

Reflex testing and interference of therapeutic monoclonal antibodies in monitoring patients with multiple myeloma

Goda Aleknavičiūtė-Valienė¹, Dalius Vitkus¹, Dovilė Karčiauskaitė¹,
Jean Deenmamode², Rėda Matuzevičienė¹

¹ Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, M. K. Čiurlionio str. 21/27, LT-03101 Vilnius, Lithuania

² JD Laboratory Consultancy, Alderley Lighthouse Laboratories, Alderley Park, Alderley edge, SK10 4TG, United Kingdom

El. paštas: goda.aleknaviciute-valiene@mf.vu.lt

Summary

Background. In recent years, monoclonal antibody therapy has become increasingly important in the treatment of multiple myeloma in Lithuania. Even it is known that low levels of these medicines can interfere electrophoresis assays and this problem is widely discussed, laboratories do not have any recommendations and often misinterpretation or inability to measure therapeutic responses happens. The aim of this article is to demonstrate the migration pattern of serum samples from patients treated with daratumumab and isatuximab in SPE and immunofixation, to describe a simple and effective method to remove daratumumab from patients' serum and to propose the use of reflex tests regarding this problem.

Methods. Data from 2 patients were retrospectively analysed. Capillary electrophoresis, immunofixation and Hydrasys 2/4 daratumumab procedures were performed.

Results. In all cases, the interference of monoclonal antibodies was established and visualised. Migration pattern was determined: isatuximab migrates to the fast gamma region in SPE, but to the mid gamma region in immunofixation, while daratumumab migrates to the slow gamma region in both assays.

Conclusion. This report highlights the importance of the ability to recognize possible interference of monoclonal antibodies by the visual electrophoresis images, which is crucial for the clinical outcome measures. It suggests including a reflex testing as a mandatory in the multiple myeloma patients who are treated with mAbs testing guidelines. Reflex testing should be performed on all patients for whom a new IgG kappa monoclonal protein is observed as well as for patients whose M-protein shares the same SPE migration as mAb.

Keywords: multiple myeloma, reflex testing, monoclonal antibody, electrophoresis, immunofixation.

INTRODUCTION

In recent years monoclonal antibody (mAb) therapy has become increasingly important in the treatment of multiple myeloma in Lithuania. Daratumumab (Darzalex) and isatuximab (Sarclisa) are the two main drugs, which were registered in Lithuania in 2016 and 2020 respectively. Although the registration date of the drugs goes several years back, their active use in clinical practice in Lithuania started just at the end of 2021. Even it is known, that low levels of these medicines can interfere electrophoresis assays and this problem is widely discussed, laboratories do not have any recommendations and often misinterpretation or inability to measure therapeutic responses happens.

The aim of this paper is to demonstrate that daratumumab and isatuximab can be detected as a monoclonal protein in serum protein electrophoresis (SPE) and immunofixation (IF) and to describe a simple and effective method to remove daratumumab from patients' serum and to propose

usage of reflex testing regarding this issue. Moreover, it is important to emphasize the need of general awareness development among clinicians and laboratory specialists that these drugs can lead to a false positive M-protein interpretation in routine clinical practice.

Therapeutic monoclonal antibodies

Daratumumab and isatuximab are humanized IgG1 kappa derived mAbs, produced from a Chinese hamster ovary (CHO) cell line. They bind with high affinity to CD38 molecules expressed on the surface of multiple myeloma cells [1]. Daratumumab treatment is administered to patients who do not have access to autologous stem cell transplant or patients who are resistant to proteasome inhibitors and immunomodulatory therapies [2]. Treatment with isatuximab is initiated for patients resistant to proteasome inhibitors and immunomodulatory therapies [3]. The principle of mAbs action is the death of tumour cells via Fc-dependent immune effector mechanisms: apoptosis, complement dependant cytotoxicity,

antibody-dependant cellular phagocytosis, and cytotoxicity [4]. The main difference between these two drugs is that isatuximab binds to a specific epitope on CD38 and selectively induces death of multiple myeloma cells [5]. Isatuximab induces direct apoptosis in the absence of cross-linking agents, and daratumumab induces apoptosis with secondary cross-linking [6]. Consequently, CD38 enzymatic activity is inhibited more efficiently by isatuximab than by daratumumab [4].

