Development of a Procedure to Resolve Daratumumab Interference in Pretransfusion Testing

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ABSTRACT

Multiple myeloma is an incurable disease characterized by the proliferation of malignant plasma cells in the bone marrow. Daratumumab (DARA), a monoclonal antibody that targets the CD38 antigen expressed on malignant myeloma cells, has been approved as a promising new treatment for patients with this disease. Although it is an effective medication, DARA presents challenges in transfusion medicine. Because normal red cells weakly express the CD38 antigen, panreactivity is observed during antibody-detection workups on patients treated with DARA; the panreactivity can mask the presence of any underlying alloantibodies. The American Association of Blood Banks (AABB) has issued a method on how to resolve DARA interference by managing reagent red cells with dithiothreitol (DTT), which cleaves the disulfide bonds of the CD38 antigen. The primary objective of this study was to develop a protocol for resolving DARA interference in pretransfusion testing at the University of North Carolina Medical Center's Transfusion Medicine Services based on the method described by the AABB. Optimal procedural conditions required 4 drops of DTT to 1 drop of packed phosphate-buffered saline–washed reagent red cells incubated at 37 °C for 45 minutes. An antibody screen using untreated and DTT-treated reagent red cells with appropriate quality-control results indicated that DTT was successful at eliminating DARA-induced panreactivity. A cost analysis was performed to consider the expenses and time required for the implementation of an in-house procedure.

ABBREVIATIONS: AABB - American Association of Blood Banks, ADCC - antibody-dependent cellular cytotoxicity, AHG - antihuman globulin, CDC - complement-dependent cytotoxicity, DARA - daratumumab, DTT - dithiothreitol, FDA - Food and Drug Administration, PBS - phosphate-buffered saline, PEG - polyethylene glycol, TAT - turnaround time, TMS - transfusion medicine services, UNC - University of North Carolina.

INDEX TERMS: antigen-antibody reactions, transfusion medicine, blood bank/methods, quality control.

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INTRODUCTION

Multiple myeloma is a neoplastic hematologic disease that involves the malignant proliferation of clonal plasma cells in the bone marrow.[1] This proliferation results in the production of a single class of immunoglobulins, which appears as a monoclonal gammopathy on immunofixation electrophoresis.[1] Clinical manifestations of multiple myeloma typically include the characteristic CRAB symptoms—hypercalcemia, renal insufficiency, anemia, and/or lytic bone lesions—all of which contribute to organ damage.[2] Current treatments, such as immunomodulatory medications and protease inhibitors, have greatly improved short-term survival, but this disease continues to show poor prognosis with a median survival rate of about 3–4 years.[1,3]

In 2015, the Food and Drug Administration (FDA) approved daratumumab (DARA), a humanized monoclonal antibody that targets a specific epitope on the CD38 glycoprotein that is highly expressed on malignant myeloma cells.[2,4] Through the mechanisms of antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), DARA is able to induce cellular apoptosis, which helps slow or stop the progression of multiple myeloma.[2]

Although DARA is a promising new treatment of multiple myeloma, panreactivity is a concern for patients in need of transfusion therapy.[4] During antibody screening workups, the anti-CD38 monoclonal antibody present in the serum of patients treated with DARA attaches to the CD38 antigen weakly expressed on the extracellular domain of normal reagent red cells, which results in 1+ or 2+ reactions with all screening cells.[5] The panreactivity observed during antibody-detection workups may persist for up to 6 months after the last administration of DARA.[6] Case studies have revealed that this panreactivity has been mistaken for a high-titer and low-avidity–like antibody

without specificity, multiple alloantibodies, or an antibody to a high-prevalence antigen.[5,7] The results from these case studies demonstrate how interference from DARA can manifest as other clinically significant antibodies and, ultimately, mask the presence of underlying alloantibodies, if present.

