CASE REPORT

Fibrinogen Interference or Monoclonal Protein? A Case Report

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ABSTRACT

A 58-year-old man presented to the hospital with back pain and unexplained weight loss. He has no known comorbid and was not on any medication. Physical examination of this patient was unremarkable; however, laboratory investigations revealed anaemia, renal impairment and hypercalcaemia. Subsequent bone marrow biopsy showed prominent plasma cells while serum protein electrophoresis (SPE) revealed two distinct bands at the gamma region. The first band, located at the mid-gamma region, corresponded to lambda (λ) light chain (LC) on immunofixation (IF) with no associated heavy chains (HC), leading to a diagnosis of λLC multiple myeloma (MM). The second band, initially suspected to be a fibrinogen band due to its fast-gamma position and absence of HC and LC, was later reinterpreted as IgD HC on repeat SPE and IF performed two-months post chemotherapy. This case report discusses an uncommon IgD MM, with atypical IF bands which were initially mistaken as fibrinogen interference.

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INTRODUCTION

Fibrinogen, a plasma glycoprotein involved in blood clotting, is one of the common interferences encountered in serum protein electrophoresis (SPE) testing. A monoclonal paraprotein band at the β / γ region on SPE may be mistaken as a fibrinogen band, and vice versa. We highlight a case of a 58-year-old man with epidemiologically rare IgD MM, complicated with atypical immunofixation (IF) bands which were initially mistaken as fibrinogen interference. Selective elimination of fibrinogen with ethanol or thrombin, or utilisation of an antibody against fibrinogen on IF are amongst effective methods to identify fibrinogen interference and avert a misdiagnosis.

CASE REPORT

A-58-year-old man presented to the hospital with non-specific back pain, lethargy and unexplained 10kg weight loss over a one-month duration. He is a smoker with no known comorbid and not on any medication. He denied weakness or paraesthesia of the lower limbs and there was no recent trauma. On admission, his vital signs were stable. Physical examination of the cardiorespiratory, gastrointestinal and central nervous system were unremarkable.

Initial laboratory investigations (Table I) revealed leukocytosis with bicytopenia. Peripheral blood film showed presence of 2% lymphoplasmacytoid cells. Hyponatraemia, hyperkalaemia and raised serum creatinine were suggestive of renal impairment. Liver function test revealed hypoalbuminaemia, a reversed albumin: globulin ratio and raised alkaline phosphatase. Non-parathyroid hormone mediated hypercalcemia and metabolic acidosis were also noted. As part of MM workup, SPE was performed using agarose gel (AG) electrophoresis (Hydrasys 2 scan, Sebia). It revealed two distinct bands at the gamma (γ) region with marked immunoparesis (Fig. 1). To identify the type of monoclonal protein, IF was carried out using agarose Hydragel 2/4 IF gels (Hydrasys 2 scan, Sebia) (Fig. 1). The first band at the mid γ region, measuring 6.7g/L on the densitogram (Fig. 1), was interpreted as a free lambda (λ) light chain (LC) because it corresponded to λ and free λ antisera on IF with no obvious corresponding immunoglobulin (Ig) G, A, M, D, E heavy chains (HC). The second band which was located at the fast γ region, measures 23.4g/L on the densitogram (Fig. 1). Since our laboratory received the SPE sample in a secondary polypropylene tube collected after centrifugation at a district hospital laboratory, we were unable to ascertain

whether a serum or plasma sample was initially sampled in this case. Judging by its electrophoretic position at the fast γ region and the lack of corresponding HC and LC on IF (Fig. 1), this band was deduced as a fibrinogen band. Urine protein electrophoresis and immunoglobulin quantification were not performed as it was unavailable at our centre. Free light chain (FLC) analysis (Optilite, The Binding Site) revealed serum free kappa (κ) at 22.91 mg/L [reference interval (RI): 3.30 – 19.4 mg/L], serum free λ at 5640.00 mg/L (RI: 5.71 – 26.3 mg/L), and a serum Free κ / λ ratio of < 0.01 (RI: 0.37 - 3.10). Ensuing bone marrow aspiration and trephine biopsy (BMAT) were suboptimal due to haemodilution and clotting. Despite this, there were areas of prominent plasma cells, with positive CD138 and tiny aggregates showing λ clonality in which myeloma needs to be considered (Table 1). A repeat BMAT was not performed as the patient developed haematoma at the biopsy site. Skeletal survey showed no radiological evidence of lytic or sclerotic lesions (Table 1). Although clonal bone marrow plasma cells >10% cannot be confirmed due to a suboptimal BMAT, the patient presented with evidence of end organ damage (hypercalcemia, anaemia, renal insufficiency), myeloma-defining events (serum involved/uninvolved FLC ratio >100) and serum monoclonal protein on SPE. With a provisional diagnosis of λ LC MM, the haematology team decided to commence the patient on treatment and repeat a BMAT later. Subcutaneous erythropoietin, intravenous pamidronate and chemotherapy with bortezomib, thalidomide, and dexamethasone (VTD regime) were started. The patient tolerated chemotherapy well and was discharged home after.

