Original article

New method for overcoming the interference produced by anti-CD38 monoclonal antibodies in compatibility testing

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Background - Plasma of patients taking anti-CD38 monoclonal antibodies (MoAbs) leads to panagglutination in the indirect antiglobulin test (IAT), that can mask clinically significant alloantibodies. Dithiothreitol (DTT) treatment of test RBCs is the more widespread method for avoiding this interference. Current DTT 0.2 mol/L method is time consuming and damages several red blood groups antigens. This study aims to evaluate low concentration DTT treatment of RBCs adapted for gel testing.

Materials and methods - Four DTT concentrations (0.01, 0.02, 0.03, and 0.04 mol/L), and three gel test brands were evaluated on six DARA patient's samples. Briefly, the method consists of pipetting 50 µL of 0.8% RBCs on AHG micro columns, followed by 25 µL of DTT, thoroughly mixing and 15 min incubation at 37 °C. Then, 25 µL of serum/plasma is added to proceed to IAT. In order to asses the effect of DTT 0.04 mol/L on different blood group antigens, serial dilutions of sera containing anti-K, -k, -Kp^b, -Lu^b, -Yt^a and anti-JMH antibodies were tested against DTT-RBCs. One sample of a DARA patient with known alloantibodies as well as samples of two patients inoculated with anti-K and anti-Fy^a were evaluated.

Results - RBCs treatment with DTT 0.04 mol/L for 15 min completely eliminated anti CD38 panagglutination in all samples studied and worked with different reactivity intensities in IAT and gel brands. The new method allowed the detection of underlying anti-D, anti-E, anti-K and anti-Fy^a alloantibodies. Titration assays demonstrated no denaturation of Kell, Lutheran, Cartwright and JMH antigens.

Discussion - The new DTT method adapted for gel testing is efficacious, simple and only adds 15 min over regular IAT. Pheno/genotyping before DARA treatment or transfusion of K negative RBCs may be unnecessary.

Keywords: *daratumumab, panagglutination, anti-CD38, DTT, gel test.*

INTRODUCTION

Plasma of patients taking an anti-CD38 monoclonal antibody (MoAb) as a treatment for multiple myeloma (MM) causes panagglutination in the indirect antiglobulin test (IAT) that can mask clinically significant alloantibodies. This is an important issue for patients that need blood transfusions since it produces a delay in releasing red blood cells (RBC). With an increasing number of patients being treated with these

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types of drugs, it is important to find the best way to avoid this interference.

CD38 is a glycoprotein found on the cellular surface of many tissues, as well as haematopoietic and immune system cells. It is also weakly expressed on normal RBC membranes. Anti-CD38 MoAbs bind cross-linked to test RBCs and cause panagglutination in IAT[1]. This pan reactivity can not be removed by regular adsorption/elution techniques[1]. There are several theoretical approaches for eliminating the interference in IAT[1-7], but up till now, the majority of transfusion services have used the Dithiothreitol (DTT) method.

Dithiothreitol, a reducing compound, denatures CD38 by cleaving the disulphide bonds[1]. As a consequence, DTT damages CD38 proteins but also other RBC antigens, resulting in the failure to detect antibodies against clinically relevant blood group systems that include: Kell, Lutheran, JMH, LW, Cromer, Indian, Knops, Dombrock, Cartwright, and Raph[8].

A detailed method for the treatment of RBCs with DTT is described in the American Association of Blood Banks (AABB) Technical Manual[9]. Around two hours are needed to complete pre-transfusion testing with DTT-treated RBCs.

Given this, we can conclude that, although DTT is effective, it is time-consuming and may miss some important alloantibodies.

Recently, Hosokawa *et al.*[4] made an important contribution to the field. By reducing the concentration of DTT from 0.2 mol/L to 0.01mol/L, and employing an IAT tube technique with an automated cell washing centrifuge, they reduced the time needed to complete the antibody screening and cross-match to around 60 min. In addition, this approach also reduces the denaturation of K antigen.

Worldwide, most blood transfusion services rely on gel testing and no longer use tube techniques or cell washing centrifuges in their routine procedures. Therefore, this study aims to evaluate a DTT technique to mitigate the panagglutination produced by anti-CD38 MoAbs therapy, adapted for gel-based IAT.

