Transfusion management for patients taking an anti-CD38 monoclonal antibody

Carolina Bonet Bub\textsuperscript{a,}*\textsuperscript{a}, Isabel Nagle dos Reis\textsuperscript{a}, Maria Giselda Aravechia\textsuperscript{a}, Leandro Dinalli Santos\textsuperscript{a}, Eduardo Peres Bastos\textsuperscript{a}, José Mauro Kutner\textsuperscript{a}, Lilian Castilho\textsuperscript{a,b}

\textsuperscript{a} Hospital Israelita Albert Einstein, São Paulo, SP, Brazil
\textsuperscript{b} Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil

\section*{Article Info}

\begin{itemize}
  \item Article history: Received 7 June 2017
  \item Accepted 18 September 2017
  \item Available online 26 November 2017
\end{itemize}

\section*{Abstract}

Introduction: Pre-transfusion tests, essential for the release of blood components, may be affected by drugs. Monoclonal antibodies represent a class of medications increasingly used in the clinical practice, with anti-CD38 monoclonal antibodies (daratumumab) being a promising resource in the treatment of refractory myeloma. This monoclonal antibody recognizes CD38 in myeloma cells and interferes with pre-transfusion tests by causing panreactivity in indirect antiglobulin tests thereby clinically masking alloantibodies. Dithiothreitol is a reagent that breaks disulfide bonds and effectively destroys antigenic sites for CD38 on red blood cells. This study reports the immunohematological findings of pre-transfusion tests of patients with multiple myeloma receiving daratumumab and solutions to prevent the interference of this monoclonal antibody.

Methods: Serum samples from five patients on anti-CD38 monoclonal antibody treatment were evaluated. Tests performed included ABO/RhD typing, indirect antiglobulin test, direct antiglobulin test and eluate test. A daily evaluation was performed to determine the shelf life of dithiothreitol-treated red blood cells when stored in Alsever’s solution.

Results: No interference in the ABO/RhD typing results was noted but in all samples, a panreactivity was observed in indirect antiglobulin tests. Regarding the direct antiglobulin test, two samples presented positive results but negative eluates. In all samples, treatment of reagent red blood cells with 0.2 M dithiothreitol offset interference by anti-CD38 monoclonal antibodies. Dithiothreitol-treated red blood cells stored in Alsever’s solution were stable for up to 15 days.

Conclusion: Treatment of reagent red blood cells with dithiothreitol can be efficient and accessible to offset the interference of the anti-CD38 drug in pre-transfusion tests. The number of costly serological workups can be reduced by having stored dithiothreitol red blood cells with this proving to be a useful reagent for investigating anti-CD38.

\section*{Acknowledgments}

© 2017 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

* Corresponding author at: Departamento de Hemoterapia e Terapia Celular, Hospital Israelita Albert Einstein, Av. Albert Einstein, 627, 3 andar, Bloco E, 05651-901 São Paulo, SP, Brazil.

E-mail address: carolina.bub@einstein.br (C.B. Bub).
Introduction

The interference of drugs in pre-transfusion tests is a well-known phenomenon in the blood bank routine. The difficulty in handling these interferents was initially described in the 1970s in respect to drugs and chemicals used as antibiotics, antihypertensives and analgesics. Over the years, the number of interfering drugs has increased in association with developments in the pharmaceutical industry.1

Among the etiological mechanisms, the immune complex formation, drug adsorption in the erythrocyte membrane, autoantibody formation and modifications of the erythrocyte membrane are described.2,3 The major concern related to the presence of these interferents is the possible impact on the transfusional management of patients using these drugs. Discrepant tests induced by the presence of interferents may generate erroneous interpretations and lead to delays in the release of blood components.4

