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# IMPORTANCE OF PHENOTYPING IN BLOOD DONORS FOR THE IDENTIFICATION OF RARE PHENOTYPES

## *IMPORTÂNCIA DA FENOTIPAGEM EM DOADORES DE SANGUE PARA A IDENTIFICAÇÃO DE FENÓTIPOS RAROS*

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**ABSTRACT**

**Introduction:** Erythrocyte alloimmunization may arise from blood group incompatibility between ethnically diverse donors and recipients. Providing blood to patients with rare or complex red cell antigen profiles poses a significant challenge. These individuals may have red blood cells that either lack high incidence antigens or possess a combination of several uncommon antigens. As a result, finding compatible blood donors becomes difficult. **Methods:** The objective was to identify extended phenotyping strategies for the various blood group systems to aid transfusion in patients with a phenotype considered rare. This is a systematic review, in which parameters were followed as a basis for the PRISMA recommendations with a focus on the search for the literature published in the last 10 years on erythrocyte phenotyping in blood donors, aiming to identify rare phenotypes for transfusion support. **Results:** We found 20 articles describing rare antigens and antibodies, reporting difficulties in finding compatible blood and the need for a registry of these blood donors. In summary, the provision of blood to patients with red cells lacking high incidence antigens or complex antigen profiles is indeed a challenging task. It necessitates specialized testing, access to rare blood donors, and alternative strategies to ensure the best possible match for transfusion.

**Keywords:** Alloimmunization, Immunohematology, Blood Group System, Hemotherapy.

***RESUMO***

***Introdução:*** *A aloimunização eritrocitária pode surgir da incompatibilidade de grupos sanguíneos entre doadores e receptores etnicamente diversos. Fornecer sangue a pacientes com perfis de antígenos eritrocitários raros ou complexos representa um desafio significativo. Esses indivíduos podem ter glóbulos vermelhos que não possuem antígenos de alta incidência ou possuem uma combinação de vários antígenos incomuns. Como resultado, torna-se difícil encontrar doadores de sangue compatíveis.* ***Métodos:*** *O objetivo foi identificar estratégias ampliadas de fenotipagem para os diversos sistemas de grupos sanguíneos para auxiliar a transfusão em pacientes com fenótipo considerado raro. Trata-se de uma revisão sistemática, na qual foram seguidos parâmetros como base para as recomendações PRISMA com foco na busca da literatura publicada nos últimos 10 anos sobre fenotipagem eritrocitária em doadores de sangue, visando identificar fenótipos raros para suporte transfusional.* ***Resultados:*** *Foram encontrados 20 artigos descrevendo antígenos e anticorpos raros, relatando dificuldades em encontrar sangue compatível e necessidade de registro desses doadores de sangue. Em resumo, o fornecimento de sangue a pacientes com glóbulos vermelhos*

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*sem antígenos de alta incidência ou perfis antigênicos complexos é de fato uma tarefa desafiadora. São necessários testes especializados, acesso a dadores de sangue raros e estratégias alternativas para garantir a melhor correspondência possível para a transfusão.*

***Palavras-chave:*** *Aloimunização, Imunohematologia, Sistemas de Grupos Sanguíneos, Hemoterapia.*

## INTRODUCTION

Although constantly updated, currently, 354 erythrocyte antigens are grouped into 44 blood group systems according to the International Society of Blood Transfusion (GASSNER *et al*., 2022). These antigens induce the formation of red cell alloantibodies that are involved in blood incompatibilities, leading to immediate and delayed hemolytic transfusion reactions and hemolytic disease of the fetus and newborn (MACHADO *et al*., 2018).

The definition of rare blood or rare donor is not well elucidated yet. In most countries, a blood donor or a patient is considered to have a rare blood type when the phenotype frequency in a given population is less than one per thousand individuals (<1:1000) (ARAÚJO *et al*., 2020). Rare phenotypes are characterized by the absence of a high-frequency antigen and/or the absence of expression of multiple antigens from the same system or other blood group systems. Patients with rare blood groups or phenotypes are usually identified after the formation of alloantibodies (HUSTINS, 2014; MUNIZ *et al*., 2020).

