Molecular matching of red blood cells is superior to serological matching in sickle cell disease patients

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Objective: To evaluate the usefulness of DNA methods to provide a means to precisely genotypically match donor blood units for the antigen-negative type of 35 sickle cell disease patients.

Methods: Red blood cell units were investigated for ABO, D, C, c, E, e, K, Fy α, Fy β, Jka, Jkb, S, s, Di α and RH variants by performing a molecular array (Human Erythrocyte Antigen BeadChip™, BioArray Solutions), polymerase chain reaction followed by restriction fragment length polymorphism analysis and sequencing of patient samples and donor units that had been serologically matched based on the ABO, Rh and K phenotypes and the presence of antibodies.

Results: Matches for 21 of 35 sickle cell disease patients presented discrepancies or mismatches for multiple antigens between the genotype profile and the antigen profile of their serologically-matched blood units. The main discrepancies or mismatches occurred in the RH, FY, JK and MNS systems. Eight Rh alloimmunized patients presented RHD and RHCE variants that had not been serologically identified. According to these results better matches were found for the patients with genotyped units and the patients benefited as shown by better in vivo red blood cell survival.

Conclusion: Molecular matching is superior to serological matching in sickle cell disease patients, decreasing the risk of transfusion reactions, especially delayed transfusion reactions to existing alloantibodies and preventing alloimmunization.

Keywords: Anemia, sickle cell; Molecular typing; Blood group antigens; Isoantibodies/blood

Introduction

Red blood cell (RBC) transfusions have played an important role in allowing sickle cell disease (SCD) patients to live longer. However, their use is complicated by the high incidence of RBC alloimmunization[1][7], making the identification of compatible RBC products difficult, and is associated with delayed hemolytic transfusion reactions (DHTRs). For it to be possible to transfuse SCD patients effectively, more effective ways to reduce risk of transfusion reactions, transfusion-associated hyperhemolysis syndrome and alloimmunization must be found.

Although transfusion services establish protocols to reduce alloimmunization, there is still no consensus on the best practical approach even though the obvious goal is to provide blood that will survive for the maximum period of time[8][10]. Three common approaches used to supply RBC products (ABO and D compatible) to SCD patients are (i) to give specific antigen-negative RBCs after the patient has made the alloantibody, (ii) to match for C, E, c, e and K antigens and (iii) to match for C, E, c, e, K, Fy α, Fy β, Jk α and Jk β antigens[11][14].

In recent years, molecular DNA-based genetic methods have greatly improved transfusion therapy for SCD patients because they can be used to genotype patients and donors and maintain an inventory of DNA-typed units to identify compatible donors. Previous studies have demonstrated the relevance of genotyping blood groups to manage multiply transfused SCD patients by allowing the determination of the true blood group genotype and by assisting the identification of suspected alloantibodies and the selection of antigen-negative RBCs for transfusion[15][18].

By testing the patient and donors it is possible to provide more extensively matched blood for patients thereby preventing additional alloimmunization. A study by Klapper et al.[17] using the human erythrocyte antigen (HEA) BeadChip™ DNA analysis and a web-based inventory management system to model donor-recipient matching showed that even with a limited donor pool, matching for Rh, Kell, Duffy, Kidd and MNS could be achieved at least 50% of the time.

Herein the use of DNA methods to precisely match donor blood units for the antigen-negative genotypes of SCD patients is reported.

Methods

Samples

DNA samples from one hundred and ten donor units from a blood bank inventory that had been serologically matched for 35 SCD patients were studied. Selected units were...
serologically matched for patients based on their ABO, Rh and K phenotypes and the presence of antibodies. Antigen-matched RBC units were investigated for recipients using blood group genotypes of the ABO, D, C, c, E, e, K, Fy*, Fy*, Jk*, Jk*, S, Lu*, Di*, Js*, Do* and Do* systems. All the patients and donors agreed to participate in this study by signing an Institute Review Board approved informed consent form.

Data processing

To perform data management, a web-hosted inventory management system was designed for this study. An electronic link using specific software to compare the blood donor units and the patients’ needs was established allowing automatic identification of the most compatible blood.

DNA preparation

The genomic DNA was extracted from 200-µL aliquots of whole blood by manual spin column separation (QIAmp, Qiagen, Valencia, CA), according to the instructions of the manufacturer and eluted into 100 µL of buffer. The DNA concentration of each sample was calculated by the measurement of optical density at 260 and 280 nm and an 8-µL aliquot, containing ~10 ng of gDNA, was transferred for the polymerase chain reaction (PCR).

Human erythrocyte antigen BeadChip™ DNA analysis

The DNA array analysis was performed using RHD and RHCE BeadChip™ analysis which uses probes directed to polymorphic sites for RHD and RHCE variants and HEA BeadChip™ containing probes directed to polymorphic sites in the RHCE, FY (including FY-GATA and FY265), DO (including HY and JO), CO, DI, SC, GYPA, GYPB (including markers permitting the identification of U-negative and U-variant types), LU, KEL, JK and LW genes and one mutation associated with hemoglobinopathies (Hb S) (BioArray Solutions, Warren, NJ, USA) for all donor and patient samples. The HEA BeadChip™ assay was performed in accordance with a previously described protocol[16,18,19].

