

Defects and countermeasures in laboratory diagnosis of rare IgE multiple myeloma

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ABSTRACT

Background: IgE multiple myeloma (MM) is a rare subtype of MM that is easily misdiagnosed. We report a rare case of IgE-MM and investigate the application of the SLiM-CRAB criteria to screen for high-risk smoldering MM (SMM) patients, so as to summarize the causes and methods used to prevent missed diagnosis or misdiagnosis in IgE-MM.

Methods: The serum monoclonal protein (M-protein) classification and IgE quantification was performed and sent to several individual institutions. The results were collected and the causes of IgE detection defects were analyzed.

Results: Upon admission to our hospital, the patient's serum free kappa light chain was 1069.9 mg/L, free lambda light chain was 9.2 mg/L, and free kappa/lambda ratio was 115.9, which met the SLiM criteria, but without CRAB features. Immunofixation electrophoresis (IF) showed "M-like protein aggregation bands" in all lanes. After pretreatment with 1% β-mercaptoethanol to depolymerize the aggregation of monoclonal protein, the "M-like protein aggregation bands disappeared. The other five institutions did not provide the correct typing results. The quantification of serum IgE was as high as 2.06×10^7 IU/mL, whereas 7 other testing institutions reported IgE levels ranging from 1.0 to 1100 IU/mL.

Conclusion: High-risk biomarkers in SLiM criteria can achieve good therapeutic effects in rare IgE-MM patients. Serum immunofixation performed without antisera against IgE, insufficient identification of the lytic bands produced by high macromolecule aggregation in IF, and the absence of a prozone effect avoidance procedure during IgE quantitative detection are the primary causes of missed diagnosis or misdiagnosis in patients with IgE-MM.

1. Introduction

Multiple myeloma (MM) is one of the most common hematological malignancies only less than lymphoma and is characterized by the presence of monoclonal immunoglobulin or light chains in the peripheral blood [1]. Immunoglobulin E (IgE) MM is a rare subtype of MM that was first recorded in 1967. Hejl et al. summarized 63 cases of IgE-MM from 1967 to 2017 [2] and only three cases of IgE-MM have been

reported since 2018 [3,4]. Despite its low incidence, IgE-MM is characterized by a higher incidence of transformation into IgE-producing plasma cell leukemia, indicating the importance of accurate classification of MM [3,5]. In the past decade, major advances have been made in the diagnosis, risk stratification, and management of MM using the SLiM-CRAB criteria [6–8]. However, the identification and quantification of monoclonal immunoglobulins, especially in the accurate classification and quantitative detection of monoclonal IgE, remained

Abbreviations: IgE, immunoglobulin E; MM, multiple myeloma; SMM, smoldering multiple myeloma; CT, computed tomography; IF, Immunofixation electrophoresis; RVD, lenalidomide-bortezomib-dexamethasone; CR, complete remission.

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stagnant. Missed diagnosis or misdiagnosis of IgE-MM is frequently reported in the literature [2,9]. In this study, we report a case of IgE-MM and investigate the typing or quantification of monoclonal IgE from several independent institutions. We initially discussed the application of SLiM-CRAB criteria in the identification of high-risk IgE SMM patients, summarized the causes and methods of preventing missed diagnosis or misdiagnosis in IgE-MM from our work experience, and provided some tips to avoid the prozone effect during IgE quantitative detection.

2. Materials and methods

2.1. Clinical characteristics of the case

A 72-year-old woman was admitted to the hospital owing to lumbar pain for >1 month. Before diagnosis, comprehensive physical examinations and relevant routine examinations were performed. The patient had a body temperature of 37 °C; respiratory rate of 16 breaths/min; heart rate of 75 beats/min; blood pressure of 120/72 mmHg; normal consciousness; absence of yellowish discoloration; no enlargement of superficial lymph nodes; normal cardiopulmonary function; flat and soft abdomen without tenderness; and no palpable liver and spleen under the ribs. A systemic bone X-ray survey revealed the absence of osteolytic lesions. Chest computed tomography (CT) revealed multiple small proliferative lesions in the right lung but no osteolytic lesions were found. Abdominal ultrasonography showed no abnormalities, while bone mineral density examination showed severe osteoporosis.

2.2. Laboratory tests

Serum protein electrophoresis and immunofixation electrophoresis (IF) were performed on an agarose gel (Hydrasys and Hydragel; Sebia). The immunoglobulin free kappa and free lambda light chain tests were performed on an Optilite nephelometer (Binding Site, Birmingham, UK) according to the manufacturer's instructions (Freelite; The Binding Site, Birmingham, UK). The normal reference interval for kappa FLC is 3.3–19.4 mg/L, the reference range for lambda FLC is 5.7–26.3 mg/L, and the reference interval for kappa/lambda ratio is 0.26–1.65. In addition, serum IgE levels were measured by solid-phase immunoassays using a Siemens-Immunitest 2000 analyzer (Siemens Healthcare Diagnostics, Flanders, USA) with a linearity range of 1–2000 IU/mL. The reference range for serum IgE level is <140 IU/mL [2].

Table 1
Routine laboratory examination results.

items	initial results without treatment	treatment course II	treatment course IV	treatment course VI	Normal reference value	Unit
White Blood Cell Count	6.9	5.37	6.2	5.63	3.5–9.5	$\times 10^9$ /L
Red Blood Cell Count	3.1	3.48	3.72	3.27	3.8–5.1	$\times 10^{12}$ /L
Platelet Count	170	205	201	213	125–315	$\times 10^9$ /L
Hemoglobin	102	120	127	110	115–150	g/L
Total Protein	111.2	57.2	64.3	59.2	65.0–85.0	g/L
Albumin	44.5	35.3	38.6	37.1	40.5–55.0	g/L
Alanine transaminase (ALT)	33	6.3	6	14	7.0–40.0	U/L
Lactic dehydrogenase (LDH)	139	133	140	151	120–250	U/L
Urea	6	4.8	3.7	6.1	3.1–8.8	mmol/L
Creatinine	91.8	57.1	57.4	59.3	35.0–123.0	umol/L
Calcium	2.5	2.3	2.2	2.2	2.1–2.8	mmol/L
Immunoglobulin G (IgG)	6	8.7	8.4	8.8	7.5–15.6	g/L
Immunoglobulin A (IgA)	0.48	1.08	1.01	0.78	0.82–4.53	g/L
Immunoglobulin M (IgM)	0.26	0.45	0.39	0.29	0.64–3.04	g/L
light chain (kappa)	67.2	7.1	6.9	/	6.3–13.5	g/L
light chains (lambda)	1.9	3.3	3.2	/	3.1–7.2	g/L
$\beta 2$ -microglobulin	6.7	1.7	/	1.8	0.6–2.4	mg/L
24 h urine protein	1625.7	44.8	/	45.5	0.0–150.0	mg/24 h

“/” represents missing value.