Table I: Blood investigation result

Analyte	On admission	Reference Interval
Haematology		
White blood cells	80	4 -10 x 10^3/μL
White blood cell differential count:		
Neutrophil	69.4	2.0-7.0 x 10^3/μL
Lymphocyte	3.8	1.0-3.0 x 10^3/μL
Basophil	0.1	0.2-1.0 x 10^3/μL
Monocyte	6.0	0.0-0.5 x 10^3/μL
Eosinophil	0.2	0.02-0.10 x 10^3/μL
Haemoglobin	7.5	13.0 - 17.0 g/dL
Platelet	33	150 - 410 x 10^3/μL

CONTINUE

Table I: Blood investigation result (CONT.)

Analyte	On admission	Reference Interval
Haematology		
Peripheral blood film	Leucoerythroblastic picture with 2% circulating lymphoplasmacytoid cells seen.	-
Bone marrow aspiration and trephine biopsy	Suboptimal sample due to haemodilution and clotting. Areas of prominent plasma cells, with positive CD138 and tiny aggregates showing λ clonality. Myeloma needs to be considered.	-
Coagulation profile		
Prothrombin Time	11	9.4-11.0 seconds
INR	1.03	0.90-1.10
APTT	30.8	22.2-31.0 seconds
Renal profile		
Sodium	125	136 - 145 mmol/L
Potassium	5.9	3.4 - 4.5 mmol/L
Urea	41.75	2.76 - 8.07 mmol/L
Creatinine	2147	62 - 106 μmol /L
Liver function test		
Total protein	61	66 - 87 g/L
Albumin	18	35 - 52 g/L
Globulin	43	20 - 36 g/L
Albumin: globulin ratio	0.4	0.8 - 2.0
Total bilirubin	17	<24 μmol/L
Alanine aminotransferase	18	10 - 50 U/L
Alkaline phosphatase	516	40 - 130 U/L
Venous blood gas		
pH	7.02	7.35 - 7.43
pCO_2	39	35 - 45 mmHg
Bicarbonate	12	22 - 28 mmol/L
Base excess	1.5	-3.0 - 3.0 mmol/L
Other biochemical tests		
Calcium	2.50	2.15 - 2.50 mmol/L
Phosphate	1.1	1.6 - 6.0 mmol/L
Parathyroid hormone	1.1	1.6 - 6.0 pmol/L
Lactate dehydrogenase	573	<250 U/L
Beta 2 microglobulin	57.3	1.09-2.53 mg/L

