Case Report

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Interference of daratumumab in monitoring multiple myeloma patients using serum immunofixation electrophoresis can be abrogated using the daratumumab IFE reflex assay (DIRA)

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Abstract: Daratumumab is a fully human anti-CD38 IgG1-κ monoclonal antibody (mAb) currently being evaluated in several Phase 2 and 3 clinical studies for the treatment of multiple myeloma (MM). In this clinical case study we demonstrate that daratumumab can be detected as an individual monoclonal band in serum immunofixation electrophoresis (IFE). M-protein follow-up by IFE is part of the International Myeloma Working Group (IMWG) criteria to assess treatment response. Therefore, it is crucial that the daratumumab band is not confused with the endogenous M-protein of the patient during IFE interpretation. Moreover, a significant number of IgG-κ M-proteins co-migrate with daratumumab. Co-migration introduces a bias in the M-protein quantification since pharmacokinetic studies show that daratumumab peak plasma concentrations reach up to 1 g/L. More importantly, co-migration can mask clearance of the M-protein by IFE which is necessary for classification of complete response by IMWG criteria (negative serum IFE). For optimal M-protein monitoring the laboratory specialist needs to be informed when patients receive daratumumab, and it is essential that the laboratory specialist is aware that a slow migrating band in the γ-region in those patients may be derived from the daratumumab. A daratumumab specific IFE reflex assay (DIRA) has been developed and can be utilized to abrogate interference. The here described mAb interference is not limited to daratumumab, and as therapeutic antibodies gain approval and enter into common clinical practice, laboratory specialists will need additional processes to characterize IFE interference and distinguish endogenous M-protein from therapeutic antibodies.

Keywords: daratumumab; daratumumab specific IFE reflex assay (DIRA); immunofixation electrophorsesis; monoclonal antibody; multiple myeloma; serum protein electrophoresis.

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Introduction

Monoclonal antibody (mAb) therapy has become increasingly important in the treatment of a variety of conditions, such as autoimmune disease and cancer [1]. It has been reported that some of these mAbs can appear as visible monoclonal protein bands in the serum IFE [2, 3]. Daratumumab is a fully human anti-CD38 IgG1-κ mAb currently evaluated in MM in several Phase 2 and 3 trials [4–6]. In this manuscript we demonstrate that daratumumab, dosed at therapeutic blood levels, can be detected as a monoclonal band in serum IFE. This 'daratumumab-band' may interfere with M-protein diagnostics, which may have serious clinical consequences as M-protein follow-up by serum protein electrophoresis (SPEP) and IFE are part of the IMWG criteria to assess treatment response in patients with MM [7, 8]. For a patient with an intact M-protein to be classified as having a complete response (CR) by IMWG

criteria, the serum and urine must be negative for M-protein, as determined by IFE and SPEP, and bone marrow plasma cells must be ≤5%. For a stringent CR (sCR), all of the criteria for CR must be met, along with a normal FLC ratio and absence of clonal plasma cells in the bone marrow measured by 2- to 4-color flow cytometry or immunohistochemistry.

We here provide information of the electrophoretic daratumumab-band characteristics and demonstrate how it may result in false M-protein interpretation in routine clinical practice.

Case presentations

Patient 1

A 79-year-old man with a 6-year history of MM was included in a daratumumab clinical study after development of progressive disease. He received daratumumab at a dose of 16 mg/kg. Before starting daratumumab treatment the IgG-κ M-protein had progressed to 20.4 g/L (Figure 1A). After two doses of daratumumab the M-protein decreased to 10.6 g/L and a second monoclonal band appeared that migrated into the slow γ-region. This additional monoclonal band was typed as an IgG-κ band which suggested it may be caused by the daratumumab treatment. Further IFE analysis of daratumumab spiked into saline, confirmed that the IgG-κ daratumumab indeed co-migrated on the exact same region of the gel as the newly appeared monoclonal band (Figure 1B). Interestingly, because of the immune-suppressed condition of patient 1, resulting in a nearly complete empty γ-region, the daratumumab-restriction can also be detected on SPEP (enlarged Figure 1B).

If the laboratory specialist had not been aware that patient one had received daratumumab, a second IgG-κ M-protein would have been reported as a non-original M-protein rather than a treatment related band of benign nature [9].

