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Original article

Functional capacity in sickle cell disease: A pilot study with 1-minute sit-to-stand test



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ABSTRACT

Background: Sickle cell disease, the most prevalent monogenic recessive genetic disorder in the world, is characterized by two main pathogenic mechanisms: vaso-occlusion and hemolysis. These characteristics lead to reduced tolerance to physical exertion and, consequently, a reduced functional capacity which can be assessed using the one-minute sit-to-stand test. Complications from sickle cell disease result in poor quality of life, increased absenteeism from school and work, and impaired social interaction.

Method: Between January 2023 and April 2024, a pilot cross-sectional study was conducted with sickle cell disease patients aged from 18 to 60 years. The one-minute sit-to-stand test, Borg's perceived exertion scale, and the SF-36 quality of life questionnaire were utilized. Patients were monitored during the test. The sample was dichotomized based on test performance and SF-36 scores. Furthermore, clinical and demographic variables were analyzed.

Main results: Fifty-eight individuals participated in the final analysis. The mean age was 29.84 ± 11.20 years; 55.1 % were men, and 79.3 % identified themselves as Black or mixed race. The most prevalent genotype was hemoglobin SS (67.2 %), and 77.5 % were taking Hydroxyurea. The group with a better performance in the one-minute sit-to-stand test showed better quality of life as assessed using the SF-36 questionnaire.

Conclusion: Functional capacity is a significant factor in the autonomy and quality of life of patients with sickle cell disease. The one-minute sit-to-stand test is a low-cost and easily applicable test, which can contribute to the assessment of functional capacity in the routine follow-up of these patients.

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Introduction

Sickle cell disease (SCD), resulting from a mutation in the hemoglobin (Hb) gene that leads to the formation of Hb S, is the most prevalent recessive monogenic disease in the world. There are multiple genotypes that cause SCD including the severe homozygous form, Hb SS, or as heterozygous forms with other hemoglobin variants, such as Hb C or Hb β -thalassemia [1].

When deoxygenated, Hb S polymerizes and modifies the morphology of red blood cells making their membrane rigid and into a sickle shape. This modified state favors the occurrence of vaso-occlusive crises (VOC), endothelial dysfunction, hemolysis, and activation of inflammatory responses through the release of cytokines and reactive oxygen species. VOC lead to ischemia and are the main factor responsible for pain crises in SCD as well as other complications, such as acute chest syndrome and osteonecrosis. Endothelial dysfunction is associated with intravascular hemolysis, nitric oxide depletion and hypercoagulability, and is the underlying mechanism of complications such as strokes, pulmonary hypertension and organ damage, especially of the kidneys and heart [1,2].

The complex physiopathology of SCD reduces tolerance to physical exertion, which is seen by a reduced functional capacity (FC). The acute complications of SCD result in school and work absenteeism, negative impacts on socialization and a low quality of life. Therapeutic exercise is an important tool for rehabilitation in SCD, recovering cardio-respiratory capacity and autonomy, and improving socialization. However, the prescription of exercise in SCD is a highly complex topic and may cause VOC if excessively intense [2].

Assessing FC is crucial for developing an appropriate exercise prescription, as a wide array of functional assessment tools, ranging from simple field tests to sophisticated laboratory procedures, is available. The cardiopulmonary exercise test (CPET) is the gold standard; however, it requires specific, costly equipment that limits its availability [3]. Other methods for functional assessment consist in field tests, which include the ergometric test, Bruce protocol, 6-Minute Walk Test (6MWT), and the 1-Minute Sit-to-Stand test (1-MSTST) [4,5]. The 1-MSTST is a submaximal test validated for assessing FC, postural control, proprioception, and lower limb strength. It is a well-tolerated, low-cost test that can be applied domestically, at the bedside and in small rooms as it only requires an armless chair, a stopwatch and minimal floor space [6,7]. To the present moment, there are no studies assessing FC in SCD patients utilizing the 1-MSTST.

Objective

The objective of this study is to assess FC in adult SCD patients using the 1-MSTST test, and to evaluate possible associations between the FC, quality of life, and clinical and demographic variables.

Method

This cross-sectional pilot study to assess FC in SCD patients using the 1-MSTST was carried out between January and April 2024. Participants were active patients in a secondary care facility specialized in hematology and hemotherapy. Adults aged between 18 and 60 years, diagnosed with SCD, who signed an informed consent form, participated in the study. Exclusion criteria were: refusal to participate; clinical events during the 1-MSTST; a drop in oxygen saturation $>4\%$ during the 1-MSTST; systolic blood pressure >180 mmHg or diastolic blood pressure >110 mmHg; unstable angina; resting heart rate >120 bpm; and orthopedic conditions that limited performance in the 1-MSTST.

The instruments utilized for this study were: the 1-MSTST test, the Borg rate of perceived exertion scale for the assessment of work of breathing, [8] and the Short Form 36 Health Survey Questionnaire, [9] which is divided in eight domains (Physical functioning; physical role limitations; bodily pain; general health perceptions; energy/vitality; social functioning; emotional role limitations; and mental health) and two summary measures (physical component summary and mental component summary). All anthropometric and clinical data were collected with standardized equipment and all tests were performed supervised by trained research staff. A brochure containing information about lifestyle changes and possible physical activities was created by the research staff and handed to the study participants.

Initially, a descriptive analysis was performed utilizing the adequate frequency and central tendency measures. The study by Strassman et al. was utilized to obtain the age-corrected target repetitions for the 1-MSTST [10] by which our sample was standardized. For the comparative analysis, the sample was divided in two groups based on the median 1-MSTST performance, and analyzed with Student's t-test and Pearson's chi-squared test. The variables included in the analysis were age, gender, ethnicity, SCD genotype, use of hydroxyurea, 1-MSTST performance, baseline and post-intervention heart rate, baseline and post-intervention peripheral oxygen saturation, and baseline and post-intervention Borg scale. The software IBM SPSS Statistics 25[®] was utilized for the statistical analysis. This study was registered and approved by the institution's research ethics committee under the number CAAE 63,424,422.9.0000.5118.

Results

In total, 85 individuals met the inclusion criteria of the study; however, 27 were later excluded, as nine had a diagnosis of hip osteonecrosis, eight did not complete the SF-36 health survey questionnaire, six presented with lower body pain during the clinical assessment and four refused to participate. After the 58 participants performed the 1-MSTST, the sample was divided in two groups based on the median performance (20.75 repetitions): 28 patients had an above median performance and 30 patients had a below median performance (Figure 1).

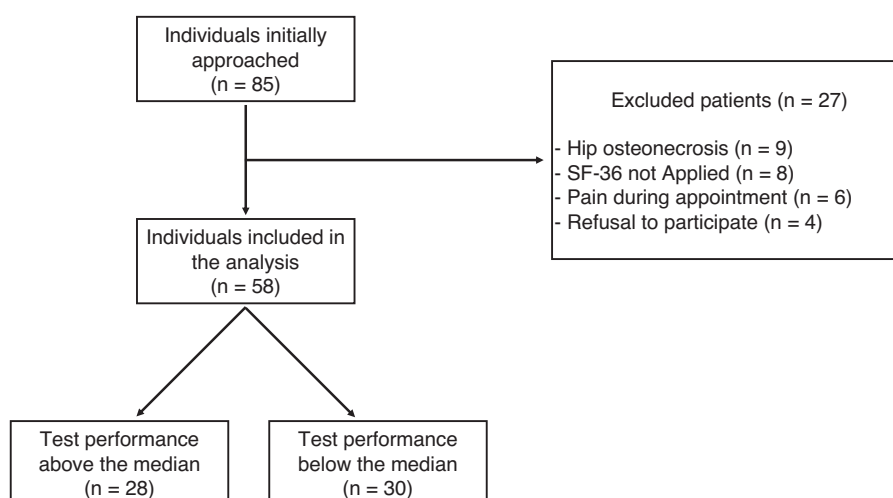


Figure 1 – Study flowchart.

Of the 58 participants, the average age was 29.84 ± 11.20 years with ages ranging between 18 and 59 years, 55.1 % were male, and 79.3 % identified themselves as Black. The most frequent genotype was Hb SS ($n = 39$; 67.2 %) and 77.5 % of the sample was taking hydroxyurea. In the comparative analysis, the group with the best 1-MSTST performance had a statistical tendency to be younger (p -value = 0.08), and had a higher post-intervention heart rate (p -value = 0.001). The other analyzed variables were not statistically significant, such as use of hydroxyurea and disease genotype (Table 1).

The data regarding the results of the SF-36 health survey analysis are found in Table 2. The group with a worse performance in the 1-MSTST had statistically significant lower scores in the physical component summary (p -value = 0.05),

mental health (p -value = 0.01) and general health perceptions (p -value = 0.02). Other SF-36 health survey dimensions, such as physical role limitations (p -value = 0.07) and physical functioning (p -value = 0.09) approached statistical significance for a worse score in the group with the worst performance.

Discussion

SCD is one the most epidemiologically relevant hematological conditions in the world. In Brazil it represents an important health burden, affecting an estimated 60,000–100,000 people [11]. Between 2014 and 2020, an annual average of 1087 people were born with the disease in Brazil, with an incidence of 3.75

Table 1 – Demographic and clinical variables of the sample categorized by 1-MSTST performance.

Variable	Total	Median performance		p-value
		<20.75 repetitions (n = 30)	>20.75 repetitions (n = 28)	
Age (mean)	29.84 ± 11.20	32.27 ± 11.95	27.25 ± 9.89	0.08
Gender, male – n (%)	32 (55.1)	15 (50)	17 (60.7)	0.41
Race, black – n (%)	46 (79.3)	24 (80)	22 (78.6)	0.57
Genotype – n (%)				0.75
Hb SS	39 (67.2)	20 (66.7)	19 (67.9)	
Hb S β ⁺	9 (15.5)	5 (16.7)	4 (14.3)	
Hb SC	9 (15.5)	4 (13.3)	5 (17.9)	
Hb S β ⁰	1 (1.7)	1 (3.3)	0 (0)	
Medication				
Hydroxyurea use	45 (77.5 %)	23 (76.7 %)	22 (78.6 %)	0.86
Average dosage (mg)	1130.16 ± 360.57	1133.70 ± 400.36	1126.45 ± 323.19	0.94
1-MSTST				
Repetitions	20.44 ± 5.88	15.96 ± 3.86	25.25 ± 3.29	<0.001*
Target/real performance ratio (%)	43.86 ± 11.79	34.94 ± 7.51	53.42 ± 7.07	<0.001*
Baseline heart rate (bpm)	78.69 ± 13.10	76.66 ± 11.28	80.87 ± 14.70	0.22
Post intervention heart rate (bpm)	121.05 ± 20.42	112.73 ± 20.84	129.96 ± 15.95	0.001*
Baseline O ₂ saturation (%)	93.68 ± 4.01	93.43 ± 4.57	93.96 ± 3.36	0.61
Post intervention O ₂ saturation (%)	92.81 ± 4.39	92.70 ± 5.22	92.94 ± 3.37	0.83
Baseline Borg	1.61 (0 - 2.12)	1.85	1.35	0.36
Post intervention Borg	5 ± 2.13	5.1 ± 2.37	4.89 ± 1.88	0.71

1-MSTST: 1-minute sit-to-stand test.

Table 2 – Results of SF-36 health survey analysis categorized by 1-MSTST performance.

SF-36	Total	Median performance		p-value
		<20.75 repetitions (n = 30)	>20.75 repetitions (n = 28)	
PCS	44.45 ± 9.74	42.12 ± 9.27	46.95 ± 9.76	0.05
MCS	47.97 ± 12.38	45.39 ± 13.23	50.73 ± 10.96	0.10
Physical functioning	72.98 ± 20.82	68.16 ± 20.11	77.50 ± 21.01	0.09
Physical role limitations	60.52 ± 44.30	52.50 ± 45.65	73.21 ± 39.63	0.07
Emotional role limitations	68.41 ± 38.03	60.00 ± 40.49	73.80 ± 37.79	0.18
Energy/vitality	59.91 ± 28.30	54.33 ± 25.68	63.39 ± 29.44	0.21
Mental health	68.70 ± 25.51	61.86 ± 25.70	76.71 ± 20.07	0.01*
Social functioning	79.60 ± 29.37	76.66 ± 30.21	83.48 ± 28.06	0.37
Bodily pain	76.57 ± 30.23	66.30 ± 33.52	78.78 ± 29.30	0.13
General health perceptions	50.70 ± 25.16	44.86 ± 20.53	59.32 ± 26.27	0.02*

SF-36: SF-36 health survey questionnaire; PCS: Physical component summary; MCS: Mental component summary.

cases per 10,000 newborns [12]. SCD represents a challenge for the Brazilian public health system due to its complex pathophysiology, need for interdisciplinary care, and socioeconomic impact. Historically, newborn screening and continued health education, in addition to regular medical care, have been effective strategies for reducing complications and increasing quality of life for SCD patients in Brazil [1].

Patients were not included in the analysis of this study if they presented with conditions that could interfere with their performance in the 1-MSTST, the most prevalent of which was hip osteonecrosis, with a frequency of 10.58 %. Osteonecrosis is a frequent complication of SCD that occurs due to VOC with the most frequent sites being the hips, vertebrae and shoulders [13]. The prevalence of osteoarticular involvement increases with age. Ouederni et al. found a prevalence of hip osteonecrosis of 18.3 % in 20-year-old SCD patients, compared to a 2.3 % hip osteonecrosis prevalence in 10-year-olds [14,15]. Daltro et al. reported a prevalence of osteonecrosis in 11.1 % of SCD patients in Brazil, most frequently of the hip joint (74.6 %), [15] a result similar to the current findings.

Regarding the SCD genotype, 67.1 % of the present sample had the Hb SS genotype, a proportion similar to other Brazilian studies, such as Silva et al., whose group identified a slightly greater prevalence of Hb SS, at 75 % [16]. Souza et al. identified a lower prevalence of 60 % in their sample with Hb SS [17]. These variations are common and reflect differences in recruiting methods and populations. Cardoso et al. found a median age of 28.6 ± 9.9 years and a 65 % prevalence of Hb SS, very similar results to this study [18]. These findings reinforce the representativeness of the current sample. The different genotypes of SCD correlate to disease severity and symptomatology: Hb S β ⁺-thalassemia and Hb SC are considered of mild severity, while Hb SS and Hb S β ⁰-thalassemia present a greater clinical relevance and severity [19]. In this study, the SCD genotype was not significantly associated with worse 1-MSTST performance, possibly due to the high proportion of Hb SS in the sample (n = 39; 67.2 %).

A worse performance in the 1-MSTST was observed among older participants (p-value = 0.08), which may be explained by the buildup of osteoarticular and inflammatory damage caused by SCD and aging. Other sociodemographic

variables, such as gender and ethnicity, were not statistically significant in this study. Hydroxyurea is the most efficient pharmacological therapy for SCD available in Brazil; its use is associated with a lower rate of degenerative damage [19,20]. In the present study, 77.5 % of the participants were in continuous use of hydroxyurea; however, its use was not associated with a better performance in the 1-MSTST (p-value = 0.86). The authors did not find any literature regarding 1-MSTST performance and use of hydroxyurea, or other drugs, among SCD patients.

No participants of this study were capable of reaching their age-corrected performance target for the 1-MSTST. Studies utilizing other methods to assess FC in SCD patients present with similar results. Cardoso et al. assessing the FC in an adult population with sickle cell anemia utilizing the 6MWT found that the average distance covered by study participants was less than the expected target distance for the Brazilian population (335.3 ± 70.6 m versus 504.7 ± 5.5 m; p-value <0.001) [21]. The 1-MSTST is a low-cost, simple assessment that can be applied in domestic and bedside environments; it provides a similar hemodynamic and ventilatory response to the 6MWT, which is considered the gold-standard field FC assessment tool. There are no studies utilizing the 1-MSTST to assess FC in SCD, which is a limiting factor of the present discussion.

The association between quality of life and FC is evident in several health conditions, including SCD, in which individuals present with decreased tolerance to physical exertion, resulting in loss of muscle mass and adverse psychosocial effects. In addition, it is common for SCD patients to be advised to avoid physical activities, leading to a sedentary lifestyle and increasing adverse psychosocial and body composition effects [22–24]. A study with SCD patients implemented an eight-week exercise program, with results showing increases in both the FC and quality of life assessed by the SF-36 health survey [23]. The present study found that a worse performance in the 1-MSTST was associated with a worse quality of life assessed by the SF-36 health survey questionnaire. Therefore, in patients with physical disabilities caused by SCD, an approach focused on mental health, in addition to physical training, is of utmost importance, as anxiety and depressive symptoms are very common in this population [24].

Conclusion

In this study, a better performance in the 1-MSTST was associated with a better quality of life assessed using the SF-36 health survey, however there was no statistical association with any of the clinical and demographic variables. The 1-MSTST, a low cost and easily applicable tool for FC assessment, could be a useful addition to the physical evaluation of SCD patients. The multidisciplinary team could use this test for triage during routine follow-ups and stimulate early physical health interventions for the purpose of reducing morbidities associated with SCD.

Data availability

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

Conflicts of interest

None.

Editor

Eduardo Rego

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Original article

Association of the ABCB1 gene polymorphism C3435T (rs1045642) with acute myeloid leukemia: A genetic study



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ABSTRACT

Introduction: The ATP Binding Cassette Subfamily B1 (ABCB1) gene is responsible for encoding the permeability glycoprotein (P-gp), a crucial protein involved in multidrug resistance. P-gp functions as an ATP-dependent efflux pump, actively removing diverse substances, including carcinogens, from cells. However, a specific genetic variation called the C3435T polymorphism of the ABCB1 gene has been linked to reduced plasma levels of P-gp substrates. This genetic variation leads to the accumulation of harmful compounds within cells, which may increase susceptibility to hematological malignancies. This study aims to determine the frequency of ABCB1 gene polymorphism C3435T (rs1045642) in acute myeloid leukemia patients at tertiary care hospitals in Lahore, Pakistan.

Methods: A cross-sectional comparative study was conducted to investigate the association between ABCB1 gene polymorphism (C3435T) and acute myeloid leukemia. A total of 100 samples (50 cases and 50 healthy controls) were genotyped using restriction fragment length polymorphism assay.

Results: The TT genotype of ABCB1 C3435T was more prevalent in cases (62%) compared to the control group (20%). In different genetic models, the TT genotype was significantly associated with acute myeloid leukemia when compared to the CC and CT genotypes.

Conclusion: This study suggests that the TT genotype of the ABCB1 C3435T gene polymorphism is more strongly associated with acute myeloid leukemia compared to controls. This specific genotype may contribute to the development or progression of this malignancy. Further research is needed to explore the functional implications of this genetic variation in the pathogenesis.

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Introduction

Acute Myeloid Leukemia (AML) is a rapidly progressing blood cancer characterized by the uncontrolled growth of myeloid blast cells in the bone marrow and peripheral blood [1,2]. The development of AML can be influenced by various factors, including exposure to radiation, certain medications, chemicals, and genetic predisposition [3]. Among the genetic factors, polymorphisms in genes involved in xenobiotic and drug transporters have been implicated as potential risk factors for AML [4].

One such gene is the *ATP Binding Cassette Subfamily B1* (ABCB1), also known as the *Multidrug Resistance 1* (MDR1) gene, located on chromosome 7. The ABCB1 gene is highly polymorphic, with over 50 reported single-nucleotide polymorphisms (SNPs) [4]. Among these SNPs, the C3435T polymorphism in exon 26 has been extensively studied [5].

The ABCB1 gene encodes a protein called permeability glycoprotein (P-gp), which functions as an ATP-dependent efflux pump, actively transporting various substances, including carcinogens, out of the cells [6]. The C3435T polymorphism of the ABCB1 gene has been associated with a significant reduction (more than two-fold) in plasma concentration of P-gp substrates [7]. Decreased expression of P-gp can lead to the accumulation of xenobiotics and toxic compounds within cells, potentially increasing the risk of hematological malignancies and other diseases [8].

Several studies have investigated the relationship between the ABCB1 gene polymorphism, C3435T, and acute leukemia in different populations. Therefore, this study aims to determine the frequency of the ABCB1 C3435T gene polymorphism in AML patients at tertiary care hospitals in Lahore, Pakistan. By examining this association, we can gain further insights into the genetic factors contributing to AML susceptibility in this population.

Material and methods

A cross-sectional comparative study was conducted involving 100 samples: 50 patients diagnosed with AML and 50 healthy controls. The samples were collected at Jinnah Hospital and Shaikh Zayed Hospital in Lahore, with the necessary approvals from ethics review committees. The research was carried out in the Hematology & Human Genetics and Molecular Biology departments at the University of Health Sciences in Lahore.

Informed consent was obtained from all AML patients who agreed to participate and 3 mL of blood were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers from each. Detailed information was collected using a specially designed proforma. AML diagnosis was based on clinical presentation, peripheral and bone marrow morphology, cytochemistry, and immunophenotyping. Age- and sex-matched healthy individuals were enrolled as a control group.

Genomic DNA extraction from frozen EDTA blood samples was performed using the phenol-chloroform-isoamyl method. The extracted DNA was stored at -20°C . The detection of the ABCB1 (C3435T) gene polymorphism was

performed using a Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay. Following genomic DNA extraction, the targeted region in exon 26 of the ABCB1 gene was amplified by PCR.

Each PCR assay was performed in a final reaction volume of 15 μL with 2.5 μL genomic DNA, 8 μL universal master mix, 0.2 μL ABCB1 C3435T forward primer, 0.2 μL ABCB1 C3435T reverse primer, and 4.1 μL distilled water. A thermal cycler was used for the PCR process, which consisted of an initial denaturation step, followed by cycles of denaturation, annealing, and extension. The final extension was performed, and the amplified PCR product, sized at 244 base pairs (bp), was obtained.

The Polymerase Chain Reaction (PCR) product was subsequently digested overnight with the MboI restriction enzyme at 37°C to identify the polymorphism. The resulting fragments were then separated by 3% agarose gel electrophoresis containing ethidium bromide and visualized under an ultraviolet transilluminator. A DNA molecular weight marker of 50 bp was used to determine the size of the PCR-RFLP products. The digestion with the MboI restriction enzyme allowed differentiation between wild and variant type alleles (homozygous and heterozygous genotypes). The CC genotype yielded two fragments of 72 and 172 bp, the TT genotype yielded one fragment of 244 bp, and the CT genotype resulted in three fragments of 72, 172, and 244 bp (Figure 1).

To confirm the genotypes observed in AML patient samples using the PCR-RFLP assay, further validation was performed through direct DNA sequencing. The 244 bp ABCB1 gene sequence was analyzed using the online insilico software Nebcutter, which confirmed the number and sizes of the fragments after MboI digestion.

Statistical analysis

Statistical analysis was conducted using SPSS version 24. The continuous variables such as age, hemoglobin level (Hb), red blood cell count (RBC), white blood cell count (WBC), platelet count, and blast percentage were reported as mean $\pm$ standard deviation (SD). Categorical variables including gender, AML subtypes, patient clinical features, and patient

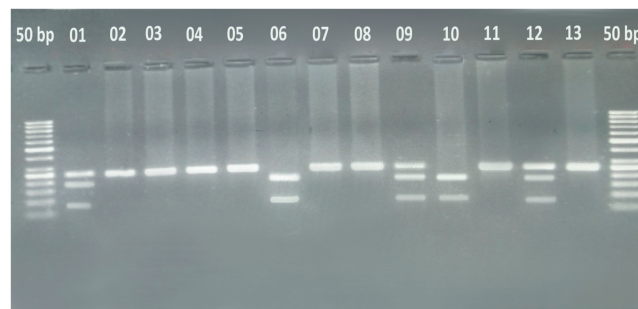


Figure 1 – A representative gel photograph of the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism assay. The gel depicts two DNA fragments in samples 6 and 10, indicating the CC genotype. Samples 1, 9, and 12 show three DNA fragments, representing the CT genotype. On the other hand, the remaining samples exhibit a single DNA fragment, indicating the TT genotype.

genotypes were presented as frequencies. The association between patient genotypes and AML was analyzed using the Chi-square test, and the odds ratio (OR) with a 95 % confidence interval (95 % CI) were calculated. A p-value <0.05 was considered statistically significant for all analyses.

Results

In this study, 50 healthy control individuals, 38 (76 %) males and 12 (24 %) females, were examined. Among the 50 AML patients diagnosed, 40 (80 %) were male, and ten (20 %) were female. A marginal higher number of male patients was observed in the AML group. PCR-RFLP analysis of the ABCB1 gene polymorphism (rs1045642) revealed the distribution of genotypes among the participants. Of the AML patients, 4 % had homozygous CC wild type alleles, 34 % had heterozygous CT alleles, and 62 % had homozygous TT alleles. The C and T allelic frequencies in the AML group were 21 % and 79 %, respectively. In the normal healthy control group, 16 % had homozygous CC wild type alleles, 64 % had heterozygous CT alleles, and 20 % had homozygous TT alleles. The C and T allelic frequencies in the control group were 48 % and 52 %, respectively.

Using the Chi-square test, a statistically significant difference was observed between the AML patients and the normal healthy controls (p-value ≤0.05). The frequency of homozygotes for the TT alleles in AML patients was significantly higher compared to normal healthy controls. Thus, the present study suggests a strong association between the homozygous TT genotype of the C3435T SNP and AML susceptibility, indicating that it may have a potential role in AML.

In the genotypic model, the TT homozygous genotype of the C3435T polymorphism was significantly associated with AML compared to the homozygous CC and heterozygous CT genotypes (OR = 16.2; 95 % CI: 0.38–12.70; p-value = 0.0001). In the dominant model, the combined CT and TT genotypes of the C3435T polymorphism showed an association with AML compared with the homozygous CC genotype (OR = 5.15; 95 % CI: 0.98–27.03; p-value = 0.032). Similarly, in the recessive model, the TT genotype of C3435T showed an association with AML when compared to the combined CC and CT genotypes (OR = 8.28; 95 % CI: 3.123–21.94; p-value = 0.0001).

By analyzing the genetic models, it was observed that the frequency of the TT genotype of the ABCB1 C3435T polymorphism was 16.24 times more prevalent than the CC genotype in the genotypic model. In the dominant model, the combined CT and TT genotypes of the ABCB1 C3435T polymorphism were 5.15 times more prevalent than the CC genotype. Furthermore, in the recessive model, the TT genotype of the ABCB1 C3435T polymorphism was 8.28 times more prevalent than the combined CC and CT genotypes. These findings suggest that the homozygous TT genotype of ABCB1 C3435T polymorphism is more associated with AML. The recessive model was identified as the best fit model for the data in the present study, which was further supported by the highly significant p-value of 0.0001 in the recessive model analysis (Table 1).

To explore potential associations, the demographic, hematological, and clinical characteristics of AML patients were stratified based on the ABCB1 gene polymorphism (C3435T).

Table 1 – Distribution of alleles and genotypes of the ABCB1 (rs1045642) gene polymorphism and its association with AML.

ABCB1 rs1045642	Frequency n (%)	
	AML cases (n = 50)	Healthy controls (n = 50)
Genotype – n (%)		
CC	2 (4)	8 (16)
CT	17 (34)	32 (64)
TT	31 (62)	10 (20)
Allele – (%)		
C	21	48
T	79	52
OR statistics		
CC versus TT (genotypic model)	OR (95 % CI)	p-value
	16.24 (2.60–101.39)	0.0001
C versus T (allelic model)	3.47 (1.86–6.46)	0.0001
CC versus CT and TT (dominant model)	5.15 (0.98–27.03)	0.032
CC and CT versus TT (recessive model)	8.28 (3.13–21.94)	0.0001
AML: Acute Myeloid Leukemia; OR: odds ratio; 95 % CI: 95 % confidence interval.		

The combined CC and CT group was compared with the TT genotype group in respect to these characteristics. Overall, there were no statistically significant differences between the two genotype groups, except for lymphadenopathy, which showed clinical significance in the combined CC and CT genotype group (p-value = 0.015 - Table 2). This indicates that the presence of lymphadenopathy may have a different clinical implication in patients with the CC and CT genotypes compared to those with the TT genotype.

Discussion

The main objective of this study was to identify the association between the ABCB1 gene polymorphism (C3435T) and AML. Using PCR-RFLP for genotype distribution analysis, a significantly higher frequency of homozygous alleles (TT) was found in AML patients (62 %) when compared to the healthy control group (20 %; p-value ≤ 0.05). These results are in line with a study conducted by Li et al. in 2016, in which the authors also observed a significantly higher frequency of TT homozygous genotypes of the ABCB1 gene polymorphism in AML patients (24.32 %) compared to healthy controls (15.56 %; p-value = 0.02) [9].

However, it is important to consider that a previous study focused on the Iraqi population and the ABCB1 SNP (C3435T) reported insignificant differences in homozygous mutant-T allele frequencies between healthy individuals (55 %) and AML patients (56 %) [10]. These discrepancies could be attributed to variations in sample selection criteria, study design, sample size, and genetic diversity between different populations, which can influence the observed associations between the ABCB1 gene polymorphism and AML susceptibility.

Table 2 – Stratification of baseline characteristics of Acute Myeloid Leukemia patients by ABCB1 (rs1045642) polymorphism.

Baseline Characteristics	ABCB1 rs1045642 (Recessive model)		p-value
	CC and CT (n = 19)	TT (n = 31)	
Demographic			
Age (years)	39 ± 10.9	35 ± 10.2	0.820
Male - n (%)	17 (89.5)	23 (74.2)	0.190
Female - n (%)	2 (10.5)	25 (25.8)	0.282
Hematological			
Hemoglobin (g/dL)	8.5 ± 1.57	9.6 ± 1.48	0.470
Red Blood Cell Count (x 10 ⁶ /μL)	3.55 ± 0.37	3.63 ± 0.38	0.953
White Blood Count (x 10 ³ /μL)	23.9 ± 7.5	22.1 ± 7.2	0.537
Platelet Count (x 10 ³ /μL)	109 ± 28	108 ± 27	0.891
Blasts (%)	65.4 ± 18.4	65.1 ± 19.2	0.821
Clinical History - n (%)			
History of Fever	16 (84.2)	26 (83.9)	0.975
History of Bruises	9 (47.4)	12 (38.7)	0.547
History of Infection	11 (57.9)	21 (67.7)	0.481
Previous History of Hematological Malignancy	3 (15.8)	5 (16.1)	0.975
Family History of Cancer	4 (21.1)	10 (32.3)	0.392
History of Pallor	14 (73.7)	27 (87.1)	0.231
History of Gum Bleeding	3 (15.8)	6 (19.4)	0.750
History of Hepatomegaly	4 (21.1)	4 (12.9)	0.445
History of Splenomegaly	12 (63.2)	17 (54.8)	0.563
History of Lymphadenopathy	5 (26.3)	1 (3.2)	0.015

To enhance the reliability of the current findings and better comprehend the role of the ABCB1 gene in AML development, further research with larger and more diverse populations is necessary.

In the present study, a significant association between the homozygous TT genotype and AML was also identified when compared to the homozygous CC and heterozygous CT genotypes. This aligns with findings from similar investigations by Feng et al., Ma et al., Li et al., and Meirav Kedmi et al. in various populations, all of which support the significant correlation between the ABCB1 C3435T polymorphism and AML [9,11–13].

However, it is noteworthy that some studies, including those by Jamrozzi et al., Rao et al., and Kaltoum et al., reported opposing results, suggesting potential variations in the impact of the ABCB1 C3435T polymorphism on AML susceptibility across different populations [14–16]. Factors such as population-specific genetic backgrounds, environmental factors, and sample sizes may contribute to these inconsistencies.

In the current study, various genetic models were applied to assess the association between the ABCB1 C3435T polymorphism and AML. The findings revealed significant associations in co-dominant, dominant, and recessive models. Specifically, the TT homozygous genotype of C3435T was associated with AML compared to the homozygous CC and heterozygous CT genotypes, with consistent ORs across the models. These results corroborate a previous study conducted by Li et al., where the authors also reported an association between the ABCB1 C3435T polymorphism and AML using similar modeling approaches [9]. The consistency between the present study and previous research highlights the robustness of the association between the ABCB1 C3435T polymorphism and AML providing further evidence of its potential role in AML.

Two shortcomings of this study are the lack of an investigation of treatment outcomes and the effect of the ABCB1 C3435T polymorphism on the prognosis of AML. While this study focused on the genetic link between ABCB1 polymorphism and AML susceptibility, future research should investigate its prognostic role to fully assess its relevance to patient outcomes.

Conclusions

In conclusion, this study investigated the potential association between the ABCB1 gene polymorphism (C3435T) and AML. A significant association was found between the homozygous TT genotype and AML, consistent with previous research. However, conflicting results from other studies suggest population-specific variations. Further research with larger and more diverse populations is needed to validate these findings. Understanding the role of the ABCB1 gene in AML development can contribute to improved detection and personalized treatment approaches.

Statements & declarations

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Author contributions

Authors Roh Ullah, Nazish Mazari, and Shagufta Khaliq contributed to the study conception and design. Material preparation, data collection and analysis were performed by Roh Ullah, Ghulam Mustafa, Asif Naveed, Aisha Hameed, Faiz Ul

Haq and Ali Amar. The first draft of the manuscript was written by Roh Ullah, Faiz Ul Haq and Asif Haleem Khan and all authors commented on the versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

The ethical committee of UHS Lahore Approved the study (erc/uhs/2018.008).

Consent to participate

Informed consent was obtained from all individual participants included in the study

Data availability

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

Conflicts of interest

The authors declare no conflicts of interest.

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Editor

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Original article

Hypoxic conditions confer chemoresistance to crizotinib but not to imatinib in chronic myeloid leukemia cells



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ABSTRACT

Introduction: Chronic myeloid leukemia is an adult leukemia, constituting 15 % of all leukemia diagnoses. The fundamental driver of disease pathogenesis is the Bcr/Abl fusion protein, characterized by dysregulated tyrosine kinase activity. Abl kinase inhibitors have become the mainstay of treatment, however, patients often develop resistance due to genetic alterations, particularly affecting the Bcr/Abl oncoprotein. The tumor microenvironment is also associated with acquired resistance to Abl kinase inhibitors in chronic myeloid leukemia.

Methods: The influence of hypoxic conditions on the development of chemoresistance to certain Abl kinase inhibitors was investigated in chronic myeloid leukemia.

Results: This study showed that hypoxia increased resistance to crizotinib, while imatinib resistance was modest. Both drugs effectively inhibited Bcr/Abl activity. Interestingly, the JAK1/2 inhibitor ruxolitinib further enhanced chemoresistance to crizotinib under hypoxic conditions. Hypoxia-inducible factor 1 α (HIF1 α) overexpression in JAK2 knockdown experiments confirmed their cooperative role in mediating crizotinib resistance. In addition, 2-methoxyestradiol, a non-estrogenic estradiol metabolite, restored crizotinib sensitivity under hypoxia and the combination of 2-methoxyestradiol with a JAK2 inhibitor showed promising results in overcoming crizotinib resistance.

Conclusion: In summary, this study shows the critical role of selective targeting of components of the HIF1 α signaling pathway for the complete eradication of chronic myeloid leukemia cells.

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Introduction

Philadelphia chromosome-positive (pH⁺) leukemia is defined by the chromosomal translocation t(9;22), which leads to the

formation of the *Breakpoint Cluster Region/Abelson proto-oncogene 1* fusion gene (*Bcr/Abl*). Persistent activation of *Bcr/Abl* leads to increased cell proliferation, decreased sensitivity to various apoptotic signals, and neoplastic transformation [1]. *Abl* kinase inhibitors (AKIs) are used to treat pH^+ leukemia. Although these treatments are initially effective [2], their clinical efficacy declines with disease progression. Patients with chronic myeloid leukemia (CML) in blast crisis or acute lymphoblastic leukemia (pH^+) rarely, if ever, benefit from AKI therapy [3]. *Bcr/Abl*-dependent or independent mechanisms may be responsible for resistance to therapy in pH^+ leukemia. A significant contributing factor to drug resistance in pH^+ leukemia is the bone marrow (BM) niche, which is a part of the tumor microenvironment (TME). The BM microenvironment is crucial for controlling stem cells and long-term hematopoiesis [4]. Nonetheless, soluble growth factors, interleukins, stromal cells, and extracellular components—all of which are found in the BM niche—may help reduce the susceptibility of cancer cells to treatment [5]. To overcome treatment failure in pH^+ leukemia, new therapeutic approaches must be developed considering these drug resistance mechanisms mediated by the BM microenvironment.

The BM microenvironment can consist of areas of hypoxia (low oxygen) and acidity, which can interfere with the ability of chemotherapy to effectively treat tumor cells. Furthermore, hypoxia in the microenvironment has been associated with metastasis, malignant invasion, and a cellular transformation known as the epithelial-mesenchymal transition (EMT).

Both malignant and normal hematopoietic stem cells, including those from CML, may encounter a hypoxic niche in the BM microenvironment. High BM hypoxia levels have been linked to resistance to therapies in several malignancies [6], minimal residual disease persistence [7], and disease progression. A major factor in the course of the disease is the interaction between leukemic cells and their surroundings.

The interaction of leukemic cells with their environment plays a significant role in the progression of the disease. *HIF-1 α* , an important regulator of the cellular response to low oxygen, is necessary for metabolic adaptability and proliferation under hypoxic conditions. *HIF-1 α* stabilizes in hypoxic conditions and suppresses programmed cell death (apoptosis) by either directly or indirectly controlling the expression of several genes that mediate chemoresistance. The genes which have been shown to be regulated by *HIF-1 α* include *Bak*, *Bax*, *Bcl-xL*, *Bcl-2*, *Bid*, *Mcl-1*, *NF- κ B*, and *p53* [8]. Notably, whereas *HIF-1 α* activation of anti-apoptotic target genes is well-established, the exact biological mechanisms underlying this phenomenon are still only partially known [9]. New data further emphasizes how the microenvironment mediates resistance to several *Abl* kinase inhibitors, including imatinib, nilotinib, and Dasatinib [10]. Furthermore, the microenvironment has been linked to the persistence of residual diseases of pH^+ leukemia [11].

Even though imatinib actively inhibits *Bcr/Abl* kinase, pH^+ leukemia cells under hypoxic conditions show partial suppression of apoptosis [12]. Given that BM microenvironment cells secrete soluble molecules including SDF-1, vascular endothelial growth factor (VEGF), and interleukin-6, which are all known to promote the survival of hematopoietic stem cells (HSCs), hypoxia may indirectly contribute to

chemoresistance [13]. Chemotherapy resistance mediated by the BM microenvironment is still a major challenge in the treatment of leukemia.

The present study investigated how hypoxia affects the efficacy of the tyrosine kinase inhibitor crizotinib by exploring its off-target activities, rather than its established function as an anaplastic lymphoma kinase (ALK) inhibitor implicated in ALK-positive non-small cell lung cancer (NSCLC). This study aims to repurpose crizotinib for an additional therapeutic indication—specifically, to overcome chemoresistance in CML which is the focus of our research program.

Materials and methods

Chemicals and reagents

Sigma Aldrich Israel Ltd. (Rehovot, Israel) provided most of the chemicals, including cobalt chloride (CoCl_2). The text includes specific sources for various compounds. 2-Methoxyestradiol (2ME2) was purchased from Cayman Chemical (Ann Arbor, MI, USA), Everolimus (Afinitor) and kinase inhibitors (Imatinib, Ruxolitinib, and Crizotinib) were obtained from Selleck Chemicals LLC (Houston, TX, USA).

Cell lines

The American Type Culture Collection (ATCC, VA, USA) provided the human CML cell lines K562 and BV173. The cells were cultured in complete RPMI 1640 media supplemented with 0.1 mg/mL streptomycin, 100 units/mL penicillin, 1 % (w/v) L-glutamine, and 10 % (w/v) fetal bovine serum (Biological Industries, Israel). All cells were maintained in a humidified incubator with 5 % CO_2 at 37 °C.

HIF1 α overexpression

Using a retrovirus harboring HA-HIF1 α P402A/P564A (Addgene plasmid #1900 [14]), K562 cells were stably transfected to overexpress HIF1 α , as previously reported [15]. In summary, puromycin selection was used to isolate stable transfected cells after K562 cells were infected with the retrovirus. Overexpression of HA-HIF1 α was detected in the transfected cells using immunoblotting with anti-HIF1 α antibodies.

JAK2 silencing in K562 cells

Using a previously established CRISPR-Cas9 method, JAK2 expression was suppressed in K562 cells [15]. In summary, lenti-cas9blast (Addgene plasmid #52,962), gRNA JAK2 (Addgene plasmid #75,728), and lentiviral packaging plasmids (pCMV-dR8.2 dvpr and pCMV-VSV-G) (Addgene plasmids #8455 and #8454) [16] were co-transfected into HEK293T cells (1.5×10^5 cells/mL) using Fugene 6 transfection reagent (Roche Applied Science, Penzberg, Germany) according with the manufacturer's instructions. The supernatant containing the lentiviral particles was collected 48 h post-transfection and utilized to infect K562 cells. Then, stably transduced clones with decreased JAK2 expression were isolated using blasticidin and puromycin selection. Western blotting was

performed to confirm the downregulation of JAK2 protein levels.

Cell viability assay

The trypan blue exclusion assay was used to assess cell viability. K562 cells were seeded at a density of 2×10^5 cells per well in six-well plates. Cells were subjected to the specified treatments following a 24-hour incubation period. Dimethyl sulfoxide (DMSO) at a concentration of 1% (w/v) was applied to the control samples. After exposure for 72 h, cells were collected, stained with a 0.4% (w/v) trypan blue solution diluted 1:1 with culture media, and then manually counted using a hemocytometer according to the previous described protocol.

Western blotting

Phosphatase inhibitors (AG Scientific, CA, USA) were used to produce cell lysates. After lysing the cell pellets in the buffer for half an hour on ice, the supernatants were separated by centrifugation. The DC™ Protein Assay (Bio Rad, USA) was used to determine the protein concentration in the supernatants (absorbance measured at 630 nm). Equal protein concentrations (50–60 µg each sample) were electrophoretically separated after being loaded onto 8–12% polyacrylamide gels. After that, the proteins were transferred onto nitrocellulose membranes (Schleicher & Schuell BioScience GmbH, Germany). To decrease non-specific antibody binding, the membranes were then blocked using 5% non-fat dried milk in Tris-buffered saline with Tween 20 (TBS-T).

To detect the presence of phospho-Abl (Tyr 245) and cleaved PARP (Poly(ADP-ribose) Polymerases - Cell Signaling Technology, MA, USA), α -tubulin (Santa Cruz Biotechnology, TX, USA), and phospho-ERK1/2 (Thr202/Tyr204) (Cell Signaling Technology, MA, USA), primary antibodies were employed. Every primary antibody incubation was carried out following the instructions recommended by the manufacturer.

Species-specific secondary antibodies combined with horseradish peroxidase (HRP), such as anti-mouse (NB7539, Novus Biologicals, CO, USA) and anti-rabbit (#7074, Cell Signaling Technology), were used to detect the bound primary antibodies. The SuperSignal™ West Pico PLUS chemiluminescent substrate (Thermo Fisher Scientific, MA, USA) was used following the manufacturer's instructions to accomplish chemiluminescent detection [17,18].

Flow cytometry analysis

The expressions of CD45 and CD44 on the cell surface were evaluated by flow cytometry, as previously reported [15]. In short, K562 cells were resuspended at a density of 1×10^7 cells/mL in phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA), together with K562/HIF1 α and K562/Si JAK2 variations. The CD45 (KRO, #A96416, Beckman Coulter) and CD44 antibodies (FITC, #9011–0441, eBioscience) were either employed at the dilutions suggested by the manufacturer or titrated in advance to maximize staining. For forty minutes, the cell-antibody combination was incubated on ice. The labeled cells were analyzed using a Beckman Coulter

Navios flow cytometer after washing with PBS mixed with 0.5% BSA.

RNA extraction and cDNA synthesis

Using Tri Reagent (Sigma), total RNA was isolated from cells following a previously described procedure [15]. The isolated RNA was then subjected to reverse transcription (RT), which produced single-stranded cDNA. In short, 15 µL of nuclease-free water (DEPC-treated) and 1 µL of oligo(dT)17 primer were added to 1 µg of RNA, and the mixture was pre-incubated for 10 min at 70 °C before being quickly cooled on ice. Second, 2 mL of 5X AMV RT reaction buffer, 2 µL of dNTP mix (25 mM), 28 units of RNasin ribonuclease inhibitor, 30 units of AMV reverse transcriptase, and nuclease-free water were added to the annealed primer-template mixture for a final volume of 10 µL. The RT reaction was incubated for 60 min at 42 °C.

Polymerase chain reaction (PCR) amplification

A commercial PCR kit (Bioline, Taunton, MA, USA) was used to amplify the generated cDNA. Specific primers for each gene of interest are specified in Table 1. Thirty-five amplification cycles (each cycle: 94 °C denaturation for 30 s, 55–60 °C annealing for 30 s, and 72 °C extension for 2 min and 30 s) proceeded after the first denaturation step at 94 °C for two minutes. The cycling program concluded with a last extension step that lasted 15 min at 72 °C. Primers specific to the housekeeping gene β -actin were used as a control. The PCR products (5 µL) were separated by electrophoresis in a 1.5% agarose gel.

Quantitative real-time polymerase chain reaction (RT-PCR)

Quantitative RT-PCR amplification of mRNA transcripts was performed using the resultant cDNA. Each 15 µL reaction mixture included 1X SYBR GREEN reaction mix (Kappa Biosystems, Wilmington, MA, USA) and 0.2 pmol/µL of forward and reverse primers. A spectrofluorometric Rotor-Gene 6000 thermal cycler (Corbett Research, Mortlake, Australia) was used to perform the PCR. The Taq DNA polymerase was first activated by a 10-minute denaturation stage at 95 °C, which was followed by 55 cycles of denaturation at 95 °C for 15 s and a one-minute combined annealing/extension step at 55–60 °C. A range of cDNA template concentrations were evaluated to guarantee amplification within the linear range. As an

Table 1 – Oligonucleotide primers for gene amplification.

Primers	Forward	Reverse
hVEGF	5'-GTCGGGCTCCGAAAC-CATG-3'	5'-CCTGGTGAGA-GATCTGGTTC-3'
hBcl2	5'-CTGGAGAGTGCTGAA-GATTGATG-3'	5'-CAATCAGCGGAA-CACTTGATTC-3'
hMcl1	5'-GATCAGTATATACACTT-CAG-3'	5'-CAGGTGCAGCCTG-TACTTGTG-3'
h β -actin	5'-GCCCTGGACTTCGAG-CAAGA-3'	5'-TGCCAGGGTA-CATGGTGGTG-3'

Sequences of the forward and reverse primers used for polymerase chain reaction along with their corresponding target genes.

internal control, the housekeeping gene β -actin was co-amplified in the same PCR reaction. The primer sequences employed in the semi-quantitative RT-PCR were the same.

Colony formation assay

A clonogenic experiment was used to evaluate the capacity of cells to form colonies on a semi-solid medium, as previously reported [19]. In summary, K562 cells were diluted in complete RPMI 1640 media with 10 % fetal bovine serum (FBS) until they reached a density of 1×10^4 cells/mL. The cell suspension was mixed with an equivalent amount of 0.6 % agar solution to reach a final agar concentration of 0.3 %. The cell-agar mixture was plated in 12-well plates over a layer of bottom agar that had already hardened and left to solidify. To maintain agar hydration after the top layer solidified, 1 mL of new medium containing the specified treatments was applied to each well. The cells were cultured for two weeks at 37 °C in a humidified environment with 5 % CO₂ to enable colony formation. A colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was used to visualize and quantify colonies. The plates were incubated for 4 h with 5 mg/mL MTT solution, followed by the extraction of the formazan product using a solubilization buffer (20 % SDS, 50 % N,N-dimethylformamide, and 25 mM HCl). Using a plate reader, the optical density of the extracted dye was measured with a plate reader at 570 nm with a reference wavelength of 630 nm.

Hypoxic conditions

A hypoxic atmosphere was created using a BioSpherix OxyCycler system (BioSpherix, Redfield, NY, USA). Cells were cultured in a custom-designed, computer-controlled incubator attached to the OxyCycler. A controlled environment of 37 °C, 5 % CO₂, and 2 % O₂ was maintained in the hypoxic chamber. The cells in the control group were cultured for the duration of the experiment in normoxic conditions (~21 % O₂ and 5 % CO₂). Twelve hours was the incubation time for both normoxic and hypoxic experiments.

Immunofluorescence staining and imaging

The cellular expression and location of phospho-c-Abl and HIF-1 α proteins in K562 cells were determined by immunofluorescence. The immunofluorescence protocol involved coating a 96-well plate with poly-L-lysine (Sigma Aldrich Israel Ltd, Rehovot, Israel) four hours before the experiment completion and allowing it to dry for two hours. Following the seeding of 5×10^5 cells in the coated wells, they were incubated for a further two hours under normoxic conditions and then 12 h of normoxic/hypoxic conditions as described above.

Following incubation, cells were washed twice with PBS and fixed with 4 % paraformaldehyde in PBS for 20 min at room temperature (RT). Following fixation, cells were washed twice with PBS and permeabilized using a solution containing 3 % FBS and 0.1 % Triton X-100 in PBS for 1 h at room temperature. Blocking was carried out for an extra one hour at room temperature using the same permeabilization solution. The primary antibody of interest (HIF1 α or phospho-c-Abl) was then diluted in 3 % FBS and 0.1 % Triton X-100 in PBS. Cells

were incubated overnight after being thoroughly washed twice with 3 % FBS in PBS. DAPI (nuclear stain) and Alexa Fluor-688 (red) (Thermo Fisher Scientific, Waltham, MA, USA), a secondary antibody, were added to the cells and incubated for 30 min the next day. The last washing step was three washes using 0.1 % Triton X-100 in 3 % FBS and PBS.

Image Acquisition used a 20x magnification with images being taken with the Zeiss Cell Discoverer 7 Imaging System. The picture capture program was Zen Blue 3.7.

Protein Expression Quantification: Immunofluorescence data were used to quantify the relative amounts of phospho-c-Abl and HIF-1 α protein expression.

Statistical analysis

Data were analyzed using Student's t-test. Statistical significance was set at a *p-value < 0.01 and **p-value < 0.001.

Results

A previous study showed that Crizotinib, an ALK and c-Met inhibitor is able to inhibit both wild-type and T315I-mutated Bcr/Abl [20]. In addition, another study reported that Crizotinib, but not Imatinib, was able to overcome soluble factor-mediated drug resistance in CML cells [21]. This was possible in part because Crizotinib could inhibit JAK2 activity. In the present study, normoxic and hypoxic conditions were used to evaluate the sensitivity of CML cell lines to Abl kinase inhibitors such as Imatinib and Crizotinib.

Induction of apoptosis in chronic myeloid leukemia cells by imatinib and crizotinib under hypoxic conditions

CoCl₂, a known inducer of HIF1, was used to simulate hypoxic conditions in the K562 and BV173 CML cell lines [22]. Immunofluorescence analysis showed that K562 cells exposed to 100 μ M CoCl₂ exhibited increased levels of HIF1 comparable to exposure to low oxygen (Figure 1A and B). Real-time PCR was also used to monitor the expression of HIF1-responsive genes such as VEGF and Bcl2 (Figure 1C) and to demonstrate the functionality of induced HIF1 in K562 cells treated with CoCl₂.

Exposure to CoCl₂ and growth of K562 under hypoxic conditions led to an accumulation of HIF1 α (Figure 1A and B), which increased the expression of VEGF and Bcl2 by more than fourfold and twentyfold, respectively (Figure 1C). Figure 1D shows that CoCl₂ treatment had no effect on Abl phosphorylation in K562. The study also showed that Imatinib and Crizotinib alone or in combination with Ruxolitinib inhibited the phosphorylation of Abl under normoxic (Figure 1D and E) and hypoxic (Figure 1F and G) conditions. Interestingly, pAbl levels were lower in hypoxic conditions (Figure 1E) compared to normoxic conditions (Figure 1G).

Next, how Imatinib and Crizotinib affected the proliferation of K562 cells in the presence and absence of CoCl₂ was examined. Figure 2A shows that exposure to imatinib inhibited the proliferation of K562 in the presence or absence of CoCl₂. In addition, a significant inhibition of cell proliferation in K562 cells treated with Crizotinib was observed. However,

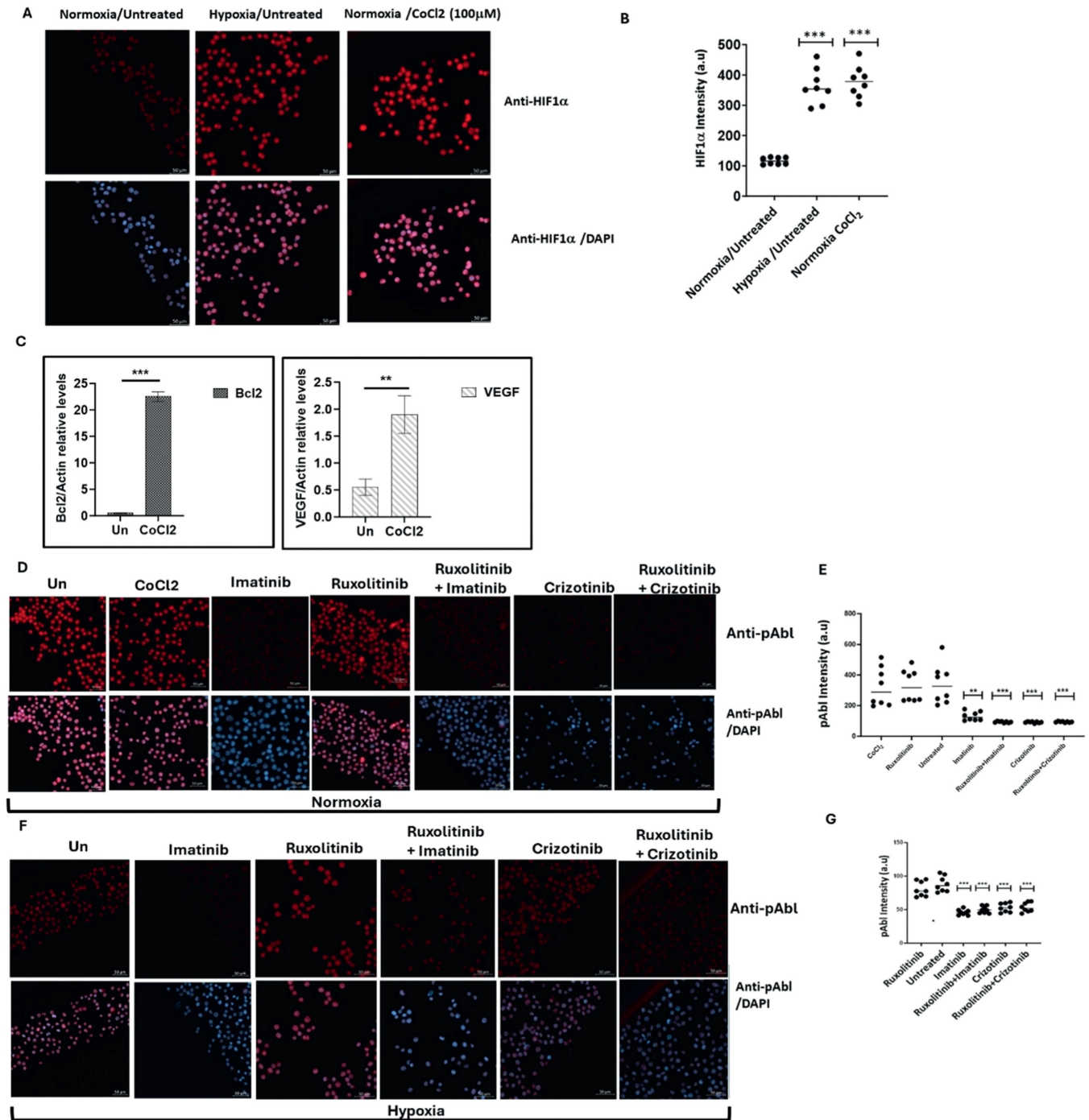


Figure 1 – Expression of HIF1α and pAbl in K562 under hypoxic conditions. K562 cells were treated with 100 μM cobalt chloride (CoCl₂) or grown under low oxygen conditions (2 %) for 12 h. (A) Induction of HIF1α in K562 cells by treatment with CoCl₂ and exposure to hypoxic conditions for 12 h. (B) Quantitation of HIF1a levels under the different conditions. (C) Real-time PCR was used to determine the relative expression of Bcl2 and VEGF in relation to β-actin in K562 cells exposed to 100 μM CoCl₂. Sequences of the primers used in this study are shown in Table 1. (D) Immunofluorescence assessment of pAbl levels in K562 cells grown under normoxic, and (E) quantitation. (F) Immunofluorescence assessment of pAbl levels in K562 cells grown under hypoxic conditions after 12 h of treatment, Imatinib, Ruxolitinib, Imatinib/Ruxolitinib, and Crizotinib/Ruxolitinib and (G) quantitation. *p-value ≤ 0.01, **p-value ≤ 0.001, *** p-value ≤ 0.0001. The experiment, performed in duplicate, was repeated, with consistent results observed across both independent experiments.

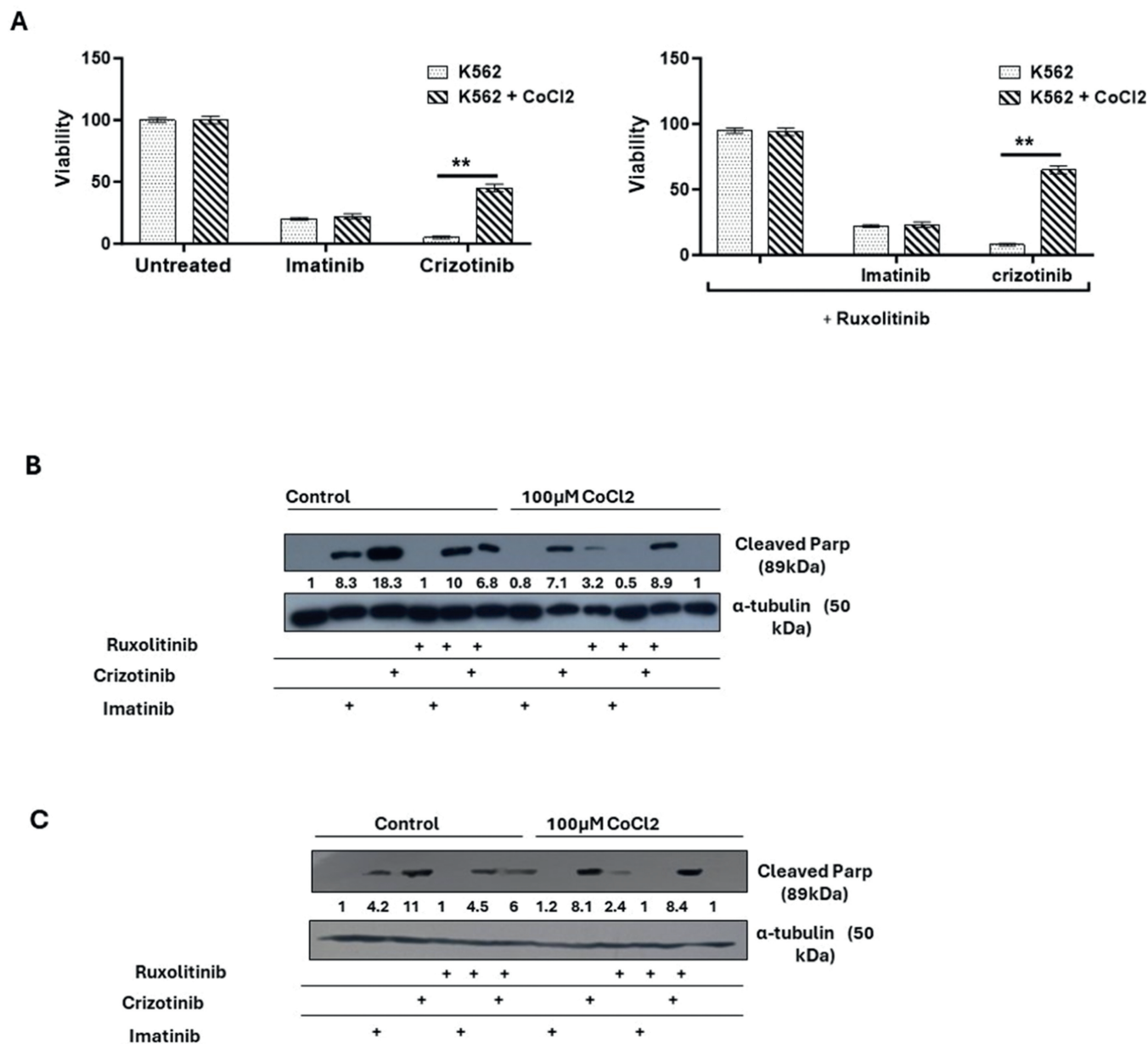


Figure 2 – Effect of cobalt chloride (CoCl₂) and ruxolitinib on the sensitivity of K562 and BV 173 cells to treatment with Imatinib or Crizotinib. K562 or BV 173 cells exposed to 100 μ M CoCl₂ and treated with 1 % DMSO, 1 μ M Imatinib, Crizotinib or Ruxolitinib for 12 h. (A) Measurement of the viability of K562 with the trypan blue exclusion assay using a two-sample t-test. Immunoblot of (B) K562 and (C) BV 173 cells exposed to 1 μ M Imatinib, Crizotinib, and Ruxolitinib in the presence or absence of 100 μ M CoCl₂. Filters were probed with anti-c-PARP and α -tubulin antibodies. The numbers below the plot represent the relative expression levels normalized to α -tubulin.

moderate drug resistance was observed in the presence of CoCl₂ in Crizotinib-treated K562 cells. This suggests that the presence of CoCl₂ blocks the inhibition of cell proliferation mediated by Crizotinib but not by Imatinib (Figure 2A). Signal Transducer and Activator of Transcription 3 (STAT3) has been associated with hypoxia-induced chemoresistance of ovarian cancer cells [23]. Therefore, the JAK1/2 inhibitor Ruxolitinib [24], which regulates the activity of STAT3, was used to investigate the role of JAK1/2 in the sensitivity of CML cells to

Crizotinib. We wondered whether the addition of Ruxolitinib to Crizotinib in the presence of CoCl₂ could restore sensitivity. Treatment with Ruxolitinib alone had no effect on the proliferation of K562 cells in the presence or absence of CoCl₂ (Figure 2B). Furthermore, there was no significant difference in the inhibition of K562 cell proliferation between the combinations of Imatinib and Ruxolitinib (Figure 2B). Interestingly, the combination of Ruxolitinib and Crizotinib did not restore the sensitivity of K562 to Crizotinib. In contrast, the inclusion

of Ruxolitinib increased Crizotinib resistance in K562 cells (Figure 2B). Comparable data were also obtained using other CML cells such as BV173 (Figure 2C).