When these mAbs were added at clinically relevant concentrations to the serum of a healthy person, the specific capillary electrophoresis migration pattern was determined: both agents have cathodal migration [7]. However, isatuximab migrates in the fast gamma region in SPE but in the mid gamma region in IF, while daratumumab migrates in the slow gamma region in both SPE and IF [8]. Therefore, it was observed that even low levels of mAb agents can be detected as a monoclonal protein and lead to a false positive SPE result and the IgG kappa monoclonal protein in IF. Furthermore, an interference is seen in the total IgG quantification measurement, especially in those patients with non-IgG monoclonal proteins [2]. The recommended doses of daratumumab and isatuximab are 16 and 10 mg/kg of body weight, respectively, and there are several dosing regimens depending on the combination with auxiliary drugs chosen. The serum half-life of daratumumab is 15–23 days and isatuximab 28 days, thus an additional monoclonal protein will remain visible during patient treatment and several weeks after the medication had been stopped. However, clearance of the drug can vary between patients due to differences in weight and renal function [7, 8].

For a patient to be classified as having a complete response (CR) according to the criteria of the International Myeloma Working Group (IMWG), the serum and urine electrophoresis results, and the immunofixation results must be negative for the monoclonal protein (M-protein) [9]. This is a serious challenge for accurate diagnosis when differentiating a CR influenced by interference of mAb therapy, since the most common type of multiple myeloma is IgG kappa [10]. mAb interference can be confused with the endogenous M-protein of the patient and lead to false positive or misleading results and can affect treatment choices. Furthermore, differentiation of endogenous M-proteins from mAb is challenging, especially in cases where both molecules co-migrate or migrate closely in the electrophoresis field [11]. Accurate identification of monoclonal proteins is essential for good patient management. If during the patient's treatment a new M-protein is detected in a different position and is identified as a new M-protein, the current treatment could be evaluated as not effective, suggesting the need for a therapy modification [12]. However, if a new M-protein is identified as daratumumab or other therapeutic mAb interference, then the current treatment should be maintained. Therefore, regarding treatment it is important to use a mAb specific IF assay for ruling out a drug interference. It is especially useful when patient's M-protein has the same isotype and migration pattern as the mAb based drug. In this case, distinguishing the two only using SPE or IF is impossible [7]. Antibodies which bind the mAb drug and separate endogenous M-protein are necessary to confirm the interference.

MATERIALS AND METHODS

There were 2 cases of different patients' data retrospectively analysed. SPE (Minicap Flex Piercing platform (Sebia, France)), IF (Hydrasys 2 Scan (Sebia, France)) testing procedures, migration and staining protocols were performed according to the manufacturer instructions. In the market there are only two manufacturer that offers commercial kits for serum electrophoresis – Sebia (France) and Helena (UK). The authors chose to use Sebia, because majority of assay in Europe is done with this manufacturer kits. Furthermore, the second reason is that at the moment Helena do not have any solutions available in Europe that deals with mAb interference and Sebia's Hydrashift 2/4 is the only commercially available method that is FDA (Food and Drug Administration) approved.

The new Hydrasys 2/4 daratumumab (Hydrasys 2 Scan (Sebia, France)) method was used. Hydrashift 2/4 daratumumab assay uses anti-daratumumab antibodies and enables the migration of a daratumumab/anti-daratumumab complex towards the α -globulin fraction on IF. Based on its charge, the daratumumab antibody migrates faster than the endogenous M-protein, and when the anti-daratumumab reagent binds to the therapeutic IgG, a complex is formed. The testing procedures, migration and staining protocols for this assay were performed according to the manufacturer's instructions.

RESULTS

Case 1

A 78-year-old man with a history of resistant anaemia treatment was admitted to the Vilnius University Hospital, Santaros Clinics. SPE and IF were performed and showed a 41.8 g/L IgG lambda M-protein (Figure 1, A). Therefore,

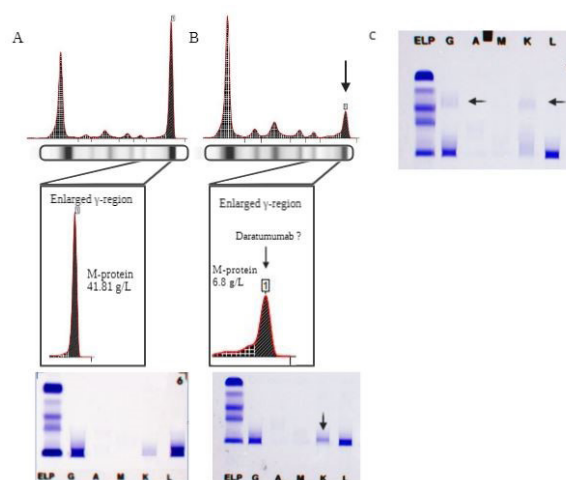


Fig 1. Detection of daratumumab by serum protein electrophoresis (SPE) and serum immunofixation (IF).

