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An unusual pattern in serum protein electrophoresis to take in mind: A case report

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ABSTRACT

Here we described a case of an asymptomatic 73 years-old female patient in geriatric routine consultation, whose laboratory testing showed hyperproteinemia with accompanying hyperglobulinemia. A diagnosis of BGUS was made only after a correlation among SPEP, densitometry tracing and IFE results was established, evidencing a second peak, that was less evident and not reported at first. These biclonal conditions are of very low incidence in the clinical laboratory, requiring the laboratory professional to have particular skills for their identification. As far as is known, clinical findings in BGUS are similar to those found in MGUS. However, they remain not well understood. Therefore, for an accurate diagnosis of BGUS, the clinical laboratory technician must be trained and sensitized to detect a second M - protein as a band or peak; taking in mind the possible different scenarios in heavy and light chain typing.

RESUMEN

Se describe el caso de paciente asintomática de 73 años de edad en consulta geriátrica de rutina, cuyos estudios de laboratorios muestran hiperproteinemia acompañada de hiperglobulinemia. Se estableció el diagnóstico de GBSI después de correlacionar entre resultados de electroforesis de proteínas, trazo de densitometría e inmunofijación en suero, los cuales evidenciaron un segundo pico monoclonal menos evidente y no reportado de primera instancia. Este tipo de condiciones biclonales son de muy baja incidencia en laboratorio clínico, lo cual requiere que profesional de laboratorio tenga ciertas habilidades para su identificación. Hasta donde se conoce, los hallazgos clínicos de GBSI son similares a aquellos encontrados en GMSI. Sin embargo, continúan sin ser bien comprendidas. Por tanto, a fin de un diagnóstico más preciso, el técnico de laboratorio debe estar entrenado y sensibilizado para encontrar una segunda proteína M como banda o pico, tomando en cuenta los diferentes posibles escenarios en la tipificación de cadenas pesadas y ligeras.

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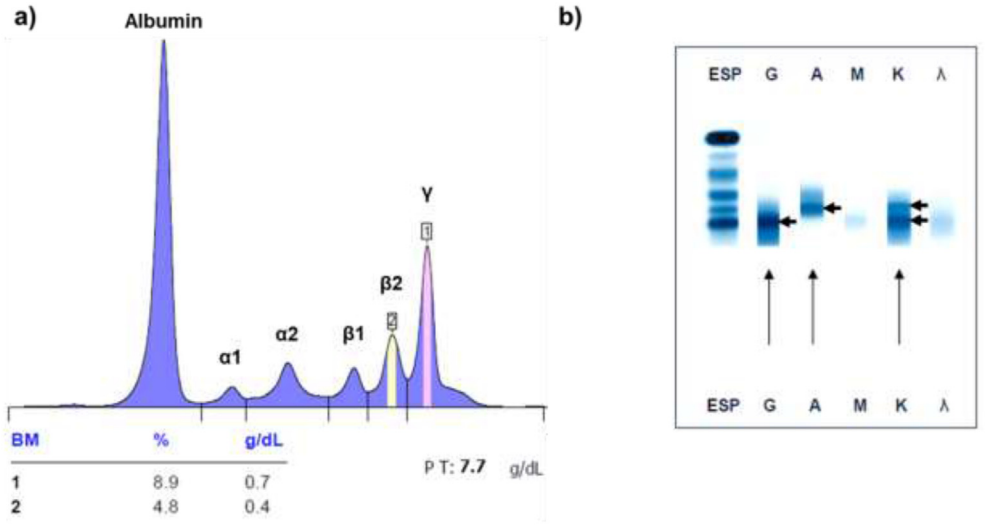
1. Introduction

Biclonal Gammopathy of Undetermined Significance (BGUS) is a plasmatic cell disorder included in the monoclonal gammopathy of undetermined significance (MGUS) condition, according to the International Myeloma Working Group classification [1]. MGUS is referred as a non-malignant state with presence of M protein, with no evidence of multiple myeloma, macroglobulinemia, amyloidosis or other lymphoproliferative disorder; and the absence of B – cell expansion related end-organ damage or tissue impairment [2]. The latter known as CRAB, acronym for hypercalcemia, renal insufficiency, anemia and lytic bone lesions. M-protein is an abnormal monoclonal immunoglobulin which is characteristic of this disorders. MGUS is estimated to occur in approximately 3–4% in general population older than 50 years [3], particularly more frequent in African-Americans than in Caucasians [4]. Approximately 3–6% of these individuals will present two different M – proteins, that supposes either the proliferation of two different clones or one clone that produces two different types of immunoglobulin (Ig) [5].