Patient 2

A 72-year-old man with a 4-year history of an IgG-κ MM was included with progressive disease into a daratumumab clinical study and received daratumumab at the pre-specified schedule at a dose of 16 mg/kg (Figure 2A). Upon therapy his IgG-κ M-protein strongly decreased and could no longer be quantified after 10 weeks of therapy. However, an IgG-κ monoclonal band remained visible

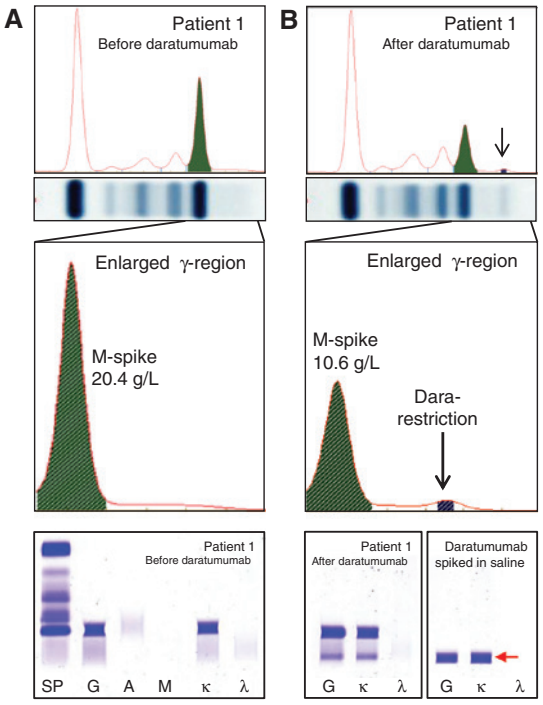

Figure 1: Detection of daratumumab by serum protein electrophoresis (SPEP) and serum immunofixation electrophoresis (IFE). (A) Baseline SPEP and IFE of patient one demonstrate an IgG-κ M-protein of 20.4 g/L. (B) After daratumumab treatment, the endogenous M-protein decreased to 10.6 g/L and a second monoclonal restriction appeared that migrated into the slow γ-region (black arrows). This second monoclonal restriction was typed as an IgG-κ band. Daratumumab spiked into saline demonstrates that this IgG-κ monoclonal biological migrates into the slow γ-region (red arrow).

(Figure 2B). As the original IgG-κ M-protein of this patient migrates into exactly the same region as a possible daratumumab-band, it is impossible in this situation to characterize this IgG-κ monoclonal band without using additional reagents or tests, such as the recently described daratumumab-specific IFE reflex assay (DIRA) [10]. This assay utilizes a daratumumab specific anti-idiotype antibody that binds daratumumab and shifts its migration pattern to distinguish between endogenous M-protein and therapeutic antibody. Figure 3 is a schematic presentation of the DIRA test. In the control lanes 3 vs. 4 the daratumumab (blue arrow) is shifted when anti-idiotype antibody is added (green arrow). The post-treatment daratumumab in the serum of patients, presented by the blue lines in lanes 7 and 11, are shifted when anti-ideotype antibody is added (green lines in lane 8 and 12). When an endogeneous IgG-κ M-protein comigrates with the daratumumab, this monoclonal band now becomes visible as a second band in the IFE gel (Figure 3A; red lines in lanes 8 and 12 indicate a positive DIRA test). With a negative DIRA

