EUROPEAN HEMATOLOGY ASSOCIATION

Challenges in blood transfusion - Section 2

Challenges in typing and matching strategies in patients with hematological malignancies in the era of immunotherapy

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Take-home messages

- CD38 is weakly expressed on human erythrocytes. Therapeutic CD38-targeting antibodies interfere with routine pre-transfusion laboratory tests, complicating the selection of compatible red blood cells (RBCs) for transfusion.
- Reported mitigation strategies to overcome the interference have different advantages and disadvantages.
- The provision of RBCs can be significantly delayed if protocols are not in place to communicate this interference with compatibility testing to patients, laboratory staff, and physicians in a timely manner.

Introduction

Multiple Myeloma (MM) is a plasma cell malignancy that represents approximately 1% of all neoplasia and about 15% of hematological cancers.[1,2] It is characterized by the proliferation of monoclonal plasma cells in the bone marrow, with a consequent increase in monoclonal immunoglobulins in the serum and/or urine and organ damage including bone lytic lesions, renal impairment, hypercalcemia or anemia.[2] Over the last decade, the survival of MM patients has significantly improved due to the application of autologous stem cell transplantation, the introduction of proteasome inhibitors and immunomodulatory drugs. However, most patients die from refractory disease.[3,4]

Innovative treatments with little toxicity and favorable tolerability are needed and immunotherapeutic strategies are emerging as therapeutic approaches in MM, with several monoclonal antibodies (mAbs) targeting cell surface markers such as SLAMF7 (CS-1) and CD38.[4] An important advantage of mAbs is their specific targeting. However, since many laboratory tests are also based on specific antibody-antigen interactions, mAb interference in laboratory medicine is considered an increasing problem.[5] In trials with the anti-CD38 mAb daratumumab, all patients demonstrated panreactivity in red blood cell (RBC) panel testing,[5,6] complicating the selection of compatible RBCs for transfusion.

Current state of the art

Daratumumab (Darzalex™), developed in 2012, is a humanized mAb that binds CD38-expressing malignant cells with high affinity, inducing tumor cell death through diverse mechanisms of action.[3,4,7] Intravenous daratumumab has been approved for patients with MM who have received at least three prior lines of therapy, including a proteasome inhibitor and immunomodulatory agent or who are double-refractory to both. In addition to daratumumab, two other CD38-specific antibodies are in clinical development: isatuximab and MOR202.[3]

Oostendorp *et al*.[5] and others[6,8] showed that treatment of MM patients with daratumumab results in false positive indirect antiglobulin tests (IATs) for 2-6 months after infusion (Figure 1). Daratumumab causes agglutination in a dose and interval dependent manner, also observed with isatuximab and MOR202.[5] This interference is due to weak expression of CD38 on erythrocytes.[5,6] Adsorptions using enzyme-treated or untreated RBCs fail to remove the interference, putatively due to low expression of intact CD38 antigen on the adsorbing RBCs.[6] Contradictory results were reported for direct coombs testing (DAT) in daratumumab-treated patients, some reporting only negative DATs[5] (suggesting that RBC's with sufficient IgG coated on the surface have been removed from the circulation) others reporting patients with IgG positive DATs.[6,8] However, no laboratory signs of chronic hemolysis are found in daratumumab-treated patients.[6]

Daratumumab infusion results in a mild, temporal hemoglobin decrease of approximately 1.6 g/dL and an increase in reticulocyte count, but no relevant anemia.[5] This is likely not due to complement-mediated lysis, but due to Fc-receptor-mediated clearance in the spleen.[5] It has been hypothesized that only a small number of RBCs have sufficient CD38 density to allow relevant daratumumab binding, resulting in *in vivo* clearance

and *in vitro* IAT interference.[5,9] Detection of irregular antibodies in the plasma of daratumumab-treated patients is masked for up to six months after the last infusion. It therefore hinders routine pre-transfusion testing and complicates the selection of suitable RBC units.[5,6] However, thus far no major transfusion related events have been observed in daratumumab-treated patients.[5]