The role of HIF1 α and JAK2 in mediating chemoresistance in chronic myeloid leukemia

The above data suggests that the sensitivity of CML cells to Crizotinib is dependent on JAK1/2 activity and hypoxic conditions (Figure 2). However, neither hypoxia nor JAK1/2 activity had a significant effect on sensitivity to Imatinib (Figure 2). HIF1 α is an important mediator of hypoxic conditions and modulates the transcription of many genes. To directly demonstrate the role of hypoxia and JAK2 in regulating sensitivity to Crizotinib, a stable mutant of HIF1 α (Pro 402 and Pro 564 were replaced by Ala) [25] was overexpressed in K562. Under normoxic conditions, the HIF1 α mutant Pro 402 Ala, Pro 564 Ala is not hydroxylated and is therefore not recognized by the von Hippel-Lindau tumor suppressor protein (VHL) for proteasome degradation [26]. Figure 3A shows that HA-HIF1 α is overexpressed in K562/HIF1 cells under normoxic conditions. CD44 is one of the genes regulated by HIF1 α [27], and monitoring of levels in K562 and K562/HIF1 α cells showed upregulation of CD44 (Figure 3B).

Next, the activity of the Abl inhibitor in K562 cells stably expressing HIF1 α was examined. The levels of phosphorylated Bcr/Abl and Abl were essentially unaffected by the overexpression of HIF1 α , as shown in Figure 3C. However, the levels of phosphorylated Bcr/Abl and Abl in K562 and K562/HIF1 α lysates were completely inhibited by Imatinib or Crizotinib alone or in combination with Ruxolitinib (Figure 3C). This suggests that the ability of Imatinib and Crizotinib to inhibit auto-phosphorylation of the Abl protein is not affected in cells overexpressing HIF1 α or exposed to Ruxolitinib. When K562 cells were exposed to Imatinib, PARP was significantly cleaved. Interestingly, the combination of Ruxolitinib and Imatinib decreased the amount of cleaved PARP in K562/HIF1 α cells but not in K562 cells. The amount of cleaved PARP was almost twice as high in Crizotinib-treated K562 cells compared to Imatinib-treated cells. In addition, the combination of Ruxolitinib and Crizotinib significantly reduced the amount of cleaved PARP in K562 by about 50 %. In contrast, neither Crizotinib nor Crizotinib in combination with Ruxolitinib resulted in cleavage of PARP in K562/HIF1 α cells. Figure 3 shows that HIF1 α is responsible for the reduction of cleaved PARP levels in K562 cells when exposed to Crizotinib, although Crizotinib still inhibits Bcr/Abl activity. In addition, it supports the notion that JAK1/2 inhibition contributes to the reduced sensitivity of Imatinib and Crizotinib.

A comparison of the effect of Imatinib and Crizotinib was also evaluated using a clonogenicity assay. Figure 3D shows that Imatinib inhibited colony formation in K562 and K562/HIF1 α cells at concentrations of 1 and 3 μ M. Crizotinib was more effective in inhibiting colony formation of K562, and significant inhibition was observed at the lowest concentration (0.3 μ M). The ability of Crizotinib to inhibit K562/HIF1 α was impaired, indicating some degree of resistance. Interestingly, the addition of Ruxolitinib reduced the ability of Crizotinib to inhibit colony formation in both K562/HIF1-positive and -negative cells. Of note, the combination of Ruxolitinib with

Crizotinib showed a minimal effect on the proliferation of K562 (Figure 2A - cells grown in two-dimensional [2D] culture) and a significant effect on K562 treated with Crizotinib plus Ruxolitinib in a three-dimensional (3D) culture (Figure 3D).

The JAK2 gene was also silenced in K562 cells (Figure 4A). The sensitivity of K562, K562/Si JAK2 and K562/ HA-HIF1 α P402A/P564A to a range of Abl kinase inhibitors (AKIs) was monitored. The results shown in Figure 4B demonstrate that the different cells have different sensitivities to the different AKIs.

Exposure of various K562 cells to AKIs resulted in a decrease in cell viability. When K562/HIF1 α cells were treated with AKIs, a marked and reproducible increase in cell viability was observed compared to K562 cells (Figure 4B), indicating partial drug resistance due to overexpression of HIF1 α (Figure 4B). The ability of Crizotinib to inhibit the proliferation of K562 cells was significantly different from that of K562/Si JAK2 and K562/HIF1 α cells. Crizotinib was less effective in inhibiting the proliferation of K562/Si JAK2 and K562/HIF1 α cells (Figure 4B). Thus, the percentages of viable cells in K562, K562/Si JAK2, and K562/ HA-HIF1 α when exposed to 500 nM Crizotinib for 72 h were 23.4, 57.9, and 63.4, respectively (Figure 4C). In addition, no significant differences in cell viability were observed between K562 cells exposed to other AKIs, including ponatinib and GNF-5 (Figure 4B). These results suggest that the reduced sensitivity to Crizotinib is significant in cells overexpressing HIF1 α or silenced with JAK2, whereas it is less evident with other AKIs.

2-methoxyestradiol restores crizotinib sensitivity of CML cells under hypoxic conditions

The data of this study suggest that HIF1 α is responsible for mediating Crizotinib chemoresistance in CML cells. Therefore, it investigated whether HIF1 α modulators can restore sensitivity to Crizotinib. Two HIF1 α modulators, 2ME2 and Everolimus (Afinitor), were used. 2ME2, a natural estradiol metabolite, has been shown to inhibit the nuclear accumulation and activity of HIF1 α in an oxygen- and proteasome-independent manner [28], while Afinitor, an mTOR inhibitor, has been shown to reduce the expression of HIF1 α and restore chemosensitivity in ALL cells [29].

Figure 5A shows that Crizotinib induces PARP cleavage in K562 cells but not in K562/HIF1 α cells. However, 2ME2 was only marginally active in inducing PARP cleavage in K562 cells, whereas Afinitor was not. The combination of Afinitor with Crizotinib had little effect on the sensitivity of K562 cells to Crizotinib, particularly in K562/HIF1 α cells. In contrast, the combination of 2ME2 and Crizotinib significantly increased the amount of cleaved PARP in K562/HIF1 α cells, suggesting that 2ME2 can restore the sensitivity of Crizotinib in K562 cells overexpressing activated HIF1 α . Thus, the decreased sensitivity of CML cells to Crizotinib under hypoxic conditions is primarily mediated by the stabilization and increased activity of HIF1 α , and inhibiting the function of HIF1 α may restore the sensitivity of Crizotinib in hypoxic CML cells.

To explore the possible mechanism by which activation of HIF1 α confers chemoresistance to CML cells despite complete inhibition of Bcr/Abl, changes in apoptosis-related gene expression were monitored in K562 and K562/HIF1 α cells

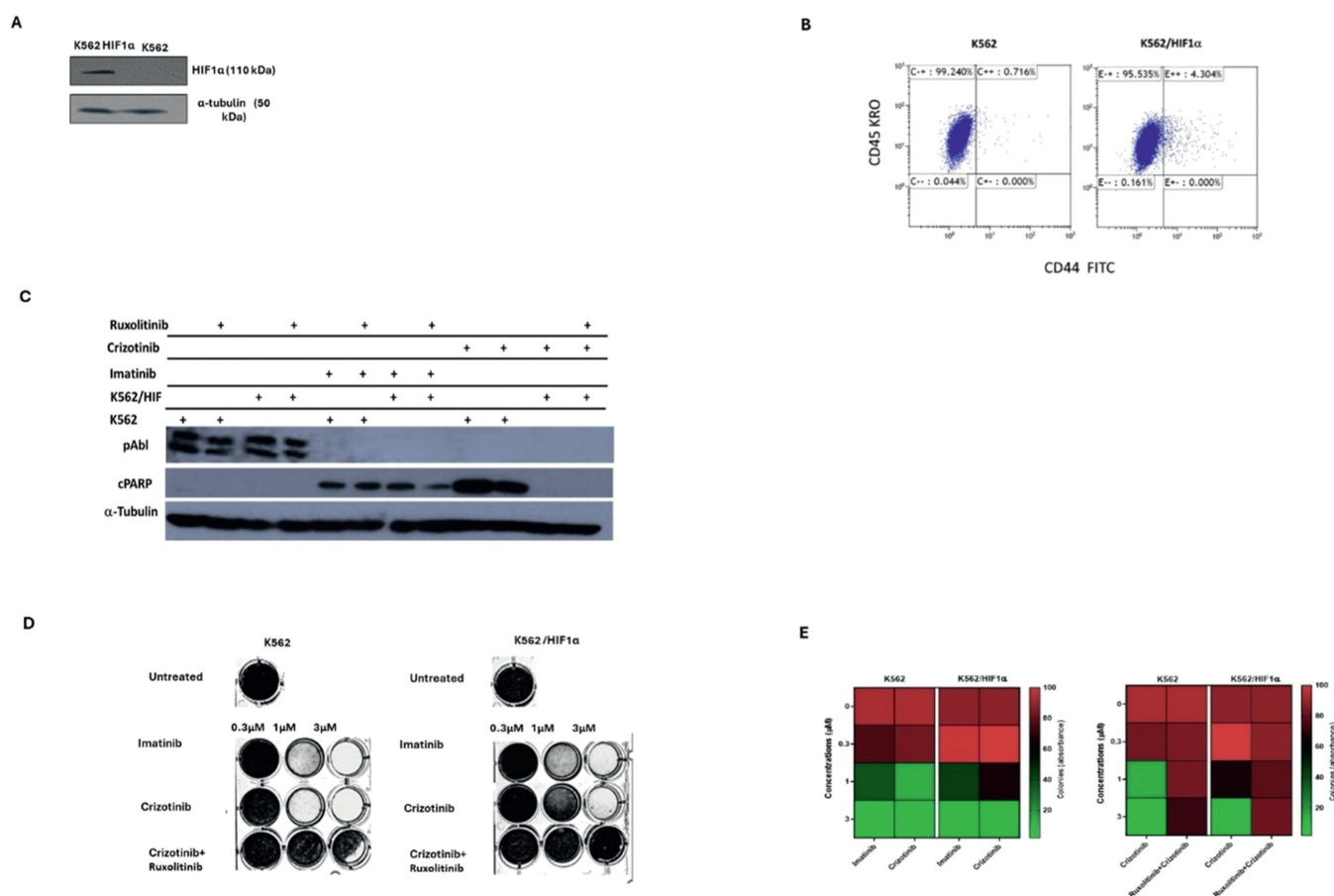


Figure 3 – Effect of overexpression of HIF1α on the chemoresistance of K562. (A) Immunoblot of HIF1α in K562 and K562/HIF1α cells. **(B)** The level of expression of CD44 in K562 and K562/HIF1α was determined by FACS analysis. **(C)** Levels of pAbl and cleaved PARP in K562 and K562/HIF1α exposed to Imatinib (1 μM), Crizotinib (1 μM), or Ruxolitinib (1 μM) for 24 h. **(D)** Clonogenicity of K562 and K562/HIF1α cells under semi-solid conditions in the presence of Imatinib (0.3, 1 and 3 μM) and Crizotinib (0.3, 1 and 3 μM) in combination with Ruxolitinib (1 μM). **(E)** Heatmap of absorbance of dye levels extracted from stained colonies in the different samples. The experiment was performed twice, yielding comparable results.

treated with Crizotinib. The expression of *Mcl1* and *Bcl2* genes in K562 and k562/HIF1α cells treated with Imatinib or Crizotinib were monitored (Figure 5B). Treatment of K562 cells with Imatinib resulted in a small reduction in the levels of both genes. Treatment with Crizotinib was more effective in reducing the levels of the *MCL1* and *Bcl2* genes. In contrast, treatment with Imatinib in K562/HIF1α led to a similar reduction in the levels of both genes as in K562 cells. However, the levels of *Bcl2* and *MCL1* were significantly higher in Crizotinib-treated K562/HIF1α cells than in Imatinib-treated cells. This suggests that the levels of the two anti-apoptotic genes *Bcl2* and *MCL1* are relatively higher in K562/HIF1α exposed to Crizotinib compared to Imatinib treatment and likely contribute to the chemoresistance to Crizotinib observed in CML cells under hypoxic conditions.

Discussion

Crizotinib inhibits native and mutant Bcr/Abl kinase activity and shows significant anti-CML activity in vitro and in vivo

[30]. In addition, Crizotinib, unlike Imatinib, can effectively overcome the TME-mediated chemoresistance in CML cells, which was attributed to the ability of Crizotinib to inhibit JAK2 activity [21].

This study investigated the anti-CML activity of Imatinib and Crizotinib under hypoxic conditions. First, it discovered that hypoxic conditions or exposure to CoCl₂, which mimics hypoxic conditions, impaired the ability of Imatinib to promote apoptosis and inhibit proliferation (Figure 2). Nevertheless, the effect of hypoxic conditions on Crizotinib-treated CML cells was profound and much stronger than that of Imatinib (Figure 2). The effect was not limited to inhibition of cell proliferation of CML, but also included the ability of treated CML cells to undergo apoptosis as measured by monitoring levels of cleaved PARP (Figure 2C and D). The observed effect was not limited to K562 cells, but also other myeloid cells, such as BV173 cells, showed a reduced ability of Crizotinib to induce apoptosis under hypoxic conditions compared to Imatinib-treated BV173 cells (Figure 2). The hypoxia-induced chemoresistance of Crizotinib in CML cells was further enhanced by the combination with Ruxolitinib (JAK1/2 inhibitor)

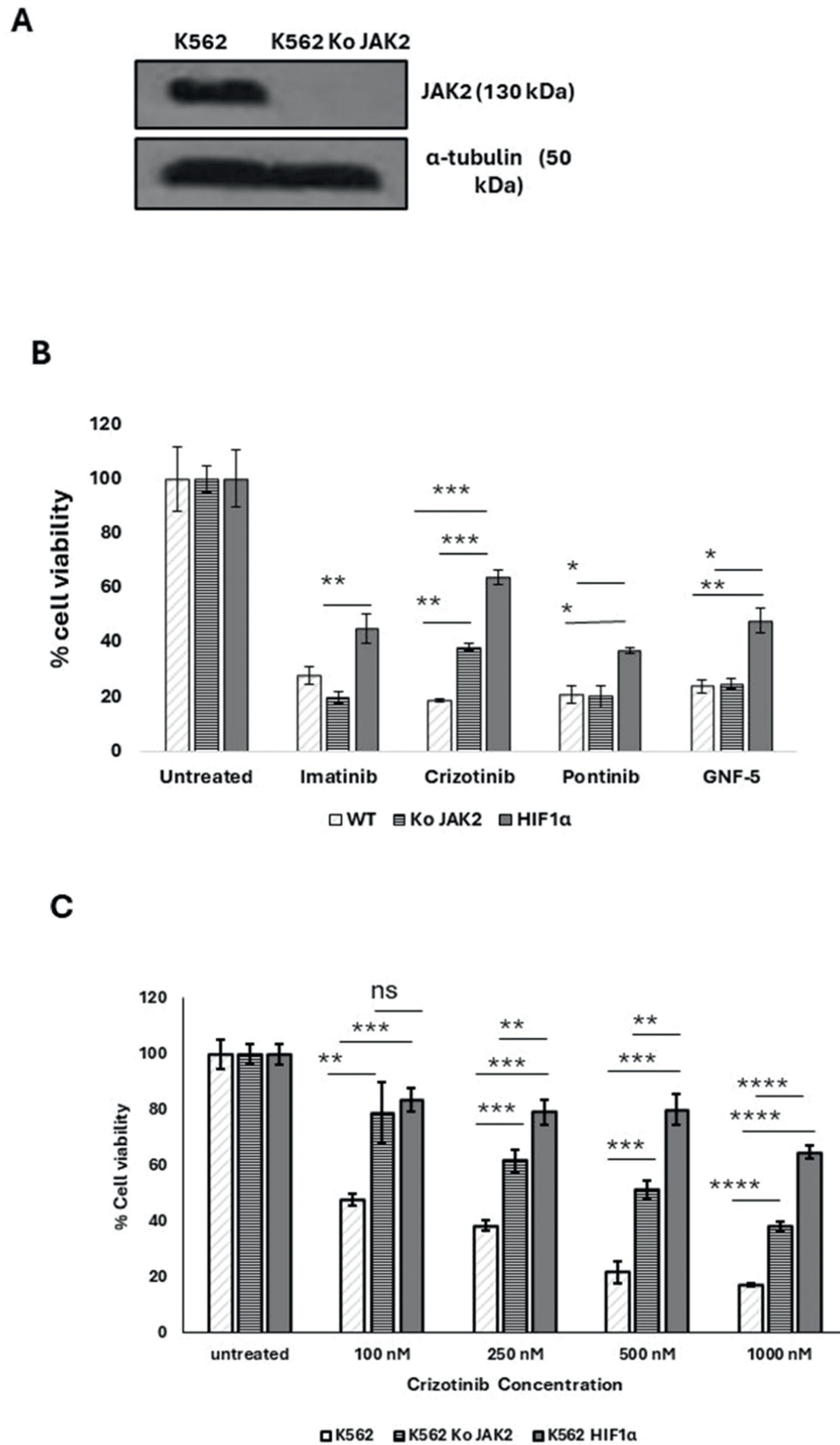


Figure 4– Effect of JAK2 silencing and HIF1 α overexpression on the sensitivity of K562 to different Abl kinase inhibitor treatments. (A) Immunoblot of JAK2 in K562 and K562 SiJAK2 cells. (B) Proliferation blot showing remaining K562, K562/Si JAK2, and

(Figure 2). These results suggest that hypoxia-mediated Crizotinib resistance in CML cells is a phenomenon that is not restricted to a specific CML cell line.

Hypoxia is one of the intrinsic features of solid tumors and is associated with aggressive phenotypes such as resistance to radiotherapy and chemotherapy, metastasis, and a poor patient prognosis [31]. The BM microenvironment is a hypoxic environment that supports hematopoietic stem cells and contributes to leukemia stem cell persistence [12]. In CML, Imatinib inhibited Bcr/Abl activity in hypoxic stem cells, but apoptosis was partially inhibited [32]. Hypoxic conditions have been reported to induce resistance to Crizotinib in NSCLC cell lines with the EML4-ALK rearrangement by activating the EMT process [32]. Hypoxia could induce drug resistance via HIF1-dependent and HIF1-independent mechanisms [33,34]. To corroborate the findings of this study and provide evidence that Crizotinib-induced chemoresistance in CML cells is dependent on HIF1 α , the mutant HIF1 α was overexpressed in K562 cell lines while silencing JAK2. The mutant HIF1 α (substitution of Pro 402 and Pro 564 by Ala) [25] is not subject to hydroxylation and proteasome degradation [26] and is therefore active under normoxic conditions. K562/HIF1 α cells were almost completely resistant to Crizotinib but not to Imatinib (Figures 2 and 4), whereas K562 Si JAK2 cells were partially resistant to Crizotinib but not to Imatinib or other AKIs (Figure 4). Remarkably, the data of the current study show that Ruxolitinib, a JAK1/2 inhibitor, enhances the chemoresistance of Crizotinib under hypoxic conditions.

Of note is the ability of Ruxolitinib to enhance the chemoresistance of Crizotinib in K562 cells grown in a 3D culture (Figure 3D), whereas the effect of Ruxolitinib in a 2D culture was minimal (Figure 2A). It is well documented that cells cultured in 3D respond differently to drugs compared to cells cultured in 2D [35]. One explanation could lie in the local pH values within 2D and 3D cells, as lower pH values were found in 3D cells [36]. It is well established that lower intracellular pH values reduce the efficacy of drugs and thus contribute to drug resistance [36]. The results of this study show that the combination of Ruxolitinib with Crizotinib enhanced the chemoresistance of Crizotinib in K562/HIF1 α as well as in K562 cultured in 3D. In contrast, in a 2D culture, the effect of the combination of Ruxolitinib with Crizotinib was only observed in K562/HIF1 α cells. The data are consistent with other observations showing that cancer cell lines growing in 3D culture are more resistant to chemotherapeutic agents than in 2D culture [37].

To elucidate a possible mechanism responsible for resistance to Crizotinib under hypoxic conditions, the levels of the anti-apoptotic genes MCL-1 and Bcl2 were measured. Imatinib inhibited the expression of both anti-apoptotic genes Bcl2 and MCL1 in both cell types, although the reduction appeared to

be greater in K562/HIF1 α (Figure 5B). In contrast, Crizotinib significantly reduced the expression of Bcl2 and MCL1 in K562 cells, while increasing Bcl2 levels in K562/HIF1 α cells (Figure 5B). It is currently uncertain whether the ability of Crizotinib to upregulate Bcl2 contributes to its ability to promote chemoresistance in K562/HIF1 α cells. Hypoxic conditions inhibit the expression of pro-apoptotic genes (Bax and Bim) and stimulate the expression of anti-apoptotic genes such as Bcl-2, Mcl-1, and XIAP [38]. An alternative explanation could be related to the ability of hypoxia to induce EMT in cancer cells [39]. Exposure to Crizotinib induces EMT in NSCLC cell lines, leading to chemoresistance [32]. Therefore, one could speculate that Crizotinib treatment in the context of hypoxia may have a stronger ability to induce EMT in K562 cells than Imatinib, leading to increased chemoresistance in treated cells. This speculation still needs to be confirmed experimentally.

The known HIF1 α inhibitor 2ME2 was used to provide further evidence for the importance of HIF1 α in mediating Crizotinib chemoresistance. Figure 5 shows that 2ME2 can restore sensitivity to Crizotinib in K562/HIF1 α cells. This result is consistent with previous findings that 2ME2 can restore the sensitivity of medullary thyroid cancer (MTC) cells [40]. In addition, 2ME2 has been shown to reverse drug resistance in human breast tumor xenografts [41] and multiple myeloma cells [42]. 2ME2 is a natural metabolite of estradiol devoted to estrogenic activity. The antitumor activities of 2ME2 were mediated by its pro-apoptotic activity, microtubule activity and superoxide production [43]. In addition, 2-hydroxyestradiol, a prodrug of 2ME2, was shown to restore the sensitivity of ovarian cancer cells to platinum, which is mediated by the TME [18,44]. These results showed that the combination of 2ME2 and Crizotinib restored the sensitivity of Crizotinib in K562 cells with active HIF1 α and JAK1/2 inhibitor treatment (Figure 5). The underlying mechanism by which 2ME2 restores the sensitivity of Crizotinib is not yet clear. The main effect of 2ME2 appears to be due to disruption of the cellular microtubules required for translocation of HIF1 α to the nucleus. Thus, 2ME2 may inhibit the accumulation and activity of HIF1 α in the nucleus in a manner that is both oxygen- and proteasome-independent [28]. Consistent with previous studies, the data of the current study show that 2ME2 induces apoptosis in K562 cells [18]. Moreover, 2ME2 inhibits the proliferation of prostate cancer cells by modulating the activity of β -catenin [45]. Furthermore, 2ME2 upregulates death receptor 5 (DR5) and induces apoptosis by activating the extrinsic pathway [46]. Recent reports suggest that the antitumor activity of 2ME2 is mediated by its ability to downregulate a number of genes, including the Bcl2, BCL-XL and c-myc genes [18,47]. Overall, 2ME2 restores the Crizotinib sensitivity of K562 under hypoxic conditions by inhibiting the activity of HIF1 α [28], which promotes apoptosis possibly mediated by the overexpression of the Bcl2, Bcl-XL and c-myc genes [18].

K562 HIF1 α cells when exposed to 1000 nM Imatinib, Crizotinib, ponatinib, or GNF-5. (C) Proliferation blot of the different K562 cells that were exposed to different concentrations of Crizotinib. Cell viability was monitored as described in Materials and Methods. *p-value ≤ 0.01 , **p-value ≤ 0.001 , * p-value ≤ 0.0001 . The experiment was run in duplicate and repeated (for a total of two independent experimental runs), with both yielding comparable outcomes.**

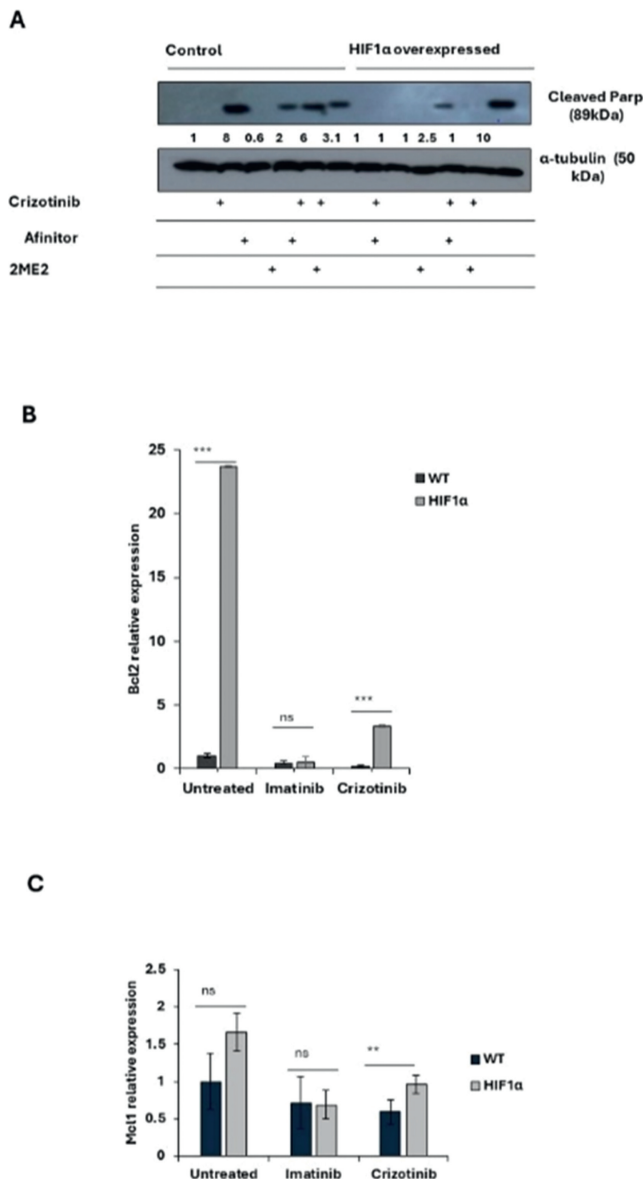


Figure 5 – Effect of 2ME2 on HIF1 α -mediated resistance to Crizotinib. K562 or K562/HIF1 α cells were treated with 1 % DMSO, Imatinib, Crizotinib, Afinitor, or 2ME2 for 24 h. (A) Immunoblot of K562 or K562/HIF1 α cells treated with 1 μ M Crizotinib, Afinitor, or 2ME2. Filters were probed with anti-c-PARP and α -tubulin antibodies. The numbers below the different lanes represent relative values normalized to α -tubulin. (B and C) Relative expressions of (B) Bcl2 and (C) Mcl1 genes compared to the β -actin gene were determined by real-time PCR using K562 and K562/HIF1 α cells treated with 1 % DMSO, Imatinib, or Crizotinib for 24 h. Sequences of the primers used in this study are shown in Table 1. The levels of PCR amplicons were analyzed using a two-sample t-test. The mean relative expression (log2) $\pm$ standard error of the mean is shown. *p-value $\leq$ 0.01, **p-value $\leq$ 0.001 and *** p-value $\leq$ 0.0001. The experiment was performed twice, yielding consistent results.

The data of the present study showed that Ruxolitinib, a JAK1/2 inhibitor, can attenuate the effect of hypoxia or HIF1 α overexpression in mediating Crizotinib resistance in CML cells. Previous studies have shown that JAK1/2 inhibitors such as AG490 promote the accumulation of HIF1 α by inhibiting its hydroxylation [48]. Specifically, JAK1/2 is involved in the activation of prolyl hydroxylase domain enzyme activity in HIF1 α , which mediates the hydroxylation of HIF1 α and its subsequent degradation by the proteasome [48]. Therefore, the JAK1/2 inhibitor Ruxolitinib may inhibit the degradation of HIF1 α and contribute to its stabilization [48]. Other studies have shown that inactivation of JAK1 promotes proliferation of endometrial cancer cells by upregulating the HIF1 α signaling pathway [49]. The authors suggested that JAK1 may act as a tumor suppressor [49], and that deletion of JAK1 activates the HIF1 α pathway [49]. Jeong et al. [50] suggested that JAK1 interacts with HIF-1/2 and reduces the expression of HIF-1/2 protein under hypoxia; therefore, silencing of JAK1 or pharmacologic inhibition of JAK1 kinase activity by Ruxolitinib leading to upregulation of transcription of HIF target genes under hypoxia. Crizotinib may affect the stability of HIF1 α in a JAK2-dependent manner, as suggested by the observation that Crizotinib inhibits JAK2 activity [50]. Since Imatinib does not inhibit JAK2, no stabilizing effect is observed in CML cells treated with Imatinib. Furthermore, the activity of Crizotinib was additive to that of Ruxolitinib, which may suggest that different mechanisms are involved in stimulating the stability and function of HIF1 α .

In conclusion, this study shows that hypoxic conditions and the presence of Ruxolitinib contribute to a substantial increase in chemoresistance of CML cells to Crizotinib. Although the ability of Crizotinib to inhibit Bcr/Abl activity remained unchanged, its ability to induce apoptosis in CML cells was diminished. It found that K562 cells overexpressing HIF1 α exhibited higher levels of the anti-apoptotic genes Bcl2 and MCL-1, suggesting a possible mechanism of action. In addition, this study showed that the inclusion of the HIF1 α inhibitor 2ME2 restored the sensitivity of Crizotinib in CML cells. The findings show that HIF1 α enhances chemoresistance of Crizotinib independent of Bcr/Abl kinase activity in CML cells. Consequently, targeting HIF1 α and the components of this pathway may be critical for overcoming chemoresistance and the complete eradication of CML. In addition, targeting the crosstalk between JAK1/2 and HIF1 α has been shown to be a promising strategy for cancer treatment. This intercellular communication could contribute to drug resistance in hypoxic tumor environments. Inhibitors of JAK1/2 or HIF1 α or drugs targeting their downstream signaling pathways have great potential for cancer therapy.

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Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Data availability

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

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Lena Avinery: Conceptualization, Investigation, Methodology, Writing – original draft. **Danielle Regev:** Investigation, Methodology, Writing – review & editing. **Hazem Khamaisi:** Conceptualization, Investigation, Methodology. **Jacob Gopas:** Conceptualization, Writing – review & editing, Formal analysis, Resources. **Jamal Mahajna:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Formal analysis, Resources.

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Original article

Early detection of cardiotoxicity in pediatric and adolescent patients with cancer treated with anthracyclines in Northeastern Brazil



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ABSTRACT

Objective: To evaluate the early detection of cardiotoxicity using echocardiography in children and adolescents with cancer treated with anthracyclines.

Methods: This cross-sectional study was conducted in a tertiary pediatric oncology center in Northeastern Brazil between January 2018 and December 2022. Eligible participants were under 19-year-old patients with cancer treated with anthracyclines presenting left ventricular ejection fraction $\geq 55\%$ (assessed using the biplane Simpson's method) and abnormal left ventricular global longitudinal strain.

Results: A total of 45 patients meeting the inclusion criteria were included. Among them, 19 patients (42.2%) showed reduced ejection fraction or left ventricular global longitudinal strain (or both) compared with baseline values, and 57.9% were asymptomatic. The most prevalent cancer was leukemia (55.5%), followed by lymphoma (20.0%). A total of 75.6% of participants were undergoing cancer treatment at the time of diagnosis of cardiotoxicity. An isolated left ventricular ejection fraction reduction occurred in 26.3% of patients, isolated left ventricular global longitudinal strain reduction in 47.4% of patients, and both alterations were experienced by 26.3% of patients.

Conclusions: Anthracycline-induced cardiotoxicity is a relevant adverse effect in cancer treatment, especially in patients with leukemia and lymphoma. Echocardiography, especially the assessment of left ventricular global longitudinal strain, plays a critical role in the early and subclinical detection of cardiotoxicity in pediatric patients.

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Introduction

Cancer during childhood accounts for about 3 % of all malignant neoplasms. According to estimates by the Brazilian National Cancer Institute, 7930 new pediatric cancer cases are expected annually during the 2023–2025 triennium [1]. Leukemia (28.0 %), central nervous system tumors (26.0 %), and lymphomas (8.0 %) are among the most prevalent types [2]. Advances in treatment have led to favorable outcomes, with survival rates reaching about 80 % in developed countries [2,3]. The increased survival has enhanced awareness of treatment-related adverse effects, particularly those affecting long-term health [4,5].

Cardiovascular complications are prevalent during cancer treatment. Cardiotoxicity is diagnosed during or after treatment by cardiovascular alterations, clinical findings, biomarkers, or imaging tests occurring after excluding other etiologies [6]. The clinical manifestations may range from asymptomatic to heart failure, arrhythmias, arterial hypertension, pericarditis, thromboembolism, or myocardial ischemia [4,6,7].

Anthracyclines are among the most widely used chemotherapeutic agents for treating pediatric cancer [2,6,8–10]. However, their use is closely associated with cardiovascular effects, particularly myocardial dysfunction [6,8–11]. Classic risk factors for cardiotoxicity include age (especially below five years), cumulative dose (CD) of anthracycline ≥ 250 mg/m², underlying heart disease, Down syndrome, electrolyte disturbances, mediastinal irradiation, cardiotoxic drugs, and female sex [2,6,8,10]. The pathophysiology of anthracycline-related cardiotoxicity is multifactorial, involving mitochondrial damage, enzymatic alterations, oxidative stress resulting from the production of free radicals, and the inhibition of topoisomerase II, which reduces myocardial contractility and leads to myocyte death [2,3,6,10,12,13].

Echocardiography is used for non-invasive monitoring of myocardial function during and after cancer treatment. The biplane Simpson's method for measuring left ventricular ejection fraction (LVEF) is widely recommended for assessing systolic function [6,7,11,14]. In addition, left ventricular global longitudinal strain (LVGLS) has emerged as a sensitive indicator for the early detection of subclinical myocardial dysfunction [2,11,12,14–19]. This study aimed to evaluate the early detection of cardiotoxicity using echocardiography in children and adolescents with cancer treated with anthracyclines.

Methods

This cross-sectional study was conducted using secondary data retrieved from the medical records of patients treated at the pediatric cardio-oncology outpatient clinic of Hospital Universitário Oswaldo Cruz (HUOC) located in Recife

(Pernambuco, Brazil) between January 2018 and December 2022. The study included under 19-year-old cancer patients treated with anthracyclines who had preserved LVEF (assessed using the biplane Simpson's method) and at least one previous echocardiogram showing abnormal LVGLS [14]. Exclusion criteria were applied and patients who did not undergo a cardiological evaluation during treatment; presented with previous left ventricular dysfunction (LVD) such as an LVEF < 55 % [14,20]; did not start chemotherapy at the institution; had a single echocardiogram; or had no LVGLS during follow-up were excluded. Only patients with at least one abnormal baseline LVGLS were included, since the study specifically aimed to monitor the evolution and early detection of subclinical myocardial dysfunction among those with initial subclinical impairment.

The variables studied included age at the onset of cancer treatment, sex, type of cancer, risk factors for cardiotoxicity, treatment initiation and completion, anthracycline CD, and patient status at the time of data collection. Left ventricular systolic function was analyzed using LVEF and LVGLS. According to current guidelines in pediatric cardio-oncology, the values considered abnormal were LVEF < 55.0 % and the absolute value of LVGLS < -18 % [14,21–24]. A LVGLS between -16 % and -17 % was considered borderline, and LVD was described by levels higher than -16 % [14].

Cardiotoxicity was diagnosed echocardiographically as an asymptomatic reduction in LVEF of ≥ 10.0 % compared with baseline, or a reduction below normal limits (< 55.0 %), or LVGLS with a reduction of ≥ 15.0 % compared with baseline [6–8]. Echocardiograms were performed by the same operator using the same equipment (CX 50 Philips® SLGVE software aCMQ 10.5.1 and Affinity 50 Philips® software aCMQ 11.0). All patients were hemodynamically stable during the echocardiogram.

Participants were divided into two groups. The overall cohort consisted of 45 eligible patients with available longitudinal data on LVEF (at baseline and at the time of dysfunction) and reduced LVGLS values. A subgroup of 19 patients was classified based on evidence of cardiotoxicity [6–8].

A descriptive analysis of the data was performed. Absolute and relative frequencies described categorical variables, while numerical variables were expressed as mean and standard deviation or median and interquartile range. The study was approved by the research ethics committee of the Instituto de Medicina Integral Prof. Fernando Figueira under the opinions 66126722.8.0000.5201 and 66126722.8.3001.5192 of HUOC. The Strobe checklist for observational studies was applied [25].

Results

A total of 141 children and adolescents were followed up at the pediatric cardio-oncology outpatient clinic of HUOC between 2018 and 2022 (Figure 1). Of these, 45 out of 141

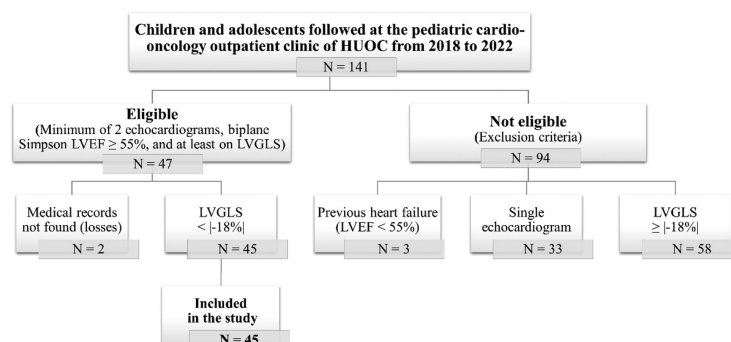


Fig. 1 – Flowchart of the recruitment of study participants.

HUOC: Hospital Universitário Oswaldo Cruz; LVEF: left ventricular ejection fraction; LVGLS: left ventricular global longitudinal strain.

patients (31.9 %) were included in this study, and 19 out of 45 patients (42.2 %) presented cardiotoxicity.

Tables 1 and 2 present the clinical variables of the full sample ($n = 45$) and the subgroup ($n = 19$), respectively. The mean age at cancer diagnosis was 10.3 ± 4.5 years, ranging from 1 to 17 years. Acute lymphoblastic leukemia was the most prevalent type of cancer among eligible patients. Of the full sample, 75.5 % of patients had hematologic cancers, comprising 55.5 % with leukemias and 20.0 % with lymphomas; the prevalence of hematologic cancers in the subgroup was 68.4 %.

Regarding the timing of cardiotoxicity diagnosis, 75.6 % of the full sample were undergoing anthracycline treatment (Table 1). Similarly, 14 patients (73.7 %) from the subgroup developed cardiotoxicity during chemotherapy, while five (26.3 %) were not undergoing therapy (Table 2). Secondary causes for LVD were excluded in all patients undergoing cancer treatment.

In the subgroup, 52.6 % of the patients were female, 15.8 % were under 5 years of age, 5.3 % had Down syndrome, and the anthracycline CD was $>250 \text{ mg/m}^2$ in 57.9 % of the cases (61.5 % had leukemia and lymphoma). No patient underwent mediastinal radiotherapy, and 57.9 % were asymptomatic for cardiovascular conditions.

A total of 10 out of 45 patients showed a reduction of $\geq 10\%$ in LVEF compared with baseline, and 35 did not have a significant change in LVEF. Of these, 31 patients (68.9 %) had borderline LVGLS, and 14 (31.1 %) met the criteria for LVD (Table 1). In the subgroup, the analysis of cardiotoxicity was progressive and confirmed by the echocardiographic criteria (Table 2). Baseline LVEF values ranged from 56.7 to 80.0 % in this group, and the post-treatment levels from 28.0 to 72.0 %. Meanwhile, the pre-treatment LVGLS values ranged from -18.0 to -23.7% , and the post-treatment values ranged from -11.0 to -17.4% . The mean time between baseline echocardiographic evaluation and the onset of cardiotoxicity was 17.3 months. Table 3 summarizes the echocardiographic findings of the subgroup. An isolated reduction in LVEF was observed in five patients (26.3 %), an isolated LVGLS reduction in nine patients (47.4 %), and a concurrent reduction in both parameters in five patients (26.3 %). During the follow-up period, 17.8 % of

patients died. Causes of death included disease progression (11.1 %), sepsis (4.4 %), and heart failure (2.2 %).

Discussion

Anthracyclines are essential chemotherapeutic agents with relevant efficacy in treating cancer during childhood. However, their use offers limitations due to substantial cardiovascular complications. The risk of cardiovascular mortality in survivors is about eightfold higher than in the general population [8].

This study describes the clinical characteristics and echocardiographic findings of 45 children and adolescents with cancer treated with anthracyclines presenting normal LVEF and reduced LVGLS at diagnosis. Nineteen patients (42.2 %) developed a reduction of $\geq 10.0\%$ in LVEF or $\geq 15.0\%$ in LVGLS compared with baseline and consequent cardiotoxicity diagnosis.

Among patients who developed cardiotoxicity, classical risk factors described in pediatric cardio-oncology, including female sex, age under five years, and Down syndrome, were proportionally more prevalent, consistent with previous studies [6,8,10].

Among the subgroup of 19 patients with cardiotoxicity, 57.9 % received an anthracycline CD $>250 \text{ mg/m}^2$. The average time between baseline and abnormal echocardiograms was 17.3 months, and the mortality rate was 31.6 %. Studies in Colombia, France, and Germany also associated high doses of anthracyclines with increased risk of cardiotoxicity [3,24,26]. However, ventricular dysfunction has also been observed with lower doses [2]. In the present study, 42.2 % of patients presented cardiotoxicity, regardless of the time between exams and despite the CD being lower than 250 mg/m^2 , which reinforces the notion that anthracyclines do not have a safe dose.

The high prevalence of hematological cancers in the present study (34 out of 45 patients - 75.6 %), with a predominance of acute lymphoblastic leukemia, is consistent with Brazilian epidemiology [1]. In the subgroup, 68.4 % had leukemia and lymphoma, and 61.5 % (8 out of 13 patients) were exposed to

Table 1 – Clinical and echocardiographic description of the 45 patients with evolutionary analysis of LVEF and primary reduction in LVGLS.

Patient (No.)	Sex	Age (years)	Type of cancer	Baseline LVEF / TT* (%)	LVGLS in TT* (%)	Δ DX CTX (months)	Status
1	F	8	Osteosarcoma	80 / 28	–13	101	OT
2	M	10	ALL	71.3 / 62	–17.4	7.5	In treatment
3	F	6	Wilms' tumor	59 / 56	–17	4	In treatment
4	M	14	Ewing's sarcoma	66 / 51	–16	10	In treatment
5	M	14	ALL	56.7 / 58	–15	14.5	In treatment
6	F	10	ALL	58 / 51	–14.8	5	Death (sepsis)
7	F	13	AML	59 / 60	–15	6	In treatment
8	M	9	ALL	65 / 53	–16.8	8.5	OT
9	F	17	Sarcoma	68 / 72	–17	3	In treatment
10	F	11	NHL	62 / 65	–16.6	2.5	In treatment
11	M	16	NHL	62.2 / 55	–17	5	Death (sepsis)
12	F	7	ALL	60 / 49.3	–12.5	3	Death (disease progression)
13	M	17	LH	60 / 50	–15	3	In treatment
14	F	4	ALL	65.6 / 58	–17.3	49	OT
15	F	14	ALL	65 / 60	–16.3	5	In treatment
16	M	3	Retinoblastoma	71 / 40	–11	63	Death (disease progression)
17	F	1	ALL	67 / 52	–14	11	Death (disease progression)
18	M	9	ALL	63 / 55	–16.5	23	OT
19	M	12	Ewing's sarcoma	61 / 50	–15.4	5.5	Death (HF)
20	M	14	Ewing's sarcoma	62 / 57	–17.2	16	Death (disease progression)
21	M	13	Osteosarcoma	65 / 60	–15	3.5	Death (disease progression)
22	M	15	ALL	60 / 55	–16	1	In treatment
23	M	15	ALL	65 / 60	–17.4	3	In treatment
24	M	8	HL	60 / 58	–14	2	In treatment
25	M	16	ALL	60 / 58	–17	21.5	In treatment
26	M	6	HL	61.4 / 57	–15.6	0.3	In treatment
27	M	1	Retinoblastoma	65.3 / 62	–17	21.5	OT
28	F	17	HL	59 / 61	–16.5	7	In treatment
29	M	11	ALL	66 / 60	–16.7	11	In treatment
30	F	15	ALL	64.2 / 63	–17	1	In treatment
31	M	16	ALL	65 / 70	–17	10	In treatment
32	M	14	ALL	55 / 55	–16	19	In treatment
33	M	14	ALL	60.3 / 55	–16	32	In treatment
34	F	14	HL	61 / 61	–16	11.5	OT
35	M	4	ALL	65 / 61	–17	49.5	OT
36	M	12	Osteosarcoma	58 / 68	–17.2	4.5	In treatment
37	F	8	ALL	57.5 / 64.8	–17.6	0.25	In treatment
38	F	7	ALL	68 / 62	–17	18	OT
39	F	18	ALL	60 / 55	–14.8	0.5	In treatment
40	F	7	ALL	65 / 61	–17.8	51	OT
41	F	10	HL	58 / 63.2	–17.8	8	OT
42	M	16	Sarcoma	60 / 55	–12.3	4	In treatment
43	M	6	ALL	62.4 / 62.4	–16.2	3	In treatment
44	F	10	ALL	60.5 / 55	–16.2	1	In treatment
45	F	17	HL	66 / 67	–17	5	In treatment

F: Female; M: Male; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; HL: Hodgkin's lymphoma; NHL: non-Hodgkin lymphoma; LVEF: left ventricular ejection fraction; LVGLS: left ventricular global longitudinal strain; Δ: time differential; DX: diagnosis; CTX: cardiotoxicity; OT: off therapy; HF: heart failure. *TT: time of acquisition of LVEF and LVGLS during or after treatment. Status: clinical situation of the patient at the time of data acquisition and death (cause of death).

anthracycline CD >250 mg/m². These findings underscore the relevance of enhanced cardiologic monitoring in pediatric patients with hematologic cancers due to the increased risk of anthracycline-induced cardiotoxicity and the consequent decreased quality of life of survivors [24,26].

Most cardio-oncology studies with children involve cancer survivors [10]. However, early detection of subclinical cardiotoxicity and intervention is crucial to prevent the progression of myocardial injury [2,10,16]. Linares et al. prospectively evaluated 112 patients with acute leukemia aged between 1 and

Table 3 – General echocardiographic evolution of the 19 patients with cardiotoxicity.

Echocardiographic variables	n (%)
LVEF	5 (26.3)
LVGLS	9 (47.4)
Both	5 (26.3)
Baseline values - median (range)	
LVEF	65 (56.7 to 80)
LVGLS	–20.2 (–18 to –23.7)
Altered values - median (range)	
LVEF	55 (28 to 72)
LVGLS	–15.4 (–11 to –17)

LVEF: left ventricular ejection fraction; LVGLS: left ventricular global longitudinal strain.

18 years [26]. The prevalence of early cardiotoxicity (first week to one year after the end of cancer treatment), identified by echocardiographic values of LVEF and LVGLS, was 17.9 %.

Early diagnosis plays a critical role in the management of cardiotoxicity. In this study, most patients were undergoing chemotherapy (75.6 %). Among the 19 patients with relevant heart failure, 73.7 % were diagnosed with cardiotoxicity during treatment, but 57.9 % of this subgroup was asymptomatic, and 47.4 % presented preserved LVEF with isolated reductions in LVGLS (Table 1), underscoring the role of early diagnosis in subclinical injury.

Wolf et al. retrospectively evaluated 79 pediatric patients with cancer who were treated with anthracyclines for ten years. Cases of acute leukemia and Hodgkin's lymphoma were the most prevalent among the sample, and 28 % had preserved LVEF but abnormal LVGLS (absolute value < -20.0 %).

The retrospective study by Rique et al. evaluated 38 children with acute leukemia treated with anthracyclines and detected alterations in LVGLS in 28.9 % of the cases, using the cutoff value of -20.0 % proposed by Levy et al. [22,24]. These studies emphasized the clinical relevance of early detection of cardiotoxicity, as the inclusion of the assessment in LVGLS in clinical practice was recommended [24]. However, the present study utilized the updated cutoff established by Mertens et al. in 2023 for pediatric cardio-oncology, defining the LVGLS criterion for LVD as an absolute value ≤ -16.0 % (borderline LVGLS) [14].

Similarly, Gunsaulus et al. reported that an abnormal LVGLS during anthracycline treatment predicts future cardiotoxicity in children, confirming the prognostic importance of early strain alterations [27].

In cases where the baseline LVGLS was unavailable, Almeida et al. suggested that absolute values lower than -17.0 %, excluding other cardiac diseases, may indicate subclinical cardiotoxicity [11]. In this study, patients with an isolated reduction of LVEF without baseline LVGLS had post-treatment values of -11.0 %, -14.0 %, and -14.8 % (Table 2). Therefore, baseline assessment also plays a critical role in the early diagnosis of cardiotoxicity.

Current Brazilian, European, and North American cardio-oncology and cardiovascular imaging guidelines recommend echocardiography as the first-line modality for cardiovascular monitoring during cancer treatment [2,6,7,14,28]. In this

study, most patients with abnormal LVGLS remained asymptomatic. Even in cases with preserved LVEF, reduced LVGLS values demonstrated the ability to detect subclinical myocardial dysfunction. The inclusion of patients with abnormal baseline LVGLS focused the analysis on early progression of subclinical myocardial injury, in accordance with the sensitivity of LVGLS for detecting anthracycline-related dysfunction even in the presence of normal LVEF [14,27,28].

This study had some limitations. First, it was conducted at a single center, which may affect the external validity of the findings. However, HUOC is a university referral center for pediatric oncology in Northeast Brazil, exclusively serving the Brazilian public healthcare system. Second, the retrospective design restricted access to some clinical data (e.g., socioeconomic data, comorbidities, anthracycline CD in all patients, and use of other chemotherapy drugs and dexrazoxane). Although dexrazoxane is part of the institutional protocol, no documentation was found in the medical records consulted. Additionally, the diastolic function was not evaluated. Last, the inclusion criterion of abnormal LVGLS likely contributed to an increased prevalence of cardiotoxicity, given the focus on the sensitivity of LVGLS even with preserved LVEF.

Although the retrospective design and small sample size limited generalizability, the internal consistency of the findings was reinforced by the fact that all echocardiograms were performed by a single operator using standardized equipment.

The findings of this study underscore the relevance of baseline echocardiographic assessment of LVEF and LVGLS for the early diagnosis of subclinical cardiotoxicity in pediatric patients with cancer treated with anthracyclines, allowing for timely cardiologic intervention and potentially improving their quality of life [28–30].

Conclusion

Anthracycline-induced cardiotoxicity is a relevant adverse effect during cancer treatment, especially in patients with leukemia and lymphoma. Early detection of cardiotoxicity in children and adolescents treated with anthracyclines is crucial to ensure the safety and efficacy of cancer treatment. Incorporating LVGLS into routine clinical practice in pediatric oncology should be encouraged, given its high sensitivity in detecting subclinical myocardial injury and its well-established prognostic value. Furthermore, baseline assessment of LVEF and LVGLS is essential for accurate diagnosis, effective monitoring, and comprehensive evaluation of cardiac function before, during, and after cancer treatment.

Author's contributions

Martins JL: Conceptualization, Formal analysis, Investigation, Writing – review and editing. Feitosa FGAM and dos Santos MVC: Conceptualization, Investigation, Writing – review and editing. Menezes TMGAL and Sena AD: Conceptualization, Formal analysis, Writing – original draft. Rodrigues EPL: Data acquisition, Investigation, Writing – review and editing. Lopes MBA and Santos JL: Data acquisition, Investigation.

Duarte MCMB: Conceptualization, Formal analysis, Investigation, Writing – review and editing. All authors approved the final version and declared no conflict of interest.

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Data availability

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

Conflicts of interest

The authors declare no conflict of interest to report.

Editor

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Original article

The impact of socioeconomic status on clinical presentation of multiple myeloma



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ABSTRACT

Introduction: The influence of socioeconomic status on cancer diagnosis, treatment, and outcomes has been studied for several decades. In multiple myeloma, many authors are investigating the impact of poverty and social inequalities, measured by indicators such as place of residence, number of residents, occupation, income and education, the incidence, stage and management of the disease, and survival, with controversial results. The aim of this study was to ambispectively analyze the association between socioeconomic status and clinical characteristics of multiple myeloma at presentation.

Methods: A total of 296 patients, diagnosed between 2015 and 2023 in three institutions in Rio de Janeiro, Brazil, were included. To assess the socioeconomic status, a social class questionnaire was administered to patients (or relatives, in the cases of death); information about educational level was collected from this interview and medical records and household income per capita were estimated, according to place of residence.

Results: Lower socioeconomic status was associated with delayed diagnosis, symptoms at presentation, advanced stage, poorer performance status, lower hemoglobin and higher calcium values.

Conclusion: These findings suggest a possible relationship between socioeconomic aspects and severity of multiple myeloma presentation in Brazil, and underscore the importance of shaping health policies to promote greater equity in cancer diagnosis and treatment access.

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Introduction

Multiple myeloma (MM) is the second most common hematological malignancy [1]. Its diagnostic criteria were updated by

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the International Myeloma Working Group (IMWG) and consist of medullary plasmacytosis $\geq 10\%$ or plasmacytoma, associated with an end-organ damage (hypercalcemia, renal dysfunction, anemia and/or bone lesion) or, plasmacytosis $\geq 60\%$, ratio of free light chains ≥ 100 or presence of more than one focal lesion on magnetic resonance imaging (MRI) [2]. Treatment outcomes for MM have evolved significantly in recent decades due to advancements in knowledge of the pathophysiology, earlier detection, and new therapeutic approaches [3–5]. Novel drugs, especially in the field of immunotherapy, have increased survival rates and improved patients' quality of life, even in advanced stages [5,6].

However, as in other neoplasms, the effectiveness of new diagnostic and treatment methods does not benefit all MM patients equally [4]. Demographic, social, and economic aspects may influence everything from disease prevention to life expectancy, as they affect exposure to risk factors, the presence and control of comorbidities, access to healthcare, quality of treatment, adherence, and even social and network support [7,8]. In general, it is reported in cancer literature that people with a low socioeconomic status (SES) have more advanced disease at diagnosis, worse general health, [9–12] less chance of being treated with modern and more efficient drugs and worst outcomes [13–16]. In MM, the influence of many of these aspects on diagnoses and outcomes has been studied in the last decades, but the conclusions are somewhat controversial [17]. Additionally, most of the studies present some limitations, mainly in the heterogeneity of SES variables, the therapeutic approaches used and outcomes, which can confound analysis [18–20].

SES can be measured in different ways, based on community or individual characteristics and data from population-based registries or hospital records; the measurement also varies according to the country/region and specific cultural aspects of the population. Common variables used as SES indicators in this kind of research include employment, family or individual income, profession/occupation, level of education, social class, place of residence, ownership of consumer goods, number of people per room/residence, water and sewage networks among others.

Many authors have also been trying to analyze the influence of race/ethnicity on the incidence of MM and outcomes, but it is difficult to know if the observed differences between racial groups are not attributable, at least partially, to socioeconomic inequalities [17,21,22]. In Brazil, assessing the impact of ethnicity is complex, mainly due to an intense population miscegenation and the known prevalence of lower SES among non-white individuals [16,23].

An advanced stage at cancer diagnosis is associated with poorer outcomes, and some studies have shown that this clinical presentation is more severe in individuals with lower SES [13,14]. Forty years ago, when assessing the impact of poverty on the presentation and treatment responses of patients with MM, Savage et al. found that individuals with lower income had more advanced disease at diagnosis and lower overall survival [17]. Since then, many studies that assessed SES aspects in MM populations and confirmed this kind of association, [24,25] but some showed different results [18,26].

The level of education, a commonly used SES indicator, impacts the treatment outcome of MM. In a Chinese study

published in 2020 that enrolled 773 MM patients, the authors showed that individuals with less education (secondary school or lower) had a higher likelihood of being elderly, unemployed, rural residents, with a low income and less access to healthcare. This group had more advanced cases (International Staging System [ISS]: III), higher lactate dehydrogenase levels and less frequently received bone marrow transplantation. Furthermore, they had inferior progression-free survival (30.6 versus 58.8 months, respectively) and overall survival (67.5 versus 122.27 months, respectively), when compared with patients with higher educational levels [27].

Therefore, the analysis of the socioeconomic impact on cancer outcomes is of great importance for patients with MM, particularly in countries with significant social inequality, as these aspects may be modifiable risk factors that can affect treatment responses [3,28].

Objective

This study aimed to evaluate the relationship between SES and clinical characteristics at MM diagnosis of patients treated over the past decade in public and private institutions in Rio de Janeiro, Brazil.

Methods

Clinical and demographic data were collected retrospectively (January 15- February 21) and prospectively (March 21-February 23) from medical records of all patients diagnosed with MM treated in two public hospitals (one university and one military) and a private institution in Rio de Janeiro, Brazil.

These patients were identified by the diagnosis of MM (ICD10 C90.0) recorded in the patient records and the myelogram registry books of the public hospitals, and by database searches in the computerized system of the private institution.

For each patient, a standardized clinical form was completed containing information on sex, age, date of symptom onset (if available), date of bone marrow (BM) aspiration, performance status (PS), Durie-Salmon (DS) stage, ISS, medullary plasmacytosis, radiologic findings, and laboratory results. Time to diagnosis was defined as the period between symptom onset to the date of diagnostic BM aspiration.

The presence or absence of the following comorbidities at diagnosis was recorded as specified in the clinical records: systemic arterial hypertension, diabetes mellitus, chronic kidney disease, other neoplasms, heart disease, autoimmune disease, psychiatric disorder, thyroid disease, dyslipidemia, chronic obstructive pulmonary disease/asthma, among other conditions. The study was approved by the Research Ethics Committees in March 2021, and all patients or their legal representatives provided written informed consent.

