

Case Report

A rare case of the IgE prozone phenomenon in IgE multiple myeloma

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ABSTRACT

IgE multiple myeloma is a rare subtype of myeloma, accounting for <0.1% of all myeloma cases. Difficulties in diagnosing and monitoring this rare myeloma type are recognized, including the need of heightened awareness for initial diagnosis, performing a reflex immunofixation test using an anti-IgE antisera, and recognizing the possibility of the prozone phenomenon. Here, we report a rare case of IgE multiple myeloma with the prozone phenomenon. This case was characterized by a paradoxical increase in IgE levels with a progressive increase in the dilution factor. Moreover, serial monitoring of IgE levels correlated with the trend in the serum free light chain ratio, especially when the monoclonal protein was no longer detectable. This case highlights the need for laboratory professionals to be vigilant about the occurrence of the prozone phenomenon in IgE multiple myeloma.

1. Case description

A 65-year-old man with hypertension and diabetes mellitus presented to an outpatient clinic with generalized weakness and loss of appetite. The results of initial laboratory tests indicated anemia (Hb, 9.0 g/dL (90 g/L)), increased Cr¹ (2.53 mg/dL (223.65 μmol/L)), and hypercalcemia (Ca, 11.8 mg/dL (2.95 mmol/L)). These signs were suggestive of the CRAB features of multiple myeloma, and the patient was admitted for further examination. Serum protein electrophoresis (SPE) was performed with the Capillarys system (Sebia, Lisses, France), and a monoclonal protein was identified in the gamma region. This monoclonal protein comprised 17.5% of the gamma region. Multiplication by the serum total protein concentration (7.4 g/dL (74 g/L)) resulted in a calculated monoclonal protein concentration of 1.3 g/dL (13 g/L). Total serum immunoglobulin levels, determined by turbidimetry, were consistently below the lower limit of the reference interval (RI): IgG 437 mg/dL (4.37 g/L) (RI: 870–1700 mg/dL (8.7–17 g/L)), IgA 17 mg/dL (0.17 g/L) (RI: 110–410 mg/dL (1.1–4.1 g/L)), and IgM 8 mg/dL (0.08 g/L) (RI: 33–190 mg/dL (0.33–1.9 g/L)). Immunotyping of the monoclonal protein, performed using immunosubtraction capillary zone electrophoresis (Capillarys) with anti-IgG, -IgA, -IgM, -kappa, and

-lambda antisera, was negative for the IgG, IgA, and IgM heavy chains, and positive only for kappa. Therefore, complementary immunofixation electrophoresis (IFE) for detecting IgD and IgE was performed using Hydrasys 2 (Sebia), which is an agarose gel electrophoresis system, and IgE-κ clonality was confirmed (Fig. 1). The serum free light chain (FLC) assay using Freelite (The Binding Site, Birmingham, UK) revealed an absolute increase in kappa FLC (4990 mg/L), while lambda FLC was below the limit of quantitation (<0.76 mg/L). In addition, urine protein electrophoresis and immunotyping results revealed the presence of urinary free kappa light chains.

The initial IgE level, measured using the Immulite 2000 analyzer (Siemens Healthineers, Erlangen, Germany) was 230 IU/mL. This technique is based on a sandwich solid-phase chemiluminescent immunoassay. The level was higher than the RI (<87 IU/mL (<208.8 μg/L)). However, we suspected that a possible prozone phenomenon led to the IgE level being falsely low; thus, we performed serial dilutions with the designated assay diluent; this resulted in a final IgE concentration of 5,543,300 IU/mL (13,303,920 μg/L). During the dilution process in all samples collected while the patient was under observation, a paradoxical increase in total IgE levels, with a progressive increase in the dilution factor, was observed (Fig. 1).

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¹ Abbreviations: Ca, calcium; Cr, creatinine; CRAB, hypercalcemia, renal failure, anemia, and bone disease; FLC, free light chain; Hb, hemoglobin; IFE, immunofixation electrophoresis; Ig, immunoglobulin; IRR, International Reference Reagent; LDH, lactate dehydrogenase; MGUS, monoclonal gammopathy of undetermined significance; SPE, serum protein electrophoresis; RI, reference interval; VGPR, very good partial response; WHO, World Health Organization

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To exclude heterophilic antibodies as a cause of positive assay interference in the assay, we pretreated patient serum samples in heterophilic blocking tubes (Scantibodies Laboratory, Santee, CA, USA) and then measured IgE levels [1]. The total IgE level obtained after pretreatment with the heterophilic blocking reagent confirmed the initial (non-pretreated) IgE level; therefore, there was no positive assay interference due to heterophilic antibodies.

