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ORIGINAL ARTICLE

Peripheral blood smear of patients with hemolytic anemia: a retrospective study in a university hospital in Rio de Janeiro

Esfregaço de sangue periférico de pacientes com anemia hemolítica: estudo retrospectivo em um hospital universitário no Rio de Janeiro

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ABSTRACT

Introduction: Hemolytic anemias are conditions characterized by a reduced eryth-rocyte half-life. Their etiology is diverse and can be suspected based on hematolog-ic alterations. The peripheral blood smear test stands out in this context due to its low cost and relative availability. **Objective:** To describe the peripheral blood smear findings in patients with hemolytic anemias followed at a university hospital in Rio de Janeiro. **Methods:** A retrospective cross-sectional study was conducted using data from the medical records of patients diagnosed with hemolytic anemias between 2018 and 2022 at the hematology clinic of a university hospital in Rio de Janeiro. **Results:** Twenty-four patients were analyzed, 66.67% (n=16) of whom were female. Peripheral blood smear findings included: codocytes in 100% (n=4) of thalassemic patients; sickle cells in 57.14% (n=7) of sickle cell disease cases; sphe-rocytes in 100% (n=1) of hereditary spherocytosis cases; spherocytes in 75% (n=4) of autoimmune hemolytic anemia due to warm antibodies; agglutinated red blood cells in 100% (n=1) of cold antibody autoimmune hemolytic anemia; schistocytes in 100% (n=2) of intravascular hemolysis cases; and spherocytes, target cells, and dacryocytes in 100% (n=2) of paroxysmal nocturnal hemoglobinuria cases. **Conclusion:** Peripheral blood smear findings were consistent with the etiological diag-noses. However, sickle cell disease was predominantly of the SC type, and there was a higher-than-expected prevalence of female patients, differing from the data reported in the literature. This study reinforces the importance of erythrocyte mor-phology evaluation as an accessible and cost-effective tool for diagnosing hemolyt-ic anemias, aiding in the selection of additional test.

Keywords: Hemolytic anemia; Abnormal erythrocytes; Hemoglobinopathies.

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RESUMO

Introdução: As anemias hemolíticas são condições caracterizadas por uma redução na meia-vida dos eritrócitos. Sua etiologia é diversa e pode ser suspeitada com base em alterações hematoscópicas. O esfregaço de sangue periférico se destaca nesse contexto devido ao seu baixo custo e relativa disponibilidade. **Objetivo:** Descrever os achados do esfregaço de sangue periférico em pacientes com anemias hemolíticas acompanhados em um hospital universitário no Rio de Janeiro. **Métodos:** Estudo transversal e retrospectivo, que utilizou dados dos prontuários médicos de pacientes diagnosticados com anemias hemolíticas entre 2018 e 2022 no ambulatório de hematologia de um hospital universitário no Rio de Janeiro. **Resultados:** Foram incluídos 24 pacientes, dos quais 66,67% (n=16) eram do sexo feminino. Os achados no esfregaço de sangue periférico incluíram: codócitos em 100% (n=4) dos pacientes talassêmicos; hemácias falciformes em 57,14% (n=7) dos casos de doença falciforme; esferócitos em 100% (n=1) dos casos de esferocitose hereditária; esferócitos em 75% (n=4) dos casos de anemia hemolítica autoimune por anticorpos quentes; hemácias aglutinadas em 100% (n=1) dos pacientes com anemia hemolítica autoimune por anticorpos frios; esquizócitos em 100% (n=2) dos casos de hemólise intravascular; e esferócitos, células-alvo e dacriócitos em 100% (n=2) dos pacientes com hemoglobinúria paroxística noturna. **Conclusão:** Os achados do esfregaço de sangue periférico foram consistentes com os diagnósticos etiológicos. No entanto, a doença falciforme foi predominantemente do tipo SC, e houve uma prevalência maior do que o esperado de pacientes do sexo feminino, divergindo dos dados descritos na literatura. Este estudo reforça a importância da avaliação da morfologia eritrocitária como uma ferramenta acessível e de baixo custo no diagnóstico das anemias hemolíticas, auxiliando na indicação de exames complementares.

Palavras-chave: Anemia hemolítica; Eritrócitos anormais; Hemoglobinopatias.

INTRODUCTION

Hemolytic anemias (HAs) are characterized by prematurely destroying red blood cells, whose normal half-life ranges from 110 to 120 days[1-3]. Clinically, they manifest when the hemolysis rate exceeds the bone marrow's compensatory capacity, even with increased erythrocyte production[2,3].

HAs can be classified based on their pathophysiology into intracorpuscular hemolysis, caused by intrinsic red blood cell defects (e.g., enzymopathies, membranopathies, hemoglobinopathies), and extracorpuscular hemolysis, resulting from external factors (e.g., immune-mediated mechanisms, drug-induced reactions, toxins, infections, or mechanical trauma)[2,3].