Currently, the number of patients requiring regular and chronic care related to red blood cell transfusion is increasing, especially in patients with sickle cell anemia (SCA), beta-thalassemia major, other hemoglobinopathies, myelodysplastic syndrome (MDS), and moderately severe hypoplastic anemia (SHAH *et al*., 2018; YOUNESI *et al*., 2016). Long-term transfusion poses a series of complications and clinical problems, including transfusion-related adverse reactions, such as the risk of transfusion-transmitted infections (Hepatitis B, Hepatitis C, HIV, among others), non-hemolytic febrile transfusion reactions (NHTR), allergic reactions, and iron overload as a result of repeated transfusions, especially in thalassemia patients (FURUSETH *et al*., 2021; HINDAWI *et al*., 2020).

The frequencies of different blood group antigens vary among distinct ethnic populations. Some of these antigens are highly immunogenic and, therefore, increase the risk of alloimmunization in blood recipients (SHASTRY *et al*., 2022). The frequency of alloimmunization depends on the homogeneity of the donor-recipient population, the coordination process of red blood cell phenotypes, and the patient's age at the start of transfusion, with clinically more significant alloantibodies being directed against antigens of the Rh, Kell, Kidd, and Duffy systems (MAKAROVSKA-BOJADZIEVA; VELKOVA; BLAGOEVSKA, 2017; ROSTAMIAN *et al*., 2022).

Various antigens corresponding to blood group systems are present on the surface of erythrocytes. Identification is carried out through an immunohematological technique, erythrocyte phenotyping, an essential and feasible tool for classifying blood groups in donor and recipient blood samples. Through the interaction between antigen-antibody, agglutination between red blood cells and specific antisera can be visualized, making it an important technique for transfusion reaction prophylaxis (LIMA *et al.*, 2022).

The current technical regulations for blood transfusion procedures recommend extended phenotyping for RhD and Kell system antigens in blood donors, and for other blood group systems such as Duffy, Kidd, and MNS in alloimmunized patients who are or may enter a recurrent transfusion scheme (BRAZIL; MINISTRY OF HEALTH, 2016).

Erythrocyte phenotyping is routinely used to identify red cell antigens and exhibits high specificity and sensitivity. However, certain limitations hinder its application, such as the presence of donor red cells in the recipient's circulation (following a recent blood transfusion), the coating of red cells by alloantibodies or autoantibodies, and the absence of commercial antisera (LANGER *et al.*, 2019). In these situations, genotyping is observed as an important tool for characterizing the genes encoding red cell antigens and their variants, enabling the inference of phenotypes with a high degree of precision (QUIRINO *et al.*, 2019).

The development of alloimmunization, an immune response after exposure to foreign antigens from genetically different cells, leads to the production of irregular antibodies. The presence of alloantibodies is clinically significant in future transfusions, as it can result in hemolytic transfusion reactions (acute or delayed) and may complicate the supply of compatible blood units (PEREIRA BUENO *et al.*, 2021). Polytransfused patients face an increased risk of producing alloantibodies due to the immune system's exposure to a multitude of foreign antigens, inducing an immediate immune response, especially after the second exposure. This results in various clinical consequences, depending on the specific blood cells and antigens involved (VALLE NETO *et al.*, 2018).

To prevent alloimmunization, extended phenotyping for major blood group systems has been recommended, especially when transfusion is necessary for patients with sickle cell anemia (SCA). This ensures that only phenotypically compatible blood is administered to this population (VIZZONI; MOREIRA, 2017).

Thus, the primary objective of this study is to identify, through a systematic literature review, expanded phenotyping strategies for considered rare phenotypes and methods for searching for compatible blood.

## METHODOLOGY

This is a systematic review, and the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were used as parameters for the study's development (PAGE *et al*., 2021).