SES was evaluated by three different measures: social class, educational level and household income per capita. To assess social class, the Brazilian Economic Classification Criteria (BECC) was administered to patients, or to a family member, in the case of death [29]. This questionnaire, developed and periodically updated by the Brazilian Association of

Research Companies, is used by the Brazilian Institute of Public Opinion and Statistics (IBOPE) in population socioeconomic surveys. The version of the BECC used in this study includes questions about the number of consumer goods (car, washing machine, DVD player, refrigerator, freezer, computer, dishwasher, microwave, motorcycle, and clothes dryer), and the number of domestic employees. It also contains information regarding details of the residence: the number of bathrooms, water supply (distribution network, water well/spring or other means), street characteristics (asphalt or dirt) and educational level of the head of the family (4 years of study or less, 5–8 years, 9 years - full elementary school, complete high/secondary school or complete university). Based on the resulting score (the sum of points obtained in each item), patients were categorized into six social classes: A (45–100 points), B1 (38–44), B2 (29–37), C1 (23–28), C2 (17–22) and DE (0–16). The information about the patient's educational level (when they were not the head of the family) was collected during an interview or from medical records, following the same categories used by the BECC questionnaire. Finally, household income per capita was estimated based on the neighborhood in which patients lived (in Rio de Janeiro city or other cities) using a computer tool designed in 2010 and available for the Brazilian territory. A current value was calculated based on 2010 and 2023 Brazilian minimum wages converted to US dollars [30,31].

To analyze the associations between SES and clinical presentation of MM, patients were categorized as from high (AB) or low (CDE) social classes, high or low educational level (≤ 9 versus >9 years of study), and with high or low income (according to the median value found). In addition to the main analyses including all patients in the study, the associations between SES and clinical presentation of MM were also specifically evaluated in the subgroup of patients prospectively enrolled.

Statistical analysis

Descriptive statistics were applied to summarize the data. Associations between social class, educational level, income, and clinical or demographic characteristics at MM diagnosis were assessed using Fisher's exact test (two-tailed) and the Mann-Whitney U test, for categorical and continuous variables, respectively. All statistical analyses were performed using the Statistical Package for Social Sciences software (version 21.0).

Results

A total of 296 patients with MM were enrolled in this study (206 retrospectively and 90 prospectively); their main characteristics are shown in Table 1. Of all the patients, 91 % ($n = 270$) had evidence of at least one target organ lesion. Specifically, 60 % ($n = 160$) had anemia, 30 % ($n = 75$) renal dysfunction, 25 % ($n = 54$) hypercalcemia, and 77 % ($n = 190$) osteolytic bone lesions, as determined by radiography, computed tomography (CT), or positron emission tomography (PET) findings. The diagnosis of MM based on new IMWG criteria,

Table 1 – Sociodemographic and clinical characteristics at multiple myeloma diagnosis.

Characteristic	Total
Number of patients - n	296
Institution - n (%)	
Private	135 (46)
University	111 (37)
Military	50 (17)
Age (median; range)	65.6 (33–93.2)
Female sex - n (%)	150 (51)
Ethnic group - n (%)	
White	81 (46)
Non-white	95 (54)
Marital status - n (%)	
Married	156 (64)
Not married	87 (36)
Performance status ≥ 2	168 (57)
Comorbidities - n (%)	222 (75)
Systemic arterial hypertension	158 (53)
Diabetes mellitus	74 (25)
Heart disease	38 (13)
Thyroidopathy	34 (12)
Other cancer	31 (11)
Classification - n (%)	172 (64)
IgG	57 (21)
IgA	35 (13)
Light chain	6 (2)
Non secretory	
Durie Salmon Staging - n (%)	
I-II	41 (15)
III	230 (85)
International Staging System - n (%)	
I-II	176 (76)
III	56 (24)
Plasmacytoma - n (%)	86 (29)
Symptoms at diagnosis - n (%)	242 (82)
Time to diagnosis in months - $n = 189$	4.7
(median; range)	(0.6–19.1)
Hemoglobin (g/dL) ($n = 265$)	9.4
(median; range)	(3.9–16.0)
Creatinine ($n = 249$)	1.1
(median; range)	(0.5–10.8)
Calcium ($n = 190$) (mg/dL)	9.9
(median; range)	(7.0–17.6)
Albumin - n (%)	
<3.5 g/dL	94 (45)
≥ 3.5 g/dL	115 (55)
$\beta 2$ -microglobulin - n (%)	
<3.5 g/dL	123 (63)
≥ 3.5 g/dL	73 (37)
Monoclonal protein ($n = 228$)	2.7
(median; range)	(0–10)
Medullary plasmacytosis - n (%)	
<10 %	15 (7)
≥ 10 %	207 (93)
Place of residence - n (%)	
Rio de Janeiro	183 (65)
Another city	98 (35)
Status - n (%)	
Alive	157 (53)
Death	139 (47)
Social class ($n = 231$) - n (%)	
AB	111 (48)
CDE	120 (52)
Education level - n (%)	
≥ 9 years ($n = 239$)	142 (59)
<9 years	97 (41)
Per capita income* ($n = 280$) - n (%)	
$>US\$ 397$	137 (49)
$\leq US\$ 397$	143 (51)

Table 2 – Number of patients (alive/deceased) at the time of socioeconomic data collection and availability of information on social class, educational level, and per capita income.

Characteristic	Total (n = 296)	University Hospital (n = 111)	Military Hospital (n = 50)	Private Institution (n = 135)
Patients alive at the time of socioeconomic status data collection	157	50	28	79
BECC* respondents (social class)	133 (85 %)	48	27	58
Education level information	134 (85 %)	49	26	59
Per capita income assessment	154 (98 %)	48	27	79
Total with SES** information (alive)	156	49	28	79
Patients deceased at the time of socioeconomic status data collection	139	61	22	56
Family members responding to the BECC*	98 (71 %)	44	19	35
Education level information	105 (76 %)	49	19	37
Per capita income assessment	126 (91 %)	56	16	54
Total with SES** information (deceased)	135	58	21	56
CCEB Respondents (Social Class)	231 (78 %)	92	46	93
Education Level Information	239 (81 %)	98	45	96
Per Capita Income Assessment	280 (95 %)	104	43	133
Cases with Socioeconomic Information	291	107	49	135

BECC: Brazilian Economic Classification Criteria; SES: Socioeconomic status.

such as medullary plasmacytosis ≥ 60 % or focal lesions on magnetic resonance image (MRI), was made in only 9 % (n = 26) of the cases. Comorbidities were present in 75 % of patients and, across all three centers, most individuals had at least one concomitant condition. Systemic arterial hypertension was the most prevalent (53 %), followed by diabetes mellitus (25 %), cardiovascular disease (13 %), thyroid disorders (12 %), and a history of another malignancy (11 %).

The BECC questionnaire, which collected information on social class, was successfully completed by 78 % of patients or their relatives. Information on educational level was obtained for 81 % of participants, and estimated household income per capita for 95 %. Table 2 summarizes the numbers of patients alive or deceased at the time of socioeconomic data collection and, within each group, the proportion for whom social class, education, or income data were available in each institution.

In cases in which at least two of these measures were available, it was observed that individuals from higher social classes had more years of education (p-value <0.001) and higher income (p-value <0.002), and also, those with higher educational level had higher income (p-value <0.001). A total

of 291 cases had at least one SES measure and were included in the association analyses.

Patients from higher social classes had a higher frequency of comorbidities (p-value = 0.048), compared to those from lower classes. However, patients from lower social classes had a longer time from first symptom to the date of bone marrow evaluation (p-value = 0.025), a higher proportion of DS stage III (p-value = 0.002) and hemoglobin values <8.5 g/dL (p-value = 0.03), as well as higher calcium values (p-value = 0.019), when compared to patients from higher social classes (Table 3).

Of the individuals who studied up to nine years, there was a higher proportion of females (p-value = 0.004), patients with PS ≥ 2 (p-value = 0.025) and delayed diagnosis (p-value = 0.011) than those with more than nine years of study (Table 4).

Individuals with higher estimated household incomes per capita were older than those with lower income (p-value = 0.009). Additionally, a higher proportion of patients with lower income presented with PS ≥ 2 (p-value = 0.011) and with symptoms at diagnosis (p-value = 0.034). Among these individuals, the median hemoglobin value was lower (p-value = 0.044), when compared to those with higher income

Table 3 – Associations between social classes and clinical presentation of multiple myeloma.

Characteristic	Total	Social classes AB	Social classes CDE	p-value
Comorbidities - n (%)	173 (75)	90 (81)	83 (69)	0.048
Durie Salmon - n (%)				
I-II	28 (13)	21 (21)	7 (6)	0.002
III	182 (87)	78 (79)	104 (94)	
Time to diagnosis in months (median; range)	n = 151 4.8 (0.6–19.1)	4.0 (0.8–16.0)	5.9 (0.6–19.1)	0.025
Hemoglobin (g/dL) - n (%)				
<8.5 g/dL	77 (37)	27 (29)	50 (44)	0.030
≥ 8.5 g/dL	130 (63)	63 (56)	67 (71)	
Calcium (mg/dL) (median; range)	10.0 (7.0–17.6)	9.6 (7.0–16.6)	10.2 (7.5–17.6)	0.019

Table 4 – Associations between level of education and clinical presentation of multiple myeloma.

Characteristic	Total	Educational level >9 years	Educational level ≤9 years	p-value
Sex - n (%)				
Male	126 (53)	86 (61)	40 (41)	0.004
Female	113 (47)	56 (39)	57 (59)	
Performance Status - n (%)				
0–1	105 (44)	71 (50)	34 (25)	0.025
≥2	134 (56)	71 (50)	63 (65)	
Time to diagnosis in months (median; range)	n = 157 4.8 (0.6–19.1)	4.2 (0.6–16.0)	6.2 (0.8–19.1)	0.011

Table 5 – Associations between income and clinical presentation of multiple myeloma.

Characteristic	Per capita income			p-value
	Total	Per capita income >US\$397	Per capita income ≤US\$397	
Age (median; range)	65.5 (33.0–93.2)	67.0 (33.0–93.0)	62.0 (35.8–93.2)	0.009
Performance Status - n (%)				
0–1	125 (45)	72 (53)	53 (37)	0.011
≥2	155 (55)	65 (47)	90 (63)	
Symptoms at diagnosis - n (%)	227 (81)	104 (76)	123 (86)	0.034
Hemoglobin (g/dL) (median; range)	9.3 (3.9–16.0)	9.8 (4.2–16.0)	9.1 (3.9–15.0)	0.044

(Table 5). No other statistically significant associations were found between SES measures and characteristics at diagnosis.

In the separate analysis of the 90 patients prospectively included from March 2021 onward, similar associations were observed between SES and characteristics of clinical presentation of MM, including age at diagnosis, presence of comorbidities, time to diagnosis, and serum calcium levels. Additionally, this analysis revealed that lower social class was associated with a higher frequency of plasmacytoma, while lower income was associated with higher calcium levels and lower serum albumin concentrations (Table 6).

Discussion

The role of specific socioeconomic factors on the clinical presentation of MM remains understudied. This research aimed to address this gap by examining the association between

social class, educational level, household income per capita, and individual characteristics at diagnosis of 291 patients followed in three onco-hematological centers in Rio de Janeiro, Brazil.

A lower prevalence of concomitant diseases, observed among patients from lower social classes, may indicate that comorbidities are underdiagnosed in these individuals, mainly due to lack of awareness of their early symptoms and to difficulty in accessing health services. Probably, patients from lower social classes seek health services more for therapeutic rather than preventive treatments. In an analysis of the sociodemographic profile of Brazilian health service users, some authors described that 58 % of public services visits were aimed at disease management, while just 29 % focused on preventive measures. On the other hand, in private services, these proportions were, respectively, 44 % and 34 % [32].

In the present study, MM patients from lower social classes were more frequently in advanced DS stage, with lower

Table 6 – Associations between SES (social classes and income) and clinical presentation of the group of multiple myeloma patients enrolled prospectively.

Characteristic	Total n = 80	Social classes AB n = 40	Social classes CDE n = 40	p-value
Age (median, variation)	64.3 (33.7–89.7)	67.3 (40.3–87.0)	59.9 (33.7–89.7)	0.031
Comorbidities	54 (68 %)	32 (80 %)	22 (55 %)	0.031
Plasmacytoma	26 (33 %)	8 (20 %)	18 (45 %)	0.031
Time to diagnosis in months (median, variation)	4.8 (0.6–19.1)	3.8 (0.9–8.1)	6.8 (0.6–19.1)	0.010
Characteristic	Total n = 88	Per capita income >US\$397 n = 45	Per capita income ≤US\$397 n = 43	p-value
Hypercalcemia	24 (34 %)	5 (15 %)	19 (53 %)	0.001
Calcium (median, variation)	10.4 (8.4–17.4)	10.0 (8.4–15.8)	11.1 (9.4–17.4)	0.005
Albumin (median, variation)	3.5 (1.8–4.8)	3.7 (2.8–4.8)	3.3 (1.8–4.6)	0.034

hemoglobin and higher calcium levels, and it took longer for them to be diagnosed, a situation that also happened with those with a lower educational level. Although there are few organized national data on the prevalence, profile and outcomes of hematological cancer patients, it has been observed that severity at diagnosis is higher than that described in developed nations and, within Brazil, it is even worse among poorer compared to richer patients. In recent publications from the Brazilian Hodgkin's Lymphoma Registry, authors described a higher prevalence of advanced stages and a longer time from the first symptom to diagnosis among patients from less favored social classes; in these studies, researchers also used the BECC questionnaire [8,33]. Similar findings are also described in non-Hodgkin's lymphoma [34] and leukemia populations [35,36].

The association observed between lower educational level and female sex probably reflects this kind of gender inequality in the older Brazilian population. According to national data from 2022, schooling rates in the country for men and women aged between 18 and 24 were 28.1 % and 32.6 %, respectively. However, when age groups similar to the sample studied here were evaluated, slightly higher illiteracy rates are observed among women (16.3 % versus 15.7 %) [37]. Furthermore, patients with less than nine years of education, as those with lower income, presented with a poorer PS (≥ 2), reflecting more limitations in work and self-care daily activities and restriction to bed for a greater percentage of time during the day when compared to those with higher educational levels and incomes.

The association between lower income and lower median age differs from Brazilian Family Budget Surveys [38]. Data from 2017/18 showed that people aged 65 or older had lower household incomes per capita than those between 25 and 64 years old. Lower income was also associated with symptoms at diagnosis and with lower median hemoglobin levels, likely reflecting the greater clinical severity of this unfavored group.

Acknowledging the limitations inherent to retrospective analyses, a specific evaluation was performed on the subset of prospectively included cases. The findings of this analysis were consistent with the overall cohort, suggesting an association between lower SES and a more severe clinical presentation of MM. Even so, further investigations using rigorous prospective designs are warranted to validate and clarify this relationship.

The results of this study constitute a meaningful advance in the Brazilian context, revealing potential associations between socioeconomic factors and critical clinical variables, including time to diagnosis, DS stage, PS, hemoglobin and calcium levels, and the presence of symptoms at diagnosis. These findings align with the perceptions of healthcare professionals caring for MM patients and are reinforced by real-world evidence from the Brazilian healthcare system.

Conclusion

The present data concerning social class, educational level, and household income per capita of MM patients and their association with the disease's clinical presentation contribute

significantly to enhancing our understanding of MM management in underdeveloped countries. This information can support discussions surrounding healthcare policies aimed at promoting equitable access to timely MM diagnosis, an essential condition for optimizing therapeutic outcomes, enhancing quality of life, and extending survival rates for affected individuals. Given that the majority of MM patients globally reside in less developed nations, tackling these concerns has the potential to notably enhance their overall health outcomes. Nonetheless, additional evaluations, preferably prospective and involving larger patient cohorts across multiple healthcare institutions, are warranted to validate these initial findings.

Data availability statement

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

Conflicts of interest

None.

Editor

Eduardo Rego

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Original article

Clinical and hematological profile of patients with philadelphia-negative myeloproliferative neoplasms: First report from the Ecuadorian registry



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ABSTRACT

Introduction: Philadelphia-negative myeloproliferative neoplasms are clonal blood disorders characterized by abnormal blood cell production. This study explores the clinical and epidemiological profiles of 111 Ecuadorian patients diagnosed with Philadelphia-negative myeloproliferative neoplasms, including polycythemia vera, essential thrombocythemia, and primary myelofibrosis, between 2014 and 2023.

Methods: Patients were treated in different institutions, with clinical data collected on disease progression, complications, and survival.

Results: Polycythemia vera was the most common subtype (45.9%), followed by essential thrombocythemia (42.3%) and primary myelofibrosis (9%). The JAK2 V617F mutation was most prevalent in essential thrombocythemia (53.2%) and polycythemia vera (41.2%). Hydroxyurea, the most widely used treatment, was prescribed to 77% of the patients. Disease progression to myelofibrosis occurred in three polycythemia vera and two essential thrombocythemia cases, meanwhile One case of primary myelofibrosis and one case of myeloproliferative neoplasm, unclassified, progressed to acute myeloid leukemia. Survival rates varied across the cohort; notably, certain patients with polycythemia vera and essential thrombocythemia achieved survival durations of up to 19 years.

Conclusion: These results reveal a relatively homogeneous epidemiological profile across the Latin American region and underscore the need for more multicenter studies to better characterize pH⁺ MPNs in Ecuador and the region, to optimize diagnostic and treatment strategies.

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Introduction

Chronic myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders characterized by bone marrow proliferation of one or more myeloid lineages, resulting in an overproduction of blood cells in the peripheral blood [1]. The most clinically relevant MPNs include chronic myeloid leukemia (Philadelphia chromosome-positive) and Philadelphia-negative chronic myeloproliferative neoplasms (pH⁻ MPNs). Three classical entities are recognized within the pH⁻ MPN category: Polycythemia vera (PV), Essential thrombocythemia (ET), and Primary myelofibrosis (PMF).

pH⁻ MPNs are defined by the presence of driver molecular alterations that affect the JAK-STAT signaling pathway and hematopoietic growth factor receptors. These mutations, which involve the JAK2, CALR, and MPL genes, are part of the current diagnostic criteria for these disorders and are typically assessed in peripheral blood and bone marrow samples [1,2].

In 2005, the somatic p.V617F mutation in the JAK2 gene was first identified in patients with MPNs. The JAK2V617F mutation is detected in 90–95 % of patients with PV, 60 % of patients with ET and 60 % of patients with PMF. The presence of the JAK2V617F mutation was incorporated by the World Health Organization (WHO) in 2008 as a major diagnostic criterion in these three entities and remains in effect, as well as in the International Consensus Classification (ICC) of 2022 [2,3].

In late 2013, mutations in the CALR gene were identified. These mutations, which consist of insertions and deletions in exon 9 (the final exon of the gene), are detected in 25–30 % of ET and PMF patients and can be found in up to 60–80 % of JAK2-negative patients [1,2].

Several mutations affecting the gene that encodes the thrombopoietin receptor, MPL, have also been described in MPNs. These mutations occur primarily in exon 10 of the gene and are reported in about 5 % of PMF and 1–3 % of ET cases [1,2].

Although studies on pH⁻ MPNs have been carried out in Europe and America, these disorders have a low global incidence, ranging from 1–5 cases per 100,000 inhabitants per year [2,3]. In Ecuador, there is a near-total absence of local data or published studies on pH⁻ MPNs, which highlights the importance of investigating their frequency, diagnosis, and treatment in the country.

This study is based on information from Ecuadorian patients diagnosed with pH⁻ MPNs from several health institutions, using data collected between 2014 and 2023. The objective is to provide a local perspective on pH⁻ MPNs in terms of frequency, clinical presentation, diagnosis and disease evolution, using appropriate statistical methods.

Methods

This is a descriptive, observational, longitudinal, and multicenter study. Data collection was carried out by hematologists

from several healthcare institutions in Ecuador between 2014 and 2023: SOLCA-Guayaquil, Quality of Care (Guayaquil), SOLCA-Quito, “José Carrasco Arteaga” Hospital (Cuenca), and Hospital “Teodoro Maldonado Carbo” (Guayaquil). The study cohort comprised 111 patients diagnosed with BCR-ABL1-negative myeloproliferative neoplasms (MPNs), specifically polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), or MPN-unclassifiable (MPN-U). Diagnoses were established according to the World Health Organization (WHO) criteria in effect at the time of each patient's initial presentation [2]. Patients who did not meet the WHO/ICC 2022 diagnostic criteria were excluded [2,3]. Data were collected using an anonymized form on a validated registry platform for this purpose (www.mpn-ecuador.com). The study was approved by the inter-institutional Ethics Committee.

Patient records included data from the date of diagnosis until death or loss to follow-up. Exclusion criteria included the absence of available bone marrow samples or missing information in the electronic registry. Histopathological analyses were performed by trained hematopathologists (FH, PS) who evaluated bone marrow biopsies when reclassification was necessary.

Epidemiological and clinical data were collected, along with information on treatments, complications, and disease progression. Statistical results are presented mainly in terms of frequencies and percentages, while quantitative variables were analyzed using univariate statistics with measures of central tendency and dispersion. A p-value of <0.05 was considered statistically significant.

Data analysis included the creation of cross-tabulation frequency tables, descriptive graphs, survival models, and Multiple Correspondence Analysis (MCA) to enable a more comprehensive exploration using multivariate visualizations. Statistical processing and analysis were conducted using R software (version 4.4.2 for Windows), available at www.r-project.org.

Analysis and results

Epidemiology

Among the 111 patients, the most frequent pH⁻ MPN subtype was PV in 51 cases (46 %), followed by ET in 46 cases (41.4 %). Eleven patients (10 %) were diagnosed with PMF, of which one was in the prefibrotic stage (PMF-P) and the others had established fibrosis (PMF-F). Additionally, three cases (2.7 %) were unclassifiable pH⁻ MPN (MPN-U). A higher proportion of male patients was observed overall ($n = 62$; 55.85 %), in particular 74.5 % ($n = 38$) patients with PV were male. Conversely, females predominated among patients with ET and PMF, with 28 (60.9 %) and six (54.5 %) cases respectively. Eighty-four patients (75.67 %) were aged 50 years or older, with a predominant subgroup of 54 patients (48.65 %) aged 65 years or older.

Table 1 – Frequency of myeloproliferative neoplasms by group.

MPN	n	%
PV	51	45.9
ET	46	41.5
PMF-F	10	9
PMF-P	1	0.9
MPN-U	3	2.7
Total	111	100

MPN: Myeloproliferative neoplasms; PV: Polycythemia vera; ET: Essential thrombocythemia; PMF-P: Primary myelofibrosis pre-fibrotic stage; PMF-F: Primary myelofibrosis fibrotic stage; MPN-U: Unclassifiable MPN.

PV cases were more prevalent in this older group, while ET was more common in patients under 50 years old. No specific age distribution pattern was observed in PMF cases. Regarding habits, six patients (5.4 %) reported alcohol consumption, and 11 patients (9.9 %) were smokers (Tables 1 and 2).

Comorbidities and clinical presentation

Regarding comorbidities, most patients ($n = 62$; 56 %) had at least one concurrent medical condition. The most common were cardiovascular diseases ($n = 36$; 32.43 %), followed by thyroid disorders ($n = 13$; 11.7 %) and diabetes mellitus ($n = 10$; 9 %).

In terms of clinical symptoms, the most frequently reported were fatigue ($n = 52$; 46.84 %), pruritus ($n = 29$; 26.12 %), weight loss ($n = 20$; 18 %), bone pain ($n = 11$; 9.9 %), diaphoresis ($n = 10$; 9 %), fever ($n = 5$; 4.5 %), migraine ($n = 1$; 0.9 %). The median spleen size of the cohort was 14 cm (range: 9–25 cm), with 61.26 % ($n = 68$) having splenomegaly.

Peripheral blood and bone marrow biopsy

Blood biometry showed hemoglobin greater than 16 g/dL in 40 cases (36.1 %), anemia in 15 (13.5 %) patients, leukocytosis greater than $10 \times 10^9/L$ in 31 (27.9 %) patients, and thrombocytosis in 56 (50.46 %) cases. Serum lactate dehydrogenase (LDH) levels were elevated in 49 patients (44.1 %). Peripheral blood analysis revealed circulating blasts (<5 %) in six patients (5.4 %), while leukoerythroblastosis was observed in eleven cases (9.9 %). Additionally, dacryocytes were identified in the peripheral smears of nine patients (8.1 %).

Bone marrow biopsy findings revealed that the majority of patients exhibited hypercellularity (mean: 59.7 %). Iron deposition was not reported in 81 (72.97 %) patients.

Table 2 – Distribution of myeloproliferative neoplasms by age.

Age	n	%
18–30 years	4	3.61
31–50 years	23	20.72
51–65 years	30	27.02
Over 65 years	54	48.65
Total	111	100

Table 3 – JAK2 V617F mutation by group.

JAK2 V617F	Present	Absent	Not performed
PV	21 (41.2 %)	10 (19.6 %)	20 (39.2 %)
ET	24 (52.2 %)	12 (26.1 %)	10 (21.7 %)
PMF	3 (30.0 %)	4 (40.0 %)	4 (30.0 %)
MPN-U	1 (33.3 %)	2 (66.7 %)	0 (0 %)

PV: Polycythemia vera; ET: Essential thrombocythemia; PMF: Primary myelofibrosis; MPN-U: Unclassifiable myeloproliferative neoplasm.

Of the remaining 27 % only two (6.7 %) had increased iron levels.

Fibrosis, assessed using Gomori's histochemical technique [4], was reported in 50 patients (45 %), in the majority, the reticulin fiber network was preserved (MF: 0) ($n = 43$; 86 %). Fibrosis Grade 1 (MF:1) was found in three patients (6 %), Grade 2 (MF:2) in none, and Grade 3 (MF:3) in four patients (8 %). Immunohistochemistry was performed in 31 patients (27.93 %), with CD34 being the most used marker. A cytogenetic study, performed in only 12 patients (10.8 %), detected monosomy 8 in one patient (8.33 %).

Presence of JAK2 V617F mutation

JAK2 V617F mutational analysis was performed in 88 (74.8 %) of the patients with the mutation identified in 50 (56.82 %) cases. The highest prevalence was in the ET group ($n = 24$; 52.2 %), followed by PV ($n = 21$; 41.2 %). However, the specific JAK2 V617F allele burden was not quantified for any of the cases in this registry (Table 3).

Treatment

The most used treatment in all MPN groups was hydroxyurea ($n = 85$; 76.6 %), followed by aspirin ($n = 55$; 49.54 %). Ruxolitinib was used in 10 patients (9 %). Approximately half of the patients with PV and ET ($n = 25$ and 22 cases, respectively) were treated with aspirin. Phlebotomy was used in 15 (29.4 %) PV cases and in one (2.17 %) ET patient. Ruxolitinib was administered in three patients with PV, one with ET and six with PMF. Two PMF patients with established fibrosis underwent allogeneic hematopoietic stem cell transplantation. Only one patient in the entire cohort underwent splenectomy. Other treatments were used in 16 % of patients, with clopidogrel being the most frequently prescribed.

Complications

Thrombotic complications were recorded in seven cases (6.3 %), while hemorrhagic complications occurred in nine patients (8.1 %). Thrombotic events were observed in two cases (4 %) with PV and four cases (8.7 %) with ET, in addition to one patient (33.3 %) with MPN-U. Hemorrhagic complications occurred in two patients (4 %) with PV, one case (2.2 %) with ET, two cases (18.2 %) with PMF, and two cases (66.6 %) with MPN-U.

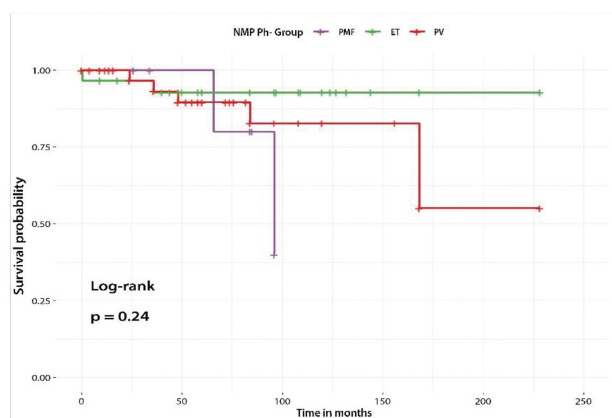


Figure. 1 – Survival curves for each group of Philadelphia-negative chronic myeloproliferative neoplasms.

Disease progression

Progression/transformation was detected in seven patients (6.3 %); five (4.5 %) with fibrotic progression and two cases (1.8 %) with leukemic transformation. Of the 51 patients with PV, three (5.9 %) progressed to secondary myelofibrosis; while of the 46 cases with ET, two (4.3 %) progressed to post-thrombocytopenic myelofibrosis. Among the 11 patients with PMF, one (9 %) case progressed to acute myeloid leukemia as did one (33.3 %) of the three cases of MPN-U.

Survival

The median overall survival was 69 months (range: 0–228). Eleven patients (9.9 %) died during the study. Survival analysis using Kaplan-Meier curves and the log-rank test showed no statistically significant differences between the three major groups (Figure 1). However, due to the smaller sample size of the PMF group, its survival curve declined more rapidly.

A Multiple Correspondence Analysis (MCA) was also performed, including clinical laboratory variables and

hematological abnormalities. The first two axes were represented in the MCA, and the area of dispersion of the points corresponding to each pH⁺ MPN subgroup was delimited by a shaded polygon to simplify visualization. When exploring the frequency tables to support the categories shown in the graph, it was observed that patients with PMF tended to present leukoerythroblasts, dysplasias, high LDH levels and low hemoglobin. This group demonstrated more diverse and high-risk characteristics. Patients with PV showed an association with elevated hemoglobin and inadequate erythropoietin, while those with ET were characterized by thrombocytosis, with the other clinical parameters remaining at normal levels in most of the patients. Although the distribution of the characteristics according to the variables were generally as expected, the total inertia captured by the plot was 29.6 %. The identification of specific trends in each group proved consistent and effective for clinicopathological characterization according to WHO/ICC 2022 criteria [2,3] (Figure 2).

Discussion

In this study, data on pH⁺ MPNs patients from five hospital centers in Ecuador were reported and analyzed comparatively with other countries in the region (Central and South America).

The reviewed studies report higher prevalences of ET and PV compared to PMF. In Ecuador, PV was the most frequent (46 %), followed by ET (42 %), which aligns with findings from Cuba, Mexico, Costa Rica, Brazil, Argentina, Colombia, and Chile, where prevalences range from 38–55 % [5–13,15–18]. Peru, in contrast, reported a higher incidence of PMF [14].

Regarding age distribution, most studies concurred with our findings: pH⁺ MPNs predominantly affect patients over 50 years old, with a peak incidence in those over 65 years in Ecuador, a pattern also reported in Chile and Brazil [5,6,11–13].

In respect to habits, 5–10 % of patients reported smoking or alcoholism in the present study and of the comorbidities, cardiovascular diseases (32 %) were the most frequently

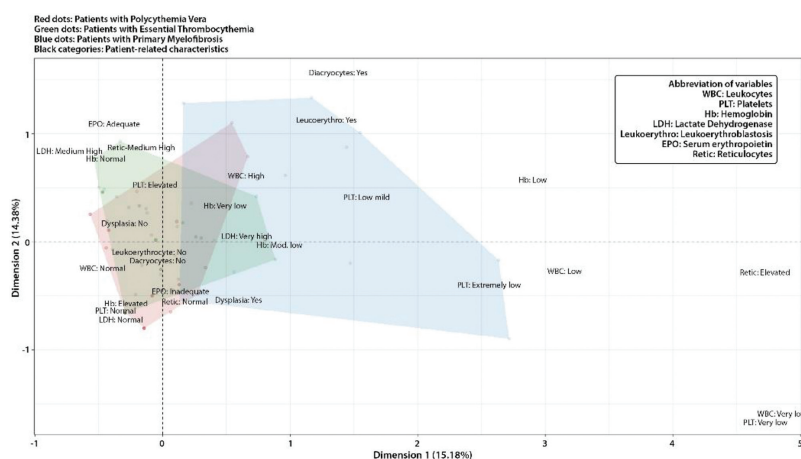


Figure. 2 – Multiple Correspondence Analysis (MCA): Relationships between characteristics and patients.

reported, with no results being found in the other countries comparing this variable.

It is important to highlight the underuse of complementary histochemical and immunohistochemical techniques. Histochemical staining should be performed on all bone marrow biopsies with suspected pH^- MPN in order to evaluate fibrosis due to its importance in differential diagnosis (ET versus PMF) and prognosis (PV) [4,19,20] although recent studies have explored the application of artificial intelligence (machine learning) directly on Hematoxylin and Eosin-stained images, potentially eliminating the need for additional staining [21].

Molecular testing for the JAK2 V617F mutation is essential for the classification and prognostic evaluation of pH^- MPNs; however, it was not consistently available to all patients [7–13]. Nationwide screening is limited by the low number of hospitals and laboratories offering JAK2 V617F, CALR, and MPL mutation testing, and by the limited financial resources of patients. Among those tested, the JAK2 V617F mutation was identified in 56.8 % of cases, a figure comparable to findings from Argentina (57 %), Chile (61 %), and Brazil (62 %) [5,6,11–13,15,16]. CALR and MPL mutations were less accessible and mostly reported in studies from Chile, Argentina, and Brazil [6,11–13,15,16].

In the setting of this study, triple-negative cases often face the additional limitation of not undergoing NGS to detect mutations such as ASXL1, EZH2, SRSF2, IDH1, IDH2, U2AF1 Q517 among others which are important to confirm clonality and assess prognostic implications [16].

The most frequent complications in the present study were thrombotic events (6.3 %) and hemorrhagic events (8.1 %), which are less common than those reported in the region: Chile (29 %), Colombia (14.7 %), and Brazil (12 %) [5–8,11–13].

The rate of leukemic transformation was low across the region, with similar figures to Brazil (2.8 %) [12,13]. Costa Rica reported the lowest progression rates [17].

Hydroxyurea, with or without aspirin, was the treatment most frequently used across the region. Notably, the use of JAK2 inhibitors in PMF patients is increasing in Brazil and Argentina [11–13,15–16].

In Ecuador, access to a broad therapeutic arsenal remains limited. Several drugs are either inaccessible or lack Ministry of Health authorization, including pegylated interferon alpha (for PV), anagrelide (for ET), and JAK inhibitors such as fedratinib and pacritinib (for PMF patients).

Five-year survival in Ecuador is approximately 90 %, like outcomes reported in Chile and other countries in the region [5,6,14,16].

Conclusions

Although the study has several limitations, such as selection bias resulting from the limited participation of most health-related institutions in the country, these data provide an initial diagnostic overview of the clinical-pathological and epidemiological status of pH^- MPNs in the Ecuadorean population. The findings highlight the prevalence of PV and ET as the most common forms of pH^- MPNs, with a notable

presence of the JAK2 V617F mutation in these subgroups. The predominant treatment was hydroxyurea. The observed survival across the different types of pH^- MPNs showed no marked variability.

These results reveal a relatively homogeneous epidemiological profile across the Latin American region and underscore the need for more multicenter studies to better characterize pH^- MPNs in Ecuador and the region in order to optimize diagnostic and treatment strategies.

Data availability

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

Conflicts of interest

The author declares no conflicts of interest.

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Original article

Mineral disorders after hematopoietic stem cell transplantation in patients receiving parenteral nutrition



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ABSTRACT

Background: Parenteral nutrition is a critical therapeutic approach for patients undergoing hematopoietic stem cell transplantation. However, parenteral nutrition is associated with complications such as mineral disorders. This study evaluated the prevalence of hypophosphatemia, hypokalemia, and hypomagnesemia in adult transplant patients before and within the first 24 days of parenteral nutrition infusion.

Patients and Methods: This retrospective cohort study included patients who underwent hematopoietic stem cell transplantation and required parenteral nutrition at a public quaternary hospital in Brazil between January 2012 and January 2022. Patients were categorized based on indications for parenteral nutrition and specific diagnoses. Laboratory monitoring of phosphorus, potassium, and magnesium levels was performed at predefined intervals during parenteral nutrition infusion, starting at 72 h and during subsequent 4-day intervals until engraftment. Hypophosphatemia, hypokalemia, and hypomagnesemia were defined as levels below established reference values. Descriptive analyses were applied, and the chi-square test, crude odds ratios, and Fisher's exact test were used to compare categorical variables between the periods.

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Results: The study revealed varying prevalences of hypophosphatemia (21.1–40.3 %), hypokalemia (10.5–31.2 %), and hypomagnesemia (50.0–62.3 %) among the 77 patients included in the study. Severe mineral disorders were observed in only a small proportion of patients. **Conclusion:** Patients undergoing hematopoietic stem cell transplantation had a high prevalence of mineral depletion throughout the engraftment period.

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Introduction

Side effects related to hematopoietic stem cell transplantation (HSCT) conditioning can lead to severe mucositis, impair oral intake, chewing, and swallowing, and suggest a need of special nutritional support for these patients [1]. Mucositis lesions are common occurrences in oncology patients, with a reported incidence of 40 % in those receiving chemotherapy and 76 % in patients undergoing hematopoietic stem cell transplantation (HSCT). This high prevalence frequently leads to poor tolerance of oral or enteral feeding routes [2] and so the parenteral route is preferred.

Parenteral nutrition (PN), although a vital lifeline for patients undergoing hematological recovery, is associated with numerous complications with mineral disorders having emerged as a prominent concern [3]. Mineral disorders in patients receiving PN predominantly arise from two key factors. First, patients undergoing HSCT often require complex regimens of medications to manage their underlying conditions [4]. These medications typically include immunosuppressants to prevent graft-versus-host disease (GvHD), antibiotics to prevent infections, and other drugs to manage post-transplant complications. Unfortunately, these drugs can significantly disrupt the delicate balance of essential electrolytes in the body, leading to imbalances in minerals such as potassium, magnesium, and phosphorus. These imbalances can have far-reaching consequences, affecting the patient's overall health and potentially hindering the success of the hematological recovery process [5]. The second factor contributing to mineral disorders in HSCT patients is the risk of Refeeding syndrome, particularly within the initial days after starting PN. Refeeding syndrome is a severe metabolic disturbance characterized by rapid shifts in mineral levels, particularly potassium, phosphorus, and magnesium, as the body adapts to increased nutritional intake after a period of malnutrition or fasting [6]. When HSCT patients, who are often in a compromised nutritional state before transplantation, begin PN abruptly, they are particularly susceptible to Refeeding syndrome. This condition can lead to various complications, including cardiac arrhythmias, respiratory distress, and neuromuscular disturbances [7]. The correct management of the mineral disorders in these vulnerable patients is paramount to ensuring successful hematological recovery while minimizing the potential complications associated with PN.

This study evaluated the prevalence of hypophosphatemia, hypokalemia, and hypomagnesemia before and within the first 24 days of PN infusion in adult patients who underwent HSCT.

Methods

Setting, study design, inclusion, and exclusion criteria

A retrospective historical cohort study was conducted of patients with malignant and non-malignant hematological diseases who underwent autologous or allogeneic HSCTs at a public quaternary hospital in Campinas, southeastern region of Brazil, over ten consecutive years. The study was approved by the local research ethics committee (#56610022.8.0000.5404).

Data from all patients who underwent HSCT and received PN were collected using a standardized form previously prepared for managing and monitoring PN patients. This form contains the total PN prescribed, details of oral and enteral intake, as well as clinical and laboratory data. All patients who underwent HSCT and required PN for at least 24 hours or until they began receiving full enteral or oral nutrition were included. Patients were classified according to their indications for PN, which included neutropenic colitis, GvHD, and grade III and IV mucositis. The data were filled in by the Nutritional Multidisciplinary Therapy Team (NMTT), composed of a dietitian, physician, nurse, and pharmacist involved in patient care. Forms with conflicting or inconsistent data were excluded.

Laboratory monitoring

Laboratory monitoring was first performed before starting PN and followed a routine based on the current protocol for the laboratory monitoring of PN [8]. In the service, the metabolic assessment was performed immediately before PN and until the day before engraftment or death. The serum levels of phosphorus, potassium, and magnesium were obtained within the first 72 h (Period 1 [P1]), and for each subsequent 4-day period (Period 2 [P2]: Days 4–7; Period 3 [P3]: Days 8–11; Period 4 [P4]: Days 12–15; Period 5 [P5]: Days 16–19; and Period 6 [P6]: Days 20–24). Energy and protein levels were evaluated throughout the period.

Hypophosphatemia, hypomagnesemia, and hypokalemia were defined as levels below the established laboratory reference values, as follows: phosphorus 2.5–4.5 mg/dL, magnesium 1.32–2.14 mEq/L, potassium 3.5–5.1 mEq/L [9–10]. A severely depleted mineral status was defined as a plasma concentration less than or equal to half the minimum reference level. In patients exhibiting severely depleted mineral levels, serum concentrations were individually corrected prior to the initiation of PN. This correction was achieved via intravenous infusion of the specific mineral at the highest

possible dose, adhering to established guidelines and pharmacokinetic compatibility considerations [11]. Any new incidence of hypophosphatemia, hypokalemia, or hypomagnesemia observed during the P2–P6 period was considered an event.

Anthropometric assessment

In the service, weight and height of all patients were measured. The technique adopted for collecting measurements was based on recommendations proposed by the Anthropometric Standardization Reference Manual [12]. Nutritional status was assessed according to the growth charts of the World Health Organization [13]. The measured weight was obtained using a digital electronic scale (Filizola®, São Paulo, Brazil), with a capacity of 150 kg and precision of 100 g, and the height was recorded using a wooden stadiometer with a precision of 0.1 cm, which was attached to a 200-cm-long flat vertical surface.

PN prescription and monitoring routine

Before the start of infusion, levels of minerals (sodium, potassium, ionic or total calcium, chloride, magnesium, and phosphorus), triglycerides, cholesterol, high-density lipoprotein (HDL), alanine aminotransferase, gamma-glutamyl transferase, hemoglobin, albumin, transthyretin, urea, creatinine, and glucose were measured in all patients. The mineral levels of potassium, magnesium, and phosphorus was measured three times a week in all the patients.

Prescriptions were formulated with the aid of the supplying pharmacy's computer software, which performs pharmacokinetics analysis to ensure compatibility of the constituent components. PN was prescribed by two physicians.

The recommendations for energy-protein and the proportions of macronutrients, minerals, vitamins, and micronutrients were prescribed according to the patient's clinical condition and the guidelines established by the American Society for Parenteral and Enteral Nutrition (ASPEN) [14]. Subsequent adjustments, where required, were made based on clinical, nutritional, and laboratory monitoring.

The initial standardized solutions, the values of phosphorus, potassium, and magnesium used for PN infusion, were those recommended by current guidelines (phosphorus: 7–10 mmol/1000 kcal/day, potassium: 1–2 mEq/kg/day, and magnesium: 8–28 mEq/day) [8,10–14].

The amount of energy, protein, and non-protein calorie/gram of nitrogen ratio were evaluated in P1–P5 until engraftment, defined as a neutrophil count $\geq 0.5 \times 10^9$ L for two consecutive days and platelet count $\geq 20 \times 10^9$ L for three consecutive days without transfusion for one week.

Energy calculation of the accepted nutrition

The total energy provided by PN was calculated based on the macronutrient content, using the following conversion factors: 4 kcal/g for amino acids, 3.4 kcal/g for carbohydrates, and 10 kcal/g for lipids. The resulting values were compared against the recommended daily requirements of 23–30 kcal/kg for energy and 1–1.5 g/kg for protein [15].

Regarding the oral diet, acceptance was analyzed in the previous day's collection chart.

Statistical analysis

The chi-square test, crude odds ratios (ORs), and Fisher's exact test were used to compare categorical variables between the groups. Confidence intervals for the proportions were calculated using the standard frequentist method. Analyses were performed using the Statistical Package for the Social Sciences version 16 (IBM Company, Chicago, IL, USA). Differences were considered statistically significant at a two-sided p-value <0.05.

Results

A total of 77 adult patients who underwent HSCT were included in this study. The mean age of the patients was 46.4 years and 50.6 % were male. Most patients had myeloid malignancies (50.6 %) and lymphoid malignancies (44.2 %). Autologous HSCT transplantation was performed in 41.6 % of cases, human leukocyte antigen (HLA) matched related and unrelated HSCT in 50.6 % and 6.5 %, respectively, and HLA-mismatched related transplantation in 1.3 %. The conditioning regimen types included high doses without total-body irradiation (81.8 %), high doses with total-body irradiation (1.3 %), and reduced intensity (16.9 %). Platelet engraftment occurred at a median of 18 days (interquartile range [IQR]: 16–22 days), and neutrophil engraftment occurred at a median of 14 days (IQR: 11–17 days) (Table 1).

The mean BMI was 25.6 kg/m² with 40.3 % of patients overweight and 13.0 % obese. Among the patients, 14.3 % died

Table 1 – Demographic and clinical data of adult patients receiving hematopoietic stem cell transplantation and parenteral nutrition.

Characteristic	n= 77
Age – years $\pm$ SD	46.4 $\pm$ 14.6
Male - n (%)	39 (50.6)
Diagnosis at Transplant - n (%)	
Myeloid malignancy	39 (50.6)
Lymphoid malignancy	34 (44.2)
Non-hematological malignancy - Others*	4 (5.19)
Donor type - n (%)	
Autologous	32 (41.6)
HLA-matched related	39 (50.6)
HLA-matched unrelated	5 (6.5)
HLA mismatched related	1 (1.3)
Conditioning regimen types - n (%)	
High dose	
Without total body irradiation	63 (81.8)
With total body irradiation	1 (1.3)
Reduced intensity	13 (16.9)
Platelet engraftment – days n (IQR)	18 (16–22)
Neutrophils engraftment – days n (IQR)	14 (11–17)

IQR: Interquartile range.

* Others included aplastic anemia, amyloidosis, polycythemia, and neutropenia.

Table 2 – Nutritional status, reasons for PN, and mucositis severity in patients receiving hematopoietic stem cell transplantation and parenteral nutrition.

Clinical data	Values
BMI - (kg/m ² ± SD)	25.6 ± 3.7
Nutritional status - n (%)	
Underweight	4 (5.2)
Normal range	32 (41.6)
Overweight	3 (40.3)
Obese	10 (13.0)
Mortality - n (%)	22 (14.3)
Time of PN	1 (1.4)
Reasons for PN - n (%)	
Neutropenic colitis	1 (1.3)
GvHD	3 (3.9)
Mucositis level III	5 (6.5)
Mucositis level IV	68 (88.3)

PN: Parenteral Nutrition; BMI: Body Mass Index; GvHD: Graft-versus-host disease.

with PN administered for a mean duration of 1.4 days. The main reason for PN indication was grade IV mucositis (88.3 %) (Table 2).

Energy intake per day varied across different periods, ranging from 23.1–36.8 kcal/kg. Protein intake per day also varied across the study period, ranging from 0.96–1.62 g/kg. The non-protein calorie/g of the nitrogen ratio was also evaluated, with values below and above the recommended target values observed during different periods (Table 3).

Table 4 provides information on the occurrence of mineral disorders during the HSCT detailed by period (P1–P6). The

prevalence of hypophosphatemia ranged from 21.1–40.3 % with the highest occurrence observed in P2. Hypokalemia was observed in 10.5–31.2 % of patients with the highest occurrence in P1. The incidence of hypomagnesemia ranged from 50.0–62.3 % with the highest incidence in P1. Severe hypophosphatemia events were observed in 1.6 % of patients in P2. Severe hypokalemia was observed in 2.6 % and 2.7 % of patients in P2 and P4, respectively. Severe hypomagnesemia occurred in 1.3 % and 1.8 % of patients in P1 and P3, respectively.

Discussion

There was a high number of mild intracellular mineral disorders. Disorders of intracellular minerals are noteworthy for their frequency. Hypokalemia, hypomagnesemia, and hypophosphatemia have been reported during the PN period; this phenomenon might be explained by the release of insulin associated with the parenteral energy infusion [16]. However, both hypokalemia and hypomagnesemia occurred in almost half of the samples during the first four days of PN when the amount of energy offered was still low; therefore, it is likely that, in addition to refeeding, there was tubulopathy caused by pre-transplant chemotherapy. A study of patients undergoing HSCT observed an incidence of acute kidney injury of 64 %, occurring more frequently in those with hypoalbuminemia [17].

Electrolyte disturbances are often associated with patients taking antineoplastic agents; this can lead to serious or fatal clinical manifestations [18]. In this study, hypokalemia and hypomagnesemia were found in 31.2 % and 62.3 % of patients,

Table 3 – Nutritional intake by oral and parenteral prescription in adult patients receiving hematopoietic stem cell transplantation and parenteral nutrition.

Variable	P1 Median (p25-p75)	P2 Median (p25-p75)	P3 Median (p25-p75)	P4 Median (p25-p75)	P5 Median (p25-p75)	P6 Median (p25-p75)
Energy per day -kcal/kg	23.1 (15.9-28.0)	35.3 (26.5-42.0)	34.8 (27.3-40.4)	36.8 (29.3-42.0)	35.9 (22.5-41.3)	27.9 (27.9-27.9)
Energy requirement (minimum) - % ^b	92.5 (63.4-112.1)	141 (106.0-168.0)	139.4 (109.4-161.7)	147.5 (117.2-168.0)	143.5 (90.0-165.4)	111.6 (111.6-111.6)
Energy requirement (maximum) - % ^c	77.0 (53.2-93.4)	117.7 (88.3-140.2)	116.1 (91.2-134.7)	122.9 (97.7-140.0)	119.7 (75.0-137.9)	93.0 (93.0-93.0)
Protein per day, g/kg	0.96 (0.69-1.14)	1.57 (1.25-1.87)	1.62 (1.40-1.93)	1.61 (1.43-1.97)	1.59 (1.22-1.79)	1.02 (1.02-1.02)
Protein requirement (minimum) - % ^d	96.1 (69.8-114.9)	157.6 (125.9-187.5)	162.4 (140.3-193.4)	162.4 (140.9-197.7)	159.7 (122.5-179.1)	102.1 (102.1-102.1)
Protein requirement (maximum) - % ^e	48.0 (34.9-57.4)	78.8 (62.9-93.7)	81.2 (70.1-96.7)	81.2 (71.9-98.8)	79.8 (61.5-89.5)	51.1 (51.1-51.1)

Variables were compared with the Mann-Whitney U test or Fisher's exact test as appropriate.

^{b,c} Energy adequacy was calculated using reference values of 25 kcal/kg/day for minimum requirements and 30 kcal/kg/day for maximum requirements.

^{d,e} Protein adequacy was calculated using reference values of 1.0 g/kg/day for minimum requirements and 2.0 g/kg/day for maximum requirements.

Table 4 – Mineral disorders in adult patients receiving hematopoietic stem cell transplantation and parenteral nutrition.

Variable	Period 1 (n = 77)	Period 2 Days 1-4 (n = 67)	Period 3 Days 4-7 (n = 55)	Period 4 Days 8-11 (n = 36)	Period 5 Days 12-15 (n = 19)	Period 6 Days 16-19 (n = 0)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Hypophosphatemia	22 (28.6)	27 (40.3)	20 (36.4)	12 (33.3)	4 (21.1)	0 (0.0)
Hypokalemia	24 (31.2)	18 (26.9)	10 (18.2)	10 (27.7)	2 (10.5)	0 (0.0)
Hypomagnesaemia	48 (62.3)	38 (56.7)	34 (61.8)	18 (50.0)	11 (57.9)	0 (0.0)

respectively. These disorders frequently occurred concomitantly, which may be explained by the association between proximal tubule damage and the blockage of the TRPM6/EGF/EGFR pathway. This blockage impairs magnesium reabsorption in the distal tubule, thereby preventing the maintenance of normal serum magnesium and calcium levels [18]. Although drug-induced tubulopathy can explain hypokalemia and hypomagnesemia, patients undergoing HSCT often experience a period of low food intake and reduced lean body mass due to processes related to the neoplasia. An underlying inflammatory process is present, characterized by anorexia and insufficient energy and protein intake. This condition is further exacerbated by the metabolic redirection of protein stores toward the synthesis of pro-inflammatory factors [19]. Thus, these factors may have contributed to hypokalemia and hypomagnesemia associated with drug tubulopathy. However, considering the prevalence of hypokalemia and hypomagnesemia during the first week of PN, it is not possible to separate the effects of tubulopathy from what can be attributed to refeeding caused mainly by PN [3].

Phosphorus is another intracellular mineral and is, therefore, characteristically depleted in the Refeeding syndrome. In the current study, the drop in this anion was most evident after the fourth day of PN (40.3 % of patients). Therefore, it can be understood that the fact that phosphorus had a more significant drop in plasma levels during the second period of PN is probably attributable to cellular Theft syndrome, given that the amount of energy administered, although without hyperalimentation, was already increased during this period [3,20]

Although there was a high prevalence of mineral depletion throughout the study, few patients had severe levels of mineral depletion. This can be directly attributed to the fact that rigorous monitoring of both the nutritional and metabolic issues occurred daily, owing to the presence of an NMTT. Notably, the patients managed to achieve their target, indicating the active involvement of the team [21].

In the 77 patients studied, Grade IV mucositis was present in 88.3 % of the cases as expected given that the patients had to receive PN as their main source of nutrition. Patients with Grade IV mucositis experience significant discomfort when eating because of intense inflammation and ulceration with necrotic lesions; however, the use of enteral tubes remains controversial [22].

In the present cohort, the use of PN was effective in meeting energy and protein needs with the amount of energy offered reaching 36.8 kcal/kg/day, and protein reaching 1.62 g/kg/day. It has been previously reported that the amount of energy needed after HSCT increases by 30–50 %, and the amount of protein should reach approximately 1.5 g/kg/day [23]. This was only possible because the place where this research was conducted by the NMTT was prepared to prescribe customized PN, with the possibility of optimizing the number of amino acids prescribed. Studies have shown that the presence of an NMTT facilitates this type of approach [24,25].

Study limitations

Given this was a retrospective cohort, the original data were recorded by many different individuals as opposed to a sole

researcher. However, to attenuate this aspect, care was taken to remove any inconsistent records. Moreover, all the charts obtained were checked by a second member of the team.

Conclusion

Patients undergoing HSCT are at risk of developing mineral disturbances during the engraftment period. Although these factors diminish over time, disorders remain prevalent in all periods. Therefore, rigorous daily monitoring of these patients by a team specializing in PN is warranted.

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Data availability

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

Conflicts of interest

None.

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Editor

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Original article

Real-world data of autologous stem cell transplantation for patients with mantle cell lymphoma in Argentina



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ABSTRACT

Background: For eligible patients with mantle cell lymphoma who respond to induction therapy, first-line consolidation with autologous hematopoietic progenitor cell transplantation remains a standard treatment. However, outcomes for these patients in Argentina have not been fully characterized. This paper aims to describe the factors linked to improved survival of patients with mantle cell lymphoma after transplantation in Argentina. In addition, the association between relapse within the first 24 months after transplant and overall survival was evaluated.

Methods: A retrospective, multicenter study was carried out. Patients over 18 years of age with a diagnosis of mantle cell lymphoma who received autologous hematopoietic progenitor cell transplantation from 2007–2023 at centers affiliated with the Argentine Group for Bone Marrow Transplantation and Cellular Therapy (GATMO-TC) were included. For the survival analysis, a landmark approach was utilized: overall survival was calculated from the date of progression for the group that relapsed within 24 months, and from the 24-month post-transplantation landmark for those who did not.

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Results: One hundred and sixty-six patients from nine Argentine centers were included, 128 of whom were men (77%). The median age at transplantation was 58 years. Eighteen (11%) had blastoid morphology. The pretransplant status was complete response in 145 (87%) patients. With a median follow-up of 38.4 months, the median overall survival and progression-free survival were 102 and 48.8 months, respectively. In the multivariate analysis, the blastoid variant, an age ≥ 55 years, and a transplant comorbidity index ≥ 2 were independent predictors of survival.

Conclusions: $>70\%$ achieved prolonged survival. Blastoid morphology, age older than 55 years, and comorbidities diminished outcomes after transplantation.

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Introduction

The current treatment of mantle cell lymphoma (MCL) for clinically suitable patients consists of a sequence that includes induction chemotherapy based on rituximab and high-dose cytarabine (HiDAC), followed by consolidation with autologous stem cell transplantation (ASCT) in those patients who achieve response with induction, and subsequently maintenance with rituximab. This sequence is associated with overall responses in $>90\%$ of patients, with complete response (CR) rates of 70% and four-year progression-free survival (PFS) of 83%. However, relapses may still occur after five years of remission [1–5].

The indication for ASCT as first-line consolidation is based on the trial by Dreyling et al., which compared consolidation with ASCT versus interferon alpha in patients with MCL after induction with a regimen similar to cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) [6]. This study demonstrated a longer PFS for patients who underwent ASCT after their first remission, and subsequently also demonstrated a longer overall survival (OS) in favor of ASCT. In addition, ASCT was associated with a low mortality rate (5%), and patients who consolidated in CR had a better PFS than those who did so in partial response (PR) [7].

Despite the clear benefit of consolidating with ASCT in first remission when using CHOP-like induction regimens, this benefit was not demonstrated in randomized clinical trials after induction with modern regimens based on rituximab and HiDAC, which are currently used as first-line treatment for MCL [7]. Moreover, a subsequent analysis of the Dreyling et al. study found no differences in PFS between the two consolidation arms (ASCT versus interferon alpha) among patients who had received rituximab in induction [7]. Recently published results of the TRIANGLE clinical trial demonstrated no inferiority in the omission of ASCT when ibrutinib is added to first-line chemimmunotherapy [8]. These are the main reasons why the percentage of patients with MCL receiving ASCT is currently decreasing [9]. However, there are Phase II and III trials that have demonstrated the effectiveness and safety of ASCT after induction with these modern regimens (rituximab and HiDAC), with very prolonged remissions, which could imply a cure in a considerable percentage of patients [2,4,5,10–12].

Given the uncertainty surrounding the outcomes of ASCT in patients with MCL and the lack of data on this topic in Argentina, a multicenter retrospective analysis was conducted. Patients diagnosed with MCL who underwent ASCT, both as first-line treatment and in the setting of relapse, at transplant centers affiliated with the Argentine Group for Bone Marrow Transplantation and Cell Therapy (GATMO-TC), were included. The objective of the study was to evaluate OS and PFS after ASCT, the cumulative incidence of relapse, and non-relapse mortality.

Patients and methods

An analytical, retrospective, multicenter study was conducted, including all patients with MCL who received ASCT in nine transplant centers affiliated with GATMO-TC. The different variables were collected from each patient's medical record by a referring physician at each hospital. Subsequently, the data were centralized and analyzed in a tertiary care hospital in Córdoba, Argentina (Hospital Privado Universitario de Córdoba). The inclusion criteria were: i) diagnosis of MCL according to WHO 2017 criteria [13], ii) age equal to or >18 years at the time of diagnosis of the disease, iii) having received ASCT, either as first-line or subsequent line therapy, between January 1, 2007, and April 30, 2023, at transplant centers affiliated with GATMO-TC. The exclusion criteria were: i) not having achieved at least a partial response prior to transplant; ii) having received an allogeneic bone marrow transplant prior to autologous transplant; iii) age equal to or >76 years.

For patients who received induction therapy at an external center, the referring physician provided a comprehensive clinical history. This summary included the diagnostic methods used at disease onset, the various treatments administered, and the subsequent response to therapy; this information was then integrated into the patient's medical record at the ASCT center. The response achieved before transplantation was assessed by physical examination and imaging, and was defined as CR if symptoms and tumor masses due to the disease disappeared, or as PR if tumor masses decreased by $>50\%$ with no new lesions. The types of conditioning used were defined by each center. Post-transplant follow-up was conducted according to institutional protocols. The frequency of surveillance imaging and the administration of rituximab maintenance therapy were left

to the discretion of the treating physicians. Relapse and progression were defined as the appearance of new biopsy-confirmed disease or a 50% increase in the size of previously known lesions in patients who had achieved CR or PR [14]. Patients who continued follow-up outside the transplant hospital were contacted by telephone to assess their current status and their post-transplant therapies, including rituximab maintenance.

This study was carried out in full accordance with the current national and international regulations: Declaration of Helsinki of the World Medical Association in its most updated version, resolution 1480/2011 of the National Ministry of Health and the Law 25,326 on Protection of Personal Data. All the study data was treated with the utmost confidentiality and anonymously, with access restricted only to authorized personnel for the purposes of the study and, once more, in accordance with the aforementioned regulations. The study was approved by the Health Research Ethics Committee of the Hospital Privado Universitario de Córdoba.

Statistical analysis

Continuous variables were expressed as median and range or mean and standard deviation according to distribution, and comparisons were performed using the Student's t-test or the Mann-Whitney test, depending on their homogeneity. Categorical variables were expressed as number and percentage and analyzed using the chi-square test or Fisher's exact test according to expected frequencies. PFS was calculated from the date of transplant to the date of relapse, progression, or death, whichever occurred first. OS was calculated from the date of transplant to the date of death or last follow-up. Survival curves were estimated using the Kaplan-Meier method and compared across strata using the log-rank test. The cumulative incidence of relapse and non-relapse mortality were analyzed by competing risks. Patients who relapsed within 24 months of ASCT were categorized as POD24-positive. For the survival analysis, a landmark approach was used: overall survival for the POD24 group was calculated from the date of relapse or progression, while for the non-POD24 group, survival was calculated starting from the 24-month post-transplant landmark. Patients who died without progression and those who did not reach 24 months of follow-up were excluded. A probability value <0.05 was considered significant. Statistical analysis was performed using the EZR statistical program (Easy R).