MGUS diagnostic criteria is based on serum M protein concentration (<3.0 g/dL), low plasmatic cells count in bone marrow (BM) (<10%), low grade infiltration in bone biopsy, absence of B – cell proliferative disease and no evidence of target organ damage [2]. Monoclonal immunoglobulins are observed in SPEP as an intense, discrete band or as a sharp peak in densitometry tracing. On the other hand, in biclonal gammopathy cases, two bands or two different sharp peaks can be observed in SPEP and in densitometry respectively. However, SPEP can also show only one discrete band that can be resolved in two bands when analyzed with IFE [6]; both cases are events of scarce incidence in the clinical laboratory.

2. Case description

A 73 years-old female attended to a geriatric routine consultation to Integral Diagnosis and Treatment Center of Médica Sur (MS) Hospital. Her laboratory tests showed in general no relevant clinical data: Red blood cells count, 4.93 × 10^6/μL (reference interval [RI]: 4.2–5.40 × 10^6/μL), with no anemia (hemoglobin, 15.4 g/dL; RI for an altitude of 2250 m above sea level: 13.0–17.0 g/dL); white blood cells count, 4.7 × 10^3/μL (RI: 4.5–11.0 × 10^3/μL), lymphocytes, 30.6% (RI: 12.0–46.0%); platelets count, 182 × 10^3/μL (RI: 150–450 × 10^3/μL). Creatinine, 0.58 mg/dL (RI: 0.44–1.03 mg/dL); eGFR, 91.7 mL/min (RI: > 60 mL/min); calcium, 10.1 mg/dL (RI: 8.9–10.3 mg/dL); lactate dehydrogenase, 165 U/L (RI: 98–192 U/L) and alkaline phosphatase, 83 U/L (RI: 32–91 U/L); the urinalysis showed no pathological data. The only altered parameters were total serum protein, 8.4 g/dL (RI: 6.1–7.9 g/dL) and globulin, 4.2 g/dL (RI: 2.3–3.8 g/dL). So, due to hyperproteinemia with accompanying hyperglobulinemia, the patient was referred to the Oncology Department for further evaluation.



Region	Reference Range %	Reference Range g/dL
Albumin	55.8 – 66.1	4.0 – 4.8
Alpha 1 (α1)	2.9 – 4.9	0.2 – 0.4
Alpha 2 (α2)	7.1 – 11.8	0.5 – 0.9
Beta 1 (β1)	4.7 – 7.2	0.3 – 0.5
Beta 2 (β2)	3.2 – 6.5	0.2 – 0.5
Gamma (γ)	11.1 – 18.8	0.8 – 1.4

Fig. 1. Patient's: a) Serum Protein Electrophoresis peaks pattern and b) Serum Immunofixation bands pattern.

After oncology consultation, the following data was added to clinical history: not known allergies; unspecified arrhythmia with not known evolution time, controlled with propafenone (150 mg/day), with an obstetric history of two pregnancies and two cesarean deliveries. She declared no ostealgia or other relevant symptoms. Additional tests showed IgA levels of 651.0 mg/dL (RI: 66.0–436.0 mg/dL); IgG, 1775 mg/dL (RI: 791.0–1643.0 mg/dL); IgM, 81.0 mg/dL (RI: 43.0–279.0); a serum protein electrophoresis (SPEP) showed an abnormal pattern which was interpreted by laboratory technician as a monoclonal band in gamma region with a concentration of 0.7 g/dL. Additionally, a band pattern consistent with IgG-kappa and IgA-kappa was observed in an immunofixation electrophoresis (IFE) (Fig. 1).