2.3. Flow cytometry assay

Bone marrow aspirate specimens collected in EDTA anticoagulant tubes were washed with PBS 3 times. The cell pellets were routinely incubated with fluorescently-labeled antibodies. After being washed with PBS and erythrocyte lysis, the cell samples were resuspended and filtered, then detected by flow cytometry (Beckman, DxFLEx). The immunophenotype of plasma cells was analyzed according to the preset flow cytometric panels. The antibodies that were used included CD56-PC7 (N901), CD19-APC-A750 (J3.119), CD45-Kro 525 (J.33), CD138-APC (B-A38), CD27-ECD (1A4CD27), CD28-APC-A700 (CD28.2), CD38-BV421 (HIT2), cyKAPPA-FITC (TB28-2), cyLAMBDA-PE (1–155-2), CD123-Pe rCP-CyTM5.5 (7G3).

3. Results

3.1. Laboratory examination

Routine laboratory examination revealed a total protein level of 111.2 g/L and a blood kappa light chain level of 67.2 g/L at initial diagnosis. The other results of routine laboratory examination were either normal or slightly changed, as shown in Table 1.

3.2. Special laboratory examination

Serum protein electrophoresis (Sebia-HYDRASYS 2) displayed an obvious monoclonal protein (M protein) in the γ -globulin fraction, and densitometric scanning (Epson-V850 Pro) confirmed that the concentration of M protein was 41.4% (46.0 g/L; Fig. 1A). Bone marrow biopsy revealed that the proportion of clonally naive plasma cells was 56.9% (Fig. 1B). Immunophenotypic profiles obtained by flow cytometry (Beckman, DxFLEx) showed that the clonally naive plasma cells expressed a high level of CD38, with uneven expression of CD138, high expression of CD56, and restricted expression of intracellular light chain kappa (Fig. 1C, S1). Results of the Binding Site Optilite analyzer showed serum free light chain kappa level of 1069.9 mg/L, serum free light chain lambda level of 9.2 mg/L, and free kappa/lambda ratio of 115.9. No lesions were found in the vertebral column or the extremities. These results indicate a diagnosis of MM based on the SLiM-CRAB criteria.

3.3. Subtype classification of M protein

For further subtype classification of the M protein, IF of the serum

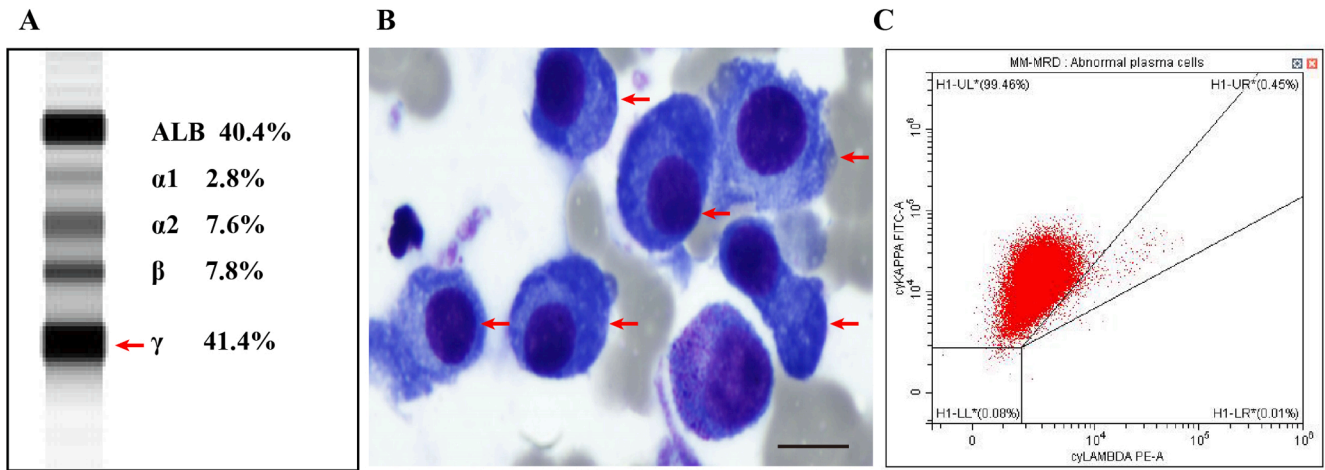


Fig. 1. Special laboratory examination. (A) The densitogram scan of serum protein electrophoresis. The red arrow shows an obvious monoclonal protein (M protein) in the γ -globulin fraction; The concentration of M protein was 41.4 % (46.0 g/L) by optical density scanning. (B) Bone marrow smear staining results, the red arrow shows naive plasma cells; (C) Immunophenotypic analysis of intracellular light chain in plasma cells of bone marrow samples, red dots indicate abnormal plasma cells with restricted expression of intracellular light chain Kappa.

was conducted. Results showed that a high concentration of the monoclonal protein remained in the polymerization state and formed “M-like protein aggregation bands” in all lanes of the gel (Fig. 2A). Therefore, 1% β -mercaptoethanol was applied to depolymerize the aggregation of monoclonal protein, and most of the “M-like protein aggregation bands” disappeared except those found in the anti-kappa and anti-IgE lanes (Fig. 2B). In the independent duplicates immunofixation assay, the IgE monoclonal bands exhibited various shapes characterized as mouth, dumbbell, vacuole, or target-like staining zone; demonstrated an antigen excess phenomenon or “hook effect”; and were unable to create large network precipitates (Fig. 2C). Therefore, the patient’s serum was diluted with normal saline 3 (as recommended by the manufacturer), 10, 20, 40, and 80 times, and subjected to a repeat immunofixation assay. The results showed a clear monoclonal band in the serum sample that was diluted 20 times, and no antigen excess effect was observed (Fig. 2D). Furthermore, the patient’s serum was sent to five individual institutions outside the hospital for subtype classification of the M protein. Results demonstrated that only one institution provided the correct typing result of IgE-kappa monoclonal, one institution provided an uncertain result of IgE-kappa monoclonal, whereas the remaining three institutions only confirmed a kappa monoclonal, without the application of antisera against IgD and IgE (Table 2).