Examination of the bone marrow revealed changes consistent with plasma cell myeloma; plasma cells comprised 84% of all nucleated cells. Chromosome analysis was unsuccessful due to inadequacy of the sample, and the skeletal survey was negative for lytic bone lesions. Based on key clinical parameters and other laboratory data (β_2 -microglobulin, 7.2 mg/L; lactate dehydrogenase (LDH), 283 U/L (4.811 ukat/L)), the patient's myeloma stage was identified as 3A according to the Durie/Salmon staging system, and stage 3, according to the International staging system.

The patient was initially treated with a modified VMP (bortezomib, plus melphalan and prednisone) regimen [2]. Four months after initiating treatment, the monoclonal IgE- κ protein could no longer be detected with SPE and IgE-specific IFE. The patient's total IgE level was 109,774 IU/mL (263,457.6 μ g/L) which equals 26.3 mg/dL in gravimetric terms (conversion factor, 1 IU/mL = 2.4 ng/mL). The patient achieved a very good partial response (VGPR) and treatment was modified to maintenance therapy with bortezomib. Serial monitoring of total IgE concentrations, the κ FLC concentrations, and monoclonal protein levels over the course of treatment are shown in Fig. 2.

2. Discussion

IgE multiple myeloma is a rare subtype of myeloma, accounting for <0.1% of all myeloma cases [3]. IgE multiple myeloma is generally associated with poor prognosis [4], although with novel agents, outcomes similar to the more common myeloma subtypes have been observed [5]. Difficulties in diagnosing and monitoring IgE multiple myeloma have been recognized since the first report of this rare subtype, including the particular caution required for initial diagnosis, the need for a reflex IFE test using anti-IgE antisera to confirm IgE heavy chain clonality [6], and awareness of the possibility of a prozone phenomenon when immunoassays show falsely low IgE levels [7].

The prozone phenomenon (hook effect) occurs when the concentration of the analyte (IgE in this case) being measured is very high compared with the fixed amount of antibody in the reagent. In the context of a sandwich immunoassay for antigen detection, each molecule of antigen must bind to one capture and one tracer (labeled) antibody. When there is an excess of antigen in the sample, formation of the antibody-antigen-antibody complex is impaired, resulting in a falsely

low signal. During monitoring of the current patient, different serum IgE assays were used, beginning with the Immulite 2000, followed by the Advia Centaur XPT, and then the Atellica IM (all from Siemens Healthineers). Internal verification showed good agreement among the assays. However, these three assays were all calibrated against the same standard, the second WHO International Reference Reagent (IRR) for serum IgE (75/502).

The prozone phenomenon was consistently observed, despite changes in the assays. A remarkable feature of the prozone phenomenon, from a laboratory perspective, is the paradoxical increase in IgE levels with a progressive increase in dilution factor (Fig. 1). This was initially puzzling because the IgE level appeared to increase by several orders of magnitude with the increase in dilution factor. For example, as depicted in Fig. 1, the increase in IgE level was 10^2 with a 1:10 dilution, and 10^5 with a 1:100 dilution. The degree of increase was erratic considering the reference range and the range of IgE levels commonly observed in patients with allergic disorders. According to a review of 63 cases of IgE monoclonal gammopathy, the reported IgE levels varied extensively, ranging from 9.38 IU/mL (22.5 μ g/L) to 17.9×10^6 IU/mL (43.0×10^6 μ g/L) (reference value < 140 IU/mL (<336 μ g/L)) [3]. The initial IgE level of 5.5×10^6 IU/mL (13.2×10^6 μ g/L) observed in our patient was near the upper limit of reported IgE levels in patients with monoclonal gammopathy, which supports the association between a high IgE concentration and the prozone phenomenon. Due to the rareness of IgE myeloma, the prevalence of the prozone phenomenon in IgE multiple myeloma is difficult to estimate. However, two cases of the prozone phenomenon associated with IgE multiple myeloma have been described, with reported IgE levels of 2.2×10^6 IU/mL (5.3×10^6 μ g/L) [8] and 5.3×10^6 IU/mL (12.7×10^6 μ g/L) [7]. Therefore, it is important to note that the IgE concentration in patients with IgE multiple myeloma or monoclonal gammopathy of undetermined significance (MGUS) [9] often exceeds the range of increased IgE concentrations observed in allergic diseases. Thus, serial dilutions should be performed whenever there is a possibility of the prozone phenomenon.

