abnormalities": AML with *PML::RARA* rearrangement, AML with *NPM1* mutation, AML with *KMT2A* rearrangement, AML with *MECOM* rearrangement, and AML with in-frame *bZIP CEBPA* (Table 1) [9]. Meanwhile, we believe that the best approach in cases of conflicting diagnoses is to treat patients according to the most appropriate available therapy, guided by the following principles: (1) avoid categorizing an aggressive neoplasm as a "low-grade" disease (for example, diagnosing MDS when AML is the most accurate diagnosis), thereby preventing undertreatment; and (2) avoid categorizing a less aggressive neoplasm as a more aggressive one (such as diagnosing AML when MDS is the most accurate diagnosis), thereby preventing overtreatment [9].

Table 1 – Preliminary proposal for a unified World Health Organization classification and International Consensus Classification.

AML with defining genetic abnormalities	Blast cutoff
APL with *PML::RARA* FUSION	Increase peripheral blood and/or bone marrow blasts
AML with *NPM1* mutation	
AML with *KMT2A* rearrangement	
AML with *MECOM* arrangement	
AML with in-frame bZIP *CEBPA* mutations	At least 10 % of blasts

Returning to the thought experiment: a single reptile can never be both an alligator and a crocodile simultaneously. Academia should return to basic logic, which states that a thing is always equal to itself, according to the *principle of identity*. In simpler terms, if something is "A," then "A" is equal to "A." Accordingly, a patient with a single disease cannot have two distinct diseases at the same time, for the simple reason that the patient has only one disease.

The purpose of classification systems is to organize and categorize objects, phenomena, information, or entities based on common characteristics, thereby facilitating their identification, study, communication, and practical application. The coexistence of the WHO and ICC systems does not make the classification of hematolymphoid tumors a simpler task, but a more complex one. The two proposals must be urgently harmonized into a single, universal classification system.

Conflicts of interest

The authors report no conflict of interest.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Letter to the Editor

Regarding "Challenges in diagnosing thrombotic thrombocytopenic purpura"

Dear Editor,

We appreciate the thoughtful critique of our recent case report reported as Images in Clinical Hematology [1] and would like to clarify points raised regarding the diagnostic criteria for immune thrombotic thrombocytopenic purpura (iTTP).

In their response, Jacobs et al. [2] express concern that our case lacked sufficient information to confirm a diagnosis of iTTP, specifically citing the absence of reported ADAMTS13 autoantibody data and genetic testing. We agree with the importance of distinguishing immune-mediated from congenital TTP, as this has significant implications for management and prognosis.

The critique reads as follows:

"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."

We would like to clarify that an ADAMTS13 inhibitor screen was indeed performed in our patient and yielded a positive result. The inhibitor titer, measured using the Bethesda assay, was 2.2 (reference <0.4), indicating the presence of a circulating autoantibody against ADAMTS13. This supports the diagnosis of acquired, immune-mediated TTP. Due to word count limitations and the case vignette format, this detail was not included in the original publication.

We agree that in the absence of detectable autoantibodies, the possibility of congenital TTP should be considered, and genetic testing may be warranted. However, in our case, the presence of a quantifiable inhibitor supports an immune-mediated process, and the clinical picture (including age of onset and concurrent autoimmune disease) makes congenital TTP unlikely.

We appreciate the ongoing discussion regarding the importance of diagnostic precision in TTP. We fully agree that thorough documentation of relevant laboratory findings is critical, not only for accurate diagnosis, but also for management and epidemiological purposes.

Conflicts of interest

No conflicts of interest to declare.

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Letter to the Editor

Fulminant hepatitis secondary to nivolumab in a patient with Hodgkin's Lymphoma after complete remission

Dear Editor,

Hodgkin lymphoma (HL) is a hematologic malignancy with a high cure rate, particularly after first-line treatment based on immunochemotherapy [1]. For relapsed or refractory (R/R) HL, which affects 10–30 % of patients depending on their initial staging, the therapeutic approach often includes second-line regimens consolidated with autologous stem cell transplantation. Immunotherapy, particularly programmed death 1 (PD-1) inhibitors, has emerged as an effective option for achieving long-term disease control in these cases [2].

Nivolumab, a PD-1 inhibitor, is an approved therapy for R/R HL and has demonstrated significant clinical efficacy. However, it is associated with a spectrum of adverse events, including fatigue, rash, loss of appetite, nausea, diarrhea, arthralgia, and elevated transaminases [3,4]. More severe toxicities, such as pneumonitis and autoimmune hepatitis, have also been reported, particularly in solid tumor oncology [5].