MATERIALS AND METHODS

Blood samples of six patients under daratumumab (DARA) therapy, submitted to our laboratory for compatibility workup, were included in the study. All samples were tested according to our routine IAT with 0.2 mol/L DTT-treated RBCs. For the study group, four different concentrations of DTT (0.01, 0.02, 0.03 and 0.04 mol/L) and three incubation times of RBCs with DTT at 37°C (15, 30 and 40 min) were assayed. All sera/plasma samples were also tested using three different brands of gel test cards.

Dithiothreitol treatment of red blood cells

One gram of DTT (Fisher Scientific, Merelbeke, Belgium) was diluted in 32 mL of phosphate-buffered saline (PBS) pH 8.0, to obtain DTT 0.2 mol/L. Aliquots of 2 mL were stocked at –20 °C until needed. Blood plasma/serum samples were submitted to standard gel IAT with 0.2 mol/L DTT-treated RBCs according to the method described in the AABB Technical Manual[9]. Briefly, this involves mixing one volume of PBS washed RBCs with four volumes of 0.2 mol/L DTT at pH 8.0, followed by incubation at 37 °C for 30-45 min. RBCs and DTT solution are manually mixed 4-5 times during incubation. After DTT treatment, RBCs are washed four times with PBS at pH 7.0, and packed. DTT-treated RBCs are then diluted to 3-5% or 0.8% with PBS, before undergoing IAT. All samples included in this study were tested in parallel following the new method.

The new method consists of treating test RBCs with low DTT concentrations directly in gel columns. RBCs along with DTT were incubated for 15-40 min at 37 °C. RBCs are not washed with PBS after DTT treatment. Plasma/serum is added to micro-columns immediately after DTT treatment to start the IAT.

Indirect antiglobulin test methods

Serum/plasma samples were screened for the presence of irregular antibodies using commercial RBC panels of 3-4 cells: ID-Diacell I-II-II (BioRad, DiaMed GmbH, Cressier FR, Switzerland), Serascan Diana 4 (Diagnostic Grifols S.A., Barcelona, Spain), and Across Gel® Cell Screen 4 (DiaPro, Istanbul, Turkey). For auto-control and cross-match RBC preparation, the corresponding LISS were used: ID Diluent 2 (BioRad, DiaMed GmbH), DG Gel Sol (Diagnostic Grifols S.A), and Across LISS Gel (DiaPro). Anti_Human Globulin (AHG) gel test cards of three different brands were used: Liss/Coombs ID Cards (BioRad, DiaMed GmbH), Anti-Human Globulin DG Gel 8 Anti-IgG (Rabbit) (Diagnostic Grifols S.A.), and Across Gel® AHG IgG+C3d (DiaPro).

Low DTT concentrations (0.01, 0.02, 0.03 and 0.04 mol/L) were prepared by diluting DTT 0.2 mol/L stock solution with PBS at pH 7.0.

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New dithiothreitol method procedure

The new method consists of pipetting 50 µL of 0.8% test RBCs to every AHG card micro-column, followed by the addition of 25 µL of low concentration DTT (0.01, 0.02, 0.03 or 0.04 mol/L) directly to the micro-column top. As a dilution factor control, in one micro-column we put 25 µL of PBS instead of DTT. After carefully mixing RBCs and DTT or PBS five times with the pipette tip, AHG cards are incubated at 37 °C for 15-30 min. Finally, 25 µL of plasma/serum is added, mixed thoroughly five times, and incubated for 15 min at 37 °C, followed by standard centrifugation and reading. Agglutination grading from 1+ to 4+ was used[10].

In order to assess the impact of this technique on DTT-sensitive blood groups, different concentrations of DTT were added to every IAT run, and three different antisera (anti-k, anti Kp^b and anti-Lu^b) were assayed in parallel to patients' sera.

Moreover, serial dilutions of commercial anti-k, anti-Kp^b, anti Lu^b and patients' sera with anti-K, anti-Yt^a, and anti-JMH alloantibodies were tested against RBCs treated with the highest DTT concentration assayed in this study (0.04 mL/L).

