Case Report

Monoclonal Gammopathy with Double M-bands Mimicking Biclonal Gammopathy: a case series

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Abstract

Background: Monoclonal gammopathies are characterised by the presence of a serum or urine monoclonal protein (M-protein), typically detected as a single narrow band on serum protein electrophoresis (SPEP). Rarely, double M-bands may appear, raising the suspicion of biclonal gammopathy. Distinguishing true biclonality from paraprotein polymerization is crucial to prevent diagnostic misclassification and inform appropriate clinical decisions.

Case: We report a case series of four multiple myeloma patients with twin M-bands who presented to XXXXX between March 2023 and March 2024. All four patients exhibited two discrete M-bands on SPEP, initially suggestive of biclonal gammopathy. However, immunofixation electrophoresis confirmed that both bands in each case belonged to the same immunoglobulin isotype and light chain, establishing a monoclonal origin. Treatment of samples with β-mercaptoethanol resulted in the collapse of double bands into a single monoclonal band, confirming paraprotein polymerization. IgA and IgM paraproteins were most frequently implicated in dual-band appearance.

Conclusion: The presence of double M-band on serum protein electrophoresis does not necessarily indicate biclonal gammopathy. Depending on SPEP results alone may lead to misclassification, and confirmatory techniques such as IFE and reducing agent studies are essential to establish the correct diagnosis.

Introduction

Monoclonal gammopathy (MG) is the presence of serum and/or urine monoclonal protein (M-protein), which is caused by the overproduction of the immunoglobulins (Igs) by a population of B-lymphocytes and/or plasma cells. This is primarily observed in Waldenstrom's macroglobulinemia, multiple myeloma (MM), and monoclonal gammopathy of unknown significance (MGUS). However, primary amyloidosis, cryoglobulinemia, lymphoproliferative diseases, solitary plasmacytoma, hepatitis C infection, POEMS syndrome, and scleromyxedema can also result in monoclonal deposition of other proteins [1]. In serum protein electrophoresis (SPEP), Longsworth et al. [2] initially reported a tall, narrow-based spike that Moore et al. [3] later referred to as the "M" component/peak. When doing SPEP, polyclonal Igs show up as a broad-based band on agarose gel, while monoclonal Igs show up as a single, discrete and intense band on agarose gel and a distinct peak in the densitometer tracing. Depending on the type of Igs produced, this band or peak may also be seen in the gamma, beta-gamma, or beta-region. Following the discovery of the "M" band or peak on SPEP, immunofixation electrophoresis (IFE) is used to definitively identify monoclonal proteins and characterize the heavy chain (HC) and light chain (LC) [4]. IFE is more sensitive than SPEP in identifying traces of "M" protein in plasmacytoma, primary amyloidosis, or treated cases of multiple myeloma/macroglobulinemia, where SPEP does not show a monoclonal band. Additionally, it helps differentiate between a monoclonal and polyclonal increase in Igs [5]. Monoclonal immunoglobulins (Igs) are produced by the growth of neoplastic plasma cells, but an increase in polyclonal Igs is usually linked to reactive or inflammatory events. Moreover, if there is a strong clinical suspicion of multiple myeloma or a similar condition in cases with normal SPEP, IFE is warranted to confirm the diagnosis [6].

A subgroup of patients with monoclonal gammopathies may have serum and/or urine containing several M-proteins. This group of gammopathies is known as "double gammopathies." In existing literature, the terms "biclonal gammopathy" and "double gammopathy" have been used interchangeably. Double gammopathy can be depicted in two different ways, wherein two different M bands or peaks on SPEP or a single M band on SPEP further resolves into two distinct bands on IFE. The variant of HC and LC found determines the further subdivision of double gammopathies. In these situations, IFE may reveal whether the monoclonal proteins differ in both HC and LC isotype (e.g IgA kappa and IgG lambda) which is a true biclonal gammopathy. In some cases the M proteins share the same HC with different LCs or different HCs with same LCs which is termed double gammopathies (IgM kappa and IgM lambda or IgA kappa and IgG kappa) [7]. Multiple myeloma, several lymphoproliferative diseases, and MGUS have been linked to biclonal/double gammopathies.