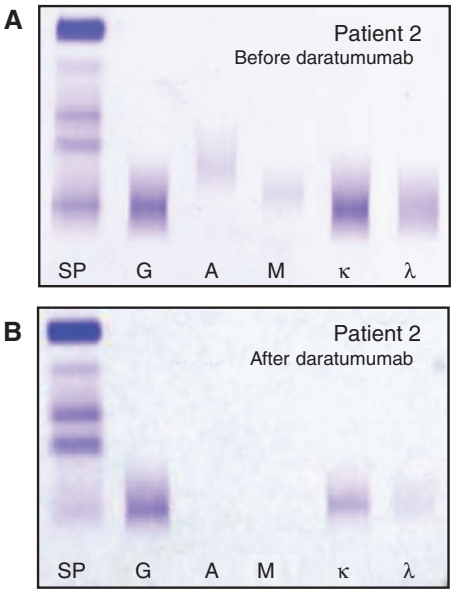

Figure 2: Serum immunofixation electrophoresis (IFE) of patient two before and after daratumumab administration.
(A) Baseline IFE of patient two demonstrates an IgG-κ M-protein. After 10 weeks of daratumumab therapy, the IgG-κ M-protein strongly decreased and could no longer be quantified. Since the endogenous IgG-κ M-protein migrates into exactly the same region as a possible daratumumab-band, it is impossible in this situation to assess whether the visible IgG-κ monoclonal band reflects the endogenous M-protein or the administered daratumumab.

test, the endogenous IgG-κ M-protein is not visible as a separate monoclonal band and only the shifted daratumumab complex can be detected (Figure 3B; green lines in lanes 8 and 12). The DIRA test in patient two was however, not performed, because this patient continued to have Bence Jones κ proteins and an abnormal FLC ratio. Based on these parameters the clinical response was classified as a very good partial response (VGPR) [7].

Patient 3

A 53-year-old man with a 3-year history of IgA-γ MM experienced progressive FLC-λ disease, so called FLC escape (Figure 4A) [11]. He was enrolled in a daratumumab clinical study and received daratumumab monotherapy at a dose of 16 mg/kg. Upon daratumumab therapy his FLC-λ M-protein disappeared on IFE after 9 weeks of treatment and a novel IgG-κ monoclonal band appeared (Figure 4B). Utilizing the DIRA test the daratumumab band was successfully shifted using an anti-idiotypic antibody (Figure 4C; lanes 8 and 12) to show this band was in fact daratumumab and not an M-protein. The FLC-λ concentration at the time of inclusion was