In order to demonstrate the ability of the new method to detect underlying alloantibodies, a serum sample of a patient on DARA therapy containing alloantibodies was included. Two more samples of patients under DARA treatment were inoculated with one specimen containing high-titre anti-K plus anti-Fy^a. Two DARA patients' samples were also inoculated with a serum specimen containing high-titre anti-K. Briefly, one volume of serum with known alloantibodies was added to two volumes of DARA patient's sera, and pan reactivity was demonstrated before submitting them for DTT testing.

RESULTS

Efficacy of different dithiothreitol concentrations in reducing daratumumab interference

There was a clear difference between gel test brands and panagglutination intensity of reaction. For every serum sample, the degree of reactivity was higher with Grifols or Accross AHG gel test, compared to BioRad.

Combinations of DTT concentrations from 0.01 mol/L to 0.04 mol/L, and incubation times with RBCs at 37 °C, from 15 to 40 min were assayed. The objective was to find a DTT concentration working for every gel test brand and a 15-min incubation time. As far as the efficacy of different DTT concentrations is concerned, samples tested with BioRad showed elimination of the interference with DTT 0.02 mol/L and 15-min incubation. Conversely, it was necessary to increase DTT concentration up to 0.04 mol/L and 15-min incubation for the complete elimination of pan reactivity of samples tested with Grifols or Across AHG gel test.

The combination of RBC treatment with DTT 0.04 mol/L directly on AHG gel cards and 15-min incubation at 37 °C was capable of eliminating the DARA interference of all patient's samples, regardless of the gel test brand used and degree of initial panagglutination.

Detection of underlying alloantibodies

One sample of a DARA patient with anti-D and anti-E alloantibodies, previously detected with the reference DTT 0.2 mol/L method, was assayed with the new method. Screening IAT showed pan reactivity with all RBCs, but two different intensities were observed: 4+ with cells I and II and 2+ with cell III (ID-Diacell panel) or III and IV (Grifols panel). Reactivity with cell III of BioRad and III and IV of Grifols disappeared after treatment with DTT 0.04 mol/L and 15-min incubation. Remaining reactivity with cells I and II revealed the presence of underlying alloantibodies. Antibody identification workup with an 11-RBC panel treated with the new DTT method revealed the presence of two alloantibodies of anti-D and anti-E specificities.

Two additional samples of patients with anti-CD38 interference, inoculated with anti-K and anti-Fy^a serum at a ratio of 2 volumes of DARA patient's sera to one volume of anti-K and anti-Fy^a serum were tested. Using DTT 0.04 mol/L and 15-min incubation, both alloantibodies were correctly identified. Two more samples of patients on DARA therapy were inoculated with serum containing high-titre anti-K. Samples were tested using 0.04 mol/L DTT and a 15-min incubation scheme and, again, alloanti-K was correctly identified.

Effect of dithiothreitol 0.04 mol/L on different blood group antigens

All low DTT concentrations tested (from 0.01 mol/L to 0.04 mol/L) preserved the antigens of different blood groups' systems known to be susceptible to high DTT (0.2 mol/L) concentrations, as shown in every run using anti-k, anti-Kp^b, and anti-Lu^b polyclonal antisera.

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Serial double dilutions of polyclonal anti-k, anti-Kp^b and anti-Lu^b antisera, as well as three patients' sera containing anti-Yt^a, anti-JMH and anti-K alloantibodies, were submitted to IAT with RBCs treated with 0.04 mol/L for 15 min at 37 °C. In parallel, the same antisera/sera dilutions were tested with untreated RBCs with the addition of 25 µL PBS to maintain the same RBC dilution factor.

These tests showed that anti-k, anti-Kp^b, anti-Lu^b, anti-K, anti-Yt^a, and anti-JMH titres with DTT-treated RBCs did not vary significantly compared to untreated RBCs, confirming that the new DTT method preserves clinically significant RBC group antigens. Even an alloantibody with a titre as low as 1 (anti-JMH) was detected with DTT-treated RBCs (**Table I**).

***Table I** - Titration of several antibodies against red blood cells untreated and treated with dithiothreitol 0.04mol/L*

Antibody	Titre without DTT	Titre after DTT
Anti-K	32	16
Anti-k	16	16
Anti-Kp^b	16	16
Anti-Lu^b	8	8
Anti-Yt^a	64	64
Anti-JMH	1	1

DTT: dithiothreitol.