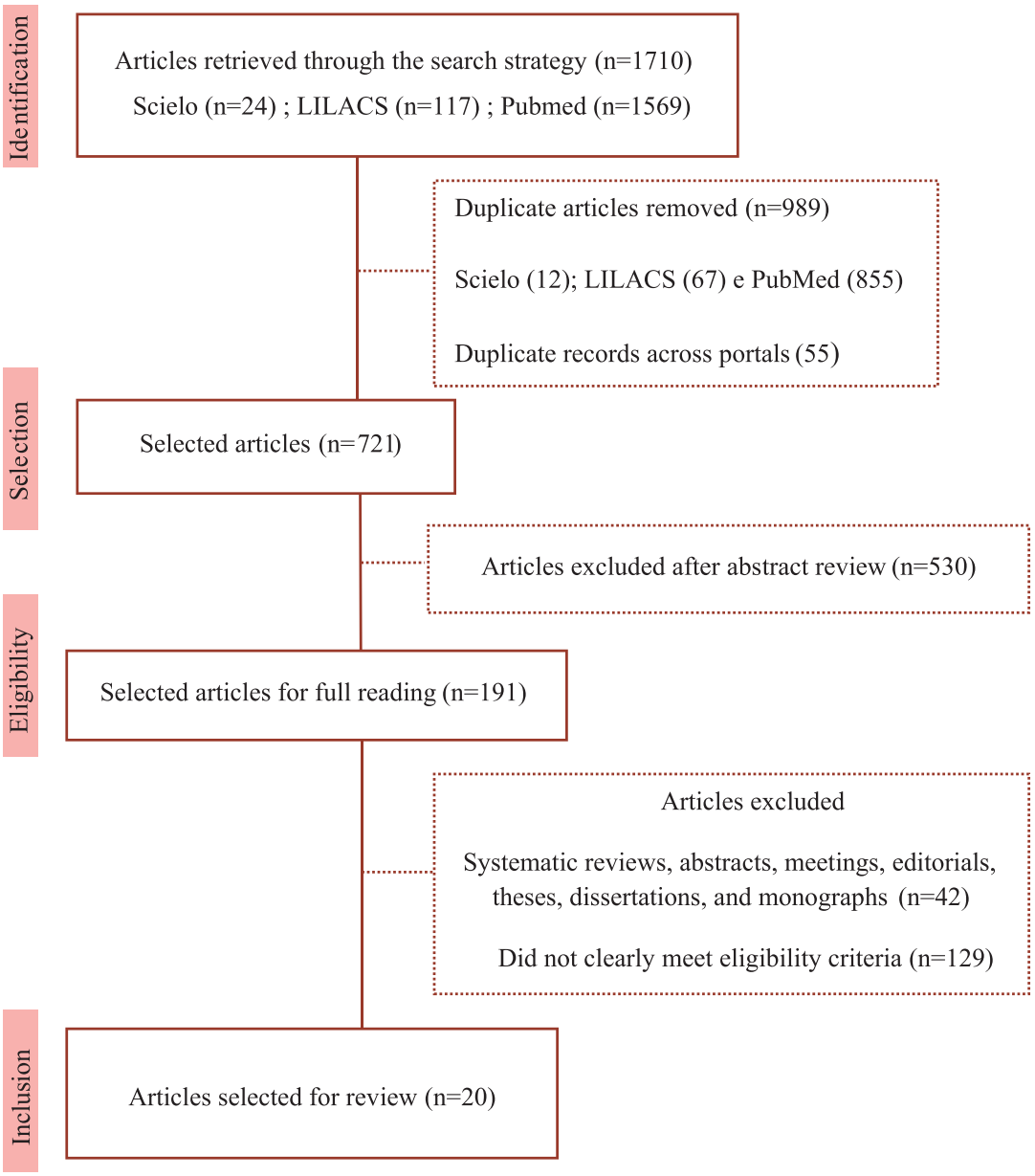
The first step involved locating articles in publications available on the databases Scielo, LILACS, and PubMed. The following inclusion criteria were established: a time span between 2013 and 2023, articles in Portuguese, English, or Spanish, and freely available online. The search commenced in January 2023 and concluded on February 24, 2023, using coordinated indexing through the use of the keywords "alloimmunization," "blood groups," and "rare phenotype," either separately or in combination, with Boolean operators AND and OR.

Following the search phase, the selection of identified materials was conducted through an exploratory and selective reading of titles and abstracts by two independent evaluators. These evaluators decided which articles to include based on predefined criteria. The eligible articles were selected to constitute the sample for the next stage, with any disagreements among researchers resolved through consensus.