A second monoclonal band in SPEP corresponding to a sharp peak in the beta-2 region with a corresponding concentration of 0.4 g/dL was later pointed out by a clinical pathologist. The laboratory report was immediately corrected to include the description of two monoclonal bands: one located in the beta-2 region and the other, in the gamma region.

2.1. Patient follow-up

A bone marrow aspiration and biopsy was suggested in order to investigate for lesion evidence. However, the patient declined to undergo this procedure and remains under observation and monitoring for condition progression.

3. Discussion

Based on the patient's asymptomatic status, no evidence of target organ damage, M – protein concentration lower than that stated as a diagnostic criterion for multiple myeloma (MM) and the observation of two monoclonal proteins in SPEP, a BGUS was diagnosed. BGUS has been related to progression to MM and symptomatic lymphoproliferative diseases in up to 6% of patients within 5.9 years since diagnosis is made, with a progression rate of 1% per year, similar to that observed in MGUS. The largest cohort to the date has indicated non-significant difference in progression or outcomes between BGUS and MGUS [7], but monitoring is still imperative. We describe a case of an asymptomatic 73 years-old female patient whose laboratory testing showed hyperproteinemia with accompanying hyperglobulinemia. A diagnosis of BGUS was made only after a correlation among SPEP, densitometry tracing and IFE results was established, evidencing a second peak, that was less evident and not reported at first.

Both analysis, SPEP and IFE are suggested to be included as an optimal and inclusive screening panel for diagnosis of monoclonal gammopathies [8,9]. In the present case, SPEP and IFE were carried out on different days and therefore the results could not be compared accurately at the same time, so the diagnosis was delayed. As a result, SPEP and densitometry tracing analyses by themselves, without taking into account IFE results, led to an error in interpretation, due to the low frequency of this finding in SPEP. The laboratory technician was not aware of a second clonal protein present in beta-2 region, which led this finding to be omitted in the laboratory report.

These biclonal conditions are of very low incidence in the clinical laboratory, requiring the laboratory technician to have particular skills for their identification, considering the still limited understanding of this gammopathy variant. As far as is known, clinical findings in BGUS are similar to those found in MGUS and respond similarly to the pharmacological therapy [5]. It is important to mention that the dominant clone in BGUS has been observed to remain as it through the course of the disease in the majority of cases, including when progressing further. However, recent evidence about a greater malignancy potential among different clones has been found [7].

On the other hand, it has been documented that after hematopoietic stem cell transplantation (HSCT) an M - protein can be transitory present. In some of these cases, densitometry tracing and band pattern looks similar to biclonal gammopathy [10,11]. This phenomenon is related to the dysregulation of the immune system caused by administration of immunosuppressive therapy or aberrant immune and hematopoietic reconstitution [10]. In patients with multiple sclerosis or primary amyloidosis undergoing HSCT, an oligoclonal pattern can be observed in IFE after transplantation. Such finding is associated with a better outcome [12–14] and may appear sometimes as a two M - protein [14].

There are two different possible scenarios to mention in SPEP: 1) Two different monoclonal bands or peaks, or 2) Only one band or peak, that need to be resolved by IFE, in where three different possible scenarios can be observed: 1) Different heavy chain with the same light chain, 2) Same heavy chain with a different light chain [6], or 3) Different heavy chain with different light chain. The last one is known as "true biclonal gammopathy" [15], due to the determining difference in antibody isotype that suppose the proliferation of two different clones.

Additionally, three particular situations deserve to be pointed out as possible false biclonal gammopathies: a) IgA type monoclonal gammopathy, in which IgA dimerization tendency generates two different bands due to a difference in migration among such form and its monomer. This phenomenon is observed in IFE as two different bands in IgA lane and two different bands in kappa and/or lambda light chain in different positions along its respective lane [6]; b) Fibrinogen interference due to anticoagulant therapy, congenital dysfibrinogenemia, an underlying acquired coagulation disorder or insufficient clotting time for the sample, that could generate a second distinct band in electrophoresis [6,16]. To the knowledge of the authors, the latter phenomenon has not been reported yet; and c) therapeutic monoclonal antibodies (t-mAbs), which must be considered since the use of these drugs are increasingly common [17,18]. Moreover, when β-2 globulin concentration is above the normal range and clinical status of the patient does not correlate with such finding (e.g. inflammation), once excluded such situations, the presence of a second monoclonal antibody should be considered and interpretation must be corroborated by IFE.

