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Treatment of multiple myeloma with monoclonal antibodies and the dilemma of false positive M-spikes in peripheral blood

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ABSTRACT

Objectives: To characterize the effect of three humanized IgG κ monoclonal antibodies (daratumumab, isatuximab, and elotuzumab) on the interpretation of results generated by protein electrophoresis, immunofixation, free light chain, and heavy/light chain assays performed on human serum.

Methods: Healthy volunteer serum and serum from multiple myeloma patients were supplemented with clinically relevant concentrations of each of the three monoclonal antibodies. These specimens then underwent analysis via serum protein electrophoresis, immunofixation, serum free light chain quantification, heavy/light chain quantification, total IgG, and total protein. In addition, serum specimens from patients who had undergone treatment with elotuzumab for multiple myeloma underwent similar analysis.

Results: Addition of the study drugs to serum from both the healthy donor as well as multiple myeloma patients resulted in a visible and quantifiable M-protein on SPEP and a visible IgGκ band by IFE. Increases were also noted in total IgG, IgGκ, and IgGκ/IgGλ-ratios. Analysis of serum from multiple myeloma patients receiving study drug showed similar findings with an additional IgGκ band and quantifiable M-protein with similar migration patterns in specimens drawn after administration.

Conclusion: The treatment of multiple myeloma patients with monoclonal antibodies results in a visible and quantifiable M-protein that has the potential to falsely indicate poor response to therapy.

1. Introduction

The initial observation from a clinical study that therapeutic monoclonal antibodies can interfere with SPEP and IFE was made in patients receiving the monoclonal antibody siltuximab which targets IL-6 [1]. Although the observation was first done in this setting, the clinical implications were limited since the drug is not FDA approved for the treatment of multiple myeloma. However, in 2015, the first monoclonal antibodies were approved by the FDA for the treatment of multiple myeloma: the humanized IgG κ monoclonal antibodies targeting CD38 (daratumumab) and SLAMF-7 (elotuzumab); and more drugs are under development (e.g., isatuximab) [2–4]. Because clinical response criteria for multiple myeloma include assessment of monoclonal-(M)-protein, the use of humanized IgG κ monoclonal antibodies has the potential to interfere with clinical response criteria. Indeed, based on current response criteria for multiple myeloma, a complete response (CR) includes the elimination of detectable M-protein in peripheral blood [5]. Therefore, the use of humanized IgG κ monoclonal antibodies in a patient with IgG κ multiple myeloma may result in a detectable M-protein which may be misinterpreted at response evaluation of peripheral blood, as residual disease. (i.e. false positive residual disease).

The purpose of our study was to characterize the effect of three humanized IgG κ monoclonal antibodies (daratumumab, isatuximab, and elotuzumab) on the interpretation of results generated by protein electrophoresis, immunofixation, free light chain, and heavy/light chain assays performed on human serum.

2. Materials and methods

Pure daratumumab, isatuximab, and elotuzumab were obtained from their respective manufacturers (Jansen, Sanofi, and Bristol Myers Squibb). Daratumumab and elotuzumab were provided in powdered form ready for clinical use and reconstituted , while isatuximab was provided from the manufacturer as a 5 mg/mL solution. Serum from a

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healthy male volunteer was obtained, and supplemented with varying concentrations of each of the three monoclonal antibodies. The concentrations that were evaluated were chosen to approximate the serum Cmax values attained by each of the drugs that were available based on available literature on Phase 1/2 studies (Daratumumab: 993 μg/mL after Dose 7 at 16 mg/kg (Dartumumab FDA submission documents), Elotuzumab: 563 μg/mL after Dose 4 at 20 mg/kg [6]). Due to the lack of publicly available information on Isatuximab, a concentration similar to that of Daratumumab was chosen. Concentrations twice the Cmax were also included to examine the effects of extremely high concentrations of monoclonal antibody on the IgG and heavy/light chain assay quantitation.

The dilute nature of the isatuximab solution provided by the manufacturer required the addition of a significant volume of isatuximab solution to the serum solution in order to attain the desired drug concentration. This resulted in the isatuximab spiked specimens that were approximately 30% PBS by volume. Therefore, an additional aliquot of volunteer serum was diluted with saline (70% serum, 30% saline) to serve as a control for the diluting effect of the isatuximab solution on the spiked specimens.

In addition, serum from two multiple myeloma patients, each with a diagnosis of IgGκ multiple myeloma, were obtained. One of the patients was in complete remission (CR), while the other had a visible M-protein. Aliquots of serum from both patients were supplemented with either elotuzumab or daratumumab. Spiking experiments were not performed for isatuximab due to the highly dilute nature of the provided antibody.

All specimens underwent analysis via serum protein electrophoresis (Sebia Capillarys 2, Sebia USA, Norcross, GA, USA), serum immunofixation (Sebia Hydrasis, Sebia USA, Norcross, GA, USA), serum free light chains and heavy/light chain assays (Freelite and Hevylite, The Binding Site, Birmingham, UK), and nephelometric IgG quantitation and total protein (Siemens USA, Tarrytown, NY, USA). All M-proteins visible by serum protein electrophoresis were quantified using the Sebia Phoresis (Sebia USA, Norcross, GA, USA). For myeloma patient specimens, M-spikes caused by the patient's clone and spikes caused by the mAb antibodies were quantitated separately.