3.4. Quantitative detection of IgE

The primary quantification of serum IgE, determined by solid-phase immunoassays using Siemens-Immulite 2000, was 116 IU/mL (1 IU/mL

Table 2

Classification results of M protein in other testing institutions.

Institution	Instruments	Results	Notes
A	Sebia-HYDRASYS 2	Kappa monoclonal	Because of the rarity, IgD and IgE types were not applied
B	Helena-Spife 3000	Kappa monoclonal	
C	Helena-Spife 3000	Kappa monoclonal	
D	Sebia-HYDRASYS 2	IgE-Kappa monoclonal (uncertained)	/
E	Sebia-HYDRASYS 2	IgE-Kappa	/

A ~ E represents different testing institutions.

= 2.4 ng/mL [10]), which was significantly lower than the monoclonal protein level measured by serum protein electrophoresis, suggesting an artifactual result owing to the excessive amount of antigen (prozone effect). Subsequently, serial dilutions of the sample were applied, and stable IgE concentration (2.06×10^7 IU/mL) was obtained after a pre-dilution of 1:20,000 (Fig. 3). The quantification (2.06×10^7 IU/mL = 49.4 g/L) is very similar to the results of the densitogram scan of M protein (46.0 g/L). Then, the serum and its 100-fold dilutions were simultaneously sent to the other seven institutions outside the hospital for IgE quantification. Results showed that six of the seven institutions provided negative results (<140 IU/mL), except for one institution that

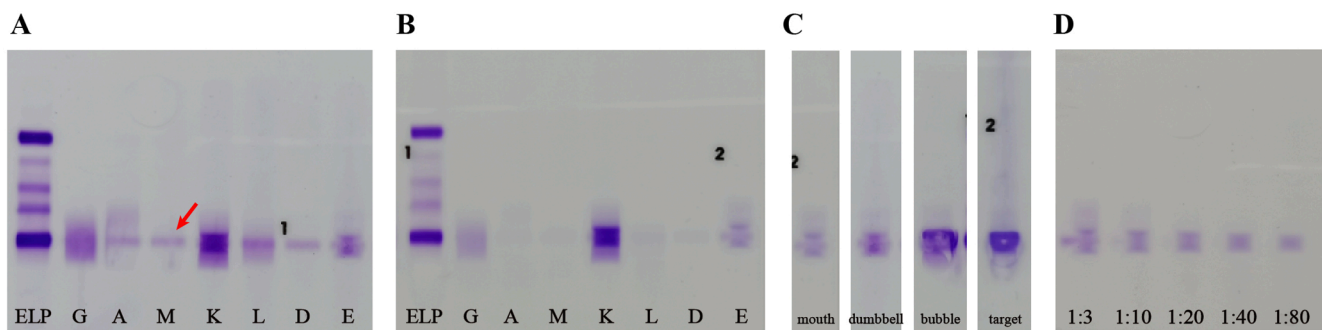


Fig. 2. Results of immunofixation. (A) Serum immunofixation carried out with antisera against IgG (G), IgA (A), IgM (M), kappa (K), and lambda (L), IgD (D), IgE (E). Red Arrow indicates “M-like protein aggregation bands”. (B) immunofixation analysis of the patient’s serum pretreated with 1 % β -mercaptoethanol; (C) Replicated immunofixation results of IgE with 1:3 dilution; (D) immunofixation analysis of the patient’s serum with the different dilutions of physiological saline.

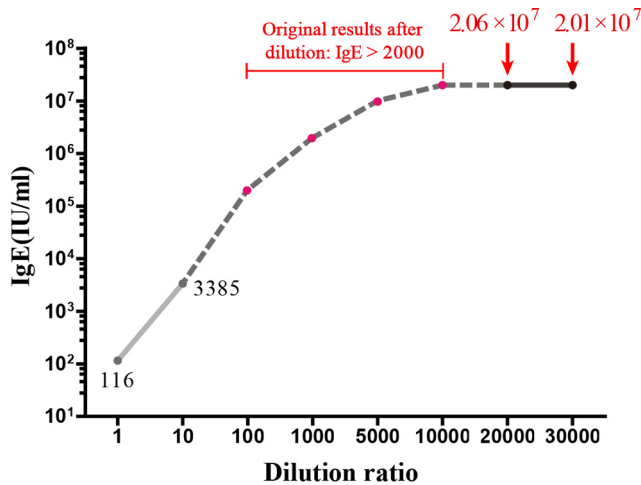


Fig. 3. IgE concentration in different diluted serum. The red dots indicate the serum is diluted 100–10000 times, with no specific result due to the IgE detection value exceeding the linear upper limit of the instrument, which gave results >2000 IU/ mL. The red arrows indicate a stable IgE concentration when the serum is diluted over 20,000-fold.

provided a positive IgE result that exceeded the upper limit of the reference interval in the undiluted serum (1100 IU/mL). A significant difference was observed in the IgE concentration between the undiluted and diluted serum obtained from the seven institutions. None of these institutions provided accurate IgE concentration values. Further investigation found that six of the seven institutions did not have the corresponding prozone effect avoidance approach in the IgE quantitative chemiluminometric analysis (Table 3).

