Research Article

Monoclonal gammopathy presenting with pseudo biclonal pattern in serum protein electrophoresis – An interesting perspective of case series

Kanmani Nethaji[1] , Karthick Elango[1*], Sathya Selvarajan[2] , Sowmya Krishnamurthy[1]

[1]*Sri Ramachandra Medical College and Research Institute, Chennai, India*
[2]*Department of Laboratory Medicine, MGM Healthcare Institute, Chennai, India*

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Corresponding Author:
E.Karthick MD., DipRCPath (London)
Postgraduate, Department of Biochemistry
Sri Ramachandra Medical College and Research Institute
Chennai, India
Telephone:7904916266
E-mail: Karthicksheela100@gmail.com
https://orcid.org/0009-0006-3358-7532

Keywords

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Abstract

Introduction: Monoclonal gammopathy (MG), arising from aberrant clonal proliferation of plasma cells, is diagnosed through the identification of an M-band via serum protein electrophoresis (SPEP), subsequently confirmed by immunofixation electrophoresis (IFE). The presence of two M-bands in SPEP is designated as double or biclonal gammopathy. Conversely, a pseudo-biclonal pattern is characterized by two M-bands on SPEP that resolve into a single immunoglobulin clone upon IFE. This case series delineates three instances of pseudo-biclonal patterns observed on SPEP in the absence of true biclonality.

Methodology: Following ethical approval, a retrospective analysis was conducted on SPEP reports from Sri Ramachandra Medical College & Research Institute, Chennai, spanning 2022 to 2024. Capillary electrophoresis was employed for SPEP, followed by immunofixation.

Results: Three cases exhibiting pseudo-biclonal patterns were identified. Case 1 involved a 73-year-old male with acute kidney injury, hypercalcemia, osteopenia, multiple fractures, and a reversed albumin/globulin ratio, where a biclonal pattern on SPEP resolved to IgA Lambda on IFE. Case 2 concerned a 57-year-old male with nodal marginal lymphoma evaluated for myeloma, whose SPEP biclonal pattern resolved to IgM Kappa with elevated polyclonal immunoglobulins on IFE. Case 3 was an 86-year-old female with acute-on-chronic kidney disease and urosepsis, where SPEP showed a biclonal pattern resolving to IgG Lambda and an additional Lambda isomer on IFE.

Conclusion: This case series highlights pseudo-biclonal patterns stemming from monoclonal gammopathy, polyclonal elevations secondary to tumor lysis syndrome, and double gammopathy due to excess free light chains. The integration of SPEP with immunofixation, serum free light chain, and serum immunoglobulin assays enhances the detection of pseudo-biclonal patterns.

Introduction

Monoclonal gammopathy (MG) arises from the overproduction of immunoglobulins due to the aberrant clonal proliferation of B-lymphocytes or plasma cells. Diagnosis of MG is established by the presence of monoclonal protein (M-protein) in serum or urine. The diagnostic workup for M-protein detection commences with the identification of an M-band on serum protein electrophoresis (SPEP), subsequently followed by serum and urine free light chain assays, immunofixation electrophoresis (IFE), beta-2 microglobulin assessment, and other relevant markers [1]. Upon performing SPEP, monoclonal immunoglobulins typically manifest as a single, intense, discrete band on the electrophoretic gel and a sharp peak on the densitometer tracing. Immunofixation electrophoresis (IFE) serves as a definitive method for identifying monoclonal proteins and characterizing the secreted heavy and light chains [2].

Based on established literature, the presence of a single monoclonal band (M-band) in SPEP predominantly indicates monoclonal gammopathy, although exceptions exist. In these less common exceptions, IFE results may reveal double bands, potentially indicative of either double gammopathy or true biclonal gammopathy contingent upon the nature of the heavy and light chains involved [2]. Double gammopathy can present as two distinct M-bands/peaks or as a single M-band on SPEP that subsequently resolves into two separate bands of either heavy or light chains upon IFE. In contrast, true biclonal gammopathy involves two distinct immunoglobulin clones, each with its own heavy and light chain, detectable as two separate bands/peaks in both SPEP and IFE. The reported incidence of double gammopathies among various cohorts in the literature is approximately 2–6% [5]. Double gammopathies are known to be associated with multiple myeloma, certain lymphoproliferative disorders, as well as with cases of monoclonal gammopathy of undetermined significance [4,5].