(A) The SPE and IF of patient 1 demonstrated IgG- λ M-protein of 41.8 g/L. (B) After six doses of daratumumab treatment, the endogenous M-protein decreased to 6.8 g/L and a second IgG- κ monoclonal protein appeared (black arrows). (C) Hydrashift 2/4 daratumumab assay confirmed that the second IgG- κ monoclonal protein is present due to daratumumab interference.

the admission diagnosis was Multiple myeloma, IgG lambda type, Revised Multiple Myeloma International Staging System (R-ISS) stage II. The patient received daratumumab treatment for one month. Daratumumab was combined with VMP (bortezomib, melphalan, and prednisone) in the pre-specified schedule at a dose of 16 mg/kg. After six doses of daratumumab the M-protein had decreased significantly to 6.8 g/L. However, the second M-protein, which could have been caused by daratumumab interference, did not appear in the SPE test. The new kappa monoclonal protein was visible only in the IF assay (Figure 1, B).

As the original IgG lambda M-protein from this patient migrated into exactly the same region as a possible daratumumab-band, in this situation it was impossible to characterize this band without using a specific assay. Therefore, another Hydrashift 2/4 daratumumab assay confirmed the co-migration of daratumumab and endogenous M-protein (Figure 1, C). The result was reported as an IgG lambda monoclonal protein with the note about daratumumab interference.

Case 2

A 57-year-old man with a 9-year history of IgG lambda multiple myeloma. Throughout the years of disease, the patient received CTD (cyclophosphamide, thalidomide, dexamethasone) therapy, two autologous stem cell transplants, VTD (bortezomib, thalidomide, and dexamethasone) therapy and bendamustine with dexamethasone therapy. After all these treatments, partial remissions were reached, but after some time, relapses occurred. Three years later, disease progression was observed and LenDex (lenalidomide and dexamethasone) treatment along with cyclophosphamide was started. After two years, carfilzomib with dexamethasone was prescribed and later PomCyDex (pomalidomide, cyclophosphamide, and dexamethasone) were prescribed. The durable response was not achieved in eight years.

In December 2021 as isatuximab became available in Lithuania, treatment with isatuximab alongside with pomalidomide and dexamethasone was started immediately. Before mAb treatment, M-protein was 32.93 g/L and monoclonal IgG lambda was observed (Figure 2, A). Unfortunately, after six doses of isatuximab, the M-protein peaked at 47.2 g/L. However, the second isatuximab peak was not seen in SPE or in IF (Figure 2, B). This could be explained by the fact that isatuximab and the endogenous patient's paraprotein comigrate at the same level. As this patient was the first and yet the only one who received isatuximab treatment, no further Hydrashift 2/4 isatuximab assay could be performed because the laboratory did not have specific antiserum. The result was reported as the IgG lambda monoclonal protein with a note about a possible isatuximab comigration.

With progression of myeloma bone marrow aspiration was performed and the V600E mutation of the BRAF gene was determined. Treatment with BRAF and MEK inhibitors – dabrafenib and trametinib – which act by inhibiting kinases further downstream of BRAF in the MAPK signaling pathway was started and after 6 months the M protein decreased to 3.3 g/L and no second M protein was visible in SPE, nor in IF (Figure 2, C). Therefore, it substantiates the hypothesis

that when the endogenous paraprotein is comigrating with isatuximab, the only option is to run a Hydrashift 2/4 isatuximab analysis.

