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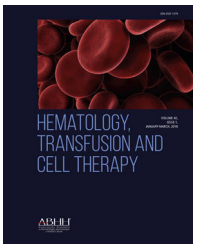

Original article

Transfusion management for patients taking an anti-CD38 monoclonal antibody

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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 on 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.

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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 myeloma[6] 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 antibodies[8] 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 cells[14] 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 QIAmp 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: $R_1{}^{W}R_1$, R_2R_2, rr and R_1R_1 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.

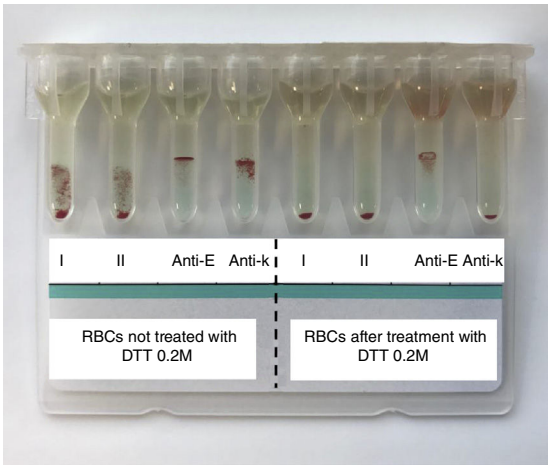

Figure 1 – Pre and post DTT treatment of reagent red blood cells. Panreactivity previously detected in the sera of these patients by IAT (samples I and II) was not observed after DTT treatment of reagent red blood cells. The efficacy of treatment was evidenced by denaturation of the k antigen in the k+ control cell, and preservation of the E antigen in the E+ control cell.

Storage survival of dithiotreithol-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.[15] 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.[16,17] 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.[6,7] 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.[8]

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.[18] 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.[5] 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.[9] 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

Table 1 – Clinical data and the results of pre-transfusion tests.

Patient (gender)	Age (years)	After anti-CD38 (days)	IAT (Gel/LISS)	Autocontrol (Gel/LISS)	DAT (Gel IgG)	Eluate	Post DTT treatment	Positive control
1 (M)	64	1	Panreactive	Positive	Positive	Negative	Negative	Negative
2 (M)	77	45	Panreactive	Negative	Positive	Negative	Negative	Negative
3 (M)	68	6	Panreactive	Positive	Negative	NP	Negative	Negative
4 (M)	52	NI	Panreactive	Negative	Negative	NP	Negative	Negative
5 (F)	68	20	Panreactive	Negative	Negative	NP	Negative	Negative

NI: not informed; NP: not performed.

the DAT with these findings being compatible with those described in the literature.

Strategies to avoid transfusion difficulties in patients receiving anti-CD38 drugs have included DTT treatment of reagent RBCs, inactivation of anti-CD38 with soluble human CD38 or the use of a cord blood panel (non-CD38 expressing cells).[10] The use of a cord blood cell panel is an efficient, low cost and non-chemical alternative, but this type of panel is not commercially available with its in-house composition making this strategy more difficult.[14] Inactivation of anti-CD38 in plasma through the use of soluble human CD38 is an easily performed commercially available strategy but soluble human CD38 is a high cost reagent.[9,11] Currently, DTT treatment of reagent RBCs is widely used and well accepted by different laboratories.[12]

This is the first study conducted in Brazil demonstrating the efficiency of DTT-treated RBCs in eliminating the interference of anti-CD38 MoAbs in pre-transfusion tests. In all the samples studied, there was a denaturation of the panreactivity and the presence of underlying alloantibodies was excluded, thus increasing transfusion safety. DTT is a reagent available in our laboratory routine but the technique although easy to standardize[12] is time consuming and denatures antigens of the Kell system, which are of great clinical importance in RBC transfusions. Thus, if the patient's Kell phenotype is not known, negative Kell blood products should be selected for transfusion. On the other hand, DTT also denatures antigens of the Lutheran, Cartwright, JMH, Knops, LW, Cromer, Indian, and Dombrock systems.[13]

This study showed that DTT-treated RBCs stored in Alsever solution maintain potency for up to 15 days with satisfactory results with positive and negative controls using a gel test. RhD antigen integrity was maintained and hemolysis did not interfere with antigen/antibody testing. Having these cells available, leads us to another aspect to be considered, the possibility of saving time and costs. An average of two RBC units every 15 days were transfused to these patients. Considering, the cost of U$22.00 per antibody screening test, U$44.00 would be saved per month using the stored DTT-treated RBCs. By the end of a year, there would be a saving of U$528.00 per patient. On the other hand, having DTT-treated RBCs available saves time as each DTT treatment takes around two hours and so better assistance would be provided to these patients related to their transfusion needs, mitigating delays or dubious results.

Blood selection for patients taking anti-CD38 MoAb has been made based on extended phenotype and/or the phenotype predicted by genotyping.[18] Pre-transfusion crossmatching of the blood units to be transfused is a step that adds safety to the transfusion process, mainly related to the risks of missing antibodies against low frequency antigens that can be erroneously ruled out and those antibodies against antigens denatured after DTT treatment. Clinical monitoring and observing post-transfusion hematimetric levels are essential for these patients.

In this context, the definition of a transfusion protocol should be established by the transfusion service and this protocol should be based on the accessibility of the technologies available in each service. Baseline determination of RBC antigens, whether done by phenotyping or genotyping, is the most effective feature in patients who already take anti-CD38 MoAbs. In subsequent transfusions, following the initiation of monoclonal therapy, it is suggested that the blood components should be compatible with previously identified phenotyping or genotyping.[19] Good communications between the transfusion service and the hematologist is also necessary for the recognition that anti-CD38 MoAbs might be the cause of interference detected in the pre-transfusion tests.

Taking into account the results obtained in this study, we can conclude that for patients who take anti-CD38 MoAbs and require transfusions, interference in pre-transfusion tests is relevant, leading to false-positive results that may mask the presence of clinically significant antibodies and cause delays in the release of blood components. DTT treatment of reagent RBCs used in the antibody screening and identification has been shown to be simple and efficient to circumvent this interference and thus to guarantee a faster and safer transfusion to these patients.

Conflicts of interest

The authors declare no conflicts of interest.

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