Biclonal/Double gammopathies can be picked up at initial presentation or after the original diagnosis has been established and the disease has progressed. The eruption of "distinct plasma cell clones that are two or more in number" producing multiple monoclonal proteins can lead to biclonal/double gammopathies[7,9]. At a later stage of disease pathogenesis, when "clonally related" plasma cells undergo class switching, it can also result in biclonal/double gammopathies. Throughout the course of the disease or after treatment, the proportion of "double" M-proteins may increase or decrease in a way that is consistent or inconsistent. The causative mechanism of this process is unclear [8]. Biclonal/Double gammopathies are reported to occur in 2-4 per cent of different gammopathy cohorts in the published literature [8,10-14]. The most prevalent combination of HC isotype, according to Kyle et al. [10], is IgG–IgA. Riddell et al. [8] reported that the most prevalent HC isotype combination was IgG–IgM. Two other studies reported that the most prevalent combination is IgG–IgG [13,14]. These findings differ from one another for a variety of reasons, including variation in racial or population distribution, variation in the sensitivity of the detection technique or selection bias in the process of case selection. Most frequently reported light chains were the kappa light chain [8,11,14]. This case series presents four multiple myeloma patients with twin M-bands on SPE who presented to XXXXX between March 2023 and March 2024. All patients had provided informed consent to the publication of their anonymised data.

Results

All four patients demonstrated double M-bands on SPEP or IFE. In all, the presence of two distinct bands initially suggested biclonal gammopathy. On subsequent IFE, both bands in each except the last case (case 4) were found to belong to the same immunoglobulin heavy and light chain type, confirming a monoclonal gammopathy with pseudo dual-band appearance. To further evaluate the possibility of polymerization or aggregation, serum samples were treated with the reducing agent β-mercaptoethanol before electrophoresis. After reduction of the sulfide bonds, the double M-bands collapsed into a single monoclonal band, supporting the interpretation that the apparent biclonal pattern was due to paraprotein polymerization giving rise to two molecules with different molecular weights (partly polymerized and partly unpolymerized) rather than the presence of two independent clones. The description of the cases is presented in Table 1. SPEP and IFE of the four cases are presented in Figures 1 to 4. The SPE and IFE were performed on a gel-based electrophoresis machine (Hydrasys 2, Sebia, France).

Table 1: Clinical features and laboratory findings of cases.

Case	Age/Sex	Clinical findings	Laboratory findings	Bone marrow findings	SPEP finding	IFE finding	Diagnosis
1	57y/M	Recurrent chest pain, shortness of breath, weakness, O/E-pallor and B/L basal crepts	Hb- 46 g/L, TP /ALB/GLOB-114.1/22.8/84.3 g/L, A/G ratio-0.35, Cr-193.6 µmol/L, Ca-2.24 mmol/L.	72% plasma cell (binucleate /multinucleate)	Two peak M band beta gamma region	IgA lambda	Plasma cell dyscrasia
2	56y/F	Fatigue, weakness, vomiting and anorexia k/c/o HTN and bronchial asthma	Hb- 68 g/L, TP /ALB/GLOB-56.7/28.7/28.0 g/L, A/G ratio-1.03, Cr-1096 µmol/L, Ca-2.45 mmol/L.	11% plasma cell (binucleate /trinucleate)	Two peak M band beta gamma region	IgG lambda	Plasma cell dyscrasia
3	75y/F	Fever, back pain, weakness O/E- pallor	Hb- 78 g/L, TP /ALB/GLOB-96.7/27.9/68.8 g/L, A/G ratio-0.41, Cr-107.0 µmol/L, Ca-2.77 mmol/L.	50% plasma cell (binucleate /trinucleate)	M band in beta gamma region	IgA kappa	Plasma cell dyscrasia
4	80y/F	Weakness, loose stool and abdominal pain, O/E-pallor	Hb-79 g/L, TP/ ALB/GLOB-87.1/33.0/54.1 g/L, A/G ratio-0.61, Cr- 158.3 µmol/L, Ca-2.22 mmol/L.	40% plasma cell (multinucleate)	M band in beta gamma region	True Biclonal gammopathy (IgG Kappa and IgA lambda)	Plasma cell dyscrasia