In the third stage, the eligible articles underwent a process of recognition, eligibility, and sorting. They were chosen for a thorough and in-depth reading, followed by a refinement in the selection. Exclusion criteria included systematic reviews, studies not in the predetermined languages, those published before the established period, and those providing only the abstract for free in the searched databases.

The search results of this review are presented in a flowchart detailing each stage of the review process and providing the obtained results (Figure 1).

**Figure 1 -** Flowchart used for the systematic review according to pre-established criteria.




Source: Developed by the authors.

## RESULTS

The initial search using predefined descriptors in the databases yielded 1710 records. Among these records, 24 (1.4%) were found in the Scielo portal, 117 (6.8%) in LILACS, and 1569 (91.8%) in PubMed. After analysis, 989 duplicates were identified, with 855 duplicates within the PubMed searches, 12 in the Scielo database, 67 in LILACS, and 55 across different portals. These duplicates were excluded, resulting in a sample of 721 remaining articles for analysis.

Following the selective reading of titles and abstracts, 191 articles were considered eligible, while others were excluded for not aligning with the theme and objectives of this review. During the full reading phase, exclusion criteria were applied, leading to the identification of twenty articles

that addressed rare phenotypes and the alloantibodies produced (Table 1). Additionally, these studies described strategies to identify and locate available blood units worldwide, aiming to meet the health-care needs of patients. Some of these studies conducted extended phenotyping of their blood donors and successfully identified extremely rare phenotypes, contributing to the establishment of rare donor banks in their respective countries.

The majority of studies were case reports (n=12; 60%); however, three cross-sectional studies (15%), four retrospective studies (20%), and one prospective study (5%) were also identified. Regarding the publication period of these studies, the highest number of articles were published in the last five years (n=13; 65%), reflecting the up-to-date nature of the addressed topic.

**Table 1 -** Research on rare blood group antigen phenotyping.

| Authors | Rare Phenotypes and/or Antibodies | Study Methodology | Main Results |
|---|---|---|---|
| Al-Riyami *et al*., 2019. | K-k-;k(a-b-); Lu(a-b-) | Prospective cross-sectional study conducted between January 2015 and December 2016, evaluating the profile of 337 blood donors from Oman, an Arab country on the southeastern coast of the Arabian Peninsula. | Donors with extremely rare phenotypes were identified, potentially forming a stock of frozen rare blood erythrocytes to facilitate the supply of compatible components to alloimmunized patients. |
| Allhoff *et al*., 2021. | Jk(a-b-) | Case report of two patients with anti-Jk3 who were serologically identified through a positive antibody screening and typed as Jk(a-b-). | Due to the rare Jk(a-b-) phenotype, red cell concentrates from homologous donors are scarce worldwide. Therefore, both patients were encouraged to donate blood for cryopreservation, contributing to the establishment of a bank of rare cells. |
| Araújo *et al*., 2020. | Kp(a+b-); Fy(a-b-); Lu(a-b-); (K+ k-) | Cross-sectional study of donors and patients in the northern region of the state of Rio Grande do Sul, Brazil, from November 2011 to December 2018. | During the study period, 17 patients and 33 blood donors with rare phenotypes were identified through extended phenotyping and/or genotyping. |
| Busani *et al*., 2015. | Anti-Tc(a) + antiJk$^a$ | The case report describes the challenges of treating a pregnant woman who had a rare case of critical placenta accreta with concomitant alloantibodies anti-Tc(a) from the Cromer system and anti-Jka. | Pregnancy is undoubtedly a risk for Cromer-sensitized patients and can be fatal when associated with obstetric abnormalities predisposing to massive bleeding. The case provides strategies that can assist clinicians in treating patients with an exceptional risk of bleeding and an inability to receive compatible blood transfusions due to alloimmunization. |
| Caudill *et al*., 2022. | anti-U | Case report featuring a healthy G10P6 woman with a known anti-U who presents for intrauterine transfusion treatment in the second trimester. | Difficulties in obtaining rare blood for the patient due to other concurrent requests involving patients with anti-U in the healthcare unit allowed for a discussion of challenges and alternatives to intrauterine transfusion when rare blood phenotypes are involved. |