Results

From January 1, 2007, to April 30, 2023, 166 patients diagnosed with MCL underwent ASCT at nine Argentine centers belonging to the GATMO-TC group. The patients' baseline characteristics are shown in Table 1.

Characteristics at the time of mantle cell lymphoma diagnosis

Seventy-seven percent ($n = 128$) were men. The median age at diagnosis of MCL was 56 years (range: 37–69 years).

Table 1 – Baseline characteristics of 166 patients who received Autologous stem cell transplantation for mantle cell lymphoma.

Characteristic	n (%)
Male	128 (77)
Female	38 (23)
Age at diagnosis - median (range) years	56 (37–69)
Stage at diagnosis	
I	4 (2)
II	6 (4)
III	22 (14)
IV	122 (73)
Unknown	12 (7)
BM compromise at diagnosis	107 (64)
B symptoms at diagnosis	62 (37)
MIPI	
Low	31 (18)
Intermediate	36 (22)
High	28 (17)
Unknown	71 (43)
MIPIb	
Low	9 (5)
Intermediate	16 (10)
High	27 (16)
Unknown	114 (69)
Initial treatment at transplant center	67 (40)
Pre-transplant induction schedules	
R-CHOP/R-DHAP	94 (57)
R-CHOP	32 (19)
R-hyper-CVAD	20 (12)
Others	20 (12)
Rituximab pre-transplant	165 (99)
High-dose cytarabine pre-transplant*	140 (84)

* Includes patients who received high-dose cytarabine prior to R-CHOP.

Of the total, 144 (87%), 107 (64%), 62 (37%), and 18 (11%) had Stage III–IV disease, bone marrow involvement, B symptoms, and blastoid morphology, respectively. Prior to transplant, 165/166 (99%) and 140/166 (84%) received rituximab and HiDAC, respectively. Other characteristics are summarized in Table 1.

ASCT: autologous hematopoietic progenitor cell transplantation; MCL: Mantle Cell Lymphoma; BM: bone marrow; MIPI: Mantle Cell Lymphoma International Prognostic Index; MIPIb: biological Mantle Cell Lymphoma International Prognostic Index; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; R-DHAP: rituximab, cisplatin, cytarabine, and dexamethasone.

Characteristics at the time of transplant

The characteristics of the patients at the time of ASCT are summarized in Table 2.

Post-transplant results

Following ASCT, 58 (35%) patients received rituximab maintenance therapy. Disease status at Day +100 post-transplant was CR ($n = 146$; 88%), PR ($n = 2$; 1%), progressive disease ($n = 4$; 2%), and unknown ($n = 14$; 9%).

Table 2 – Characteristics at the time of transplant.

Characteristic	
Age at transplant - median (range) years	58 (37–69)
Transplant comorbidity index - median (range)	0 (0–9)
Pre-transplant lines - median (range)	1 (1–5)
Pre-transplant disease status	
Complete response - n (%)	145 (87)
Partial response - n (%)	17 (10)
Unknown - n (%)	4 (2)
Transplant in first remission - n (%)	139 (83)
Transplant in second remission - n (%)	27 (16)
Conditioning	
BEAM - n (%)	98 (59)
CBV - n (%)	31 (19)

BEAM: carmustine, etoposide, cytarabine, and melphalan; CBV: cyclophosphamide, carmustine, and etoposide.

Overall and progression-free survival post-transplant

After a median follow-up of 38.4 months (interquartile range: 14.2–71.2), the median OS and PFS were 102 (range: 85.5–not reached months) and 48.8 months (range: 41.2–74 months), respectively. The estimated 3-year OS and PFS were 74.9% (95% confidence interval: 66.5–81.5) and 62.1% (95% confidence interval: 53.1–69.8), respectively. In the multivariate analysis, the blastoid variant (Hazard ratio [HR]: 5.81; p-value <0.001), age ≥ 55 years (HR: 3.95; p-value = 0.006) and transplant comorbidity index ≥ 2 (HR: 3.09; p-value = 0.008) were independent predictors of OS (Figs. 1–3). The 3-year OS for those transplanted in CR was 76% versus 55% for those transplanted in PR (p-value = 0.064). While OS was similar for those who received rituximab maintenance after ASCT versus those who did not, the 3-year PFS for those who received rituximab maintenance was 66% versus 49% for those who did not (p-

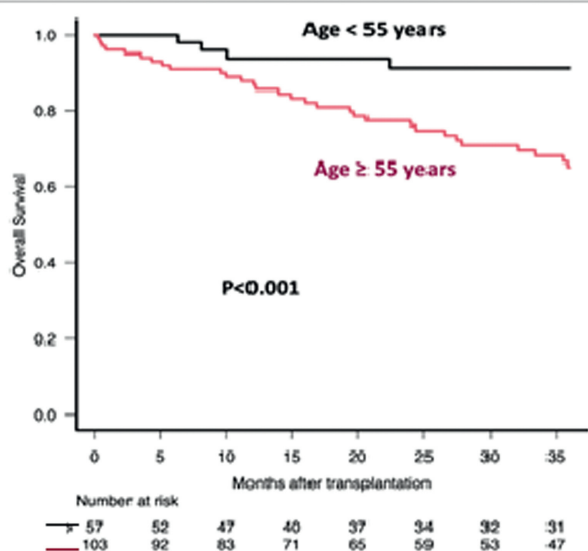


Fig. 1 – Overall survival (OS) after Autologous Hematopoietic Progenitor Cell Transplantation (ASCT) according to age at transplantation. Comparison between patients aged ≥ 55 years and those aged <55 years.

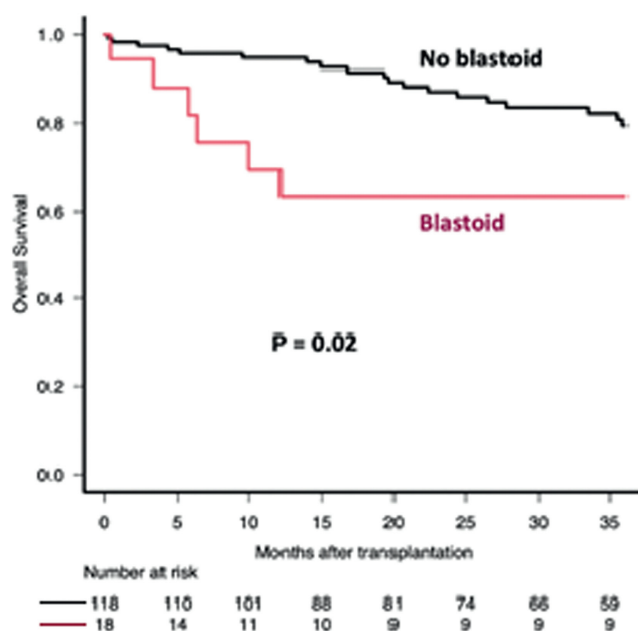


Fig. 2 – Overall survival (OS) after Autologous Hematopoietic Progenitor Cell Transplantation (ASCT) according to morphology. Comparison between patients with and without blastoid morphology.

value = 0.016). Induction and conditioning regimens, pre-transplant response (CR versus PR), B symptoms, elevated mantle cell lymphoma international prognostic index, and Stage III-IV did not affect OS or PFS.

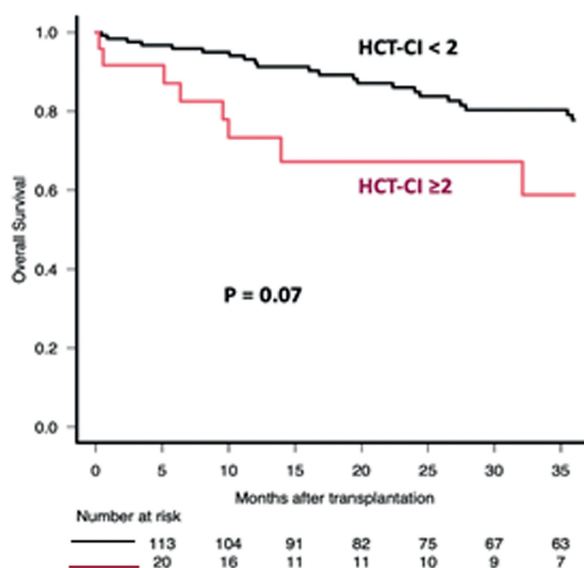


Fig. 3 – Overall survival (OS) after Autologous Hematopoietic Progenitor Cell Transplantation (ASCT) according to HCT-CI at transplantation. Comparison between patients with an Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) ≥ 2 and those with an HCT-CI <2.

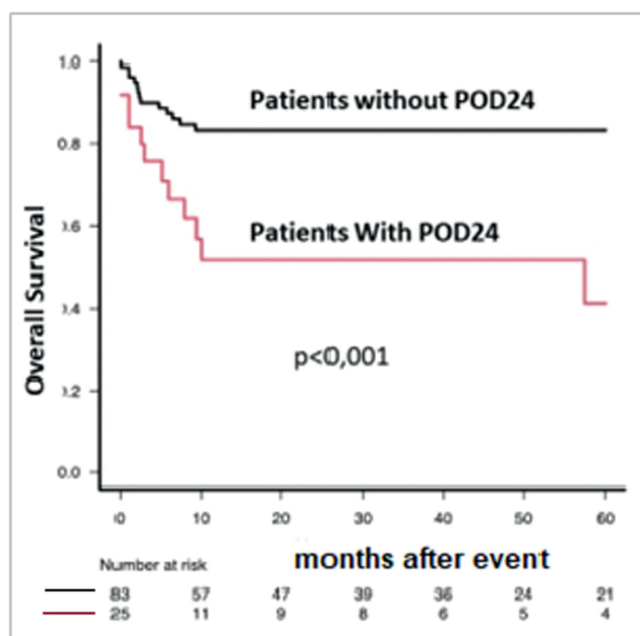


Fig. 4–Overall survival (OS) according to progression of disease within 24 months (POD24) status. Comparison between patients with and without relapse within 24 months after autologous hematopoietic progenitor cell transplantation (ASCT). For this landmark analysis, OS was calculated from the date of relapse or progression for the POD24 group and from the 24-month post-ASCT landmark for the group without POD24.

Post-transplant relapse

Of the total number of patients, 56 (34%) relapsed. The cumulative incidences of relapse at 12, 24, and 36 months were 9.1% (5.2–14.4%), 17.4% (11.7–24.1%), and 25.3% (18.2–33%), respectively. In the multivariate analysis, the only factor independently associated with a higher cumulative incidence of relapse was having undergone ≥ 2 lines of therapy prior to ASCT (p -value ≤ 0.001). Within the first 24 months post-ASCT, 25 patients relapsed (POD24), of whom 14 died. The 1-year and 5-year overall survival rates were significantly lower for the POD24 group (51.6% and 41.3%) compared to those without a POD24 event (83.3% and 83.3%, respectively (p -value < 0.001 ; Figure 4).

Post-transplant mortality

Of the total, 51 (31%) patients died, with 24/51 in remission. The cumulative incidence of non-relapse mortality at 3, 6, and 12 months was 3% (range: 1.1–6.5%), 4.9% (range: 2.3–9.9%), and 6.3% (range: 3.2–10.8), respectively. In multivariate analysis, blastoid morphology (HR: 3.28; p -value = 0.014) and age ≥ 55 years (HR: 2.63; p -value = 0.013) were predictors of non-relapse mortality. The causes of non-relapse mortality were: sepsis ($n = 7$), COVID-19 ($n = 6$), infectious pneumonia ($n = 2$), acute pulmonary thromboembolism ($n = 1$), infectious meningoencephalitis ($n = 1$), candidiasis ($n = 1$), glioblastoma ($n = 1$), and unknown ($n = 5$). One case of acute myeloid

leukemia and one case of myelodysplastic syndrome following ASCT were reported. More than five years after ASCT. Twenty-three patients are alive and relapse-free.

Discussion

The outcomes with MCL after ASCT in Argentina are presented in this retrospective study.

The baseline characteristics of the cohort were similar to those reported in different studies of patients with MCL who were candidates for ASCT, with a median age at diagnosis of approximately 56–58 years, a high predominance of males, and Stage III–IV disease with bone marrow involvement. In line with other studies, B symptoms were present in 37%, blastoid morphology in 11%, and high mantle cell lymphoma international prognostic index in 17% of cases [2,4]. Unlike other studies, the induction regimens used did not impact post-ASCT OS. This may be because the majority of the patients received HiDAC prior to ASCT (84%). Moreover, unlike the study by Delarue et al. [5], patients who presented with B symptoms at diagnosis did not have worse OS in the present study.

With a median follow-up of 38 months, the 3-year PFS and OS were 62% and 75%, results similar to those observed by Touzeau et al. [15], but somewhat lower than those of Merryman et al. [4], who reported 3-year PFS and OS of 83% and 92%, respectively after induction with rituximab-bendamustine alternating with rituximab-cytarabine and subsequent consolidation with ASCT. Similarly, the current OS results were lower than those of Hermine et al. [11] after a long-term follow-up of patients who underwent R-CHOP/R-DHAP followed by ASCT. These authors reported a five-year OS of 76%. We believe the differences in OS observed in the present study are associated with a higher transplant-related mortality, which may be explained by the inclusion of patients over an extended period of time. The lower transplant-related mortality observed in trials is likely attributable to strict inclusion criteria, which may not accurately reflect the complexities of a real-world patient population. In this regard, in a real-world report of patients with MCL who underwent ASCT, survival rates were similar to those found in the present series [16]. Although the results of the TRIANGLE clinical trial [8] demonstrated that adding ibrutinib to induction chemotherapy, followed by ASCT and then maintenance employing ibrutinib for two years, provides survival benefits, it has not yet been demonstrated that, with the addition of ibrutinib, ASCT can be omitted. The use of ibrutinib in first-line treatment was associated with increased hematologic and infectious adverse events [8].

As in most studies that include rituximab and HiDAC in induction regimens, most of the current patients were transplanted in CR which was associated with longer OS. As in other studies [15], the blastoid variant and age were clear predictors of OS.

For indolent lymphoma, such as follicular lymphoma, progression or death within 24 months of treatment initiation, is an indicator of poor survival [17]. In MCL, retrospective studies identified POD24 as associated with short OS [18,19]. However, the predictive value of POD24 for OS still needed to be

assessed, using data from patients included in clinical trials. Recently, an analysis of previously untreated MCL patients enrolled in six multicenter trials conducted in France demonstrated that POD24 status is strongly associated with subsequent OS in MCL and that rituximab maintenance provided significant protection against the risk of POD24 [20]. In the present study, the OS of patients with POD24 was much lower than those who did not experience the event.

Regarding relapse, the cumulative incidences of relapse of this study were 9% and 25% at 12 and 36 months, respectively, both values lower than those reported in other studies [15]. The non-relapse mortality in the present series was 6% at one year and the main causes of non-relapse mortality were infections, as was reported in a Spanish study [21].

A positive feature of this study is the large number of patients analyzed. However, primarily due to its retrospective design, this study has several limitations, most notably missing data for variables such as Ki-67. Consequently, the biological Mantle Cell Lymphoma International Prognostic Index could not be calculated for the majority of the study population.

Conclusion

These results of ASCT for MCL in Argentina are encouraging, with >70% of patients achieving prolonged survival. Therefore, we believe this therapy still has a key role as a first-response consolidation therapy in the country. Certain factors, such as the number of prior lines ≥ 2 , age over 55 years, and comorbidities, diminish the results after ASCT. The benefit of consolidation with ASCT is less in patients with blastoid morphology, thus another more aggressive consolidation strategy should be sought for these patients. Early post-transplant relapse (POD24) confers a poor prognosis with these patients urgently requiring aggressive strategies.

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Data availability

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

Conflicts of interest

None to declare.

Editor

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Original article

Comparison of nutritional risk index and body mass index in predicting survival outcomes in patients undergoing allogeneic hematopoietic stem cell transplantation: a retrospective study



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ABSTRACT

Introduction: Allogeneic hematopoietic stem cell transplantation is a viable therapeutic option for several serious diseases however it is a high-risk procedure because it involves high-toxicity protocols with many adverse effects. Existing factors, such as the underlying disease and nutritional status, may influence the outcome. The objective of this study was to evaluate the Nutritional Risk Index as a prognostic tool by correlating it with body mass index, nutritional status, and clinical outcomes in patients undergoing hematopoietic stem cell transplantation.

Methods: This single center retrospective study was conducted collected sociodemographic, anthropometric, biochemical, and clinical data before conditioning and 30 days post-transplantation. Statistical analyses were performed using the Mann-Whitney test and Spearman's correlation. Overall survival was estimated using the Kaplan-Meier method, with comparisons conducted via the Gehan-Breslow-Wilcoxon test. A Cox Proportional Hazards regression analysis was employed to identify factors associated with mortality; variables demonstrating a p-value ≤ 0.20 in the univariate analysis were included in the multivariate model. For all analyses, statistical significance was defined as a p-value < 0.05 .

Results: Seventy-seven participants were included, with an average age of 41 years. According to the nutritional risk index, the entire sample was classified as having severe nutritional risk. The body mass index showed that 6.4 % were malnourished, 19.4 % were obese, and 12.9 % had hypoalbuminemia. The estimated survival curve identified a significant difference for patients aged < 45 years with survival being significantly longer (p-value = 0.01).

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Higher albumin levels (≥ 3.5) after transplantation were associated with longer survival (p -value = 0.04). Sex, body mass index, albumin level before conditioning, and graft-versus-host disease showed no significant differences in terms of survival. Albumin levels ≥ 3.5 g/dL after transplantation were marginally associated with a lower mortality risk and malignant disease showed a trend toward increased mortality.

Conclusion: These findings underscore the clinical utility of prognostic indices, such as the Nutritional Risk Index and albumin levels, during the pre-transplant period, emphasizing the necessity for early nutritional interventions in hematopoietic stem cell transplantation patients.

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Introduction

Hematopoietic stem cell transplantation (HSCT) is a curative therapeutic option for several onco-hematological diseases [1]. In recent years, an increase in this procedure has been observed even with the COVID-19 pandemic: 3391 HSCT performed in Brazil in 2022 and 1026 in the first quarter of 2023 [2].

Although HSCT represents a viable therapeutic option for some serious diseases, it is a high-risk procedure, as it involves high-toxicity protocols, with many adverse effects such as mucositis, febrile neutropenia, and infection [1]. Some existing factors, such as the underlying disease, number of associated comorbidities, and nutritional status, may influence the treatment outcome; others directly related to HSCT, such as the intensity of the conditioning regimen and the occurrence of graft-versus-host disease (GvHD), have important nutritional repercussions such as malabsorptive conditions and deficiencies, malnutrition and obesity especially during corticosteroid therapy, changes in metabolic energy, such as an increase or decrease in basal energy expenditure, and various biochemical changes [1,3]. However, the nutritional assessment of patients before transplantation is not an easy task [4]. Often, weight gain due to drugs such as glucocorticoids results in camouflaging changes in metabolism and tissue loss, especially muscle mass [5].

The use of the Nutritional Risk Index (NRI) is expanding because of its ease of use and good correspondence as a predictor of the interference of nutritional status on the patient's clinical outcome in different clinical situations [6]. NRI is a low-cost instrument as it uses data from biochemical tests that are routinely used in transplant centers and easy-to-obtain measurements, such as weight [7].

There is still no consensus in the literature regarding the optimal nutritional assessment method for this population. However, some studies indicate that due to treatment characteristics (such as glucocorticoid use and high-dose chemotherapy) and disease factors (including increased basal metabolic rate and hyporexia), weight fluctuations can mask the true deterioration of a patient's nutritional status. The objective of this study was to correlate NRI classification data with nutritional status, and clinical outcomes of patients undergoing HSCT [1].

Methods

This retrospective, descriptive, and exploratory study analyzed data from the electronic medical records of patients who underwent HSCT from January 2015 to December 2019 in a single center in Brazil. The study was approved by the institution's research ethics committee under the number 3.819.250.

The cohort included over 18-year-old individuals of both sexes diagnosed with neoplastic and non-neoplastic hematological diseases. The exclusion criteria were age under 18 years old, those receiving autologous transplantations and those who underwent more than one transplant.

Sociodemographic, anthropometric, biochemical, and clinical data were collected for the study population. The time points evaluated were at the beginning of conditioning (T0) and 30 days post-transplantation (T1). The following formula was used to calculate the NRI [7]:

$$NRI = (1.519 \times \text{serum albumin(g/L)} + 41.7 \\ \times (\text{present weight/usual weight})$$

The following classifications were used to stratify the risk of malnutrition: no nutritional risk ($NRI \geq 100$), mild risk ($97.5 \leq NRI < 100$), moderate risk ($83.5 \leq NRI < 97.5$), and severe risk ($NRI < 83.5$). Considering data from previous studies, it was assumed that $NRI < 97.5$ would indicate clinically significant malnutrition [6,8,9]. In this study, patients did not receive standardized nutritional support protocols. Parenteral nutrition was initiated only in specific clinical situations, particularly in cases of severe hyporexia and Grade 4 mucositis in which oral intake is not feasible. None of the patients received enteral nutrition during the study period. Therefore, nutritional management was individualized based on clinical judgment and patient tolerance. The primary outcomes were the NRI classifications before and after HSCT. These classifications were analyzed in relation to sex, etiopathogenesis (neoplastic versus non-neoplastic), and clinical outcomes, including GvHD, disease recurrence, and mortality.

Statistical analyses were performed using the STATA software employing the Mann-Whitney non-parametric test for numerical variables (NRI, albumin, and body mass index [BMI]). The non-parametric Kruskal-Wallis test was used to

analyze the albumin levels at different time points. For NRI and BMI, the Wilcoxon signed-rank test was applied to paired samples. The correlation between NRI (before and after) and albumin level (before and after) was assessed using Spearman's rank correlation coefficient.

Overall survival curves were estimated using the Kaplan-Meier method and compared between groups using the Gehan-Breslow-Wilcoxon test, considering variables such as age, sex, BMI, albumin levels, and disease type. In addition, Cox proportional hazards regression was performed by including variables with p -values ≤ 0.20 in the univariate analysis. Statistical significance was defined as p -value < 0.05 for all analyses.

Results

Table 1 summarizes the clinical characteristics of the study population. During the study period, a total of 151 transplants were performed at the center. After the exclusion of 48 pediatric patients and 41 patients who underwent a second transplant or autologous HSCT, the final cohort comprised 77 participants with a mean age of 41 years. Hematological neoplasms accounted for 77 % of the sample. Additionally, over

80 % of transplants were performed using related-donor grafts, with 67 % of stem cells being derived from bone marrow.

Data on the conditioning regimen are presented in Table 1. About 65 % of the sample received the myeloablative protocol. Approximately 28 % of patients were diagnosed with GvHD, with the highest incidence (13 %) being skin GvHD. Approximately 71 % of the patients were in remission and 28.6 % died or relapsed.

The NRI values were significantly below the clinical threshold, classifying all patients as having a severe risk of malnutrition at baseline (T0; mean: 49.37). These values remained low at the subsequent assessment (T1; mean: 47.54), showing a significant decline (p -value = 0.003). Similarly, the BMI values differed significantly (p -value = 0.01) between T0 (mean: 25.44) and T1 (mean: 25.08). Despite this decrease, the participants remained classified as overweight at both time points (Table 2).

The mean albumin values at T0 and T1 were 3.89 ± 0.39 and 3.88 ± 0.60 , respectively. Table 3 shows the correlation between NRI and albumin levels. The NRI T1 score was positively correlated with the T1 albumin level ($r = 0.485$; p -value = 0.000).

On comparing albumin levels with the outcome, significantly higher albumin values at T0 were found in the remission group (p -value = 0.048). The NRI score and BMI did not differ significantly between the outcomes (Table 4).

When comparing albumin levels with the GvHD site, significantly higher albumin values at T0 were found in the two or More-site group (p -value = 0.056). The NRI score and BMI did not differ significantly between the GvHD site groups (Table 5).

The estimated survival curves (Figure 1) demonstrated that an age of < 45 years was significantly associated with a higher survival rate (p -value = 0.01). Higher albumin levels (≥ 3.5 g/dL) at T1 were associated with longer survival (p -value = 0.04), whereas malignant diseases were associated with shorter survival (p -value = 0.006). Conversely, sex, body mass index (BMI), albumin levels at T0, and GvHD did not show any significant association with survival outcomes.

In the multivariate Cox regression analysis, sex (p -value = 0.36) and age (p -value = 0.42) were excluded from the model because of the lack of statistically significant associations with the outcome. Among the variables retained in the model, albumin levels ≥ 3.5 g/dL at time point T1 showed a marginal association with a lower risk of death (p -value = 0.07; OR = 0.40; 95 % CI: 0.15–1.07), suggesting a trend toward a 60 % reduction in the odds of mortality compared to lower levels. Similarly, the presence of malignant disease was associated with a trend toward increased risk of death (p -value = 0.06;

Table 1 – Sample characterization.

		n	%
Sex	Female	34	44.2
	Male	43	55.8
Illness	Malignant	59	76.6
	Not Malignant	18	23.4
BMI	Normal	57	74
	Underweight	5	6.4
	Obese	15	19.4
NRI	Severe	77	100
	Moderate	0	0
	Mild	0	0
Albumin	No risk	0	0
	> 3.5	67	87
	< 3.5	10	12.9
Progenitor cell source	Bone Marrow	52	67.5
	Peripheral blood	24	31.2
	Umbilical cord	1	1.3
Related Donor	Related	62	80.5
	Unrelated	15	19.5
Conditioning Regimen	Myeloablative	50	64.9
	Non-myeloablative	5	6.5
	Reduced Intensity	22	28.6
GvHD	Yes	28	36.4
	No	49	63.6
GvHD site	Skin	10	13.0
	Mouth	2	2.6
	Gastrointestinal tract	2	2.6
	Liver	1	1.3
	≥ 2 sites	13	16.9
Outcome	Remission	55	71.4
	Others*	22	28.6

GvHD, graft-versus-host disease; BMI, body mass index; NRI, nutritional risk index;

* Note: Other outcomes included relapse and death.

Table 2 – Comparing nutritional risk indexes and body mass indexes at the beginning of conditioning (T0) and 30 days post-transplantation (T1).

Index	T0 (n = 77)	T1 (n = 77)	p-value
NRI	49.29 ± 7.57	47.54 ± 4.76	0.003
BMI (kg/m ²)	25.44 ± 5.23	25.08 ± 4.89	0.01

NRI: Nutritional risk index; BMI: Body mass index.

Table 3 – Correlation of nutritional risk index with albumin level.

	NRI T0 (n = 77)	NRI T1 (n = 77)
Albumin T0	r = 0.148 p-value = 0.199	r = 0.35 p-value = 0.766
Albumin T1	r = 0.160 p-value = 0.166	r = 0.485 p-value = 0.000

NRI: Nutritional Risk Index.

Table 4 – Comparison of outcomes with albumin, nutritional risk index and body mass index.

	Remission (n = 55)	Other outcomes (n = 22)	p-value
Albumin T0 (g/dL)	3.96 ± 0.36	3.74 ± 0.44	0.048
Albumin T1 (g/dL)	3.97 ± 0.46	3.64 ± 0.83	0.102
NRI T0	49.92 ± 8.19	47.96 ± 4.92	0.420
NRI T1	49.98 ± 5.68	46.50 ± 4.25	0.470
BMI T0 (kg/m ²)	25.59 ± 5.61	25.05 ± 4.25	0.866
BMI T1 (kg/m ²)	25.16 ± 5.18	24.88 ± 4.20	0.752

NRI: Nutritional risk index; BMI: Body mass index.

*Note: Other outcomes were relapse and death.

OR = 7.03; 95 % CI: 0.89–55.44), corresponding to approximately sevenfold higher odds of mortality compared to other conditions, although this result was not statistically significant.

Discussion

This study highlights the importance of using instruments for the early assessment of nutritional status combining biomarkers with anthropometric data, such as the NRI. Despite the sample exhibiting normal mean serum albumin levels and a mean BMI categorized as overweight prior to transplantation, severe nutritional risk was identified across the entire cohort. These findings reinforce the importance of selecting accurate predictive tools and the early initiation of nutritional monitoring.

Albumin and NRI data at T1 seem to be associated with difficulties in maintaining an adequate nutritional intake in this treatment period [1,10,11]. During conditioning, patients

experience hyporexia, nausea, and mucositis, which limit satisfactory energy and protein intake [11]. Therefore, the positive and significant correlation in this period reflects difficulties related to nutritional support.

Regarding the comparison between NRI and BMI, the data showed a significant difference between time points for both indexes, but did not indicate differences in the respective classifications compared to the beginning of treatment. It is worth noting that comparing these data with those of Sagou et al., higher mean BMI and lower NRI values were observed, signaling different characteristics in the samples regarding nutritional status [7]. This finding may reflect population characteristics, such as a difference in the overweight and obese numbers in the Brazilian population, as well as a worsening of the nutritional quality of food associated with the consumption of ultra-processed foods [12].

Patients whose outcome was remission showed significantly higher serum albumin levels in the initial assessment when compared to the other outcome group (death & relapse). This finding may be associated with the mobilization of acute-phase response proteins during inflammation, indicating the clinical role of albumin as a biomarker [13].

With respect to survival, it is noteworthy that serum albumin at T1 was associated with greater survival, probably not only because it is a nutritional marker but also because it indicates inflammation. The post-transplant period is characterized by inflammation and, as such, there is a re-prioritization of the hepatic protein metabolism, which can result in reduced serum levels of negative acute-phase proteins, such as albumin.

The findings regarding albumin levels provided an important insight into potential prognostic factors in the transplant population, although the associations observed did not reach the conventional levels of statistical significance. The trend toward a protective effect of higher albumin levels (≥ 3.5 g/dL) on mortality aligns with previous studies that highlighted serum albumin as a surrogate marker of nutritional and inflammatory status, both of which are known to influence clinical outcomes in critically ill and immunocompromised patients [14,15]. Although the association was marginal (p-value = 0.07), the observed 60 % reduction in the odds of death suggests that albumin may play a clinically relevant role in risk stratification and should be further explored in larger, adequately-powered cohorts.

Similarly, the association between malignant disease and a seven-fold increase in the odds of mortality (p-value = 0.06) underscores the severity and complexity of outcomes in

Table 5 – Comparison of graft-versus-host disease site with albumin, nutritional risk index and body mass index.

	Skin (n = 10)	Two or more sites (n = 13)	Other sites* (n = 5)	p-value
Albumin T0 (g/dL)	3.79 ± 0.30	3.89 ± 0.40	3.80 ± 0.53	0.056
Albumin T1 (g/dL)	3.62 ± 0.26	3.70 ± 0.37	4.00 ± 0.55	0.533
NRI T0	52.72 ± 13.19	46.94 ± 5.27	48.84 ± 3.71	0.592
NRI T1	47.06 ± 2.98	45.92 ± 2.40	47.59 ± 1.00	0.325
BMI T0 (kg/m ²)	27.95 ± 6.96	23.29 ± 3.87	26.96 ± 7.61	0.284
BMI T1 (kg/m ²)	26.55 ± 6.22	23.32 ± 3.73	26.56 ± 6.63	0.490

NRI: Nutritional risk index; BMI: Body mass index.

* Note: Other sites included the mouth, gastrointestinal tract, and liver.

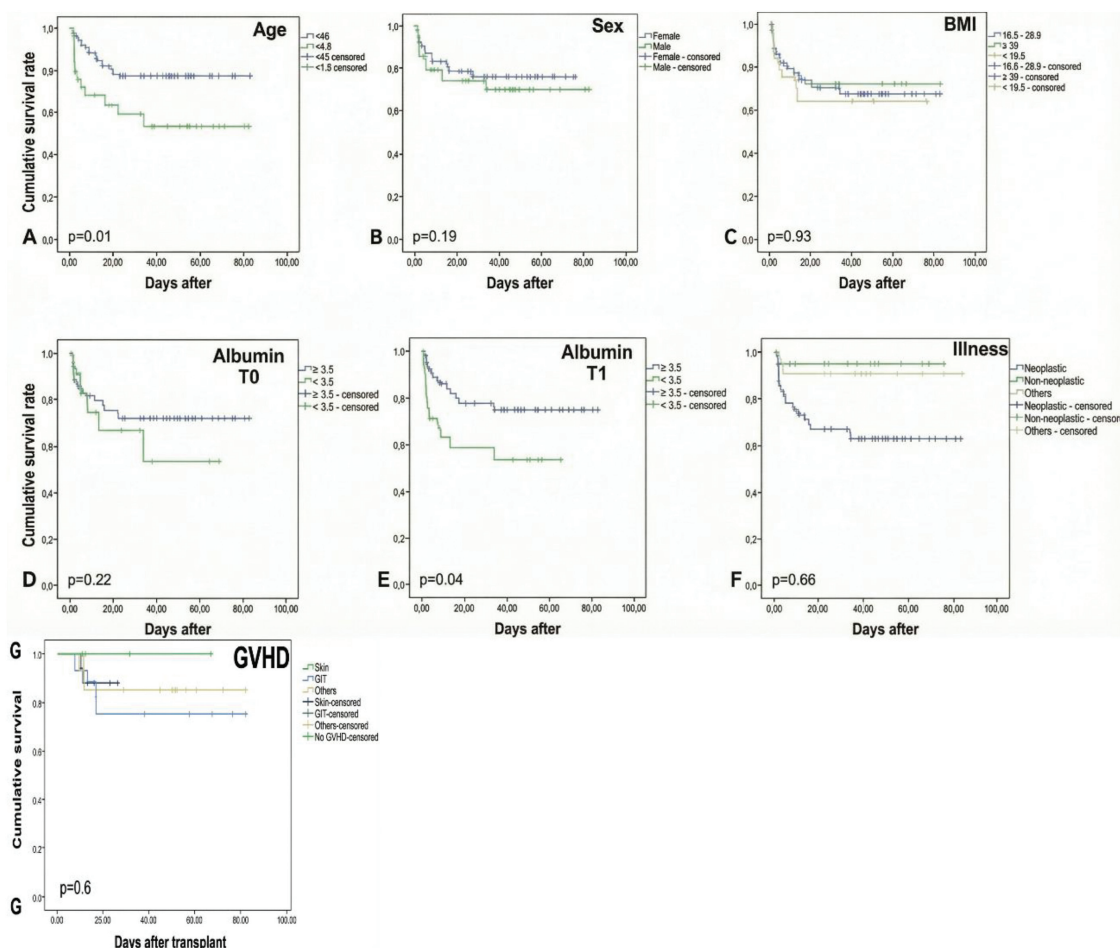


Fig. 1 – Survival curves. A: Estimated survival curve according to age. B: Estimated survival curve according to sex. C: Estimated survival curve based on body mass index. D: Estimated survival curve according to albumin level at T0. E: Estimated survival curve according to albumin level at T1. F: Estimated survival curve according to disease type. G: Estimated survival curve based on graft-versus-host disease

patients with underlying cancer, although this finding was not statistically significant. The wide confidence interval (0.89–55.44) reflects sample size limitations and heterogeneity but may indicate a meaningful trend. These results support the hypothesis that disease type, particularly malignancy, may significantly affect post-transplant prognosis, reinforcing the need for disease-specific approaches in clinical management and risk modeling.

This study has several limitations that should be considered when interpreting the findings. First, the potential for selection bias cannot be excluded, given that participants were included based on clinical and available nutritional data, which may have led to an underrepresentation of patients with more severe conditions. Second, the unicentric nature of the study may limit the generalizability of the results, as institutional protocols, patient profiles, previous nutritional conditions, and care practices may differ between centers. Additionally, the heterogeneity of the sample, which included both malignant and non-malignant conditions, may have introduced confounding effects owing to differences in disease trajectory, conditioning regimen intensity, and

nutritional risk. Although these factors reflect the real-world complexity of clinical practice, they may have influenced the observed associations and should be addressed in future multicenter studies with more homogeneous populations and standardized protocols.

Despite this, the NRI appears to be an efficient instrument for screening patients who will undergo HSCT, allowing teams to organize themselves according to levels of nutritional care, ensure adequate nutritional intervention strategies, and contribute to better treatment outcomes.

Data availability

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

Conflicts of interest

The author declares no conflicts of interest.

Editor

Eduardo Rego

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Original article

Evaluation of vaccine-induced antibody responses against SARS-CoV-2 in multiple myeloma patients from the northeastern region of Mexico



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ABSTRACT

Objective: To evaluate immunoglobulin G antibody responses against Severe Acute Respiratory Syndrome Coronavirus 2 in multiple myeloma patients in the northeastern region of Mexico following different COVID-19 vaccination regimens.

Methods: This retrospective study included 33 multiple myeloma patients, and 28 healthy controls vaccinated with mRNA (BNT162b2, mRNA-1273) or adenovector (AZD1222) vaccines. Anti-spike receptor-binding domain (RBD) and anti-nucleocapsid immunoglobulin G levels were measured. Laboratory parameters, including monocyte and lymphocyte counts and serum-free light chains, were analyzed. Statistical comparisons used ANOVA, Kruskal-Wallis, and logistic regression.

Results: No significant differences in anti-spike receptor-binding domain immunoglobulin G levels were observed between multiple myeloma patients and healthy controls after one vaccine dose. Multiple myeloma patients who received ≥ 3 doses showed higher anti-spike receptor-binding domain immunoglobulin G levels compared to single-dose recipients. Anti-nucleocapsid antibodies (indicative of prior subclinical infection) were detected in 51.5% of multiple myeloma patients and correlated with elevated anti-spike receptor-binding domain levels. Patients in the third anti-spike immunoglobulin G quartile (10,427.9–23,954.0 AU/mL) had higher monocyte counts compared with patients in the lower quartiles. No correlations were found between anti-spike immunoglobulin G and lymphocyte counts, serum proteins, or gamma globulins. Abnormal kappa/lambda light chain ratios did not impair antibody responses in nucleocapsid-positive patients.

Conclusion: Multiple myeloma patients achieved comparable immunoglobulin G responses to healthy controls after one vaccine dose, with enhanced responses following ≥ 3 doses.

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Subclinical infections may augment humoral immunity. Elevated monocyte counts are indicative of innate immune activation following the administration of the vaccine. These findings support prioritizing booster doses for multiple myeloma patients despite immunosuppressive therapies.

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Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by the clonal expansion of plasma B cells that are committed to producing a specific type of immunoglobulin, which can serve as a tumor antigen. The disease is also marked by a heterogeneous accumulation of tumor cells within the bone marrow, leading to clinical complications such as anemia, lytic bone lesions, hypercalcemia, renal failure, and increased susceptibility to infections. This heightened vulnerability to infections is directly associated with the aberrant expression of transcription factors and the impaired co-stimulatory signaling between B cells and T helper cells during antigen presentation [1].

The immunocompromised state in MM patients, further exacerbated by immunomodulatory treatments and, more recently, by using anti-CD38 monoclonal antibodies, can significantly impair adaptive immunity and increase the risk of infections. During the COVID-19 pandemic, vaccination was strongly recommended for all hematological patients, as they are a high-risk group for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection and develop severe disease [2]. Although some MM patients may exhibit reduced responses to vaccines, vaccination still provides potential benefits in this vulnerable population [3]. Immunosuppression in MM patients affects all effector cells of the immune system [1,4]. Given this immunological landscape, which impairs effective antigen presentation and hinders the development of adaptive immunity and long-term protection against infections, it is crucial to assess the long-term efficacy of vaccines in these patients to establish the optimal vaccination schedule.

Immunological traits, which are associated with a low specific antibody response to vaccination, are commonly found in MM patients. These traits include older age, kidney function alteration, lymphocytopenia, low immunoglobulin levels, and the need for a second line of treatment, and also in patients not in complete remission [5,6].

Therefore, this study aims to evaluate the anti-spike receptor-binding domain (RBD) immunoglobulin G (IgG) response in MM patients who followed different vaccination regimens during the 2020 COVID-19 pandemic. The patients were attended at the University Hospital in the Northeast of Mexico.

Patients and methods

Study group

Patients with confirmed diagnosis of MM and who were being treated in the University Hospital were invited to participate.

Patients previously vaccinated against SARS-CoV2 with mRNA vaccines (BNT162b2 and mRNA1273) or the adenovector vaccine AZD1222 vaccine were eligible if they were aged 18 to 65 and had an Eastern Cooperative Oncology (ECOG) performance status score between 0 and 2 (on a 5-point scale, where higher scores represent increased disability). Individuals were excluded if they had active disease, central nervous system involvement, primary amyloidosis, or showed inadequate hematologic, hepatic, renal, or cardiac function. The enrolled patients and controls should have at least received a single dose of one of the following three COVID-19 vaccines authorized for emergency or full use during the study period (2020–2021): the mRNA vaccines BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna), and the adenoviral vector vaccine AZD1222 (AstraZeneca-Oxford).

Healthy controls were recruited through convenience sampling from hospital staff and patient companions. The following exclusion criteria were applied specifically to the control group: immunosuppressive conditions (including autoimmune diseases, immunomodulator use, or HIV infection), active infection at recruitment, history of hematologic malignancies, and prior organ or stem cell transplantation. All controls received their SARS-CoV-2 vaccinations according to the national guidelines during the same epidemiological period as patients, ensuring comparable exposure to circulating variants.

Patients and controls provided both written and verbal informed consent for trial participation. Patient clinicodemographic characteristics and outcomes were retrospectively gathered from their electronic medical records. No interventions were conducted as part of this study. This research adhered to institutional guidelines and received approval from the Institutional Review Board (IN23–00,003) and the School of Medicine Ethics Committee. Additionally, the study followed Good Clinical Practice standards Practices.

Assessment of serological response

This study included 33 patients with MM and 28 healthy controls. Blood samples were obtained from each patient and control; the sera were recovered by centrifugation and stored at -80°C until use. Total IgG antibodies directed against the RBD of the SARS-CoV-2 spike protein were quantified using the SARS-CoV-2 IgG II Quant assay (Abbott). Additionally, qualitative detection of IgG directed against the nucleocapsid protein was performed using the SARS-CoV-2 IgG assay (Abbott), in accordance with the manufacturer's established protocols. The cutoff for RBD IgG antibodies was ≥ 250 AU/mL according to previous reports [5] and the cutoff for the

nucleocapsid antibodies index was ≥ 1.40 S/C, according to the manufacturer's guidelines.

Laboratory findings

A complete blood count and routine serological assessment of MM patients, including total proteins, gamma globulin, and serum-free chains, were performed along with the serum sample for IgG antibody determination with the results being obtained from electronic health records following the attainment of informed consent from all participants and formal approval from the Institutional Review Board.

Statistical analysis

Statistical analyses were performed using Graph Pad Prism-8 (La Jolla, GraphPad Software). Normality was analyzed using Shapiro-Wilk test. The differences between the group means were evaluated using one-way ANOVA followed by Tukey's post hoc test, or Kruskal-Wallis followed by Dunn's post hoc test for multiple comparisons. P-values < 0.05 was considered statistically significant.

Results

A total of 33 patients and 28 healthy controls were included in the study. The patient group comprised 20 males (60.6 %) with a mean age of 65.76 ± 11.2 years, while the healthy control group consisted of nine males (32.1 %) with a mean age of 47 ± 22.0 years.

Patient characteristics

Patients were treated with bortezomib, lenalidomide and dexamethasone (VRD) induction therapy. Nineteen patients (57.6 %) underwent autologous stem cell transplant (ASCT) with the antibody levels being obtained at least six months post-transplant. Maintenance treatment included lenalidomide 10 mg/day or bortezomib 1.3 mg/m² every other week. All patients were in very good partial response or better at the time of study.

The evaluation of serum immunoglobulins and light chains demonstrated a notable predominance of IgG, accounting for 71.4 % (95 % confidence interval [95 % CI]: 52.9–84.9 %) of the cases analyzed. Remarkably, even at the lower limit of the confidence interval, IgG constituted the most prevalent isotype, appearing in more than 50 % of the cases. In the analysis of light chains, kappa was observed to have a slightly higher prevalence, represented in 53.6 % (95 % CI: 35.8–70.5 %), compared to lambda, which accounted for 46.4 %. However, this difference lacked statistical significance.

A distinct trend was evident in the immunoglobulin A (IgA) cases, where lambda light chains observed in 75 % of these cases (6 out of 8), predominated. Nonetheless, the limited sample size of IgA patients led to a considerable degree of uncertainty in this estimate (95 % CI: 10.0–40.1 %).

Patient characteristics, vaccination status, anti-SARS-CoV-2 antibody levels, and laboratory findings are summarized in Table 1. Notably, only one patient tested negative for IgG

spike-RBD antibodies, with a 2.2 level of AU/mL. Additionally, 17 patients (51.5 %) were positive for IgG nucleocapsid antibodies. Regarding vaccination status, 13 patients (39.4 %) had received a single vaccine dose. Of these, 12 (92.3 %) were vaccinated with BNT162b2, while only one (7.7 %) received AZD1222. The only patient who was negative for spike RBD antibodies received one dose of the BNT162b2 vaccine. In respect the administration of multiple doses of the vaccine, no patients reported the use of two doses. Twelve patients (36.4 %) reported they had received three doses, consisting of various combinations of BNT162b2 (B), AZD1222 (A), and mRNA-1273 (M). Of these, six (50 %) received three doses of AZD1222, two (16.7 %) received the BBA combination, and the remaining patients received the following regimens: BAA (8.3 %), ABB (8.3 %), BBB (8.3 %), and MMM (8.3 %). Seven patients (21.2 %) had received four doses of the vaccine: six (85.7 %) received four doses of AZD1222, and one patient (14.3 %) received four doses of mRNA-1273. Finally, one patient had received five doses, with the vaccination scheme being BBMMB.

Control characteristics

A total of 28 healthy controls were included for the assessment of antibody response after a single dose of the mRNA vaccines BNT162b2 and mRNA1273. The healthy control group consisted of 28 participants, with nine males (32.1 %) and a mean age of 47 years (standard deviation ± 22.0). All controls received mRNA vaccines as their most recent dose, with Pfizer-BioNTech (BNT162b2) being the most common (64.3 %; $n = 18$), followed by Moderna (mRNA-1273 - 35.7 %; $n = 10$). Serological analysis revealed robust immunogenicity, with 92.9 % ($n = 26$) showing positive anti-spike-RBD IgG responses. The median antibody titer was 8201 AU/mL (inter-quartile range [IQR]: 1354–19,219), indicating substantial variation in individual humoral responses. Only two participants (7.1 %) had negative antibody results, potentially reflecting individual differences in immune reactivity to vaccination.

Serum immunoglobulin G response to the severe acute respiratory syndrome coronavirus 2 vaccination

The IgG levels of anti-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) were compared between healthy controls (with a single dose of the vaccine when they were included in the study) versus MM patients (with a single dose and ≥ 3 doses). A significant difference was found between the study groups in the non-parametric analysis (p-value < 0.05); however, in the multiple comparison analysis no differences were identified between the study groups (p-value > 0.05) (Figure 1a). Additionally, the group receiving three or more doses showed significantly elevated levels of anti-nucleocapsid antibodies above the cutoff point of 1.4 S/C (p-value < 0.001), thus suggesting an unreported prior subclinical infection (Figure 1b). No significant differences were observed between the groups in other laboratory findings. The patients were divided into groups based on their vaccination schemes and anti-nucleocapsid serological status. The group of MM patients that received three or more doses and with anti-nucleocapsid antibodies showed higher levels of IgG against

Table 1 – Descriptive statistics for the study population.

Total patients	33
Males - n (%)	20 (60.6)
Age (years) - mean (SD)	65.76 (11.2)
Vaccination status (n = 33)	
Total number of vaccines received - median (IQR)	3 (1–3.5)
Time elapsed since last vaccination - n (%)	
<1 year	5 (15.1)
>1 year	18 (54.5)
Not reported/ Does not remember	10 (30.3)
Brand of the last dose received - n (%)	
Pfizer (BNT162b2)	22 (66.6)
AstraZeneca (AZD1222)	9 (27.3)
Moderna (mRNA-1273)	2 (6.1)
Modality of the last dose received - n (%)	
mRNA	24 (72.7)
Adenovector	9 (27.3)
Anti SARS-CoV2 antibodies	
IgG anti-spike-RBD (AU/mL) - median (IQR)	10,428 (2323–26,048)
IgG anti-spike response - n (%)	
Positive	32 (96.97)
Negative	1 (3.03)
IgG anti-nucleocapsid (S/C index) - median (IQR)	1.71 (0.08–5.68)
IgG anti-nucleocapsid response - n (%)	
Positive	17 (51.5)
Negative	16 (48.5)
Laboratory findings	
Hemoglobin (g/dL) (n = 32) - mean (SD)	12.66 (1.80)
White blood cell count ($\times 10^3/\mu\text{L}$), (n = 32) - mean (SD)	5.56 (1.98)
Platelets ($\times 10^3/\mu\text{L}$) (n = 32) - median (IQR)	191.00 (151.0–261.8)
Lymphocytes ($\times 10^3/\mu\text{L}$) (n = 32) - mean (SD)	1.71 (0.71)
Monocytes ($\times 10^3/\mu\text{L}$) (n = 32) - median (IQR)	0.61 (0.422–0.80)
Neutrophils ($\times 10^3/\mu\text{L}$) (n = 32) - mean (SD)	2.78 (1.21)
Eosinophils ($\times 10^3/\mu\text{L}$) (n = 32) - median (IQR)	0.15 (0.09–0.22)
Basophils ($\times 10^3/\mu\text{L}$) (n = 32) - median (IQR)	0.02 (0.01–0.04)
Serum total proteins (g/dL) (n = 32) - mean (SD)	6.50 (0.83)
Serum gamma globulin (g/dL) (n = 31) - median (IQR)	0.80 (0.40–1.20)
Serum kappa free light chains (mg/L) (n = 27) - median (IQR)	31.27 (15.44–68.08)
Serum lambda free light chains (mg/L) (n = 27) - median (IQR)	21.55 (13.58–35.70)
Serum kappa/lambda ratio (n = 27) - median (IQR)	1.36 (0.84–4.12)
Characteristics of controls	
Total (n)	28
Males - n (%)	9 (32.1)
Age (years) - mean (SD)	47 (22.0)
Brand of the last dose received - n (%)	
Pfizer (BNT162b2)	18 (64.3)
Moderna (mRNA-1273)	10 (35.7)
Modality of the last dose received n (%)	
mRNA	28 (100)
Anti SARS-CoV2 antibodies	
IgG anti-spike-RBD (AU/mL) - median (IQR)	8201 (1354–19,219)
IgG anti-spike response - n (%)	
Positive	26 (92.9)
Negative	2 (7.1)

mRNA: Messenger Ribonucleic Acid; Adenovector: Adenovirus vector; IgG: Immunoglobulin G;.
 *Data is presented as mean $\pm$ standard deviation (SD) for normally distributed variables and as median (interquartile range, IQR) for non-normally distributed variables.

spike RBD compared to those who had lower values of anti-nucleocapsid antibodies (p-value = 0.0024) (Figure 1c).

In a logistic regression model, this study aimed to predict whether high levels of Anti-spike RBD IgG were associated with the presence of anti-nucleocapsid antibodies. Although a significant relationship was found between these variables

(p-value = 0.0007), the practical impact was minimal, as indicated by the odds ratio (1.000) and a small coefficient B (–0.000138). Therefore, anti-spike RBD levels are not a strong predictor of subclinical infection in this patient cohort. Additionally, non-significant correlations were observed between serum anti-spike RBD IgG levels and various laboratory

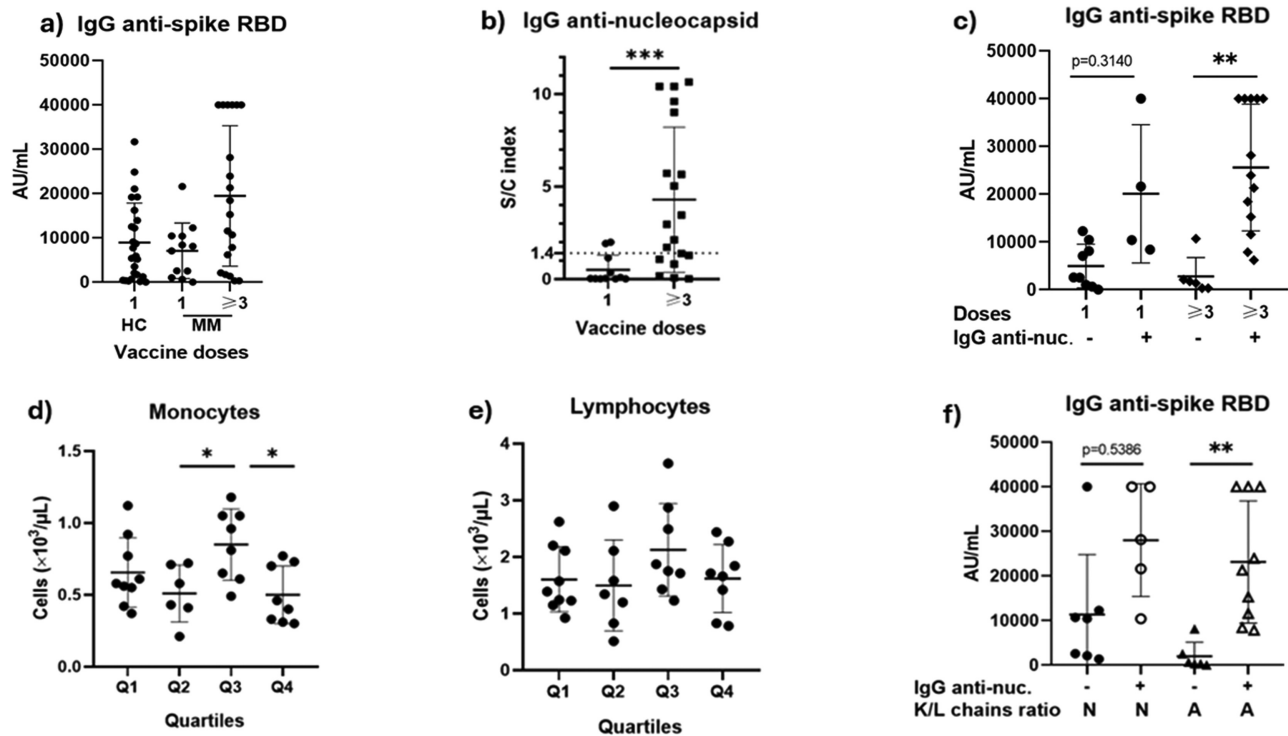


Figure 1 – Evaluation of anti-spike receptor-binding domain (RBD) immunoglobulin G (IgG) concentrations in patients with multiple myeloma (MM). a) Serological assessment of levels of anti-spike RBD IgG in healthy controls and MM patients, and b) anti-nucleocapsid IgG of patients who received 1 dose of anti-SARS-CoV-2 vaccine and three or more doses; c) Comparison based on the vaccination doses and anti-nucleocapsid IgG positive or negative status; d) Assessment of monocytes and e) Lymphocytes in the complete blood count of peripheral blood according quartile classification of anti-spike RBD IgG antibody levels (Q1 ≤ 2523.2 AU/mL, Q2 = 2523.2–10,427.9 AU/mL, Q3 = 10,428.0–23,954.0 AU/mL, and Q4 $\geq 23,955.0$ AU/mL); f) Analysis of patients with a subclinical infection and normal (N) or abnormal (A) kappa/lambda serum free chain ratio. The differences between the groups means were evaluated using one-way ANOVA followed by Tukey's post hoc test, or Kruskal-Wallis followed by Dunn's post hoc test for multiple comparisons. P-value < 0.05 was considered statistically significant. * P-value < 0.05 , ** P-value < 0.01 , and *** P-value < 0.001 .

parameters, including the lymphocyte count (Pearson's: $r = 0.0310$; p-value = 0.8650), monocyte count (Spearman's: $r = -0.0930$; p-value = 0.6120), total serum proteins (Pearson's: $r = 0.1790$; p-value = 0.3280), and serum gamma globulins (Spearman's: $r = 0.1980$; p-value = 0.2860).

The levels of anti-spike RBD antibodies were classified in MM patients into quartiles to assess potential differences in laboratory findings. The quartile ranges were as follows: Q1 ≤ 2523.2 AU/mL, Q2 = 2523.3–10,427.9 AU/mL, Q3 = 10,428.0–23,954.0 AU/mL, and Q4 $\geq 23,955.0$ AU/mL. MM patients with anti-spike RBD antibodies in Q3 had an elevated number of monocytes compared to Q2 (p-value < 0.05) and Q4 (p-value = 0.0216) (Figure 1d). No other significant changes were observed regarding lymphocytes (Figure 1e) or other laboratory findings. Additionally, based on the normal Serum-Free Kappa/Lambda (κ/λ) light chain ratio reference range (0.26–1.65), patients were grouped to assess whether abnormal ratios were associated with significant changes in the anti-spike RBD IgG levels in patients with a subclinical infection (Figure 1f). Among patients exhibiting an abnormal κ/λ ratio, those with detectable Anti-nucleocapsid IgG demonstrated significantly higher RBD IgG titers (p-value < 0.01). These data

suggest that despite the presence of underlying monoclonal plasma cell proliferation, these individuals maintained a robust humoral response following subclinical SARS-CoV-2 infection. There are no significant differences in any other laboratory findings between the groups with an abnormal κ/λ ratio compared with those who had a normal ratio.

Additionally, the antibody response was evaluated in the patients who had undergone ASCT compared to those who had not. No statistically significant differences were observed in anti-spike RBD IgG levels (p-value = 0.8709) or anti-nucleo-protein antibodies (p-value = 0.1011).

Discussion

Patients with MM often exhibit compromised immune responses due to impaired antigen presentation and antibody production. In this cohort, no significant differences were observed in anti-spike RBD IgG antibody levels between the healthy control group (individuals who had received a single vaccine dose) and MM patients who had received one or three doses. This contrasts with a previous study reporting that

smoldering and symptomatic MM patients had a lower antibody response compared to healthy individuals [6]. The findings of this study suggest that, in this cohort, the protective anti-SARS-CoV-2 immune response was not significantly affected by MM treatment. It is important to mention that patients who received three or more vaccine doses tended to have higher antibody levels compared to the patients who received a single dose; this finding suggests that multiple vaccine doses may help overcome these limitations, enhancing vaccine immunogenicity. This is supported by a study showing that a third dose of the mRNA COVID-19 vaccine significantly improved the humoral response, even in individuals who did not respond to the first or second dose [5]. It is important to mention that the presence of anti-spike antibodies in serum is not an indicator of neutralizing antibodies, as up to one third of MM patients did not present virus-neutralizing antibodies, even after two doses of the vaccine, depending on their race, disease status and treatment [7].

Regardless of the inclusion criteria considered, some patients were found to have a possible subclinical infection identified through the presence of positive IgG antibodies against the viral nucleocapsid. Patients with higher levels of anti-nucleocapsid antibodies also showed elevated anti-spike antibody levels; this association was statistically significant among those with more than three vaccine doses. The elevated levels of antibodies, anti-nucleocapsid and anti-spike RBD, were also reported after six months of acute SARS-CoV-2 infection in 9/10 MM patients [8], indicating that the presence of anti-nucleocapsid antibodies in asymptomatic MM patients suggests prior exposure to SARS-CoV-2.

The present analysis revealed no significant differences in anti-spike RBD IgG or anti-nucleocapsid antibody levels between ASCT recipients and non-recipients. This finding suggests that prior ASCT may not independently impair humoral responses to COVID-19 vaccination in MM patients, at least among those with adequate immune reconstitution (e.g., vaccinated ≥ 6 months post-transplant). Notably, all ASCT recipients in the current cohort were on lenalidomide or bortezomib maintenance, which may have supported immune recovery [5]. However, the small sample size limits definitive conclusions, and larger studies stratifying patients by time since transplantation, conditioning regimens, and maintenance therapies are warranted.