Time-to-test results

The turnaround time (TAT) of the new method for an antibody screening with four cells was 45 min compared to the 120 min needed for the current DTT 0.2 mol/L technique; this is 75 min quicker. Therefore, the new method takes only 15 min more than a regular IAT.

DISCUSSION

This study demonstrates that the elimination of anti-CD38 interference can be perfectly accomplished with lower DTT concentrations (0.04 mol/L), while preserving Kell and other blood groups' antigens and reducing the TAT of pre-transfusion compatibility tests. From an economic point of view, this method significantly reduces the costs of the reagents and of laboratory technical staff.

One of the major problems for hospital transfusion services in performing compatibility tests for patients on anti-CD38 MoAbs therapy, is the long TAT of the current DTT 0.2 mol/L method. Furthermore, the current DTT method prevents detection and identification of alloantibodies of several blood group systems. DTT 0.2 mol/L also damages RBC membranes, producing visible haemolysis, and causing some RBC stroma to be trapped on top of gel columns, giving a false positive image.

The new method uses lower DTT concentrations so it does not cause as much damage to RBC membranes, thus preventing false positive images and haemolysis. Another advantage is the elimination of washing RBCs before and after DTT treatment, which significantly reduces the TAT.

Other methods have been proposed for eliminating the interference[1-7], such as anti-idiotype antibodies and CD38 soluble antigens; however, these are still not commercially available[2].

Recent research has been directed to the development of DARA Fab fragments[6,7] that block CD38 epitopes on the RBC membranes, overcoming DARA interference. DARAex (Imusyn; patent-pending) is a new technology based on DARA Fab fragments that mask CD38 antigens in RBCs. It is a very promising approach that also preserves the integrity of red blood group antigens. However, the major drawback of this method is the price, which makes it unaffordable for most blood transfusion services. Furthermore, DARAex pre-incubation of RBCs before IAT is about 30 min, while our method only needs 15 min. In addition, the usefulness of DARAex is probably restricted to patients taking DARA, and it is not known whether it works in patients treated with Isatuximab, MOR202, or other anti-CD38 MoAbs. On the other hand, our low concentration DTT method works with every anti-CD38 MoAb because it is based on direct CD38 glycoprotein denaturation and, therefore, is not affected by different MoAb binding affinities. Furthermore, the new method described here, is safe, fast and inexpensive.

By using this new DTT method, clinically significant blood group antigenicity will be preserved and complementary preventive strategies, such as full phenotyping/genotyping before daratumumab treatment, or the use of K negative RBCs[10], may not be necessary.

The Osaka Hospital group[4] was the first to describe the benefits of reducing DTT concentration for RBC treatment to overcome DARA interference. Although their DTT 0.01 mol/L method significantly reduces TAT compared to regular DTT 0.2 mol/L treatment, it is not useful for

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hospitals working exclusively with gel test technology. In Spain, many transfusion services no longer have tube technology in place, nor Coombs washing centrifuges in their laboratories. This situation is actually limiting their ability to appropriately manage transfusion of DARA patients.

Compared to our method, the TAT of the Osaka method is 15 min longer. Moreover, our study demonstrates that not only K antigen is unaffected by DTT 0.04 mol/L treatment, but also k, Kp^b, Lu^b, Yt^a, and JMH antigens are not denatured.

One limitation of our study is that we were unable to test the integrity of other red blood cell antigens because of the scarcity of sera of anti-LW, -Dombrock, -Knops, -Indian and -Raph specificities. Another limitation is that incubation of DTT 0.04 mol/L along with patient's sera, where the final DTT concentration reaches 0.01 mol/L, may diminish the ability to detect IgM alloantibodies; but this is only a theoretical inconvenience as long as compatibility testing is performed in IAT to look for clinically significant, IgG, alloantibodies.

CONCLUSIONS

The new method described here fulfils the needs of many transfusion services in eliminating the anti-CD38 interference while maintaining the ability to detect the vast majority of blood group alloantibodies. Following on from the Osaka method, we would like to name the method described in this article as the Valencia method.

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AUTHORSHIP CONTRIBUTIONS

EC developed the study concept and design, conducted the study, and also drafted the manuscript. dML and LL contributed to the revision and correction of the manuscript. CA is responsible for the critical revision of the final manuscript.

The Authors declare no conflict of interests.

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