There is a current discussion regarding the role of autologous transplantation in patients receiving PD-1 inhibitors (e.g., nivolumab, pembrolizumab). Specifically, the debate centers on the optimal time to discontinue therapy in two distinct patient groups: those who do not undergo consolidation therapy and those who achieve only partial remission after two years. The case reported here serves as a basis to discuss at what point we should start worrying about the extent of long-term treatment with PD-1 and its risks, especially in patients who are in complete remission.

Case presentation

A 28-year-old man was diagnosed with advanced-stage classical HL with a high International Prognostic Score (IPS >2) in January 2017. This patient received five irregular cycles of first-line eBEACOP-D (escalated bleomycin (Bleomycin), etoposide (Etoposide), Doxorubicin, Cyclophosphamide, Vincristine, Procarbazine, and Prednisone plus dacarbazine) therapy with poor adherence and presented with a primary refractory disease. Subsequent salvage therapies with IGEV (Ifosfamide, Gemcitabine, Vinorelbine, and Prednisolone), DHAP (Dexamethasone, High-dose Ara-C [cytarabine (Cytarabine)], and Platinol [Cisplatin]), and brentuximab showed suboptimal responses due to poor adherence. In 2020, nivolumab was initiated as monotherapy, and, after six cycles, he finally achieved complete remission. Despite irregular follow-up, clinical remission was maintained while continuing monthly nivolumab therapy. Since the patient did not undergo imaging to assess disease response after two years, nivolumab therapy was continued.

In October 2023, three years after achieving complete remission, the patient suddenly started with nausea, vomiting, right upper quadrant abdominal pain, dyspnea, and myalgia, necessitating hospitalization. Laboratory tests revealed fulminant hepatitis with markedly elevated transaminases (AST 3108 U/L, ALT 2380 U/L), canalicular enzymes (alkaline phosphatase 312 U/L, GGT 238 U/L, total bilirubin 20.4 mg/dL), and coagulopathy (INR: 4.49).

The patient underwent a series of laboratory tests that showed no sign of psychoactive substance use, alcohol abuse, use of other medications, or any concomitant infectious condition. The hepatology team described the main hypothesis in this case as an autoimmune fulminant hepatitis secondary to the use of nivolumab. Despite supportive care, he progressed to liver failure, multiorgan dysfunction, and refractory shock, leading to death within days after admission. The patient's response status at the time of death was not assessed.

Discussion

In 2019, Martins et al. [6] published a review on the adverse effects of the use of checkpoint inhibitors, demonstrating that the frequency of immune-related adverse events related to this kind of medication depends on the agents used, exposure time and the dose. Hepatitis was described as the second most common fatal adverse effect, along with pneumonitis

and colitis in patients using PD-1 inhibitors. The review does not describe cases of fulminant autoimmune hepatitis. Nivolumab has also been associated with a well-documented risk of adverse events, including Grade 3 or higher toxicities in approximately 10 % of cases, often necessitating treatment discontinuation [6].

No alternative etiology for the acute liver failure was identified, strongly implicating nivolumab as the causative agent. To our knowledge, this represents the first reported instance of nivolumab-induced fulminant hepatitis in a patient with HL in remission. This case demonstrates that adverse effects from nivolumab can occur, persist, and manifest as severe, life-threatening events even in patients with sustained remission.

Conclusion

This case underscores the importance of monitoring hepatic function in patients undergoing nivolumab therapy for HL, even those in remission. Early identification of liver dysfunction and prompt intervention are critical to prevent fatal outcomes. High-risk patients receiving immune-checkpoint inhibitors should be regularly monitored by specialized multidisciplinary teams for treatment-related complications, ideally using a personalized surveillance strategy.

This serves as a warning to restart discussions on prolonged therapies with PD-1 inhibitors, the need for 'chemotherapy holidays' even for hematologic malignancies and the importance of consolidation as a mark of the end of treatment.

Conflicts of interest

Authors have no interests that are directly or indirectly related to the work submitted for publication.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Images in Clinical Hematology

Howell-Jolly-like inclusions in granulocytes of a liver transplant recipient

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A 38-year-old woman with history of a liver transplant performed four months earlier, presented with fever and multiple lymphadenopathies. She was taking mycophenolate, tacrolimus and prednisone for chronic rejection, lamivudine because of hepatitis B virus serology, and valganciclovir due to recent reactivation of cytomegalovirus.