In the context of MM, it is relevant to explore whether these patients rely on cellular immunity to compensate for their limited humoral response due to their immunocompromised status. To address this, the cellular response was analyzed depending on the levels of anti-spike RBD antibodies. This showed that patients with antibody levels in the third quartile, displayed elevated monocyte counts. The increase in monocytes within this group may indicate innate immune activation related to vaccination or a prior subclinical infection. In MM patients, monocytes might play a compensatory role in innate immunity due to deficits in adaptive responses. Alternatively, this could represent a marker of mild systemic inflammation, as the SARS-CoV-2 virus can infect monocytes via Fc γ -receptors, activating NLRP3 inflammasomes, resulting in the secretion of IL-18 and IL-1 β , cell death by pyroptosis, contribute to systemic inflammation, severe COVID-19, and

dissemination of the virus from the lungs [9,10]. The main limitations of this study are the small cohort size, the lack of an alternate method to measure anti-nucleocapsid IgG antibodies, and the potential for previous infection and neutralizing antibodies were not determined.

On the other hand, anti-RBD IgG levels provide valuable insights as they do not fully capture functional immunity and thus cannot be utilized as a marker of serological response [11]. Neutralizing antibodies and T-cell responses are critical for protection, especially in immunocompromised cohorts. Future work should integrate viral neutralization tests and Interferon-gamma release assays to evaluate functional immunity.

This study also reflects the reality of COVID-19 vaccination in Latin America, where heterogeneous vaccine regimens, including mixed platforms (mRNA and adenovector) and variable dosing intervals, were implemented due to supply constraints [12]. While this diversity limits direct comparisons of immunogenicity across specific vaccine types, it enhances the generalizability of our findings to real-world clinical practice in resource-variable settings. Notably, the data of this study suggest that the number of doses had a greater impact on anti-spike IgG levels than the vaccine platform itself. However, unmeasured confounding variables (e.g., interval between doses and prior undiagnosed infections) may influence these results. Future studies in similar contexts should stratify analyzes by both vaccine type and dosing intervals to better isolate these effects.

Conclusion

Similar Anti-spike RBD IgG levels were found in patients with MM compared to controls and patients with three doses of anti-SARS-CoV-2 vaccine showed higher anti-spike RBD antibodies, suggesting the treatment did not affect the antibody response in these patients.

Clinical practice points

- Prioritize ≥ 3 COVID-19 vaccine doses for MM patients to enhance antibody responses.
- Monitor for subclinical SARS-CoV-2 infections via anti-nucleocapsid antibody testing in vaccinated MM cohorts.
- Consider innate immune markers (e.g., monocytes) as potential indicators of vaccine-related immune activation.
- Reassess vaccination schedules in MM patients on immunomodulatory therapies to optimize long-term protection.

Data availability

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

Conflicts of interest

The author declares no conflicts of interest.

Editor

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Review article

Acute fibrinous and organizing pneumonia after bone marrow transplantation, an underrecognized, severe condition



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ABSTRACT

Introduction: Allogeneic bone marrow transplantation can lead to various pulmonary complications, including acute fibrinous organizing pneumonia. This condition is rare and presents with aggressive clinical features, distinct from other forms of organizing pneumonia, such as cryptogenic organizing pneumonia.

Method: A literature review using the PubMed, Embase, Lilacs, and Cochrane databases was conducted to analyze cases of acute fibrinous organizing pneumonia following transplantation focusing on clinical features, therapeutic approaches, and outcomes.

Case Report: The case of a 62-year-old female who developed acute fibrinous organizing pneumonia after transplantation for acute myeloid leukemia is presented. Despite an initial absence of infectious agents, parainfluenza virus was later identified in a bronchoalveolar lavage. The patient progressed to severe hypoxemic respiratory failure and was unresponsive to corticosteroids and rituximab, ultimately dying seven months post-transplant.

Conclusion: This is a rare and severe complication following allogeneic bone marrow transplantation. Early diagnosis, histopathological confirmation, and prompt initiation of corticosteroid therapy are critical for improving outcomes. Patients diagnosed before Day +100 generally have a better response to treatment and more favorable clinical outcomes. The

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

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need for a more effective and targeted treatment strategy remains an unmet challenge in managing this condition.

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Introduction

Long-term disease-free survival and the possibility of cure are goals for patients who undergo allogeneic bone marrow transplantation (allo-BMT). However, despite improvements in supportive care, early and late transplant-related complications remain a significant cause of morbidity and mortality.

Pulmonary infectious and noninfectious complications are reported in 30–60 % of all allo-BMT recipients and carry high morbidity and mortality rates [1,2].

Pulmonary complications encompass a heterogeneous group of conditions including graft versus host disease (GvHD), frequently manifested as bronchiolitis obliterans (BO), organizing pneumonia (OP) – which in the post-bone marrow transplant context is also known as cryptogenic organizing pneumonia – pulmonary edema, diffuse alveolar hemorrhage and idiopathic pneumonia syndrome [1].

Acute organizing fibrinous pneumonia (AFOP) is a rare presentation of acute lung injury that resembles OP. This disease was first described in 2002 as a unique histological pattern of acute lung injury that is histologically different from diffuse alveolar damage, eosinophilic pneumonia [3]. Although the pathology of diffuse alveolar damage and OP may be similar to AFOP, these are distinct conditions [4].

In lung transplant patients, chronic lung allograft dysfunction shares striking similarities with the pathogenesis and underlying immunopathology of lung GvHD after allo-BMT [5]. AFOP has been identified as a novel form associated with a significantly poorer prognosis than other forms of allograft dysfunction [4,6].

In this report, we present a case of AFOP following allo-BMT, potentially exacerbated by parainfluenza virus infection, which was refractory to both corticosteroids and rituximab. Furthermore, our aim was to review the characteristics of the major non-

infectious organizing pneumonias that occur post-bone marrow transplantation (Table 1). Additionally, a literature review of AFOP following allo-BMT is provided, detailing the therapeutic approaches employed and the outcomes of each case (Table 2).

Methods

A literature review was performed of cases of acute fibrinous organizing pneumonia in allo-BMT patients. The PubMed, Embase, Lilacs, and Cochrane databases were searched using the following terms “acute fibrinous and organizing pneumonia” AND “hematopoietic cell transplantation”. Table 2 presents reports of acute fibrinous and organizing pneumonia following allo-BMT between 2009 and 2024 [7–9].

Case report

A 62-year-old female patient was diagnosed with acute myeloid leukemia with low cytogenetic risk (normal cytogenetics; *NPM1* positive, *FLT3ITD/TKD* negative). She achieved complete response after receiving induction chemotherapy using the 7 + 3 regimen (cytarabine (Cytarabine) 100 mg/m² Days 1–7 and daunorubicin 90 mg/m² Day 13) followed by three cycles of consolidation with high doses of cytarabine (Cytarabine). Six months after remission, molecular relapse (*NPM1*) was diagnosed. She underwent two cycles of azacitidine (75 mg/m² Days 1–5) and venetoclax (400 mg/daily), again achieving complete response. She underwent allo-BMT with a reduced intensity conditioning regimen (fludarabine, cyclophosphamide, total body irradiation 4 Gy). The donor was her 33-year-old son, with 6 × 10⁶ CD34⁺ cells/kg from a peripheral blood source. As GvHD prophylaxis, she received cyclophosphamide, cyclosporine (cyclosporine

Table 1 – Characteristics of non-infectious pneumopathies after bone marrow transplantation.

Non-infectious pneumopathy	Pathological findings	Radiological findings	Clinical course
Organizing pneumonia	Loose organizing fibroblastic plugs: loose plugs of fibromyxoid tissue within alveolar ducts, surrounding alveoli, and focal peribronchiolar and mild-to-moderate interstitial chronic inflammation	Single or multiple, sometimes migratory alveolar opacities; ground-glass pattern; consolidations; reversed halo sign	Pneumonia-like presentation with fever, malaise, cough, and dyspnea. Usually responsive to corticosteroids, in some cases immunosuppressants may be needed. Can be recurrent
Acute fibrinous organizing pneumonia	Reactive pneumocytes and presence of fibroblastic plugs associated with intra-alveolar fibrin deposition, often organized into balls. Type II pneumocyte hyperplasia. Prominent fibrin deposition in alveolar spaces and sparse granulation tissue in the form of fibromyxoid plugs	Diffuse bilateral opacities; consolidations; nodular opacities; more diffuse distribution than classic organizing pneumonia	Acute or subacute: fever, cough, progressive dyspnea, hypoxemia, rapid progression in some cases. Poorer outcome, high mortality, may have fulminant course, unresponsive to high dose corticosteroids and immunosuppression

(cyclosporine (Cyclosporine capsules) capsules) capsules) and mycophenolate mofetil (CellCept) post-transplant.

As post-infusion complications, she presented severe bloodstream sepsis due to *Rothia sp* and *Corynebacterium*, diarrhea due to enteropathogenic *Escherichia coli* and acute kidney injury requiring dialysis support for seven days. She presented late neutrophil engraftment on the 38th day after transplantation. The MMF was suspended on Day +30. She presented mild acute GvHD of the skin which was successfully treated with topical corticosteroid therapy.

Four months after transplantation, she was hospitalized due to cough and dyspnea. A computed tomography (CT) scan showed diffuse ground glass opacities and alveolar consolidations compatible with organizing pneumonia. A bronchoalveolar lavage was performed without evidence of pathogenic agents by molecular and usual microbiological and culture techniques. Prednisone (1 mg/kg) was started, together with fluticasone, montelukast and azithromycin three times a week. The patient was discharged after two weeks and continued as outpatient for one month until she was readmitted due to worsening of the pulmonary symptoms. Another bronchoalveolar lavage was then performed that proved positive for parainfluenza virus in a respiratory virus polymerase chain reaction (PCR) panel (which had not been performed with the previous bronchoalveolar lavage). A CT scan showed worsening of the lung parenchymal opacities, and the patient had worsening of respiratory symptoms and hypoxemia. Given her clinical and radiological deterioration and the fact that the new bronchoalveolar lavage only revealed the presence of parainfluenza virus, a lung biopsy was deemed necessary for better etiological definition approximately one week after hospitalization. A CT-guided lung biopsy revealed moderate to severe fibrinoleukocyte exudate, frequent neutrophils, and areas of neutrophilic debris. The biopsy also showed organizing fibrinoid aggregates in the alveolar spaces, as well as histiocytes containing

hemosiderin. These findings were compatible with fibrinous and organizing interstitial pneumonia (Figure 1).

The patient presented progressive hypoxemic respiratory failure, requiring mechanical ventilation ten days after admission. Methylprednisolone (2 mg/kg/day) was initiated for seven days followed by methylprednisolone pulse therapy (500 mg/daily) for five days with transient, non-sustained clinical improvement. A dose of Rituximab (375 mg/m²) was then administered in the context of clinical refractoriness to corticosteroids. Despite intensive support and medication, the patient was refractory to treatment and died of intractable hypoxemic respiratory failure, approximately one month and ten days after admission and seven months after the allo-BMT.

Discussion

Table 1 shows the clinicopathological differences between the most well-known entity, OP, and AFOP. OP is characterized by intra-alveolar granulation tissue with myofibroblasts and connective tissue, while AFOP exhibits intra-alveolar fibrin aggregates with patchy distribution, organizing connective tissue, mild interstitial inflammation, and type 2 pneumocyte hyperplasia. The main clinical difference is that AFOP typically follows a much more aggressive and acute course. In the present report the diagnosis of AFOP was made in the pathology department by a pathologist specialized in respiratory diseases, who highlighted a pattern similar to that of organizing pneumonia (OP) but with some striking differences (mainly the presence of intra-alveolar fibrin deposits). The case was discussed in a multidisciplinary meeting involving a pulmonologist, radiologist and pathologist, as is recommended for the evaluation of interstitial lung diseases. This diagnosis is, indeed, challenging for pathologists without expertise in interstitial lung diseases.

To date, four cases of AFOP following allo-BMT have been described in the literature, with patient ages ranging from 25–62 years. Compared to older patients, those under the age of 60 had better outcomes, and patients diagnosed before Day +100 generally had a better response to treatment and more favorable clinical outcomes [8,9]. Regarding hematological diseases, two of the patients had leukemia and the other two had lymphoma. All underwent allo-BMT and subsequently developed AFOP, with corticosteroid therapy as part of the initial treatment in all four cases [8,9].

Simmons et al. described AFOP as a disease with a very aggressive course, associated with a high level of morbidity [9]. They reported the case of a 52-year-old male patient diagnosed with angioimmunoblastic T-cell lymphoma who developed AFOP on Day +325 after allo-BMT. He presented with a late form of AFOP, concomitant with chronic GvHD of the skin and eyes. The clinical presentation was severe, requiring mechanical ventilation and high-dose immunosuppression with methylprednisolone (250 mg q6h) and tacrolimus. Due to the refractoriness of the condition, etanercept (25 mg twice weekly for eight doses) was introduced. The authors suggested that the response to etanercept could highlight the role of tumor necrosis factor-alpha in a cytokine-modulated immune response causing inflammation and fibrosis [9].

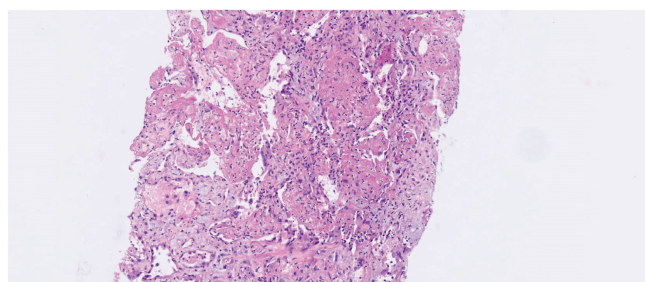


Figure 1 – Fibrinous and organizing interstitial pneumonia with neutrophilic exudate - histological sections of the lung parenchyma showing moderate to severe exudate fibrinoleukocyte with frequent neutrophils and areas of neutrophilic debris. Presence of fibrinoid aggregates in organization in the alveolar spaces, with histiocytes containing hemosiderin. Pulmonary interstitium with mild edema and immature fibroplasia (focal myofibroblastic aggregates). No granulomas or malignancy were identified in the sample. Acid-resistant bacilli, bacteria and fungi by Ziehl-Neelsen stains, Brown-Breen, PAS and Grocott were negative. The immunohistochemical investigation of Herpes, CMV and Adenovirus viruses, resulted negative.

Only one of the reports mentions concomitant GvHD (acute or chronic). In the current case, the patient presented with acute skin GvHD, which was resolved with topical corticosteroid therapy; AFOP developed after the resolution of the GvHD.

As AFOP after allo-BMT is a rare pulmonary disease manifestation, no consensus exists as to the optimal therapeutic approach. Beasley et al. reported a 40 % mortality rate for AFOP following allo-BMT, and severity markers such as the need for mechanical ventilation were present in all patients [3,7–9]. With improvements in antibiotic prophylaxis, infection-related mortality has decreased, making noninfectious pulmonary complications increasingly significant as causes of transplantation-related mortality. These complications are prevalent in 30–40 % of patients, with an overall mortality of 30 % [10–12]. Unfortunately, the underlying pathophysiology of many of these conditions remains poorly understood. While corticosteroids and immunosuppressive therapy remain the cornerstone of treatment for most noninfectious pulmonary complications after allo-BMT, the poor outcomes highlight the need for more effective and targeted therapies, as well as improved strategies for mitigation and prevention [13].

Concomitance of AFOP with infectious agents were not present in any of the reported cases. In the present case, the parainfluenza virus was isolated through the Respiratory Virus PCR Panel for the bronchoalveolar lavage. Although this viral infection alone would not account for the histopathological findings, we hypothesize that the presence of the virus may have triggered the development of AFOP.

Since AFOP is characterized by nonspecific radiological findings and specific histopathological features, a lung biopsy should be considered for patients with severe non-infectious respiratory failure after allo-BMT, especially when the cause is unclear or when a clinical diagnosis is elusive. Early initiation of corticosteroids is crucial for better outcomes. Other immunosuppressive agents may be considered, but given the scarcity of literature, further research is needed to optimize management strategies and improve outcomes.

Conclusion

AFOP is a rare and severe complication following allo-BMT. Early diagnosis, histopathological confirmation, and prompt initiation of corticosteroid therapy are critical for improving outcomes. Patients diagnosed before Day +100 generally have a better response to treatment and more favorable clinical outcomes. The need for a more effective and targeted treatment strategy remains an unmet challenge in managing this condition.

Data availability

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

Conflicts of interest

None.

Editor

Eduardo Rego

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Review article

Is asciminib an effective tyrosine kinase inhibitor for chronic myeloid leukemia patients with tyrosine kinase inhibitor resistance?



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ABSTRACT

Asciminib represents a significant advancement in the treatment of chronic myeloid leukemia, establishing a novel therapeutic paradigm by specifically targeting the ABL1 myristoyl pocket, a mechanism distinct from that of conventional adenosine triphosphate-competitive inhibitors. Such a selective inhibitor offers an alternative treatment strategy for patients with chronic myeloid leukemia who have developed resistance to previous tyrosine kinase inhibitor therapies. Although asciminib demonstrates a superior safety profile, primarily characterized by a reduction in cardiovascular adverse events associated with prior tyrosine kinase inhibitors, its clinical significance extends further. The effectiveness of asciminib, combined with its capacity to overcome resistance through combination strategies with adenosine triphosphate-binding site tyrosine kinase inhibitors, establishes it as a focal point in emerging chronic myeloid leukemia treatment approaches. It remains essential to continue research and clinical trials to enhance the therapeutic efficacy of asciminib and manage its associated side effects.

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Overview of chronic myeloid leukemia

Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm genetically defined by the presence of the Philadelphia chromosome (pH). This chromosome results from a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;

q11), wherein the ABL1 gene from chromosome 9 is fused with the BCR gene on chromosome 22, leading to the formation of the BCR::ABL1 fusion gene (Figure 1) [1]. The BCR::ABL1 fusion gene is responsible for the production of constitutively active BCR::ABL1 tyrosine kinase which is considered to be the core of CML pathogenesis due to its function of activating essential signaling pathways including the PI3K/AKT, RAS/RAF/MEK/ERK, and JAK/STAT pathways [1]. These pathways contribute to uncontrolled cell growth, proliferation, and survival, underlying the disease's escalating nature. The fusion

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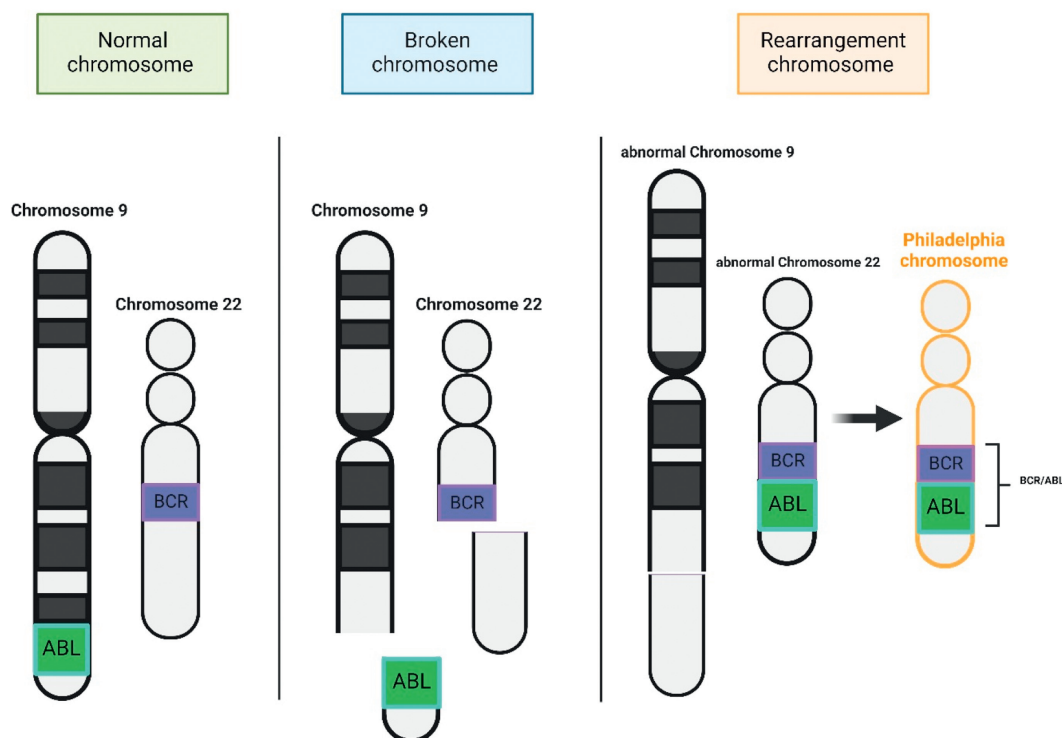


Figure 1 – Schematic representation of the t(9;22)(q34;q11) translocation, which facilitates the formation of the Philadelphia chromosome. The ABL1 gene is located on the long arm of chromosome 9, while the BCR gene is situated on chromosome 22. This reciprocal translocation results in the formation of the BCR::ABL1 fusion gene on the derivative chromosome 22, commonly referred to as the Philadelphia chromosome.

of BCR and ABL genes disrupts the normal regulation of ABL1 kinase activity, leading to the malignant transformation of myeloid cells [1].

CML represents approximately 15 % of all leukemia cases, predominantly affecting adults with a median age of 55 years at diagnosis [2]. It progresses through three phases: chronic, accelerated, and blast crisis, with most diagnoses occurring in the chronic phase [2]. Initially, the disease is characterized by a bone marrow expansion of myeloid cells, causing fatigue, splenomegaly, and anemia. If left untreated, CML may progress to more aggressive phases, culminating in a blast crisis characterized by increased blast cells and genetic instability, often resulting in death [2].

Tyrosine kinase inhibitors (TKIs), such as imatinib, nilotinib, and dasatinib, have revolutionized CML treatment, greatly improving survival rates. These drugs specifically inhibit the BCR::ABL1 tyrosine kinase, leading to remission in the majority of patients. However, further research for new therapeutic targets and strategies is essential because long-term use has revealed issues with drug resistance [3].

The treatment of CML has evolved over recent decades. Initially, physicians would conduct observations of the patient's condition, the symptomatic presentation of the disease and how it progressed as well as alternative treatment paths involving combining chemotherapy with medication [4]. Stem cell transplants are another treatment path. Some circumstances necessitate the administration of interferon-alpha (IFN α) and TKIs. Frequently, practitioners combine therapies to enhance the effectiveness of treatment approaches.

As there is no cure for CML to date, these approaches help to manage the disease [4].

Predicting treatment outcomes remains challenging, despite the availability of highly efficacious therapeutic options. Current predictive models do not fully account for the long-term response of individual molecular markers to treatment. Furthermore, there is a lack of comprehensive data regarding the kinetic profiles of these markers or the associated risk of molecular relapse for individual patients. Even the kinetics of BCR::ABL1 transcripts, which provide insights into molecular responses, are limited by the fact that samples are typically derived from peripheral blood. Consequently, these data may not accurately reflect the levels of minimal residual disease within the bone marrow. This discrepancy is primarily attributable to the difficulty of directly accessing and monitoring the leukemic stem cell compartment within the bone marrow environment [5].

A major advancement in the management of CML was the development of first-generation TKIs such as imatinib, which achieve high rates of sustained cytogenetic responses. [6] A significant challenge, however, is resistance [7]. Second-generation tyrosine kinase inhibitors were developed to overcome imatinib resistance and intolerance. An example is nilotinib, which exhibits significantly higher potency against the BCR::ABL1 oncoprotein and possesses a distinct profile of adverse events compared to first-generation therapies [8]. The composition of nilotinib is similar to that of imatinib, but modifications were made to enhance activity and selectivity improve its efficacy against most imatinib-resistant BCR::ABL1 mutations, except for the T315I mutation [9,10].

The recognition of the role of T315I mutation in resistance led to the development of third-generation TKIs like ponatinib and asciminib. Ponatinib was the first third-generation TKI designed to overcome resistance attributable to the T315I mutation through its inhibitory action facilitated by the creation of an ethynyl bond [11]. Ponatinib incorporates a carbon-carbon triple bond (ethynyl linker) that allows the molecule to bypass the steric hindrance caused by the T315I mutation [11].

Asciminib in chronic myeloid leukemia management

Asciminib is still considered an investigational drug. However, it is gaining popularity because of its capacity to specifically inhibit the tyrosine kinase activity of native ABL1 and that of the chimeric BCR::ABL1 oncoprotein responsible for CML [12]. It was granted breakthrough therapy designation by the U.S. Food and Drug Administration (FDA) on 9 February 2021 to treat patients with chronic phase Philadelphia chromosome positive chronic myeloid leukemia (pH⁺ CP-CML) who had been treated with two or more TKIs and pH⁺ CP-CML patients with the T315I mutation [12]. Figure 2 shows the chemical structure of asciminib.

Unlike tyrosine kinase inhibitors that target adenosine triphosphate (ATP)-binding sites, asciminib, a Specifically Targeting the Abelson Myristoyl Pocket (STAMP) inhibitor, binds to the myristoyl pocket of the Abelson tyrosine kinase 1 protein. This agent was granted accelerated approval in the treatment of CML by the FDA in October 2021 [14]. The pharmacological action of the drug impedes the interaction between the myristoyl group and the myristate pocket of the ABL1 protein [12]. This obstruction precludes the regulatory function of the myristoyl group upon its conjugation with BCR, thereby inhibiting subsequent enzymatic activity [12]. Figure 3 shows the mode of action of asciminib.

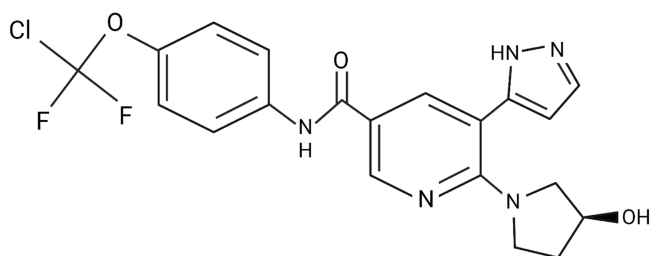


Figure 2 – Chemical structure of asciminib, a first-in-class Specifically Targeting the ABL Myristoyl Pocket inhibitor. Structurally, asciminib features a dichlorodifluoromethoxyphenyl moiety coupled to an amide-linked central phenyl ring. This central scaffold is conjugated to a pyrimidine ring, which is further substituted with a piperidine ring bearing a terminal hydroxy group. Unlike traditional ATP-competitive TKIs, asciminib functions through allosteric inhibition by mimicking the regulatory myristoylated N-terminus of ABL1. This restores the autoinhibitory conformation of the kinase, a mechanism typically lost following the BCR::ABL1 oncogenic fusion, thereby providing a potent therapeutic alternative for patients with acquired TKI resistance [13].

No clinically significant adverse outcomes have been reported regarding its off-target activity. This favorable safety profile is likely attributable to the high specificity of the inhibitor toward cells expressing the BCR::ABL1 oncoprotein, alongside its negligible impact on non-kinase targets in comprehensive biochemical screens [16]. Without binding to the ATP-binding site, asciminib is mainly focused on inhibiting kinase domain mutations that lead to the drug resistance seen in earlier generations of ATP-competitive drugs [16]. Binding to the ABL1 myristoyl binding pocket is vital to enable asciminib to inhibit BCR::ABL1 in a non-ATP competitive way. The drug has an almost non-overlapping point mutation-driven resistance profile which is superior to ATP-competitive TKIs [17]. Nevertheless, studies have identified specific BCR::ABL1 mutations that diminish the anti-proliferative activity of the drug, with certain alterations leading to clinical resistance. Despite these challenges, asciminib maintains high potency against the majority of known BCR::ABL1 mutations, offering a robust therapeutic option for patients who have developed resistance to other inhibitors [17]. However, this does not guarantee that it will always be like that. There are emerging mutations, including V468F, I502L, P465S, and A337V/T, for which the drug is ineffective. These mutations are localized within the myristoyl-binding pocket. Consequently, they confer resistance to asciminib, rendering the treatment [17]. As a result of resistance to mutations, transformations from the accelerated phase to the blast phase are recorded in 4.6 % and 7.3 % of patients on dasatinib and imatinib, respectively [18].

Asciminib may suit patients with significant vascular risk factors or a history of cardiovascular diseases, as well as patients with documented toxicities thought to be related to the class of ATP-competitive TKIs including nilotinib and ponatinib [14]. The drug may also be the choice for patients who do not achieve time-dependent molecular targets but are TKI-responsive [14]. Patients are prone to adverse events due to intolerance and resistance that often lead to dose interruptions or reductions. The current data suggest that the safety profile of asciminib is relatively favorable but the view is expected to keep evolving as long-term data become available to reflect the true incidence of toxicities [14].

Mechanism of action of asciminib

Asciminib serves as a selective allosteric inhibitor of BCR::ABL1 by mimicking the natural autoinhibitory mechanism of ABL1. In the native ABL1 protein, the N-terminal myristoyl group binds to a specific hydrophobic pocket to maintain the kinase in an inactive state. However, this autoinhibitory capacity is lost upon the formation of the BCR::ABL1 fusion protein [14]. The binding of asciminib mimics the natural interactions within the myristoyl pocket, thereby inhibiting BCR::ABL1 and restoring allosteric control over its kinase activity. This mechanism establishes asciminib as a first-in-class inhibitor targeting the ABL1 myristoyl pocket. The molecule was developed through the structural optimization of GNF-2 and GNF-5; the former served as a pivotal tool compound in validating the allosteric inhibition of BCR::ABL1 and demonstrating that targeting this site effectively suppresses

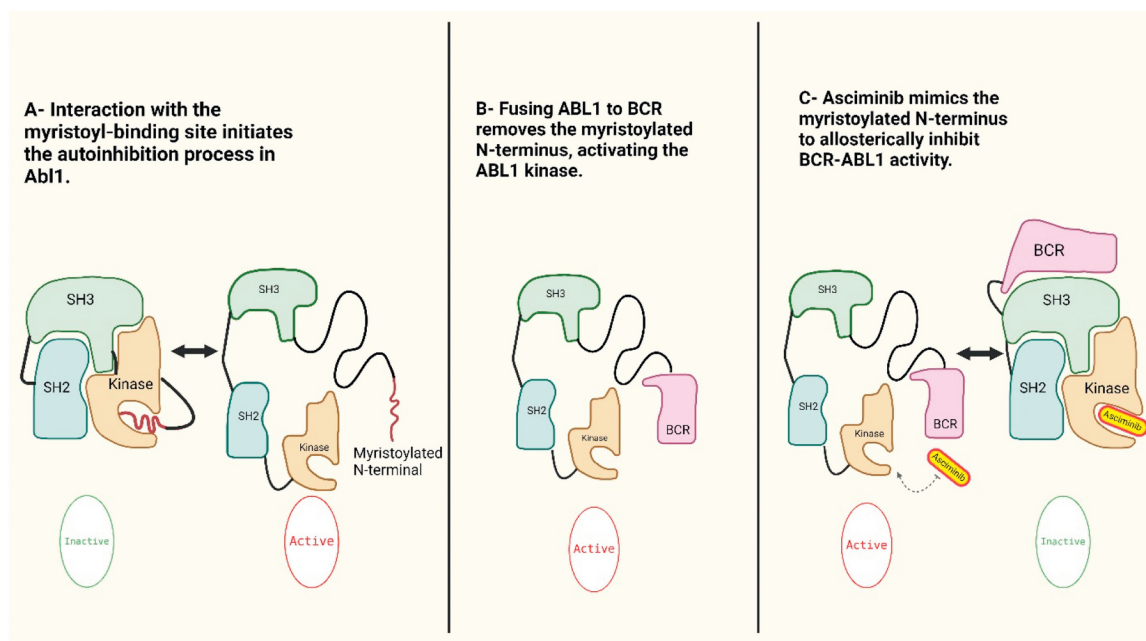


Figure 3 – Asciminib binds specifically to the allosteric myristoyl pocket of the BCR::ABL1 oncoprotein. Under physiological conditions, autoinhibition of the ABL1 kinase is achieved through the binding of the myristoylated N-terminus to the myristoyl-binding site (A). This regulatory motif locks the ABL1 kinase in its inactive conformation (B). However, the oncogenic fusion of BCR and ABL1 results in the loss of the myristoylated N-terminus, leading to constitutive kinase activation (B). Asciminib mimics the natural myristoyl ligand by binding to this allosteric site, thereby restoring the autoinhibited state and inhibiting BCR::ABL1 kinase activity (C) [15].

kinase activity. This proof-of-concept was fundamental to the subsequent development of asciminib. Although asciminib shares the non-ATP-competitive biochemical kinetics characterized by GNF-2, it represents a significant clinical advancement, exhibiting approximately 100-fold greater potency [17].

The myristoylic domain physiologically serves as a negative control for kinase activity. Its function is lost in CML. The limited availability of the myristoyl pocket in other kinases means that the molecule is highly selective for BCR::ABL1. The highly selective nature of asciminib for ATP site mutants such as T315I and unmutated BCR::ABL1 is inhibited by the ABL1 and ABL2 kinases [14]. Asciminib helps to recover the original autoregulatory mechanism that induces the inactive conformation and inhibits downstream signaling [14].

Comparing asciminib with other tyrosine kinase inhibitors

Asciminib demonstrates a favorable safety profile alongside statistically significant and clinically meaningful superior efficacy compared to ATP-competitive inhibitors such as bosutinib [19]. These findings support its utilization as a preferred therapeutic strategy for CML, particularly for patients who have failed to respond to conventional ATP-binding site inhibitors. The unique mechanism of action differentiates it from ATP-competitive TKIs. First, it works by specifically targeting the ABL myristoyl pocket. Furthermore, ABL1 under normal conditions is regulated by the binding between the myristoylated N-terminal and the myristoyl-binding pocket, which renders the kinase inactive. Another factor that

differentiates asciminib is the loss of autoregulation in CML due to the formation of the constitutively active BCR::ABL1 fusion oncoprotein. Binding to the myristoyl-binding pocket restores the inactive conformation leading to the inhibition of ABL1 kinase [16,17].

Adverse events of asciminib

It has been reported that asciminib can cause a variety of adverse events, including pancreatitis and elevated blood pancreatic enzyme levels, both of which are serious, but relatively uncommon. It is necessary to monitor blood counts regularly due to myelosuppression, which can manifest as thrombocytopenia, neutropenia, and anemia [20]. In the ASCSEMBL trial, Grade ≥ 3 neutropenia occurred in approximately 32 % of patients, thrombocytopenia in 24 %, and anemia in 5 % [15]. According to the Australian Scemblix product information, Grade 3 or higher adverse events included thrombocytopenia (18.5 %), neutropenia (15.7 %), elevated pancreatic enzymes (12.4 %), hypertension (8.4 %), and anemia (5.3 %). The most recent FDA Prescribing Information (2024) reported higher incidences of Grade 3 or higher neutropenia (13 %), thrombocytopenia (17 %), anemia (4 %), and elevated pancreatic enzymes (9 %), based on large pooled data sets. These data confirm the reproducible hematologic profile of asciminib while indicating a lower incidence of severe off-target toxicity compared to ATP-binding TKIs. In the presence of elevated liver enzymes, liver function abnormalities suggest potential hepatotoxicity and should be monitored closely [8]. As well as fatigue, patients have reported musculoskeletal

pain as well as gastrointestinal disturbances like diarrhea and dermatological reactions such as rashes [20]. It shows that the adverse cardiovascular events of asciminib are strikingly different to ATP-pocket TKIs, such as ponatinib and nilotinib, offering therapeutic advantage to patients with compounded risk of cardiovascular disease.

Management of adverse events

To advance CML management by decreasing or even eliminating the adverse events of asciminib requires a dynamic and integrative process [15]. Patients with pancreatitis and elevated pancreatic enzymes require close monitoring for abdominal signs and symptoms; accordingly, asciminib therapy may need to be interrupted or discontinued based on the severity of the condition [20]. Monthly full blood counts are needed to investigate myelosuppression with dose adjustment or temporary discontinuation of therapy being required if a cytopenia is identified [20]. For early detection of liver toxicity, biochemistry profile tests including liver function tests should be conducted on a regular basis, and the dose of asciminib should be adjusted or the drug discontinued as possible interventions [21]. Medications such as analgesics can be used as part of musculoskeletal pain management. Moreover, rehabilitation exercises can often be useful to improve physical function. In the case of gastrointestinal side effects, such as diarrhea, dietary changes, hydration, and antidiarrheal medications can all be helpful. A topical treatment may be used to address dermatological reactions, whereas systemic therapies may be used if the reaction is severe [22]. In order to take action early, patients must be educated to recognize symptoms and report them in a timely manner. Finally, the treatment plan should consider a cardiovascular risk assessment, given the favorable profile of asciminib [17].

Comparison with other chronic myeloid leukemia treatments

When compared to other treatments for CML, asciminib represents a significant advancement [17]. Unlike imatinib, a first-generation TKI notorious for gastrointestinal reactions, fluid retention, cramps, and rash, asciminib has other adverse events, such as myelosuppression and liver enzyme elevations [11,17].

The combination of asciminib with second-generation TKIs such as nilotinib and dasatinib is safer than second-generation TKIs alone, which can result in serious cardiovascular toxicities like QT prolongation, arterial occlusive disorders, pleural effusions, and pulmonary hypertension [21]. Patients at risk of or with existing cardiovascular diseases find its lower rate of cardiovascular adverse events particularly attractive [17,21].

Asciminib is also safer than the third-generation TKI ponatinib, which is associated with an elevated risk of arterial thrombotic events [17]. In patients with cardiovascular risk factors or a history of vascular complications, asciminib is likely a better choice than ponatinib since it requires less cardiovascular monitoring [17].

Resistance to asciminib in chronic myeloid leukemia treatment

The primary cause of BCR::ABL1 resistance to asciminib is the emergence of specific genetic mutations within the kinase domain. This underscores the mechanistic distinction between asciminib and conventional ATP-competitive TKIs; specifically, asciminib binds to the myristoyl pocket, representing a novel allosteric approach to inhibiting the BCR::ABL1 oncoprotein. While mutations such as T315I confer resistance to many ATP-site inhibitors, they do not typically affect the myristoyl-binding site. However, novel missense mutations within the C-terminal lobe can disrupt the architecture of the pocket, thereby preventing drug binding and leading to clinical relapse [17]. Nevertheless, mutations that affect the structure and orientation of the myristoyl pocket are responsible for loss of affinity with asciminib which consequently causes resistance. Furthermore, resistance can also be caused by BCR::ABL1 independent mechanisms, for example, altered efflux transport or alternative signaling pathway activation which mediate resistance.

Detection and monitoring of resistance

The most frequently applied genetics and molecular methods used to recognize and track asciminib resistance are Next Generation Sequencing (NGS) and Quantitative Polymerase Chain Reaction (qPCR). These advanced techniques detect possible mechanisms of resistance due to mutations within the BCR::ABL1 gene. By routinely tracking BCR::ABL1 transcripts, early resistance to treatment can be recognized and the effectiveness of treatment assessed.

Impact on treatment strategy

The emergence of resistance to asciminib significantly impacts CML therapeutic strategies. To address resistance mediated by specific mutations, combination therapies targeting multiple signaling pathways or employing diverse mechanisms of action may be required for patients with advanced-stage CML. Utilizing agents that target downstream signaling or combining asciminib with other TKIs represents a viable approach to overcoming such resistance [23]. Furthermore, upon the detection of resistance, treatment must be tailored to the patient's specific mutational profile. Potential interventions include switching to an alternative TKI, dose optimization, or considering allogeneic stem cell transplantation in advanced disease states [23]. Ultimately, prioritizing the understanding and management of asciminib resistance through ongoing research and innovative strategies is essential to optimize clinical outcomes.

Combination strategies with asciminib

Asciminib demonstrates a favorable safety profile as well as statistically significant and clinically meaningful superior

efficacy compared to ATP-competitive inhibitors such as bosutinib [19]. These findings support the use of asciminib as the preferred therapeutic option, particularly for patients who have demonstrated resistance or intolerance to previous TKIs. Its unique mechanism of action, characterized by specifically targeting the ABL myristoyl pocket, distinguishes it from conventional ATP-competitive TKIs. Under physiological conditions, ABL1 activity is regulated by the binding of its myristoylated N-terminus to the myristoyl-binding pocket, which maintains the kinase in an autoinhibited, inactive state. Asciminib restores this native autoinhibitory function by mimicking the binding of the myristoyl group, a regulatory mechanism that is otherwise lost following the oncogenic fusion of BCR and ABL1 [16,17].

Rationale for combination therapy

The main reasons combination treatment with asciminib is chosen to treat CML patients are to increase treatment efficacy, overcome treatment resistance, and decrease adverse events. Asciminib targets the ABL myristoyl pocket by inhibition and can be combined with different TKIs which target different pockets or stages in the proliferation of cancer cells. The rationale behind this strategy is to provide a more comprehensive blockade of BCR::ABL1 oncogenic signaling with this strategy potentially leading to a deeper molecular response, and fewer resistance mechanisms as well as better flexibility in terms of addressing different patient requirements and disease characteristics [23].

Current research on combination therapies

The combination using asciminib along with an ATP-pocket inhibitor such as imatinib, nilotinib, or ponatinib is a key

interest of ongoing research. Initial results indicate that this combination is beneficial for single-agent TKI resistance such as imatinib resistance as well as in CML patients with complex pathophysiology [23].

The use of asciminib along with other TKIs significantly inhibits the expression of resistance point mutations in Ba/F3 [17]. Ba/F3 as laboratory-grown cells, are a useful tool in experimental combination trials. It is possible to genetically manipulate these cells to have particular mutations of the BCR::ABL1 gene. By testing Ba/F3 cells it is possible to evaluate the efficacy of asciminib to target various BCR::ABL1 mutations. This enables an understanding of how effective combinations of drugs will be in patients with certain genomic characteristics of leukemia especially in respect to drug resistance [17]. Combination strategies show more effective management in CML patients than the use of only one TKI. Even if a compound mutation confers resistance to either drug separately, combining asciminib with ponatinib is effective against Ba/F3 cells that harbor BCR::ABL1 compound mutations at therapeutically-relevant concentrations [17]. Apart from prolonging survival, the combination inhibits hematological malignancy in a T315I-inclusive compound mutant model. The complementary resistance profiles of asciminib and nilotinib facilitate the complete regression of hematologic malignancies when administered in combination. In contrast, resistance mediated by specific point mutations persists when either agent is utilized as a monotherapy [17]. Mutations within the myristoyl and ATP-binding sites can collectively confer resistance to both asciminib and nilotinib. Emerging evidence suggests a significant overlap between the specific residues involved in ATP-binding site resistance and those that impact the efficacy of established ATP-competitive TKIs [17]. Figure 4 illustrates the mutational landscape of resistance to asciminib and nilotinib; notably, the myristoyl pocket mutations associated

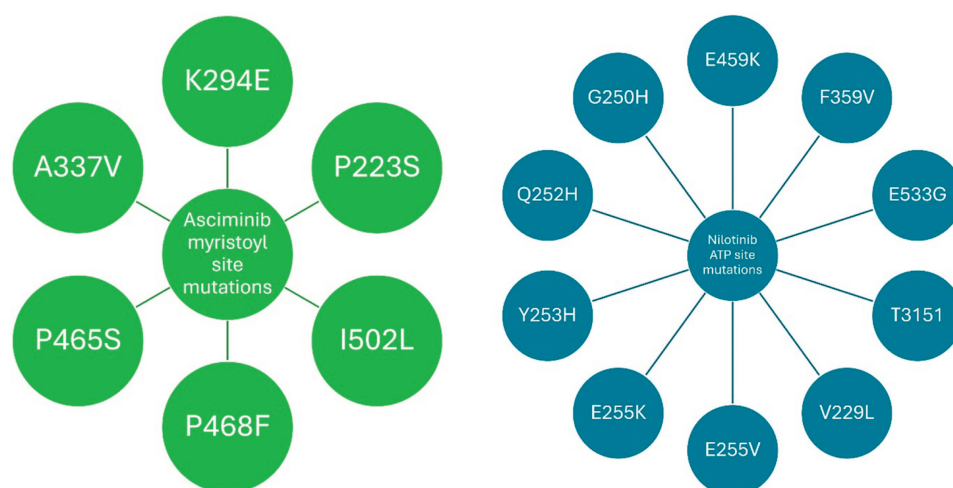


Figure 4 – Resistance mutations for asciminib and nilotinib. Green circles denote mutations within the myristoyl pocket that confer resistance to asciminib. These are distinct from the blue circles on the right, which represent ATP-binding site mutations conferring resistance to nilotinib. Because these mutations occur in spatially separate domains of the BCR::ABL1 protein, they highlight the lack of cross-resistance between allosteric and ATP-competitive inhibitors, providing a mechanistic rationale for combination therapy.

with asciminib resistance are spatially and mechanistically distinct from the ATP-binding site mutations that confer resistance to nilotinib [17].

As García-Gutiérrez et al. noted, the mechanism of action that targets the myristoyl pocket, when combined with TKIs targeting the ATPase domain of BCR::ABL1, helps to suppress the emergence of resistance [24]. However, dose escalation with ponatinib is not a feasible option because of dose-limiting toxicities [17]. Combining asciminib with ponatinib allows for lower doses of ponatinib to target highly-resistant BCR::ABL1 compound mutations [17].

Efficacy and safety of combination approaches

Early clinical trials demonstrated that combination therapies involving asciminib can achieve deeper and more durable responses in CML [23]. However, it is essential to note that combination therapies may exacerbate existing adverse events or induce novel toxicities in CML patients. Future research aimed at optimizing CML management through combination strategies must carefully balance therapeutic efficacy with the safety profile of the treatment [17].

Clinical trials of asciminib as a monotherapy

Asciminib monotherapy is frequently indicated for patients who have exhausted conventional therapeutic options or those harboring the T315I gatekeeper mutation. Its unique mechanism of action (specifically targeting the ABL1 myristoyl pocket) enables continued efficacy in cases where traditional ATP-competitive TKIs fail due to binding-site alterations [16]. For instance, a Phase 1 study of asciminib as monotherapy was used to demonstrate significant efficacy in patients suffering from CML who had shown resistance to at least two TKIs. In the same study, a major molecular response was observed in 48 % of patients, and 57 % of patients achieved complete cytogenetic response (CCyR) at 24 weeks of treatment [25]. Similarly, the hematological remission rate, used to determine the normalization of blood counts in another study, was achieved in 95 % of the patients. Two ongoing trials (ASC4START and ASC4FIRST) further investigate the efficacy of monotherapy as a first-line treatment for patients newly diagnosed with CML in the chronic phase [26]. Therefore, the studies above reveal the efficacy of asciminib as a monotherapy in patients with heavily pretreated CML. Thus, it provides a promising alternative, especially for patients who have exhausted other treatment options.

Clinical trial in combination with adenosine triphosphate-pocket inhibitors

In instances where monotherapy proves insufficient due to emergent resistance or suboptimal molecular response, combining asciminib with ATP-competitive TKIs may be necessary. This dual-targeting approach represents a promising strategy to circumvent TKI resistance and achieve deeper molecular responses in CML patients [27]. For instance,

asciminib was evaluated in combination with ATP-pocket inhibitors in a Phase 3 clinical trial. It yielded improved outcomes compared to using ATP-pocket inhibitors as a monotherapy. In one such study, 72 % of patients receiving asciminib in combination with imatinib achieved a major molecular response at 24 weeks, compared to 51 % of patients in the control group receiving imatinib monotherapy. In yet another study, combination therapy yielded a better response, with 65 % of patients achieving a deep molecular response, defined as a BCR::ABL1 transcript level of ≤ 0.01 %, compared to 42 % in the control group [17].

Conclusion

Asciminib, characterized as an allosteric myristoyl pocket inhibitor rather than a conventional ATP-competitive inhibitor, offers superior management compared to earlier CML therapies. A primary advantage of asciminib is its ability to circumvent the resistance and adverse events associated with traditional TKIs, particularly in patients with advanced-stage CML. The development of asciminib addressed a critical clinical need for advanced management strategies that surpass the efficacy of previous approaches to improve patient outcomes and quality of life. Asciminib serves as a vital alternative for patients experiencing relapse, intolerance, or resistance to prior TKIs. Furthermore, the strategic combination of asciminib with other TKIs represents a promising approach to mitigating adverse effects and enhancing therapeutic depth. The preliminary success of these combination regimens has solidified the role of asciminib in the evolving landscape of CML treatment.

The future role of asciminib in CML management remains contingent upon the outcomes of extensive longitudinal research. It is imperative to conduct long-term clinical trials to comprehensively evaluate the efficacy and safety profile of asciminib in both monotherapy and combination settings. Such research is critical to identify optimal therapeutic protocols, elucidating emergent resistance mechanisms, and devising strategies to mitigate them. Furthermore, exploring synergistic interactions between asciminib and novel therapeutic modalities will be pivotal to maximizing the clinical potential of STAMP inhibition.

Data availability

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

Conflicts of interest

None.

Editor

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Review article

Warm autoimmune hemolytic anemia in adults in Latin America: A scoping review



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ABSTRACT

Background: Autoimmune hemolytic anemia is a rare disorder characterized by the autoimmune-mediated destruction of red blood cells. The condition is classified into subtypes based on the thermal reactivity of the autoantibodies, with warm autoimmune hemolytic anemia representing the most common form in adults. This review aimed to summarize the clinical evidence regarding warm autoimmune hemolytic anemia generated in adult patients within the Latin American region.

Methods: A literature search was conducted in Embase, Medline, Lilacs, Cochrane Library, Epistemonikos, and Value in Health in April 2023. Gray literature was also consulted. Records were eligible for inclusion if they presented data on the epidemiology, diagnosis, treatment, or healthcare resource utilization among adult warm autoimmune hemolytic anemia patients in Latin American countries. Clinical practice guidelines on the condition issued in Latin American were also eligible.

Results: Nine records were included: seven retrospective studies (six single-center), one national clinical guidelines (Mexico), and one therapeutic protocol (Brazil). Corticosteroids are the cornerstone of first-line treatment, reported in 97.7 % of 354 patients enrolled in the studies, primarily as monotherapy (91 %). While response rates were high (69–84 %), a considerable proportion of patients (15–40 %) required second-line therapies. The use of splenectomy varied widely between studies, from being the preferred second-line option to not being performed in any patient. Access barriers contribute to a limited use of rituximab.

Conclusion: Compared to other hemolytic disorders, warm autoimmune hemolytic anemia studies are lacking in Latin American and consist primarily of single-center,

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retrospective case series. Further research is warranted to understand management strategies in refractory disease.

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Introduction

Autoimmune hemolytic anemia (AIHA) is a rare disorder characterized by an autoimmune-mediated destruction of red blood cells (RBCs) [1]. Its epidemiology is not well described, but a study in Denmark estimated 1.77 new cases per 100,000 person-years from 2008 to 2016 [2]. Despite being a rare disease, AIHA has a relevant impact on both patients and society and is associated with important healthcare resource utilization, namely due to highly expensive drugs, hospital admissions, and frequent outpatient visits and blood transfusions [3].

The clinical picture in AIHA varies widely, ranging from no symptoms to severe life-threatening hemolysis [4]. AIHA is classified according to the temperature reactivity of the pathogenic antibodies as warm AIHA (wAIHA), cold AIHA, or mixed AIHA [5]. wAIHA, in which autoantibodies target RBC surface antigens at body temperature and trigger hemolysis [6], accounts for the majority of all AIHA cases in the adult population (60–70 %) [1,7]. wAIHA is further categorized as primary (idiopathic) or secondary depending on whether there is an underlying cause. Approximately half of all wAIHA cases are attributed to lymphoproliferative neoplasms, autoimmune and infectious diseases, immunodeficiencies, solid tumors, transplants, or drugs [1,7]. Thrombotic events and infections secondary to immunosuppressive interventions are known complications of wAIHA and can potentially be fatal [8–10].

The direct antiglobulin test (DAT) is the keystone of AIHA diagnosis with its findings being critical to distinguish between the various subtypes [11–13]. In wAIHA, the DAT is typically positive for immunoglobulin (Ig)G only, but it may also show positivity for both IgG and complement component 3d (C3d) or, less commonly, C3d alone. Cold agglutinin disease is characterized by DAT positivity for C3d only. Mixed forms of AIHA demonstrate DAT positivity for both IgG and C3d, with coexistence of warm autoantibodies and a high titer of cold agglutinins. Patients may also present with DAT-negative AIHA, in which testing fails to detect IgG on RBCs despite ongoing hemolysis. This is most commonly due to levels of RBC-bound IgG falling below the detection threshold of standard DAT methods. Less frequently, it may result from low-affinity IgG or from RBC-bound immunoglobulins of other classes, such as IgA or, more rarely, IgM [13].

DAT-negative AIHA occurs in 5–10 % of patients with unequivocal evidence of the disease [7,13]. Consequently, a negative DAT result is insufficient to exclude a diagnosis of wAIHA [7]. The diagnosis is supported by a combination of clinical symptoms, including fatigue, syncope, chest pain, weakness, and exertional dyspnea, and laboratory evidence of hemolysis, specifically anemia, jaundice, and splenomegaly. Characteristic biochemical findings include elevated indirect bilirubin, increased lactate dehydrogenase (LDH), reticulocytosis, and depleted serum haptoglobin levels [5,14–16].

The goal of wAIHA treatment is to reduce the degree of hemolysis, consequentially increasing hemoglobin (Hb) levels and improving symptoms. Management of the underlying cause is crucial in secondary wAIHA, either by treating the associated lymphoproliferative or autoimmune disease or by stopping the drugs that might be causing the disease [17]. Evidence on the treatments used for wAIHA is limited, with a scarcity of randomized prospective clinical trials. As a result, treatment has historically been based on expert opinions and retrospective studies [18–22].

Corticosteroids are the mainstay first-line treatment for wAIHA, with a response rate of 70–80 % [23,24]. However, between 20 and 35 % of patients fail to respond to corticosteroid treatment or require excessively high maintenance doses to control hemolysis [25]. Published relapse rates in wAIHA after first-line corticosteroid therapy vary widely, ranging from 10.5–68 % among patients who initially respond to treatment [26–28]. In such cases, second-line treatments are employed, mainly splenectomy, rituximab, and immunosuppressants.

The present review aimed to evaluate and summarize the available evidence on adult patients with wAIHA in Latin America. Specifically, this study intended to assess the epidemiology, patient journey (diagnosis, treatments, and outcomes), clinical burden (morbidity and mortality), and healthcare resource utilization among patients with wAIHA, as well as to identify any unmet needs in the management of this condition.

Materials and methods

This scoping review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) [29].

Literature search

A literature search was carried out in Embase, Medline, Lilacs, Cochrane Library, Epistemonikos, and Value in Health in April 2023. The search terms aimed at retrieving literature focused on wAIHA in Latin America. Detailed information on the search strategy is provided in Supplementary Table 1. Gray literature was also consulted. The reference lists of selected articles were searched for additional records.

Selection criteria

Studies were eligible for inclusion in the present literature review if: 1) data were provided on the epidemiology, diagnosis, treatment, or healthcare resource utilization of patients with wAIHA and 2) results were available for adult patients (≥ 18 years) in Latin American countries. Review articles, case

reports, letters to editor, and commentaries were excluded. Gray literature was eligible if published within the last ten years. This period was extended to 15 years for any other publication. Any clinical practice guidelines on wAIHA issued in Latin America was eligible for inclusion in the review. Only reports in English, Portuguese, or Spanish were considered.

The Rayyan platform was used to screen the titles and abstracts of records retrieved from the literature search. The full-text versions of all those complying with the pre-established criteria were obtained and reviewed for eligibility. Records that did not meet with the selection criteria were excluded. Two reviewers selected records for inclusion, with disagreements settled by a third reviewer.

Data extraction process

Data were extracted from studies into an electronic data extraction form. These data included the primary author, year of publication, country, study type, number and characteristics of enrolled wAIHA patients, treatments for wAIHA, and reported outcomes.

Results

Study selection

The literature search retrieved a total of 321 records (Figure 1), of which 303 were identified via databases and registers and

18 from gray literature. Six records were duplicates and were removed. Forty-four of the records identified using the database search potentially met eligibility criteria following screening of the titles and abstracts using Rayyan. The review of the full-text versions led to the exclusion of 37 records, resulting in seven eligible records. Two of the 18 records retrieved from gray literature were also eligible, giving a total of nine records included in the present review. Overall, the included records consisted of seven retrospective studies, one national clinical guidelines, and one therapeutic protocol.

Characteristics of included studies

Overall, seven retrospective analyses of series of patients treated for wAIHA in Latin American countries (three in Cuba, two in Mexico, one in Chile, and one in Colombia) were included. Of these, six were single-center studies. No randomized clinical trials met the eligibility criteria.

A total of 354 wAIHA patients were enrolled in these studies. The characteristics of the studies and enrolled populations are summarized in Table 1.

None of the included studies reported prevalence or incidence data for wAIHA in Latin American countries.

Diagnosis

Inclusion was limited to patients with a positive DAT in three of the studies (Table 1) [5,14,30]. The rate of DAT positivity ranged from 91.4–100 % in the remaining four studies [31–34].

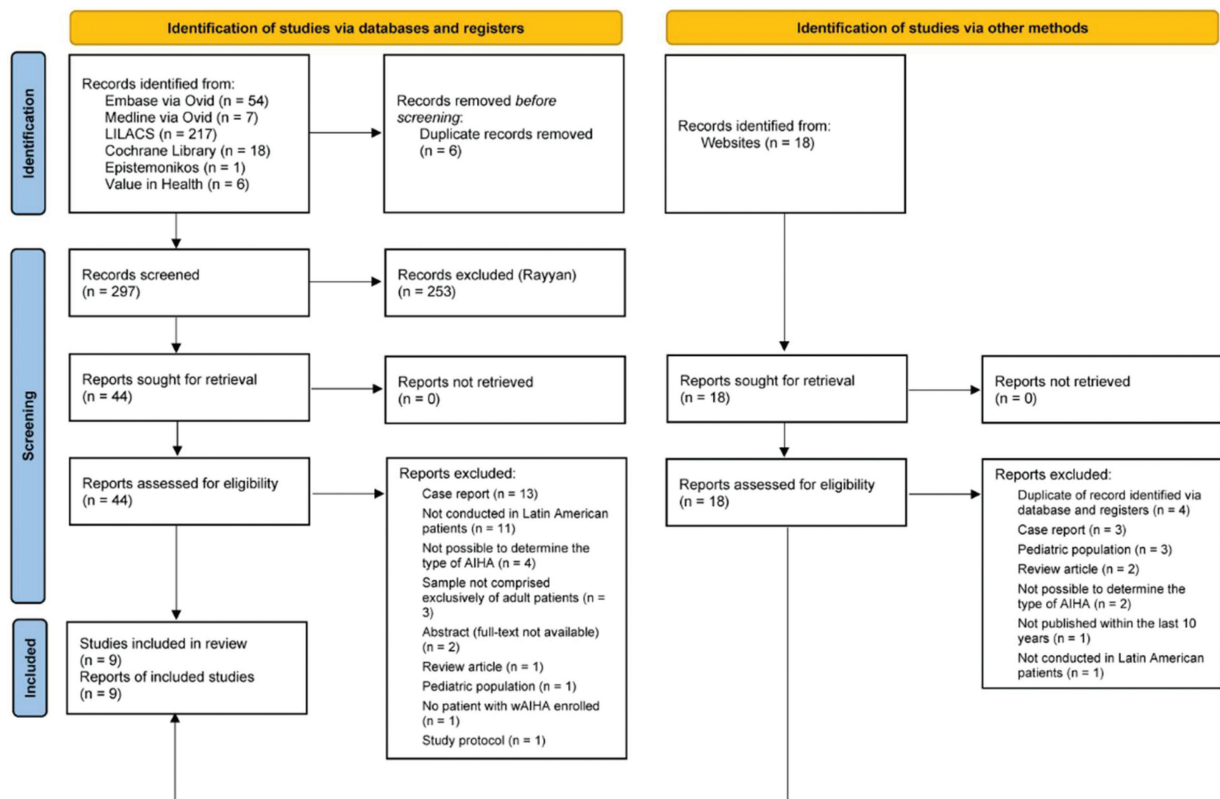


Figure 1 – Flow diagram of literature search. Modified from the Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2020 flow diagram for new systematic reviews.

Table 1 – Summary of included studies.