Interestingly a pseudo-biclonal pattern can happen in rare cases and is characterized by the presence of two M-bands on SPEP (suggesting a biclonal pattern) that resolve into a single immunoglobulin clone upon IFE [6]. The published literature on such cases remains sparse. Accurate identification of double gammopathy or pseudo-biclonal patterns is crucial for timely diagnosis and appropriate therapeutic intervention. This case series presents three cases exhibiting pseudo-biclonal patterns on SPEP, where the subsequent IFE revealed either double gammopathy or monoclonal gammopathy, thus highlighting the absence of true biclonality.

Materials and Methods

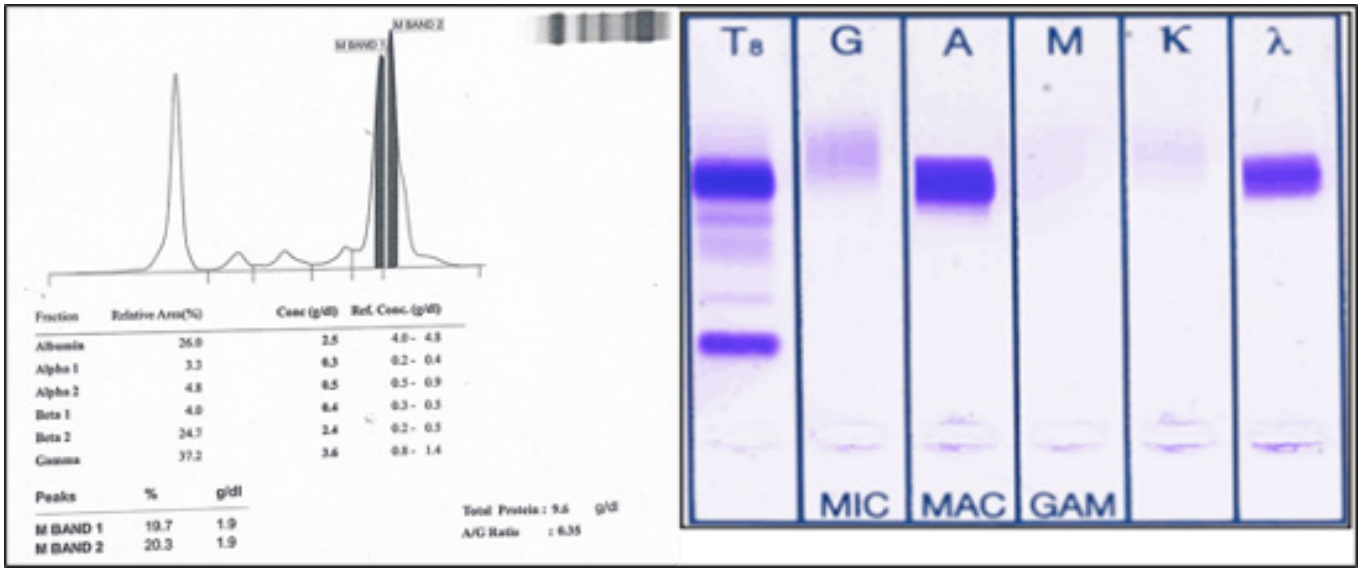
This retrospective case series was conducted using SPEP reports obtained from the Department of Biochemistry at Sri Ramachandra Medical College & Research Institute, Chennai, Tamilnadu, India. Ethical approval, with a waiver of consent due to the retrospective nature of the study, was granted by the Institutional Ethics Committee. SPEP was performed using capillary electrophoresis, followed by immunofixation electrophoresis. The study period encompassed the years 2022 to 2024, and included reports demonstrating a biclonal pattern on SPEP that did not correspond to true biclonality on IFE, instead revealing double gammopathy or monoclonal gammopathy.