Fortunately, the use of dithiothreitol (DTT) to manage screening red cells is a known method to resolve the interference seen in pretransfusion testing. DTT disrupts the disulfide bonds of the CD38 antigen expressed on red cell surfaces.[8] Once DTT denatures extracellular CD38, DARA in the patient's serum is unable to bind to the treated reagent red cells, thus eliminating the weak reactions seen during antibody-detection procedures.[8] This allows for the detection of underlying alloantibodies, except for antibodies in the Kell and Knops blood group system and most examples of anti-LW^a, -Yt^a, -Yt^b, -Do^a, -Do^b, -Gy^a, -Hy, and -Jo^a, because DTT also cleaves the bonds of the corresponding antigens.[9]

The American Association of Blood Banks (AABB) has issued a method that outlines the use of DTT to eliminate DARA interference.[9] Previous studies have corroborated the suitability of the AABB's method and the effectiveness of DTT in serologic problem solving. Clinical sites participating in 2 such studies observed in vitro panreactivity during antibody screening with untreated screening cells.[8,10] When screening cells were managed with DTT, panreactivity was eliminated, and personnel were able to identify underlying alloantibodies. The use of flow cytometry indicated that instances of DARA binding to DTT-treated CD38$^+$ red blood cells were significantly reduced.[8,10]

The implementation of in-house DTT testing for pretransfusion workups is critical in patient-care improvement and turnaround time (TAT). Prior to the completion of this study, the Transfusion Medicine Services (TMS) at University of North Carolina (UNC) Medical Center protocol required establishment of a baseline antibody status for each patient prior to the first administration of DARA. If a patient had been transfused within the last 3 months, their sample was sent out to a reference laboratory for a molecular genotype. After DARA administration, the panreactivity seen in the gel and polyethylene glycol (PEG) antibody screens was identified as a nonspecific antibody, and patients were transfused at the discretion of their physician.

The nonspecific antibody identification does not allow for differentiation between DARA panreactivity and potential underlying alloantibodies. Therefore, this protocol is inadequate and limited by any transfusions that patients treated with DARA receive after the antibody baseline is established. Implementing an in-house DTT-treatment protocol would eliminate this challenge. The primary objective of this study was to develop an in-house protocol for the TMS laboratory at UNC Medical Center based on the method outlined by the AABB. Additionally, a cost analysis was performed to evaluate the expenses and time required for the implementation of such a procedure.

MATERIALS AND METHODS

Treatment of Cells with DTT

The method of DTT treatment outlined in the 19th edition of the AABB technical manual was followed.[9] Commercially available 0.2M DTT was purchased and prepared by dissolving 1g of DTT powder into 32 mL of phosphate-buffered saline (PBS). There were 6 drops each of Immucor 3% screening cells I, II, and III added to 3 different test tubes and washed once with PBS. Each of the PBS-washed screening cells had 1 drop transferred to another appropriately labeled test tube. To obtain a 4 to 1 ratio of DTT to screening cells as described by the AABB, 4 drops of DTT were added to each tube. The DTT cell solutions were incubated at 37 °C with inversion every 5 minutes. There were 2 runs completed with varied incubation times (30 and 45 minutes). Once incubation was complete, the samples were washed 4 times with PBS. After the last wash, the cell buttons of the DTT-treated screening cells I, II, and III were resuspended in PBS to obtain a 3% cell suspension.[9]

PEG-Antibody Screen

Antibody screening with serum from the patients treated with DARA was performed using both DTT-treated and untreated Immucor reagent screening red cells. In a 12×75-mm tube, 1 drop of untreated 3% reagent red cells was added to 2 drops of patient serum and 2 drops of Immucor PEG potentiating medium. Tubes were incubated at 37 °C for 15–30 minutes and washed 3 times with physiologic saline. There were 2 drops of Immucor antihuman globulin (AHG) added, and the tubes were centrifuged and read macroscopically for agglutination and hemolysis. For quality-control purposes, check cells were added to all tubes that exhibited negative reactions in the AHG phase. Subsequently, the same antibody screening procedure was followed using the DTT-treated 3% reagent red cells. An autocontrol composed of patient serum and untreated 3% patient red cells was also included.