The role of IgE quantitation in monitoring IgE multiple myeloma has not been established [10]. As far as the prozone phenomenon is concerned, others have referred to the unreliability of quantitative IgE levels in assessing the response of IgE multiple myeloma to treatment [7], in addition to the risk of inappropriate management. Although monoclonal protein quantification has been recommended as an alternative measure for monitoring patient response, not all IgE multiple myeloma cases can be monitored using monoclonal protein levels. In our case, monoclonal protein was undetectable after 4 months of therapy. Furthermore, IgE monoclonal proteins are often small and may escape detection with capillary electrophoresis [3]. In our case, total IgE levels

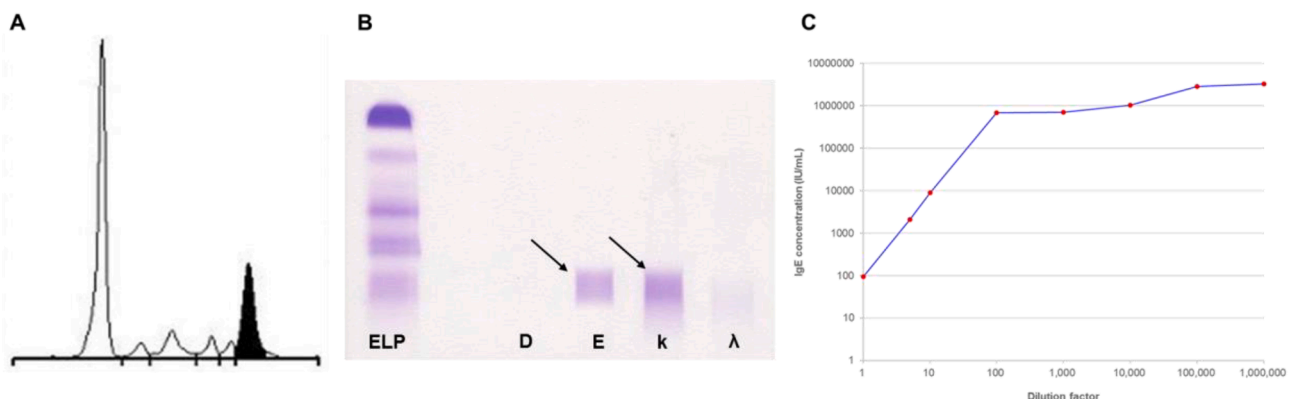


Fig. 1. SPE shows a monoclonal peak migrating in the gamma region (A). Serum IFE (B), carried out using anti-IgD, -IgE, -kappa, and -lambda antisera, shows bands positive for IgE and kappa. The monoclonal peak in the gamma region corresponds to an IgE- κ monoclonal peak. IgE concentrations are shown against serial dilutions, indicating the prozone phenomenon (C). A paradoxical increase in the IgE concentration with a progressive increase in the dilution factor (up to 1:100) can be seen. Both axes are in logarithmic scales. Abbreviations; SPE: serum protein electrophoresis, IFE: immunofixation electrophoresis, ELP: reference line.