Additionally, they can be categorized by the site of hemolysis as intravascular or extravascular[1,3,4]. Intravascular hemolysis occurs within blood vessels and is associated with conditions such as glucose-6-phosphate dehydrogenase (G6PD) deficiency, paroxysmal nocturnal hemoglobinuria (PNH), ABO incompatibility, trauma, and infections[1,4]. Extravascular hemolysis occurs in the spleen or liver and includes disorders such as pyruvate kinase deficiency, thalassemia, sickle cell disease (SCD), hereditary spherocytosis (HS), autoimmune hemolytic anemia (AIHA) caused by warm antibodies, drug-induced hemolysis, and bartonellosis[1,4].

Clinical manifestations range from mild anemia to severe hemolysis requiring urgent intervention[2]. Despite

their rarity, HAs have a significant clinical impact, often necessitating hospitalizations, surgeries, and blood transfusions, which burden healthcare systems and affect patients' quality of life[1,5].

Diagnosis is challenging due to the diverse etiologies and overlapping clinical and laboratory features[1,2]. It requires a stepwise approach, including clinical history, peripheral blood smear examination, and the direct antiglobulin test (DAT), complemented by specific tests when needed[2]. However, limited resources, especially in the public health system (SUS), can hinder access to specific methods[1,2,5,6]. In this context, it is crucial that physicians efficiently use available resources, such as the peripheral blood smear, due to its low cost and relative availability[5,6].

The peripheral blood smear is a valuable, low-cost tool that provides information on the shape, size, color, presence of inclusions, and other characteristics and abnormalities of blood cells, including red blood cells, white blood cells, and platelets and is also useful for identifying any other coexisting hematological or malignant disorders[1,2,5-7]. In HAs, this analysis can identify specific alterations that aid in the etiological diagnosis and consequently faster treatment[1,4,5]. Spherocytes are related to membrane defects, as in HS, or to the repetitive removal of the membrane by macrophages, as in immunological HAs[1]. Schistocytes are cellular fragments associated with intravascular destruction, typical of microangiopathic hemolytic anemia (MHA) syndromes[1]. Bite cells and blister cells result from partial phagocytosis, often linked to oxidative causes, such as G6PD deficiency[1,4].

Considering that peripheral blood smear is an accessible and relatively available method that, together with clinical and laboratory signs of hemolysis, offers valuable information for the etiological diagnosis and for directing the investigation of HAs[1,4-7], this study aims to describe peripheral blood smear findings in patients with different types of HAs, diagnosed at a university hospital in Rio de Janeiro and correlate these findings with their underlying etiologies[1,4-7].

Methods

This is a cross-sectional and retrospective study, carried out with data extracted from the medical records of 24 patients diagnosed with HA, treated at the hematology outpatient clinic of a university hospital in Rio de Janeiro.

The sample consisted of 24 patients, who were diagnosed with HA, regardless of the etiology of the disease, between January 1, 2018, and July 27, 2022. Patients under 18 years of age, who did not have peripheral blood smear at diagnosis, or those who were not in adequate condition for evaluation were excluded. The analysis of the peripheral blood smear was performed by two independent hematologists to minimize observer bias. In cases of discrepancy, the opinion of a third hematologist was requested.

The peripheral blood smear studied were frosted lapidated slides (26 mm x 76 mm), which were stained using the May-Grunwald and Giemsa methods, following the Romanowsky technique[8]. These peripheral blood smears were evaluated and their images were captured with an optical microscope, using 40x and 100x objectives (obj), the latter under immersion oil. Data collection began after the project was approved by the local Research Ethics Committee (opinion number: 7,196,228). The Informed Consent Form was waived due to the impossibility of contacting most of the patients, who were no longer being followed up at the outpatient clinic or had died. Data regarding sex, gender, age, diagnosis, and outcome were compiled in a Microsoft Excel spreadsheet for statistical analysis.

Results

During the study period, 44 patients had a confirmed diagnosis of HA, of which 17 were excluded because the peripheral blood smear was not performed at the time of diagnosis. Among the remaining 27 patients, 3 were excluded because the peripheral blood smear were not in adequate condition for evaluation. Thus, the study sample consisted of 24 individuals, 66.67% (n=16) female and 33.34% male.

Of these, 29.17% (n=7) were diagnosed with SCD, 20.84% (n=5) AIHA, 16.67% (n=4) thalassemia, 12.5% (n=3) HA with negative DAT, 8.34% (n=2) PNH, 8.34% (n=2) intravascular HA, and 4.17% (n=1) HS (Table 1).