Author, Year	Country	Study type	Date of wAIHA diagnosis	Patient characteristics	DAT positivity and immunoglobulin pattern	Secondary wAIHA	Clinical presentation
Jaime-Pérez et al. 2019 [14]	Mexico	Single-site, retrospective, cohort study	2002 - 2017	64 patients 60.9 % female Median age at diagnosis: 37 (range: 16 to 77 years)	100 % DAT positivity ^a	33 patients (51.6 %) • Systemic lupus erythematosus (21.9 %) • Evans syndrome (18.8 %) • Antiphospholipid syndrome (3.1 %) • Hyperthyroidism (3.1 %) • Chronic lymphocytic leukemia (1.6 %) • Hodgkin lymphoma (1.6 %) • Rheumatoid arthritis (1.6 %)	Anemic syndrome (100 %) ^b
López-Vidal et al. 2019 [31]	Chile	Single-site, retrospective, cohort study	2010–2018	36 patients 72 % female Mean age at diagnosis: 55 years (range: 22 to 81 years)	97.7 % DAT positivity	24 patients (66.7 %) • Lymphoid neoplasia (38.9 %) • Mesenchymopathy (16.7 %) • Other neoplasia (11.1 %)	No information on clinical presentation
Hernandez-Compan et al. 2017 [5]	Mexico	Single-site, retrospective, cohort study	1992 - 2015	89 patients 73 % female Median age at diagnosis: 36 years (range: 17 to 86 years)	100 % DAT positivity ^a Isolated IgG (71.9 %) IgG + C3d (28.1 %)	49 patients (55.1 %) • Systemic lupus erythematosus (34.7 %) • Primary antiphospholipid syndrome (30.6 %) • Hepatic disease (10.2 %) • Thyroid disease (6.1 %) • Solid malignancy (6.1 %) • Sjogren's disease (2.0 %) • Psoriasis (2.0 %) • Non-Hodgkin lymphoma (2.0 %) • HIV infection (2.0 %) • Rheumatic fever (2.0 %) • Ingestion of allopurinol (2.0 %)	• Jaundice (78.7 %) • Anemic syndrome (71.9 %) • Splenomegaly (33.7 %) • Choleluria (23.6 %)
Agramonte et al. 2015 [32]	Cuba	Single-site, retrospective, cohort study	2011–2013	15 patients 53 % female Median age at diagnosis: 59 years (range: 34 to 75 years)	93.3 % DAT positivity Isolated IgG (46.7 %) IgG + C3 (33.3 %) C3 (20.0 %)	2 patients (13.0 %) • Systemic lupus erythematosus (50.0 %) • Chronic lymphocytic leukemia (50.0 %)	• Jaundice (73 %) • Diffuse joint pain (20 %) • Splenomegaly (13 %) • Thrombotic events (13.3 %)

[illegible]

new AIHA, warm autoimmune hemolytic anemia; DAT, direct antigen test.

^a Inclusion in the study was limited to patients with a positive DAT per the eligibility criteria.

No information on other symptoms was provided.

One patient also presented antiphospholipid antibody syndrome.

One patient also presented Sjogren's disease.

^a Inclusion in the study was limited to patients with idiopathic wAIHA per the eligibility criteria.

Inclusion in the study was limited to patients with IgG antibodies per the eligibility criteria.

Two of the 35 patients enrolled in the study had cold AIHA.

In two of the three studies where at least one patient had a DAT-negative wAIHA result, enzyme immunoassays were employed to confirm the presence of immunoglobulins on the surface of RBCs [32,33]. The isolated IgG pattern was the most common in three of the four studies with available data, accounting for 46.7–71.9 % of patients [5,32–34].

Sociodemographic characteristics and clinical presentation

wAIHA disproportionately affected women in the studies (Table 1), with all seven studies presenting study populations primarily composed of female patients (53–73 %). The median age at diagnosis ranged from 36–59 years.

Inclusion was limited to patients with primary wAIHA per the eligibility criteria in one of the studies [34]. Of the remaining six studies, the proportion of patients with secondary wAIHA varied from 13.0–66.7 %. Systemic lupus erythematosus was the most common underlying cause of wAIHA in five of these studies, accounting for 21.9–50.0 % of patients with secondary wAIHA [5,14,32,33]. López-Vidal et al. reported the most common cause was lymphoid neoplasia (38.9 % of

patients) [31]. wAIHA secondary to drugs was observed in a single case in the seven studies (allopurinol) [5].

“Two studies reported symptoms at the time of presentation; jaundice was the most prevalent symptom in both, occurring in 73.0 % and 78.7 % of patients.” [5,32].

The laboratory results of Hb, indirect bilirubin, LDH, and reticulocytes reported are summarized in Table 2. Hb was considerably reduced among patients with wAIHA, with the mean/median values falling below the cut-off (<8 g/dL) thereby defining severe anemia in five of the six studies with available information [35]. Conversely, hemolytic markers (indirect bilirubin, LDH, and reticulocytes) were generally elevated.

Response and remission criteria

Six studies evaluated response rates to wAIHA treatments. Of these, four detailed the criteria considered to define treatment response (Table 3). The two studies evaluating disease remission rates specified the definition used.

The lack of a current consensus on the definitions of complete and partial response to wAIHA treatments is evident in

Table 2 – Summary of laboratory data at patient presentation.

Author, Year	Hemoglobin, g/dL	Indirect bilirubin, mg/dL	LDH, U/L	Reticulocyte, %
Jaime-Pérez et al. 2019 [14]	6.7 (1.0–10.8)	1.7 (0.4–9.8)	434 (4–2938)	8.2 (0.0–65.0)
López-Vidal et al. 2019 [31]	5.8	Not reported	449	Not reported
Hernandez-Company et al. 2017 [5]	6.4 (2.0–14.0)	2.18 (0.2–9.7)	437 (140–4124)	5.5 (0.5–20.0)
Agramonte et al. 2015 [32]	6.9 (4.2–10.0)	0.3 (0.2–0.4)	890 (450–1120)	Not reported
Quintero 2015 [30]	9.1 (n = 70)	2.5 (n = 50)	Not reported ^a	3.5 (n = 43)
Hernández et al. 2013 [34]	6.9 g/L (2.8–10.2)	Not reported	Not reported	13.4 (4.0–24.0)

LDH, lactate dehydrogenase.
Results are presented as median (minimum-maximum) except for López-Vidal et al. [31] and Hernández et al. [5] – mean (minimum-maximum).
^a Results on LDH were provided qualitatively: 52 patients (73.2 %) had elevated LDH and data were missing for the remaining 14 patients.

Table 3 – Criteria used to define treatment response and disease remission.

Author, Year	Definitions
Treatment response definitions	
Jaime-Pérez et al. 2019 [14]	CR: Hb ≥ 12 g/dL in women and ≥ 13 g/dL in men PR: Hb ≥ 10 g/dL with persistent hemolysis or with a ≥ 2.0 g/dL increment in basal Hb at diagnosis without reaching normal values
Hernandez-Company et al. 2017 [5]	CR: Hb > 12 g/dL and a normalization of all hemolysis markers PR: Hb > 10 g/dL or an increment of 2 g/dL over the basal Hb without the need for transfusions
Quintero 2015 [30]	CR: Hb > 11 g/dL in primary wAIHA and normal Hb levels according to age and sex in secondary wAIHA with negative hemolysis markers and a negative DAT PR: Hb > 9 g/dL without the need for transfusions
Hernández et al. 2013 [34]	Treatment response to corticosteroid treatment: patients who received one to two cycles of prednisone at a dose of 60 mg/day for 3 months and who had the dose gradually decreased while maintaining hemoglobin and reticulocyte counts within normal values without treatment or with maintenance doses of 5–15 mg per day
Disease remission definitions	
López-Vidal et al. 2019 [31]	Complete remission: Hb ≥ 12 g/dL without signs of hemolysis (normal reticulocytes, bilirubin, and lactate dehydrogenase) or need for treatment Partial remission: Hb ≥ 12 g/dL and need to maintain corticosteroid therapy
Hernández et al. 2013 [34]	Disease remission: patients with Hb levels and reticulocyte count within normal values, with a negative DAT, and without immunosuppressive treatment for at least one year

CR: complete response; PR: partial response; Hb: hemoglobin; DAT: direct antigen test.

Table 3. Though the definitions used by Jaime-Pérez et al. [14] and Hernandez-Company et al. [5] were similar, slight differences are still observed. For instance, the latter required the normalization of all hemolysis markers and made no distinction between sexes regarding the Hb target (>12 g/dL) in the definition of complete response (CR), and considered that patients receiving blood transfusions were not partial responders.

Treatment

Corticosteroids

Corticosteroids were used for the first-line treatment of nearly all patients enrolled in the studies (346 out of 349 patients - 99.1 %) with available data. The chosen corticosteroid (prednisone, methylprednisolone, or dexamethasone), posology, and corresponding treatment outcome are summarized in Table 4.

The great majority (90.8 %) of the 349 patients received corticosteroids alone as the first-line treatment for wAIHA. Response rates ranged from 68.6–84.3 % in the included studies. The timing of treatment response assessments was specified by two studies at three and four weeks after starting treatment in the studies by Hernandez-Company et al. [5] and Agramonte et al. [32], respectively. Two studies computed median times to response: 11.5 days (range: 1–30 days) [14] and 27 days (range: 9–44 days) [5]. The proportion of patients attaining disease remission with first-line corticosteroid treatment was 75.0 % and 92.0 % in the studies that assessed this outcome [31,34]. Long-term outcomes were evaluated by two studies. The median duration of response, defined as the time from response to relapse or death, was 22 months (range: 11–31 months) in the study by Jaime-Pérez et al. [14]. In addition, the median relapse-free survival was 81.7 months in this study [14] and 11.7 months for primary wAIHA and 6.6 months for secondary wAIHA as reported by Hernandez-Company et al. [5]. The former study defined relapse as a reemergence of disease, while the latter considered it a decrease in the Hb level or the appearance of hemolytic markers after patient response.

Combined corticosteroid therapy was used as the first-line treatment in 29 of the 349 patients (8.3 %). In the study by Jaime-Pérez et al. [14], 18 patients received low-dose rituximab (100 mg/week for four weeks) plus high-dose dexamethasone (HDD) as front-line therapy for wAIHA at a reference center in Mexico. Of these 18 patients, two (11.1 %) attained a CR and 16 (88.9 %) achieved a PR, with a median time to response of 14 days (range: 3–30 days). The median duration of response was 16.5 months (range: 1–39 months). No statistically significant differences in relapse-free survival were observed between patients treated with low-dose rituximab plus HDD and those treated with corticosteroids alone at the same center. In a study conducted in medical institutions in Bogotá, Colombia, treatment outcomes were not available for ten patients treated with corticosteroids plus azathioprine and one patient with corticosteroids plus an alkylating agent [30].

Corticosteroid use beyond the first-line treatment was reported by only one study, where four relapsing patients received a new cycle of corticosteroids in the second line, resulting in a 100 % remission rate [31].

Splenectomy

Splenectomy was not used for the treatment of wAIHA in two studies. In a study conducted at Hospital del Salvador, Chile, none of the 36 patients diagnosed with wAIHA between 2010 and 2018 underwent this surgical procedure [31]. The authors based their decision on the fact that splenectomy is not as effective in secondary wAIHA, which accounted for most patients at the center. Similarly, in a study conducted in Cuba, none of the four patients requiring second-line treatment underwent splenectomy [32].

In contrast, splenectomy was the only second-line treatment used in a referral center in Mexico City and the most common second-line treatment in another study conducted in Cuba (Table 5) [5,34]. In the former study, 36 patients with wAIHA (corresponding to 40.4 % of all those enrolled) underwent splenectomy, 61.1 % of whom experienced a CR and 33.3 % a PR. Although splenectomy was deemed safe and effective, study authors underlined that this procedure should be deferred in patients with secondary wAIHA in light of the associated risks of developing infectious or thrombotic complications [5]. In the study conducted in Cuba [34], seven of ten patients (70.0 %) who required second-line treatment underwent splenectomy, corresponding to 15.9 % of included patients. The disease remission rate was 30.0 %. In five of these seven patients, other treatments were administered in addition to splenectomy including intravenous immunoglobulin ($n = 3$), azathioprine (Imuran), cyclophosphamide ($n = 2$), danazol ($n = 1$), and vincristine ($n = 1$).

Use of splenectomy for wAIHA treatment was also observed in the remaining three studies, primarily in the second-line therapy. In a study conducted in Cuba, 4 of 11 patients (36.4 %) who did not successfully respond to first-line corticosteroid treatment underwent splenectomy as the second-line therapy (response rate of 100 %) [33]. Four of 28 patients (14.3 %) who required second-line treatment for wAIHA underwent splenectomy in a study conducted in Colombia [30]. In a study conducted at a reference center in Mexico, seven patients (10.9 % of the study sample) underwent splenectomy, either as their second-, third-, or fourth-line treatment. Though a 100 % response rate was obtained, all patients subsequently relapsed. The authors of this study reported that most patients declined to undergo splenectomy [14].

Rituximab

Data on the use of rituximab for the treatment of wAIHA are scarce in Latin America. Excluding first-line treatment, a total of 17 patients received rituximab in these studies (Table 5). Two patients were treated with rituximab as second-line therapy in the study by López-Vidal et al. [31] (remission rate of 100 %) and another two received this monoclonal antibody as third-line therapy in the study by Hernandez-Company et al. [5] (treatment outcome not reported). Low-dose rituximab (100 mg/week) for four weeks plus HDD was administered to eight patients refractory to several lines of therapy (range: 2–4) in the study by Jaime-Pérez et al. [14]. CR and PR rates were 37.5 % and 62.5 %, respectively with the median time to response being 16 days. The response was maintained for an average of 81.9 months. At the same center, five refractory patients who relapsed following treatment with cyclophosphamide (in the

Table 4 – Summary of first-line treatments and outcomes.

Author, Year	First-line treatment	Number of patients	Drug and posology	Outcomes	Median (min-max) time to response
Jaime-Pérez et al. 2019 [14]	Corticosteroids	46	Prednisone (1–2 mg/kg/day) for 28 days Dexamethasone (40 mg/day) for 4 days	Response rate: 78.0 % CR: 35 % PR: 43 %	11.5 days (1–30 days)
	Rituximab plus corticosteroids	18	Rituximab (100 mg/week) plus high-dose dexamethasone for 4 weeks	Response rate: 100 % CR: 11.1 % PR: 88.9 %	14 days (3–30 days)
López-Vidal et al. 2019 [31]	Corticosteroids	36	Prednisone (1 mg/kg/day) for at least 3 weeks Dexamethasone (40 mg/day) for 4 days Methylprednisolone (500 mg/day) for 3 days	Remission rate: 92.0 %	Not reported
Hernandez-Company et al. 2017 [5]	Corticosteroids	89	Methylprednisolone (1 g/day) for 3 days followed by prednisone (1 mg/kg/day) for 30 days	Response rate: 84.3 % CR: 50.6 % PR: 33.7 %	27 days (9–44 days)
Agramonte et al. 2015 [32]	Corticosteroids ^a	15	Not reported	Response rate: 73.3 % CR: 33.3 % PR: 40.0 %	Not reported
Quintero 2015 ^b [30]	Corticosteroids Combined corticosteroid therapy (10 with azathioprine and 1 with an alkylating agent) Other	52 11 3	Not reported Not reported Not reported	Response rate (n = 40): 90.0 % ^c CR: 16.7 % PR: 83.3 %	Not reported
Hernández et al. 2013 [34]	Corticosteroids	44	Prednisone (one or two cycles with a 60 mg/day dose) for 3 months	Response rate: 77.3 % ^d Remission rate: 75.0 %	Not reported
Valdés et al. 2009 [33]	Corticosteroids	35	Not reported	Response rate: 68.6 % ^e	Not reported

Min: minimum; max: maximum; CR: complete response; PR: partial response.

^a Two patients required the administration of intravenous immunoglobulin.

^b Information on first-line treatment was missing for five patients.

^c Response rate was reported for 40 of the 71 patients (56.3 %) included in the study and corresponds to the overall response observed across first-line treatments.

^d Five of the patients (14.2 %) responded after a second cycle of prednisone.

^e Response rate was reported for the total of 35 patients included in the study, two of whom had cold AIHA.

Table 5 – Summary of treatments and outcomes in refractory wAIHA.

Author, Year	Treatment	Number of patients	Treatment line	Outcome
Hernandez-Company et al. 2017 [5]	Splenectomy	36	Second	Response rate: 94.4 % CR: 61.1 % PR: 33.3 %
Hernández et al. 2013 [34]	Splenectomy ^a	7	Second	Remission rate: 30.0 %
Valdés et al. 2009 [33]	Splenectomy	4	Second	Response rate: 100 %
López-Vidal et al. 2019 [31]	Corticosteroids	4	Second	Remission rate: 100 %
López-Vidal et al. 2019 [31]	Rituximab	2	Second	Remission rate: 100 %
López-Vidal et al. 2019 [31]	Immunosuppressants • Cyclophosphamide (n = 1) • azathioprine (Imuran)	2	Second	Remission rate: 100 %
Valdés et al. 2009 [33]	Immunosuppressants • Cyclophosphamide (n = 1) • azathioprine (Imuran)	7	Second	Response rate: 100 %
Hernández et al. 2013 [34]	Immunosuppressants (azathioprine)	1	Second	Remission: No
	Cyclophosphamide, vincristine, and intravenous immunoglobulin	1	Second	Remission: Yes
Quintero 2015 [30]	Intravenous immunoglobulin	1	Second	Remission: Yes
	Immunosuppressants (azathioprine)	16	Second	Response rate (n = 16): 75.0 % ^b CR: 31.3 % PR: 43.7 %
	Splenectomy	4	Second	
	Alkylating agents	2	Second	
	Danazol	2	Second	
	Intravenous immunoglobulin	2	Second	
	Other	2	Second	
Hernandez-Company et al. 2017 [5]	Rituximab	2	Third	Not reported
Hernandez-Company et al. 2017 [5]	Immunosuppressants (azathioprine, cyclophosphamide, and vincristine)	30	Third	Response rate: 93.7 % ^c CR: 43.7 % PR: 50.0 %
Jaime-Pérez et al. 2019 [14]	Splenectomy	7	Second to fourth	Response rate: 100 % ^f
Jaime-Pérez et al. 2019 [14]	Immunosuppressants (cyclophosphamide)	5	Second to fourth	Not reported
Jaime-Pérez et al. 2019 [14]	Intravenous immunoglobulin	2	Second to fourth	Not reported
Jaime-Pérez et al. 2019 [14]	Danazol	2	Second to fourth	Not reported
Jaime-Pérez et al. 2019 [14]	Rituximab (100 mg/week) plus high-dose dexamethasone for 4 weeks	8	Third to fifth ^d	Response rate: 100 % CR: 37.5 % PR: 62.5 %
Jaime-Pérez et al. 2019 [14]	Immunosuppressants (cyclophosphamide)	5	Third to fifth ^d	Transient response followed by relapse
Jaime-Pérez et al. 2019 [14]	Low dose rituximab	5	Fourth to sixth ^e	Response rate: 100 %

CR: complete response; PR: partial response.

^a In five patients, other treatments were administered in addition to splenectomy: intravenous immunoglobulin (n = 3), azathioprine (Imuran), cyclophosphamide (n = 2), danazol (n = 1), and vincristine (n = 1).

^b Response rate was reported for 16 of the 28 patients (57.1 %) who received second-line treatments and corresponds to the overall response observed in the second line.

^c The rate was computed for a total of 32 who required third-line treatment at the site – two of whom received low dose rituximab plus high-dose dexamethasone.

^d These patients were part of a subgroup of ten patients with refractory disease who had received a median of 2.5 (2–4) lines of treatment, including steroids (100 %), intravenous immunoglobulin (20 %), cyclophosphamide (50 %), danazol (20 %), and splenectomy (70 %).

^e Patients who relapsed after cyclophosphamide received low dose rituximab.

^f Followed by relapse.

third to fifth lines) successfully responded to low-dose rituximab, with a median response maintenance of 100 months.

Immunosuppressants

All six studies that characterized treatments beyond the first line recorded the use of immunosuppressants for wAIHA

management. Overall, 67 patients received immunosuppressants in the included studies, primarily as second- and third-line therapies (over 85.1 % of patients) (Table 5). Of the 30 patients treated with immunosuppressants as the third-line therapy, 43.7 % experienced a CR and 50.0 % a PR. Of note, this rate was computed for a total of 32 patients who required

third-line treatment at the center, two of whom received low-dose rituximab plus HDD.

Other treatments

Treatments other than corticosteroids, splenectomy, rituximab, and immunosuppressants were rarely administered, occurring in only 15 patients across all included studies (Table 5). These included intravenous immunoglobulin (nine patients), danazol (four patients), and alkylating agents (two patients).

Three studies evaluated transfusion requirements among patients with wAIHA, with the proportion of patients receiving blood transfusions during the follow-up varying between 19.1 % and 39.4 % [5,14,30].

Safety data

Whereas all seven studies evaluated the effectiveness of wAIHA treatments, safety data were reported by only one study. Jaime-Pérez et al. [14] reported that no adverse effect was observed among the 18 patients who received low-dose rituximab plus HDD as first-line therapy for wAIHA.

Mortality

Two studies reported mortality data. López-Vidal et al. [31] found a mortality rate of 20.9 % in a sample of 43 patients with AIHA (36 with wAIHA [83.7 %] and the remaining seven with cold AIHA [16.3 %]) followed up for an average of 38 months. None of the patients died due to the anemia but as a result of the secondary causes: gastric cancer ($n = 3$), chronic lymphocytic leukemia ($n = 3$), and non-Hodgkin lymphoma ($n = 1$). No cause of death was reported for two of the patients. Jaime-Pérez et al. [14] reported mortality results exclusively for wAIHA patients. In this study, two out of 64 patients with wAIHA (3.1 %) died during long-term follow-up. One death was secondary to Kaposi sarcoma in an HIV-positive patient while the other was a result of an ischemic stroke associated with a hemolytic episode.

Unmet needs

In the study by Hernández et al. [34], rituximab was not available at the center during the analyzed period (1997–2006). Financial barriers further limit access to rituximab, even in centers where this drug is available. In a referral center in Mexico City, only two out of 32 patients who required a third-line treatment were able to receive rituximab due to economic constraints [5]. Importantly, this was the study where the second-line treatment was comprised exclusively of splenectomy. Though not stated by the authors, the lack of other treatment options owing to financial barriers might have contributed to the frequent use of splenectomy at the center. Additionally, in a different center in Mexico, authors reported that low-dose rituximab was administered due to financial restrictions in the low-income, uninsured patient population treated at the center [14].

Clinical practice guidelines and healthcare policy documents

The literature search retrieved a single national clinical guidelines for AIHA issued in Mexico [36]. In addition, a Clinical Protocol and Therapeutic Guidelines (CPTG) on AIHA was issued in 2018 by the Ministry of Health in Brazil [17]. This document, intended primarily as a healthcare policy aimed at guiding clinical-decision making and resource allocation within the public health system, does not constitute a national clinical guidelines.

Both the Mexican guidelines and the Brazilian CPTG provide guidance on the diagnosis and treatment of AIHA, including specific recommendations for wAIHA. Both documents recommend corticosteroids, namely prednisone and methylprednisolone, as the first-line treatment for wAIHA. Additionally, both include splenectomy as a second-line treatment for patients who either do not respond to corticosteroids or require high maintenance doses to control the disease. However, while splenectomy is the sole second-line treatment presented in the Mexican guideline, the Brazilian CPTG further states that immunosuppressants (cyclophosphamide or cyclosporine) can also be administered for patients refractory to corticosteroids. In the third line, the Brazilian CPTG details that immunosuppressants may be used in patients refractory to splenectomy. The Mexican guidelines provide several treatment options for patients with Hb levels below 10 g/dL after splenectomy. These include rituximab, immunosuppressants (cyclophosphamide, cyclosporine, azathioprine, and mycophenolate mofetil), and alemtuzumab. Immunoglobulin is recommended in both documents only in select cases, namely in severe patients with Hb below 7 g/dL in Brazil, and in those with cardiac or pulmonary disease who require transfusions and in refractory chronic patients in Mexico. The Brazilian CPTG also establishes recommendations on the monitoring and post-treatment follow-up of AIHA patients.

Discussion

The present scoping review aimed to evaluate and summarize the available evidence reported on adult patients with wAIHA in Latin America. A thorough literature search was performed, encompassing various databases and gray literature. Therefore, we are confident that we have identified the majority of available literature on wAIHA in Latin America.

As is the case outside Latin America, studies on wAIHA are lacking and the scarce evidence available is primarily derived from retrospective single-center case series of patients.

As expected, the results show that women are disproportionately affected by wAIHA. This is likely explained by the fact that women have an increased susceptibility to autoimmune diseases [5], and is aligned with published literature [23,26]. The present review also confirms that systemic lupus erythematosus is a frequent underlying cause of wAIHA, being the most common in five of six studies that included patients with secondary wAIHA. Literature data show that AIHA occurs in 5–10 % of patients with systemic lupus erythematosus [37].

Corticosteroids are the mainstay first-line treatment for wAIHA, being recommended as such in both the Mexican guidelines and the Brazilian CPTG [17,36]. Accordingly, all but three of the 349 patients with non-missing information enrolled in the included studies received corticosteroids as the first-line treatment, in the great majority of cases as monotherapy (90.8 %). Prednisone, methylprednisolone, and dexamethasone were used.

Response rates to first-line treatments based on corticosteroids alone ranged from 68.6–84.3 % in these studies. Differences in response rates between studies might partly result from the variability in doses and durations of corticosteroid treatment and definitions of treatment response. These findings align with published literature reporting that between 70–80 % of patients generally respond to corticosteroid treatment [23,24]. The attainment of a CR to first-line corticosteroid treatment is particularly important, as this is associated with long-term remission. In a study by Roumier et al. [24], long-term remission was recorded in 92 % and 53 % of patients with a CR and a PR to first-line corticosteroids, respectively. Moreover, patients with PR may still present mild to moderate anemia, as the cut-off points defined for Hb in the response criteria were >9 g/dL and ≥ 10 g/dL in the three included studies that specified this information [5,14,30]. The CR rates observed in the studies included in this review (33.3–50.6 %) are also consistent with published literature [24,26]. The median relapse-free survival after first-line corticosteroid treatment differed significantly between the two studies that reported this outcome: 11.7 and 6.6 months in primary and secondary wAIHA [5], respectively, versus 81.7 months in a study where approximately half of the patients had secondary wAIHA [14]. This discrepancy may be, at least in part, a result of differences in relapse definitions, study populations, and type and duration of first-line corticosteroid treatment between the studies.

Though response rates to corticosteroid treatment were high, one should note that a considerable proportion of patients required second-line therapies (15.6–40.4 %). These findings converge with those reported by other studies, which showed that the rate of long-sustained remission after tapering and discontinuation of first-line corticosteroids is low (30–40 % after one year) [22,38,39]. Additionally, other literature found proportions of patients requiring second-line treatments after an initial response to corticosteroids (28.3 % and 52.2 %) similar to those in the present review [24,26]. Information on corticosteroid dependency was scarce in the included studies. Published data show that a high proportion of corticosteroid responders become corticosteroid dependent. In a study conducted in France, the rate of corticosteroid dependency among patients initially responding to corticosteroid treatment was above 60 % [24]. In another study of 44 patients receiving first-line prednisone, nine (20.5 %) required long-term maintenance immunosuppression. Of these nine individuals, seven also required the introduction of second-line agents [34].

While the first-line treatment of wAIHA was based almost exclusively on corticosteroids, the second line was characterized by various treatment options. Second-line treatment of wAIHA has classically been based on splenectomy, the effectiveness of which is similar to that of rituximab while providing slightly longer remissions [7]. Nevertheless, reports

outside Latin America have described a gradual decline in the use of this invasive procedure, which is currently employed in <10 % of cases [7,40]. This decline has mostly been attributed to the resulting increase in infection risk and thrombotic complications. Moreover, splenectomy entails some additional downsides, namely the lack of reliable predictors of outcome and other potential surgical complications [7,40]. We found different practices regarding the use of splenectomy as the second-line treatment of wAIHA in Latin American centers. These practices ranged from a complete absence of splenectomies in two of the studies (in Chile [31] and Cuba [32]) to splenectomy being the sole second-line treatment in a referral center in Mexico City [5].

European data have demonstrated an emergence of rituximab as the preferred second-line option in the last decades [24]. Our findings show that rituximab was used in only two out of the 93 patients (2.2 %) who received second-line treatments in the included studies. Moreover, due to the limited number of studies and countries contributing data, these results may not accurately reflect the current use of rituximab in the management of wAIHA throughout Latin America. One of the possible reasons behind the limited use of this monoclonal antibody is the fact that rituximab is not currently approved for the treatment of wAIHA. Reasons reported by the retrieved studies included the unavailability of rituximab at some centers, as well as economic constraints limiting its use for the treatment of wAIHA [5,34].

The healthcare burden among relapsing patients is significant and underestimated [3]. These patients have a higher transfusion burden and a higher rate of alloimmunization [41]. Currently, the treatment of patients with relapsing disease and unfit for or refusing splenectomy constitutes an important clinical challenge [42,43]. Treatment options for such patients are few (namely azathioprine, cyclophosphamide, and cyclosporine) and of limited efficacy. Novel agents currently being studied in wAIHA, such as bortezomib, nipocalimab, rilzabrutinib, and ialalumab, might become important future treatment options for patients with relapsing disease [42–46].

The present literature review shows that several data gaps and challenges related to wAIHA currently exist in Latin America. First, epidemiological and healthcare resource utilization data are scarce, and no data were retrieved on patient-related outcomes, quality of life, or perspectives on wAIHA and its daily burden. In addition, whereas all seven studies evaluated the effectiveness of treatments given to wAIHA patients, only one reported safety results, specifically evaluating the short- to long-term safety of low-dose rituximab plus HDD as first-line therapy. Furthermore, data on blood transfusions among these patients were provided by only three of the studies and were limited to the proportion of those receiving transfusions during follow-up. Additional details, such as the number of transfusions per patient, patient response to transfusion, or the setting in which the transfusion was performed (inpatient versus outpatient), which would be essential to fully characterize transfusion burden in wAIHA patients, were not reported. Second, all studies determined response and/or remission rates as a means of evaluating the effectiveness of wAIHA treatments. However, other important treatment-related outcomes, such as time to response and relapse-free survival, have seldom been reported. Third, there

are currently no standardized criteria to evaluate response (complete or partial) to wAIHA treatments, as is the case internationally [47,48]. The variability in the definitions used impairs the validity of cross-study comparisons. Lastly, information on patients refractory to wAIHA treatment, particularly to third line therapies and beyond, is scarce. However, the data reported by Jaime-Pérez suggest that an appreciable proportion of wAIHA patients (over 15 %) require more than two lines of treatment [14]. Further research is warranted to understand the management strategies and unmet needs of patients who fail to control the disease after several lines of treatment.

This study is not without limitations. Only four Latin American countries were represented in the present literature review, with studies conducted in Cuba accounting for 42.9 % of those included. As a result, our findings might not be representative of the reality throughout Latin America. In addition, most studies were small case series (four included <50 patients), and all were retrospective.

Though the population of interest in this review were adult patients with wAIHA, we decided to include studies that did not report data exclusively for patients ≥ 18 years or with the wAIHA subtype. Three of the studies, Quintero [30], Jaime-Pérez et al. [14], and Hernandez-Company et al. [5], presented data for samples of wAIHA patients aged ≥ 15 , ≥ 16 , and ≥ 17 years, respectively. In the study by Valdés et al. [33], two of the 35 enrolled patients had cold AIHA. The decision to not exclude these studies from the review was based on the scarcity of available data on wAIHA patients in Latin America. The impact of this limitation is expected to be minimal, as the study samples were still comprised almost exclusively of adult patients with wAIHA.

As previously shown, the definitions of CR and PR varied. Moreover, the timing of the assessment of treatment response differed between both studies that provided this information: three and four weeks after the start of first-line treatment with corticosteroids in the studies by Hernandez-Company et al. [5] and Agramonte et al. [32], respectively. The full extent of this limitation is difficult to ascertain as information on the timing of treatment response assessment was not available in the remaining studies. Both these factors might influence response rates (e.g., more stringent response criteria and earlier assessment of response to treatment may both lead to lower response rates) and should thus be taken into account when comparing the results of these studies.

The treatment outcome rates (response or remission) reported by the individual studies, particularly for treatments beyond the first line, should be interpreted cautiously considering the small number of treated patients.

Conclusion

The present review summarized the available evidence on adult patients with wAIHA generated in Latin America. Studies on wAIHA are lacking and the scarce evidence available is primarily derived from retrospective case series of patients conducted at single centers. Corticosteroids were part of the first-line treatment of wAIHA in virtually all patients enrolled in these studies, mainly as monotherapy (91 %). Response

rates were high (69–84 %), but a considerable proportion of patients required second-line treatments (15–40 %). Treatment approaches beyond first-line therapies varied significantly between countries and institutions, likely due to a lack of disease-specific therapeutic options, the absence of standardized guidelines throughout Latin America, and barriers to access newer therapies. Data on patients refractory to several lines of treatment are currently very limited.

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Author contribution

Yudy Medina, Renato Watanabe de Oliveira, and Pamela Villanova developed the protocol outline and search strategy. Yudy Medina and Renato Watanabe de Oliveira conducted the literature search, which was reviewed by Diogo Ribeiro. Yudy Medina and Diogo Ribeiro performed the data extraction. Diogo Ribeiro drafted the manuscript draft, and all other co-authors contributed to writing, reviewing, and editing. All authors read and approved the final manuscript.

Data availability

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

Conflicts of interest

Juan Jiménez and Sandra Gualandro report no conflicts of interest. Yudy Medina, Renato Watanabe de Oliveira, and Pamela Villanova are employees of Johnson & Johnson, the study sponsor. Diogo Ribeiro is an employee of CTI Clinical Trial & Consulting, which is a consultant to Johnson & Johnson, the study sponsor.

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Supplementary materials

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Review article

Anti-Kp^a antibody: Getting to know a strange and dangerous specificity



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ABSTRACT

Anti-Kp^a is an irregular antibody of clinical significance directed against the red blood cell antigen Kp^a of the Kell system. It is rare in the general population and, therefore, uncommon as a cause of transfusion or hemolytic complications. It has been documented in isolated cases, mainly in the context of alloimmunization after transfusion exposure or during pregnancy; its incidence in clinical practice is limited and can be difficult to detect in routine pre-transfusion testing, which could lead to failure to identify the antibody prior to transfusion.

The production of Anti-Kp^a antibodies is less common than antibodies against other Kell system antigens, such as anti-K. However, when generated, these antibodies can mediate hemolytic reactions in patients receiving incompatible blood and can cause hemolytic disease in the fetus since in addition to immune destruction of red blood cells, Kell system antibodies such as Anti-Kp^a can cause suppression of fetal erythropoiesis, resulting in severe anemia.

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Introduction

Erythrocyte alloimmunization is a complex immunological process that occurs in patients who have been exposed to foreign erythrocyte antigens, either through blood transfusions or gestation, and who develop irregular antibodies against these antigens [1]. This phenomenon is particularly relevant in patients who require repeated transfusions, such as those with chronic anemia, as the presence of clinically significant antibodies can complicate transfusion management and increase the risk of hemolytic reactions [1].

The Anti-Kp^a antibody is a rare specificity within the Kell blood group system. Due to its low prevalence in the general population, it is an infrequent cause of hemolytic transfusion reactions or hemolytic complications [2]. This antibody, directed against the Kp^a antigen, has been documented in isolated cases, mainly in the context of alloimmunization following transfusion exposure or during pregnancy, but its incidence in clinical practice is limited [2].

The aim of this review is to provide comprehensive and up-to-date information on the Anti-Kp^a antibody and its relevance in transfusion medicine.

Relevant characteristics of the Kp^a antigen

The Kp^a antigen is part of the Kell blood group system (ISBT 006). This system comprises 38 antigens carried by a 732-amino acid transmembrane glycoprotein known as CD238.

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The nomenclature derives from 'Kelleher', the first producer of an anti-K antibody, and 'p' for 'Penney,' the name of the first patient identified as an Anti-Kp^a producer [3]. This antigen, first discovered in 1957, has gained notoriety due to its rarity and its involvement in transfusion reactions and hemolytic disease of the fetus and newborn (HDFN) [3].

The Kp^a antigen, referred to by the International Society of Blood Transfusion (ISBT) as KEL3, is an allelic variant of the protein encoded by the KEL gene, located on chromosome 7q32-q36 [3]. This gene gives rise to the Kell glycoprotein, a metalloenzyme expressed in the erythrocyte membrane that performs several functions such as regulation of cell growth and interaction with other membrane proteins [3].

The variation in Kp^a is due to a polymorphism in the KEL gene, which generates an amino acid difference in the resulting protein [3]. This makes Kp^a a low frequency antigen in most populations, with an estimated prevalence of <2 % in Caucasians and virtually absent in other ethnic groups. Conversely, its antithetical partner, the Kp^b antigen (KEL4), is a high-frequency antigen, appearing in >99 % of individuals [3].

Table 1 illustrates the phenotypes of the Kell system, frequency in European and African populations and the reported clinical significance:

Information on the frequencies of KEL:3,4 phenotypes in patients with hemoglobinopathies and blood donors in South America is scarce due to the rarity of these specific variants in the general population and the limited specific genetic research in this region [3,4]. Thus, genetic studies focused on local populations, especially in polytransfused patients and blood donors, is necessary [3,4].

Relevant characteristics of anti-Kp^a

The low frequency of the Kp^a antigen reduces the likelihood of sensitization in the general population [4]. However, when it does occur, the formation of anti-Kp^a antibodies can have serious clinical implications [4]. Sensitization to Kp^a occurs primarily through blood transfusions or, less frequently,

during pregnancy when a Kp^a-negative mother is exposed to Kp^a-positive fetal red blood cells [4].

The production of anti-Kp^a is less common than that of other Kell system antibodies, such as anti-K. The latter is known to suppress erythropoiesis by an immunologic mechanism that affects both the production of erythroid progenitors in the bone marrow and the survival of mature erythrocytes in the circulation [4]. Anti-K can bind to erythroid progenitor cells expressing the Kell antigen in the bone marrow and trigger mechanisms such as macrophage-mediated phagocytosis or functional interference where antibodies block signals essential for the proliferation and maturation of these cells, directly affecting erythropoiesis [4].

Alloimmunization against the Kp^a antigen is extremely rare, even in polytransfused patients, such as those with hemoglobinopathies [5]. Anti-Kp^a can mediate hemolytic reactions in patients receiving incompatible blood by causing extravascular hemolysis through opsonization of erythrocytes leading to destruction in the reticuloendothelial system primarily in the spleen and liver [5]. Fortunately, due to the low prevalence of Kp^a, the risk of developing such antibodies and having a transfusion reaction is relatively low compared to other blood systems [5].

Anti-Kp^a can be difficult to detect in routine pre-transfusion testing, which could lead to failure to identify the antibody prior to transfusion [5]. In addition to immune destruction of red blood cells, anti-Kp^a can possibly cause suppression of fetal erythropoiesis with consequent severe anemia as reported by Tuson et al. where the first case of probable erythropoiesis suppression attributable to anti-Kp^a is described in a twin pregnancy where only one of the twins was affected [6].

Anti-Kp^a is an immunoglobulin G (IgG), predominantly of the IgG1 subtype. While naturally occurring antibodies are extremely rare, they have been reported in a 12-month-old infant with recurrent infections as a cross-reaction to bacterial capsule antigens [7].

Table 1 – Kell system phenotypes, reported frequencies in European and African populations and their clinical significance.

Phenotype (ISBT Nomenclature)	Antigen	Reported Frequency	Clinical Significance
K:-1,2	k (006,002)	91 % European and African Populations	The most common phenotype; anti-K antibodies (006,001) can cause severe reactions.
K:1,2	K (006,001)	9 % European and African Populations	Compatible with most transfusions; risk with anti-K antibodies.
K:1,-2	k (006,002)	0.2 % European and African Populations	Rare phenotype; can cause significant incompatibility due to the absence of k
Kp(a + b-) (K:-3,4)	K (006,001)	0.1 % European and African Populations	Very rare phenotype; anti-Kp ^b antibodies (006,004) may be clinically significant.
Kp(a-b+) (K:3,-4)	Kp ^a (006,003)	97.9 % European and African Populations	Common phenotype; anti-Kp ^a antibodies are important in transfusion medicine.
Kp(a + b+) (K:3,4)	Kp ^b (006,004)	2 % European and African Populations	Compatible with most transfusions.
Js(a + b-) (K:-5,6)	Kp ^a (006,003) Kp ^b (006,004)	0.01 % European; 20 % African	Very rare in Europe; common in populations of African descent. Anti-Js ^b (006,006) antibodies are relevant.
Js(a-b+) (K:5,-6)	Js ^a (006,005)	81 % African	Anti-Js ^a antibodies may cause hemolytic reactions, especially in Africans.
Js(a + b+) (K:5,6)	Js ^b (006,006)	19 % African	Compatible with some donors, but there may be incompatibility in specific cases.

Case reports related to hemolytic disease of the fetus due to anti-Kp^a

Alloimmunization against the Kp^a antigen is extremely rare; the medical literature documents very few cases of perinatal anemia or miscarriages associated with anti-Kp^a antibodies [3–5]. Most studies and reports focus on antibodies against other Kell system antigens, such as K1 (KEL1), due to their higher prevalence [3–5].

The 2013 report by Rossi et al. documents a case of HDFN caused by the anti-Kp^a antibody [8]. In this case, a pregnant woman developed anti-Kp^a antibodies resulting in severe fetal anemia. The diagnosis was confirmed by serologic testing and prenatal imaging studies that revealed signs of hydrops fetalis, a severe complication in HDFN. This antibody was initially identified as part of an investigation for parvovirus B19-induced fetal anemia. Clinical management included multiple intrauterine transfusions to correct the fetal anemia, which allowed the pregnancy to be prolonged to a point where delivery was viable. The newborn, although affected by anemia, was stabilized after birth, but required additional monitoring and treatment. This case underscores the importance of early detection and proper management of alloimmunization by antibodies such as anti-Kp^a, because of the possibility of serious consequences if not treated early [9]. However, the paucity of specific reports makes it difficult to accurately assess the frequency and severity [9].

Erythrocyte phenotyping and transfusion impact

Extended erythrocyte phenotyping facilitates the identification of anti-Kp^a in patients requiring transfusion. This is particularly critical for polytransfused patients and women of childbearing age, as alloimmunization within the Kell system can significantly impact future pregnancies [10]. In the laboratory, identification of an anti-Kp^a antibody is carried out through serological testing, using techniques such as the indirect antiglobulin test (IAT) [10]. In cases where serological analysis is inconclusive, genetic testing may be useful to detect polymorphisms in the KEL gene [10].

In transfusion situations, it is suggested to administer Kp^a-negative red blood cell units to alloimmunized patients, although some studies suggest that serologically compatible units may also be adequate, given the low relative risk of severe hemolysis [11]. However, in scenarios such as hemolytic disease of the newborn, anti-Kp^a may generate fetal anemia, although to a lesser degree than other Kell system incompatibilities [11].

For patients requiring transfusions, the general recommendation is to use blood units that are compatible by cross-matching at 37 °C in the antiglobulin phase without the need to routinely select Kp^a-negative units due to the low prevalence of the antigen in the population [12]. However, in situations where anti-Kp^a has been identified, for example in HDFN and sickle cell disease, it is essential to ensure strict compatibility to prevent severe complications [12].

Conclusions

Appropriate management of patients with anti-Kp^a antibodies requires an individualized approach, with special attention to

the compatibility of transfused units and erythrocyte phenotyping. Alloimmunization, although rare, underlines the need to implement preventive strategies and accurate diagnosis to avoid hemolytic complications both in transfusions and in the perinatal context. This type of finding, although infrequent, highlights the relevance of detecting irregular antibodies in pregnant women, as well as the need to perform specific compatibility tests prior to transfusions to ensure the safety of both the mother and the fetus.

It is also important to perform genetic studies focused on local populations, especially in polytransfused patients and blood donors, since information on the specific variants and the frequency of alloimmunization and transfusion and gestational hemolysis due to these antibodies is scarce in the Americas.

Data availability

The data used to support this study's findings are included within the article.

Conflicts of interest

The author declares no conflicts of interest.

Editor

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Review article

Time-dependent trade-offs among intravenous iron formulations for iron-deficiency anemia: A longitudinal systematic review and network meta-analysis

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ABSTRACT

Intravenous iron formulations differ in benefits and risks that evolve across clinically relevant timepoints. A comprehensive network meta-analysis of randomized trials compared different intravenous iron formulations with oral iron and with each other. In addition to overall estimates, results are presented by predefined timepoints (4, 8, 12 and 24 weeks) for hemoglobin, ferritin, transferrin saturation, serious adverse events, and hypophosphatemia. Over 4-8 weeks, ferric carboxymaltose more consistently increased iron stores (ferritin/transferrin saturation) compared with the alternatives at the cost of a higher risk of hypophosphatemia. In 12-24 weeks, differences in iron stores attenuated and safety considerations became the main driver of choice, with ferric derisomaltose and iron sucrose generally favored when mineral safety is prioritized (e.g., for chronic kidney disease and inflammatory bowel disease). Effects on hemoglobin were broadly comparable between ferric carboxymaltose and ferric derisomaltose across most timepoints. These findings support conditional, scenario-specific decisions rather than a single 'best' formulation: ferric carboxymaltose when rapid repletion is critical and monitoring for hypophosphatemia is feasible; ferric derisomaltose and iron sucrose when safety predominates, or longer-term maintenance is planned. This windowed presentation facilitates pragmatic evidence translation for clinical decision-making while maintaining transparency through standard network meta-analysis diagnostics and certainty assessments.

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Introduction

Iron-deficiency anemia (IDA) is a common and significant cause of global morbidity, affecting more than 1.9 billion people (~27 % of the population) [1,2]. Common etiologies include

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gastrointestinal disorders, malignancies and inflammatory bowel disease (IBD) [3].

Diagnosis and monitoring consider hemoglobin (Hb), transferrin saturation (TSAT) and ferritin levels, which reflect both circulating and stored iron. Their combined interpretation aids differentiation of etiologies and treatment decisions [4].

Multiple intravenous (IV) iron formulations are available, each with distinct pharmacokinetic and tolerability profiles. Compared with oral iron, IV formulations provide faster and more complete repletion of iron stores, particularly in patients with inflammation, intolerance, or poor absorption [5–7].

However, the temporal dynamics of response and safety, critical for therapeutic planning, are rarely analyzed. This systematic review and network meta-analysis (NMA) compared IV formulations across clinically relevant decision time intervals (4, 8, 12, 24 weeks) to inform time-sensitive and context-specific clinical choices.

Methods

Study design and reporting

This systematic review was designed in accordance with the guidelines of the Cochrane Handbook and reported following the PRISMA 2020 and PRISMA-NMA guidelines (Supplement 1) [8,9]. The protocol was registered in PROSPERO (CRD420251065465).

Research question and eligibility criteria

PICO framework: In adults with IDA (P), how do intravenous (IV) iron formulations (I), compared with other IV formulations or oral ferrous sulfate (C), affect hemoglobin, ferritin, transferrin saturation, hypophosphatemia, any adverse events, and serious adverse events (O) when summarized within pre-specified clinical decision time intervals at 4, 8, 12, and 24 weeks (T)?

Randomized controlled trials of adults with IDA were included in this study to compare iron dextran, sodium ferric gluconate complex (SFG), iron sucrose (ISC), ferumoxytol (FXM), ferric carboxymaltose (FCM) and ferric derisomaltose (FDI) (versus oral ferrous sulfate and with each other). The primary outcome investigated was the hemoglobin level; secondary outcomes included ferritin, TSAT, adverse events, serious adverse events, and hypophosphatemia. Trials reporting obstetric and surgical IDA were excluded.

Data sources and search strategy

A systematic search was performed in February 2025 of PubMed/MEDLINE, the Cochrane Library, and Embase using structured descriptors to target the six IV iron formulations [10]. No language or date restrictions were applied (Supplement 2).

Study selection and data extraction

Two reviewers independently screened and extracted data using the Rayyan platform [11]. Extracted variables included baseline characteristics, intervention details, and outcomes at all reported timepoints. Discrepancies were resolved by consensus. Calculations and imputations followed the guidelines of the Cochrane Handbook [9].

Risk of bias and certainty of evidence

Risk of Bias Tool (RoB 2) [12] was used to assess the risk of bias at the outcome level. Certainty for NMA estimates used CINeMA [13,14] (overall and subgroups) and GRADE (high/moderate/low/very low) was used for pairwise analyses [15]. Downgrading reasons are detailed in Supplement 3.

Data synthesis and analysis

Mean differences and risk ratios (RRs) were estimated with 95 % confidence intervals (CIs). For connected networks, frequentist random-effects NMAs were performed using NMA Studio; otherwise, random-effects pairwise meta-analyses were conducted in R (version 4.2.3) [16].

Analyses covered overall, renal, and gastrointestinal patient populations across all timepoints; oral ferrous sulfate (OFS) results were reported as a common comparator, though the focus remained on IV formulations.

Multi-arm trials were handled via variance-covariance modeling to prevent double-counting.

Network geometry, transitivity, consistency

Nodes represented iron formulations, and edges indicated direct comparisons. The network geometry is shown in Supplement 3.

Transitivity was evaluated a priori by comparing baseline Hb, TSAT, and ferritin distributions across studies (Supplement 10).

Consistency was tested using design-by-treatment (global) and node-splitting (local) approaches in NMA Studio with p-values <0.05 indicating inconsistencies (Supplement 11).

Decision time intervals and visualization

Timepoints at 4 and 8 weeks (early response), 12 weeks (intermediate, when available), and 24 weeks (maintenance/safety) were prespecified to reflect time-sensitive decisions.

When trials reported additional timepoints (e.g., 1–3–5–6–10 weeks), each value was mapped to the nearest time interval using a $\pm$ two-week tolerance rule for the early timepoint and $\pm$ four-week tolerance for 24 weeks.

The RON-AI visualization tool was used to display week-by-week trends derived from these analyses without performing additional computations (Figure 1).

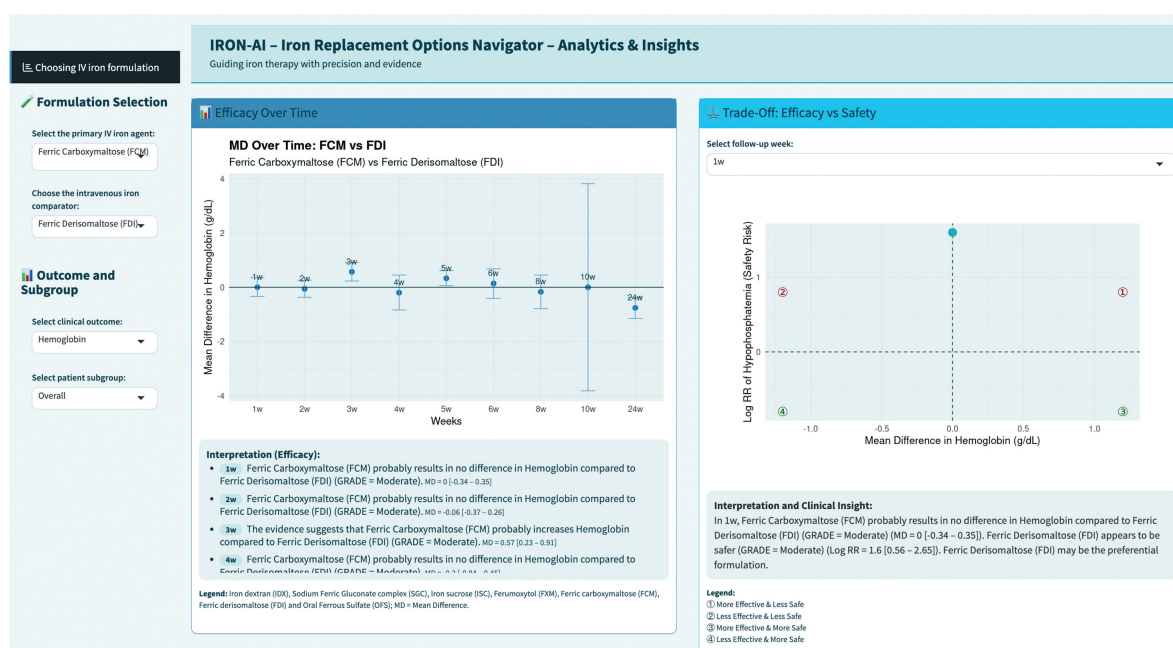


Fig. 1 – IRON-AI week-by-week visualization and trade-off. The interface displays weekly trajectories of comparative effects. Interpretation follows prespecified timepoints at 4, 8, 12, and 24 weeks adopted in the NMA. Visualization only; no additional analyses are performed.

Results

Search results and study characteristics

Fifty-five randomized controlled trials ($n = 23,302$) published between 2005 and 2024 were included in this study. Six IV formulations were evaluated. Baseline characteristics and risk-of-bias summaries are provided in Supplement Tables 5-8; the PRISMA flow diagram is shown in Figure 2.

Studies were categorized into overall, renal, and gastrointestinal subgroups (Supplement Tables 6 and 7).

Time-windowed findings

At 4-8 weeks, FCM more consistently increased ferritin/TSAT than alternatives but with a higher hypophosphatemia signal; at 10-24 weeks, differences in stores attenuated and safety considerations predominated, with FDI and ISC generally preferable. Hemoglobin effects were broadly similar between FCM and FDI across most timepoints (Tables 1–3).

The 12-week timepoint contained fewer direct observations in several head-to-head contrasts; as a result, estimates relied more on indirect evidence and were downgraded for imprecision (Tables 1–3; Supplement Tables).

Change in hemoglobin levels

Across timepoints, FCM versus FDI showed small and variable mean differences, with no consistent clinically important differences. Early contrasts favored FCM over some alternatives

at isolated timepoints (e.g., weeks 3-5), while FDI versus ISC showed modest early gains (week 1-3).

By 12-24 weeks, between-formulation differences generally attenuated. Full estimates by timepoint/comparator and certainty are shown in Table 1 (overall) and corresponding Supplement Tables 12 and 13.

Change in Ferritin levels

At 4-8 weeks, FCM consistently increased ferritin versus OFS and most IV comparators; FDI and ISC also outperformed OFS at several timepoints. Some head-to-head contrasts (e.g., FCM versus FDI and ISC) favored FCM early, but differences narrowed by 10-24 weeks. Timepoint-specific mean differences and certainty are shown in Table 2 (overall) and Supplement Tables 14 and 15.

Change in transferrin saturation levels

FCM showed early increases compared to several comparators (Weeks 1–5), while FDI > ISC at some early timepoints. By 8-12 weeks, differences attenuated. Full estimates and certainty by timepoint and comparator are provided in Table 3 (overall) and Supplement Tables 16 and 17.

Serious adverse events

Differences were observed only in the renal subgroup: FCM was associated with a higher risk compared to OFS (RR = 1.27; 95 % CI: 1.04-1.56), whereas FDI suggested a lower risk

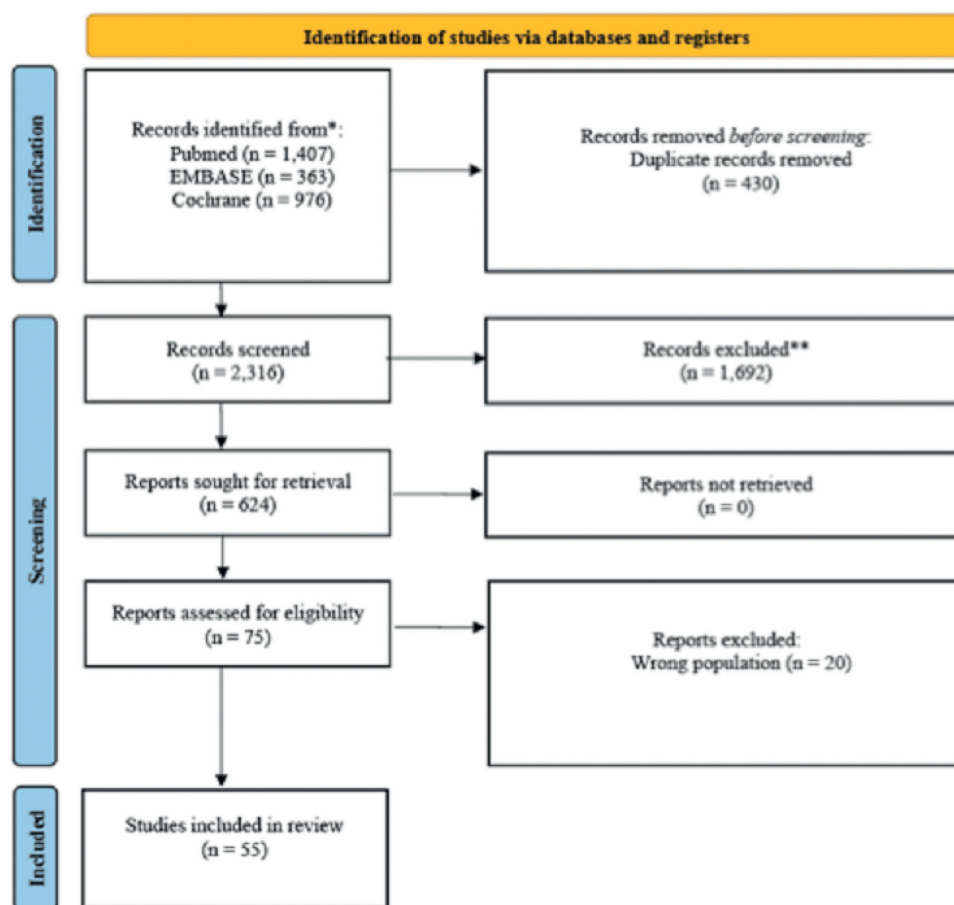


Fig. 2 – PRISM flow diagram showing selection process.

compared to iron dextran (RR = 0.10; 95 % CI: 0.01-0.97) (Supplement Tables 21–23).

Hypophosphatemia

FCM was associated with a higher risk of hypophosphatemia, especially in the renal and gastrointestinal subgroups. FDI and ISC had more favorable profiles: in gastrointestinal trials, FDI versus FCM (RR = 0.21; 95 % CI: 0.10-0.46) and ISC versus FCM (RR = 0.04; 95 % CI: 0.02-0.09) indicated substantially lower risks (Supplement Tables 25 and 26).

Heterogeneity and inconsistency

Localized inconsistency (p-value <0.05) occurred in some pairwise contrasts, largely due to baseline heterogeneity. Global coherence remained acceptable, supporting pooled estimates.

IRON-AI

Figure 1 illustrates the IRON-AI interface displaying week-by-week efficacy–safety trade-offs aligned with NMA-derived decision time intervals.

Discussion

This network meta-analysis indicates time- and scenario-dependent differences: early gains in iron stores with FCM coincide with a higher hypophosphatemia signal, whereas FDI and ISC provide a more favorable mineral safety profile for maintenance horizons; Hb effects were small and variable across timepoints.

It is worth noting that a prior study reported a more favorable safety profile for FCM, contrasting with the present results. However, that study lacked a formal assessment of evidence certainty and included a narrower population scope [17].

Importantly, this analysis highlights persistent concerns regarding hypophosphatemia, a known adverse event associated with FCM, which occurred more frequently than with other agents [18–20]. This review extends current knowledge by evaluating outcomes across distinct subgroups and multiple timepoints. Few previous studies have used direct or indirect network meta-analysis in patients with different anemia etiologies [20–23].

The present results also suggest that FXM and SFG may be viable alternatives when safety is prioritized, despite modest gains [24]. Some formulations, however, did not

Table 1 – Comparative efficacy of iron formulations on hemoglobin levels by timepoint (Overall analysis).