3.5. Clinical diagnosis, treatment, and curative effect monitoring

According to the guidelines and modified international staging system [11,12], the patient was diagnosed with IgE-kappa-type MM (stage II), and a lenalidomide-bortezomib-dexamethasone (RVD) treatment regimen was administered. After 3 courses of RVD treatment, serum M protein was undetectable on protein electrophoresis and IF assays (Fig. 4A, S2). After the fourth course of treatment, the total protein (64.3 g/L), hemoglobin (127.0 g/L), light chain kappa (6.9 g/L), free light chain kappa (20.96 mg/L), free light chain kappa/lambda ratio (1.07), urinary protein levels, and other routine laboratory examination results returned to normal. The serum IgE concentration was reduced to 2090 IU/mL (10,000-fold decrease; Fig. 4B, C). MRD assayed by flow cytometry was negative, and normal mature plasma cells accounted for 0.355 % with undetectable naive plasma cells in the bone marrow. The clinical evaluation showed complete remission (CR). At present, the patient has completed 6 courses of RVD treatment and is still in a CR

state.

4. Discussion

IgE-MM is a rare subtype of MM. Morris et al. analyzed the data of 22,244 MM patients in the European Group for Blood and Marrow Transplantation Myeloma Database, of whom 13 (0.006%) were diagnosed with IgE-MM [11]. Patients with IgE-MM have the shortest median survival compared with those with other types of myeloma, such as IgG-MM. Hejl et al. and Pandey et al. concluded that patients with IgE-MM had a higher incidence of chromosomal translocations and plasma cell leukemia after a comprehensive analysis of IgE-MM cases [2,5]. Although the survival duration is short, the prognosis of such rare type of myeloma has improved with the advent of new drugs and autologous transplantation. Hence, early detection and standardized treatment will benefit patients, although IgE-MM is rare and fatal.

4.1. Application of MM high-risk biological markers in rare IgE-MM

SMM is an asymptomatic clonal plasma cell disorder between monoclonal gammopathy of undermined significance (MGUS) and MM, with no evidence of end-organ damage included in the CRAB criteria [13]. After the early diagnosis of SMM, therapy was only provided when symptoms have developed, because of the inability to accurately identify the subset of patients who have biological malignancy and will consistently progress within a short period. This conundrum makes high-risk SMM patients unable to receive early therapy to prevent organ damage. Therefore, the International Myeloma Working Group reached a consensus on the SLiM-CRAB criteria and recommended the implementation of three validated biomarkers in an attempt to distinguish high-risk patients early [6]. The high-risk makers include clonal bone marrow plasma cell percentage of >60% (S), involved/uninvolved serum free light chain (SFLC) ratio of >100 (Li), and >1 focal lesion >5 mm as determined by magnetic resonance imaging (M) [14–16]. High-risk SMM patients who met the SLiM criteria defined only by biomarkers were allowed to receive treatment without developing other symptoms that correspond to the conventional CRAB criteria [6]. Currently, the criteria have been accepted and applied by most countries, such as China [12], United States [17], and Europe [18].

In this case, initial laboratory investigations showed a bone marrow clonal plasma cell percentage of 56.9%, a serum M protein level of 46.0 g/L, hemoglobin level of 102 g/L, and normal blood calcium level and glomerular filtration rate. Laboratory and imaging examinations indicated insufficient evidence of MM-defining events (MDEs) to meet the CRAB criteria. However, the free light chain kappa level was 1069.9 mg/L, free light chain lambda level was 9.2 mg/L, and free kappa/lambda ratio was 115.9, which fulfilled the SLiM criteria. Considering the age of the patient (72 years old), she underwent six courses of RVD chemotherapy regimen in accordance with the 2020 Chinese Guideline for the

Table 3
IgE quantitative results from 7 other different institutions.

Institution	IgE (undiluted, IU/ml)	IgE* (100-fold dilution, IU/ml)	Instruments	Methodology	Prozone effect avoiding program [#]
A	<17.8	80,100	Siemens-BNII	Time scattering turbidimetry	no
B	<5.0	3210	Beckman-IMMAGE800	Rate scattering turbidimetry	no
C	5.0	44,500	Beckman-AU5800	Transmission turbidimetry	no
D	8.7	98,060	Roche-e602	Electrochemiluminescence	no
E	8.8	111,100	Roche-E411	Electrochemiluminescence	no
F	<1.0	13,530	Snibe-MAGLUMI2000	Chemiluminescence	no
G	1100	>500000	Thermo-ImmunoCAP ^{&}	Enzyme immunoluminescence	yes

* IgE results in this column have been multiplied by the dilution, The large difference between the diluted and undiluted samples suggests the existence of the band effect.

[&] Although the instrument has the procedure to avoid the prozone effect in IgE quantification, the prozone effect is still caused by incomplete washing.

[#] In the turbidimetric method, the prozone effect avoidance program is the over antigen monitoring program; and in the luminescence method, it is the excessive antigen washing procedure after the solid-phase antibody binds to the antigen.