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| Derouich *et al*., 2016. | anti-Tja ($PP_1P^k$) | Case report of a 38-year-old Tunisian woman with a history of 11 recurrent spontaneous abortions, all occurring between 10-11 weeks of amenorrhea. | The identification of alloantibodies revealed the presence of anti-Tja (PP1Pk). Family investigation unveiled a single compatible blood donor: compatible with ABO group and Tja negative (Tja- phenotype is extremely rare, 1/100,000 to 1/1,000,000). Maternal anti-PP1Pk then leads to trophoblastic lysis and early termination of pregnancy. |
| Langer *et al*., 2019. | Lu(a+b-) | Cross-sectional study where 251 blood donors in the southwest region of the state of Paraná (Brazil) were phenotyped for the Lua and Lub antigens. Subsequently, genotyping of these donors was conducted, including antigens from the Dombrock system (Doa and Dob). | Blood units from this rare Lu(a+b-) phenotype, constituting 0.004%, could be suitable for patients with the anti-Lub antibody. In Brazil, the Lua/Lub phenotype is not routinely investigated, and the risk of alloimmunization should be considered, as antigen incompatibility in this system can lead to delayed hemolytic reactions. |
| Levitt *et al*., 2018. | anti-$Ge^3$ | Case report of perinatal hemolytic disease due to the maternal phenotype Ge:-2,-3,4 and anti-Ge3 alloimmunization, which was molecularly investigated and successfully managed through maternal blood transfusion. | Emphasis on the importance of early recognition of rare alloantibodies in pregnancy and the need to have a system in place when blood matched with specific antigens is not readily available. Additionally, highlighting the use of molecular diagnostics in rare alloimmunizations. |
| Machado *et al*., 2018. | (K+k-) | Retrospective study covering the period from 2005 to 2015, involving blood phenotyping for the Kell, Duffy, and Kidd systems of 1,759 blood donors from the Apucarana Blood Center. | Three donors with the rare K+k- phenotype (0.17%) were identified. The importance of implementing extended blood donor phenotyping is emphasized, aiming to identify rare phenotypes that should be confirmed through molecular methods. |
| Mani *et al*., 2021. | anti-Rh17 ($Hr_o$) | Case report of a woman during her second pregnancy who gave birth to a child with severe Rh hemolytic disease of the newborn (DHPN). Detailed serological analysis revealed that the mother has the rare Rh phenotype D--/D-- and none of the CcEe antigens were present on the red blood cell membrane. | Rare phenotypes like D--/D--, especially if the patient is alloimmunized, are a significant concern during periods of transfusion demands. The proper identification of these individuals is crucial not only to contribute to a rare donor pool but also for the appropriate management of patients' blood. Effective collaboration through a rare donor registry should be established nationally and internationally, aiming to mobilize rare blood units worldwide when needed. |
| Moise *et al*., 2017. | anti-Vel | Case report of a 31-year-old patient, G2P1, who gave birth to a newborn with Hemolytic Disease of the Newborn due to anti-Vel antibodies. | The high prevalence of the Vel antigen makes it extremely challenging to find Vel- donors. The patient was evaluated before pregnancy, and no siblings or family members were identified as potential donors. A search conducted by the regional blood center found only one eligible donor. The patient was instructed to undergo autologous donations early in pregnancy and was able to donate 4 units. The neonatal course was benign, requiring only phototherapy treatment for 3 days. The newborn was discharged on the 7th day of life. |