Intravenous iron agent	Comparator	Timepoint (Week)	Estimative	Hemoglobin MD (95% CI)	Intravenous iron agent	Comparator	Timepoint (Week)	Estimative	Hemoglobin MD (95% CI)
FCM	FDI	1	Network	0.00 (-0.34, 0.35)	FDI	ISC	1	Network	0.26 (0.15, 0.36) *
		2	Network	-0.06 (-0.37, 0.26)			2	Network	0.13 (-0.13, 0.39)
		3	Network	0.57 (0.23, 0.91) *			3	Network	0.37 (0.02, 0.76) *
		4	Network	-0.20 (-0.84, 0.45)			4	Network	0.15 (-0.33, 0.63)
		5	Network	0.33 (0.06, 0.61) *			5	Network	0.19 (-0.11, 0.49)
		6	Network	0.14 (-0.41, 0.68)			6	Network	0.02 (-0.49, 0.54)
		7	NR	NR			8	Network	0.42 (-0.01, 0.84)
		8	Network	-0.17 (-0.79, 0.45)		FXM	2	Network	-0.17 (-0.75, 0.41)
		10	Direct	0.00 (-3.82, 3.82)			3	Network	0.07 (0.44, 0.58) *
		24	Network	-0.76 (-1.15, -0.37) *			4	Network	0.05 (-0.80, 0.90)
	ISC	1	Network	0.26 (-0.10, 0.62)			5	Network	-0.01 (-0.32, 0.30)
		2	Network	0.08 (-0.28, 0.43)		IDX	3	Network	0.57 (-0.07, 1.21)
		3	Network	0.94 (0.49, 1.39) *		SFG	6	Network	0.00 (-0.87, 0.88)
		4	Network	-0.05 (-0.65, 0.56)		OFS	1	Network	0.30 (-0.03, 0.63)
		5	Network	0.52 (0.23, 0.82) *			2	Network	0.11 (-0.24, 0.47)
		6	Network	0.16 (-0.23, 0.55)			3	Network	0.30 (-0.07, 0.68)
		8	Network	0.24 (-0.28, 0.77)			4	Network	0.27 (-0.23, 0.77)
		12	Network	-0.50 (-6.17, 5.17)			5	Network	0.67 (0.22, 1.11) *
FXM	FXM	2	Network	-0.22 (-0.85, 0.41)	ISC		6	Network	0.20 (-0.37, 0.77)
		3	Network	0.64 (0.08, 1.20) *			8	Network	0.11 (-0.37, 0.60)
		4	Network	-0.15 (-1.07, 0.78)			24	Network	1.00 (-0.71, 1.29)
		5	Network	0.33 (0.06, 0.59) *		FXM	2	Network	-0.30 (-0.82, 0.22)
	IDX	3	Network	1.14 (0.46, 1.82) *			3	Network	-0.30 (-0.63, 0.03)
		12	Network	1.20 (1.02, 1.38) *			4	Network	-0.10 (-0.80, 0.60)
	SFG	6	Network	0.14 (-0.63, 0.91)			5	Network	-0.20 (-0.37, -0.02) *
	OFS	1	Network	0.30 (-0.18, 0.78)		IDX	3	Network	0.20 (-0.31, 0.71)
		2	Network	0.06 (-0.33, 0.45)			12	Network	1.70 (-3.97, 7.37)
		3	Network	0.87 (0.45, 1.29)		SFG	6	Network	-0.02 (-0.76, 0.72)
		4	Network	0.08 (-0.52, 0.67)		OFS	1	Network	0.04 (-0.30, 0.39)
		5	Network	1.00 (0.65, 1.35) *			2	Network	-0.02 (-0.44, 0.41)
		6	Network	0.34 (-0.04, 0.72)			3	Network	-0.06 (-0.32, 0.19)
		8	Network	-0.06 (-0.65, 0.54)			4	Network	0.12 (-0.49, 0.74)
		12	Network	0.90 (-4.24, 6.04)			5	Network	0.48 (0.02, 0.93) *
		24	Network	0.24 (-0.25, 0.73)			6	Network	0.18 (-0.14, 0.50)
		52	Network	0.40 (-2.97, 3.77)			8	Network	-0.30 (-0.81, 0.21)
	IDX	3	Network	0.50 (-0.10, 1.10)			12	Network	1.40 (-6.25, 9.05)
FXM	OFS	2	Network	0.28 (-0.39, 0.95)			20	NR	NR
		3	Network	0.24 (-0.18, 0.65)			52	NR	NR
		4	Network	0.22 (-0.71, 1.15)	IDX	OFS	3	NR	NR
		5	Network	0.67 (0.24, 1.35)	SFG	OFS	6	NR	NR

IDX: Iron dextran; SFG: Sodium ferric gluconate complex; ISC: Iron sucrose; FXM: ferumoxylol; FCM: Ferric carboxymaltose; FDI: Ferric deriso-maltose; OFS: Oral Ferrous Sulfate; NR: Not reported; 95 % CI: 95 % Confidence interval.

Asterisk indicates statistically significant estimate. Shading of the cells indicates the certainty of evidence as follows: green (high); yellow (moderate); orange (low) and red (very low).

Table 2 – Comparative efficacy of iron formulations on ferritin levels by timepoint (overall analysis).

Intravenous Iron Agent	Comparator	Timepoint (Week)	Estimative	Ferritin MD (95% CI)	Intravenous Iron Agent	Comparator	Timepoint (Week)	Estimative	Ferritin MD (95% CI)	
FCM	FDI	1	Network	96.76 (-17.43, 210.95)	FDI	ISC	1	Network	268.88 (168.21, 369.55) *	
		2	Network	79.17 (-109.30, 267.64)			2	Network	131.31 (-27.27, 289.0)	
		3	Network	175.78 (-29.48, 381.05)			3	Network	218.85 (-48.48, 486.17)	
		4	Network	-9.92 (-199.15, 179.32)			4	Network	62.06 (-22.98, 147.10)	
		5	Network	59.21 (-4.68, 123.10)			5	Network	111.87 (12.60, 211.14)	
		6	Network	59.10 (-356.52, 474.72)			6	Network	57.87 (-421.54, 537.28)	
		7	Direct	-20.70 (-32.77, 74.17)			8	Network	33.88 (-13.47, 81.23)	
		8	Network	-19.61 (-116.02, 76.80)		FXM	2	Network	54.48 (-263.02, 371.97)	
		10	Direct	8.80 (-57.95, 40.35)			3	Network	62.35 (-370.30, 494.99)	
		24	Network	316.70 (271.82, 361.58) *			4	Network	17.66 (-210.32, 245.63)	
	ISC	1	Network	365.64 (213.41, 517.87) *		5	Network	59.59 (-46.22, 165.41)		
		2	Network	210.48 (1.12, 419.85) *		IDX	3	Network	216.15 (-210.41, 642.70)	
		3	Network	394.63 (85.72, 703.54) *		SFG	6	Network	-73.36 (-712.76, 566.05)	
		4	Network	52.14 (-123.56, 227.83)		OFS	1	Network	383.38 (246.2, 522.55) *	
		5	Network	171.08 (64.24, 277.93) *			2	Network	179.16 (-48.58, 406.91)	
		6	Network	116.97 (-121.97, 355.91)			3	Network	227.82 (2.67, 452.96) *	
		8	Network	14.27 (-71.54, 100.08)			4	Network	63.84 (-58.75, 186.42)	
		12	Network	-26.30 (-34.18, -18.42)			5	Network	208.83 (76.33, 341.33) *	
	FXM	2	Network	133.65 (-169.32, 436.62)			6	Network	102.74 (-380.65, 586.13)	
		3	Network	238.13 (-221.38, 697.64)			8	Network	27.69 (-27.08, 82.46)	
		4	Network	7.74 (-267.24, 282.71)			24	Network	-13.60 (-70.68, 43.48)	
		5	Network	118.80 (21.40, 216.20)		FXM	2	Network	-76.84 (-381.39, 227.72)	
	IDX	3	Network	391.93 (-61.85, 845.71)			3	Network	-156.50 (-496.68, 183.68)	
		12	Network	48.00 (43.38, 52.62) *			4	Network	-44.40 (-255.92, 167.12)	
	SFG	6	Network	-14.26 (-500.16, 471.64)			5	Network	-52.28 (-158.61, 54.05)	
	OFS	1	Network	481.14 (333.15, 629.13) *		IDX	3	Network	-2.70 (-335.10, 329.70)	
		2	Network	258.34 (-8.95, 525.62)			12	Network	74.30 (65.16, 83.44) *	
		3	Network	403.60 (161.53, 645.67) *		SFG	6	Network	-131.23 (-613.25, 350.79)	
		4	Network	53.92 (-141.58, 249.43)		OFS	1	Network	115.50 (-55.45, 286.45)	
		5	Network	268.04 (151.96, 384.12) *			2	Network	47.85 (-195.78, 291.49)	
		6	Network	161.84 (-84.99, 408.68)			3	Network	8.97 (-252.43, 270.37)	
		8	Network	8.08 (-87.55, 103.71)			4	Network	1.78 (-125.45, 129.01)	
		12	Network	16.50 (-3.21, 36.21)			5	Network	96.96 (-60.81, 254.72)	
		24	Network	303.10 (230.49, 375.71) *			6	Network	44.87 (-194.23, 283.97)	
		52	Network	314.00 (288.91, 339.09) *			8	Network	-6.19 (-57.69, 45.31)	
	FXM	3	Network	153.80 (-321.82, 629.42)			12	Network	42.80 (21.57, 64.03) *	
		OFS	2	Network			124.69 (-243.94, 493.32)	20	Direct	65.00 (33.73, 96.27) *
			3	Network			165.47 (-263.54, 594.48)	52	NR	NR
			4	Network		46.18 (-200.66, 293.02)	IDX	3	Network	11.67 (-411.20, 434.54)
			5	Network		149.24 (-2.29, 300.77)	SFG	6	Network	176.10 (-283.44, 673.73)

IDX: Iron dextran; SFG: Sodium ferric gluconate complex; ISC: Iron sucrose; FXM: ferumoxytol; FCM: Ferric carboxymaltose; FDI: Ferric deriso-maltose; OFS: Oral Ferrous Sulfate; NR: Not reported; 95 % CI: 95 % Confidence interval.

Asterisk indicates statistically significant estimates. Shading of cells indicates the certainty of evidence as follows: green (high), yellow (moderate), orange (low) and red (very low).

demonstrate superiority over OFS, underscoring the need for cautious clinical interpretation and corroboration in future trials [25–27].

Some pairwise contrasts showed local inconsistency (p-value <0.05), likely reflecting heterogeneity in baseline characteristics. Despite this, overall network coherence was acceptable, supporting the pooled estimates and their interpretation [28].

Limitations include heterogeneous regimens and follow-ups, sparse timepoints for some comparisons, and higher measurement bias risk for adverse events. Interpretation of hypophosphatemia is limited by heterogeneous thresholds across trials (e.g., <2.0 versus <2.5 mg/dL) and outcome ascertainment; standardization in future randomized controlled trials is warranted. These factors may attenuate precision and generalizability for specific comparisons.

Table 3 – Comparative efficacy of iron formulations on transferrin saturation by timepoint (Overall analysis).

Intravenous Iron Agent	Comparator	Timepoint (Week)	Estimative	Transferrin Saturation MD (95% CI)	Intravenous Iron Agent	Comparator	Timepoint (Week)	Estimative	Transferrin Saturation MD (95% CI)
FCM	FDI	1	Network	-1.35 (-5.52, 2.81)	FDI	ISC	1	Network	9.83 (6.69, 12.98) *
		2	Network	5.44 (2.30, 8.57) *			2	Network	2.73 (-2.40, 5.14)
		3	Network	7.10 (3.35, 10.85) *			3	Network	6.22 (2.30, 10.14) *
		4	Network	2.85 (-0.91, 6.61)			4	Network	1.26 (0.13, 2.40) *
		5	Network	3.02 (-0.90, 6.95)			5	Network	2.81 (-1.80, 7.43)
		6	Network	0.70 (-8.48, 9.86)			6	Network	9.56 (0.65, 18.47) *
		7	Direct	2.10 (-3.80, 8.00)			8	Network	0.65 (-0.35, 1.65)
		8	Network	2.72 (-0.39, 5.84)		FXM	2	Network	-3.71 (-8.22, 0.79)
		10	Direct	2.10 (-1.11, 5.31)			3	Network	3.90 (-0.14, 7.94)
		24	NR	NR			4	Network	-0.14 (-5.24, 4.96)
	ISC	1	Network	8.48 (3.26, 13.70) *			5	Network	0.36 (-4.64, 5.37)
		2	Network	8.17 (4.74, 11.59) *		IDX	3	Network	5.52 (-1.23, 12.27)
		3	Network	13.32 (8.92, 17.71) *		SFG	6	Network	1.67 (-8.08, 11.42)
		4	Network	4.11 (0.39, 7.83) *		OFS	1	Network	6.01 (1.65, 10.36) *
		5	Network	5.84 (0.83, 10.84) *			2	Network	1.36 (-2.16, 4.87)
		6	Network	10.26 (5.07, 15.46) *			3	Network	0.51 (-3.37, 4.39)
		8	Network	3.38 (0.18, 6.58) *			4	Network	1.73 (-0.69, 4.15)
		12	NR	NR			5	Network	15.68 (8.48, 22.88) *
	FXM	2	Network	1.73 (-2.43, 5.88)			6	Network	7.07 (-1.52, 15.64)
		3	Network	11.00 (6.05, 15.95) *			8	Network	2.00 (-0.06, 4.05)
		4	Network	2.71 (-3.50, 8.92)			24	Network	NR
		5	Network	3.39 (-1.23, 8.01)		FXM	2	Network	-6.44 (-10.78, -2.11)
	IDX	3	Network	12.62 (5.58, 19.65) *			3	Network	-2.32 (-6.55, 0.00, 1.91)
		12	NR	NR			4	Network	-1.4 (-6.37, 3.57)
	SFG	6	Network	-0.43 (-5.62, 4.75)			5	Network	-2.45 (-6.42, 1.53)
	OFS	1	Network	4.65 (0.37, 8.94) *		IDX	3	Network	-0.70 (-6.20, 4.80)
		2	Network	6.80 (2.79, 10.80) *			12	Network	NR
		3	Network	7.61 (4.15, 11.07) *		SFG	6	Network	-7.89 (-13.29, -2.50) *
		4	Network	4.58 (1.33, 7.83) *	ISC	OFS	1	Network	-3.83 (-9.20, 1.54)
		5	Network	18.70 (12.66, 24.74) *			2	Network	-1.37 (-5.14, 2.40)
		6	Network	4.97 (2.65, 7.29) *			3	Network	-5.71 (-9.29, -2.16)
		8	Network	4.72 (2.12, 7.32) *			4	Network	0.47 (-1.96, 2.89)
		12	NR	NR			5	Network	12.86 (5.02, 20.71) *
		24	NR	NR			6	Network	-2.49 (-5.25, 0.27)
		52	Direct	1.40 (-5.15, 7.95)			8	Network	1.34 (-0.90, 3.59)
							12	Network	NR
	FXM	3	Network	1.62 (-5.32, 8.56)			52	NR	2.10 (-1.11, 5.31)
	OFS	2	Network	5.07 (-0.17, 10.31)			20	NR	NR
		3	Network	-3.39 (-7.89, 1.20)			52	NR	2.10 (-1.11, 5.31)
		4	Network	1.87 (-3.66, 7.40)	IDX	OFS	3	NR	NR
		5	Network	15.31 (7.71, 22.92) *		OFS	6	NR	5.40 (0.76, 10.04) *

IDX: Iron dextran; SFG: Sodium ferric gluconate complex; ISC: Iron sucrose; FXM: ferumoxytol; FCM: Ferric carboxymaltose; FDI: Ferric deriso-maltose; OFS: Oral Ferrous Sulfate; NR: Not reported; 95 % CI: 95 % Confidence interval.

Asterisk indicates statistically significant estimates. Shading of the cells indicates the certainty of evidence as follows: green (high), yellow (moderate), orange (low) and red (very low).

Clinically, these results emphasize the trade-off between efficacy and safety that should guide individualized decision-making. FCM demonstrated superior improvements in ferritin and TSAT, with sustained effects for up to two months, making it appropriate for rapid iron repletion or severe anemia. However, its higher risk of hypophosphatemia warrants caution in patients at risk of mineral metabolism disorders. FDI and ISC offer balanced efficacy with better safety profiles and may be preferable for maintenance or in vulnerable populations.

This review underscores the need for high-quality, head-to-head randomized trials with standardized safety outcomes - particularly for serious adverse events and hypophosphatemia - and the inclusion of cost-effectiveness and quality-of-life assessments to inform clinical and policy decisions.

Conclusion

In summary, the current findings support time- and scenario-dependent choices rather than a single preferred formulation. When rapid repletion over 4-8 weeks is clinically valuable (e.g. active IBD), FCM may be considered for its more consistent gains in ferritin/TSAT provided that serum phosphate is proactively monitored given the risk of hypophosphatemia. In safety-first or maintenance horizons (12-24 weeks), particularly in chronic kidney disease and inflammatory bowel disease, FDI or ISC are generally preferable owing to more favorable mineral safety profiles with broadly comparable hemoglobin effects. From a formulary stewardship perspective, restricting FCM to contexts where short-term repletion and reliable monitoring are feasible, listing FDI and ISC as options where safety predominates, and avoiding routine escalation from oral iron in the absence of a clear indication appear reasonable.

Data availability statement

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

Conflicts of interest

No conflicts of interest related to the design, conduct, or reporting of this study.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2026.106435.

Editor

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Special article

Criteria for referring pediatric and adult patients with hematological diseases to palliative care: Consensus of the Brazilian Association of Hematology, hemotherapy and cell therapy (2025)



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ABSTRACT

Palliative care is, according to the World Health Organization (WHO), “a global ethical responsibility” and crucial to person-centered healthcare, for the relief of physical and psychological symptoms and social and spiritual suffering, impacting the improvement of the quality of life not only of pediatric and adult patients dealing with life-threatening illnesses, but also of their families. Despite the growing recognition of palliative care in the hematological setting and the increase in the number of publications on the subject, the available data are still scarce and limited. The objective of this consensus is to establish recommendations for the referral of pediatric and adult hematological patients to specialized palliative care teams and the possible integration of these two specialties.

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Introduction

In 1990, the World Health Organization (WHO) published the first definition of Palliative Care (PC). Subsequently, in 1998, it addressed the topic with a particular focus on children, and since then, this issue has undergone several reformulations and updates. According to a revision in 2018, the WHO states that PC is “an approach that improves the quality of life (QoL) of patients (adults or children) and their families facing problems associated with life-threatening illness. PC prevents and alleviates suffering through early investigation, correct assessment, and treatment of pain and other physical, psychosocial, or spiritual problems.” [1].

It is important to emphasize that QoL is a subjective assessment that goes beyond the concern of eradicating diseases and their symptoms, as it is also directly related to the effects caused by health interventions on all aspects of an individual's life, within their culture, the value systems in which they live, and in relation to their goals, expectations, standards, and concerns [2].

Complementing the WHO definition, the International Association for Hospice and Palliative Care (IAHPC) stated that PC is active care, especially, but not only, for those in the final stages of life [3]. Given its inherent complexity, PC should be delivered by a multidisciplinary team comprising a minimum core of specialized professionals, including a physician, nurse, psychologist, or social worker. The interdisciplinary framework may further incorporate other specialists, such as nutritionists, physiotherapists, occupational therapists, and chaplains, among others [1]. The composition of the team and the duties and responsibilities of its members vary according to the patient's needs, available resources, and the social context.

Evidence shows that early intervention with PC significantly improves QoL, symptoms, and satisfaction of patients with advanced cancer, and may even prolong survival [4,5]. Despite this evidence, it is well known that the integration of PC in hematological diseases has lagged behind that of solid

oncology for numerous reasons. The stigmatized association between this treatment and end-of-life care is responsible for many patients with advanced hematological diseases still not receiving PC [6]. Routinely, data already consolidated for solid tumors are extrapolated to hematology, [4,7] and, knowing that the trajectories and prognostic estimates are quite different in these scenarios, this model of reasoning is an obstacle to the integration of PC services. Prognostic uncertainty and the use of intensive treatments, which can result in a cure while still presenting a high risk of mortality, make it difficult to define referral criteria for hematological malignancies (HM) and end-of-life indicators in hematology [8,9].

The availability of effective and safe targeted therapies and immunotherapies for a variety of HM has increased the expectation of cure; however, this is sometimes at the cost of significant morbidity and distressing symptoms, since a significant proportion of patients will not respond to or tolerate these new therapies [10,11]. Estimating prognosis and envisioning outcomes are challenging when faced with new therapeutic possibilities; they also reduce the chances of discussing the risks and benefits of a new treatment, addressing care preferences, and defining the care plan for the end-of-life phase.

Most patients with HM want to participate in decision-making regarding their treatments, but consider the information they receive to be insufficient or unclear, as exemplified in different studies by the significant discrepancy between the expectations of doctors and patients regarding both treatment responses and chances of cure, as well as diagnostic understanding and disease trajectory [12–14]. Patients with a high risk of mortality, but who maintain a small chance of cure, should be informed about possible outcomes, from the best to the worst scenarios. Early integration of PC is recognized as an ideal way to allow patients and families to be more aware of the possible prognoses and cope with the psychosocial sequelae and uncertainties inherent in therapies, enabling greater patient involvement in decisions about their treatment [8,11,15,16]. Ultimately, the unique and close bond between hematologists and their patients, often rooted in

deep trust and strong clinical rapport developed throughout the treatment process, can create barriers to the integration of other professionals into the care team [17].

In an attempt to define the optimal timing for the integration of PC and to contribute to care planning, Shaulov et al [18] proposed specific trajectories for hematological diseases, categorizing them into three distinct groups:

- a) aggressive diseases (e.g., acute leukemias, aggressive lymphomas) and bone marrow transplantation: life-threatening diseases with a high risk of morbidity associated with the possibility of long-term treatment, often with cure being a reasonable and achievable goal until days before death;
- b) indolent diseases (e.g., chronic lymphocytic leukemia, indolent lymphomas, and multiple myeloma [MM]): incurable diseases with progressive loss of functionality due to multiple relapses and different lines of treatment, associated with periods of prolonged remission and preserved performance status;
- c) bone marrow failure (e.g., myelodysplastic syndrome, myelofibrosis, aplastic anemia [AA]): a group characterized by cytopenias secondary to bone marrow failure, leaving the patient dependent on blood transfusions and susceptible to recurrent bacterial and fungal infections, in which the prognosis is challenging as death results from acute complications of cytopenias, and not from disease progression [18].

Based on an adaptation of these models and the publication by Murray et al., [19] we suggest the following trajectories for benign and malignant hematological diseases (Figure 1).

Fortunately, there has been an increase in the number of publications on PC in hematology, demonstrating the impact that this partnership can generate in all dimensions of suffering [20–22]. Following this trend, the 2024 update of the American Society for Clinical Oncology (ASCO) guidelines recommends referral to PC specialists for all patients with HM [23].

Despite the growing recognition of PC and the number of publications in the literature, including randomized trials that confirm the benefits of PC integrated with HM, the available data are still scarce and limited, especially when considering benign diseases. The objective of this consensus is, therefore, to establish criteria that encourage the possible integration and referral of pediatric and adult hematological patients to a specialized PC team, seeking a joint action for the benefit of patients in their entire environment, including their support network and healthcare team.

Specifics in pediatrics

Influenced by the *hospice* movement in adults, pediatrics has adopted a more holistic approach, transforming and expanding awareness of multidimensional suffering in children and their families. Pediatric and adolescent patients cannot be considered separately, since their families are directly or indirectly involved in patient care, and thoughts about the possibility of death and the end of life vary among them. Care and

communication should be patient-centered and family-focused [24,25]. Most pediatricians aspire to promote the physical and emotional well-being of their patients' development, hoping to offer a long and healthy life, but a portion of cases will not achieve this.

In 1979, Smith et al. described an approach that provided total care for children with cancer and their families, aiming to meet physical, emotional, and financial needs [26,27]. In 1983, Lewis argued that the principles of alleviating suffering should be applied to all seriously ill children as ongoing care, and not only to those who are dying [26,28,29]. Until then, the management of pain and end-of-life symptoms, which were limited to one paragraph, began to be studied by small groups of specialists with the collaboration of parents, aiming at improving care [26].

In general, children and adolescents undergo painful procedures such as bone marrow aspiration, lumbar punctures, and venous catheter insertions, both at diagnosis and during treatment, and sometimes after remission. This exposes them not only to a high burden of physical symptoms, but also psychological, social, and spiritual ones. Chemotherapy protocols, in most cases, do not facilitate the outpatient administration of medications, and due to age, side effects, bureaucratic issues, or complications, patients remain hospitalized for long periods, isolated from their family, school, and friends. Visual and bodily changes, especially in adolescence, secondary to hair loss and weight gain, impact self-esteem, triggering greater social isolation, depressive episodes, and anxiety. Denial, or the desire to be like other adolescents, can lead patients to engage in risky behaviors and abandon medical treatments, with serious consequences. No less important, as they become young adults, they must leave the familiar environment of pediatric doctors and nurses who have cared for them so far in their lives and establish a relationship with an unfamiliar care team, with health care often being transferred from parents to the patients themselves [25,30].

Studies show that children with advanced cancer, including those with HM, are more likely to receive intensive end-of-life treatment compared to adults. Furthermore, they are more likely to receive intravenous chemotherapy (CT) in the 14 days preceding death, even in advanced stages of the disease [31]. Mortality rates in patients with malignant HM are higher than those with solid tumors due to treatment-related complications. Consequently, there are higher rates of admission to the pediatric intensive care unit (ICU) for acute respiratory failure, septic shock, and severe hemorrhage [31]. Due to the more effective response to CT, there is a high number of different protocols, including for relapse, resulting from technological advances in specific therapies, the possibility of hematopoietic stem cell transplantation (HSCT), and chimeric antigen receptor T cell (CAR-T) therapy, making prognostic estimation and the decision-making process more complex [31,32].

A study analyzed PC opportunities in different childhood cancer diagnosis groups and identified that children with solid tumors and central nervous system tumors have more opportunities for PC compared to those with leukemia or lymphoma [33]. This suggests that early identification of PC opportunities can guide the use of these services to alleviate

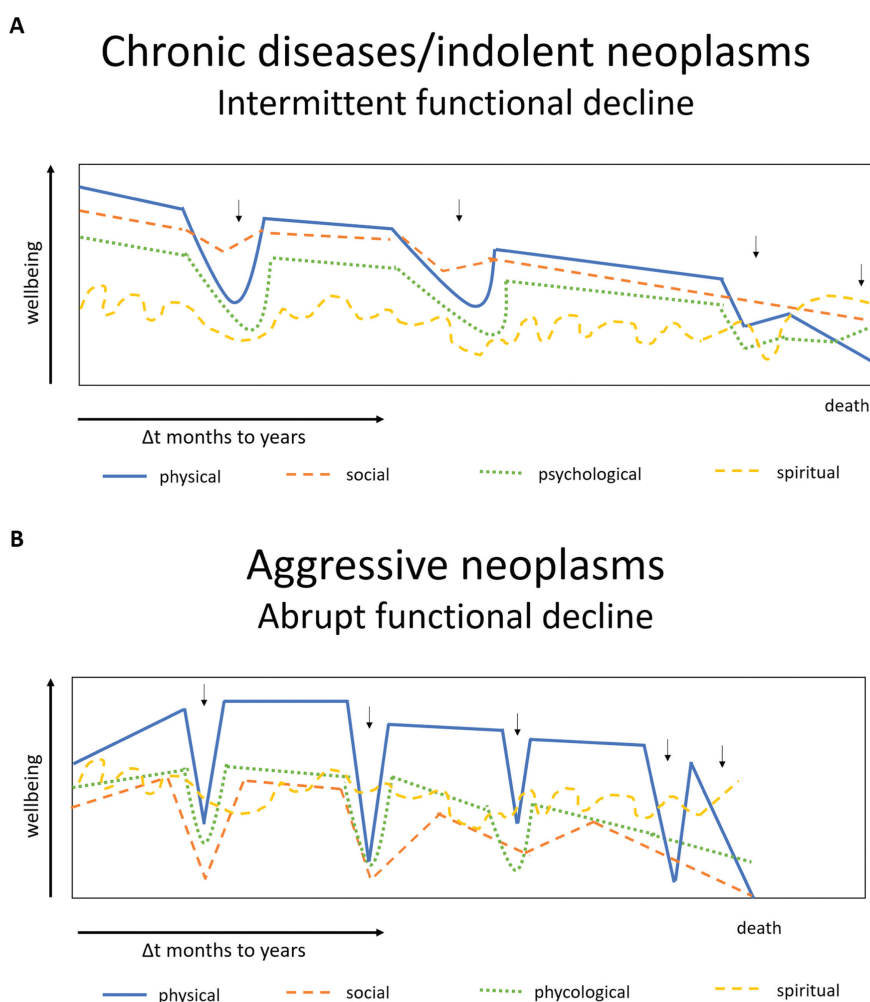


Fig. 1 – Trajectories of hematological diseases across the four dimensions of care: physical, social, psychological, and spiritual. (A) Indolent hematological neoplasms, bone marrow failure, and benign chronic diseases – patients in this group are frequently followed for months to years in hematology services, possibly with alternating periods of disease-modifying treatment and periods of expectant management. During follow-up, various acute events (small arrows) (e.g., hospitalizations, infections, hemorrhages, worsening anemia, etc.) or the accumulation of comorbidities resulting from chronic disease combined with aging can lead to a worsening of physical and psychological symptoms. At these times, impairment of social capacity is frequent, while spirituality is variable, depending on the characteristics of each individual. After the resolution of acute events, incomplete recovery of health conditions is common, with progressive loss of functionality over time. The development of prognostic awareness deepens over the follow-up period as functionality is lost. (B) Aggressive hematological neoplasms (acute leukemias, high-grade lymphomas) and bone marrow transplantation – in addition to the manifestations caused by the neoplasm itself, patients in this group are frequently subjected to aggressive treatments, resulting in potentially life-threatening acute complications (small arrows) (e.g., sepsis, hemorrhages, gastrointestinal symptoms, tumor lysis, etc.), which can lead to abrupt loss of functionality in the physical, social, and psychological dimensions, with or without subsequent recovery. Spirituality is variable, depending on the individual characteristics of the patients. Given the aggressiveness of the HM in this group, the mortality rate within weeks to months is substantial, and establishing earlier prognostic awareness is fundamental.

the stress of patients and their families [33]. PediQUEST, an important trial in children with advanced cancer, evaluated the use of a computerized tool for assessing patient-reported outcomes (PROs) [34]. The primary objective was to verify whether providing feedback to healthcare providers and families with respect to PROs could improve health-related QoL and reduce suffering and symptom burden. The secondary objective was to assess the level of agreement between parents and healthcare providers regarding patient outcomes.

Children with advanced cancer aged two years or older, or their parents, completed the PediQUEST questionnaire weekly, including adapted versions of the Memorial Symptom Assessment Scale (MSAS) and the Pediatric QoL Inventory 4.0 (PedsQL4.0), and a general question about the disease [35]. While there was no feedback in the control group, in the intervention group, oncologists and family members received printed reports summarizing the PROs, and emails were sent to oncologists when certain scores were exceeded. The results

showed that feedback did not significantly affect the mean trends in MSAS and PedsQL4.0 scores or disease progression [34]. However, exploratory analyses suggested improvements in emotional QoL in specific subgroups, such as children aged eight years or older who survived for 20 weeks. Furthermore, feedback from PROs was valued by children, parents, and providers, contributing to the initiation of psychosocial consultations in indicated cases. The importance of strategies to overcome the 'normalization' and undervaluation of symptoms, which prevent the use of appropriate interventions to alleviate suffering in childhood cancer, was also highlighted. This support is crucial for improving QoL and can be integrated by PC teams [36,37].

Methods

This consensus was written by hematologists specializing in PC, members of the PC Committee of the Brazilian Association of Hematology, Hemotherapy and Cell Therapy (ABHH), in collaboration with members of the respective specialty committees of the ABHH. The criteria of the Grading of Recommendations Assessment, Development and Evaluation (GRADE) [38] working group were used to define the levels of evidence and strength of the recommendations. Since scientific production on PC in hematological diseases is limited, the levels of evidence for the recommendations are weak. Therefore, to construct referral criteria that are in accordance with the principles of PC and the particularities of each group of hematological diseases, the recommendations were developed by the PC committee and established after discussion with specialists in each area covered in this Consensus through the respective specialty committees of the ABHH.

This document was developed after a systematic search of scientific articles related to the topic. References were searched in databases (PubMed, SciELO, Web of Science, ScienceDirect, Latin American and Caribbean Literature [LILACS], and SCOPUS) with no limit on publication date or format. The following keywords were used: "palliative care", "hospice care", "symptom management", "symptom burden", "quality-of-life", "end-of-life", "caregiver outcome", "advance care planning", "treatment decision-making", "health service utilization", "place of death", "prognostic tools", "prognostic scores", "predictors", "acute leukemia", "lymphoma", "lymphoproliferation", "multiple myeloma", "amyloidosis", "myelodysplastic syndrome", "myelofibrosis", "myeloproliferative neoplasm", "chronic myeloid leukemia", "essential thrombocythemia", "polycythemia vera", "bone marrow transplant", "CAR-T cells", "sickle cell", "thalassemia", "hemoglobinopathies", "hemophilia", "thrombosis", "bone marrow failure", "neutropenia", "pediatrics", and "children". Articles with abstracts related to the theme of this consensus were evaluated in full.

Palliative care in hematology—General criteria of referral

According to a 2015 publication by the American Society of Hematology (ASH), referral to a specialized PC team should be considered in some general scenarios, regardless of the patient's diagnosis and age.

Recommendations

General criteria for referring hematological patients to palliative care

1. Patients with a high symptom burden or symptoms refractory to established treatments; impaired QoL and significant psychological distress (mood disorders or existential suffering); sequelae and late side effects related to established treatments. (Level of recommendation: B)
2. Patients with difficulty understanding diagnosis and prognosis; those with significant or persistent misperceptions about the trajectory of their disease and overall prognosis. (Level of recommendation: B)
3. Patients experiencing difficulties coping with their illness; need for communication adjustments; patient involvement in the shared decision-making process and in defining end-of-life care preferences; assistance in conflict resolution. (Level of recommendation: D)
4. Support for distressed family members/caregivers. (Level of recommendation: D)
5. Reserved prognosis or limited life expectancy (surprise question: "Would you be surprised if your patient died within 1 year?"). (Level of recommendation: B)
6. Patients with recurrent unplanned hospitalizations and loss of performance status/functionality. (Level of recommendation: D)

Palliative care in hematology—Referral criteria according to diagnosis

• Acute leukemias

Acute leukemias are malignant clonal disorders of hematopoietic stem cells, classified according to the origin of the abnormal cells. They are among the most distressing cancer diagnoses, because very soon after receiving the diagnosis, most patients will face a prolonged hospital stay, experiencing a short transition between feeling healthy and having a life-threatening illness [39].

Acute myeloid leukemia (AML) is a predominantly adult disease, with a median age at diagnosis of 68 years; [26] it accounts for approximately 15 % of acute childhood leukemias [31,40]. In pediatrics, only one-third of patients achieve remission and are cured, with an overall five-year survival rate of 82 % for those classified as low risk, while high-risk patients still have very poor outcomes, with a survival rate of approximately 46 % [31,40]. Acute promyelocytic leukemia (APL) and AML associated with Down syndrome have favorable prognoses [41]. In the adult age group, with standard treatment (intensive chemotherapy '7 + 3'), long-term survival for AML patients is 35-45 % for patients younger than 60 years and 10-15 % for those older than 60 years [26]. Despite many recent advances, the prognosis for over-60-year-old patients remains poor, with up to 70 % of patients aged 65 or older dying within one year of diagnosis [26]. Newer regimens and targeted therapies have reduced treatment-related toxicity and increased remission rates, offering hope to a greater

proportion of older patients with AML. However, these approaches have not significantly improved long-term cure rates, often extending median survival only by weeks or months [42,43]. This was demonstrated by the VIALE-A clinical trial (Venetoclax and Azacitidine), which reported a median overall survival of 14.7 months [44].

Acute lymphoblastic leukemia (ALL) is traditionally considered a pediatric disease, as it is the most common and prevalent neoplasm in this age group. Even so, about half of all diagnoses occur in approximately equal proportions in younger adults (18-49 years) and older adults (50 years and older) [30]. ALL accounts for 30 % of malignancies between 0 and 14 years of age, with a high incidence especially in children aged 1 to 4 years, representing about 75-80 % of acute leukemias. ALL presents significant racial and ethnic disparities, including lower five-year survival rates in black children [40]. These disparities may be influenced by socioeconomic factors, access to healthcare, and biological differences in disease presentation. Patients with Down syndrome have a greater predisposition to infections and, therefore, need to be treated with reduced CT regimens [40,45]. In adolescents and young adults (15-39 years old), ALL is still prevalent, but the survival rate is lower compared to under-15-year-old patients, reflecting not only the biological complexity but also differences related to treatment [40,45]. The follow-up of adolescents and young adults with ALL presents a significant challenge due to non-adherence to treatment regimens and high rates of absenteeism from appointments, related to emotional factors (such as depression and low self-esteem), patient health beliefs, and their family environment [46]. Unfortunately, the high cure rates seen in children have not been replicated in older cohorts. Young adults have over 70 % long-term survival with the use of protocols inspired by pediatric treatments, while older adults (aged 55 to 60 years or older) have a less than 20 % cure rate [46]. Older adults, in addition to comorbidities, present greater toxicity secondary to CT, treatment delays, high rates of early mortality, and treatment-related mortality in those in remission [46,47]. The main unfavorable prognostic factors are positive minimal residual disease and relapsed or refractory (R/R) disease. Immunotherapy has emerged as a revolutionary form of treatment, often reducing the need for allogeneic HSCT, [40] but despite advances, a portion of patients will not be cured [31,48].

To better understand the burden of symptoms presented by patients diagnosed with acute leukemia, Zimmermann et al [49] followed patients with newly diagnosed disease or recent relapse, demonstrating a median of nine physical symptoms (range: 0-22) and two psychological symptoms (range: 0-6), among which the most prevalent were: fatigue (79 %), drowsiness (56 %), xerostomia (54 %), weight loss (54 %), and pain (49 %). The most prevalent psychological symptoms were insomnia (55 %), worry (43 %), difficulty concentrating (39 %), and sadness (36 %). Fatigue, insomnia, and pain were the most intense symptoms, with those presenting them being more likely to report intense worry and sadness [49]. A substantial drop in QoL scores (measured by the Patient Care Monitor v2.0 (PCM), the Functional Assessment of Cancer Therapy-Leukemia [FACT-Leu], and the National Comprehensive Cancer Network [NCCN] distress thermometer [DT]) was observed in a cohort of AML patients within the

first two weeks of starting induction CT. These scores did not return to baseline levels by Week 4, with the greatest reductions occurring in physical and functional well-being [50]. Depressive symptoms such as hopelessness and sadness were reported by 15 % and 16 % of patients, respectively [50]. To minimize the burden of disease-related symptoms, a randomized phase II clinical trial evaluated an intervention named 'Emotion and Symptom-Focused Engagement' (EASE). This intervention was delivered in a hospital setting to patients with a recent diagnosis of acute leukemia, specifically within one month of their initial diagnosis [51]. This intervention integrates supportive psychotherapy combined with trauma-focused cognitive behavioral therapy (CBT), in addition to systematic screening for physical symptoms. Patients were referred early for PC when symptom intensity reached a score of 4 or greater. The results demonstrated that an integrated intervention for the control of psychological and physical symptoms led to significant reductions in symptoms of traumatic stress and acute stress disorder (DSM IV criteria), pain intensity, and pain interference in daily activities, when compared with usual care [51]. Additional secondary outcomes, including the severity of physical symptoms and resulting distress, depressive disorders, satisfaction with care, and overall QoL, showed non-statistically significant trends favoring the intervention group [51].

Aiming to evaluate the early integration of PC in hospitalized patients with AML receiving intensive CT, a randomized clinical trial was conducted as follows: patients were seen by the PC team (physician or nurse) at least twice per week starting within 72 h of randomization and followed up during all subsequent hospitalizations for up to one year. The results showed that patients seen by the PC team experienced substantial improvements in QoL, with a reduction in symptoms of depression, anxiety, and post-traumatic stress disorder (PTSD) during hospitalization, with the impacts lasting up to six months after diagnosis. Moreover, the patients who died were more likely to discuss their end-of-life care preferences with their physicians and were also less likely to receive chemotherapy in the last 30 days of life. To investigate the effect of PC interventions on coping with the diagnosis among patients with AML, Nelson et al [53] reported that the joint action of the PC team facilitated a greater use of coping strategies (acceptance, positive reframing, and emotional support), while simultaneously reducing the reliance on avoidance strategies (behavioral disengagement, denial, and self-blame). These changes in coping were associated with improvements in QoL and a reduction in symptoms of depression and anxiety in Week 2 of treatment, a time when patients are presumed to feel worse at the nadir of CT [53]. Another randomized controlled trial evaluated the impact of monthly outpatient PC, supplemented by at least two visits per week during hospitalization, in a cohort of 115 patients with high-risk AML and Myelodysplastic Syndromes (MDS) undergoing non-intensive CT [54]. Patients who were followed by the PC team showed higher rates of discussion and documentation of end-of-life care preferences, a longer interval between documentation and death, and a greater propensity to discuss care with their attending physicians [54]. There was a reduction in the hospitalization rate in the last 30 days

of life, and, in three months of follow-up, patients showed better QoL, with no differences in the depression and anxiety rates [54].

Recommendations

Criteria for referring patients with acute leukemia to palliative care

1. AML patients aged 18 years or older receiving intensive chemotherapy in a hospital setting: Early integration of PC with admission by the PC team within 72 h of the start of chemotherapy and at least two visits per week during hospitalization and subsequent hospitalizations. (Level of recommendation: A)
2. AML patients receiving non-intensive chemotherapy: PC integrated early with admission by the PC team within 30 days of treatment initiation, with at least one monthly outpatient visit, and at least twice a week during each hospital stay. (Level of recommendation: A)
3. ALL patients aged 18 years or older receiving intensive chemotherapy in a hospital setting: extrapolating the evidence from PC to AML patients, we suggest early integration of PC with admission by the PC team within 72 h of the start of chemotherapy and at least two visits per week during hospitalization and subsequent hospitalizations. (Level of recommendation: D)
4. Pediatric patients with AML or ALL: Extrapolating the evidence to patients aged 18 years or older with AML, we suggest early integration of PC with admission by the PC team within 72 h of the start of chemotherapy and at least two visits per week during hospitalization and subsequent hospitalizations. (Level of recommendation: D)
5. Newly diagnosed patients with acute leukemia requiring only best supportive treatment. (Level of recommendation: D)
6. Patients with acute leukemia and a high symptom burden, refractory symptoms, moderate to intense psychological or existential distress, in end-of-life care, or presenting any of the general criteria for referral of hematological patients to PC. (Level of recommendation: D)

Myelodysplastic neoplasms

MDS comprise a heterogeneous group of myeloid disorders characterized by ineffective erythropoiesis, morphological cellular dysplasia in the bone marrow, cytopenias in peripheral blood, and a risk of progression to AML [55]. They affect men more frequently from the seventh decade, [56] with the main risk factors being a history of chemotherapy or radiotherapy and exposure to chemical agents such as benzene [57]. In adults and the elderly, the most common manifestation is anemia, with the bone marrow evaluation generally being hypercellular. Children diagnosed with MDS may present with frequent bleeding and infections resulting from severe thrombocytopenia and neutropenia; isolated anemia is less common. A bone marrow evaluation may show hypocellular bone marrow. As MDS is a diagnosis

of exclusion, patients should be evaluated for secondary causes [40].

Both the MDS Revised-International Prognostic Scoring System (IPSS-R) [58] and the more recent Molecular International Prognostic Scoring System (IPSS-M), which incorporates molecular data into risk classification [59], are utilized to stratify the risk of leukemic transformation and estimate overall survival. For low-risk groups, the estimated median survival is up to ten years, with most deaths unrelated to leukemic transformation. In high-risk groups, there is a high rate of progression to AML in a short period of time, with a median overall survival of three years [56]. Patients with low-risk MDS may present with minimal symptoms and may not require CT or serial transfusions, and may receive specific therapies targeted at clinically relevant cytopenias, such as transfusion support, growth factors, immunomodulators (e.g., lenalidomide), transforming growth factor-beta (TGF- β) neutralizers (e.g., luspaterecept), and immunosuppressants (e.g., cyclosporine). Patients with high-risk MDS experience high morbidity and mortality rates, high transfusion demands, bleeding, recurrent infections, and progression to AML, with a very unfavorable prognosis. Treatment with disease-modifying drugs aims to improve overall survival, such as the use of hypomethylating agents, intensive chemotherapy, and HSCT, the only potentially curative treatment, with disease-free survival rates between 30–50 %. Determining the best treatment for these patients takes into account age, comorbidities, clinical performance, availability of treatments, and a suitable bone marrow donor [60–62].

Patients with MDS present a high symptom burden with consequent impairment of health-related QoL [61]. Lack of recognition of these symptoms can lead to inadequate management, potentially reducing patient functionality and consequently impacting adherence to and tolerability of disease-modifying and supportive treatments [61]. The symptoms most frequently reported by patients are fatigue (89 %), insomnia, daytime sleepiness, and dyspnea [63]. The estimated incidence of frequent bleeding, bone pain, unwanted weight loss, and recurrent infections is 55 %, 39 %, 25 %, and 20 %, respectively [64]. Although fatigue is a constitutional symptom that correlates weakly with anemia rates, it significantly compromises QoL. Specifically, it impairs work capacity and reduces participation in leisure activities [65]. Patients who develop transfusion dependence experience up to a 50 % reduction in QoL scores [66]. A retrospective study of patients with HM, 67 % of whom had a diagnosis of MDS, was conducted to evaluate the impact of early CP on symptom control. This study demonstrated that, after the fourth CP intervention visit, there was a statistically significant reduction in pain, depression, anxiety, and anorexia [67]. An evaluation of an extensive retrospective database of over seven thousand elderly patients with MDS demonstrated that 28 % of patients were admitted to the ICU and that transfusion dependence correlated with lower rates of transfer to hospices and higher rates of ICU admission in the end-of-life phase [68]. The main causes of death among patients with MDS relate to transformation to AML (46 %), infection (27 %), and bleeding (10 %); other common causes are heart failure and secondary hemochromatosis [69].

A randomized clinical trial that included high-risk AML and MDS patients showed that patients who received early integrated PC had a longer time between the documentation of care preferences and death, were more likely to discuss their end-of-life care preferences, and had lower rates of hospital admission in the last 30 days of life, with a significant improvement in QoL [54].

Recommendations

Criteria for referring patients with myelodysplastic syndromes to palliative care

1. Patients with high-risk MDS receiving non-intensive chemotherapy: Early integration of PC with admission by the PC team within 30 days of starting treatment, with at least one monthly outpatient visit, and at least twice a week during each hospital stay. (Level of recommendation: A)
2. Patients with MDS receiving intensive chemotherapy in a hospital setting: extrapolating the evidence from PC to patients with AML, we suggest early integration of PC with admission by the PC team within 72 h of the start of chemotherapy, and at least two visits per week during hospital stays. (Level of recommendation: D)
3. Patients with intermediate-high, high-risk, and very high-risk MDS who are indicated for best supportive care only. (Level of recommendation: D)
4. Pediatric patients diagnosed with MDS: we suggest early integration of PC, regardless of the chosen treatment strategy. (Level of recommendation: D)
5. Patients with MDS and a high symptom burden, refractory symptoms, moderate to intense psychological or existential distress, or the presence of any of the criteria described in the general referral criteria of hematological patients to PC. (Level of recommendation: D)

Chronic myeloproliferative neoplasms

Chronic myeloproliferative neoplasms (CMPNs) constitute a heterogeneous group of diseases affecting one or more myeloid lineages, resulting from aberrant clonal proliferation. The current WHO classification subdivides the group into seven categories based on distinct clinical, molecular, and prognostic characteristics, but with a burden of symptoms and complications that often overlap [70]. Studies in clinical practice divide them into two major groups: chronic myeloid leukemia (CML) and Philadelphia-negative CMPNs.

CML is rare in childhood, with an incidence in children and adolescents of less than 5 % [45]. In Western countries, the average age of CML patients is around 57 years [61]. Patients with chronic phase CML treated with tyrosine kinase inhibitors (TKIs) usually have a mean survival similar to the general population [71,72]. However, the good prognosis coexists with the prospect of long-term treatments resulting in repercussions on physical, psychosocial, and financial conditions, [71,73,74] which contribute to the manifestation of

depression, anxiety, and lack of self-recognition by the patient [75]. Fatigue is the most frequent and most intense physical symptom (51 %), but nausea, diarrhea, cramps, and muscle pain are also common [71,72,75,76]. Discontinuation of TKIs in patients with deep molecular responses appears to result in symptom control and improvement in QoL in young patients, but with minimal effect in over 60-year-old patients [77]. It is estimated that 25 % of patients may need to switch TKIs due to resistance or intolerance, with an increasing rate of therapeutic failure as subsequent line changes become necessary [78]. A suboptimal response to two or more CT treatments should prompt immediate consideration of HSCT.

Patients with advanced CML at diagnosis should be treated as high-risk, becoming eligible for HSCT if response to CT is not optimal. Progression to the accelerated phase has become a rare event, and HSCT should be considered immediately if it occurs during treatment [79]. Blastic crises maintain a guarded prognosis with survival generally less than one year due to bleeding or infections; [76] the treatment plan is like that of acute leukemias, culminating in allogeneic HSCT when disease remission occurs [79]. In an analysis of Medicare end-of-life care, it was found that, among patients with CML, 52 % were in the ICU in the last 30 days of life and 22 % received CT within the last 14 days [80]. Hospice care significantly reduced these numbers to 25 % and 9 %, respectively, as well as healthcare spending in the last 30 days of life (US \$31,400 versus US\$ 18,500), [80] suggesting a potential benefit of integrating PC into the usual care of patients with CMPNs.

Among Philadelphia chromosome-negative MPNs, the most common are Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Primary Myelofibrosis (PM). Fatigue is the predominant symptom, reported in 99 %, 92 %, and 90 % of patients, respectively [81]. This symptom significantly impacts functionality and social life, often requiring assistance with work and activities of daily living [82,83]. Other recurrent symptoms include pruritus, night sweats, early satiety, thrombosis, bleeding, and sexual dysfunction, with a high incidence of symptoms related to distress (40 %), anxiety (31 %), and depression (12.5-23 %) [84]. Proactive symptom assessment is recommended at all consultations, preferably using validated tools such as the Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) [85]. With proven clinical applicability, the MPN-SAF TSS is a questionnaire that measures symptom burden in patients with MPN using a numerical scale from 1 to 10 (a score of 7 or higher indicates severe symptoms) [86]. The frequency varies according to the MPN subtype, although consistently, patients with MF had the highest symptom burden [87].

Patients with MF have a life expectancy that can vary from months to years, so risk stratification models seek to refine the prognosis and assist in decision-making, especially regarding the timing of allogeneic HSCT. Despite currently being associated with a rate of at least 30 % of procedure-related deaths or severe morbidity, HSCT is the only treatment modality capable of prolonging survival with curative potential [88]. The International Prognostic Scoring System (IPSS), Dynamic International Prognostic Scoring System (DIPSS), and the Refined Dynamic International Prognostic Scoring System (DIPSS-plus) are considered adequate to characterize the prognosis of these patients [70]. The median

survivals according to the DIPSS-plus risk categories range from 15.4 years (low risk) to 1.3 years (high risk). More recently, molecular assessment has been integrated into the development of three new prognostic models for myelofibrosis (MF): the Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients (MIPSS70), the Mutation and Karyotype-Enhanced International Prognostic Scoring System version 2.0 (MIPSS70+ v2.0), and the Genetically Inspired Prognostic Scoring System (GIPSS) [88].

The evolution of PV and ET to secondary myelofibrosis is part of the natural history of these diseases, with an average transformation time estimated to be between 7 and 10 years [89]. The rate of transformation to AML within the first decade following diagnosis varies between 10 % and 20 % in patients with MF, while it is approximately 2.3 % in those with PV and less than 1 % in ET; notably, such transformation is associated with a dismal prognosis with average survival ranging from 1.5-2.5 months when on supportive treatment, and 3.9-9.4 months among those who receive intensive treatment [90]. The three-year survival rate after HSCT varies between 16 and 33 % [90].

Recommendations

Criteria for referring patients with chronic myeloproliferative neoplasms to palliative care

1. Patients with chronic phase CML who have failed or are intolerant to two or more TKIs or have progressed to an advanced phase and are candidates for allogeneic HSCT. (Level of recommendation: D)
2. Patients with CML blast crisis or CMPN progressing to acute leukemia on an intensive chemotherapy treatment strategy: extrapolating the PC evidence to patients with AML, we suggest early integration of PC, with admission by the PC team within 72 h of the start of chemotherapy and at least two visits per week during hospitalizations. (Level of recommendation: D)
3. Patients diagnosed with primary myelofibrosis and DIPSS-plus intermediate-2 and high-risk. (Level of recommendation: D)
4. Patients diagnosed with progression from PV or ET to secondary myelofibrosis. (Level of recommendation: D)
5. Patients with CMPN and an indication for only best supportive treatment. (Level of recommendation: D)
6. Patients with CMPN and high symptom burden, refractory symptoms, moderate to intense psychological or existential distress, or the presence of any of the criteria described in the general referral criteria of hematological patients to PC. (Level of recommendation: D)

Lymphoproliferative neoplasms

Lymphoproliferative neoplasms constitute a heterogeneous group of malignant diseases of the lymphatic tissue with a

broad prognostic spectrum. There are more than 60 types and subtypes of lymphomas according to the histological classification [91]. The third most common cause of death in children and adolescents, [40] lymphomas represent about 4 % of all cancers and 3.3 % of cancer deaths in the USA [92]. In Brazil, it is estimated that more than five thousand deaths occur annually due to lymphoma [92]. They are classified as Hodgkin's lymphoma (HL) and Non-Hodgkin lymphoma (NHL) and develop from the accumulation of acquired somatic mutations, with viral infections (Epstein-Barr, hepatitis C, and HIV) among their main risk factors, although, for most cases, there is no evident etiological factor [93].

HL is most common among people aged 15 to 40, with a second peak in incidence after 60 years old. The combination of CT, radiotherapy, and monoclonal antibodies allows for a cure in about 80 % of HL patients under 60 years of age [93]. When relapse or refractoriness occurs, patients should receive high-intensity CT followed by autologous HSCT, which can be challenging in elderly and frail patients [94].

NHLs account for approximately 60 % of lymphoma cases. The classic division into high-grade (aggressive) and low-grade (indolent) lymphomas is found in the 2022 classification and helps to interpret clinical behavior and define the need for treatment. Differences in biological behavior, pathophysiological mechanisms, and cytogenetic/molecular alterations result in very varied prognoses [91]. High-grade lymphomas have a high rate of cell replication and growth, presenting symptoms such as pain, superior vena cava compression syndrome, fatigue, weight loss, and fever. Most types are sensitive to chemotherapeutic and immunotherapeutic treatments, with potential for cure. In patients with aggressive R/R lymphomas, prognostic uncertainty is exacerbated by recent therapeutic advances and the increasing attainability of a cure [95]. Indolent lymphomas, on the other hand, are characterized by slow and progressive growth and may not require immediate treatment, but are managed using a 'watch and wait' strategy. They are considered an incurable disease with periods of clinical remission interspersed with periods of relapse, a high symptom burden during disease activity, and the need for subsequent lines of treatment with a progressive accumulation of toxicities [95]. The rapid changes in the clinical conditions of patients and the possibility of functional recovery, even in advanced disease scenarios, pose a challenge to prognostic definition and the consequent early referral to PC [96]. Patients present high rates of pain, dyspnea, fatigue, depression, and intense and limiting pruritus, all of which tend to worsen near the end of life [95,97]. Although disease-modifying therapies have shown exponential evolution in the last decade, a significant proportion of patients still die from disease progression and refractoriness or suffer from uncontrolled physical and emotional symptoms [98].

In childhood, the most common NHLs are Burkitt lymphoma, lymphoblastic lymphoma, and diffuse large B-cell NHL, which exhibit aggressive or very aggressive behaviors [40]. The primary clinical presentation consists of mediastinal widening, with or without associated pleural effusion, which

may rapidly progress to superior vena cava syndrome. This condition constitutes an oncological emergency and may occur alongside infiltration of the bone marrow and the central nervous system (CNS) [40,99,100]. In the adult population, the most common subtype of high-grade NHL is diffuse large B-cell NHL. The complete response rate after treatment with CT and monoclonal antibodies is 70 %, with event-free survival around 50 %. However, 10 % of patients will be primarily refractory, and between 30 and 40 % will experience disease relapse.[78]. For these R/R patients, five-year survival is less than 50 %, even after salvage chemotherapy and autologous HSCT. CAR-T cell therapy has emerged as a potential alternative to induce prolonged remissions or even curative treatment in a subgroup of patients [101]. A study with 267 patients with diffuse large B-cell NHL evaluated the relationship between sarcopenia and prognosis. The findings showed that patients with an intermediate and low sarcopenia index (CXI) had a worse prognosis compared to those with a high CXI, [102] suggesting that this score may be a patient-related prognostic biomarker, reflecting the impact of frailty syndrome on the survival of patients with lymphoma, and a potential referral criterion for PC [103].

Although there are few specific studies of PC in patients with lymphoma, research that has evaluated hospice-associated lymphoma, including lymphomas, has demonstrated a reduction in futile end-of-life interventions, improved alignment of medical goals with care preferences of patients, and increased referral to hospice-type end-of-life care [104–106]. Referral of patients with lymphomas to PC should be less related to prognosis and more to patient needs (e.g., in aggressive T-cell R/R lymphomas, the likelihood of a durable response is typically even more somber than for B-cell lymphomas, and predictors of sustained response are unclear) [77]. Refractoriness of symptoms, emotional distress, difficulty understanding the disease, the need for complex discussions regarding care goals, and the possibility of serious complications or death within one year should be triggers for PC referral [98].

Recommendations

Criteria for referring patients with lymphoproliferative malignancies to palliative care

1. Pediatric and adult patients with R/R lymphomas. (Level of recommendation: D)
2. Adult patients with aggressive lymphoproliferative malignancies and frailty syndrome, low functionality, or sarcopenia. (Level of recommendation: B)
3. Pediatric and adult patients with lymphoproliferative malignancies and an indication for best supportive care only. (Level of recommendation: D)
4. Pediatric and adult patients with lymphoproliferative malignancies and a high symptom burden, refractory symptoms, moderate to intense psychological or existential distress, or the presence of any of the criteria described in the general referral criteria of hematological patients to PC. (Level of recommendation: D)

Monoclonal gammopathies

Among the Monoclonal Gammopathies, there are Monoclonal Gammopathies of Undetermined Significance (MGUS), asymptomatic or smoldering MM (MMS), and symptomatic MM, the latter being of greatest interest due to the high prevalence of symptom burden, mainly pain, and the incurable nature of the disease, directly impacting QoL and causing an immense psychological burden even in its stable phases [107–109].

A cross-sectional analysis of patients with MGUS and MMS assessed symptom burden and psychological distress; the results were not significantly different between participants, and, to a certain point, similar to those found in previous studies of patients with MM [110]. The prevalence of anxiety, depression, and symptoms of PTSD were 27 %, 14 %, and 24 %, respectively. Overall, 31 % of patients reported moderate to severe symptom burden and the presence of fatigue, malaise, drowsiness, and pain. It is not possible to conclude whether the described physical symptoms are attributable to MGUS or MMS [110].

The median age at diagnosis of MM is 69 years, and associated with a high prevalence of frailty and comorbidities. This demographic profile increases the challenges regarding treatment and symptom management, as the symptom burden is often greater in both frequency and intensity than in other HM [107]. Due to population aging associated with the availability of new therapies, there is a significant upward trend in the global incidence among the elderly, from 6.7 million in 2012 to an expected 14 million in 2035 [107,108]. A study in Denmark showed that 50 % of patients have symptoms considered intense, with high levels of pain, fatigue (98 %) and constipation (66 %) Patients also have problems related to physical function and social role, with high rates of depression (26 %) and anxiety (23 %), and low scores in functional scales [111,112]. Symptoms are related to the underlying disease, treatment-related side effects, and cumulative toxicity, particularly in the context of disease relapse or continuous therapy. Consequently, patients endure prolonged periods of physical symptoms and psychological dysfunction, which may lead to treatment discontinuation and, subsequently, disease progression and compromised clinical outcomes [112,113]. Furthermore, family members are shown to be as psychologically burdened as the patients stemming from extensive caregiving responsibilities related to medication, mobility, and assistance with activities of daily living [109].

In the literature, there are few recommendations regarding PC in MM, with most focusing on symptom control: bone pain, anemia, peripheral neuropathy, constipation, infection, and thrombosis prophylaxis [114]. It is believed that PC should be integrated as soon as possible, according to service availability, mainly with the aim of controlling clinical signs and symptoms and other comorbidities that interfere with the patient's well-being [115,116]. In a retrospective analysis, 55 patients with MM received early PC, while 231 received usual hematological care [117]. Patients managed by the PC team experienced superior pain control, characterized by the prolonged use of strong opioids and higher rates of symptom

management, as well as greater psychological support and more frequent and earlier discussions regarding goals of care. Furthermore, there was a trend toward fewer aggressive end-of-life interventions, including anti-MM treatment within 14 to 30 days of death, and cardiopulmonary resuscitation, or orotracheal intubation within the last 30 days. Notably, these patients also had fewer hospitalizations exceeding seven days, and fewer than two hospital admissions or emergency department visits in their final 30 days, while maintaining equivalent survival rates [117].

