Management of daratumumab interference in Portuguese blood transfusion medicine: A literature review about unmet needs and challenges

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ABSTRACT

Multiple Myeloma is a malignant disease of the bone marrow plasma cells that mainly affects older people, and whose prognosis is reserved on relapsing or refractory disease. The arrival of CD38 monoclonal antibody daratumumab into the market allowed an increase in overall survival rate, with limited associated toxicity, for pretreated multiple myeloma patients. However, this antibody interferes with pre-transfusion tests since erythrocytes also express, even at reduced levels, CD38 receptors. A literature review and discussion about this topic was performed by a board of Portuguese Immuno-hemotherapy experts. Based on the literature review, the mechanisms by which daratumumab interferes with pre-transfusion tests and may mask the presence of irregular antibodies in plasma of treated patients, which may result in difficulties in preparing and delivering results and blood components to be transfused, were identified and discussed. In addition, the applicable strategies in clinical practice to mitigate this interference were identified, namely the denaturing method of CD38 with dithiothreitol, considered an inexpensive, reproducible, reliable and validated technique, although with logistic limitations and should not be ignored. The experts also assessed and discussed the main concerns and priorities of transfusion medicine services, considering the limitations of this technique and its implication in routines for transfusional support. The experts agreed on the importance of communication between physicians, hematologists, immuno-hemotherapy specialists, nurses and patients, as well as the need to align all methodologies to develop global and universal protocols to the various clinical analysis laboratory centers.

Keywords: Blood tests, Daratumumab, Dithiothreitol, Transfusion Medicine

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INTRODUCTION

Multiple myeloma (MM) is a malignant blood cancer that results from the infiltration of abnormal clonal plasma cells into the bone marrow and immune evasion [1]. Risk factors include increased age, male gender, family background and mostly genetic modifications, such as chromosomal translocations and hyperdiploidy [1, 2]. In patients with disease double refractory to a proteasome inhibitor (PI) and an immunomodulatory drug (IMiD), life expectancy is around nine months [3].

Over the last years, researchers have put great effort on the clinical development of innovative and more efficacious drugs, such as immunomodulators (e.g. lenalidomide), proteasome inhibitors (e.g. bortezomib), histone deacetylase inhibitors (e.g. panobinostat), and monoclonal antibodies (e.g. daratumumab, elotuzumab) [3, 4]. Many studies have focused on CD38, a 46-kDa type II multifunctional transmembrane glycoprotein that plays an important role in hematopoiesis, from both lymphoid and myeloid origin, such as chronic lymphoproliferative leukemia and myeloma [5, 6]. However, CD38 receptors are also present, with lower expression, in normal cells, such as red blood cells (RBCs), myeloid cells and platelets [7, 8]. Human CD38 acts as an enzyme with cyclase and hydrolase activities, mediates lymphocyte endothelial adhesion and promotes proliferation of both T and B immune cells [3, 9, 10]. Thus, the presence of this receptor is recognized as a prognostic factor and a potential therapy target as its overexpression is associated with decreased immune function and disease progression in patients with myeloma [11, 12].

Daratumumab, a fully human IgG1 monoclonal antibody, approved by the US Food and Drug Administration (FDA) as monotherapy for the treatment of heavily pretreated adult patients with RRMM, [13] and in combination with lenalidomide and dexamethasone or bortezomib and dexamethasone, for the treatment of adults’ patients with multiple myeloma who have received at least one prior therapy. Lately, it has been detected that daratumumab interferes with laboratory tests, such as blood compatibility testing (direct and indirect antiglobulin testing) and immunofixation electrophoresis (IEF) [3, 12]. Currently, other CD38 monoclonal antibodies are under clinical development (e.g. isatuximab and MOR202) and as these bind CD38 receptors, the same interference is expected/observed [3, 12].

Due to the aggressive nature of MM, transfusions are frequently at therapeutic option for patients with relapsed/ refractory disease. Therefore, interference with blood compatibility tests is a concern among health care professionals, mainly Clinical Hematologists and Oncologists, as it may lead to delays in the delivery of results and conditions for transfusion. This led to an intense search for strategies and methods that can mitigate those interferences. The aim of this review is to present the current “state of art” on this topic and contribute to a more informed discussion and decisions about management of daratumumab interference in blood transfusion tests.