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| Pytel *et al.*, 2020. | Fy(a-b-) + S-s-U-; Lu (a+b-); K+k-; RH:-18 (Hr); RH: 32,-46 (Sec); Fy(a-b-) + anti-e | Retrospective study of records of pregnant patients referred for rare blood group screening and alloimmunization during the period from 2010 to 2018 at Beaujon Hospital, France. | Rare blood types were diagnosed in the advanced stages of pregnancy, most often due to a lack of obstetric consultations. None of the patients reported a previous transfusion, but 38.5% had a gynecological history of early pregnancy. The management of rare blood groups in obstetrics requires excellent interdisciplinary coordination. |
| Ristovska *et al.*, 2022. | (K+k-); RH:-1,2,-3,-4,5 | Retrospective study of 75,528 blood donors registered during the period from 2016 to 2021. Frequencies of ABO, Rh (D, C, E, c, e), and Kell (K, k) system antigens were analyzed using data available in the donor computerized system. | The K+k- phenotype is considered rare, with a frequency of < 1%. In the studied population, the presence of this phenotype is 0.06%. There are 21 regular blood donors typed as having the K+k- phenotype across all ABO blood groups in the donor registry. The rare ddCcEe phenotype was also identified in 0.007% of donors. |
| Rodrigues *et al.*, 2022. | anti-K11 + anti-Jk$^{a}$ | Case report of a 39-year-old woman with multiple comorbidities, including amputation of the lower right extremity below the knee, who developed aggressive osteomyelitis associated with continuous bleeding, leading to anemia. To address these issues, limb amputation was necessary. The patient exhibited anti-K11 and anti-Jka antibodies. | he presence of anti-K11, an extremely rare antibody, along with anti-Jka, posed a significant challenge as no compatible blood units were available. Transfusion of incompatible units with crossmatching was deemed a high-risk proposition. Following an unsuccessful search in the U.S. rare blood program, two units of irradiated red blood cells, group O, K0 (Kell null), Jk(a–), were identified in Japan and considered compatible with the patient's plasma. These units were successfully acquired and infused without evidence of adverse reactions. The patient subsequently underwent amputation. |
| Romphruk *et al.*, 2019c, E, e. | Jk(a-b-) | Retrospective study examining red blood cell phenotypes in 13,597 Thai voluntary blood donors from 2013 to 2017. | The Jk(a-b-) phenotype was significantly less frequent (0.07%) compared to Chinese individuals in Malaysia (3.50%), mainland China (0.50%), and North Indian individuals (0.39%). Individuals with the Jk(a-b-) phenotype may develop a rare anti-Jk3 antibody, likely leading to difficulties in finding compatible blood. |
| Santos *et al.*, 2019. | anti-Holley (Hy) | Case report of a 46-year-old female patient diagnosed with hemoglobinopathy, who experienced a symptomatic drop in hemoglobin levels (5.3 g/dL) after blood transfusion, suggestive of a transfusion reaction. The patient's blood type was O+. Irregular antibody screening was positive, showing panreactivity against all tested red blood cells but not reactive to dithiothreitol. | Holley is a high-prevalence antigen in the Dombrock blood system, and its negative phenotype is extremely rare in all populations, being associated with hemolytic transfusion reactions. This antibody is challenging to identify, as laboratories need expertise in resolving complex cases, a large inventory of rare sera and red blood cells, along with other tools such as enzymes, thiol reagents, and molecular tests. |