Porta-Sales analyzed 67 patients referred to a PC clinic with an average time of 355 days between diagnosis and first consultation. The study demonstrated a significantly lower proportion of patients with moderate to severe pain; less interference of pain in general activities, sleep, and mood; and a lower rate of depression [116]. A multicenter study evaluated 557 patients in various stages of the disease: 1) newly diagnosed (pre-treatment or first-line treatment); 2) treatment-free interval (stable disease with no evidence of disease progression); and 3) relapsed/progressive disease (second-line or higher-level therapy or progression in treatment). In this study, the most prevalent symptoms were pain, fatigue, memory changes, shortness of breath, peripheral neuropathy, and reduced mobility. Among the concerns regarding QoL were "problems performing usual activities," "concern that the disease might worsen," and "not having enough information about what might happen in the future." [118]. The burden of symptoms and concerns about QoL is greater in those with relapsed/progressive disease due to the fear of manifesting previous symptoms, followed by those with newly diagnosed disease, who worry about what might happen, the impact of the new treatment, and the occurrence of side effects. Symptom scores tend to be higher in patients receiving treatment due to the presence of side effects associated with underlying comorbidities; however, those with stable disease also face a multitude of physical and psychosocial challenges, as many live with chronic pain, peripheral neuropathy, or associated organ dysfunctions, in addition to the fear of relapse [118]. When the disease becomes refractory to available therapies, early care planning should focus on transitioning from drug treatment to symptom control and clarifying end-of-life care preferences [115].

Another important monoclonal gammopathy is systemic light chain (AL) amyloidosis, which can culminate in rapidly progressing, often devastating, multi-organ dysfunction through amyloid deposits [119]. Clinical manifestations are deceptive and sometimes recognized only at an irreversible stage with an adverse prognosis. Complications include heart failure, hypotension, pleural effusions, renal involvement, including nephrotic syndrome with peripheral edema, gastrointestinal symptoms leading to anorexia and cachexia, complex pain syndromes, and mood disorders [120]. Cardiac involvement has the greatest impact on determining survival in these patients, followed by renal involvement [121]. Patients and their families should have a realistic understanding of the disease and the goals and limitations of treatments so that they can make informed decisions about medical therapy and supportive care, as well as plan for end-of-life care. Given such a complex presentation and high morbidity and mortality rates, establishing a PC program in clinical management is a means to improve QoL, reduce emergency room visits, readmissions, and hospital mortality [120].

Recommendations

Criteria for referring patients with monoclonal gammopathies to palliative care

- 1) Patients with symptomatic MM or medullary/extramedullary plasmacytoma: patients with moderate to severe physical or psychosocial symptoms at any stage of the disease; moderate to severe treatment-related side effects or toxicity, and impaired QoL. (Level of recommendation: D)
- 2) Patients with systemic AL amyloidosis: patients with moderate to severe physical or psychosocial symptoms at any stage of the disease; moderate to severe side effects or toxicity related to treatment, and impaired QoL. (Level of recommendation: D)
- 3) Patients with MM or systemic AL amyloidosis and an indication for only best supportive treatment. (Level of recommendation: D)
- 4) Patients with MM or systemic AL amyloidosis and high symptom burden, refractory symptoms, moderate to intense psychological or existential distress, or the presence of any of the criteria described in the general referral criteria of hematological patients to PC. (Level of recommendation: D)

Bone marrow transplantation and cell therapy

Hematopoietic stem cell transplantation

HSCT is an intensive and potentially curative therapy for various hematological diseases, both malignant and non-malignant. Its high toxicity and morbidity are fully justified by the ultimate goal: the pursuit of restoring a normal life free from the underlying disease. It is divided into two modalities: (1) autologous, where the source of the graft is the patient's own stem cells collected before the conditioning protocol; and (2) allogeneic, in which the stem cells come from a donor [74]. There are several differences between pediatric and adult HSCT recipients. These include the biology of the diseases; variations related to age and body size; body composition; physiological differences in organ function and metabolism; and psychosocial factors affecting family dynamics, neurodevelopment, and resource availability [99]. Prolonged hospitalizations and social isolation are associated with decreased patient-reported QoL, elevated levels of anxiety and depressive symptoms, including pronounced anhedonia [74]. Despite its clear indication, it is a procedure with significant risks, including side effects from conditioning chemotherapy, cytopenias, and acute and chronic graft-versus-host disease (GvHD) [122–124].

Studies suggest that the intensity of physical symptoms experienced during HSCT may be a factor in greater psychological distress over time, [125] with psychological stressors also being associated with higher risks of GvHD and decreased overall survival [74]. GvHD is generally a mild condition, but it can sometimes be fatal. Psychosexual dysfunction is common in HSCT recipients, as are neuro-hormonal

toxicities related to chemotherapy, negative body image, and financial toxicity [126]. The presence of PTSD symptoms is commonly described [127,128]. According to the guidelines of the American Society for Transplantation and Cellular Therapy (ASTCT), [99] early consultation with the PC team is essential to establish a multidisciplinary relationship and discuss care goals, including pain management and coping strategies [32,41].

A randomized study comparing standard treatment (HSCT alone; $n = 79$) with integrated care (HSCT combined with twice-weekly PC consultations; $n = 81$) demonstrated that the integrated approach resulted in a significantly smaller decline in QoL from baseline to week two, that is, the peak symptomatic period. Furthermore, patients receiving integrated care experienced a lower increase in symptom burden and significantly lower rates of depression and anxiety compared to the standard treatment group [22]. Three months after HSCT, patients maintained higher QoL scores and lower rates of depressive symptoms [22]. These data align with those described by El-Jawahri et al., with lower rates of depression and PTSD six months after HSCT, with clear benefits for survivors [129]. In line with this study, the literature highlights the importance of early integration of PC in pediatric transplants, [41,48,130,131] highlighting the high receptivity by patients and their parents, and the perception that attention to QoL should be a priority from the beginning of treatment [48,130,131].

An exploratory analysis of two randomized clinical trials [22,132] evaluated the specific factors associated with the magnitude of patient response to CP intervention. Participants were categorized as either 'high' or 'low' responders based on the variation in their QoL scores from admission to two weeks after HSCT. The analysis revealed that belonging to a racial minority, having a lower baseline QoL, and experiencing a higher burden of physical symptoms or PTSD were all associated with a greater likelihood of being a high responder. Furthermore, these high-responding patients reported significantly greater use of coping strategies at the two-week, three-month, and six-month marks post-HSCT [133].

Regarding the clinical consultations themselves, high-responder visits placed a heavier emphasis on disease education, HSCT-related information, and support for coping and symptom control. In contrast, visits for low-responding patients focused predominantly on symptomatic management. These findings suggest that a CP approach to education and coping reduces disease-related uncertainty, thereby improving QoL. Notably, no differences in response were observed regarding the underlying disease, the type of HSCT, or the conditioning regimen used [133].

Caregivers of patients undergoing HSCT face a prolonged and intense caregiving burden, which negatively impacts QoL, physical well-being, and mood. In the pre-HSCT period and during the procedure, suffering is high, as their loved one experiences treatment of toxicities, physical and psychological symptoms, prolonged hospitalization, and prognostic uncertainty [74]. In the post-HSCT period, home settings will presumably require caregivers to assume greater functional demands and social problems, such as isolation, financial demands, and family tension, compromising their well-being [134].

Current evidence points to the need for integration between HSCT and PC services; however, specialized PC interventions evaluated in the included clinical trials were limited to the inpatient setting and were designed to improve QoL during hospitalization after HSCT. However, events experienced by the patient after HSCT, such as GvHD, infections, and disease recurrence, also negatively affect QoL [133]. There is still no ideal format described in the literature for establishing this partnership, but it is already known, through well-designed studies, that the presence of PC services in HSCT units contributes to the lasting relief from suffering for both patients who progress to end-of-life care and survivors [22,52,124].

Cell therapy (chimeric antigen receptor T cells)

Cell therapy is based on the use of CAR-T cells: genetically modified T cells capable of recognizing and fighting a specific tumor [105]. In Brazil, this treatment is approved by the National Health Surveillance Agency (ANVISA) for the following R/R HM with the potential to induce prolonged remissions and be a curative alternative for this subgroup of patients: diffuse large B-cell NHL, B-cell ALL, follicular NHL, and MM [105]. Although it presents good tolerability, the toxicity of CAR-T therapy can appear up to 30 days after the infusion, [135] with the most serious and frequent toxicities being cytokine release syndrome (CRS) and CAR-T-related encephalopathy [136]. CRS can appear between the first and twelfth day after infusion, with severe cases (Grade III or IV toxicity) in 20 % of patients [105,136]. Both SLC and CAR-T therapy-related encephalopathy are reversible if managed appropriately, with deaths rarely documented [137,138]. Secondary neoplasms after CAR-T therapy represent a small fraction of reported adverse events, suggesting an increased risk for myeloid and T-cell malignancies [139].

To date, there are few studies evaluating the benefits of partnering with PC teams. However, given the nature of the procedure, which is very similar to that of HSCT, its indications in R/R diseases, the prognostic uncertainty, and the resulting need for symptom management and psychosocial support, we believe it is possible to extrapolate the indications for PC in HSCT to CAR-T. The involvement of the PC team in this setting, in addition to facilitating communication between teams, patients, and families, also enables the planning of care objectives [99].

Recommendations

Referral criteria for patients undergoing hematopoietic stem cell transplantation or chimeric antigen receptor T cell therapy for palliative care

1. Patients aged 18 years or older admitted for HSCT: PC integrated early, with admission by the PC team within 72 h of hospital admission and at least two visits per week during hospitalization and subsequent hospitalizations. (Level of recommendation: A)
2. Pediatric patients hospitalized for HSCT: extrapolating data from the adult population, we suggest early

integration of PC, with admission by the PC team within 72 h of hospital admission and at least two visits per week during hospitalization and subsequent hospitalizations. (Level of recommendation: D)

3. Adult and pediatric patients with Grade II to IV GvHD: outpatient follow-up by the PC team. (Level of Evidence D)
4. Adult and pediatric patients hospitalized for CAR-T cell therapy: extrapolating the evidence from PC for HSCT, we suggest early integration with admission by the PC team within 72 h of hospital admission and at least two visits per week during hospitalization and subsequent hospitalizations. (Level of recommendation: D)
5. Adult and pediatric patients eligible for CAR-T cell therapy may be referred to outpatient PC for care plan alignment. (Level of Evidence D)
6. Adult and pediatric patients undergoing HSCT or CAR-T cell therapy with a high symptom burden, refractory symptoms, moderate to intense psychological or existential distress, or the presence of any of the criteria described in the general referral criteria of hematological patients to PC. (Level of recommendation: D)

Congenital bone marrow failure, aplastic anemia, and paroxysmal nocturnal hemoglobinuria

Bone marrow failure syndromes encompass a heterogeneous group of diseases characterized by bone marrow insufficiency, resulting in an inadequate production of blood cells and consequent isolated cytopenias or pancytopenia. These conditions may have an immune (acquired) etiology, as occurs in idiopathic AA, or a genetic (congenital/hereditary) etiology as in Fanconi anemias, congenital dyskeratosis, and telomeropathies, among others. Although they share the same hematological phenotype – failure of hematopoiesis – the pathophysiological mechanisms are distinct, being auto-immune in acquired forms and related to genetic defects in cell maintenance or repair in congenital forms [140].

Hereditary forms are usually identified in childhood and adolescence, but may only be diagnosed in adulthood [40,140]. Fanconi anemia, a progressive bone marrow failure in the presence of multiple congenital abnormalities, is predisposed to transformation into acute leukemias, MDS, and head and neck tumors [40,140–142]. Dyskeratosis Congenita is a rare disease with progressive bone marrow failure and is recognized by a classic triad: skin hyperpigmentation, nail dystrophy, and leukoplakia [40,143]. Shwachman-Diamond syndrome manifests as exocrine dysfunction, skeletal abnormalities, cytopenias, and possible progression to MDS and acute leukemias [143,144]. Although the only treatment is HSCT, the results are unsatisfactory [40,143]. Blackfan Diamond anemia is a congenital red blood cell hypoplasia that occurs in early childhood; approximately 50 % of cases present with congenital physical abnormalities, absence of red blood cell precursors, and a predisposition to cancer. Treatment up to one year of age is limited to blood transfusions and corticosteroid therapy; however, in the absence of response, allogeneic HSCT is indicated [143,145].

Congenital amegakaryocytic thrombocytopenia (CAMT), a severe thrombocytopenia that occurs from birth, is a diagnosis of exclusion. It does not usually respond to corticosteroids, immunoglobulin, or splenectomy, and HSCT is indicated in cases where platelet counts are lower than $50 \times 10^9/L$ [146]. Among severe neutropenias, Kostmann syndrome is characterized by severe congenital neutropenia with treatment generally involving the use of granulocyte colony-stimulating factors (G-CSF) to increase neutrophil counts and reduce the risk of infections; [147] its only curative therapy to date, indicated for patients with a high risk of malignancy, is HSCT [147].

Acquired AA and paroxysmal nocturnal hemoglobinuria (PNH) are diseases belonging to the group of bone marrow failure syndromes and should be considered as two distinct manifestations of a common pathophysiology, but that are interrelated. They frequently affect young patients, although the age distribution for AA shows a bimodal trend with a peak in young adults, while the average age of diagnosis in PNH peaks between 30 and 45 years. Both require long-term care and have high morbidity, despite therapeutic advances [148]. Furthermore, patients with AA and PNH have a risk of developing MDS and AML, with a ten-year incidence of 15-20 % and 2-6 %, respectively [149].

Prospective studies demonstrate that between 69 % and 89 % of AA cases are classified as severe or very severe. These cases are associated with higher morbidity and mortality and a higher incidence of infections compared to non-severe AA. Treatment options include HSCT, immunosuppressive therapy, thrombopoietin receptor agonists, and clinical trials, depending on patient age, clinical performance, and donor availability for HSCT [148]. Although HSCT has high overall survival rates, the availability of compatible donors and patient age are obstacles to its widespread implementation [148]. In patients with moderate to severe anemia, periodic transfusions may be necessary, affecting QoL due to time spent on healthcare and the risk of side effects [150]. Complications related to cytopenias (e.g., symptomatic anemia, febrile neutropenia, sepsis, hemorrhages requiring medical intervention) and secondary to transfusions (e.g., iron overload and iron chelation) are common [148,151,152].

A study conducted with 74 patients diagnosed with AA demonstrated a higher frequency and intensity of psychosocial symptoms in these patients. Symptoms included worries and uncertainties about the future, the need for continuous monitoring, insecurity in planning activities, and fear regarding the worsening of their blood levels. Interestingly, the complications frequently feared and highlighted by attending physicians, such as susceptibility to infections and bleeding tendency, had a small impact on the QoL of patients [153].

In PNH, the lack of complement regulatory proteins (CD55 and CD59) leads to intravascular hemolysis, hemoglobinuria, and an increased frequency of thrombotic complications, which are the main cause of death [154,155]. The greatest risk factor for PNH is immune-mediated AA. Factors such as the size of the PNH clone and the degree of hemolysis may correlate with the intensity of symptoms, which range from asymptomatic to significant impairment of QoL [148,154]. Symptoms related to hemolysis episodes include fatigue, back and abdominal pain, headache, dysphagia, and erectile

dysfunction [154,155]. Increased hemolysis can occur during acute events such as infection, surgery, and transfusions [155]. The most common symptom in PNH is fatigue, present in 80.9 % of patients, impacting overall well-being, interfering with daily and work activities, cognitive and psychological functions, and causing demotivation, sadness, frustration, and mental exhaustion [155]. Treatment with complement inhibitors is the choice for patients with high hemolytic activity associated with clinical repercussions, bringing patient survival closer to that of healthy individuals of similar ages [148,155]. However, some patients using C5 complement inhibitors still have residual anemia and fatigue, which limit their ability to perform daily activities (e.g., work, school, and hobbies) [154].

A systematic review found no articles on the role of PC in these diseases. Given the complexity of the symptoms presented by patients with bone marrow failure, the possible referral for HSCT, the recurrent risk of hospitalization, and high morbidity and mortality, we suggest integrating PC similarly to what is recommended for acute leukemias. For patients with PNH, we recommend integration according to the presentation of symptoms, such as more severe life-threatening events or unsatisfactory response to the proposed treatment.

Recommendations

Referral criteria for patients with congenital bone marrow failure, acquired aplastic anemia, and paroxysmal nocturnal hemoglobinuria to palliative care

1. Pediatric patients diagnosed with congenital bone marrow failure: due to high morbidity and mortality and physical, psychological, and social complications, we suggest referral to PC centers preferably from the time of diagnosis, or in the case of failure of the therapeutic strategy, indication for recurrent blood transfusions, or referral for allogeneic HSCT. (Level of recommendation: D)
2. Patients diagnosed during hospital admission with severe or very severe AA: extrapolating the evidence of PC to AML patients, we suggest early integration of PC at diagnosis. (Level of recommendation: D)
3. Patients diagnosed with severe or very severe AA undergoing immunosuppressive therapy and outpatient follow-up, requiring recurrent blood transfusions or regular visits to healthcare services. (Level of evidence D)
4. Patients diagnosed with NPH: unsatisfactory response to disease-modifying treatment and persistence of symptoms despite targeted treatment. (Level of recommendation: D)
5. Patients with recurrent unplanned hospitalizations or due to acute events with physical, psychological, or social impact. (Level of recommendation: D)
6. Patients with a high symptom burden, refractory symptoms, moderate to intense psychological or existential distress, or the presence of any of the criteria described in the general criteria for referring hematological patients to PC. (Level of recommendation: D)

Congenital anemias and hemorrhagic diseases

Sickle cell disease (SCD) is the most prevalent monogenic disorder in the world [2]. It results from a complex pathophysiological process involving tissue damage mediated by ischemia and hemolysis. Patients experience, in addition to the typical picture of intense acute pain episodes, high morbidity resulting from chronic target organ damage (e.g., retina, lungs, heart, brain, kidneys, and bones) [156]. Pain episodes are the main cause of visits to the pediatric emergency room and hospitalizations [157]. Dactylitis affects children between six months and two years of age, while in older children, crises usually occur in the long bones of the lower limbs, thoracic, and abdominal regions [40,157]. Acute chest syndrome is the second most frequent complication and is the leading cause of death; priapism can lead to sexual impotence; while stroke is a medical emergency with a high risk of mortality and serious sequelae, both physical and cognitive-behavioral [157]. Furthermore, children and adolescents may face insomnia, daytime sleepiness, episodes of anxiety, and depressive states due to social isolation resulting from repeated hospitalizations, in addition to physical impairment and the various stigmas associated with SCD [157,158]. While advances in the diagnosis and early management of SCD complications have increased survival rates, this population now faces a prolonged burden of chronic pain and comorbidities. These factors lead to significant impairment of QoL, characterized by motor limitations and high rates of absenteeism from occupational and educational environments [159]. Clinical complications interfere not only with the patient's life but also with that of their families. Parents or caregivers are subjected to high levels of stress and sometimes manifest mental health problems [157,158].

A study conducted by Toro et al. at a leading Brazilian institution demonstrated that patients with SCD present a high burden of limiting symptoms, including fatigue, depression, hyporexia, and chronic pain [160]. Furthermore, the presence of other comorbidities and social and economic factors, particularly low family income and low education, negatively impacts symptom scores. The prevalence of exposure to interpersonal episodes of violence (physical, emotional, or sexual) is increased and is associated with a higher frequency of chronic pain episodes, regular opioid use, depression, and anxiety [161]. Inadequate analgesic treatment can result in hypersensitivity and amplified responses to subsequent pain episodes, lack of trust in health services, and absenteeism [159]. Recurrent pain episodes and consequent social isolation, associated with cognitive impairment resulting from ischemic stroke events, lead to higher rates of depression and anxiety [162,163]. Sometimes, chronic complications can lead to an unpredictable decline in health conditions, causing patients and, occasionally, family members, to make complex decisions, such as defining end-of-life care [164].

In childhood SCD, some randomized studies, which specifically analyzed pain management, should be considered, since pain relief is understood within the pillars of PC [165,166]. Strategies such as CBT for pain through digital intervention in adolescents aged 12 to 18 years significantly

reduced the average intensity of pain and the number of days with pain over six months when compared to the educational control group [143,158,165–167]. Individualized pain management plans and biofeedback programs can significantly reduce hospital stays and readmission rates, as well as generate substantial savings in hospital costs [168]. Al Zahrani et al. demonstrated that systematic multidisciplinary team assistance for patients with SCD and a history of opioid abuse, by incorporating educational activities, access to PC or pain clinics, rational opioid protocols, and chemical dependency approaches, was able to reduce costs and emergency service visits while significantly decreasing opioid consumption [169]. The combination of psychological treatments can reduce the intensity of chronic pain and disability [170]. A systematic review found that some non-pharmacological interventions for pain management (CBT and biofeedback), both in outpatient and home settings, and during hospital stays (yoga sessions, massage, and virtual reality), significantly reduced the need for analgesics and contributed to more holistic patient care [167].

Individuals with SCD and their families deal with repeated losses throughout their lives, such as physical functionality, the ability to provide for their family, and absenteeism from work/school/family life. The grief of these losses needs recognition and support [25]. Despite the evidence, it is known that patients with SCD have less access to PC [171] and, given the high complexity of the symptoms, most professionals are not accustomed to treating them adequately, resulting in unsatisfactory control of acute and chronic pain, inadequate management of psychosocial and spiritual issues, and a lack of advanced end-of-life care planning [162].

Thalassemias are a heterogeneous group of hereditary diseases that result in distinct phenotypes depending on the partial decrease or absence of synthesis of alpha or beta chains of hemoglobin. Patients may be asymptomatic, with only the thalassemia trait present, or they may present with severe clinical manifestations, including death or severe anemia, requiring regular transfusion regimens and iron overload, with HSCT being the only curative treatment option [40].

Coagulopathies encompass qualitative and quantitative alterations in coagulation factors, potentially leading to mild to severe bleeding disorders depending on the degree of factor deficiency. Bleeding manifestations can be frequent and spontaneous, and, in hemophilia, they occur mainly in the joints, causing deformities and physical sequelae, as well as acute and chronic pain. From birth, there is a high risk of severe bleeding. Currently, prophylaxis and immunotolerance are part of the treatment and monitoring of these patients [172]. Hereditary deficiencies of factors I (fibrinogen), II, V, VII, X, XI, and XIII, as well as combined deficiencies of factors such as V and VIII, are rare, but these disorders can cause potentially fatal bleeding episodes, such as intracranial hemorrhages, especially in the neonatal period [172].

Except for the limited evidence of the applicability of PC in patients with SCD, our systematic review found no articles on PC in other benign hematological diseases, also known as classic hematological diseases. These include a range of different clinical manifestations and the possibility of chronicity, with substantial physical and psychosocial sequelae, such as fatigue, pain secondary to post-thrombotic syndrome,

chronic arthropathy, cognitive disorders, and physical injuries from ischemic and/or hemorrhagic strokes, cardiovascular events, organ dysfunction, frequent blood transfusions and intravenous infusions, and occasional hospitalizations, with a consequent impact on the QoL of the patient and their family. PC should, if possible, be integrated into early childhood and continued throughout life, offering support to the patient and their families, assisting them in navigating the complexities of the disease and in making decisions about its treatment, focusing on education about the pathology and its prognostic limitations, genetic counseling, defining a therapeutic plan, controlling physical and psychological symptoms, and assisting in modifying environmental factors that impact treatment (e.g., social, educational, pedagogical, and economic vulnerability factors) [25]. PC can facilitate advanced care planning, assist during hospitalizations, and help align patient expectations with clinical reality. PC also plays a critical role in supporting the complex transition from pediatric to adult-centered care.

Recommendations

Criteria for referring patients with sickle cell disease, thalassemia, congenital anemias, and hemostasis disorders to palliative care

1. Patients with symptoms that are difficult to manage in their different dimensions (physical, social, psychological, and spiritual) and that are refractory to interventions by the referring care team. (Level of recommendation: D)
2. Patients with chronic organ dysfunction or progressive multi-organ impairment; physical and/or cognitive sequelae, progressive loss of functionality, and impairment of QoL. (Level of recommendation: D)
3. Patients with high transfusion needs/chronic transfusions or who require regular visits to health services. (Level of recommendation: D)
4. Patients with recurrent unplanned hospitalizations or hospitalizations due to acute events with physical, psychological, or social impact. (Level of recommendation: D)
5. Patients with a high symptom burden, chronic pain, refractory symptoms, moderate to intense psychological or existential distress, or the presence of any of the criteria described in the general referral criteria of hematological patients to PC. (Level of recommendation: D)

Final considerations

Although in recent years there has been a growth in publications and studies on PC in hematology, scientific evidence is still scarce. The integration between PC and hematology seeks more effective symptom control, adaptation, and rehabilitation of the patient in the face of functional and social losses; family support in the face of illness and bereavement; alignment of care objectives with the patient's values; and the development of advanced end-of-life care planning [173]. Despite this panorama, the term 'palliative care' is frequently misunderstood by healthcare professionals as synonymous with end-of-life care, or as only offered when there are no more disease-modifying treatment options, thereby delaying

PC referrals and hindering patients' understanding of the importance and scope of this care. A public opinion survey in the United States showed that most laypeople have never heard the word 'palliative' and do not know what it means. On the other hand, as soon as they learn more about PC, they become interested in receiving it for themselves or a loved one [174,175].

Internships in PC during hematology training or continuing education programs are strategies to expand the competencies and skills of hematologists in primary PC, since these are critical components of care for a large proportion of hematological patients. Specialist-level involvement in PC can be restricted to more complex scenarios, being episodic and intermittent, or otherwise continuous, longitudinal, and co-managed [173,174].

Therefore, we believe that the publication of this consensus will allow for the expansion of the discussion on the interface between PC and Hematology, inspiring the integration of these specialties and the search for answers to so many questions and doubts for better care of hematological patients.

Authors' contributions

All authors contributed to the literature review, development of recommendations, and writing of the text. APSF and PMC created the figures, edited the complete text, and submitted the final version for approval by the other authors.

Data availability

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

Conflicts of interest

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

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Editor

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Case Report

Myeloid neoplasm with eosinophilia associated with FIP1L1–PDGFRA presenting as femoral avascular necrosis: A rare case report



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Introduction

Myeloid neoplasms with eosinophilia and abnormalities of the *platelet-derived growth factor receptor alpha* (PDGFRA) gene are rare hematologic malignancies driven by fusion genes that encode constitutively active tyrosine kinases [1]. Although they most commonly present as chronic eosinophilic leukemia, these disorders may also manifest as acute myeloid leukemia or T-lymphoblastic leukemia/lymphoma [1].

Clinically, these disorders are characterized by multisystem involvement resulting from the infiltration of tissues by

clonal eosinophils. The heart, lungs, skin, gastrointestinal tract, and central or peripheral nervous system are among the most frequently affected organs [2–6]. The *Factor Interacting with PAPOLA and CPSF1* (FIP1L1)-PDGFRA fusion gene, resulting from a cryptic microdeletion on chromosome 4q12, plays a central role in disease pathogenesis and predicts a remarkable therapeutic response to imatinib, which has significantly improved outcomes for affected patients [7].

Despite the broad range of organ involvement described, skeletal manifestations remain exceptionally uncommon [8,9]. Only isolated case reports have documented bone infiltration or lytic lesions associated with FIP1L1-PDGFRA-positive neoplasms, underscoring the rarity of this presentation [8,9]. Notably, avascular necrosis has not been previously reported as an initial manifestation of this disease.

We describe a rare case of a FIP1L1-PDGFRA-rearranged myeloid neoplasm presenting initially with femoral avascular

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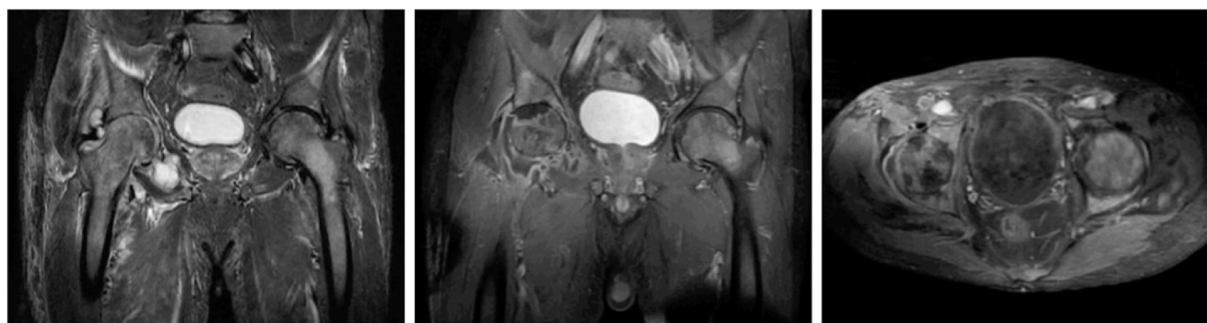


Fig. 1 – Magnetic resonance imaging of the pelvis showing avascular necrosis of the right femoral head, chondral thinning and narrowing of the right hip joint space, large right hip joint effusion with distension of the articular recesses and capsular enhancement after contrast administration; in addition to edema in the gluteal muscles and the proximal right thigh, along with signs of bone marrow infiltration of the pelvis, sacrum, studied vertebral bodies, and bilaterally of the femurs.

necrosis, highlighting the diagnostic challenges, imaging findings, and response to targeted therapy.

Case presentation

A 59-year-old man with no relevant medical, family, social, or occupational history presented with progressive asthenia and severe right hip and lower-limb pain lasting five months. The pain gradually worsened, compromising ambulation. He also reported an unintentional weight loss of approximately 20 kg during this period. He initially sought emergency care, where he required a transfusion of red blood cell concentrate, and was subsequently referred to a tertiary university hospital for further evaluation.

Physical examination revealed intense pain on mobilization of the right lower limb. Initial laboratory testing showed hemoglobin of 8.2 g/dL, leukocyte count of $67.2 \times 10^9/L$ with an absolute eosinophil count of $18.141 \times 10^9/L$, platelet count of $107,000/mm^3$, and erythrocyte sedimentation rate of 111 mm/h.

Abdominal computed tomography demonstrated splenomegaly (16 cm longitudinal axis). Magnetic resonance imaging of the pelvis and right knee revealed avascular necrosis of the right femoral head without loss of sphericity, associated with chondral thinning and joint space narrowing. A large right hip joint effusion was present, with distension of the articular recesses and capsular enhancement after contrast. Edema of the gluteal muscles and proximal thigh was noted, along with diffuse bone marrow infiltration of the pelvis, sacrum, evaluated vertebral bodies, and both femurs (Figure 1).

Bone marrow immunophenotyping showed eosinophilia (22 %) with predominance of granulocytic lineage. Tests for BCR::ABL1 and JAK2 were negative, while fluorescence in situ hybridization (FISH) revealed the FIP1L1-PDGFR α rearrangement in 98 % of analyzed nuclei (Figure 2). A biopsy of the right femur confirmed bone infiltration compatible with chronic eosinophilic leukemia.

The patient initially received hydroxyurea for cytoreduction. After five months, therapy was switched to imatinib (400 mg/day), resulting in marked clinical improvement and

the normalization of hematologic parameters. Complete radiologic resolution of bone lesions was achieved after six months of imatinib treatment.

Discussion

Myeloid and lymphoid neoplasms associated with eosinophilia and the FIP1L1-PDGFR α rearrangement are rare hematologic malignancies that typically present with persistent eosinophilia and multisystem involvement, most commonly affecting the heart, lungs, skin, and central or peripheral nervous system [1–3,5,7]. In contrast, skeletal involvement is exceptionally uncommon, with only isolated case reports describing bone lesions secondary to these neoplasms [8,9].

Recent literature suggests that bone infiltration may occur more frequently than previously recognized, although still representing a minority of cases. Lv et al. [8] described a

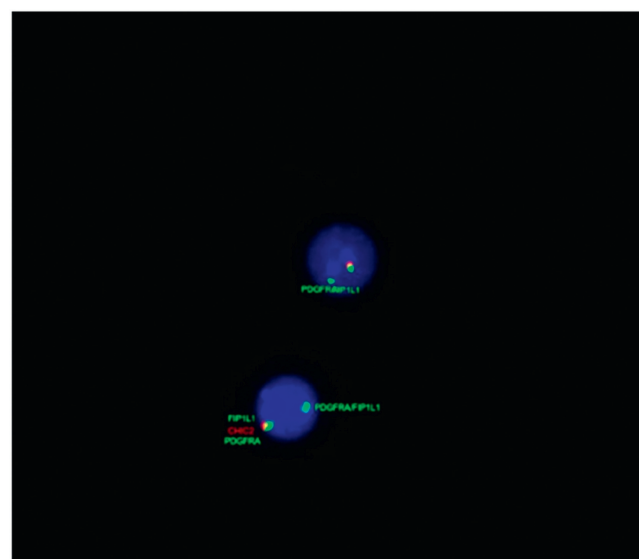


Fig. 2 – Fluorescence in situ hybridization for 4q12 (FIP1L1/PDGFR α) on chromosome 4 - rearrangement of FIP1L1-PDGFR α was detected in 98 % of the analyzed nuclei.

young adult with multiple osteolytic vertebral lesions at presentation, while McGregor et al. [9] reported a pathological fracture due to a lytic lesion of the femoral neck in a patient with the *FIP1L1*-*PDGFRA* rearrangement identified in the tumor tissue despite negative bone marrow findings. These reports, together with the present case, indicate that skeletal manifestations should be considered within the clinical spectrum of *FIP1L1*-*PDGFRA*-positive neoplasms.

The mechanisms underlying bone involvement remain unclear. It has been proposed that eosinophilic infiltration may directly contribute to bone damage, given the release of pro-inflammatory cytokines and cytotoxic mediators by neoplastic eosinophils. Additionally, cooperating genetic alterations, such as *RUNX1* loss-of-function mutations, may enhance osteoclast activity and pathological bone resorption [8]. This aligns with the established concept that osteoclasts originate from erythroid-myeloid progenitors and can be replenished by monocyte-lineage cells derived from hematopoietic stem cells, suggesting a potential for lineage plasticity among malignant cells [8].

To our knowledge, avascular necrosis has not previously been described as an initial manifestation of the *FIP1L1*-*PDGFRA*-rearranged disease. In this case, the avascular necrosis likely reflected extensive marrow infiltration and local tissue damage, expanding the recognized clinical spectrum of these neoplasms. This underscores the importance of considering clonal eosinophilic disorders in patients with unexplained eosinophilia and atypical bone findings.

Diagnosis requires exclusion of secondary causes of eosinophilia, followed by molecular testing for the *FIP1L1*-*PDGFRA* fusion gene, an alteration undetectable by standard cytogenetics and requiring techniques such as FISH or reverse transcription polymerase chain reaction (RT-PCR) [1]. In our patient, the rearrangement was detected in 98 % of nuclei by FISH, confirming the diagnosis and guiding targeted therapy.

Imatinib mesylate has dramatically transformed the prognosis of patients with *FIP1L1*-*PDGFRA* rearrangements. Bacarani et al. [4] demonstrated universal hematologic and molecular responses, even at low doses, and similar findings were described by Müller et al. [5] and Hilal et al. [7], including cases with significant organ involvement. Consistent with these observations, our patient exhibited marked clinical, radiologic, and hematologic improvements following treatment with imatinib, reinforcing its role as first-line treatment regardless of disease presentation or site of involvement.

This case emphasizes the necessity of considering myeloid neoplasms with eosinophilia in the differential diagnosis of unexplained avascular necrosis, especially when associated with marked eosinophilia. Early identification of the *FIP1L1*-*PDGFRA* rearrangement enables timely initiation of targeted therapy, which is frequently associated with favorable outcomes even in cases with atypical presentations.

Conclusion

Myeloid neoplasms with eosinophilia and the *FIP1L1*-*PDGFRA* rearrangement are rare disorders that should be considered in patients presenting with eosinophilia and evidence of target-organ damage. This case highlights an atypical and

previously unreported presentation characterized by femoral avascular necrosis associated with diffuse marrow infiltration. Early recognition of this unusual manifestation is essential, as timely molecular diagnosis enables initiation of targeted therapy with imatinib, which has been consistently associated with favorable clinical outcomes.

Author contributions

KGF: Conceptualization; Data curation; Writing – original draft. ICRD: Investigation; Visualization; Data curation. PGMF: Visualization; Writing – review & editing; Validation. VLA: Writing – original draft; Writing – review & editing. JRD: Methodology; Writing – original draft; Writing – review & editing. PSB: Methodology; Writing – original draft; Writing – review & editing. JBT: Methodology; Writing – original draft; Writing – review & editing. NLF: Investigation; Validation; Supervision. VRGAV: Conceptualization; Project administration; Supervision; Writing – review & editing.

Ethics approval statement

The study was approved by the local institutional ethics committee (CAAE: 81,586,924.4.0000.5238; number: 7.095.793).

Data availability

The authors confirm that the data generated and analyzed in this study are included in this published article. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of interest

No conflicts of interest declared.

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Case Report

A mysterious foe in a case of pancytopenia with splenomegaly: *Plasmodium vivax* gametocytes in the bone marrow



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Introduction

Plasmodium vivax, the most widely distributed human malaria parasite, poses a significant disease burden [1]. *P. vivax* usually causes a less severe form of malaria compared to *Plasmodium falciparum*, as it is rarely associated with severe complications but it can cause severe anemia and severe thrombocytopenia [1,2]. Pancytopenia with splenomegaly as an initial manifestation of acute *P. vivax* malaria is extremely uncommon; it is mainly reported after chronic or repeated exposure to *P. vivax*, as a manifestation of hypersplenism associated with hyperactive malarial splenomegaly syndrome. Pancytopenia with splenomegaly as a primary presentation is more often associated with *P. falciparum*.

Diagnosis of *P. vivax* malaria typically involves a peripheral blood smear (PBS) to detect the parasite. Even though *P. vivax* can be found in the bone marrow, its presence in this tissue without being present in the peripheral blood has rarely been documented [3]. To the best of our knowledge, there are no documented cases of pancytopenia with splenomegaly

associated with acute *P. vivax* malaria with the presence of its gametocytes only in the bone marrow and not in PBSs.

Recognizing the unusual presentation of *P. vivax* malaria is essential for early diagnosis and management. We report a rare case of acute *P. vivax* malaria presenting with fever, pancytopenia and splenomegaly which incidentally showed gametocytes of *P. vivax* in a bone marrow smear without any parasite stage in PBS. The patient had a full recovery from fever, pancytopenia and splenomegaly with oral chloroquine and primaquine. We believe that physicians from endemic countries for malaria such as India should be aware of such a presentation.

Case report

A 47-year-old woman from western India, a malaria endemic region, with unremarkable medical history and in overall good health initially visited her local hospital for primary care due to a high-grade fever of one week duration. Routine laboratory investigations were performed, including a rapid diagnostic test (RDT) and microscopic examination of PBS; both were negative for malaria parasites, and no hematological abnormalities were detected. Symptomatic medications and

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antibiotics were given, but the fever persisted for more than three weeks. During this period, she developed easy fatigability, and clinical examination revealed a palpable spleen. Subsequent laboratory evaluations showed persistent pancytopenia despite serial RDTs and PBS remaining negative for malaria. Due to the suspicion of a hematological malignancy, the patient was referred to our center for further diagnostic evaluation. Upon presentation, she was febrile with a temperature of 39 °C (102.3°F). She was very pale and had a palpable splenomegaly (7 cm below left costal margin) on the physical examination. The rest of the physical examination was unremarkable. Her initial laboratory findings revealed pancytopenia with hemoglobin (Hb) of 5.3 g/dL, total leukocyte count of $2.4 \times 10^3/\mu\text{L}$ with absolute neutrophil count of $0.8 \times 10^3/\mu\text{L}$, and a platelet count of $27 \times 10^3/\mu\text{L}$. She had a reticulocyte count of 4.5 % with a corrected reticulocyte count of 1.9 %. The PBS showed predominantly microcytic hypochromic erythrocytes, leucopenia (neutropenia and lymphopenia), and reduced platelets on smear, without any evidence of ring forms, trophozoites or schizonts of *P. vivax*. Biochemical tests revealed normal levels of serum ferritin (131.7 ng/mL), vitamin B12 (490 pg/mL) and triglyceride (110 mg/dL) with a mildly raised serum lactate dehydrogenase level

(227.15 U/L). Her plasma fibrinogen level was normal (420 mg/dL) and glucose-6-phosphate dehydrogenase activity was normal (32.31 U/g Hb). A direct Coombs test was negative. Renal and liver function tests were normal. Serologic tests for hepatitis B and C virus, human immunodeficiency virus, and cytomegalovirus were negative. Blood and urine cultures showed no growth. Her abdominal ultrasound confirmed a moderate splenomegaly (spleen size 17.8 cm). Since there were signs and symptoms mimicking a hematological malignancy, a bone marrow examination (aspirate and biopsy) was performed which surprisingly revealed *P. vivax* gametocytes in the bone marrow smear with mild erythroid hyperplasia and dyserythropoiesis, normal myelopoiesis, and an adequate number of functioning megakaryocytes (Figure 1). Based on the clinical, hematological and biochemical parameters, and bone marrow findings, a final diagnosis of pancytopenia with splenomegaly as a result of *P. vivax* infection was established. This is a rare case because acute *P. vivax* malaria presented with pancytopenia and moderate splenomegaly along with the presence of *P. vivax* gametocytes in the bone marrow without any evidence of the parasite in the initial work-up, particularly in the PBS. Antimalarial treatment was administered following the standard guidelines (1500 mg of oral chloroquine over three

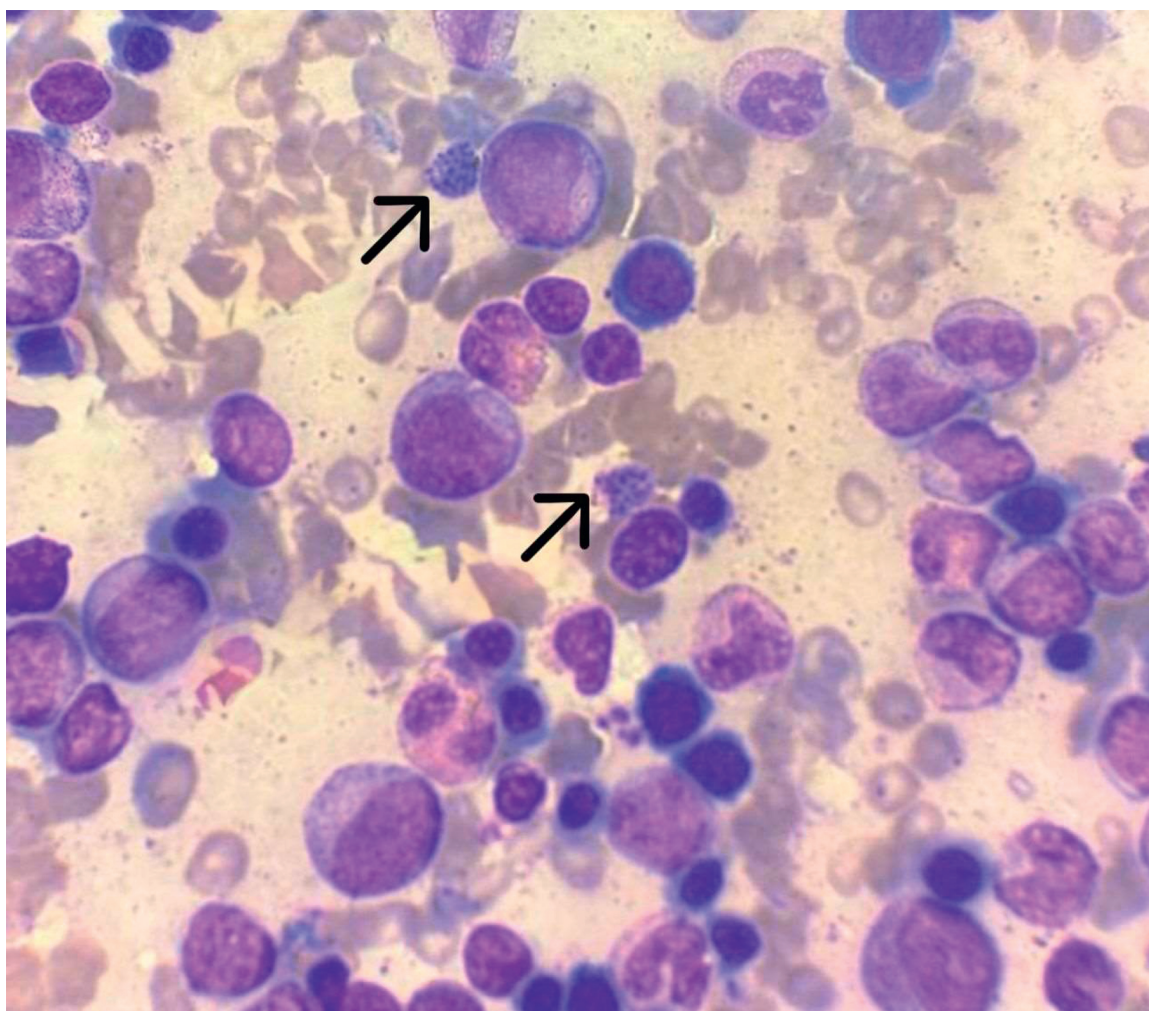


Figure 1 – Presence of *Plasmodium vivax* gametocytes in a bone marrow aspirate.

Table 1 – Hematological parameters at baseline and at two and six weeks of follow-up.

Parameter	Baseline	2 weeks	6 weeks
Hemoglobin (g/dL)	5.3	8.0	12.5
Hematocrit (%)	18.6	24.6	41.3
Total erythrocyte count ($10^6/\mu\text{L}$)	3.36	3.98	4.65
Mean corpuscular volume (fL)	55.4	61.8	81.9
Mean corpuscular hemoglobin (pg)	15.8	20.1	26.9
Mean corpuscular hemoglobin concentration (g/dL)	28.5	32.5	32.8
Total leukocyte count ($10^3/\mu\text{L}$)	2.40	3.92	8.93
Absolute neutrophil count ($10^3/\mu\text{L}$)	0.80	1.86	6.30
Platelet count ($10^3/\mu\text{L}$)	27	80	371
Reticulocyte count (%)	4.5	4.2	1.3
Corrected reticulocyte count (%)	1.94	2.30	1.19
Reticulocyte production index	0.8	1.1	1.2

days and 15 mg of primaquine per day for 14 days: the primaquine was administered for radical treatment of *P. vivax* along with iron supplements. Her condition improved rapidly after starting the treatment with fever subsiding after three days of chloroquine. During her follow-up visits, her hematologic parameters had normalized. The pancytopenia and splenomegaly were fully resolved by the 28th day after discharge. At six weeks after treatment, repeat bone marrow aspirate and biopsy samples were obtained for comparison with the initial findings. A microscopic bone marrow smear examination was negative for gametocytes. Hematological parameters at baseline and follow-ups are shown in Table 1.

Discussion

P. vivax malaria is a significant global health issue in many countries, particularly in tropical and subtropical regions, including sub-Saharan Africa, Asia, and Latin America [1,4]. Contrary to the general perception that *P. vivax* malaria usually results in minimal complications, a series of reports have demonstrated that the infection can cause multiple-organ dysfunction and severe life-threatening complications such as severe anemia, hepatic dysfunction and jaundice, acute lung injury, acute respiratory distress syndrome and pulmonary edema, shock, acute kidney injury, severe thrombocytopenia and splenic rupture [1,2].

This case presented with a prolonged fever, splenomegaly, pancytopenia, and easy fatigability, the signs and symptoms mimicking a hematological malignancy. *P. vivax* was not identified in the preliminary work-up (RDT and PBS examinations); quantitative polymerase chain reaction is not a routine work-up for *Plasmodium* suspected patients in our country. The patient underwent extensive work-up to exclude other infectious diseases. Our patient incidentally demonstrated *P. vivax* gametocytes in the bone marrow smear, indicating that the parasites can invade the bone marrow, leading to pancytopenia and other hematological abnormalities.

Pancytopenia due to *P. vivax* malaria is extremely rare and so far has been documented in 0.9 % of confirmed *P. vivax* cases [4]. Pancytopenia as a primary presentation is uncommon in *P. vivax* malaria; it is more often associated with *P. falciparum*. *P. vivax* malaria may cause pancytopenia through

several mechanisms, including hemophagocytic lymphohistiocytosis, myelosuppression, hypersplenism, or tumor lysis by infection-related steroid release [4]. However, the underlying pathogenic mechanisms of pancytopenia remain largely unresolved in our case as all these mechanisms are unlikely. Further research is needed to understand the infection-related reduced blood cell counts.

The spleen plays a crucial role in filtering the blood and removing infected red blood cells, leading to its enlargement during malaria infection. Splenomegaly, one of the most common features of malaria, is directly related with severity [5]. Spleen enlargement occurs if an individual experiences parasitemia for a period exceeding two weeks. Thereafter the degree of spleen enlargement depends upon the duration of exposure and severity of parasitemia [5]. Splenomegaly during acute malaria infections is more common with *P. falciparum* than with *P. vivax*, as the former is more frequently associated with high parasite densities, leading to increased clearance of both parasitized and non-parasitized erythrocytes [5]. Soni & Jalaly observed splenomegaly in 41 % of *P. falciparum* and 20 % of *P. vivax* patients [5]. Strickland et al. observed that greater splenic volume was positively correlated with the likelihood of *P. falciparum* infection, whereas *P. vivax* was more frequently associated with mild splenomegaly [6]. Splenomegaly in acute *P. vivax* malaria can be associated with a higher risk of spontaneous splenic rupture, a serious and potentially life-threatening complication [1]. The spleen could be a niche for *P. vivax* leading to splenomegaly and maintenance of a low peripheral parasitemia [1].

In the present case, repeated thin and thick PBSs and RDTs failed to demonstrate *P. vivax*. Because the clinical presentation was suggestive of a hematological malignancy, a bone marrow examination was performed as part of the diagnostic evaluation. *P. vivax* parasites can be found in the bone marrow during an active infection. The presence of *P. vivax* in the bone marrow was first noticed in the late 19th century [3,7]. Sternal bone marrow aspirate examinations used to be performed as an accessory to peripheral blood examination in malaria [8]. Baro et al. showed that *P. vivax* gametocyte stage-infected cells are enriched in the bone marrow compared to peripheral blood during the acute infection [3]. These data suggest that the bone marrow could also be a reservoir for gametocytes during *P. vivax* infections. Whether the bone marrow functions as a niche for gametocyte production or maturation, and whether these forms sequester there as observed in *P. falciparum*, warrants further investigation in future studies. Our case suggests that bone marrow aspiration is of value in the diagnosis of malaria.

In the present case study, the repeated RDTs for the malarial antigen were negative, and the *P. vivax* was also not detected in repeated PBS examinations. RDTs for *P. vivax* have relatively poor performance compared with those for *P. falciparum* as uptake is slow and inconsistent. This is due to a combination of lower parasite density in *P. vivax* infections, lower expression of the parasite lactate dehydrogenase, and poorer performance of the reagents used for this specific antigen. As a consequence, many RDTs might fail to detect *P. vivax* in samples containing ≤ 200 parasites/ μL [9]. The negative results of serial RDTs in this case was possibly due to false-negative results. On the other hand, the negative PBS for *P. vivax* may be attributed to the pretreatment with

antimalarial drugs in inadequate doses, causing partial clearance of the parasite, low levels of parasitemia not detected by conventional microscopy or by sequestration of the parasitized cells, in deep vascular beds [4].

Conclusion

P. vivax malaria should be considered in the differential diagnosis in all people from endemic areas who present with fever, pancytopenia and splenomegaly. A diagnostic bone marrow examination should be recommended in patients from endemic regions with negative results for *P. vivax* in serial RDTs and microscopic PBS examinations after all other possible infectious causes are excluded, since early diagnosis and treatment of malaria are crucial to prevent serious complications and mortality.

Authors' contributions

Both authors meet the ICMJE authorship criteria. GRP designed the study, collected data, and contributed to writing, reviewing and editing the manuscript with overall supervision. VS collected raw patient data, obtained the patient consent and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Ethics approval

Ethical approval for this case report was given by the Institutional Ethics Committee.

Consent for publication

The patient provided written informed consent for the publication of this case report.

Data availability

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

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Conflicts of interest

The authors declare no competing financial or other conflicts of interest.

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Editor

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Letter to the Editor

Comment on “Risk factors associated with the use of red blood cells in elective cardiac surgeries”

Dear Editor,

We herein discuss the publication on “Risk factors associated with the use of red blood cells in elective cardiac surgeries: A patient blood management (PBM) view”. [1] This article investigates the risk variables linked with the use of packed red blood cells (PRBC) in patients undergoing elective heart surgery in Brazil.

While stepwise logistic regression results show that female gender, poor hematocrit level, diabetes, and extracorporeal circulation duration of more than 90 min are significant risk variables, this analysis is statistically limited. As this is a retrospective study, it is susceptible to bias from data collection or sample selection. Furthermore, stepwise logistic regression may result in overfitting and variable selection based on the specific data set, restricting its applicability to other hospitals or populations.

Furthermore, underlying heart disease severity, changes in surgeon treatment, surgical technique, or anticoagulant use can all have an impact on the accuracy of the outcomes as these variables may not be recorded or controlled in the analysis. Furthermore, the identification of female gender as a high-risk factor could be explained by variations in body weight or total blood volume, however the paper did not account for these variables in its analysis. This may confound the relationship.

New interpretations may concentrate on synthesizing findings for PBM guidelines. Preoperative hematocrit assessment and improvement of anemia, diabetes management, and surgical planning to limit cardiopulmonary bypass time can all help to lessen the requirement for PRBC. Furthermore, including risk prediction models that take into account characteristics such as gender, body weight, disease severity, and laboratory results might improve accuracy and simplify effective blood use planning.

To broaden the topic, consider the following research questions:

- 1) Can this retrospective study be transformed into a prospective or multicenter study to corroborate the relationship?

- 2) How much can enhancing PBM with preoperative optimization techniques reduce PRBC use and clinical outcomes?
- 3) How do individual characteristics, such as body mass index and female gender, affect overall blood volume?
- 4) Can multivariate machine learning algorithms accurately predict the requirement for PRBC in cardiac surgery patients?

These questions may lead to applicable research as well as safe, resource-saving strategies in Brazil's health-care system.

Data availability statement

there is no new data generated

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there is no funding

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not applicable

Patient consent statement

not applicable

Permission to reproduce material from other sources

not applicable

Clinical trial registration

not applicable

Author contributions

HP 50 % ideas, writing, analyzing, approval

VW 50 % ideas, supervision, approval

AI declaration

the authors use computation tool in language checking and editing.

Conflicts of interest

the authors declare no conflict of interest

Editor

Eduardo Rego

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Letter to the Editor

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

To the editor:

We eagerly read the paper entitled “Clinical characteristics and outcomes of non-tuberculous mycobacterial pulmonary infections after hematopoietic stem cell transplantation: A retrospective cohort study” by Zahia Esber [1] with great interest. The authors assessed the clinical manifestations, diagnosis, and consequences of lung infections caused by nontuberculous mycobacteria (NTM) in patients who received hematopoietic stem cell transplantation (HSCT). The study offers evidence-based recommendations for improving NTM infection diagnosis, risk assessment, and management in a susceptible post-transplant population.

As the authors point out, one of the study’s main limitations is the difficulty in distinguishing between colonization and actual NTM pulmonary infection. One third (35.3 %) of the cases were categorized as “possible” infections (positive cultures alone) despite the application of the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) criteria, which recent systematic reviews indicate may not necessarily imply clinically severe disease needing treatment. For example, single positive test results frequently indicate colonization, but ≥ 2 positive cultures are linked to a 98 % chance of *Mycobacterium avium* complex (MAC) pulmonary illness. Also according to the study’s findings, the majority of patients (76.5 %) had positive results even though they did not receive therapy [1–3]. But the authors could make their arguments stronger by specifically citing this kind of research to put their management choices in perspective. Also, the lack of rapidly growing mycobacteria (RGM) instances (such as *Mycobacterium abscessus*) restricts the generalizability to more aggressive NTM species, a situation that calls for additional multicenter research.

There is a lack of a control group of HSCT patients with no NTM infection, which is another limitation of this study.

Without this comparison, it is challenging to identify the clinical characteristics, risk factors, or results that are directly related to NTM infection as opposed to HSCT or related issues such as immunosuppression or graft-versus-host disease (GvHD). This makes it difficult to draw conclusions about causality and restricts the capacity to pinpoint the main risk factors or results linked to NTM. Liu et al., in their cohort analysis, addressed this restriction by comparing HSCT patients with and without mycobacterial infections. This made it possible to more clearly identify independent risk factors and outcomes linked to NTM infection. Their research highlights the significance of including a control group that is not infected in order to improve the validity and generalizability of results [4].

The authors in this study noted that 86.3 % of patients with NTM infections were on steroids and immunosuppressive therapy for GvHD. However, they did not conduct a thorough statistical analysis to determine whether GvHD and its treatment independently increased the risk of NTM infections. For example, a retrospective cohort study by Upton et al. found that chronic GvHD and cytomegalovirus (CMV) viremia were significantly associated with a higher risk of NTM disease, with hazard ratios of 1.99 and 5.77, respectively. Although routine NTM screening may be warranted in high-risk subgroups, such as those with GvHD or CMV coinfection, its usefulness should be substantiated by quantitative analysis of independent risk [5].

According to von Elm et al. in the STROBE Statement [6], “authors should report efforts to address potential sources of bias, such as design strategies, statistical adjustments, or sensitivity analyses.” The current study lacks any such reporting, making it difficult to assess the robustness of its conclusions. The authors should explicitly identify and discuss potential sources of bias inherent in the retrospective design, such as selection and information bias. The inclusion of strategies to minimize bias—such as blinded image review or comparison

to a control group—would strengthen the study's credibility and align it with best practices in observational research.

In contrast, Kontoyiannis et al. effectively handled bias in a similar HSCT cohort study by using matched controls, stratification, and acknowledging the limitations of their retrospective design [7]. Their approach enhances internal validity and should serve as a methodological benchmark.

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Ethical consent

Not applicable.

Author contributions

Both authors contributed to the conceptualization, writing, and critical review of the manuscript. Both authors reviewed and approved the final version of the letter. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

None.

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Letter to the Editor

Donor DNA: Embedding self-regulation into blood donation culture



Dear Editor,

I read with great interest the article by Ranjbar Kermani et al. on the development and validation of the Self-Regulation of Blood Donation Scale (SRBDS) for Iranian blood donors [1]. This pioneering work adds depth to our understanding of donor motivation by contextualizing it within the self-determination theory (SDT) framework. While the scale demonstrates strong psychometric properties, I believe its implications extend far beyond measurement. In this correspondence, I propose several novel avenues to translate self-regulation science into actionable strategies for donor recruitment and retention.

1. Digital Behavioural Nudges and Gamification

The SRBDS highlights intrinsic and integrated regulation as key drivers of sustained donation [1]. Embedding these dimensions into digital blood donor apps could transform engagement. For instance, gamified features that reward streaks of consistent donation, peer challenges, and personalized motivational feedback could amplify intrinsic satisfaction, as supported by SDT research in other health behaviors [2].

2. Culturally Adapted Messaging Beyond Iran

The study reflects the importance of religious and cultural motivations in Iran [1]. However, cross-cultural adaptation is vital. In Western settings, altruism and community solidarity dominate donor narratives, while in parts of Asia, familial duty or reciprocity may prevail [3]. A modular, culturally sensitive adaptation of the SRBDS could help global blood services tailor campaigns to local motivational landscapes.

3. Integrating Psychometrics into Donor Management Systems

Routine use of the SRBDS during donor registration could generate valuable motivational profiles. Linking these profiles with donor return data in electronic donor management systems would allow predictive analytics to identify 'at-risk' lapsed donors and target them with personalized interventions [4]. This approach shifts from reactive donor recruitment to proactive donor relationship management.

4. Motivational Interviewing as a Retention Tool

Evidence from other domains suggests motivational interviewing enhances internalization of health behaviors [5]. Training donor recruiters to use brief motivational interviewing techniques, rooted in SRBDS dimensions, could reinforce identified and integrated regulation, thereby promoting long-term donor identity formation.

5. Research on Motivation Transitions Over Time

While the SRBDS validation was cross-sectional, longitudinal research could uncover how motivations evolve with donor experience. Do donors shift from external to intrinsic regulation as they internalize a "donor identity"? Understanding these transitions could inform stage-specific retention strategies, from first-time donor encouragement to legacy donor recognition.

In conclusion, the SRBDS represents more than a psychometric advance; it is a roadmap to reimagine donor retention strategies grounded in behavioral science. By embedding self-regulation principles into digital tools, cultural tailoring, donor management systems, and motivational interviewing, transfusion services can transform donor motivation into mobilization.

Data availability statement

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

Conflicts of interest

There are no conflicts of interest.

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Editor

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