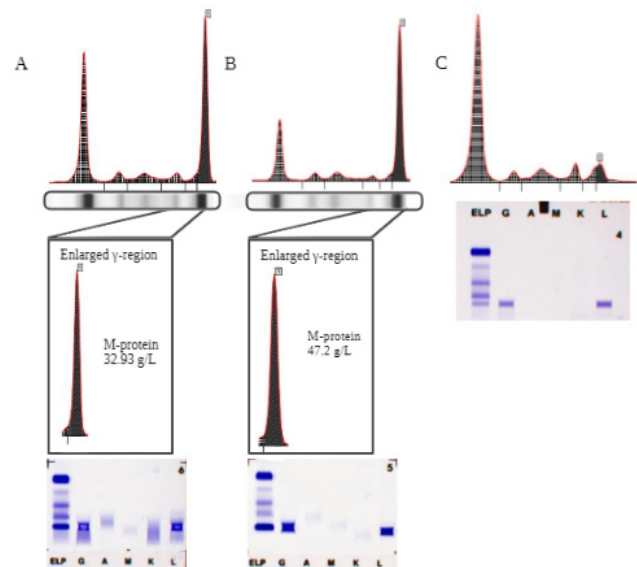


Fig 2. Detection of isatuximab by serum protein electrophoresis (SPE) and serum immunofixation (IF). (A) SPE and IF of patient 2 demonstrated IgG-λ M-protein of 32.93 g/L. (B) After six doses of isatuximab treatment, the endogenous M-protein peaked at 47.2 g/L, but no second IgG-κ monoclonal protein appeared. (C) After further treatment with BRAF and MEK inhibitors, no second monoclonal IgG-κ protein was visible in SPE and IF.

DISCUSSION

In the future many more patients will be treated with the various combinations of mAb therapy, and thus the evaluation of M-protein by electrophoresis assays will become more challenging. The incorrect interpretation of the response to treatment in the SPE / IF analysis due to interference usually ranges from a misclassification between CR and a very good partial response [9]. However, there exists no established standard practice for determining the necessity of distinguishing between multiple myeloma disease and mAb therapy, thus decision is usually laboratory depending.

The mAb interference could be suspected from either the distinct migration of the M-protein SPE (if the migration profile is the same as the endogenous M-protein, then suspicion is not possible) or the different isotype ascertained by IF. However, the migration pattern alone is not strong enough evidence that a band is formed by mAb interference [13]. Therefore, effective collaboration between clinicians and laboratory medicine specialists is necessary for optimal monitoring of M proteins. In many cases, the clinical laboratory specialists do not receive detailed clinical and medication information, particularly about antibody therapy, and can misinterpret the external mAb as the endogenous M-protein, and thus report *false positive* results. Alternatively, if accessible, authorised laboratory specialists should be able to search electronic medical records of the patient's treatment history.

It is important to note that IF is not ordered routinely; therefore, it is necessary to establish a follow-up reflex test procedure, especially for patients whose M-protein shares the same migration of SPE as mAb. The purpose of reflex testing is to provide more efficient and accurate results by performing additional tests that are relevant to the initial test results. This type of test is carried out without the intervention of the ordering physician when an initial test result is inconclusive without a follow-up test. One of the most important points of reflex testing is that no additional patient sample is needed. Furthermore, it further identifies valuable diagnostic information and improves patient care due to shorter turnaround times (TAT) and significantly higher accuracy. Consequently, reflex testing can improve the accuracy and efficiency of diagnostic testing and healthcare providers can quickly arrive at an accurate diagnosis and provide appropriate treatment for patients. What is more important, each laboratory creates its own criteria for reflex testing, but it requires clinical judgment and experience of laboratory medicine specialist. Also, physicians must know all protocols for performing reflex testing and must be able to reject follow-up tests at the time the initial test is ordered. However, reflex testing will result in additional charges. Therefore, it should be considered to start using IF and Hydrashift 2/4 tests as reflex testing, especially for patients whose M-protein shares the same SPE migration as mAb. In this way, it will be possible to distinguish external mAb from the endogenous M-protein. This is well illustrated in our case 2. Furthermore, according to the IMWG criteria of, a test to distinguish between monoclonal protein and therapeutic antibody should be mandatory in all clinical trial settings [9]. The Hydrashift 2/4 method has a short turnaround time without the need for additional instrumentation or a standardised and automated test and since there are other mAbs for the treatment of multiple myeloma, this simple technique can be adopted for the detection of other interfering mAb drugs using the respective antibodies [10]. However, it has several drawbacks, such as being a nonquantitative assay; interpretations can be complicated in cases of hypergammaglobulinemia [10]. In the future there will be more and more newly approved therapeutic mAb and the commercial kits will not be available as fast as it needed. Anti-CD38 can prevent interference and can be used with different therapeutic mAbs. This antigen-specific therapeutic mAbs depletion assay (ASADA) is based on magnetic beads coated with specific antigens of the therapeutic antibodies, thus depleting the mAbs in the sample [14]. The advantage of this method is that it is not require for specific antisera for different mAbs. However, disadvantages, such as it is non standardised, non-FDA approved and usually it is in-house method, outweigh the advantage. Another point of view is that more generalised method such as matrix-assisted laser desorption ionisation–time of flight mass spectrometry (MALDI-TOF MS) could be more expedient [13]. On the other hand, limited by the economic conditions, not all laboratories could use this technique.