On admission the complete blood count findings included: hemoglobin 9.7 g/dL, platelets 260 $\times$ 10^9/L, leukocytes 1.5 $\times$ 10^9/L with 0.2 $\times$ 10^9/L neutrophils and elevated C-reactive protein (120 mg/L).

Peripheral blood examination showed hyposegmentation in neutrophils with Howell-Jolly body-like inclusions (Figure 1).

Blood cultures for bacteria and fungus did not support growth of any organism and serologic tests were negative. Additionally, lymph node aspiration cytology did not reveal tumoral cells however, a polymerase chain reaction-based assay to detect *Mycobacterium tuberculosis* in the ganglion was positive. With the diagnosis of ganglionic tuberculosis, the patient received treatment with isoniazid, pyrazinamide, myambutol and levofloxacin. After one year of treatment, the leukocyte count is normal and the adenopathies have disappeared in a full body scan.

Howell-Jolly body-like inclusions in granulocytes are small dense basophilic inclusions similar to Howell-Jolly in erythrocytes. Their appearance in neutrophils may indicate a nuclear fragmentation induced by antiviral treatment with nucleoside analogs, which act on viral DNA. They arise secondary to stressed granulopoiesis often induced by immunosuppressive states including congenital conditions or acquired due to drugs for HIV infection or chemotherapy [1,2]. They are also been described in patients with *Mycobacterium avium* infection and more rarely in myelodysplastic syndromes [3]. These inclusions must be differentiated from other neutrophil inclusions such as those observed in intracellular bacterial infections, those found in genetic conditions such as Chédiak-Higashi syndrome, or Döhle bodies [1].

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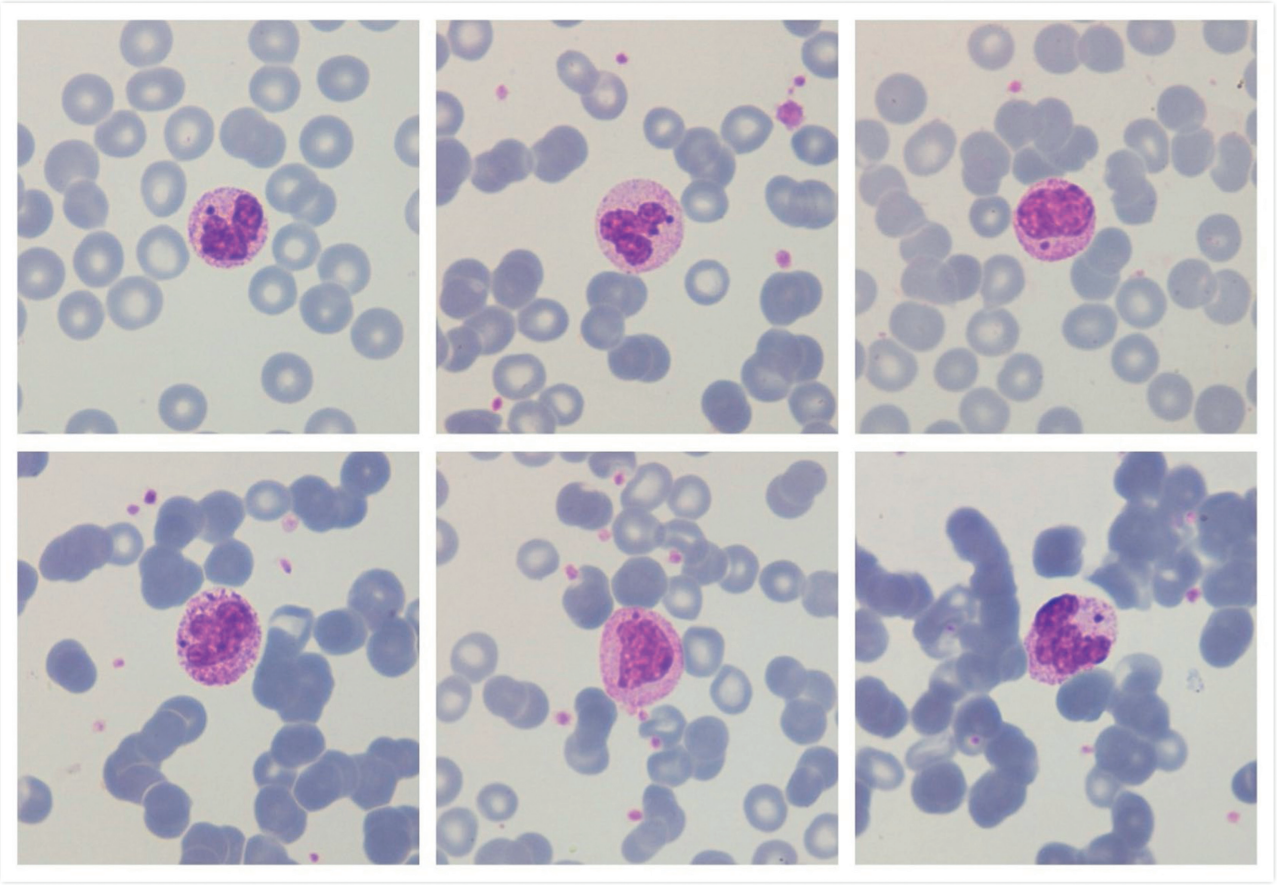