Regarding the peripheral blood smear findings in the microscopic analysis (Table 2), anisocytosis was the most prevalent finding in the individuals studied, present in 50% (n=12) of the patients. Polychromatophilia was observed in the peripheral blood smear of 29.17% (n=7) of the patients, anisochromia in 25% (n=6), and hypochromia in only one case (4.17%).

As for the patients diagnosed with thalassemia, target cells were seen in all peripheral blood smears. In addition, 75% of the thalassemic patients presented elliptocytes; 50% dacryocytes; and 50% acanthocytes. Drepanocytes, schizocytes, spherocytes, bite cells, and other poikilocytes were also observed (Figure 1).

Among the patients diagnosed with some type of SCD, target cells were found in the peripheral blood smear of all patients; in 57.14% of them, sickle cells were observed; in 42.86%, elliptocytes; and in 71.43%, other poikilocytes were noted. Spherocytes, bite cells, anisochromia, and polychromatophilia were also observed (Figure 2). All patients diagnosed with SCD SS presented sickle cells, while in those diagnosed with SCD SC, this finding was seen in 50%, and no sickle cells were seen in the only patient diagnosed with hemoglobinopathy S/β-thalassemia.

Table 1. Distribution of different hemolytic anemias, according to sex.

Hemolytic anemia	Sample percentage
Thalassemia	16.67% (F=50%; M=50%)
Beta-thalassemia	8.33% (F=50%; M=50%)
Alpha-thalassemia	8.33% (F=50%; M=50%)
Sickle cell disease	29.17% (F=85.7%; M=14.3%)
S/β-thalassemia	4.17% (F=100%)
Sickle cell disease SC	16.66% (F=75%; M=25%)
Sickle cell disease SS	8.34% (F=100%)
Hereditary spherocytosis	4.17% (F=100%)
Autoimmune hemolytic anemia	20.84% (F=60%; M=40%)
Warm antibodies	16.67% (F=50%; M=50%)
Cold antibodies	4.17% (F=100%)
Paroxysmal nocturnal hemoglobinúria	8.34% (M=100%)
Intravascular hemolytic anemias	8.34% (F=50%; M=50%)
Prosthetic valve	4.17% (F=100%)
Microangiopathic hemolytic anemia	4.17% (M=100%)
DAT-negative hemolytic anemias	12.5% (F=100%)

Legend: F = Female; M = Male; DAT = Direct antiglobulin test.
Source: By the authors, 2024.

Table 2. Peripheral blood smear findings found in each type of hemolytic anemia.

Hemolytic anemia	Hematoscopic finding
Thalassemia	
	target cell (100%)
	elliptocytes (75%)
	dacryocytes (50%)
	echinocytes (50%)
	polychromatophilia (50%)
	spherocyte, sickle cell, schizocyte, bite cell, hypochromia (25%)
SCD	
	target cell (100%)
	sickle cell (57,14%)
	elliptocyte (42,86%)
	anisocytosis (42,86%)
	spherocyte, bite cell, anisochromia, polychromatophilia (14.29%)
HS	
	esferócito (100%)
	policromatofilia, anisocitose (100%)
AIHA warm antibodies	
AIHA cold antibodies	
	anisocytosis (100%)
	spherocyte (75%)
	anisochromia (75%)

	polychromatophilia (50%)
	dacryocyte (25%)
AIHA cold antibodies	
	agglutinated red blood cells (100%)
PNH	
	target cells (100%)
	spherocyte (100%)
	dacryocyte (100%)
	anisocytosis (100%)
	microspherocyte, elliptocyte, polychromatophilia (50%)
Intravascular HAs	
	schizocyte (100%)
DAT-negative HAs	
	target cells (66.67%)
	acanthocytes (66.67%)
	anisocytosis (66.67%)
	anisochromia (66.67%)
	elliptocyte (33.33%)
	spherocyte (33.33%)
	multisegmented neutrophil (33.33%)

Caption: SCD = Sickle cell disease; HS = Hereditary spherocytosis; AIHA = Autoimmune hemolytic anemia; PNH = Paroxysmal nocturnal hemoglobinuria; HAs = Hemolytic anemias; DAT = Direct antiglobulin test.
Source: By the authors, 2024