Results

Case 1

A 73 year old male presented with slurred speech, decreased appetite and generalized fatigue. His baseline investigations revealed Hemoglobin-7.9 g/dl (peripheral smear- normocytic normochromic anemia with neutrophilia), Total count – 11,780 cells/cu.mm; BUN – 26 mg/dl; Creatinine – 2.3 mg/dl and Calcium – 14.5 mg/dl. LFT showed Total protein – 9.7 gm/dl; Albumin - 2.5 g/dl; Globulin – 7.2 g/dl with reversal of A:G Ratio along with diffuse osteopenia and multiple fractures of varying degree in CT scan of thorax. In view of AKI, hypercalcemia, osteopenia with multiple fractures and A:G ratio reversal, Myeloma workup was performed. Bone marrow biopsy showed myelomatous marrow with 27% plasma cells. Serum protein electrophoresis detected hypoalbuminemia along with two distinct M-bands in the gamma region (biclonal pattern) that subsequently resolved into Ig A and Lambda in IFE (monoclonal gammopathy) as depicted in Figure 1. These findings were further corroborated by elevated levels of IgA - 4666 mg/dl; Lambda - 198 mg/l; Beta-2-microglobulin - 9234 ng/ml confirming the diagnosis of Multiple myeloma. The Patient was started on VCD (Bortezomib, cyclophosphamide, Dexamethasone) regimen chemotherapy.

Figure 1: shows the SPEP & IFE reports of Case 1.

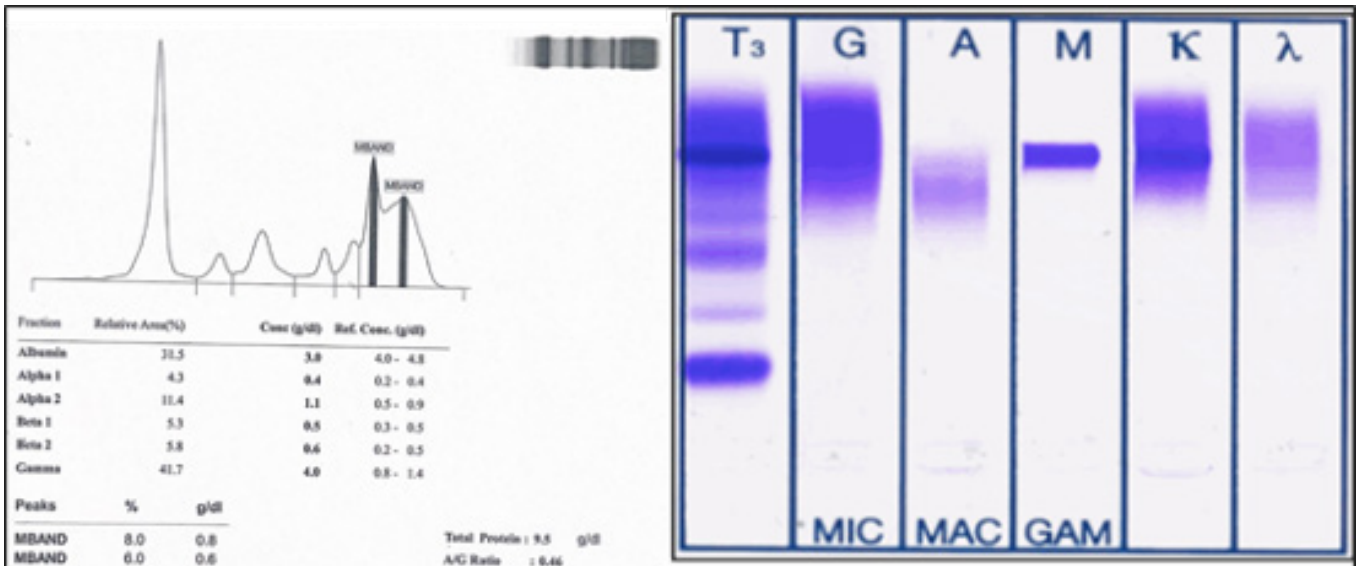


In Case 1 the above SPEP (left) demonstrates two distinct M bands in the gamma region which upon IFE (right) resolved into IgA- Lambda.