Quality Control

The DTT-treatment method outlined in the AABB technical manual states that DTT-treated K$^+$ red cells should be tested with anti-K antisera for quality-control purposes because DTT can destroy the K antigen of the Kell system.[9] This procedure used 3 reagent red cells: a panel cell heterozygous for K (K$^+$k$^+$) as the positive control, a panel cell negative for K (K$^-$k$^+$) as the negative control, and a screening cell heterozygous for K (K$^+$k$^+$). Screening cell II (K$^+$k$^+$) was treated with DTT, and then a phenotype for the K antigen was calculated using the positive and negative controls according to the manufacturer's instructions. One drop of Immucor anti-K antisera was mixed and incubated for 5 minutes at room temperature with 1 drop of the appropriate cell suspension. After incubation,

the tubes were centrifuged, resuspended, and read macroscopically for agglutination.

Cost Analysis

A cost analysis was performed to determine the overall expenses and time of the DTT-treatment protocol. The volume of each reagent and the number of consumables required were considered. Once these data were obtained, the breakdown per drop of reagent and per consumable were calculated from the price information available in the UNC TMS laboratory. Indirect costs were also included and accounted for approximately 30% of the total cost of reagents and consumables.

RESULTS

PEG-Antibody Screen

DTT-treated and untreated screening cells were tested in the PEG-antibody screen for comparison purposes. Many of the results from testing serum of patients treated with DARA showed panreactivity with untreated cells and negative reactions with DTT-treated cells in the AHG phase (Table 1). Other results were not entirely panreactive with the untreated cells, but differences in reactivity patterns were seen with the DTT-treated screening cells (Table 1). The autocontrol had negative results for all patients treated with DARA. All reactions with check cells were appropriate.

Table 1. Summary of antibody screening of patients treated with DARA

Patient	Antibody Screen With Untreated Reagent Cells (AHG Phase)		Antibody Screen With DTT-treated Reagent Cells (AHG Phase)		Autocontrol
1	SC I	1+	SC I	Negative	Negative
	SC II	1+	SC II	Negative	
	SC III	1+	SC III	Negative	
2	SC I	1+	SC I	Negative	Negative
	SC II	Negative	SC II	Negative	
	SC III	1+	SC III	Negative	
3	SC I	Negative	SC I	Negative	Negative
	SC II	Weak	SC II	Negative	
	SC III	1+	SC III	Negative	
4	SC I	1+	SC I	Negative	Negative
	SC II	1+	SC II	Negative	
	SC III	1+	SC III	Negative	

SC, screening cell.

Quality Control

The DTT-treated screening cell II (K^+k^+) showed no reaction with anti-K antisera when the cells were incubated with DTT for 45 minutes. The controls exhibited appropriate reactions (Table 2) and were valid for patients 1–4. However, when screening cells were incubated with DTT for 30 minutes, a weak positive reaction was seen (Table 3). Patient samples that were tested with DTT-treated screening cells and were incubated for only 30 minutes were not included as part of the data because the quality-control outcome invalidated those results.

The DTT-treatment protocol outlined in the AABB technical manual provided the most reliable guideline for this workup. Based on the results of the quality-control procedure, it was determined that 4 drops of DTT to 1 drop of packed PBS-washed screening cells incubated at 37 °C for 45 minutes was the optimal set of conditions needed for complete effectiveness. Under these conditions, hemolysis was not observed during any part of the procedure.

Cost Analysis

The cost analysis was divided into reagent costs (Table 4) and supply costs (Table 5). After considering the price of reagents, consumables, and indirect expenses (30% of direct costs), the total cost of performing the DTT-treatment procedure was about $54. The procedure took approximately 1 hour and 53 minutes to perform in its entirety.

DISCUSSION

Multiple myeloma involves the malignant proliferation of clonal plasma cells and is a difficult neoplastic disease to

Table 2. Quality-control results with anti-K, 45-minute incubation with DTT

Anti-K Plus	Reaction	Interpretation
Positive control (K^+k^+)	4+	Positive
Negative control (K^-k^+)	0	Negative
DTT-treated SC II (K^+k^+)	0	Negative

SC, screening cell.