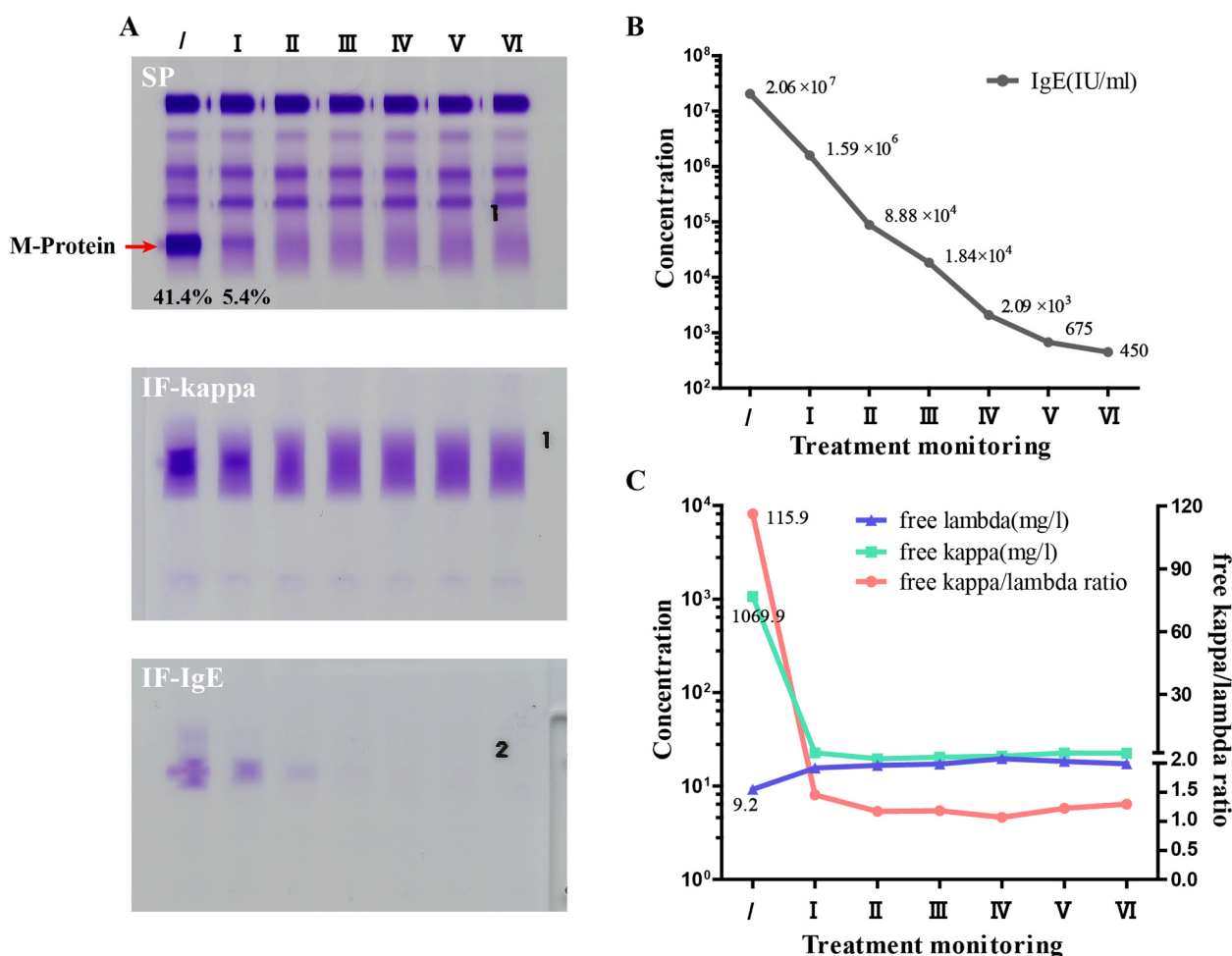


Fig. 4. Therapeutic effect monitoring. A. The results of protein electrophoresis and IF assay, “/” represents the initial results without treatment, “I” to “VI” represents the course of RVD treatment, and “SP” represents serum protein electrophoresis. “IF-kappa and IF-IgE” are the IF assay results with antisera against Kappa and IgE, respectively. B. The concentration of IgE. C The concentration of free light chain and free kappa/lambda ratio was monitored during the treatment course.

Diagnosis and Treatment of Multiple Myeloma. The patient achieved CR after four courses of treatment and remained in the same state at present. This is the first study to report the application of SLiM criteria in diagnosing a rare type of IgE-MM, which achieved good therapeutic results.

4.2. Defects of IgE-MM in typing detection

According to the 2003 IMWG criteria, 3% of MM patients had no detectable serum or urinary monoclonal protein, making the presence of monoclonal protein not mandatory for the diagnosis of MM [6,19]. However, M protein detection still preserves value in the confirmation, classification, and evaluation of secretory MM [17]. Therefore, M protein is a significant factor in the diagnosis and treatment of MM [18]. The IF technique is the primary technique for the typing of M protein, in which IgG, IgA, IgM, kappa, and lambda are detected using routine typing tests, while IgD and IgE are not commonly included [20]. In China, considering the high incidence of IgD-MM (6.5%) [21], the 2020 China Guideline for the Diagnosis and Treatment of Multiple Myeloma states that IF typing detection should include the determination of IgD when necessary. Insufficient attention has been paid to the detection of IgE. The same situation also occurs in other countries worldwide. Jurczynszyn et al. conducted a case-control study of 18 cases of IgE-MM from 9 testing centers in Hungary, Italy, Israel, Poland, and the United States; they concluded that many testing centers did not detect the presence of IgE, and some IgE-MM cases were misdiagnosed as light-chain MM [22]. In the present study, three of the five institutions (A,

B, and C) did not perform the IF assay with antisera against IgD and IgE. Additionally, all institutions provided a false result for kappa monoclonal, confirming that it is common for laboratory specialists to underestimate the possibility of IgE-MM and prozone effect in the antigen–antibody reaction-based methods. Therefore, the presence of IgD and IgE monoclonality should be further identified in patients with monoclonal light chains but without IgG, IgA, and IgM monoclonal antibodies.

In the IF assay, high concentrations of macromolecules such as rheumatoid factor, cold agglutinin, or autoantibodies may stay in the lane to form an “M-like protein aggregation band.” Typically, the IgE heavy chain has four constant domains with a large molecular weight (190 kDa) [23], and the monoclonal IgE harbors a richer and more complex glycosylated side-chain structure in order to increase its molecular weight [24]. The patient tested negative for rheumatoid factor, cold agglutinin, or autoantibodies, indicating that high molecular weight monoclonal IgE remained in polymerization and formed aggregation bands in all the lanes of the IF assay. “M-like protein aggregation bands” are easily misdiagnosed in daily work; hence, the results should be verified further by pretreatment of β -mercaptoethanol or by conducting a repeat IF assay using diluted serum. Additionally, excessive concentrations of IgE cannot form large network precipitates with the antisera against IgE, resulting in the prozone effect, manifesting as multiple lytic bands, such as mouth shape, dumbbell shape, target shape, and vacuolar shape in the gel [25]. The different characteristics of lytic bands are easily misdiagnosed as false negatives, while the

increase in either κ or λ FLC along with abnormal κ/λ ratios provide an indicator of clonality.