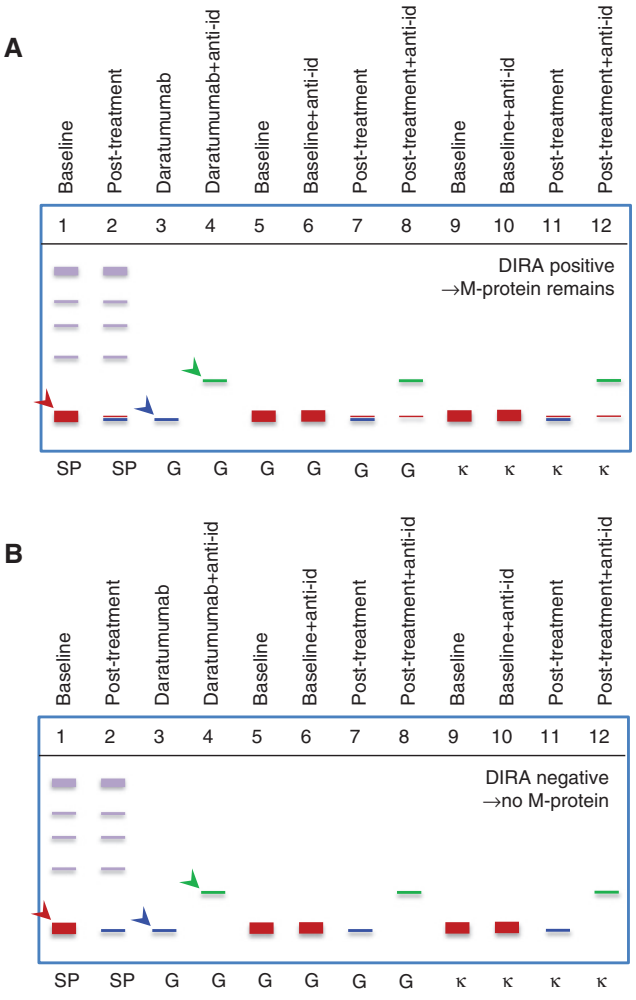

Figure 3: Schematic presentation of Daratumumab IFE Reflex Assay (DIRA).
The endogenous IgG-κ M-protein (in red+arrow lane 1) comigrates with the daratumumab monoclonal band (in blue+arrow control lane 3). The daratumumab specific anti-idiotype antibody binds daratumumab and shifts its migration pattern (in green+arrow control lane 4) to distinguish between endogenous M-protein and therapeutic antibody. The IgG (lanes 5–8) and κ (lanes 9–12) are separately analyzed and should in theory show the same results in patients with an endogenous IgG-κ M-protein. (A) With residual IgG-κ endogenous M-protein, a second band in the IFE gel becomes visible (red lines in lanes 8 and 12) which results in a positive DIRA test. (B) When the endogenous M-protein is no longer visible as a separate monoclonal band, the only bands visible are the shifted daratumumab complexes (green lines in lanes 8 and 12) which is indicative of a negative DIRA test. SP, total serum protein fixation; G, anti-IgG antisera; κ, anti-κ antisera.

528 mg/L and within 9 weeks completely normalized to 10 mg/L. Urine analysis at this time showed no Bence Jones proteinuria and bone marrow analysis demonstrated absence of clonal cells. In combination with the negative DIRA test classified this patient as a stringent complete responder [7].

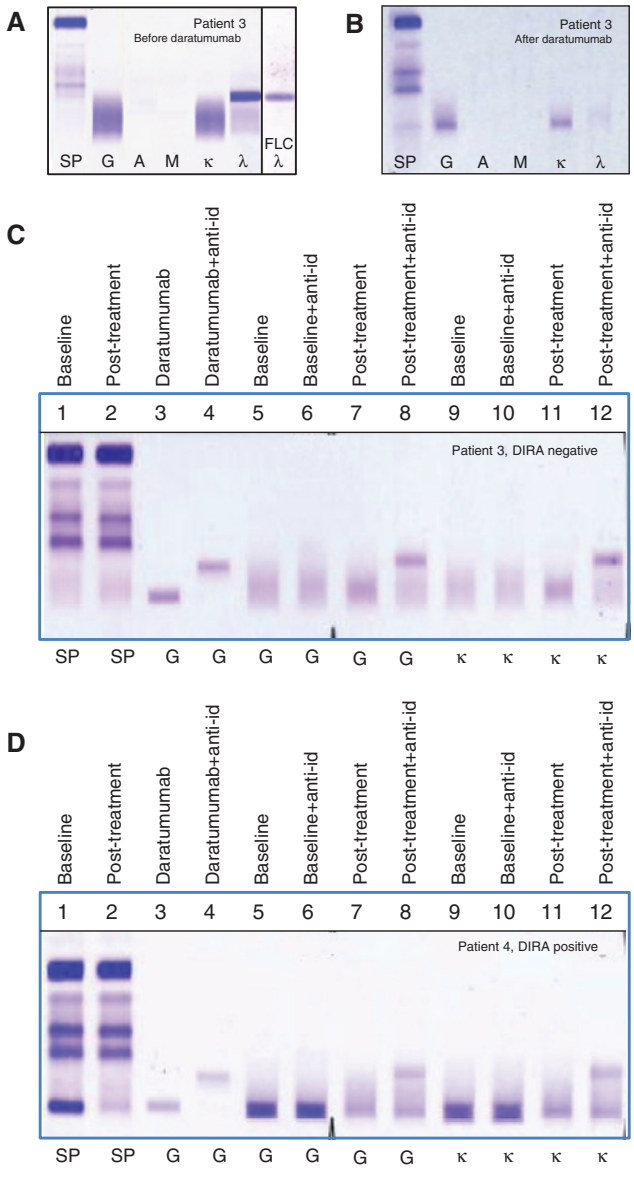

Figure 4: Daratumumab IFE reflex assay (DIRA) in clinical practice. (A) Baseline IFE of patient three demonstrates a free light chain (FLC) λ monoclonal gammopathy. (B) Upon daratumumab therapy a novel IgG-κ monoclonal band appeared in the serum of patient three and the endogenous FLC-λ M-protein disappeared. (C) Negative DIRA test for patient three in which the IgG-κ band was completely shifted using the anti-idiotypic antibody demonstrating that this band was in fact daratumumab and not an M-protein. (D) Positive DIRA test of patient four, demonstrating that the endogenous IgG-κ M-protein is still present after daratumumab administration. FLC, free light chain; SP, total serum protein fixation; G, anti-IgG antisera; κ, anti-κ antisera.

