



Case Report

Application of Monocyte Monolayer Assay technique to predict hyperhemolysis in patients with sickle cell disease

Dahra Teles Cruz ^{*}, Marina C.V. Conrado , Alfredo Mendrone, Carla L. Dinardo

Fundação Pró-Sangue, São Paulo, SP, Brazil

ARTICLE INFO

Article history:

Received 30 December 2022

Accepted 7 July 2023

Available online 23 August 2023

Introduction

Red blood cell (RBC) transfusions are an important part of the treatment of sickle cell disease (SCD) patients indicated for acute chest syndrome, stroke (treatment or prevention) and severe pain episodes. However, transfusions may be associated with delayed hemolytic transfusion reactions, RBC alloimmunization, acute lung transfusion reactions, transfusion-transmitted infections and transfusion-induced iron overload. RBC alloimmunization rates in SCD vary between 7 and 44%, while in the general population it is around 2–18%. The high incidence in SCD patients mainly due to the strong background inflammation and the racial disparity between transfusion donors and recipients.^{1,2}

RBC alloimmunization may cause serious hemolytic transfusion reactions (HTRs), sometimes fulfilling the criteria for hyperhemolysis syndrome (HS), which is potentially lethal. HS is one of the most severe manifestations of DHTR in

patients with SCD, in which both transfused and autologous red blood cells (RBCs) are destroyed leading to devastating consequences for the patients. Cases of HS are typically not associated with the development of new alloantibodies or with recrudescence of previous alloantibodies. In the scenario of hyperhemolysis, further RBC transfusions should be suspended, erythropoiesis should be stimulated with erythropoietin-stimulating agents (ESAs), and immunomodulatory drugs should be employed.³

Antibody identification can be challenging in the context of SCD, mainly because multiple antibodies are involved and the patients are multi-transfused, hampering the application of some serological immunohematological tools. Predicting HS would be extremely valuable for transfusion practice, considering this is a life-threatening complication. Monocyte Monolayer Assay (MMA) was described as the gold standard technique for assessing the clinical importance of irregular antibodies and can be used to evaluate which of the transfused RBC units has deflagrated a HTR reaction.^{4–6}

We report the case of a patient with SCD with severe pain episode that presented risk of hemolysis detected by the MMA in spite of the selection of phenotype compatible and

^{*} Corresponding author.

E-mail address: dahra.teles@upe.br (D.T. Cruz).

<https://doi.org/10.1016/j.htct.2023.07.004>

2531-1379/© 2023 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

IAT-compatible RBC unit. The patient evolved with HS, as predicted by the cell assay. After this episode, MMA was used as second crossmatch for all subsequent transfusions.

Case presentation

Female patient with SCD (HbSS), presenting with vaso-occlusive episode, and stemming from another country (Angola). Initial immunohematological evaluation showed anti-C, anti-E, anti-S, anti-Jkb, auto-cryoagglutinin, warm auto-antibody and another antibody that was initially classified as antibody of undetermined specificity (AUS). The patient had been transfused 30 days before the episode and the predicted phenotype derived from RBC genotype performed by conventional molecular methods was C-, c+, E-, e+; K-; Fy(a-b-); Jk(a+b-); S-s+. Direct antiglobulin test (DAT) was positive and panagglutination was detected in the eluate. A phenotype-matched unit was selected for transfusion and MMA was performed to evaluate the clinical significance of the AUS.

The erythrocytes derived from the unit selected for transfusion were incubated with monocytes obtained from healthy donors and the phagocytosis index was determined according to the method described elsewhere. The resulting monocytic index was 10% (percentage of monocytes with red blood cells from the donor inside or attached to their membrane), demonstrating that the antibody had relevant clinical value (>5%).⁷

Due to the worsening of the patient's clinical condition, the medical team decided to transfuse the MMA-incompatible RBC unit. Pre-transfusion hemoglobin was 6,3 mg/dL and there were no acute complications during the infusion. About 7 days after transfusion, the patient was admitted to the emergency department with a significant drop in hemoglobin (Hemoglobin = 1,5 mg/dL) and reticulocytopenia, confirming the diagnosis of HS. She was medicated with Intravenous Immunglobulin (IVIG), evolving with progressive improvement in the hematimetric indices. The immunohematological evaluation presented with the same antibodies as before. The DAT kept positive and panagglutination was still observed on the eluate.

One month after discharge, RBC transfusion was requested for the patient again. However, now an anti-e like was observed in the antibody identification panel and the AUS was not detected. Investigation for RH variants was indicated and performed by Sanger sequencing, indicating the presence of RHD*DAR and RHCE*ceAR in homozygosity. The specificity of the alloantibody was confirmed as anti-hrS. Phenotype-compatible units were selected for further transfusions. The MMA performed was negative and the transfusion was safe without intercurrent.