Polymerase chain reaction-restriction fragment length polymorphism

PCR followed by restriction fragment length polymorphism (RFLP) was used to identify the RHCE*ceBI and RHD*DOL variants prevalent in African descendents. Screening of samples was carried out by analyzing RHCE*818 and RHCE*1132 in standard PCR products generated from genomic DNA using RHCE-specific-primers, followed by RFLP using the Mwol and Tsp45I restriction enzymes, respectively[20]. Genomic DNA was amplified using RHD-specific primers flanking exon 4 and exon 8, with the products being submitted to sequencing to determine the presence of the RHD*DOL (nt 509T>C in exon 4) and RHD*DOL-2 alleles (nt 509T>C in exon 4 and nt 1132C>G in exon 8). PCR products were sequenced in both directions.

Results

Molecular matching

Of the 35 SCD patients studied, 21 presented discrepancies or mismatches for multiple antigens between their extended HEA (xHEA) profile and the antigen profiles of their serologically-matched blood units. The main discrepancies or mismatches occurred in the RH, FY, JK and MNS systems. Discrepancies between the previous phenotype and genotype-derived phenotype were found in 14 alloimmunized chronically transfused patients (Table 1) who were not having good in vivo RBC survival and mismatches for multiple antigens were found in 17 patients receiving blood units matched for ABO, Rh and K. Eight Rh alloimmunized patients presented RHD and RHCE variants that had not been serologically identified or that had been misinterpreted as autoantibodies. Of these eight patients, two had the RHCE*ceAR associated with the RHD*DAR variant, two had the RHCE*ceBI associated with the RHD*DOL variant, two had RHCE*ce48C,733G and two had RHCE*ce48C,733G,1006T, both associated with the RHD*DIIIa variant (Table 2). According to these results, better matches were found for the patients in the institution’s DNA-typed units, and in the majority of cases, the degree of matching was enhanced and the patients benefited by receiving transfusions that provided better in vivo RBC survival. The transfusion timeline before genotyping was about 1 week, and with extended genotyped-matched blood this changed to 30 days. These patients were followed up for one year and have not developed other antibodies.
These results were also very important to clarify and confirm the specificities of alloantibodies and were essential to distinguish between alloantibodies and autoantibodies, particularly in patients with RH variants.

Discussion

The provision of antigen-negative blood forms the basis for safe blood transfusions by decreasing the risk of hematolytic transfusion reactions and preventing new instances of alloimmunization. High-throughput genotyping based on DNA arrays is a very feasible method to obtain a large pool of well-typed blood donors and can contribute to the management of transfusions in SCD patients by allowing a more accurate selection of donor units to reduce transfusion requirements. The ability to test patients and a large number of donors simultaneously for several antigens, together with computer analysis and interpretation of data, facilitates the matching of RBC components to the recipients’ blood type making the process feasible and easily increasing the inventory of donor units for SCD patients. Klapper et al. concluded that if patients and donors are extensively DNA typed, it is feasible to provide units for transfusion that are more extensively matched than in the current standard of practice.

In the Blood Center of UNICAMP, SCD patients who require multiple transfusions are placed on chronic prophylactic transfusion protocols. For the effective application of genotyping of these patients it is important that suitably phenotyped/genotyped donors are available.

When molecular-matching was applied to 35 SCD patients, discrepancies or mismatches were found for multiple antigens between their xHEA profile and the antigen profile of their serologically-matched blood units. Additionally, RH variants that would never be identified by serology were found in eight patients and alloantibodies were distinguished from autoantibodies. It was possible to find a better match in our xHEA-typed units for these patients, enhancing the degree of matching and reducing the risk of alloimmunization and delayed transfusion reactions.

The patients who benefited from receiving antigen-matched RBCs based on genotyping (as shown by better in vivo RBCs survival) had increased hemoglobin levels and diminished frequency of transfusions. These patients presented autoantibodies that masked clinically significant alloantibodies and the use of this DNA approach was essential to distinguish between autoantibodies and alloantibodies. Alloimmunization may cause several problems ranging from inconvenience due to delay in obtaining matched blood to DHRs. The benefits that donor-recipient blood group genotype matching would have on reducing the incidence of delayed transfusion reactions were demonstrated in this study. Extended matching of donors and SCD patients for the ABO, D, C, c, E, e, K, Fy, Fy, Jkb, Jk, Jk, K, e, K, S, and Dr antigens and RH variants would also reduce the development of the majority of alloantibodies that currently exist in more than 90% of the immunized SCD patients.

It is not a feasible goal to provide an antigen-match blood transfusion to all SCD patients for all antigens. However, extensive DNA-based blood group typing of donors and patients has shown to be a cost-effective procedure and now chronically SCD patients can be screened for at least the lack of the C, c, E, e, K, S, Fy, Fy, Jkb and Jk antigens.

The reliable prediction of xHEA phenotypes offers a potential alternative to the current serologic methods of donor unit screening and transfusion recipient typing. DNA array technology can contribute to the management of transfusions in SCD patients by facilitating transfusion support with antigen-matched blood. It has the potential to replace the routine blood group phenotyping with a reduction in costs as well as the workload involved in donor and patient antigen typing.

Conclusion

In summary, the implementation of molecular matching can decrease the risk of transfusion reactions, especially delayed transfusion reactions to existing alloantibodies, and prevents alloimmunization. Additionally, the degree of enhanced matching was higher than that which occurred by random based matching of only the ABO, Rh and K systems. Matching at the DNA level may provide an added level of safety and efficacy by reducing transfusion requirements, decreasing the risk of transfusion-related acute lung injury and potential exposure to infectious diseases.

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