SPEP: serum protein electrophoresis, IFE: immunofixation electrophoresis, M: Male, F: Female, Hb: Hemoglobin, TP: Total Protein, ABL: Albumin, GLOB: Globulin, A/G: Albumin/Globulin, Cr: Creatinine, Ca: Calcium, O/E: on examination, HTN: hypertension, B/L: bilateral, k/c/o: known case of, IgA: Immunoglobulin A, IgG: Immunoglobulin G, M band: monoclonal band, g/dl: grams per liter; µmol/L: micromoles per liter; mmol/L: millimoles per liter

Figure 1: Serum protein electrophoresis and immunofixation of Case 1 before and after β-mercaptoethanol treatment.

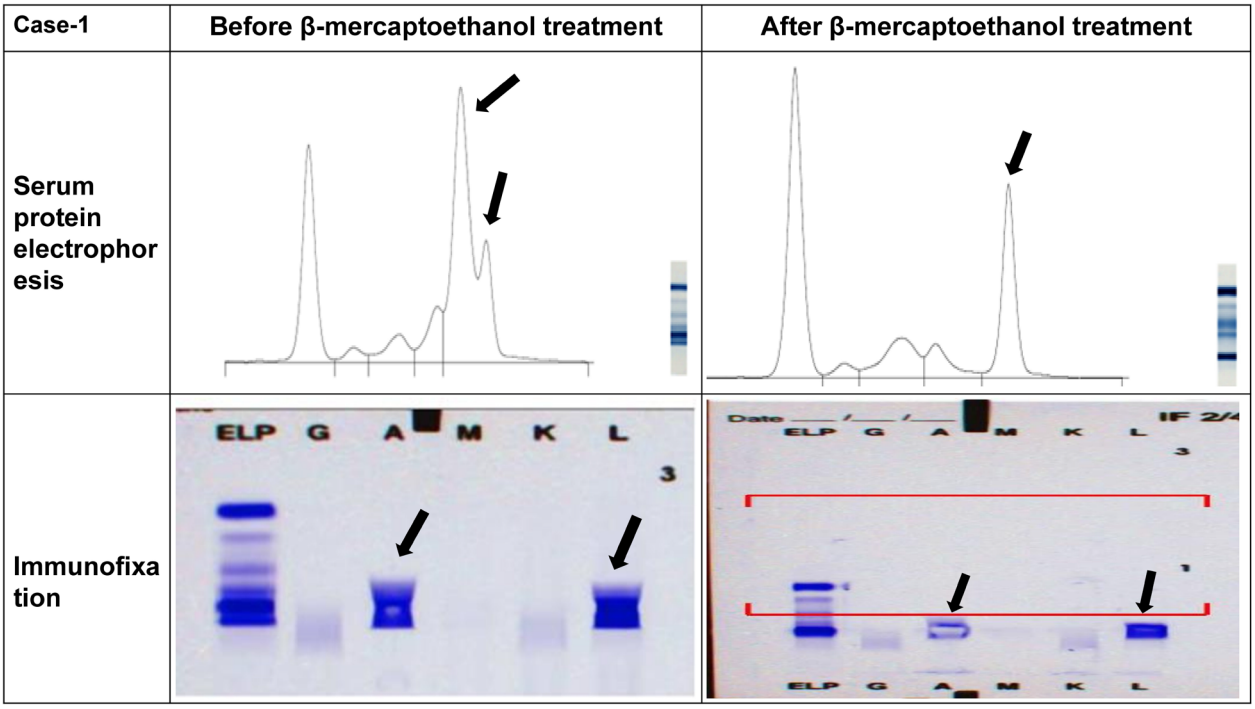


Figure 2: Serum protein electrophoresis and immunofixation of case 2 before and after mercaptoethanol treatment.