METHODS

The present report resulted from a bibliography review and further discussion in an expert meeting held in Lisbon, in September 2016. Portuguese consultant members of Immuno-hemotherapy field, from reference hospitals and clinical analysis laboratories in Portugal, took part in this meeting. Literature review allowed the collection of information for the discussion preparation, mainly about MM (i.e., epidemiology, clinical manifestations, natural history of the disease and currently available therapeutic options), as well as treatment with daratumumab (i.e., drug class, mechanism of action, efficacy and safety data, and interference with blood compatibility testing).

RESULTS

The hemotherapy experts focused the discussion on the mechanisms by which daratumumab interferes with blood compatibility tests and the possible methods and challenges to counteract this interference, bringing the following questions to discussion:

1. Which analysis should be done before treatment with daratumumab?
2. Which analysis should be done during treatment with daratumumab, and before transfusion?
3. What information should be shared with the transfusion medicine services?
4. What information should be shared with Hematologists/ Clinicians?
5. Should the centers that operate with dithiothreitol (DTT) method be centralized?

Multiple Myeloma: Epidemiology, clinical presentation and available treatments

MM accounts for 0.8% of all cancers, with an incidence of 114,000 cases and 80,000 deaths worldwide [14]. MM is a very complex and heterogeneous cancer, which involves several genetic pathways, responsible for its progression and poor prognosis. For example, deregulation of G1/S transition and of tumor suppressor genes, like the loss of TP53 gene, contribute to MM progression and aggressiveness [1, 2]. Disease prognosis is based on fluorescence in situ hybridization (FISH)
technique to evaluate genetic events, such as gain of 1q, loss of 17p and the adverse translocation groups t(4;14), t(14;20) and t(14;16) [1, 2]. In terms of clinical presentation, the bone marrow microenvironment is affected by an abnormal infiltration of clonal plasma cells, deregulating the immune system [2]. The clinical symptoms of MM include lytic bone lesions, anemia, hypercalcemia and renal failure [1].

Currently, there are several drugs available for newly diagnosed MM and relapsed disease due to the advances in clinical research and to a deeper knowledge of the underlying biology of multiple myeloma. Briefly, the most representative groups of drugs for MM are alkylating agents, corticosteroids, immunomodulatory agents, proteasome inhibitors, histone deacetylase (HDAC) inhibitors and monoclonal antibodies. In fact, monoclonal antibodies have demonstrated to be a successful target therapy for MM while promoting immunomodulatory effects that prevent cancer recurrence, however with an high heterogeneity of results [3, 11]. In particular, the fully human anti-CD38 daratumumab, which binds strongly to a specific epitope of this overexpressed receptor in myeloma cells, has been clinically assessed, successfully, in monotherapy for patients with relapsed and refractory MM, as well as in combination with bortezomib and dexamethasone or lenalidomida and dexamethasone for patients who have received one or more previous lines of therapy [15, 16].

Daratumumab main mechanisms of action are: complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), direct tumor apoptosis and modulation of the enzymatic activity of CD38 [11, 12, 17]. Moreover, daratumumab shows immunomodulatory effects through reduction of CD38-positive regulator immune suppressor cells with subsequent induction of T Cell expansion [12].

Usmani and colleagues hypothesized that daratumumab may enhance the patients response to subsequent treatments. An additional possibility is that, due to the tolerability of daratumumab, patients may be afforded an opportunity to recover from the stress of prior therapies, allowing them to receive subsequent therapies at higher doses or in a more aggressive dosing schedule [14]. Additionally, this drug can be used in special populations, such as patients with liver or renal impairment, since it is not metabolized by the kidney and no dose modifications are necessary in mild hepatic disease [14].

Anti-CD38 monoclonal antibodies appear to be a great opportunity for MM targeted treatment, as well as for other hematologic malignancies (e.g., Non-Hodgkin’s lymphoma). While several of these are being developed, and are under preclinical and clinical studies, it has been determined that some monoclonal antibodies (e.g., siltuximab, rituximab, infliximab, cetuximab, trastuzumab, bevacizumab, adalimumab and ofatumumab) can interfere with serum protein electrophoresis (SPEP), immunofixation electrophoresis (IFE), flow cytometry and blood compatibility tests [3, 18]. Particularly, it has been observed that CD38 monoclonal antibodies, such as daratumumab, can interfere with blood compatibility testing, such as, direct and indirect antiglobulin tests (DATs and IATs), causing false-positives due to the antibody direct binding to the low-expressed CD38 receptors on RBCs (in vitro pan-reactivity) [8].