The MMA tests started to be used as a second crossmatch for this patient along with conventional tests every transfusion to ensure a safety, even with red blood cells phenotype compatible with the patients.

Discussion

RBC alloimmunization remains a frequent and potentially serious complication of transfusion in patients with SCD. RBC phenotype differences between Afro-descendants patients

and Caucasian-descendants blood donors, mainly in the RH system, are thought to contribute to the high frequency of antibody development in SCD, together with a high inflammation background presented by the patients.⁸ Also, the RH complexity of RH locus presented by SCD patients is high, underlying the development of antibodies derived from partial RH antigens and antibodies directed to RH high frequency antigens.⁸

In the presented case, the multi-transfused patient was transferred to our service presenting with several alloantibodies and both warm and cold autoantibodies. At his moment, one antibody was classified as AUS, which was later classified as anti-hrS. However, the indication of MMA to evaluate the clinical relevance of the AUS proved extremely useful, because the result shed to light the possibility of HTR and, even if the specificity of the antibody could not be determined at that moment, incompatible transfusion could not be indicated, unless with pre-administration of immunosuppression therapy.⁹

According to MMA literature data the percentage of erythro-phagocytosis measured as Monocyte Index (MI) less than or equal to 5% was associated with little risk of clinical hemolytic reactions, in between 5.1% and 20% was associated with 33% risk of overt clinical hemolysis and above 20% was associated with 64% risk of overt clinical hemolysis if incompatible RBC units were transfused.¹⁰ In the presented case, the MI was 10%, suggesting that antibody would probably lead to post-transfusion hemolysis. The MMA assay can be used as the main laboratory support to assess the clinical significance of AUS.^{5,7} The application of the MMA technique on patients with antibodies against antigen high frequency is already well described, but in this paper we discussed the use of MMA as a complementary test in alloimmunized patients with AUS and predisposition to evolve the HTRs/HS.

After implementing the MMA technique as a second crossmatch, we increased the transfusion safety of patients with the possibility or history of hyperhemolysis, transfusing only when the MMA is negative and closely monitoring the post-transfusion period.

This particular patient continued to be transfused safely, without further episodes of hyperhemolysis until death from complications of the disease.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

None.

REFERENCES

1. Chou ST, Liem RI, Thompson AA. Challenges of alloimmunization in patients with haemoglobinopathies. *Br J Haematol*. 2012;159(4):394–404.
2. Dezan MR, Oliveira VB, Bianchi JVS, Rodrigues V, Solano JH, Gomes FC, et al. Effectiveness of a red cell antigen-matching

- transfusion protocol in sickle cell disease patients. *ISBT Sci Ser.* 2016;11(3):132–9.
3. Dean CL, Maier CL, Chonat S, Chang A, Carden MA, El Rassi F, et al. Challenges in the treatment and prevention of delayed hemolytic transfusion reactions with hyperhemolysis in sickle cell disease patients. *Transfusion.* 2019;59(5):1698–705.
 4. Conrado MCAV, D’Avila AN, Vieira JB, Bonifacio SL, Gomes FCA, Dezan MR, et al. Defining the clinical relevance of red blood cell autoantibodies by Monocyte Monolayer Assay. *J Clin Lab Anal.* 2017;32(2):e22274.
 5. Conrado MA, Leão Bonifácio S, Nogueira FM, Saraiva-Filho JCP, Dezan MR, Chinoca KZ, et al. Massive autoimmune hemolysis documented by monocyte monolayer assay in a multiply transfused patient using reticulocytes isolated by simple centrifugation in microhematocrit tubes. *Transfusion.* 2018;58(7):1578–9.
 6. Noumsi GT, Billingsley KL, Moulds JM. Successful transfusion of antigen positive blood to alloimmunised patients using a monocyte monolayer assay. *Transfus Med.* 2015;25(2):92–100.
 7. Nance S, Arndt P, Garratty G. Predicting the clinical significance of red cell alloantibodies using a monocyte monolayer assay. *Transfusion.* 1987;27(6):449–52.
 8. Dezan MR, Ribeiro IH, Oliveira VB, Vieira JB, Gomes FC, Franco LAM, et al. RHD and RHCE genotyping by next-generation sequencing is an effective strategy to identify molecular variants within sickle cell disease patients. *Blood Cells Mol Dis.* 2017;65:8–15.
 9. Chou ST, Alsawas M, Fasano RM, Field JJ, Hendrickson JE, Howard J, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. *Blood Adv.* 2020;4(2):327–55.
 10. Hendrickson JE, Chadwick TE, Roback JD, Hillyer CD, Zimring JC. Inflammation enhances consumption and presentation of transfused RBC antigens by dendritic cells. *Blood.* 2007;110(7):2736–43.