Table 3. Quality-control results with anti-K, 30-minute incubation with DTT

Anti-K Plus	Reaction	Interpretation
Positive control (K^+k^+)	3+	Positive
Negative control (K^-k^+)	0	Negative
DTT-treated SC II (K^+k^+)	Weak	Positive

SC, screening cell.

Table 4. Reagent cost breakdown

Reagent	Reagent Cost	Unit Volume (mL)	Drops per Unit	Cost per Drop	Number of Drops Used	Volume Used (mL)	Total Cost
DTT	$53.58	2	40	$1.40	12	N/A	$16.80
SC I	$60.00	10	200	$0.30	7	N/A	$2.10
SC II	$60.00	10	200	$0.30	7	N/A	$2.10
SC III	$60.00	10	200	$0.30	7	N/A	$2.10
PEG	$21.00	10	200	$0.11	14	N/A	$1.47
Anti-immunoglobulin G	$28.50	10	200	$0.14	14	N/A	$1.96
Check cells	$32.00	10	200	$0.16	3	N/A	$0.48
Anti-K antisera	$250.00	5	100	$2.50	3	N/A	$7.50
K^+ panel cell	$30.00	5	100	$0.30	1	N/A	$0.30
K^- panel cell	$30.00	5	100	$0.30	1	N/A	$0.30
PBS	$20.00	1000	N/A	N/A	N/A	226	$5.42
Total	N/A	N/A	N/A	N/A	N/A	N/A	$40.53

N/A, not applicable; SC, screening cell.

Table 5. Supply cost breakdown

Supply	Cost per Individual Supply	Number of Supplies Used	Total Cost
12×75-mm test tubes	$0.02	13	$0.26
Transfer pipets	$0.03	21	$0.63
Total	N/A	N/A	$0.89

N/A, not applicable.

treat.[1] Current medications have greatly improved short-term survival, but patients with this disease continue to exhibit a poor prognosis with a median survival rate of 3–4 years.[1,3] With its FDA approval in 2015, DARA has proven to be a viable treatment option. This humanized monoclonal antibody targets the CD38 antigen highly expressed on malignant myeloma cells and induces apoptosis through ADCC and CDC.[2,4] However, DARA presents an unanticipated interference in that it attaches to the CD38 antigen weakly expressed on normal red cells. This results in panreactivity during pretransfusion workups on patients treated with DARA.[4,5] Fortunately, the use of DTT to manage reagent red cells irreversibly reduces the disulfide bonds of the CD38 antigen to free sulfhydryl groups, which effectively eliminates DARA's attachment.[9] Implementation of an in-house DTT-treatment protocol would resolve the interference that DARA presents during pretransfusion testing.

The primary aim of this study was to develop a protocol for resolving DARA interference in UNC Medical Center's TMS laboratory based on the method described by the AABB. As outlined by the AABB, 4 drops of DTT to 1 drop of packed PBS-washed screening red cells was the optimal ratio; no hemolysis was observed visually. The incubation time of 45 minutes ensured complete uptake of DTT, as seen from the negative reactions with DTT-treated screening cells and the expected results of the quality control (Tables 1 and 2). Cells incubated at 30–35 minutes produced invalid quality-control results, in which phenotyping reactions with the K^+ screening cells were positive (Table 3).

The quality-control procedure was most significant in ensuring all results were valid. Because DTT cleaves the disulfide bonds of the K antigen, reactions with K^+ DTT-treated cells and anti-K antisera should be negative. A negative result enables one to make the highly supported assumption that DTT was also effective at disrupting the disulfide bond of the CD38 antigen on reagent red cell surfaces. Phenotyping the K^+ DTT-treated screening cell showed that the K antigen was no longer present on the extracellular surface; the positive and negative controls validate that claim (Table 2). From the quality-control results, it can be assumed that the elimination of panreactivity in the PEG-antibody screen with DTT-treated cells was because of the removal of the CD38 antigen.