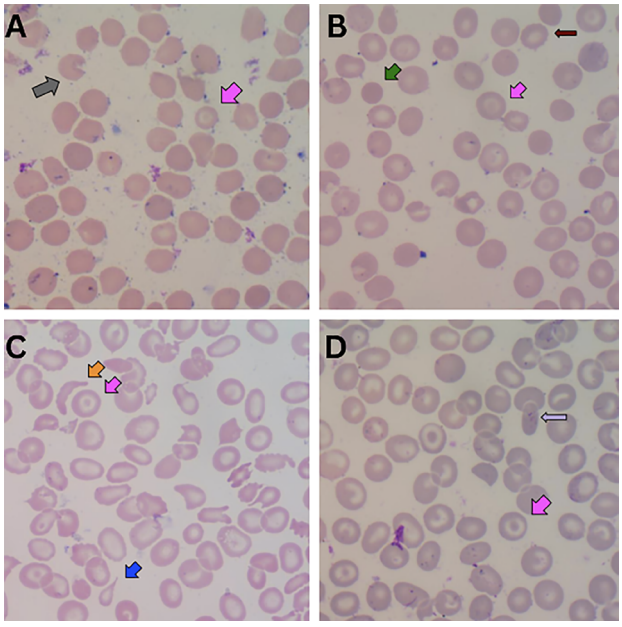

Figure 1. Alterations found in peripheral blood smear of patients diagnosed with thalassemia. **A.** βthalassemia minor: polychromatophilia, target cells (pink arrow) and bite cells (gray arrow) in obj 100x; **B.** β-thalassemia: target cells (pink arrow), spherocytes (green arrow) and acanthocytes (brown arrow) in obj 100x; **C.** α-thalassemia intermedia: target cells (pink arrow), sickle cells (orange arrow), dacryocytes (blue arrow), schistocytes (purple arrow) and other poicilocytes in obj 100x; **D.** α-thalassemia: target cells (pink arrow), elliptocytes (purple arrow) and other poicilocytes in obj 100x.

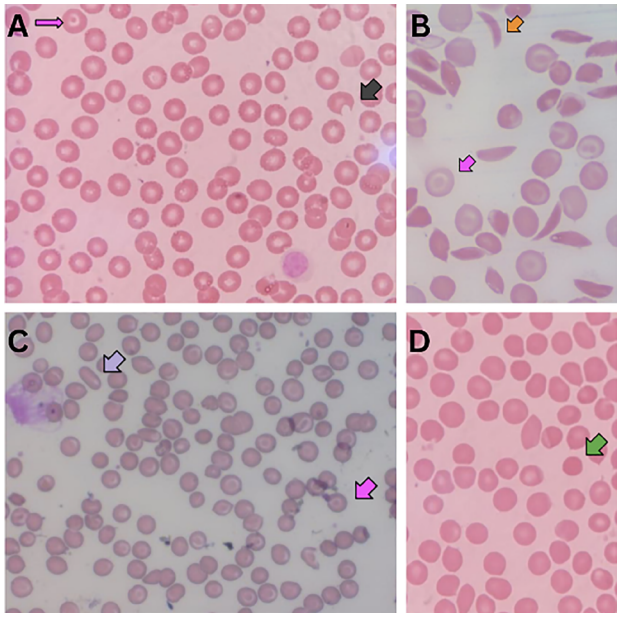

Figure 2. Alterations found in peripheral blood smear of patients diagnosed with sickle cell disease. **A.** S/βthalassemia: target cell (pink arrow) and bite cell (gray arrow) in obj 100x; **B.** Sickle cell disease SC: polychromatophilia, target cell (pink arrow), sickle cell (orange arrow) and other poicilocytes in obj 100x; **C.** Sickle cell disease SC: anisochromia, target cells (pink arrow), elliptocyte (purple arrow) and other poicilocytes in obj 40x; **D.** S/β-thalassemia: anisocytosis, spherocytes (green arrow) and other poicilocytes in obj 100x.

As for the only individual diagnosed with HS, spherocytes were observed in his microscopy, in addition to polychromatophilia and anisocytosis (Figure 3).

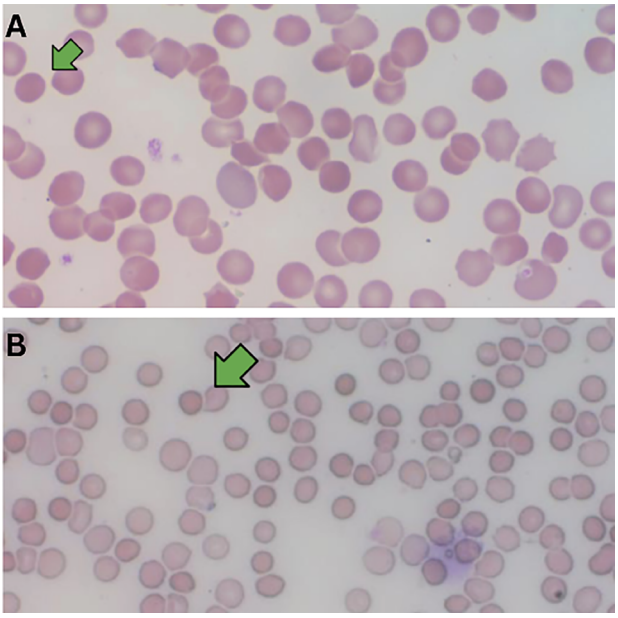

Figure 3. A-B: Peripheral blood smear from a patient diagnosed with hereditary spherocytosis, showing the presence of polychromatophilia, anisocytosis and spherocytes (green arrow) in obj 100x.