Case 2

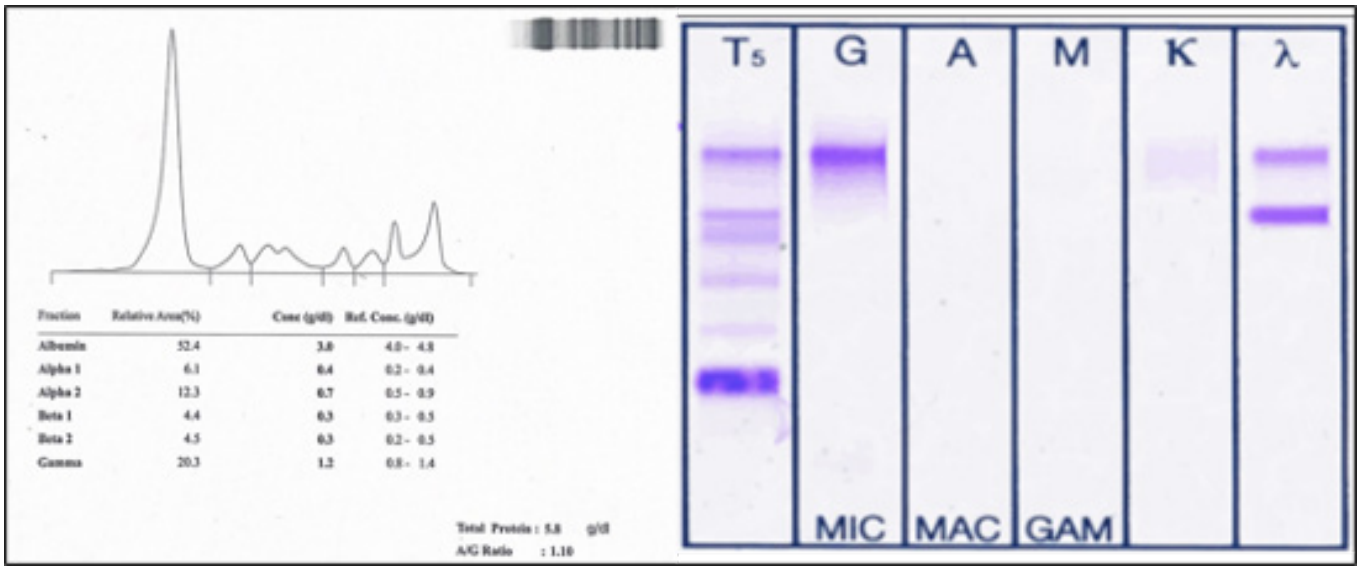
A 57-year-old male was referred with a provisional diagnosis of Nodal marginal lymphoma / lymphoplasmacytic cell lymphoma. His basic investigation revealed Hemoglobin-7.4 g/dl; total count-7,700 cells/cu.mm; platelets- 4.25 lakhs; calcium- 7.90 mg/dl; BUN-28 mg/dl; creatinine-1.21 mg/dl; and albumin-2.50 g/dl. PET CT scan reported multiple enlarged nodes in the abdomen, neck, axilla, mediastinum, pelvis and inguinal region. Histopathology of cervical lymph nodes and bone marrow aspiration biopsy showed features suggestive of lymphoproliferative disorder. In view of lymphoma, serum protein electrophoresis was done. SPEP showed hypoalbuminemia and elevated levels of alpha1, alpha2, beta1, beta2 globulins. Two discrete M bands were observed in the gamma globulin region (biclonal pattern), which further resolved into IgM and Kappa (monoclonal gammopathy) upon IFE, along with elevation of other immunoglobulins as depicted in Figure 2. These findings were further confirmed by quantifying serum immunoglobulins, which revealed elevated IgG - 3928 mg/dl; IgM - >3300 mg/dl; IgA - 365.80 mg/dl; Kappa - 280 mg/l; Lambda - 174 mg/l and Beta-2-microglobulin - 10884 ng/ml. The patient was started on chemotherapy cycles with Bendamustine.