4.3. Defects in the quantitative detection of IgE

Generally, the IgE heavy chain gene contains an abnormal poly-adenosine signal, leading to the low expression of membrane-bound form IgE (mIgE) [26], which results in an extremely low concentration of IgE in the circulation compared with other types of immunoglobulins [23]. Although the IgE concentration is quite low, it plays an important role in mediating the pathogenesis of allergic diseases [27] and is widely applied in the diagnosis of various allergic diseases [28]. IgE is usually present at low concentrations in patients with allergic diseases. Ibrahim et al. reported that the IgE concentrations in patients with atopic dermatitis ranged from 4 to 1300 IU/mL, which is mainly within the range of 4–393 IU/mL [29]. For patients with IgE-MM, Hejl C et al. indicated that the distribution of IgE concentrations in patients varied dramatically from 9.38 IU/mL to 1.79×10^7 IU/mL, and it reached 2.06×10^7 IU/mL in this case [2].

IgE is generally detected by immunoturbidimetry, electrochemiluminescence, or enzyme immunoluminescence assays. In the external quality assessment (EQA) carried out by the National Center for Clinical Laboratories of China, a total of 853 laboratories participated in the EQA of IgE, 596 (70%) of which detected IgE using different immunoturbidimetry techniques, while 232 (27%) of which quantified the IgE levels using immunoluminescent techniques. The above two detection techniques accounted for 97% of all IgE detection techniques.

Immunoassay measurement of the analyte requires proper proportion to form an analyte-antibody complex that is forceful enough to provide a detection signal. With excessive amounts of analyte, the reaction antibody is saturated by the analyte molecules and fails to form a large network complex, resulting in an underestimation of the analyte concentration. This phenomenon is generally described as the prozone effect or “hook effect,” which almost occurs in virtually any type of immunoassay and represents one of the most common reasons for reporting false-negative test results [25]. Increased insight into the hook effect mechanisms and the availability of tools to prevent it could reduce the false negative in daily laboratory practice. For example, the IMMAGE 800 analyzer (Beckman Coulter) can perform a prozone effect avoidance procedure, which involves adding a small amount of standard antigen at the end of the detection and observing the change in reaction rate. From the laboratory perspective, the possibility of a prozone effect should be taken into account, as the detection kits are designed to measure IgE levels much lower than those in the IgE-MM. Currently, most IgE kit manufacturers have abandoned the prozone effect avoidance approach to pursue high analytical speed and high-throughput detection, thus resulting in the occurrence of the prozone effect. In the current case, three institutions reported negative results for IgE detection as the prozone effect avoidance procedure was no longer performed, although different detection instruments and immunoturbidimetric techniques were applied.

In solid-phase immunoassays, if the excessive antigens in the serum are rinsed thoroughly, the zone effect could be significantly alleviated. However, if the manufacturers added the luminescence-labeled antibody without adequate rinsing to increase the detection speed, both detection and capture antibodies will be saturated and cannot form sandwich complexes. In this case, the Siemens-Immolute 2000 analyzer was used for quantitative detection of IgE level, which added the detection and capture antibodies with the serum sample in just a single step simultaneously, resulting in a significantly low IgE level (116 IU/mL). Although the detection institutions D, E, and F employed different detection instruments and immunoluminescence techniques, they may cause severe zone effects for their detection steps were similar to those of the Siemens-Immolute 2000 analyzer. The IgE detection procedure used by institution G included washing steps to remove the excess analyte, thus significantly alleviating the prozone effect and obtaining the IgE

level at the upper detection limit. Therefore, several factors originating from antigen excess should be suspected: (i) the results from the diluted samples are significantly higher than the undiluted sample, (ii) the immunoassay kinetics is abnormal; (iii) the test results are not in line with other laboratory findings, and (iv) the test result is conflicting with the clinical condition of the patient.

In the past few years, with the development of biotechnology, new detection techniques, such as paper-based upconversion fluorescence aptasensor [30] and graphene aptasensor technology [31], have been applied for the quantitative detection of IgE. However, these technologies are not only complex and expensive but also remain in the research stage. Moreover, these technologies focus on the diagnosis and therapeutic monitoring of the low concentration of IgE in patients with allergic diseases, and there remains a lack of corresponding technologies that can be used for the diagnosis and therapeutic monitoring of IgE monoclonal antibodies in MM. We have summarized the causes and troubleshooting of preventing missed diagnosis and misdiagnosis in IgE-MM from our work experience (Table 4).

In recent years, serum FLC and FLC ratios have been included in various guidelines and standards for their advantages in diagnosis, risk stratification, and prognosis assessment. FLC measurement not only benefits the diagnosis and management of MM, but also implicates the diagnosis of oligosecretory myeloma, light-chain amyloidosis, light chain deposition disease and plasmacytoma. If the amount measured by densitometry of the electrophoretogram and simultaneously obtained FLC results are discordant, one should always look for an atypical heavy chain or chain fragment causing the increase in the protein amount of M-band. Patients with IgE-MM have a shorter median survival time

Table 4
Causes and troubleshooting of missed diagnosis/misdiagnosis in IgE MM.

Causes	Troubleshooting
Neglect of IgE in guidelines or criteria	Guidelines or criteria should pay attention to the typing detection of IgE
Presence of a sole light chain monoclonal band without further detection for IgD and IgE monoclonal	IgD and IgE detections should be performed with the sole presence of a light chain monoclonal
Insufficient identification of the “M-like protein aggregation bands”	High concentrations of rheumatoid factor and cold agglutinin may cause “M-like protein aggregation bands”. Pretreated the sample with β -mercaptoethanol
Insufficient identification of the various lytic bands	Vacuolar-shaped, mouth-shaped, dumbbell-shaped, and target-shaped bands may appear at high concentrations of M-protein, and clear clonal bands can be obtained by diluting serum samples
Narrow linearity range of IgE quantitative detection kits	Manufacturers could produce IgE quantitative detection kits with a more wide linearity range
The absence of antigen excess monitoring procedure	It is necessary to introduce the corresponding prozone effect avoidance program
No washing steps after the binding of capture antibody and IgE in the luminescence immunoassay.	Analyzers using luminescence immunoassay should wash sufficient to remove the excessive IgE
Insufficient work experience of laboratorians	A contradictory result between IF typing and immunoassays based quantification should be suspected The diluted sample is higher than the undiluted sample should be suspected Abnormal immunoassay kinetics should be suspected The test result is not in line with other lab findings should be suspected The test result is conflicting with the clinical condition of the patient should be suspected A significant difference between the current result and the previous result should raise doubts Record the abnormal samples and cases to attract attention

compared with those with other types of myeloma, such as IgG-MM. However, there is currently no special treatment regimen for IgE MM, because of the low incidence and lack of cohort studies. In China, the latest version of guidelines for the diagnosis and management of multiple myeloma does not distinguish the difference between IgE MM and other types of MM. RVD is the initial treatment recommended by the guidelines. Other scholars have also reported that IgE-MM may be responded well to VMP (Bortezomib-melphalan-prednisone) regimen [32]. To definitively determine which treatment regimen is superior, further studies need to be conducted. But it is especially important for the accurate diagnosis of IgE multiple myeloma.