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| Setya *et al.*, 2020. | Bombay(Oh); D--; (K+k-) | A cross-sectional observational study was conducted in the Department of Transfusion Medicine at a large tertiary hospital in India over 20 months, from October 2016 to May 2018. The study population included 6,678 healthy blood donors of both genders who voluntarily donated and agreed to participate in the study. | Four rare donors with the following phenotypes were identified: 1 D- - donor (0.01%), 2 Bombay donors; Oh (0.03%), and 1 K+k- donor (0.02%). The outcome is an enhanced patient safety, which is the primary motto of any healthcare institution. The study can contribute to increasing the confidence of blood banks in finding appropriate units for patients with unexpected antibodies or rare phenotypes. |
| Shahverdi *et al.*, 2019. | Rh Null (RH29) | Case report of a 43-year-old woman with severe anemia after splenectomy, classified as A RhD negative. A blood sample was sent to the Reference Laboratory in Immunohematology of the Iranian Blood Transfusion Organization, Tehran, for ABO/Rh(D) typing. | In this study, the patient's life was saved by compatible Rh null blood donated by her brother. After consulting with her brother, he consented to being added to the national rare donor registry database in Iran. The Rh null blood type is a rare blood group worldwide, and consanguineous marriage plays a significant role in the development of this blood type. Due to the prevalence of consanguineous marriage in Iran, it may be more common in Iran compared to other regions of the world. |
| Win *et al.*, 2018. | anti-E + anti-N + anti-U; anti-$Jr^a$ | Case report of two individuals: a 25-year-old female patient with hemoglobinopathy and antibodies anti-E + anti-N + anti-U, and a 54-year-old male patient, a victim of a traffic accident, with anti-Jra antibodies. Both received transfusions of incompatible red blood cells, along with intravenous immunoglobulin (IVIG) units. | U- donors, the majority of whom are of African Black descent, are registered in the international rare donor panel, and U- units are rare with limited availability in the UK. Blood with the E-, N-, U- phenotype is extremely rare, with 0% from white blood donors and 0.4% from donors typed as M+N-s-S-U-. In the UK, only one donor is compatible with the first patient. Jra is the sole antigen in the JR blood group system. While the frequency of Jra is over 99% in all populations, the Jr(a-) phenotype has been reported as the highest in the Japanese population. |
| Yousuf *et al.*, 2014. | anti-$Jk^3$ | Case report of a 47-year-old Malaysian woman, P4G1, with a known medical history of hypertension for 10 years, presented in the Emergency Department with severe symptomatic anemia secondary to menorrhagia caused by uterine fibroids. | The antibody reacted equally with cells from the Jk(a+b-), (a-b+), and (a+b+) panels. However, anti-Jk3 was suspected and confirmed by the patient's phenotype as Jk(a-b-). Subsequently, the patient's sample was sent to the Reference Laboratory, and the antibody was confirmed as anti-Jk3. As Jk(a-b-) blood is very rare, the patient's sample was sent to the Reference Laboratory to request two units of Jk(a-b-) cells. The procedure was successfully carried out, and the patient was transfused with the 2 units of compatible Jk(a-b-) blood. The postoperative recovery was irregular, but the patient was discharged on the eighth day after the operation. |

Source: Developed by the authors.

## DISCUSSION

A rare blood donor is defined as one found with an incidence of 1:1000. Donors without a high-incidence antigen in red blood cells in a specific population or those lacking multiple common antigens are also considered rare donors (RATURI *et al.*, 2023). Recognizing that a patient needs rare blood is often the initial factor for a series of events that can extend beyond the local blood supplier and involve national or international searches for suitable rare blood. Over the years, a network of national and international rare donor panels and banks of cryopreserved red blood cell units has been established to facilitate the supply of compatible blood for these patients (NANCE *et al.*, 2016).

It has been a challenge for blood centers in the country to identify and confirm rare phenotypes in the donor and patient populations, especially in regions distant from the capitals, where molecular methods are not widely available (ARAÚJO *et al.*, 2020). Extended phenotyping, currently economically viable for erythrocyte alloimmunization prophylaxis, proves to be more cost-effective compared to molecular methodologies using PCR-RFLP (QUIRINO *et al.*, 2019).

The risk of alloimmunization is high in patients receiving multiple transfusions, such as sickle cell anemia patients (CAMPBELL-LEE *et al.*, 2018; LINDER; CHOU, 2021; VIZZONI; MOREIRA, 2017), severe thalassemia, aplastic anemia, hematologic malignancies (TARIQ *et al.*, 2022), chronic renal failure, and cancer patients undergoing chemotherapy (MANGWANA; KACKER; SIMON, 2019).

The current scenario in the public blood services in Brazil demonstrates an increase in phenotyping for blood groups with higher transfusional importance. However, few scientific data are disclosed in the literature, making the topic less attractive to young researchers and allowing for new population-based studies (LIMA *et al.*, 2022).

Araújo and colleagues (2020) demonstrated that some rare blood types found in the southern region of Brazil correspond more to those found in European populations than in other states and regions of Brazil, where the mixing of Europeans, Africans, and Indigenous people is higher. This study highlighted the complexity of transfusion support in patients with rare phenotypes and emphasized the importance of continuous investment in phenotyping and genotyping in various regions of Brazil. Through this investment, accurate information about the antigens present in blood donors' red blood cells can be obtained, allowing better compatibility with recipients with rare phenotypes. This enhanced approach to blood supply contributes to ensuring safe and effective transfusion, avoiding complications, and ensuring proper treatment for patients with specific transfusion needs.