Figure 2: shows the SPEP & IFE reports of Case 2.



In Case 2 the above SPEP (left) shows two M-Bands in Gamma region which resolved upon IFE(right) as IgM-Kappa along with increase in other Ig lanes.

Figure 3: shows the SPEP & IFE reports of Case 3.



In Case 3 the above SPEP (left) shows two M-Bands in the Gamma region which resolved upon IFE (right) as IgG-Lambda-Lambda.

Case 3

An 86-year-old woman, with history of Acute on chronic kidney disease, coronary artery disease and systemic hypertension was admitted for Urosepsis with persistent hyperkalemia. Baseline investigations revealed Hemoglobin - 7.8 gm (Peripheral smear - normocytic normochromic anemia); total count – 3350 cells/cu.mm; platelet – 2.47 lakhs. LFT showed total protein – 6.6 g/dl; Albumin – 3.6 g/dl; Globulin -2.9 g/dl; A:G ratio- 1:2 and Serum calcium-9 mg/dl. CT scan of the abdomen revealed multiple lytic lesions with an unknown primary. Serum protein electrophoresis showed the presence of two discrete bands in the gamma region (biclonal pattern) which further resolved into IgG Lambda and one more Lambda isomer in IFE as depicted in Figure 3. These findings were further confirmed by elevated levels of serum Immunoglobulins IgG - 1045 mg/dl; Lambda - 10297.4 mg/l and beta 2 microglobulin - 17142 ng/ml, confirming the diagnosis of multiple myeloma with excess free light chains.

Discussion

A pseudo-biclonal pattern, as observed in SPEP, is characterized by the presence of two discrete or non-discrete peaks that subsequently resolve upon IFE into either monoclonal gammopathy or double gammopathy, without exhibiting true biclonality [5,6]. The terms Double gammopathy (DG) and Biclonal gammopathy (BG) are often used interchangeably in the literature. True biclonal gammopathy may arise from the division of a single B-lymphoid cell clone into two distinct clones following antigenic selection or from the neoplastic proliferation of two independent malignant plasma cell lines [4]. In contrast, double gammopathies can originate from two or more distinct, yet clonally related, plasma cell lines, resulting in the production of multiple monoclonal proteins [4,6]. Double gammopathies have been observed in various plasma cell disorders, including monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma (MM), and Waldenström's macroglobulinemia. They are also frequently associated with leukemia, lymphoproliferative disorders, primary amyloidosis, cryoglobulinemia, solitary plasmacytoma, POEMS syndrome (a rare condition), and certain infections, such as Hepatitis C virus (HCV) infection [6].

The literature provides limited elucidation regarding the underlying causes of pseudo-biclonal patterns. A common misinterpretation involves the presence of a fibrinogen band in the beta-gamma region, resulting from inadequate clotting of the blood sample. As IFE does not employ specific antisera for fibrinogen, this band will not be apparent upon immunofixation [2,3]. Another recognized cause is the propensity of immunoglobulins, originating from the same plasma cell lines, to polymerize and form aggregates. IgA and IgM are the immunoglobulins most commonly implicated in forming such atypical electrophoretic patterns [7]. The pseudo-biclonal pattern observed in Case 1 (Figure 1) can likely be attributed to the polymerization of IgA, leading to the appearance of an additional M-band near the beta region, which subsequently resolved as IgA Lambda upon IFE. The quaternary structure of IgA allows for polymerization, with or without subsequent light chain (LC) production due to the sequestration of LC epitopes. Owing to their low isoelectric pH, these aggregates can exhibit more anodal migration, appearing as separate bands in proximity to the beta region. IgA can also form dimers, potentially resulting in two M-bands due to variations in electrophoretic mobility [7] . However, IFE in such instances reveals a single band in the IgA and its corresponding light chain lanes, consistent with monoclonal gammopathy. In rare cases, Polymerized IgA structure hinders the reaction between LC epitopes and its antibodies, producing a condition termed