The results of the PEG-antibody screen on untreated vs DTT-treated screening cells revealed the true underlying antibody status. Once DTT eliminated DARA-induced panreactivity, the patients showed negative reactions with screening cells I, II, and III, indicating that no alloantibodies were present in the patient serum (Table 1). It is significant to note that patient 2 and patient 3 did not show initial panreactivity with the untreated screening cells; however, it cannot be assumed that this pattern is a result of the presence of alloantibodies to any of the screening cells because the DTT-treated results show negative reactions (Table 1). Differences in initial reaction strengths of patients 2 and 3 may be because of a lower concentration of DARA present in these patients' serums, as compared with the serums of patients 1 and 4 who exhibited

panreactivity with untreated reagent red cells. The autocontrols of all patients had negative results, but—based on the panreactivity observed with untreated red cells—it is likely that one would predict a positive result for the autocontrol. Although this phenomenon is not completely understood, there is evidence to suggest that DARA is able to induce CD38 loss on the patients' red cell surfaces, which results in the negative autocontrol.[11]

The secondary goal of this study was to perform a cost analysis for the implementation of an in-house protocol. Cost considerations of this study suggested that additional expenses of this procedure were relatively low, with a total cost of approximately $54 and a total performance time of under 2 hours. Prior to implementing the DTT method, protocol required a molecular genotype and fully phenotypically matched donor cells. With the implementation of the DTT method, compatibility testing is done with treated donor cells and is expected to be compatible. If a patient is K^- or a K phenotype is unavailable, then antigen negative units are selected. The in-house DTT-treatment protocol improves TAT and hospital care for patients treated with DARA. Without this protocol, send-outs and physician-approved transfusions are the alternatives. Send-outs for molecular genotypes with patients who have been recently transfused delays treatment. Results from send-outs may take several hours or even days to receive, as opposed to the 1 hour and 53 minutes that in-house testing requires. Additionally, physician approval is required when compatibility testing is compromised by DARA-induced panreactivity, in which a nonspecific antibody is identified as the underlying cause. This may negatively impact the care that patients receive, and the patient may be at risk of developing an alloantibody or experiencing an adverse transfusion reaction because the panreactivity masks alloantibodies that may be present.

Limitations of this study include narrow testing of the optimal incubation time and lack of data about the patients' DARA concentrations. The incubation time to determine complete cellular uptake of DTT was only examined using 30- and 45-minute time periods. To obtain a more accurate picture of optimal procedural conditions, future studies should include a time-dependent experiment that explores several different incubation periods in relation to the effectiveness of DTT's ability to denature the CD38 antigen. Second, DARA concentrations in the patients' serum were not measured to account for differences in reaction strengths among patients (Table 1). Hemagglutination discrepancies have been observed when investigators detected dose-dependent reaction-strength patterns in the AHG phase of antibody testing on human serum spiked with varying concentrations of DARA.[6] In future studies, a timeline of DARA administration and subsequent dosing could help establish a broader clinical picture of the relationship between concentration and reaction strengths seen during pretransfusion workups on patients treated with DARA.

Although DTT is a viable resolution to pretransfusion antibody discrepancies, the cost analysis performed in this study is only specific to the UNC TMS laboratory and may vary among institutions. In addition, this procedure cannot be used to rule out antibodies to antigens in the Kell and Knops blood group system and most examples of LW^a, Yt^a, Yt^b, Do^a, Do^b, Gy^a, Hy, and Jo^a because DTT cleaves the disulfide bonds of these glycoproteins.[9]

CONCLUSION

The introduction of DARA into medicine has presented challenges for pretransfusion testing. However, a method for using DTT to manage reagent red cells provides an effective guideline for hospitals and laboratories seeking to implement an antibody-detection methodology that eliminates DARA-induced panreactivity. With the addition of novel monoclonal-antibody treatments for multiple myeloma, interferences in clinical laboratory testing may become more frequent. For example, other therapeutic monoclonal-antibody medications, such as elotuzumab, may interfere with serum immunofixation results.[2] It is up to the laboratory personnel to find procedural techniques to help resolve interferences seen in clinical testing, so that patients receive accurate test results and the highest quality care.

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