Agglutinated red blood cells were observed on the peripheral blood smear of the only patient with AIHA by cold antibody, but they were not seen in any of the patients with AIHA by warm antibody. In 75% of the peripheral blood smear of patients with AIHA by warm antibody, spherocytes were observed, and dacryocytes were observed in 25% (Figure 4).

As for the two patients diagnosed with PNH, both presented target cells, spherocytes, and dacryocytes, while only one of them presented microspherocytes and elliptocytes (Figure 5).

In patients who presented some type of intravascular HA, the most prevalent finding was the presence of schistocytes, which was present in the peripheral blood smear of both patients with this diagnosis (Figure 6). Among patients with DAT-negative HA, target cells, acanthocytes, and other poikilocytes were observed in 66.67%. Elliptocytes and spherocytes were seen in the peripheral blood smear of 33.33% of these patients. The presence of a slide with multisegmented neutrophils was also observed, however, the patient had associated anemia due to vitamin B12 deficiency.

Discussion

Although rare, HAs can have significant clinical repercussions, requiring hospitalization, surgery, and transfusion of blood concentrates[2,3]. With multiple causes, etiological diagnosis can be challenging due to the high cost

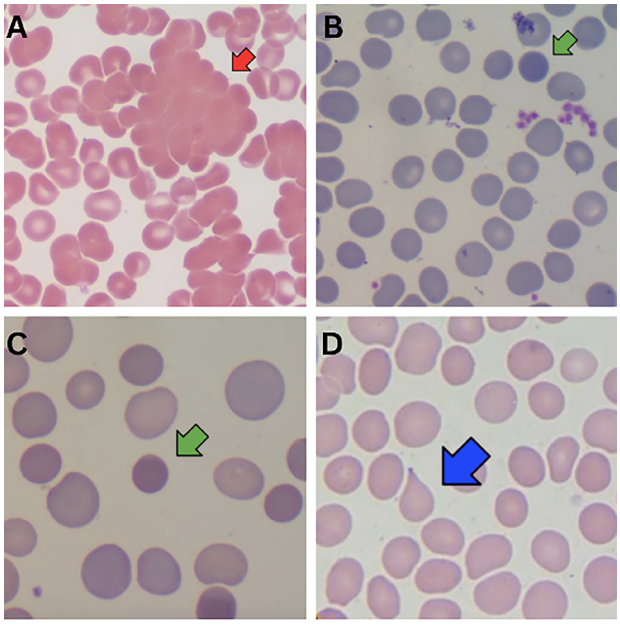

Figure 4. Alterations found in peripheral blood smear of patients diagnosed with autoimmune hemolytic anemia. **A.** Cold antibody autoimmune hemolytic anemia: agglutinated red blood cells (red arrow) at 100x magnification; **B.** Warm antibody autoimmune hemolytic anemia: anisochromia, anisocytosis, and spherocytes (green arrow) at 100x magnification; **C.** Warm antibody autoimmune hemolytic anemia: polychromatophilia, anisochromia, anisocytosis, and spherocytes (green arrow) at 100x magnification; **D.** Warm antibody autoimmune hemolytic anemia: anisocytosis, anisochromia, and dacryocytes (blue arrow) at 100x magnification.

and limited availability of specific methods, especially in rare or difficult-to-identify cases[1,2,5,6]. These characteristics directly impact public health service resources, requiring attention from health professionals and managers to optimize diagnosis, treatment, and efficient use of available resources[5,6].

Given the limited access to some diagnostic methods, it is essential to know how to use all available tools[5,6]. The peripheral blood smear test stands out due to its low cost and relative availability[1,2]. In addition, certain morphological abnormalities of red blood cells can only be detected through peripheral blood smear analysis[2,5-7]. Although some alterations may be nonspecific, others are of great value in the etiological identification of HA, guiding the investigation and often sufficient to identify the cause of hemolysis[2,6].

Among the many causes of HA, thalassemias affect approximately 1.5% of the global population, with no gender preference[9]. They result from quantitative alterations in the globin chains and are classified according to the polypeptide chain affected (alpha or beta)[2,4,9,10]. The α-thalassemia syndromes present four clinical forms and may be completely asymptomatic, or even incompatible with life, depending on the number of genes involved in the production of the α chain: α-thalassemia minima (one gene affected), α-thalassemia minor or α-thalassemia trait (two genes affected), α-thalassemia intermedia or hemoglobin