**Daratumumab interference with laboratory testing**

As aCD38 monoclonal antibody, daratumumab binds to erythrocytes, as these also express CD38 surface cell antigen, although at lower levels [19]. Therefore, binding of daratumumab to RBCs may mask the presence of irregular antibodies in patients’ plasma, such as the ones for K and E antigens [8]. Also, clinically non-significant decreases of Hb have been reported, although adverse events encountered with daratumumab infusion did not include anemia or hemolysis and patients did not require blood transfusion. This may be due to rapid clearance of a subpopulation of daratumumab-coated erythrocytes presumably via the spleen [18].

Antiglobulin test (also known as Coombs test) is important in transfusion medicine to identify blood compatibility features and avoid complications during transfusions [20]. Based on both daratumumab clinical trials (GEN501 and SIRIUS), all patients previously phenotyped were assessed for changes in hemoglobin levels, transfusion outcomes and clinical sites experience. As conclusion, none of the transfusions were associated with complications, but many patients, after treatment with daratumumab, demonstrated in vitro panreactivity on RBCs panel testing [21]. This interference may persist for up to six months after the last daratumumab infusion [18].

While conducting a clinical trial with daratumumab, Schmidt and colleagues found that patients negative antibody screens for RBCs alloantibodies; however, after starting daratumumab, patients rapidly developed positive antibody screenings. The authors reported difficulties in determining the presence of clinically significant RBCs antibodies due to the interference of daratumumab with DATs and IATs [22]. In spite of this, it should be noted that there is no interference with the identification of ABO / RhD antigens, and that no transfusion-related cases of hemolysis were identified in the global safety database for daratumumab [7, 23].

**Challenges and methodologies in medicine transfusion**

According to recent studies, several methods have been developed to mitigate the CD38 antibodies interference. International researchers have analyzed and discussed extensively this topic and came to three
main solutions: Chemical denaturation/reduction of CD38 disulfide bonds with DTT or trypsin; Patients’ RBCs baseline phenotyping or genotyping, before starting treatment with daratumumab; and Neutralization of anti-CD38 antibodies in patients’ plasma, with an anti-idiotype antibody that blocks daratumumab and CD38 interactions or with a soluble recombinant CD38 (sCD38) [8]. Other solutions were also presented, such as the use of a screening panel, obtained with cord blood cells (which show low expression of CD38 receptors), to determine the clinically significant alloantibodies in daratumumab-treated patients [22]. However, most routine laboratories, especially in Europe, do not have access to cord blood samples neither to typed cord RBCs, compromising this strategy’s applicability in daily practice [24]. Although all developed strategies show some limitations, CD38 denaturation seems to be the most suitable choice to apply as a gold-standard technique in laboratory testing for patients treated with daratumumab.

Researchers have conducted a validation study for DTT method in multiple academic medical centers and blood center reference laboratories worldwide (DARA-DTT Study Group) [19]. The study was conducted in 25 different countries, not including Portugal. All sites followed a defined protocol for two coded plasma samples, one containing only daratumumab and the second one spiked with daratumumab and unknown antibodies. Agglutination was eliminated using DTT treated RBCs in 100% of the sites and it was possible to identify correctly the unknown antibodies. Furthermore, many laboratory professionals agreed that DTT denaturation is simple to use and easy to apply as standard procedures in laboratory [19]. The main disadvantage is that DTT also affects other blood group antigens, such as Kell, Cartwright (Yt), John Milton Hagen (JMH), Knops (KN), Landsteiner-Wiener (LW), Lutheran (LU), Dombrock (DO) and Cromer (CR); most importantly, the laboratory must confirm if the patient’s sample is negative for Kell antigen [21].

Additional recommendations include phenotyping, before the patient is treated with CD38 monoclonal antibody, while genotyping can be performed before or after drug administration [21]. In fact, although transfusions could be based on antigen phenotyping and/or genotyping, these procedures are very time-consuming, expensive and limited to the available number of matching donors. To address this issue two potentially neutralizing agents were analyzed, in order to restore the reliability of the antiglobulin tests. Addition of an excess of anti-idiotype antibodies or sCD38 protein to the test abrogated CD38 MoAb interference and successfully restored irregular antibody screening and identification [18]. Although neutralization of anti-CD38 antibodies allow the correct identification of irregular antibodies from K and E antigens, it is a very expensive method and lacks an approved supplier, as well as reproducibility.

**DISCUSSION**

In line with international studies on this matter, Portuguese experts have identified the main challenges and proposed possible solutions, including methodologies to hinder daratumumab interference with laboratory testing.