Figure 1 – Peripheral blood smear showing atypical inclusions ("Howell-Jollylike-bodies") in granulocytes

(Optical microscopy images using May-Grünwald Giemsa stain - x1000 magnification).

Conflicts of interest

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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HEMATOLOGY, TRANSFUSION AND CELL THERAPY

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Images in Clinical Hematology

VEXAS syndrome: more than just vacuoles

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A 78-year-old man with an autoimmune disorder (vasculitis) and a recent diagnosis of VEXAS syndrome, confirmed by next generation sequencing (presence of the p.Met41Thr variant of the *UBA1* gene with a variant allele frequency of 56.4 %) was admitted to hospital.

Analytically he presented: erythrocytes 3.75 × 10^{12}/L (reference values [RV]: 4.5–5.9 × 10^{12}/L), hemoglobin 132 g/L (RV: 130–175 g/L), mean corpuscular volume 103.6 fL (RV: 80–97 fL), leucocytes 3.9 × 10^{9}/L (69 % neutrophils, 20 % lymphocytes), and platelets 197 × 10^{9}/L (RV: 150–450 × 10^{9}/L).

The bone marrow aspirate smears were normocellular, with a myeloid to erythroid (M:E) ratio of 2:1. The smears also revealed 20 % myeloid precursors (all stages) and 30 % proerythroblasts with cytoplasmic vacuoles. Other notable findings included pseudo-Pelger-Hüet anomalies and megaloblastic precursors. Most of the megakaryocytes exhibited a high nuclear-to-cytoplasmic ratio; specifically, 50 % of total megakaryocytes (TM) were monolobated with eccentrically placed nuclei, and other megakaryocytes showed a wreath-like rearrangement of nuclear lobes. Additionally, multinuclear megakaryocytes (3 % TM) and megakaryocyte emperipolesis (3 % TM) were observed (Figure 1). There was no increase in blasts. Storage iron was decreased with no ring sideroblasts.

VEXAS syndrome (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) was first reported by Beck et al. in 2020. Vacuoles are observed in erythroid and myeloid precursor cells [1]. The E1 enzyme is related to the ubiquitin activating enzyme encoded by the *UBA1* gene, an X-linked gene [1]. Mutation in this gene is responsible for an autoinflammatory disease (characterized by recurrent fevers, cytopenias, chondritis, vasculitis, pulmonary inflammation, and neutrophilic dermatoses) as the result of somatic mutations in the blood [1].

The most frequent mutations are p.Met41Thr (49 %), p.Met41Val (26 %) and p.Met41Leu (19 %) [2].

In VEXAS syndrome the bone marrow is usually hypercellular with an increased M:E ratio (>4:1 in >70 % of cases) [2]. The presence of ≥10 % of myeloid precursors with >1 vacuole can be both sensitive and specific for VEXAS syndrome, [3] however cytoplasmic vacuolization of myeloid and erythroid precursors can be found in other clinical settings: alcohol abuse, copper deficiency, treatments (chemotherapy and antibiotics), zinc toxicity, myelodysplastic syndrome, lymphoproliferative disorders, multiple myeloma, myeloproliferative neoplasms and acute myeloid leukemia, or as an artifact of sample preparation [2,3]. Furthermore, some atypical *UBA1* variants can present absence of precursor vacuolization in the bone marrow [2].

Storage iron is usually increased with no significant number of ring sideroblasts (<10 % of cases) [2].

