

further analysis. Laboratory and clinical findings were also used to evaluate the clinical significance of the alloantibodies produced. **Results:** Partial D phenotypes were found in 25/79 (31.6%) of these patients and included: DAR, DAU0, DAU3, DIVa, DIIa, DVa, Weak partial D 4.0. Eight (32%) patients produced anti-D: 1 DAR, 1 DAU3, 1 DIVa, 1 DIIa, 1 DVa, 3 DAU0. Laboratory and clinical evidence of a delayed hemolytic transfusion or decreased survival of transfused RBCs were associated with five anti-D produced in patients with partial D (DAR, DAU3, DIVa, DIIa, DVa). Anti-D produced by the patients with partial D DAU0 were not clinically significant and patients with weak partial D 4.0 did not develop anti-D even with more exposures of D+ RBC units than those with the other partial D types. Unexpected anti-D was also found in 5/54 (9.3%) patients with conventional D type as a result of D+ RBC transfusions from donors with altered Rh epitopes. Anti-D was more persistent in patients with partial D DAR, DAU3, DIVa, DIIa, DVa compared to those with DAU0 and conventional D. **Conclusions:** Patients with partial D are more likely to develop clinically significant and more persistent anti-D than patients with conventional D and therefore may benefit from prophylactic D- RBC units or RHD genotype-matched transfusions. Our results also suggest that patients with partial D DAU0 do not develop clinically significant anti-D and patients with weak partial D 4.0 are not at risk of developing anti-D.

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EVALUATION OF THE COST AND EFFECT OF PROPHYLACTIC GENOTYPIC MATCHING ON ALLOIMMUNIZATION AND AUTOIMMUNIZATION IN PATIENTS WITH SICKLE CELL DISEASE (SCD)

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Background: Blood transfusion is one of the mainstays of treatment for patients with SCD but despite of the beneficial effects, transfusion therapy can lead to red blood cell (RBC) alloimmunization with serious complications for the patient including life-threatening delayed hemolytic transfusion reactions and the hyperhemolysis syndrome. In addition, the presence of alloantibodies, which is often associated with the concomitant presence of autoantibodies, can lead to difficulty in finding compatible units, which can cause transfusion delays. To reduce alloimmunization some strategies have been implemented to provide antigen matched RBC transfusions to patients with SCD who need chronic transfusion support. In this context, phenotypic and genotypic matching protocols have been used. Based on this, the aim of this study was to evaluate the cost and the effect of prophylactic RBC transfusion genotypic matching on alloimmunization and autoimmunization in patients with SCD. **Methods:** Our study included 79 (33 male and 46 female) patients with SCD, homozygous for HbS, on chronic RBC transfusion therapy receiving prophylactic genotypic matching RBC transfusions in the last

two years. In this period, patients received RBC units genotypic matched for Rh, K, Fya/Fyb, Jka/Jkb, S/s, Doa/Dob and Dia antigens. Molecular matching was performed according to previous molecular typing results performed by the HEA BeadChip (Immucor) in patients and donors. Adsorption onto autologous RBC was also performed to aid the differentiation of autoantibodies from alloantibodies. The cost and availability of compatible units by performing genotypic matching were compared to serologic matching. **Results:** The patients received a range of 5-215 units. And the median age in this group was 39 years old. Of the 79 patients, 17 (21.5%) were alloimmunized before they started receiving genotypic matched RBC transfusions. During this study-period only 2 patients (2.5%) with Rh variants developed alloantibodies (1 anti-C, 1 anti-e) and no patient produced autoantibody. Although the availability of compatible units has decreased when compared to serological matching, it was possible to find compatible units for most patients. A cost reduction of 30% was observed in the genotypic matching compared to the serological matching for the selected matched antigens. **Discussion and conclusion:** RBC transfusions with extended genotypic matching had significant effects on autoimmunization and alloimmunization rates in chronically transfused patients with SCD over two years. Alloimmunization rates have shown to decrease from a range of 21.5 to 2.5% in these patients. SCD patients may benefit from receiving prophylactic genotypic matching RBC transfusions as demonstrated by the reduction on the rates of alloimmunization, autoimmunization and cost.

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ALLOIMMUNIZATION PREVALENCE IN PATIENTS WITH SICKLE CELL DISEASE (SCD): ASSESSMENT OF THREE TRANSFUSION PROTOCOLS

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Background: RBC alloimmunization is a major complication associated with RBC transfusion in patients with SCD with serious complications for the patient including life-threatening delayed hemolytic transfusion reactions and an increase in the risks inherent to transfusion such as TRALI, infectious diseases and the hyperhemolysis syndrome. In addition, the presence of alloantibodies, which is often associated with the concomitant presence of autoantibodies, can lead to difficulty in finding compatible units, which can cause transfusion delays. The prevalence of alloimmunization vary according to the transfusion protocols used and may be reduced by matching for RBC antigens. Based on this, the aim of this study was to assess the prevalence of RBC alloimmunization in patients receiving transfusions with ABO and D matching, with phenotype matching for C, E and K antigens and with extended minor RBC antigen matching. **Methods:** A total of 368 patients with SCD, homozygous for HbS were enrolled. In this period, 81 patients received RBC units matched for ABO and RhD, 188