Monoclonal antibodies (MoAbs) represent a class of therapy that is increasingly used in a variety of pathological conditions, including solid tumors, leukemia, and infections.5 Daratumumab (anti-CD38) is an immunoglobulin (Ig)G1 MoAb that is indicated in the treatment of relapsed/refractory multiple myeloma6 and has shown high efficiency and safety as described by Dimopoulos et al. in the POLLUX study.7 Daratumumab is directed to the CD38 portion of malignant cells; however, this drug reacts with the red blood cell (RBC) reagents used in pre-transfusion tests which also express CD38 on their cell surface complicating the identification of clinically significant RBC antibodies8 since the plasma/serum will be panreactive with IAT screening and panel cells. Mild hemolysis has been associated with this drug, with a maximum hemoglobin drop of 1.0 g/dL (related to splenic sequestration of RBCs with surface-bound anti-CD38). These alterations identified in pre-transfusion tests may persist for months making transfusional management of these patients even more difficult.9 The American Association of Blood Banks (AABB) issued a bulletin suggesting strategies for blood banks to effectively bypass daratumumab panreactivity findings that include chemical treatments of panel cells, the use of a cord blood cell panel and inactivation of anti-CD38 in the patient’s plasma through the use of soluble human CD38.10

Dithiotreithol (DTT), a disulfide-bridging reductant reagent is commonly used in blood banks and has been applied as an affordable and efficient resource to resolve this identified panreactivity through the destruction of antigenic sites for CD38 on red blood cells.11,12 However, DTT is known to denature antigens from the Kell blood group system and other blood group antigens found less commonly in the population.13 Other alternative and promising solutions, such as the use of cord blood cells14 and, neutralization of free daratumumab in plasma, are not yet widely available.9,11 Assertive strategies should be applied for the safe release of blood components and to avoid possible predictable transfusion complications.

This study describes the immunohematological findings of pre-transfusion tests on patients receiving anti-CD38 MoAbs and evaluates the use of DTT as a laboratory strategy to eliminate the interference of this drug by removing CD38 from the RBC surface. Although DTT may be effective, it is time-consuming and therefore this study also aimed to evaluate the storage survival of DTT-treated reagent RBCs to mitigate the laborious work of DTT cell treatment prior to each transfusion.

Methods

Patient samples

Pre-transfusion samples of five hematological patients with diagnosis of multiple myeloma receiving anti-CD38 drugs were sent to the immunohematology reference laboratory for serologic testing from January to December 2016. Information regarding the gender and age of the patient and the time between anti-CD38 MoAb infusion and sample collection were also sent to the laboratory.

Serologic testing

Serologic testing included ABO/RhD typing, antibody screening, RBC panel, direct human antiglobulin test (DAT), and antihuman globulin (AHG) cross-match in gel test (Grifols, Spain). If the DAT was positive, an eluate test was performed.

Antibody identification was performed using commercial panels of 11 cells previously phenotyped for the main erythrocyte antigens (Bio-Rad, Brazil; Grifols, Spain). DAT was performed with polyspecific and monospecific testing for IgG and C3 (DC screening, Grifols, Spain). Eluates were prepared from patient samples with a positive DAT using an acid elution technique (Diacidel, Bio-Rad, Switzerland).

Serum samples showing panreactivity with IAT screening and panel cells were further tested with reagent RBCs treated with 0.2 M DTT solution. Briefly, 0.2 mol/L DTT was prepared by diluting 1 g DTT (Sigma–Aldrich, São Paulo) in 32 mL of phosphate buffered saline (PBS – pH: 8.0). RBCs positive for the k+ and E antigens were used as positive and negative controls to verify the efficacy of DTT treatment. A volume of reagent RBCs were washed three times with saline, diluted to a 3% suspension with PBS (pH: 7.3), mixed with four volumes of 0.2 mol/L DTT and incubated at 37 °C for 20 min. RBCs were then subjected to a final wash sequence with PBS for subsequent testing.

Molecular testing

DNA was extracted from whole blood using the QIAamp DNA blood mini-kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. Molecular tests were performed on all samples using the human erythrocyte antigen (HEA) BeadChip™ assay (Bioarray Solutions, Immucor, NJ, USA). Genotypes and predicted phenotypes were determined.

Storage survival of dithiotreithol-treated red blood cells

A daily evaluation was performed in order to determine the shelf life of DTT-treated reagent RBCs when stored in Alsever solution. The study was conducted over a period of 15 days. Four screening RBCs with the Rh phenotypes: R1(R), R2(R), r and R1R1 from Grifols (Serascan Diana 4 reagent) were treated...
with 0.2 M DTT. A starting volume of 1 mL of treated RBCs was diluted to 1% in Alsever solution and a daily evaluation was performed. The evaluation consisted of (1) visual grading of hemolysis (mg/dL) comparing treated and untreated RBCs using a standard hemolysis chart, (2) antigen testing of DTT-treated RBCs to evaluate Kell and RhD antigens, (3) indirect antiglobulin test (IAT) by gel test with DTT-treated and untreated RBCs using a serum of a patient receiving daratumumab therapy, a serum containing anti-k (positive control) and a serum containing anti-D (negative control).