During the discussion, Portuguese experts have defined the use of DTT for CD38 denaturation as the only available, cost-effective, reproducible, reliable and validated method; however, they also agreed that it is a time-consuming technique that requires specific laboratory conditions and qualified operators. Daratumumab anti-idiotype was considered as an expensive and highly specific technique but not available. The application of sCD38 was also considered a very expensive technique. The experts mentioned the possibility of using umbilical cord cells for mitigating daratumumab interference, as well as patients’ phenotyping and genotyping.

The Portuguese experts also reinforced the need to align all methodologies according to technical sections described in daratumumab SmPC, such as “4.4 - Special warnings and precautions for use” and “4.5 - Interaction with other medicinal products and other forms of interaction”. Hence, it will be possible to establish global protocols and follow consolidated procedures.

When answering to the questions raised regarding daratumumab interference with laboratory testing, it was agreed that before starting treatment with daratumumab patients’ ABO/Rh groups should be identified, as well as a broad phenotyping (such as Kell, Duffy, Kidd or MNSs). Thus, techniques such as DATs (or Direct Coombs test) and irregular antibodies screening should be applied. In addition, patients’ samples during pre-treatment with daratumumab should be frozen and stored for further evaluation, as a valid comparator.

During treatment with daratumumab, and before blood transfusions, patients should be proposed for pre-transfusion testing, ABO/ RhD grouping and irregular antibodies screening. If this result is positive, patients, and antibody screening panel RBCs, should be treated with DTT and, afterwards, confirm the antibody presence through screening. In presence of negative results, there is no need for further tests.

Furthermore, some cases were pointed out as relevant and should be considered during the assessment of patients’ samples in the laboratory. On the one hand, patients treated with daratumumab, or other CD38 antibodies, who had never been transfused or were transfused for more than 3 months, should be screened for ABO grouping and Rh, Kell, Duffy, Kidd, MNSs phenotyping, because these are the systems that most often have clinically significant antibodies. The erythrocytes to be transfused should be ABO compatible and negative for the patient’s negative antigens, to avoid de formation of antibodies. On the other hand, patients without irregular antibodies and who have been treated with more than one...
line of treatment, should be assessed at least for AB0/ Rh grouping and Kell antibody screening, while the blood compatibility test should be conducted with DTT-treated samples. Also, for these patients, the procedure should be repeated before each transfusion.

In addition, the experts highlighted the importance of communication between Hematologists, Oncologists, Nurses, Professionals in Blood Transfusion Centers and Patients to generate and share reliable and prompt information. As mentioned by De Vooght and co-authors, communication between health care professionals prevents delays and superfluous costs on reagents and resources to repeat laboratory tests [8]. As final suggestion, patients should have a blood group card informing about the treatment with aCD38 monoclonal antibody and physicians should notify blood bank professionals. On the other hand automatic alerts could be created in the laboratory information systems or national databases for patients with irregular antibodies, as the one existing in the Netherlands [8, 24].

Thus, the patients' clinical information, such as diagnosis, beginning of daratumumab treatment date and previous history of transfusion, should be given to Transfusion Medicine services before the patient starts the treatment. Information about daratumumab treatment should include the date of the first and latest infusion. Moreover, information containing the laboratory tests results, positive antibodies screening and duration of the analysis should be provided to medical doctors, to emphasize the need of requesting exams soon enough hand to guarantee that all health care professionals are in possession of equal knowledge about the patients.

In respect to DTT technique, the experts believe that centralized analysis in reference centers can become an opportunity to implement a unique, detailed and validated procedure for all patients’ laboratory testing. Therefore, it is crucial to understand which centers are interested to have available identical phenotype blood units of Rh, Kell, Duffy, Kidd or MNs antigens and communication between health professionals in Hematology, Oncology and Transfusion Medicine services, is crucial to generate and share reliable and prompt information, in order to guarantee that all stakeholders involved have equal knowledge about the patients’ clinical and treatment conditions.

CONCLUSION

Daratumumab, is used in monotherapy as rescue therapy for heavily treated patients with relapsed/ refractory disease and in combination for patients who have received at least one prior therapy. However it can interfere with laboratory assays. Therefore, health care professionals have gathered efforts to discuss this and develop strategies to mitigate those confounding results. CD38 denaturation with DTT has been considered a cost-effective, simple and reproducible method. It’s implementation in reference centers for centralized analysis could be an opportunity to produce unique, detailed and high quality validated procedures. Finally, coordination with blood centers to have available

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Conflict of Interest

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