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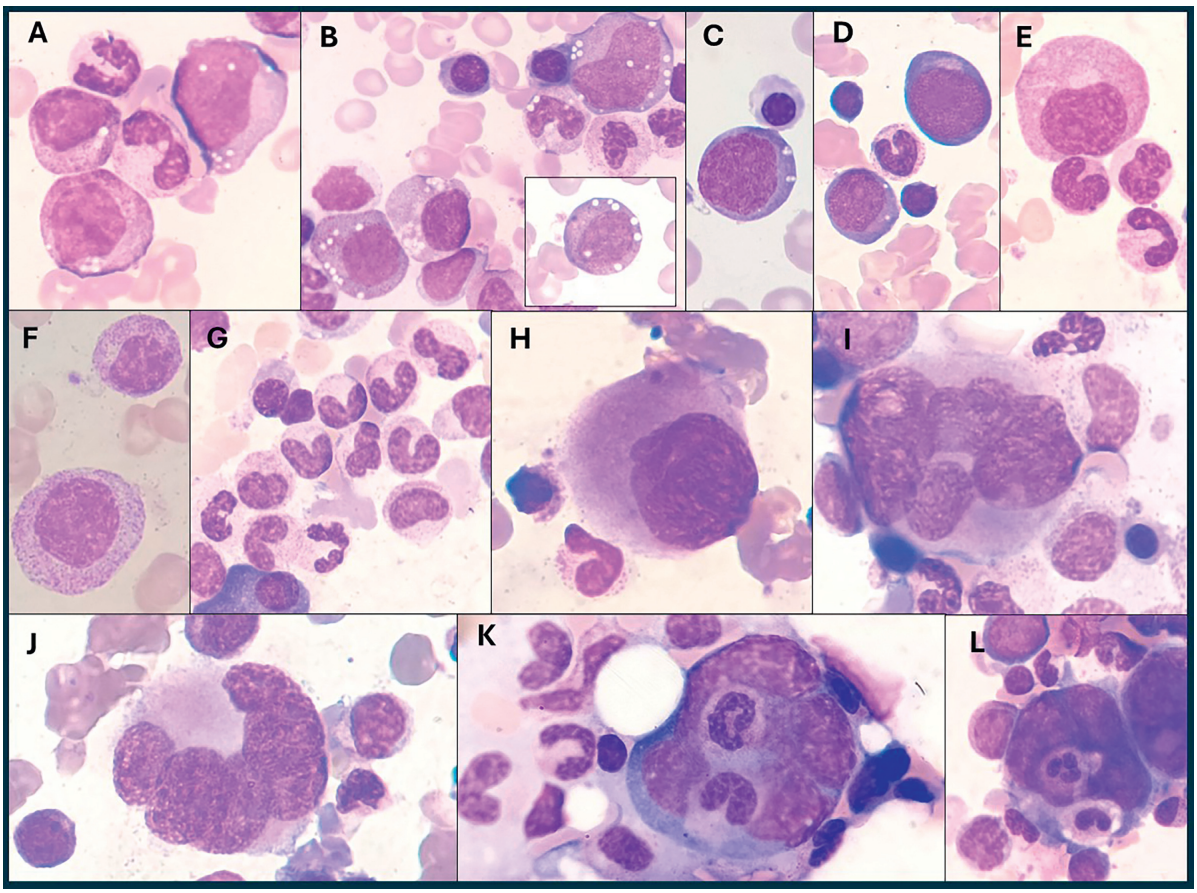

Figure 1 – Bone marrow aspirate (May-Grünwald-Giemsa stain, ×100 objective): Myeloid precursors with vacuoles (A, B); proerythroblasts with vacuoles (C, D); megaloblastic precursors (D, E, F); pseudo-Pelger-Hüet (G); monolobated megakaryocytes (H); megakaryocytes with a wreath-like rearrangement of nuclear lobes (I, J); megakaryocytes emperipolesis (K, L).

The full blood count can show: macrocytic anemia (90–100 %), lymphopenia (60–80 %), monocytopenia (50 %), neutropenia (<30 %) and thrombocytopenia (45–69 %) [2].

The *UBA1* gene mutation is also a predisposing factor for myelodysplastic syndromes, plasma cell proliferation disorders (monoclonal gammopathy of undetermined significance, multiple myeloma) or both [2].

Conflicts of interest

The author declares no conflicts of interest.

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