Since the first reports on anti-CD38 interference, different solutions have been presented, each with its own (dis)advantages.[10] Oostendorp *et al*.[5] reported the use of an in-house developed sCD38 extracellular domain protein (sCD38) as a generic mitigation option to prevent false-positive IATs. sCD38 was shown to block daratumumab and interference by other anti-CD38 mAbs, and allowed correct identification of known irregular antibodies. Addition of excess in-house developed anti-idiotype daratumumab antibody to both daratumumab-spiked plasma and plasma of daratumab-treated patients, also abrogated the interference and successfully restored antibody screening and identification.[5] An advantage of these neutralization methods is that, if freely available, they provide a fast and uniform way to deal with the interference. Suitable for every laboratory, since routine techniques for antibody screening, identification and crossmatching can be used. Disadvantages are higher reagent costs and yet a lack of widespread availability of the reagents.[6] In addition, a more thorough clinical validation of these assays is needed.

CD38 on RBCs is sensitive to denaturation by dithiothreitol (DTT) and Chapuy *et al.*[6] showed that treating reagent RBCs with DTT negates the daratumumab interference and allows alloantibody identification. Because DTT also denatures Kell antigens, K negative units should be selected for these patients.[6] An advantage of this method is that DTT is inexpensive and already used in immunohematological reference laboratories.[6] Drawbacks are the disruption of a limited number of blood group antigens[6] and difficulties performing this method in routine laboratories.[8]

Schmidt *et al*.[11] were able to rule out significant RBC antibodies in daratumumab-treated patients by use of cord RBC panels. They concluded that these cells have extremely low CD38 on their membrane and are thus useful for antibody screening