In summary, the application of the SLiM-CRAB criteria to screen high-risk SMM patients with early therapy could achieve a good therapeutic effect. Although the quantitation of monoclonal Ig is not essential for the diagnosis of MM, the concentration of MM protein is important for monitoring the patient's response to therapy. In addition, the type of M protein influences the risk of progression in MM. Therefore, more attention should be paid to the quantitation and typing of monoclonal immunoglobulin. Insufficient identification of the lytic bands produced by high macromolecule aggregation in IF and the lack of a prozone effect avoidance procedure during IgE quantitative detection are the primary causes of missed diagnosis or misdiagnosis in IgE-MM. The following three strategies are effective countermeasures to address the dilemma: (i) IgD and IgE detection should be performed in the presence of a light chain monoclonal band alone, (ii) the lytic bands should be efficiently identified and treated, and (iii) the corresponding zone effect avoidance procedure should be included during IgE quantitative detection.

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CRedit authorship contribution statement

Yongjian Chen: Conceptualization, Supervision, Funding acquisition, Writing – original draft. **Yuzhou Chen:** Writing – original draft. **Yanping Zhou:** Data curation, Visualization. **FeiFei Zhao:** Data curation, Visualization. **Sumei Wang:** Data curation, Visualization. **Sujie Zheng:** Data curation, Visualization. **Yuhuan Shen:** Data curation, Visualization. **Xiangmin Tong:** Conceptualization, Supervision, Funding acquisition, Writing – original draft. **Jing Du:** Conceptualization, Supervision, Funding acquisition, Writing – original draft. **Yanchun Li:** Conceptualization, Supervision, Funding acquisition, Writing – original draft.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cca.2022.05.011>.