It is worth mentioning that commercial systems for M – protein detection by capillary electrophoresis are available and they allow heavy and light chain immunotyping with comparable effectivity as IFE [19]. Such procedure is performed by immunosubtraction method, that use specific anti-sera directed against both heavy and light chain isotype to subtract out monoclonal immunoglobulin

signal from electrophoresis trace. Monoclonal antibody isotype is qualitatively determined by overlaying the original electrophoresis trace with those resulting from mixing patient serum with one of each 5 different anti-sera. Disappearance of one peak on any resulting trace determines the isotype. This represents a methodological alternative that could be useful in cases like the one described here, by discarding a false BGUS due to IgA dimerization or serum masking proteins in β2 like fibrinogen. Nonetheless, t-mAbs continue to be worrisome because they could be detected by immunosubtraction as endogen M – protein [20].

Recently, Mass Spectrometry (MS) methods have been adapted to measure monoclonal antibodies in clinical samples. They have proved to be useful for t-mAbs differentiation from endogenous monoclonal protein in serum [21]. Moreover, MS methods can identify and measure M - protein with higher sensitivity and specificity than traditional methods (i.e. IFE and SPEP) [22]. A study reanalyzing 226 negative samples for MGUS by SPEP evidenced sensitivity differences by finding 10.6% positives with IFE, 50% with Matrix-Assisted Laser Desorption/Ionization – Time of Flight - Mass Spectrometry (MALDI-TOF-MS) and 65.9% by Micro Liquid Chromatography – Electrospray Ionization - Time of Flight - Mass Spectrometry (micro LC-ESI-TOF-MS) [23]. Unfortunately, MS is not routinely used in most of clinical laboratories at present and is out of possibilities for some of them, particularly in developing countries. However, this is an innovative approach that could be considered as reference method in the future. On the other hand, protein electrophoresis and IFE are increasingly more common and some helpful measures to avoid t-mAbs interferences in traditional methods for monoclonal protein detection can be used [24].

4. Conclusion

For an accurate diagnosis of BGUS, the clinical laboratory technician must be trained and sensitized to detect a second M - protein as a band or peak. It is important to keep in mind the possible different scenarios in heavy and light chain typing, as well as the use of efficient tools for a timely and accurate laboratory report, such as combining SPEP and IFE, and using methods like capillary electrophoresis, immunosubtraction or even MS. Also, is of great importance to remember that correlation with clinical data is the key for an adequate interpretation of laboratory tests as well as having the advice of an experienced pathologist for the issuance of informative and descriptive notes. Additionally, communication among clinical laboratory and physicians must be encouraged in order to have an accurate diagnosis. Some mechanisms, such as internal policies or inclusion of a statement to waiting for IFE in situations like those discussed here, might be helpful. Although, BGUS have been described in many reports, these findings must be further studied in order to better understand this phenomenon and its possible consequences.

Credit author statement

All the authors included in this research contributed to the intellectual content of this paper and have met the following requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article. This is a product of equal efforts and all authors here listed have contributed significantly to this project in the following way:

José María Gastélum Cano (JMGC) Has contributed with bibliographic revision, writing, edition and sending the present paper, as well as in retrospective research, data collection and problem solution here described. Also, he has participated in figures edition.

Jaime Fragoso Flores (JFF) Has participated in writing, edition and technical revision of the present work. Besides, he has participated in problem solving.

Victor Manuel Noffal Nuño (VMNN) Has supported this work with writing, edition, technical revision and also with clinical case solving and data collection.

Marcela Deffis Court (MDC) Is the attending doctor, who have contributed with clinical data and with technical revision as hematology specialist.

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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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