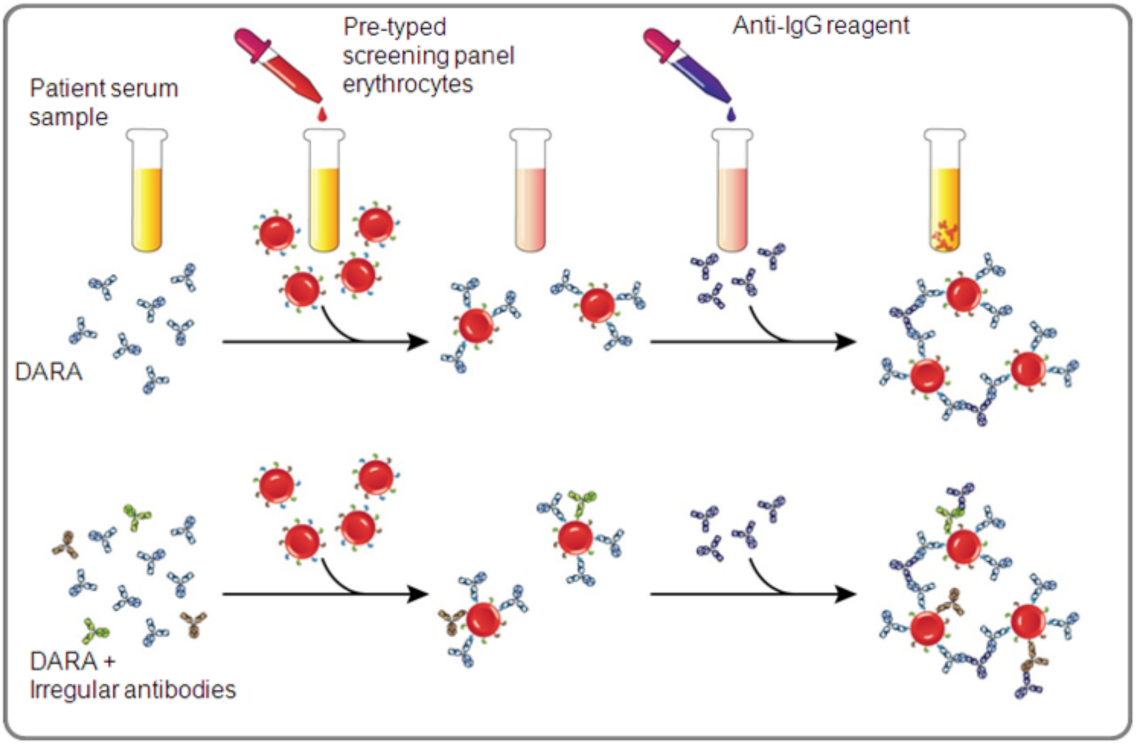

Figure 1. Interference of daratumumab in indirect antiglobulin test. Daratumumab (DARA) in the patient's serum binds to the test RBCs. After adding the anti-IgG reagent, RBC agglutination is observed, thereby generating a false positive result. The presence of irregular antibodies is masked by the presence of daratumumab.[5] Reproduced from Oostendorp *et al.* Transfusion 2015;55:1555-62; with permission.

in daratumumab-treated patients.[11] However, this method depends on the availability of cord blood RBCs, which could be a problem in some countries.[9]

Hannon *et al*.[8] described the successful application of antigen typing, allowing for selection of antigen-matched units, in a clinical daratumumab trial with six patients requiring transfusion. Although this strategy prevents mismatching for and irregular antibodies against the most common blood groups, it is time consuming. Often only a limited number of matching donors are available, resulting in shortage of compatible RBC units if the blood loss is too extensive. Furthermore, the presence of other irregular antibodies cannot be excluded due to anti-CD38 mAb induced positive cross-matching results.[10]

Besides implementation of mitigation strategies in the laboratories, patients should be provided with a blood group card alerting physicians on their anti-CD38 use to prevent unnecessary delays.[5,6,9,11,12] It may be prudent to have clinicians notify the blood bank (whether or not by HIS/LIS connection) when patients receive daratumumab, to prevent the laboratory from spending unnecessary time and resources in evaluating these samples.[11] In addition, before starting daratumumab, a serum screen for irregular antibodies is recommended.

Future perspectives

Immunotherapeutic strategies are emerging as promising therapeutic approaches in MM, with several monoclonal antibodies being in advanced stages of clinical development. CD38-targeting antibodies interfere with blood compatibility testing and thereby complicating safe transfusion. The development and availability of a neutralization reagent will probably help routine laboratories most, since it can be integrated into standard serological techniques. The provision of RBC units can be significantly delayed if protocols are not in place for communicating this interference to patients, laboratory staff, and physicians in a timely manner. Additionally, laboratories should have a protocol on how to deal with the interference and select compatible RBC units. As CD38 antibodies may have a role in the treatment of diseases beyond hematological malignancies, including solid tumors and antibody-mediated autoimmune diseases[3], many physicians and laboratory staff are likely to encounter this issue in the near future if they have not done so already.

References

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This is an excellent and extended review on CD38 as an emerging therapeutic target for the treatment of hematological malignancies, in particular multiple myeloma. The expression of CD38 in disease, as well as the use of CD38 mAbs, their mode of action, their use in treatment of multiple myeloma, clinical studies and the interference they cause in clinical laboratory assays are discussed.

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This is the first study, together with the back-to-back in Transfusion published report of Chapuy et al.,[6] reporting on the interference of anti-CD38 mAbs with blood compatibility testing. The authors show that this interference can be diminished by use of neutralizing reagents, i.e. anti-CD38 specific anti-idiotype antibody and the more generic solution recombinant soluble CD38.

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In this excellent review the clinical efficacy of mAbs targeting CD38 and SLAMF7 in MM is summarized. In addition, the emerging laboratory interferences they cause, with respect to immunofixation, serum protein electrophoresis assays, flow cytometric evaluation and blood compatibility testing are extensively discussed.