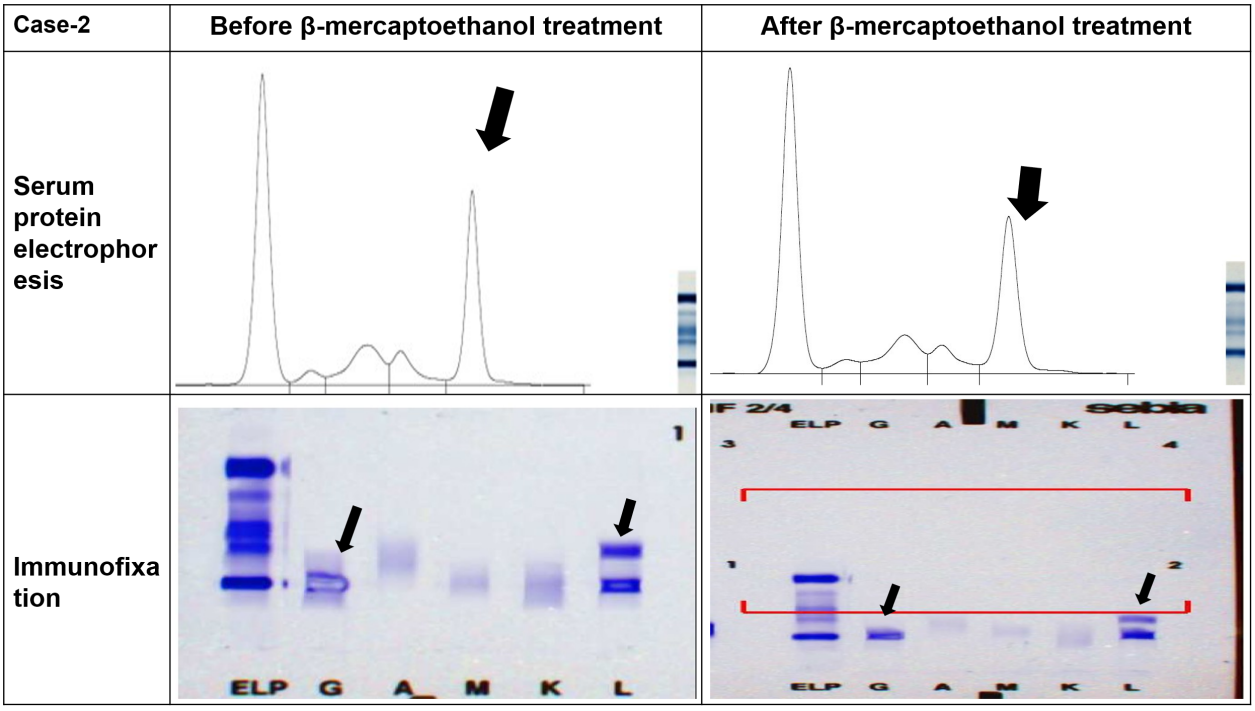


Figure 3: Serum protein electrophoresis and immunofixation of Case 3 before and after β-mercaptoethanol treatment.