as 'IgA with no apparent light chain attached' [6,8]. Clinically, the polymerization of IgA may contribute to hyperviscosity syndrome and potentially lead to the overestimation of serum calcium levels or underestimation of the hemoglobin levels. This spurious increase in calcium occurs due to its binding to the secreted paraprotein, necessitating careful evaluation [9]. If the polymerization effect is due to IgM (pentamer), it can result in the appearance of two or more bands on SPEP [7].

Polyclonal increase in Immunoglobulins can occur in various conditions such as chronic inflammation, chronic liver disease, systemic lupus erythematosus, rheumatoid arthritis, cystic fibrosis etc. [10]. Such polyclonal elevations are usually depicted in SPEP as hypoalbuminemia alongside elevation of alpha, beta and gamma globulins without a discrete monoclonal band. Interestingly, a Polyclonal elevation of Immunoglobulins may coexist with a monoclonal gammopathy in the context of metastatic hematological malignancy, underlying chronic infection, inflammation or autoimmune disorders [11]. In our Case 2, the patient had chronic cervical lymphadenopathy, multiple lymph node metastasis along with features of Tumor lysis syndrome. These changes have contributed to the elevation of all globulin fractions (alpha, beta and gamma). The observed pseudo-biclonal pattern in Case 2 is thus attributed to the polyclonal immunoglobulin elevation combined with increased acute phase reactants due to Tumor lysis syndrome complicating splenic marginal zone lymphoma. This was confirmed by IFE revealing IgM kappa and elevated serum levels of IgG, IgA, and free light chains (kappa and lambda). Tumor Lysis Syndrome (TLS) is a common complication in hematological malignancies occurring either during disease progression or following chemotherapy. TLS can cause an increase in serum free light chains, acute phase reactants and also hyperuricemia, which can influence the migration of proteins during SPEP [12,13]. These metabolic changes can induce Pseudo-biclonal patterns as seen in Case 2.

In our Case 3, the biclonal pattern resolved into IgG-Lambda-Lambda (LC isotype-matched). This finding is probably because of the asynchronous production of excess free light chains (serum Lamba-10297.4 mg/L), which can migrate faster and produce an additional M-band on SPEP. Double gammopathy (DG) occurs only in clonally related plasma cells, of which IgG-IgA, IgG-IgM and IgG-IgG are reported in various literatures [12,13]. These can occur with either heavy chain or light chain isotype matching, attributable to two primary mechanisms. The first mechanism involves the molecular process of antigenic diversity that allows clonally related plasma cells to undergo class switch recombination. This can be seen during disease progression or following treatment. The levels of such Double M proteins can rise or fall either concordantly or discordantly [16]. Molecular analysis of these DGs has frequently demonstrated isotype switches in heavy chains, where different heavy chains can be encoded from the same IGHV, IGHD, and IGHJ genes with varying degrees of somatic mutations, resulting in identical immunoglobulin heavy chain variable regions (identical amino acid sequences) [17]. Yet such class switch was not observed in the kappa and lambda chains, rather low abundance of Plasma cells for light chain proliferation was reported in molecular analysis [16,17]. The second common cause is the asynchronous production of excess free light chains along with Ig heavy chains. This excess free LC migrates faster than HC producing a biclonal pattern [18]. In addition to IFE, the measurement of serum free light chains and serum IgA, IgG, and IgM levels is valuable in the diagnosis of these DGs. In case 3, the serum lambda levels (serum lambda - 10297.4 mg/l) was disproportionally elevated compared to the heavy chains (serum IgG - 1045 mg/dl). Other less common factors that can lead to biclonal patterns include renal impairment, which can cause elevated serum free light chains, and oligoclonality following stem cell transplantation [19]. Among these causes renal failure was also observed in this patient. Renal impairment can lead to decreased clearance of free light chains and an increased molecular half-life of light chains. The increase in free light chains secondary to renal failure has been identified as a poor prognostic factor in myeloma patients [20,21].