Furthermore, to expand our work and to include in vivo samples from patients treated in our clinic, we assessed serum specimens drawn from patients ($N = 3$) who had undergone treatment by elotuzumab for multiple myeloma. All three patients were on a protocol in which they received 10 mg/kg of elotuzumab once a week. These specimens were drawn at various points during the course of their treatment. Patient clinical information was deidentified for this study, and use of the data was approved by our institutional review board.

3. Results

3.1. In vitro studies

Analysis of the healthy donor serum demonstrated no visible monoclonality by either protein electrophoresis or immunofixation (Fig. 1a). Addition of clinically relevant concentrations of either daratumumab (Fig. 1b), isatuximab (Fig. 1c), or elotuzumab (Fig. 1d) to the serum specimen, all resulted in a visible and quantifiable M-protein by protein electrophoresis as well as a visible monoclonality by immunofixation. The addition of any of the three monoclonal antibodies to healthy donor serum resulted in noticeable increases in total IgG, and IgG κ via heavy/light chain assay as well as IgGκ/IgGλ-ratios relative to the unspiked serum specimens (Table 1), however, the increases did not result in an abnormal IgGκ/IgGλ ratio. No clinically significant increases were noted in the quantitation of kappa free light chains (Freelite), or free κ/free λ-ratios.

Serum from a multiple myeloma patient in CR with no visible monoclonality by immunofixation or protein electrophoresis (Fig. 1e), as well as a patient with a visible M-spike and monoclonal band (Fig. 1h), were analyzed by protein electrophoresis and immunofixation after addition of daratumumab or elotuzumab. The specimens supplemented with daratumumab (Fig. 1f) as well as elotuzumab (Fig. 1g) demonstrated monoclonal bands visible by both protein electrophoresis and immunofixation in serum from the remission patient. In serum from the patient with a pre-existing M-protein, the specimen supplemented with daratumumab showed a slight increase in the M-protein peak (Fig. 1i, Table 1), while the specimen supplemented with elotuzumab (Fig. 1j) showed two distinct M-protein peaks by protein electrophoresis and two monoclonal bands by immunofixation. Quantitation of total IgG, IgG κ, IgG κ/IgG λ-ratios, free light chains, and free light chain-ratios resulted in findings similar to that found in supplemented healthy human serum (Table 1).

3.2. In vivo studies

To expand our work to include in vivo samples, stored serum specimens from three patients receiving elotuzumab were analyzed by protein electrophoresis and immunofixation. Patient A, a multiple myeloma patient with free κ light chain-myeloma, had a visible IgGκ band as well as a corresponding M-protein that was not caused by the monoclonal free κ light chain band at both 1 week post dose 4 (Fig. 2a) as well as at 1 week post dose 12 (Fig. 2b). Patient B, with IgAλ disease, had an additional faint IgGκ band visible by immunofixation at both 1 week post dose 3 (Fig. 2c) and at 1 week post dose 6 (Fig. 2d). Patient C, with IgAλ disease, had no additional visible M-protein or monoclonal bands prior to receiving elotuzumab (Fig. 2e), but had an additional M-protein by protein electrophoresis and an IgGκ band visible by immunofixation at both 1 week post dose 3 (Fig. 2f) and at 1 week post dose 4 (Fig. 2g). The electrophoretic pattern of the additional visible M-proteins on both SPEP and IFE were noted to be similar to the electrophoretic pattern seen in the in vitro spiking studies on healthy serum. Also worth noting, the visible bands caused by elotuzumab became more pronounced with increasing numbers of doses, indicating accumulation of elotuzumab in the patient's peripheral blood.

The reporting of an additional M-protein along with the appearance of an additional IgGκ band by immunofixation (Fig. 2a-b) had no effect on the clinical management of the Patient A. Patients B and C were taken off protocol shortly following dose 6 and 4, respectively, due to progression despite treatment with elotuzumab. Both patients had progressive increases in their M-spike values (Table 2) which were determined not to be due to elotuzumab based on review of SPEP and immunofixation results (Fig. 2c-g).

4. Discussion

Current International Myeloma Working Group (IMWG) Uniform response criteria for multiple myeloma [5] state that in order for patients to be classified as being in CR, laboratory testing should show absence of M-protein by immunofixation in serum and urine, while classification into very good partial response (VGPR) requires serum and urine M-protein detectable by immunofixation but not on electrophoresis. The results from our study clearly demonstrate that all three of the new therapeutic monoclonal antibodies for the treatment of multiple myeloma are capable of generating M-protein detectable by protein electrophoresis and immunofixation. This leads to a dilemma in the assessment of treatment response. To highlight these issues, a clarification of the definition of CR was recently proposed by the IMWG [7]. The updated definition stipulates that the presence or absence of monoclonal bands by immunofixation or SPEP refers only to the patient's original M-protein secreted by the pathologic plasma cells.

Serum protein electrophoresis and immunofixation results are typically reviewed by a pathologist or doctoral level scientist prior to release into a patient's medical record. However, there is significant variation in how results are reported due to lack of standardization [8]. One such example of relevance to this study is that many laboratories report out all visible M-protein values and monoclonal bands without reference to

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