If the laboratory specialist had falsely interpreted the novel monoclonal band as a new M-protein, the clinical response could have been falsely classified as a VGPR instead of a stringent complete response.

Patient 4

A 73-year-old man with IgG-κ MM entered a daratumumab clinical study and received 16 mg/kg daratumumab. Patient had a good clinical response, however, the IFE remained positive for IgG-κ after treatment for several months, indicating potential daratumumab interference on the IFE. In this case, the DIRA test is useful for distinguishing patients who are still IFE positive for endogenous M-protein (DIRA positive) vs. those who have only daratumumab and no endogenous M-protein remaining (DIRA negative). DIRA testing was performed and demonstrated co-migration of daratumumab and endogenous M protein (Figure 4D). In this case, not all M protein was shifted with the anti-idiotype antibody (lanes 8 and 12) indicating that endogenous M protein was still remaining (DIRA positive). Based on these results, the patient was classified as VGPR and was not assessed for further response criteria.

Learning points

In this paper we demonstrate that daratumumab, dosed at therapeutic blood levels, can be detected as a monoclonal band in serum IFE. The daratumumab mAb migrates into the cathodal end of the γ-region. Whether or not a daratumumab-band is visible on IFE depends mainly on three parameters. First, the serum concentration of daratumumab at the time of IFE testing, which may be higher than 500 μg/mL during the intense weekly and bi-weekly dosing. Second, the amount of polyclonal immunoglobulins in the patient's serum. Immunosuppressed patients have a hypogammaglobulinemia, and small monoclonal bands can be detected with better sensitivity in these patients. And last, the presence of the endogenous M-protein or treatment related secondary oligoclonal bands that may obscure the daratumumab band.

Therapeutic antibodies may interfere with M-protein follow-up that is part of the IMWG criteria to assess treatment response in patients with MM [7, 8]. Incorrect response assessment due to interference in SPEP/IFE assays, most often misclassification between VGPR and CR, may lead to underestimation of prognosis and possible overtreatment of individual patients. In addition, this hampers outcome evaluation in clinical studies with therapeutic antibodies. M-protein monitoring is most difficult in patients with an IgG-κ M-protein that co-migrates with the daratumumab-mAb. In this situation the two bands cannot be differentiated from each other which makes valid clinical response calls that meet the IMWG

criteria impossible without additional reflex testing to distinguish the therapeutic from the endogenous M-protein. It is expected that up to 50% of the IgG-κ M-proteins co-migrate with the daratumumab-mAb or at least partially overlap in the slow γ-region [12, 13]. Daratumumab does not affect the interpretation of IgA and IgM M-proteins as these bands migrate mostly into the β-region and the fast γ-region [13]. McCudden et al. recently reported the development of a clinical assay to mitigate the daratumumab interference in IFE [10]. To mitigate interference, they utilized an antibody that binds daratumumab and thereby the complex migration shifts on IFE.

To date, over 50 patients with suspected daratumumab interference have been DIRA tested. Daratumumab was detected in >90% samples, but those in which it was not still had M protein that flagged DIRA testing. One third of cases tested were DIRA negative, indicating that only daratumumab was detectable and the remaining M-protein was not present. These patients were then assessed for additional IMWG response criteria including FLC and percentage of bone marrow plasma cells.

Daratumumab will also overestimate M-protein quantification by SPEP if the patient's M-protein co-migrates with the daratumumab mAb. The maximal overestimation will be 1 g/L, since pharmacokinetics studies show that daratumumab (16 mg/kg, weekly) mean peak plasma concentration is 915 mg/L [10]. Therefore, it is not anticipated that daratumumab presence would promote the clinical response call of progressive disease.

In the course of MM, patients may develop an M-protein band different from the original one. This so called secondary monoclonal gammopathy of undetermined significance (MGUS) is often a treatment related phenomenon of benign nature. It develops more frequently in patients who achieve a deep response and represents oligoclonal immune reconstitution [9]. If a daratumumab-band is not recognized as such, it may be interpreted as secondary MGUS.

To anticipate possible interference of daratumumab treatment in M-protein monitoring it is important that the laboratory specialist is informed of the use of daratumumab, and the use of monoclonal biologicals in general.

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