SPEP alone exhibits limited sensitivity in detecting pseudo-biclonal patterns. The diagnostic accuracy for pseudo-biclonal patterns can be enhanced by the combined use of SPEP with immunofixation electrophoresis, serum free light chain assays, and serum immunoglobulin assays. In limited resource settings or as a cost-effective strategy, reducing agents like beta-mercaptoethanol and dithiothreitol can be used to differentiate between true biclonality and polymeric forms [2,3]. Pre-treatment of serum with beta-mercaptoethanol prior to SPEP depolymerizes polymeric immunoglobulins by disrupting their disulfide bonds, resulting in a single M-band [4]. However, it is important to note that reducing agents cannot distinguish between true biclonal gammopathy and biclonal patterns arising from excess free light chains, necessitating IFE for definitive confirmation.

Conclusion

While no major differences may exist in the clinical presentation, treatment strategies, or outcomes among biclonal/double or monoclonal gammopathy, the accurate identification of these atypical patterns is crucial for improving diagnostic precision and laboratory quality indices. Double gammopathy/ Biclonal gammopathy are commonly associated with leukemia and lymphomas other than multiple myeloma, for which early diagnosis can help in timely treatment. Similarly, in cases where multiple myeloma is diagnosed based on double gammopathy or biclonal gammopathy, monitoring the synchronous response of all clones to treatment is critical, as it may necessitate modifications to the treatment regimen. The identification of double gammopathies, particularly those involving different heavy chains, may provide valuable insights into the molecular mechanisms underlying these conditions, potentially shedding light on the genetic evolution and genomic alterations in multiple myeloma. Our case series has primarily focused on pseudo-biclonal patterns arising from monoclonal gammopathy,

polyclonal elevation due to TLS and Double gammopathy due to excess free light chains. Although literature exists regarding polymerization and excess free light chains, the significance of our series lies in highlighting the rarely reported TLS-induced pseudo-biclonal pattern. SPEP followed by IFE remains a necessary approach for the accurate identification of such pseudo-biclonal patterns.

Author contributions

Conceptualization: Kanmani N, Karthick E, Sathya Selvarajan, K Sowmya; Methodology: Kanmani N, Karthick E, Sathya Selvarajan, K Sowmya; Material preparation, data collection: Kanmani N, Karthick E, Sathya Selvarajan, K Sowmya; Formal analysis and investigation: Kanmani N, Karthick E, Sathya Selvarajan, K Sowmya;Writing - original draft preparation: Kanmani N, Karthick E, Sathya Selvarajan, K Sowmya; Writing - review and editing: Kanmani N, Karthick E, Sathya Selvarajan, K Sowmya; Resources: Kanmani N, Karthick E, Sathya Selvarajan, K Sowmya; Supervision and final approval: Kanmani N, Karthick E, Sathya Selvarajan, K Sowmya; Accountability for the research: Kanmani N, Karthick E, Sathya Selvarajan, K Sowmya.

Ethical approval

The study was approved by Sri Ramachandra Institute of Higher Education and Research Institutional Ethics Committee (CSP-MED/24/AUG/107/270). Waiver of consent was provided pertaining to the nature of study. This study was conducted in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Data availability

The datasets used and/or analysed during the current study are not available because of the Institutional policy.

Conflict of interest

The authors declare that there is no conflict of interest concerning this study.

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