Since daratumumab or isatuximab interference electrophoresis tests, screening for every new IgG kappa monoclonal protein is impractical and expensive, all laboratories should request clinicians to state the antibody drug and rely on a simple protocol for patients treated with mAb [13, 15]:

1. It is recommended that the blood draw should be done before the next mAb drug infusion and it is important to ensure that blood is not drawn proximal to an active infusion site or right after the infusion;

2. It is crucial to inform a laboratory specialist about the patient's treatment with mAb;

3. If suspected interference is seen in SPE an IF, laboratory reflex tests should be performed: Hydrashift 2/4 IF should be performed to confirm that the new IgG kappa monoclonal protein is from the drug;

4. If the mAb drug is present, the result should be given with the appropriate comment about the interference;

5. If the band is not from the mAb drug, then clinicians must be notified about the implications of a new clone.

CONCLUSIONS

Due to the increasing use of monoclonal antibodies, such as daratumumab and isatuximab, for the treatment of multiple myeloma, both laboratory specialists and clinicians should be aware of possible interference of these drugs in electrophoresis assays. Therefore, this report highlights the importance of a careful and accurate interpretation of the results of SPE and IF, which is crucial for the clinical outcome measures. Moreover, it suggests to include a reflex testing as a mandatory in the MM patients who are treated with mAbs testing guidelines. Reflex testing should be done, if possible, for all patients for whom a new IgG kappa monoclonal protein is observed as well as for patients whose M-protein shares the same SPE migration as mAb. In order to facilitate selection of patients for whom reflex testing is needed, the usage of laboratory software programme is recommended. The majorities of laboratories have an opportunity to check patient chart for each sample to see previous results. Furthermore, the cooperation between laboratory specialists and clinicians is crucial in order to pursue accurate results. It is recommended that clinicians always inform laboratory about patients who are receiving mAbs. On this wise laboratory specialists could evaluate results more carefully.

Gauta: 2024 05 10

Priimta: 2024 08 30

Santrauka

TERAPINIŲ MONOKLONINIŲ ANTIKŪNŲ INTERFERENCIJA IR PAPILDOMI DAUGINĖ MIELOMA SERGANČIŲ PACIENTŲ TYRIMAI

**Goda Aleknavičiūtė-Valienė, Dalius Vitkus,
Dovilė Karčiauskaitė, Jean Deenmamode,
Rėda Matuzevičienė**

Įvadas. Pastaraisiais metais monokloninių antikūnų terapija Lietuvoje tampa vis svarbesnė gydant dauginę mielomą. Nors ir žinoma, kad mažas šių vaistų kiekis gali

trukdyti elektroforezės tyrimams, ir ši problema yra plačiai aptariama, tačiau laboratorijos neturi jokių rekomendacijų ir dažnai pasitaiko klaidingų interpretacijų ar netinkamai įvertinto terapinio atsako. Šio straipsnio tikslas – parodyti pacientų, gydytų daratumumabu ir izatuksimabu, serumo mėginių migracijos modelį atliekant kapiliarinę elektroforezę ir imunofiksaciją, aprašyti paprastą ir veiksmingą daratumumabo pašalinimo iš pacientų serumo metodą bei pasiūlyti šiuo tikslu naudoti papildomus tyrimus.

Metodai. Dviejų pacientų duomenys buvo analizuojami retrospektyviai. Atlikta kapiliarinė elektroforezė, imunofiksacija ir Hydrasys 2/4 daratumumabo procedūros.

Rezultatai. Visais atvejais buvo aptikta ir vizualizuota monokloninių antikūnų interferencija. Nustatytas migracijos modelis: izatuksimabas migruoja į greitąją gama sritį atliekant kapiliarinę elektroforezę ir į vidutinę gama sritį imunofiksacijos metu, o daratumumabas migruoja į lėtąją gama sritį abiejuose tyrimuose.