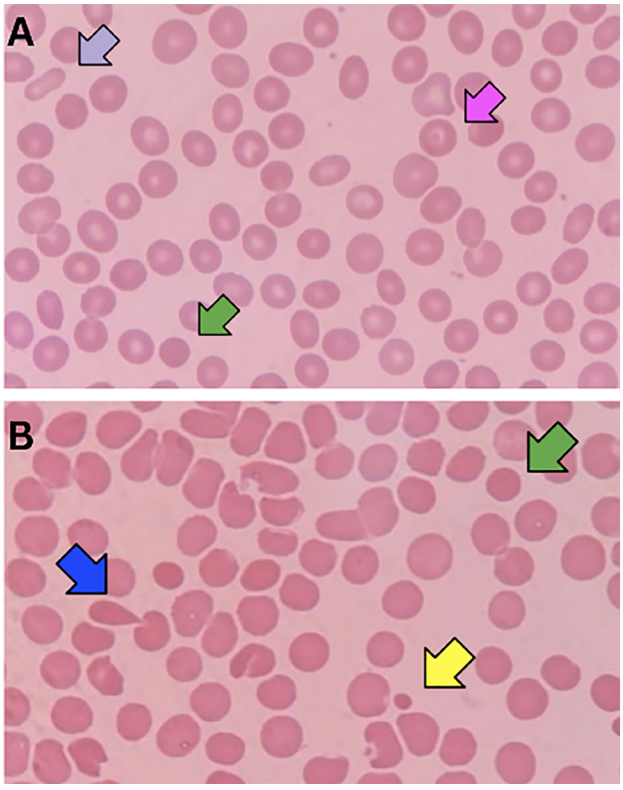

Figure 5. A e **B.** Changes found in peripheral blood smear of patients diagnosed with paroxysmal nocturnal hemoglobinuria: anisocytosis, elliptocyte (purple arrow), target cell (pink arrow), spherocyte (green arrow), microspherocyte (yellow arrow), dacryocyte (blue arrow) and other poicilocytes in obj 100x.

H disease (three genes affected) and α-thalassemia major (four genes affected)[2,10]. The α-thalassemia minor and hemoglobin H disease are the only forms responsible for HA during adulthood[2]. The β-thalassemia syndromes also present distinct clinical forms, which can be classified as: β-thalassemia major, intermedia, and minor (β-thalassemia trait)[10].

The diagnosis of thalassemias can be challenging, especially because hemoglobin electrophoresis may show normal results and genetic testing is not widely available[2]. Peripheral blood smear plays a fundamental role in this context and may show varying degrees of microcytosis, hypochromia, anisocytosis, polychromatophilia, and significant poikilocytosis. Red blood cells of various shapes, such as target cells, dacryocytes, elliptocytes, and schistocytes, may be found[7]. In addition, coarse basophilic stippling, Heinz bodies, Howell–Jolly bodies, and hemoglobin H inclusion bodies are present in α-thalassemia intermedia[2,7]. In this study, the search for bodies was limited by the staining technique used. However, target cells were observed in all patients with thalassemia in this study, and elliptocytes and dacryocytes in 75% and 50%, respectively, of β-thalassemia cases. Furthermore, there was no difference in prevalence between genders, corroborating data from previous literature. It is important to emphasize that even a probable diagnosis of thalassemia by peripheral blood

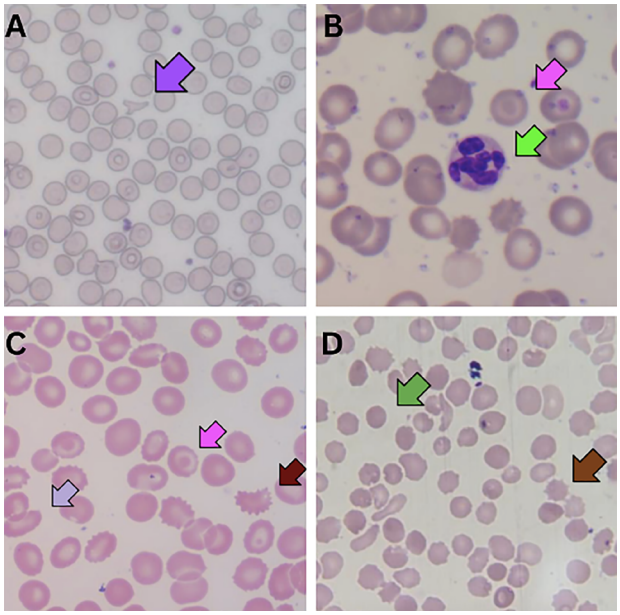

Figure 6. Changes found in peripheral blood smear of patients diagnosed with mechanical intravascular hemolytic anemia and TDA-negative hemolytic anemia. **A.** Mechanical intravascular hemolytic anemia due to mitral valve prosthesis: schistocytes (purple arrow) in obj 100x; **B.** TAD-negative hemolytic anemia associated with B12 deficiency: target cells (pink arrow) and multisegmented neutrophil (light green arrow) in obj 100x; **C.** TAD-negative hemolytic anemia: anisocytosis, anisochromia, target cells (pink arrow), elliptocytes (purple arrow), acanthocytes (brown arrow) and other poicilocytes in obj 100x; **D.** TAD-negative hemolytic anemia: anisocytosis, anisochromia, spherocytes (green arrow), acanthocytes (brown arrow) and other poicilocytes in obj 100x.

smears should still be confirmed by high-performance liquid chromatography (HPLC) of hemoglobin (Hb), molecular testing, or some other reliable method[7].