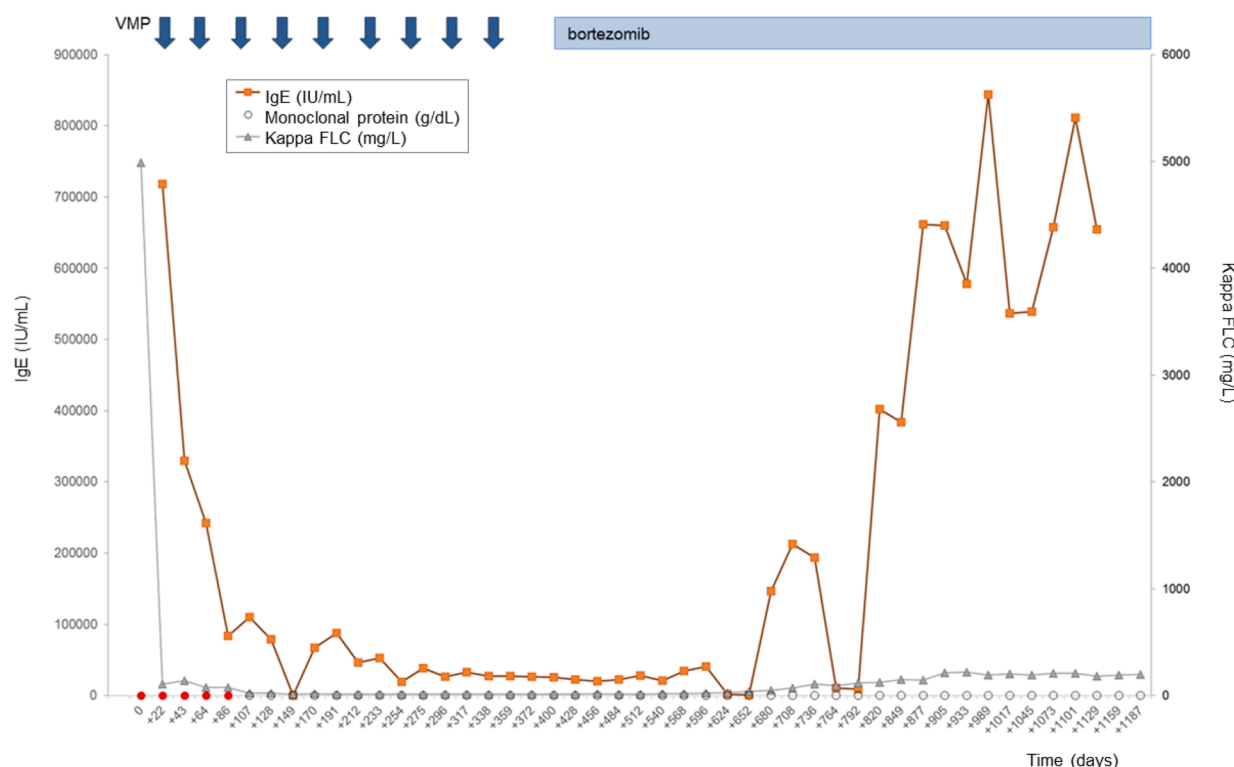


Fig. 2. Total IgE concentrations (orange squares), serum kappa FLC concentrations (gray triangles), and monoclonal protein measurements (circles) over the course of treatment. The patient presented with an IgE level of 5,543,300 IU/mL (13,303,920 μ g/L), serum κ FLC concentration of 4990 mg/L, and IgE- κ monoclonal protein level of 1.3 g/dL (13 g/L) (red filled circles). After the first course of modified VMP, total IgE, serum kappa FLC, and monoclonal protein levels started to decrease (red filled circles). The monoclonal protein could no longer be detected (empty circles) 4 months after initiating treatment (after 3 courses of VMP). Nine months from presentation, serum kappa FLC levels started to increase above the RI, as well as total IgE levels. The monoclonal peak has not reappeared until the time of this writing. Abbreviations: FLC, free light chain; RI, reference interval; VMP, bortezomib, plus melphalan and prednisone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

showed a reasonable correlation with the κ -to- λ FLC ratio, and correlated well with the kappa FLC concentrations. To the best of our knowledge, this is the first IgE multiple myeloma case showing the results of serial monitoring of total IgE and FLC levels. Given the lack of laboratory markers for monitoring the response of IgE multiple myeloma to treatment, certain cases may benefit from serial monitoring of involved FLC concentrations or FLC ratios, as shown in the present case.

In conclusion, the importance of IgE measurements in assessing IgE myeloma, as demonstrated in this case, is twofold. First, serial dilutions are mandatory in the presence of the prozone phenomenon, and second, the trend in serial IgE levels correlates with the trend in the serum FLC ratio, especially when the monoclonal protein is undetectable. This case highlights the need for laboratory professionals to be vigilant for occurrence of the prozone phenomenon, and to perform appropriate serial dilutions when IgE levels are discordant with other available laboratory and clinical information in cases of IgE multiple myeloma.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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