References

- [1] N. Gupta, A. Sharma, A. Sharma, Emerging biomarkers in Multiple Myeloma: a review, *Clin. Chim. Acta*; *Int. J. Clin. Chem.* 503 (2020) 45–53.
- [2] C. Hejl, R. Mestiri, T. Carmoi, S. Bugier, D. Chianca, C. Renard, P. Vest, IgE monoclonal gammopathy: a case report and literature review, *Clin. Biochem.* 51 (2018) 103–109.
- [3] N. Galakhoff, C. Leven, J.R. Eveillard, A. Tempescul, H. Kerspern, C. Aubron, C. Buors, E. Lippert, J.L. Carre, M. Padelli, A case of IgE myeloma transformed into IgE-producing plasma cell leukaemia, *Biochimica Medica* 30 (1) (2020), 010801.
- [4] R. Brivio, A. Cappellani, M. Carpenedo, V. Minolfi, J. Intra, R. Romano, N. Spinoni, P. Brambilla, IgE monoclonal gammopathy: the clinical relevance to perform the immunofixation using IgE antisera, *Int. J. Laboratory Hematol.* 42 (5) (2020) e237–e239.
- [5] S. Pandey, R. Kyle, Unusual myelomas: a review of IgD and IgE variants, *Oncology (Williston Park, NY)* 27 (8) (2013) 798–803.
- [6] S.V. Rajkumar, M.A. Dimopoulos, A. Palumbo, J. Blade, G. Merlini, M.-V. Mateos, S. Kumar, J. Hillengass, E. Kastritis, P. Richardson, O. Landgren, B. Paiva, A. Dispenzieri, B. Weiss, X. LeLeu, S. Zweegman, S. Lonial, L. Rosinol, E. Zamagni, S. Jagannath, O. Sezer, S.Y. Kristinsson, J. Caers, S.Z. Usmani, J.J. Lahuerta, H. E. Johnsen, M. Beksac, M. Cavo, H. Goldschmidt, E. Terpos, R.A. Kyle, K. C. Anderson, B.G.M. Durie, J.F.S. Miguel, International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma, *Lancet Oncol.* 15 (12) (2014) e538–e548.
- [7] S.V. Rajkumar, Myeloma today: disease definitions and treatment advances, *Am. J. Hematol.* 91 (1) (2016) 90–100.
- [8] R. Al Hamed, A. Bazarbachi, F. Malard, J. Harousseau, M. Mohty, Current status of autologous stem cell transplantation for multiple myeloma, *Blood Cancer J.* 9 (4) (2019) 44.
- [9] G. Talamo, W. Castellani, N.G. Dolloff, Prozone effect of serum IgE levels in a case of plasma cell leukemia, *J. Hematol. Oncol.* 3 (2010) 32.
- [10] A. Caldini, F. Balboni, P. Parronchi, S. Scoccianti, T. Biagioli, A. Terreni, B. Morrocchi, M. Brogi, M. Berardi, M. Graziani, A rare condition: IgE type monoclonal gammopathy of undetermined significance, *Clin. Chem. Lab. Med.* 52 (8) (2014) e183–e185.
- [11] C. Morris, M. Drake, J. Apperley, S. Iacobelli, A. van Biezen, B. Björkstrand, H. Goldschmidt, J.-L. Harousseau, G. Morgan, T. de Witte, D. Niederwieser, C. Gahrton, Myeloma subcommittee of chronic leukaemia working party of E. Efficacy and outcome of autologous transplantation in rare myelomas, *Haematologica* 95 (12) (2010) 2126–2133.
- [12] [The guidelines for the diagnosis and management of multiple myeloma in China (2020 revision)]. *Zhonghua nei ke za zhi* 59 (5) (2020) 341–346.
- [13] S. Rajkumar, O. Landgren, M. Mateos, Smoldering multiple myeloma, *Blood* 125 (20) (2015) 3069–3075.
- [14] E. Kastritis, E. Terpos, L. Mouloupos, M. Spyropoulou-Vlachou, N. Kanellias, E. Eleftherakis-Papaikovou, M. Gkotzamanidou, M. Migkou, M. Gavriatopoulou, M. Roussou, A. Tasidou, M.A. Dimopoulos, Extensive bone marrow infiltration and abnormal free light chain ratio identifies patients with asymptomatic myeloma at high risk for progression to symptomatic disease, *Leukemia* 27 (4) (2013) 947–953.
- [15] J.T. Larsen, S.K. Kumar, A. Dispenzieri, R.A. Kyle, J.A. Katzmann, S.V. Rajkumar, Serum free light chain ratio as a biomarker for high-risk smoldering multiple myeloma, *Leukemia* 27 (4) (2012) 941–946.
- [16] E. Kastritis, L.A. Mouloupos, E. Terpos, V. Koutoulidis, M.A. Dimopoulos, The prognostic importance of the presence of more than one focal lesion in spine MRI of patients with asymptomatic (smoldering) multiple myeloma, *Leukemia* 28 (12) (2014) 2402–2403.
- [17] S.V. Rajkumar, Multiple myeloma: 2020 update on diagnosis, risk-stratification and management, *Am. J. Hematol.* 95 (5) (2020) 548–567.
- [18] J. Caers, L. Garderet, K.M. Kortum, M.E. O'Dwyer, N. van de Donk, M. Binder, S. M. Dold, F. Gay, J. Corre, Y. Beguin, H. Ludwig, A. Larocca, C. Driessen, M. A. Dimopoulos, M. Boccadoro, M. Gramatzki, S. Zweegman, H. Einsele, M. Cavo, H. Goldschmidt, P. Sonneveld, M. Delforge, H.W. Auner, E. Terpos, M. Engelhardt, European Myeloma Network recommendations on tools for the diagnosis and monitoring of multiple myeloma: what to use and when, *Haematologica* 103 (11) (2018) 1772–1784.
- [19] R.A. Kyle, M.A. Gertz, T.E. Witzig, J.A. Lust, M.Q. Lacy, A. Dispenzieri, R. Fonseca, S.V. Rajkumar, J.R. Offord, D.R. Larson, M.E. Plevak, T.M. Therneau, P.R. Greipp, Review of 1027 patients with newly diagnosed multiple myeloma, *Mayo Clin. Proc.* 78 (1) (2003) 21–33.
- [20] T. Morrison, R.A. Booth, K. Hauff, P. Berardi, A. Visram, Laboratory assessment of multiple myeloma, *Adv. Clin. Chem.* 89 (2019) 1–58.
- [21] J. Lu, J. Lu, W. Chen, Y. Huo, X. Huang, J. Hou, Chinese Medical Doctor Association Hematology B. Clinical features and treatment outcome in newly diagnosed Chinese patients with multiple myeloma: results of a multicenter analysis, *Blood Cancer J.* 4 (2014) e239.
- [22] A. Jurczyszyn, J.J. Castillo, D.H. Vesole, J. Liu, I. Avivi, A. Waszczuk-Gajda, E. Lech-Maranda, M. Gentile, G. Mikala, T. Guerrero-Garcia, A. Suska, M.A. Gertz, Clinical characteristics and treatment outcomes in IgE multiple myeloma: a case-control study, *Am. J. Hematol.* 93 (9) (2018) E238–E241.
- [23] B.T. Kelly, M.H. Grayson, Immunoglobulin E, what is it good for? *Ann. Allergy Asthma Immunol.: Official Publ. Am. College Allergy Asthma Immunol.* 116 (3) (2016) 183–187.
- [24] R. Plomp, P.J. Hensbergen, Y. Rombouts, G. Zauner, I. Dragan, C.A. Koeleman, A. M. Deelder, M. Wührer, Site-specific N-glycosylation analysis of human immunoglobulin e, *J. Proteome Res.* 13 (2) (2014) 536–546.
- [25] J.F. Jacobs, R.G. van der Molen, X. Bossuyt, J. Damoiseaux, Antigen excess in modern immunoassays: to anticipate on the unexpected, *Autoimmun. Rev.* 14 (2) (2015) 160–167.
- [26] A. Karnowski, G. Achatz-Straussberger, C. Klockenbusch, G. Achatz, M.C. Lamers, Inefficient processing of mRNA for the membrane form of IgE is a genetic mechanism to limit recruitment of IgE-secreting cells, *Eur. J. Immunol.* 36 (7) (2006) 1917–1925.
- [27] K. Ishizaka, T. Ishizaka, Identification of IgE, *J. Allergy Clin. Immunol.* 137 (6) (2016) 1646–1650.

- [28] R.G. Hamilton, W. Hemmer, A. Nopp, J. Kleine-Tebbe, Advances in IgE testing for diagnosis of allergic disease, *J. Allergy Clin. Immunol. Practice* 8 (8) (2020) 2495–2504.
- [29] H.M. Ibrahim, M.A. El-Taieb, M.H. Hassan, A.A.E. Mohamed, E.A. Kotop, O. H. Abd-Allah, E.M. Hegazy, Relations between vitamin D3, total and specific IgE for house dust mites in atopic dermatitis patients, *Sci. Rep.* 10 (1) (2020) 21000.
- [30] M. He, N. Shang, Q. Zhu, J. Xu, Paper-based upconversion fluorescence aptasensor for the quantitative detection of immunoglobulin E in human serum, *Anal. Chim. Acta* 1143 (2021) 93–100.
- [31] X. Wang, Y. Zhu, T.R. Olsen, N. Sun, W. Zhang, R. Pei, Q. Lin, A graphene aptasensor for biomarker detection in human serum, *Electrochim. Acta* 290 (2018) 356–363.
- [32] H. Sato, Y. Komiya, S. Hoshino, Bortezomib plus melphalan and prednisone (VMP regimen) as the initial treatment for IgE multiple myeloma: a case study, *Int. J. Hematol.* 99 (6) (2014) 786–789.