In a population with diverse ethnicities like Brazil, blood group antigens vary significantly in their frequency. Genotyping studies in this population have shown that blood group polymorphisms differ significantly from other populations (CALDAS *et al.*, 2022; CASTILHO, 2016a), and studies within the country have also shown a heterogeneous distribution of blood group alleles and variants among people from different regions (ARNONI *et al.*, 2015).

The implementation of automation in the extended erythrocyte phenotyping methodology for blood donor samples has shown a significant increase in the number of processed samples compared to exclusively manual methods. This automation enables the detection of donors with rare phenotypes in the population much more broadly. Moreover, this approach considerably increases the availability of compatible red blood cell units for these rare phenotypes, facilitating transfusions for patients with specific needs. This improvement in the detection and supply of phenotype-compatible blood bags is crucial for ensuring safe and effective transfusion, contributing to proper treatment of patients requiring blood transfusions (CARNEIRO; JUNIOR; AMARAL, 2022).

A study on extended phenotyping with 5407 blood donor samples in the Himalayan region of Uttarakhand, India, managed to identify rare phenotypes (Jka-Jkb-; S-s-, Oh) among the local population, creating a registry of rare blood donors. This registry will be of great use for the timely notification of blood donors with rare phenotypes and the use of rare blood in alloimmunized patients, demonstrating that serological methodology (phenotyping) can substantially contribute (RATURI *et al.*, 2023).

To meet the demand for transfusion support for patients, Brazil implemented a national rare blood registry (CNSR) in 2014. This registry centralizes information about blood donors considered rare across the country. Through this initiative, it is possible to manage demands related to rare phenotypes that arise in different regions of Brazil (ARAÚJO *et al.*, 2020; CASTILHO, 2016b).

In the current scenario, the CNSR has a record of more than 8,880 registered rare blood donors in public blood centers in Brazil. It is important to highlight that the Southeast Region of the country has a significant representation in the CNSR, with over 99% of the total donor records. However, other regions of Brazil have minimal representation in the registry. While the concentration of records in the CNSR is predominantly in the Southeast Region, continuous efforts are essential to expand the registry and include donors from all regions of the country. This will ensure greater diversity and availability of rare blood donors nationwide (BRASIL, 2022).

Expanding the registry beyond the Southeast Region is important to meet the specific transfusional demands of patients with rare phenotypes in different parts of Brazil. The inclusion of donors from other regions in the CNSR will allow more equitable access to compatible blood bags, improving transfusion support for patients across the country. Therefore, continuous expansion of the CNSR is essential, encouraging the participation of blood centers from all regions of Brazil to increase the

representativeness and coverage of the registry, aiming to comprehensively address the transfusion needs of patients with rare phenotypes nationwide (BRASIL, 2014; CARNEIRO *et al.*, 2022).

It is true that, despite current regulations for guidance and identification of rare phenotypes in blood donors (BRASIL, 2016), the number of donors is still low compared to emergency demands. Identifying rare phenotypes is not sufficient to fully meet these needs. Therefore, it is crucial for Brazilian hemotherapy to concentrate efforts on promoting extended phenotyping in blood donors, especially in remote regions of the country.

An objective to be achieved is to expand the capacity to perform extended phenotyping in blood donors, even in the most distant locations in Brazil (CASTILHO, 2016b). This involves a concentrated effort to train healthcare professionals and provide the necessary resources for conducting these tests in different blood centers and transfusion services throughout the country. Promoting extended phenotyping in blood donors in geographically isolated areas is essential to identify and include donors with rare phenotypes in the national registry. This will help increase the availability of compatible blood bags and address emergency transfusion needs for patients with specific transfusion needs nationwide (CARNEIRO *et al.*, 2022).

Therefore, a joint effort of regulatory bodies, healthcare institutions, and professionals in the field is needed to promote the expansion of extended phenotyping in blood donors, aiming to address emergency demands and ensure adequate transfusion support in all regions of Brazil.

Finally, the authors acknowledge that the use of molecular biology for confirming phenotypes is necessary. However, as this technology is not available in all blood transfusion services, extended phenotyping should be an initial step for creating a local rare blood database. Institutional partnerships should be established for molecular diagnostic confirmation.

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