The presence of echinocytes was also observed in 50% of the peripheral blood smears. Echinocytes are erythrocytes that have several surface projections, are regularly distributed, and are often a technical artifact, especially when the blood sample is stored in ethylenediaminetetraacetic acid (EDTA) for prolonged periods, or when there is excess EDTA, as well as excessively acidic or basic dyes[7].

In Brazil, SCD is one of the most common hereditary diseases, whose incidence and prevalence vary substantially between regions in Brazil, as does access to health care[11]. In this study, SCDs accounted for the majority of cases of hemolytic anemia, representing 29.17% of cases. Among the types of FD, the most common is SS[12], which is different from the data observed in this study. The slight predominance of female patients is described in the literature, but in the results of this study, a much higher proportion than that described was observed[11-13]. This may have occurred due to the small sample size, as this hospital is not a referral center for these diseases, and there are other nearby centers.

A typical peripheral blood smear in SCD reveals anisocytosis, anisochromasia, sickle cells (sickle red

blood cells), boat-shaped red blood cells, target cells, polychromatophilia, basophilic stippling, erythroblasts and spherocytes[2,6,7]. In addition, signs of hyposplenism, such as Howell-Jolly bodies, Pappenheimer bodies, and acanthocytes[2,6,7], may be detected. The diagnosis is confirmed by hemoglobin electrophoresis, which shows hemoglobin S in a concentration greater than 50%, or by Hb HPLC, considered the preferred method because it allows the differentiation between Hb SS and heterozygous forms[2,7], however, it is available in only a few reference services in the SUS.

In S/β-thalassemia, there are fewer sickle cells, a greater number of target cells, microcytosis, hypochromia and Pappenheimer bodies[2,6,7]. On the other hand, SC hemoglobinopathy presents fewer sickle cells, less polychromatophilia, a scarcity of circulating erythroblasts and reduced or absent signs of hyposplenism, due to the late development of this condition in these patients[2,6,7]. The findings of this study reinforce the characteristics described in the literature. Target cells were identified in all patients with sickle cell anemia. Drepanocytes were observed in all cases of SCD SS, in 50% of cases of SC hemoglobinopathy, and in no case of S/β-thalassemia.

HS is characterized by a defective erythrocyte cytoskeleton that leads to deformation of red blood cells as they pass through the splenic circulation[14]. The diagnosis is based on clinical and family history, negative DAT and the presence of spherocytes in the peripheral blood smear[2,7]. In typical cases, additional tests are not necessary[2]. In the peripheral blood smear, reticulocytosis, polychromatophilia and anisocytosis[2-4,15] can also be observed, as evidenced in the only case described in this study. Methods such as the osmotic fragility test, the eosin-5'-maleimide (EMA) binding test using flow cytometry, and ektacytometry can confirm the diagnosis[2,4]. However, the osmotic fragility test may present normal results or results altered by other membranopathies, making identification difficult[2,4]. Ektacytometry has limited availability in Brazil, and the EMA test, although useful, is expensive and unavailable in the SUS[2]. Although this study addressed only HS, other membranopathies present specific peripheral blood smear findings, such as elliptocytes in elliptocytosis, ovalocytes in ovalocytosis and stomatocytes in stomatocytosis, reinforcing the importance of the peripheral blood smear in the diagnostic investigation of HAs[1,2].

AIHAs are acquired disorders characterized by the presence of autoantibodies that attack the erythrocyte membrane, resulting in hemolysis[1,15]. They can be primary or secondary to other diseases and are classified into two types: AIHA by warm antibodies, which act at temperatures above 37 °C (Celsius), and by cold antibodies, which generally act between 0-4 °C[16]. A slight predilection for females is described in the literature, at least in part due to the association with autoimmune diseases[13]. This is in line with the data from this study, in which the only case in this study with this diagnosis was female. AIHA due to warm antibodies accounts for approximately 75% of all AIHAs in adults[15] according to data found in this study. The diagnosis is based mainly on the detection of autoantibodies and/or complement components on the surface of red blood cells, through the DAT, which detects autoantibodies or complement fractions adhered to red blood cells, indicating hemolytic anemias of immune origin when positive[1,2], in addition to the exclusion of other causes of hemolytic anemia, in which the peripheral blood smear can be an important resource[2]. The DAT is a very sensitive test, but its interpretation must take into account the context and the findings on the blood smear[2].