Išvados. Šie klinikiniai atvejai įrodo terapinių monokloninių antikūnų interferencijos svarbą vizualiniams elektroforezės tyrimų rezultatams. Papildomi privalomi tyrimai, tokie kaip terapinių monokloninių antikūnų pašalinimas iš mėginio pacientams, gydomiems šiais vaistais, turėtų būti atliekami norint tinkamai įvertinti gydymo atsaką. Ypač didelė svarba turi būti teikiama tiems, kam aptinkamas naujas IgG kapa monokloninis baltymas, bei tiems, kurių kapiliarinės elektroforezės M baltymo migracijos profilis toks pat kaip terapinių monokloninių antikūnų.

Reikšminiai žodžiai: dauginė mieloma, monokloniniai antikūnai, elektroforezė, imunofiksacija.

REFERENCES

1. van de Donk NW, Otten HG, El Haddad O, Axel A, Sasser KA, Croockewit S, et al. Interference of daratumumab in monitoring multiple myeloma patients using serum immunofixation electrophoresis can be abrogated using the daratumumab IFE reflective assay (DIRA). *Clin Chem Lab Med* 2016; 54: 1105–9.
2. Sheldon J, Wheeler RD, Powles R. Electrophoretic patterns post daratumumab. *Ann Clin Biochem* 2018; 55: 299–301.
3. Hulin C, Beksac M, Goodman HJ, Spicka I, Alegre A, Prince M, et al. Antibody interference and response kinetics of isatuximab plus pomalidomide and dexamethasone in multiple myeloma. *Blood Cancer J* 2021; 11: 169.
4. Martin TG, Corzo K, Chiron M, van de Velde H, Abdessa G, Campana F, et al. Therapeutic Opportunities with Pharmacological Inhibition of CD38 with Isatuximab. *Cells* 2019; 8: 1522.
5. Finn G, Macé S, Campana F, Le-Guenec S, Muccio S, Tavernier A, et al. Evaluating Isatuximab Interference with Monoclonal Protein Detection By Immuno-Capture and Liquid Chromatography Coupled to High Resolution Mass Spectrometry in the Pivotal Phase 3 Multiple Myeloma Trial, Icaria-MM. *Blood* 2019; 134 Suppl 1: S3143.
6. Mikhael J, Belhadj-Merzoug K, Hulin C, Vincent L, Moreau P, Gasparetto C, et al. A phase 2 study of isatuximab monotherapy in patients with multiple myeloma who are refractory to daratumumab. *Blood Cancer J* 2021; (5): 89.
7. Tang F, Malek E, Math S, Schmotzer CL, Beck RC. Interference of Therapeutic Monoclonal Antibodies With Routine Serum Protein Electrophoresis and Immunofixation in Patients With Myeloma: Frequency and Duration of Detection of Daratumumab and Elotuzumab. *Am J Clin Pathol* 2018; 150(2): 121–9.
8. Wheeler D, Costa M, Crichlow A, Willis F, Reyat Y, Linstead SE, et al. Case report: Interference from isatuximab on serum protein electrophoresis prevented demonstration of complete remission in a myeloma patient. *Ann Clin Biochem* 2022; 59(2): 144–8.
9. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol* 2016; 17(8): e328–46.
10. Vakili H, Koorse GS, Dong X, Kansagra A, Patel H, Muthukumar A, et al. Complete Depletion of Daratumumab Interference in Serum Samples from Plasma Cell Myeloma Patients Improves the Detection of Endogenous M-Proteins in a Preliminary Study. *Diagnostics (Basel)* 2020; 10(4): 219.
11. Caillon H, Irimia A, Simon J, Axel A, Sasser K, Scullion M, et al. Overcoming the Interference of Daratumumab with Immunofixation Electrophoresis (IFE) Using an Industry-Developed Dira Test: Hydrashift 2/4 Daratumumab. *Blood* 2016; 128(22): 2063.
12. Liang S, Feng W, Ma H, Zhang L, Jia C. False positive results: a challenge for laboratory physicians and hematologists in treating multiple myeloma with daratumumab. *Hematology* 2022; 27(1): 332–6.
13. Mills JR, Murray DL. Identification of Friend or Foe: The Laboratory Challenge of Differentiating M-Proteins from Monoclonal Antibody Therapies. *J Appl Lab Med* 2017; 1(4): 421–31.
14. Liu L, Shurin R, Wheeler E. A novel approach to remove interference of therapeutic monoclonal antibody with serum protein electrophoresis. *Clin Biochem* 2020; 75: 40–47.
15. Lynch C, Yen T, Trambas C, Louey W, Sikaris KA. New laboratory protocol to identify iatrogenic IgG kappa monoclonal bands due to daratumumab therapy. *Pathol* 2019; 51 Suppl 1: S113.