patients were matched for C, E and K and 99 patients were matched for ABO, Rh, K, Fya/Fy/b, Jka/Jkb and S/s antigens. Transfusion and antibody histories were obtained from our records, and antibody screening was done to assess the alloimmunization prevalence. Molecular typing was performed in all patients who had recent transfusions or a positive direct antiglobulin test by Laboratory Developed Tests (LDT) and HEA BeadChip (Immucor) to predict their antigen profile. RH variant alleles were determined in patient's antigen-positive who developed Rh antibodies using RHD and RHCE BeadChips (Immucor). Adsorption onto autologous RBC was also performed to aid the differentiation of autoantibodies from alloantibodies. **Results:** Of the 368 patients evaluated, 128 were male and 240 female. Mean age was 37.4 years (range 18-60) and they received an average of 74 RBC units. Prevalence of alloimmunization in the SCD patients with ABO/RhD matched only units (n=81) was 54%. For individuals transfused with C, E and K matched units (n=188), alloimmunization prevalence was 13% and patients transfused with extended-matching units (n=99) the prevalence of alloimmunization was 0.8%. Antibody specificities developed by the patients receiving Rh and K matched units were: Anti-e, -D, -Jka, -Jkb, -S, -Dia, -V and by the patients receiving extended matching were anti-C, -e, -V, -VS. The variant RHCE*ceS, RHCE*ceMO, RHD*DIIIa-CE(4-7)-D and RHD*DAU3 alleles were associated with the production of Rh antibodies in these patients and 9.8% of the alloimmunized patients developed autoantibodies. **Discussion and conclusion:** RBC alloimmunization rates shown to decrease to 24 % in patients with SCD transfused with RBCs matched for C, E and K antigens and to 1.5 % to patients with extended matching. Provision of RBC transfusions with limited and extended antigen matching is essential to reduce the rates of alloimmunization and hemolytic transfusion reactions in patients with SCD.

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APLICAÇÃO DA GENOTIPAGEM DE GRUPOS SANGÜÍNEOS EM CASOS COMPLEXOS DE IMUNO-HEMATOLOGIA

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Introdução: Os sistemas de grupos sanguíneos eritrocitários são representados por antígenos localizados na superfície das hemácias, os quais quando transfundidos a um receptor que não os possui, podem resultar em aloimunização e causar reações transfusionais hemolíticas agudas e tardias, Doença Hemolítica do Feto e do Recém Nascido (DHFRN), danos a tecidos transplantados, e menor número de doadores compatíveis. Para evitar tais complicações, recomenda-se a transfusão de concentrados de hemácias compatíveis com o receptor, principalmente quando se tratam dos antígenos dos sistemas de grupos sanguíneos mais imunogênicos, como Rh

(E,e,C,c), Kell, Kidd, Duffy e MNS. A genotipagem dos grupos sanguíneos pode ser utilizada como técnica auxiliar quando as técnicas convencionais apresentam limitações, seja devido a expressão fraca ou alterada dos antígenos, população mista de células em pacientes politransfundidos, teste de antiglobulina direta positivo, falta de anti soros comerciais disponíveis, ou na busca de doadores com fenótipos raros em larga escala.

Objetivo: Realizar a genotipagem de grupos sanguíneos de amostras de sangue de pacientes atendidos pelo Centro de Hematologia e Hemoterapia do Paraná (HEMEPAR), que necessitam de transfusão e cujas fenotipagens foram inconclusivas ou incompletas. **Material e métodos:** As amostras de sangue total foram obtidas do HEMEPAR, e o DNA genômico foi extraído utilizando-se kit de extração por coluna. Os fragmentos de interesse foram amplificados por Reação em Cadeia de Polimerase - Sequência Específica de Primers (PCR-SSP) para os antígenos eritrocitários: RHCE (RHCE*C, RHCE*c, RHCE*E e RHCE*e), Kell (KEL*01, KEL*02), Duffy (FY*01, FY*02) e Kidd (JK*01, JK*02), além da pesquisa pela mutação do gene GATA (FY*02N.01). Os produtos amplificados foram separados por meio de eletroforese em gel de agarose a 1% com revelador GelRED, e posteriormente revelados em fotodocumentador.

Resultados: Foram analisadas 12 amostras de pacientes politransfundidos, sendo encontradas discrepâncias entre fenotipagem e genotipagem para 1 amostra no sistema Rh (E) e para 4 amostras no sistema Kidd. Além disso foram encontradas 9 amostras com a mutação em GATA. **Discussão:** A maior quantidade de discrepâncias foi observada em Kidd, além disso foram encontrados 9 casos de silenciamento do antígeno Fyb atribuídos à mutação em GATA, que se apresentou positiva em todos os indivíduos com fenótipo Fy(a-b-). As técnicas utilizadas também solucionaram as fenotipagens inconclusivas causadas por duplas populações, as quais não interferem nos métodos moleculares. **Conclusão:** Os resultados obtidos demonstram a contribuição da genotipagem de grupos sanguíneos como técnica auxiliar, principalmente em casos complexos de imuno-hematologia, otimizando a liberação de bolsas de sangue adequadas à segurança transfusional, além da ampliação do número de doadores compatíveis para pacientes com o fenótipo Fy(a-b-) relacionados com o alelo FY(02N.01).

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PERFIL FENOTÍPICO DE DOADORES DO GRUPO O DE UM HEMOCENTRO NO NORTE DO BRASIL

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Objetivos: Traçar o perfil fenotípico dos doadores do grupo sanguíneo O, avaliando a presença dos antígenos D, C, “c”, E, “e”, K, “k”, Kpa, Kpb, Fya, Fyb, Jka, Jkb, M, N, S, “s”, Lea, Leb, Lua, Lub e P1 identificando o perfil fenotípico dos doadores de sangue do serviço de hemoterapia. **Material e métodos:**