**Results**

Four of the five tested patients were male and one was female. The ages ranged from 52 to 77 years and the number of days after anti-CD38 MoAb infusion ranged from one to 45 days, although in one case this information was not reported.

All patients had a positive IAT in gel, with agglutination intensities ranging from 2+ to 3+ even in the case where anti-CD38 MoAb had been administered 45 days prior to sample collection. Autocontrol and DAT were positive in 2/5 cases with IgG detected and negative in 3/5 cases. Eluate tests performed for two RBC samples were negative.

Sera of all patients were tested with a panel of RBCs treated with 0.2 M DTT by IAT. The efficacy of treatment was evidenced by denaturation of the k antigen in the k+ control cell, and preservation of the E antigen in the E+ control cell. Panreactivity previously detected in the sera of these patients by IAT was not observed after DTT treatment of reagent RBCs, demonstrating that interference of the anti-CD38 MoAb was eliminated (Figure 1). No alloantibody was identified in the patients’ sera but all had been transfused with extended genotype-matched units for clinically-relevant RBC antigens that included the Rh, K, Jk, Fy and S antigens, to avoid alloimmunization and to ensure a safer transfusion. Table 1 shows the clinical data and the results of pre-transfusion tests.

### Storage survival of dithiotreitol-treated red blood cells

The results of this study showed that hemolysis did not affect expected performance of reagent cells. RBCs gave negative results for the k antigen and reacted (4+) to the D antigen after DTT treatment throughout the study and were acceptable for use as CD38 negative cells. A gel test with low-ionic-strength saline (LISS) showed negative results with the sera of patients receiving daratumumab and positive results with the positive control throughout the evaluation. No false positive reactions were observed with the negative control. This study showed that DTT-treated RBCs stored in Alsever solution are stable for up to 15 days.

### Discussion

Multiple myeloma is a malignant plasma cell disorder characterized by bone, renal, hematological, and infectious complications due to the accumulation of these cells in the bone marrow. Although the survival of patients with this pathology has improved with new classes of drugs (e.g. proteasome inhibitors and immunomodulatory drugs) along with hematopoietic stem cell transplantation, most patients still die of refractory disease. New options of treatment have emerged for patients with multiple myeloma refractory to the use of proteasome inhibitors and immunomodulatory drugs. A novel MoAb directed against CD38 (expressed on the cell surface of diseased plasma cells) was evaluated in these patients with monotherapy using the anti-CD38 MoAb or associated to other drugs demonstrating encouraging efficacy and favorable safety in this patient population. However, RBCs also express the CD38 antigen on their cell membrane, and the drug binds cross-linked to these cells, which may ultimately interfere with pre-transfusion testing.

The use of this MoAb associated with mild hemolysis has already been described in the literature, with a drop of at most 1 g/dL caused by the splenic sequestration of the RBCs with anti-CD38 bound on their cell surface; this is not associated with major clinical manifestations. Another alteration described is an interference in antibody screening and identification represented by a serum panreactivity with all the RBCs used in the panel with this panreactivity persisting for up to six months after anti-CD38 MoAb administration. No interference is associated with ABO/RhD typing tests and interference in the DAT is variable. These tests are of paramount importance in the pre-transfusion analysis of the patient, and any interference can induce a false interpretation of results and impact on the transfusion management of patients, with delays in the release of the blood component to be transfused or even a hypothesis of alloimmunization that, in reality, does not exist. In all cases herein described there was a panreactivity identified in the IAT. The longest date between drug administration and sample collection was 45 days with interference being detected in this sample. There was a variation between the findings of the autocontrol and
The use of a cord blood cell panel is an efficient, low time consuming and denatures anti-CD38. Inactivation of anti-CD38 in plasma through the use of soluble human CD38 is an easily available, leads us to another aspect to be considered, the possibility of interference detected in the pre-transfusion tests. Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES