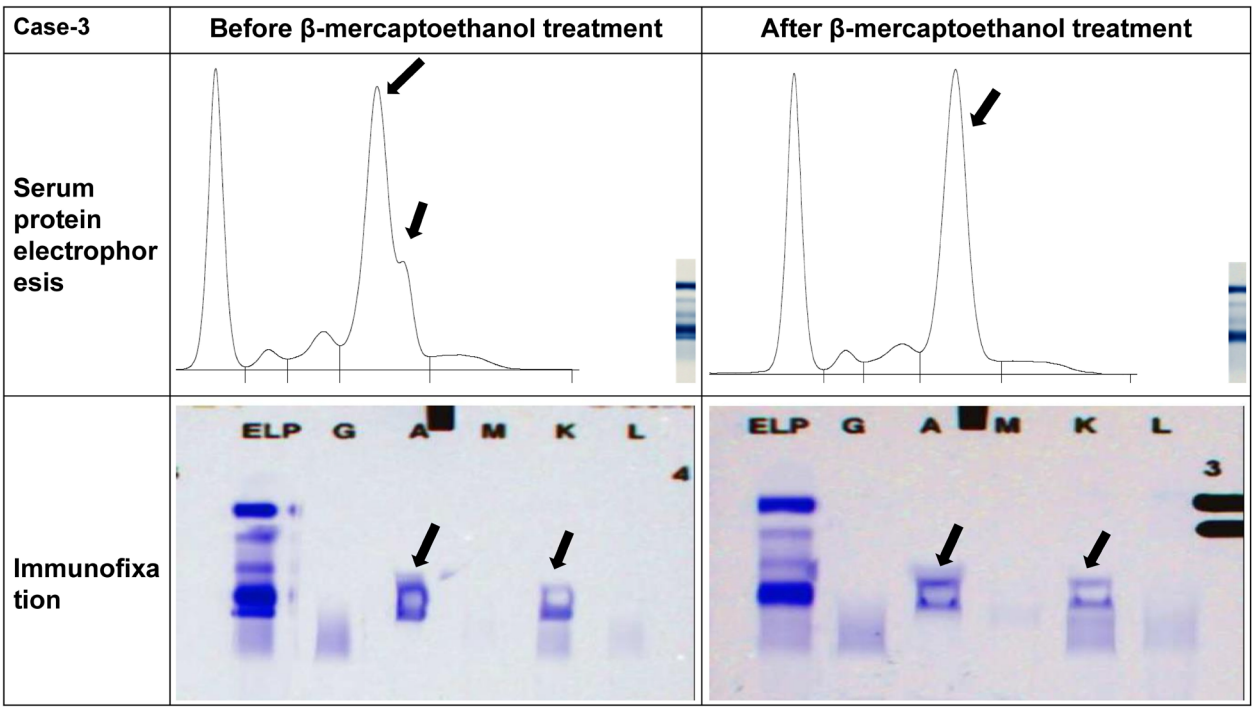
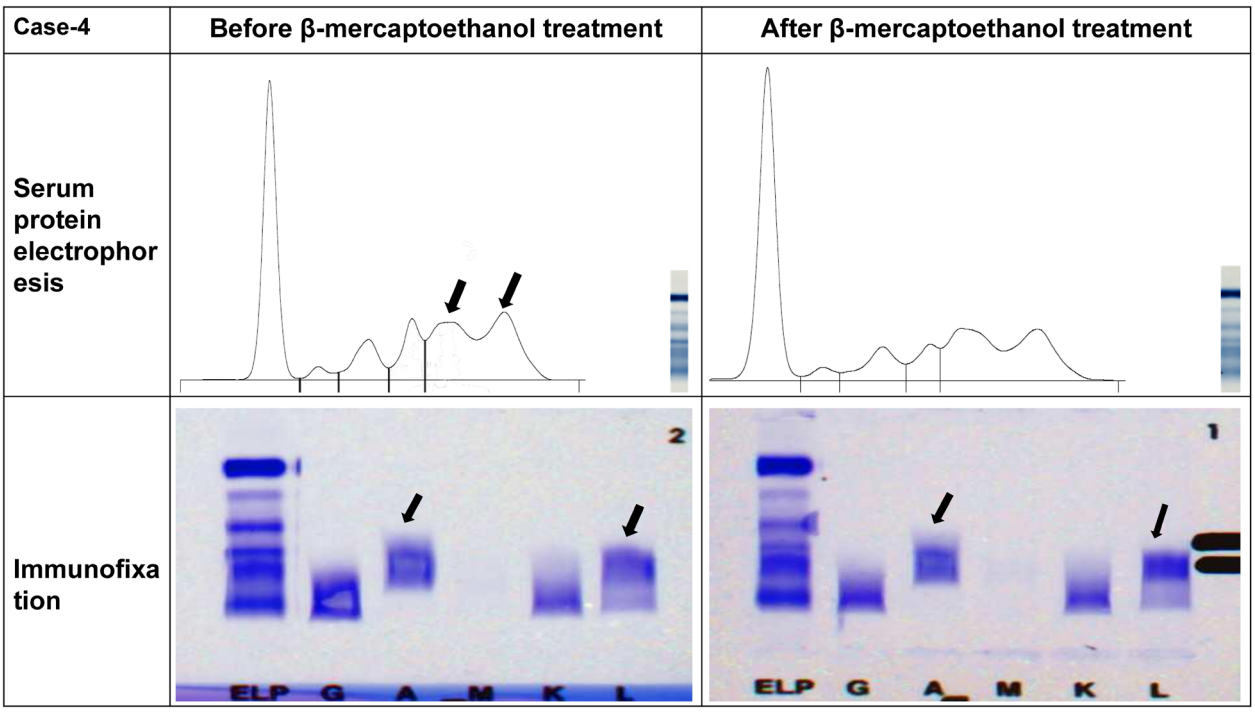


Figure 4: Serum protein electrophoresis and immunofixation of Case 4 before and after β-mercaptoethanol treatment.



Discussion

Multiple myeloma is the second most prevalent hematological malignancy, contributing to nearly 2% of cancer-related mortality worldwide [15]. SPEP is an essential diagnostic tool in the initial laboratory evaluation and characterization of monoclonal proteins. Monoclonal immunoglobulins usually appear on SPEP as a sharp band within the gamma-globulin region. However, due to their unique physicochemical properties, monoclonal IgM andespecially IgA may migrate into the beta region [16]. Often, IgA-type M proteins exhibit unusual and atypical electrophoretic patterns, which can complicate interpretation. These include a tendency for more anodal migration toward the β-region due to their lower isoelectric point. IgA has a quaternary structure that permits polymerization, with or without subsequent light chain (LC) production, due to the sequestration of LC epitopes, thereby hindering the reaction between LC epitopes and their antibodies.

This produces a condition termed 'IgA with no apparent light chain attached', an apparent lack of detectable light chains on IFE. Another recognized cause of pseudo-biclonal gammopathy is the tendency of immunoglobulins to polymerize and form aggregates. IgA and IgM are the immunoglobulins most commonly involved in polymer formation. In such cases, the polymerized form has a different molecular weight as compared to the unpolymerized form, leading to different localization on SPE due to a difference in relative migration. A possible interference from a fibrinogen band leads to a common misinterpretation of biclonal gammopathy due to the presence of an additional band in the gamma region, in addition to a single M band in the case of true monoclonal gammopathy. Fibrinogen is noted in the gamma region on SPE when a plasma sample is used instead of serum due to probable contamination with EDTA/heparin or other anticoagulants. Subsequent IFE rules out the probability of biclonal gammopathy as it does not employ antisera targeted to fibrinogen. Some cases with more than one clone of M protein are also noted in patients with remission of monoclonal gammopathy following treatment or during progression from less aggressive to more aggressive forms and renal failure [17,18].

In this study, on SPE, cases 1 and 2 revealed a bifurcation of the sharp band obtained in the beta-gamma region, hinting at the presence of a biclonal gammopathy. IFE revealed IgA lambda and IgA kappa monoclonal gammopathy, respectively. The pseudo-biclonal pattern observed in cases 1 and 2 (Figures 1 and 2) on SPE was most likely due to polymerization of IgA, leading to the appearance of an additional M-band near the beta region, which subsequently resolved as IgA Lambda upon IFE. Upon BME treatment, polymers collapsed into monomers, resulting in a single band if it is a true monoclonal protein representing a single clone (like polymerized IgA).

In Case 3, a single monoclonal band was observed in the gamma region on SPE. However, the biclonal pattern resolved into IgG-Lambda-Lambda (Light Chain isotype-matched). This finding is probably because of the uncoordinated production of excess free light chains in serum, which can migrate faster due to lower molecular weight and produce an additional band in the lambda light chain lane. In Case 4, two peaks were observed in the gamma region on SPE. On IFE, two separate clones were identified, namely IgG kappa and IgA lambda, indicating a biclonal gammopathy. Further on treatment with BME, the same bands persisted, indicating a true biclonal gammopathy of IgG Kappa and IgA lambda type.

True biclonal gammopathy occurs only in clonally related plasma cells, of which IgG-IgA, IgG-IgM and IgG-IgG are reported in various literature [12,13]. These are caused by two main mechanisms, either heavy chain or light chain isotype matching. Distinguishing true biclonality from polymerization-related artefacts is essential, as misinterpretation can result in overestimation of disease severity, unwarranted investigations, or inappropriate treatment decisions. In genuine biclonal gammopathy, two discrete monoclonal proteins are typically produced by separate plasma cell clones. On rare occasions, however, a single clone may generate two M-proteins through mechanisms such as class-switch recombination or dual isotypeexpression events, that, while uncommon, are documented in the literature [19]. Conversely, particularly in the case of IgA, a single monoclonal protein may undergo polymerization and produce multiple migrating bands on electrophoresis, thereby simulating biclonality [20]. Further, a pentameric IgM may dissociate into 7S subunits and present on electrophoresis as one or more additional monoclonal bands. These bands are generally attributable to alternate molecular forms of a single monoclonal protein produced by the same plasma cell clone [1]. In our cases, this mechanism was substantiated, as treatment with β-mercaptoethanol unmasked a single IgA lambda component in three cases of false biclonal gammopathy.

Chemical reduction with β-mercaptoethanol serves as a useful diagnostic approach in such contexts. By cleaving the disulfide bonds that join immunoglobulin monomers into dimers or higher-order polymers, β-mercaptoethanol dissociates these complexes into their monomeric form, which subsequently appears as a single, uniform peak on SPEP [17]. In our cases, reduction with β-mercaptoethanol resulted in the convergence of the two discrete bands into a single band, thereby substantiating IgA polymerization as the underlying mechanism rather than true biclonality.

Beyond mere detection, IgA polymerization carries important implications for disease monitoring. Polymeric IgA can be erroneously interpreted as two distinct M-proteins, resulting in an overestimation of disease burden. Evidence suggests that patients with polymeric IgA often demonstrate apparently higher levels of M-protein compared to those with monomeric IgA [7,17]. Additionally, IgA polymers have been linked to clinical complications such as hyperviscosity syndrome and laboratory artefacts, including spuriously raised calcium levels due to calcium binding by IgA complexes [16,20]. While our cases did not develop hyperviscosity syndrome or hypercalcemia, these potential risks highlight the clinical relevance of identifying IgA polymerization. Although IFE is considered to be gold standard for paraprotein characterization, it may not single-handedly suffice in distinguishing co-migrating polymers and may sometimes lead to inconclusive results [11]. In our cases, IFE was performed both before and after pretreatment with β-mercaptoethanol to aid in clarification.

Conclusion

This case series highlights that the presence of double M-band on serum protein electrophoresis does not necessarily indicate biclonal gammopathy. In all our patients, immunofixation electrophoresis confirmed a single monoclonal protein, and in selected cases, the use of reducing agents such as β-mercaptoethanol further demonstrated that the dual bands were due to polymerization of the same paraprotein rather than two independent clones. The observation was most frequently associated with IgA paraproteins, which are inherently prone to form polymeric structures and exhibit atypical migration patterns. Therefore, reliance on SPEP alone may lead to misclassification, and confirmatory techniques such as IFE and reducing agent studies are essential to establish the correct diagnosis. Accurate interpretation is crucial for appropriate patient management and to avoid over-reporting of biclonal gammopathy.

Conflict of Interest
None.

Ethical Approval
Ethical clearance is waived off for case series from our institute. The study was done in accordance with the Declaration of Helsinki.

Author's contributions
All authors fulfil the criteria for authorship as set out by the ICMJE and as recommended by the Committee on Publication Ethics (COPE).

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Data availability
All data related to the case series has been submitted along with the manuscript.
All reagents and instrument for running SPE and IFE have been provided by SEBIA, France.
All Clinical chemistry assays have been provided by Beckman Coulter, USA.

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