The peripheral blood smear examination usually reveals varying degrees of anisocytosis, polychromatophilia and spherocytes (present in approximately 40% of cases), with few or no schistocytes[2,13]. In AIHA caused by cold antibodies, red blood cell agglutination is a common feature on the peripheral blood smear, in addition to polychromasia, anisocytosis, poikilocytosis, spherocytes and erythrophagocytosis[2,7,13]. In this study, 100% of patients with AIHA caused by cold antibodies presented agglutination, while this alteration was not observed in patients with AIHA caused by warm antibodies. Spherocytes were detected in 75% of cases of AIHA caused by warm antibodies. Nonspecific alterations, such as anisocytosis, anisochromia and polychromatophilia, were also identified. In addition, peripheral blood smears can help identify secondary causes through alterations in other hematologic lineages[13].

AIHAs can also be presented with a negative DAT[2,13]. Patients with DAT-negative AIHA may present with IgA or IgM autoantibodies that are not routinely detected, requiring a specific DAT with anti-IgA and/or anti-IgM[2,13]. DAT may also be falsely negative when there is a very low number of antibodies bound to the surface of red blood cells or when the antibody affinity is low[2,13]. The peripheral blood smear is a critical step in the evaluation of DAT-negative HAs[3]. Although rare, the diagnosis of DAT-negative AIHA should be considered only after other causes have been excluded[2]. In addition, patients without access to specific tests or without conclusive results are grouped as cases of DAT-negative HA, which reflects the diagnostic difficulties due to the high cost and low availability of specific methods, especially in rare or difficult-to-identify cases[5,6].

MHAs are forms of intravascular hemolytic anemia caused by shearing and fragmentation of erythrocytes as they pass through turbulent flows in prosthetic or defective valves, or due to microvascular damage[1,7]. These conditions may occur after mitral valve repair or in cases of native valve disease[2,7]. The diagnosis of MHA, in particular, is clinically urgent, as patients can progress rapidly[2,6]. The presence of fragmented erythrocytes (schistocytes) on microscopy is an important indicator for diagnosis[6]. In addition to schistocytes, microspherocytes, anisocytosis, poikilocytosis, and polychromasia may be observed[6]. In this study, schistocytes were identified in two patients with intravascular hemolytic anemia, one case associated with a prosthetic valve and the other with MHA.

PNH is a rare clonal disorder of hematopoietic stem cells caused by an acquired somatic mutation in the PIGA gene on the X chromosome, which leads to a deficiency of glycosylphosphatidylinositol (GPI) and its anchored proteins, and can affect not only erythrocytes, but also granulocytes, monocytes, lymphocytes, and platelets[2]. On peripheral blood smears, patients with PNH present only nonspecific findings[2,4]. A study that described the peripheral blood smear of 20 patients with PNH showed that the main alteration was the presence of elliptocytes (50%) followed by dacryocytes (25%) or teardrop-shaped cells, which are frequently associated with bone marrow failure, target cells (20%), basophilic stippling (15%), schistocytes (10%), frequently associated with thrombotic events, and spherocytes (5%)[17]. In our study, nonspecific alterations such as anisocytosis and polychromatophilia were observed in 100% and 50% of the individuals, respectively. In addition, the presence of spherocytes, dacryocytes, and codocytes was noted in both patients, and microspherocytes and elliptocytes were noted in one of them. The confirmatory diagnosis is made by flow cytometry, which detects the absence of surface proteins anchored to blood cells, which became available within the SUS for PNH only in 2019[18].

The limitations of this study include the small sample of patients, since the hospital is not a reference center for these diseases, with other centers nearby. In addition, it is impossible to investigate corpuscles due to the staining technique used.

Conclusion

The alterations identified in the peripheral blood smears were compatible with the etiological diagnosis of the patients. There was a predominance of cases in women, and SCDs were the most prevalent HAs, in agreement with Brazilian literature. However, hemoglobinopathy SC was the most common form of SCD, and the predominance of females in SCDs was higher than that reported in the literature, possibly due to the small sample size.

The data from this study highlight the importance of the peripheral blood smear, a low-cost and relatively available test, to identify unique or unusual morphological findings, combined with blood count data and clinical characteristics, in the diagnosis of HAs and in the efficient and economical direction of additional laboratory tests.

Authors' Contributions:

We describe contributions to the papers using the taxanomy (CRediT) provided above:

Conceptualization, Investigation, Methodology, Visualization & Writing - original draft: FOC Souza; YN Setti; ICR Diogo; JRS Evangelista. *Project administration, Supervision & Writing - review & editing*: MC de Magalhães; VRGA Valviesse. *Validation & Software*: EB Riscarolli. *Data curation & Formal Analysis*: KG Frigotto.

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