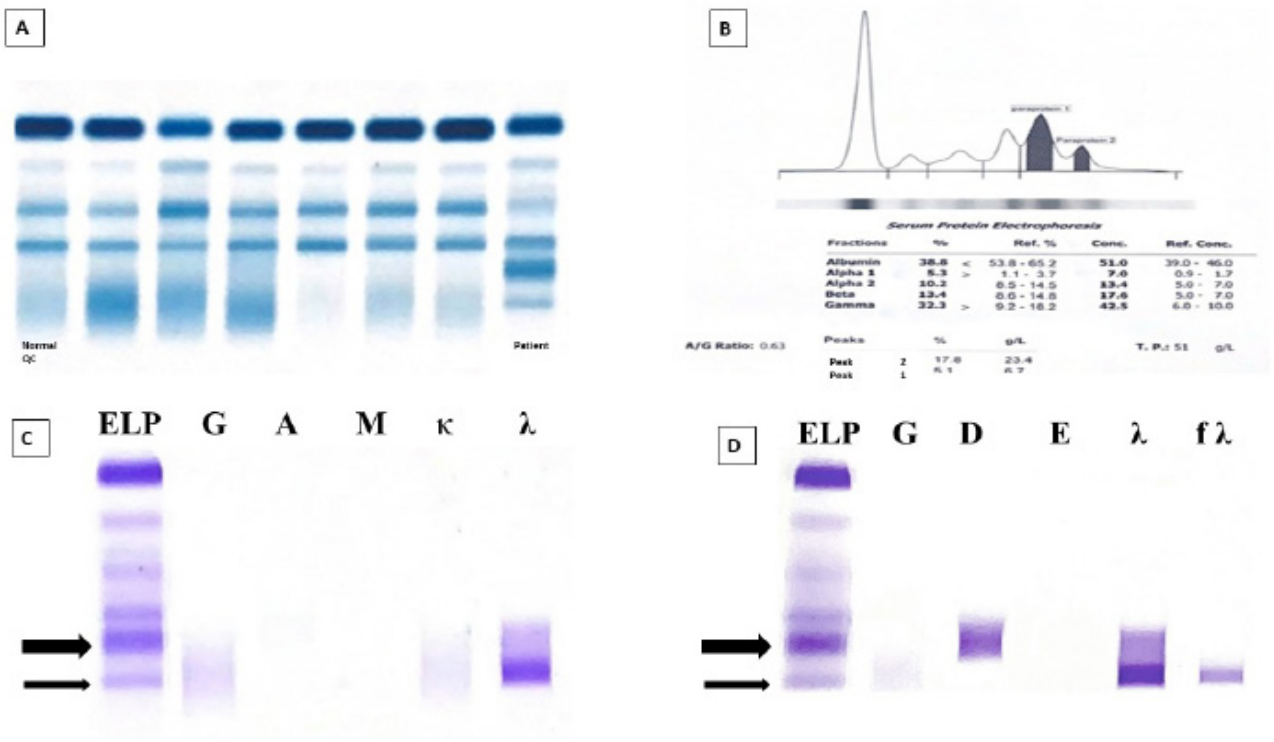

Fig.1: Paraproteins detected by electrophoresis (Hydrasys 2 scan, Sebia) during initial diagnosis.
A: The patient's serum protein electrophoresis showed two discrete bands located at the mid γ region and fast γ region.
B: Densitogram showed two peaks at the γ region, measuring 6.7 g/L and 23.4g/L respectively. C: Serum immunofixation using the anti-Ig G, IgA, IgM, total κ and total λ antisera showed two discrete bands. The first band at the mid γ region (denoted by the thin arrow) had a corresponding λ light chain but no corresponding heavy chain band. The second band at the fast γ region (denoted by the thick arrow) had no corresponding heavy and light chains. D: Serum immunofixation using the anti-IgG, IgD, IgE, total λ and free λ antisera showed two discrete bands. The first band at the mid γ region (denoted by the thin arrow), corresponded to λ and free λ antisera, as evidenced by a sharp, well-demarcated and dense band at the λ and free λ lane. Corresponding HC and LC for the second band at the fast γ region (denoted by the thick arrow) were not considered because the IgD and λ lanes were less-densely stained, broad, with an observed gradual and smooth reduction in the colour density towards the edges of the lane without any obvious restrictions or sharp borders as would be visualised with a monoclonal band.

A follow up SPE (Hydrasys 2 scan, Sebia) was performed two months later (after two cycles of chemotherapy). The first monoclonal band of free- λ type at the mid γ region was no longer detected (Fig. 2). Interestingly, despite using serum sample this time, the second band at the fast γ region which was previously identified as a fibrinogen band, was still present. Although the concentration had decreased markedly and was not detected on SPE, it was faintly visible on IF (Hydrasys 2 scan, Sebia), corresponding to IgD HC with a concentration of <1.0 g/L (Fig. 2). The IgD band had a greater intensity than the λ LC, which appeared as a very faint band. FLC analysis (Optilite, The Binding Site) revealed concomitantly raised serum free κ of 143.75 mg/L (RI: 3.30 – 19.40 mg/L) and serum free λ of 103.29 mg/L (RI: 5.71 – 26.3 mg/L), with a normal κ / λ ratio of 1.39 (RI: 0.37 - 3.1). In keeping with the changes in SPE and IF, the patient's diagnosis was revised to IgD λ MM with excess λ LC. His haematology and biochemical parameters also improved. The haematology team decided not to repeat a BMAT as the patient responded well to treatment. He then went on to complete his chemotherapy regime the next four months. However, after completion of chemotherapy, his disease progressed rapidly and he succumbed to death within a year from diagnosis.

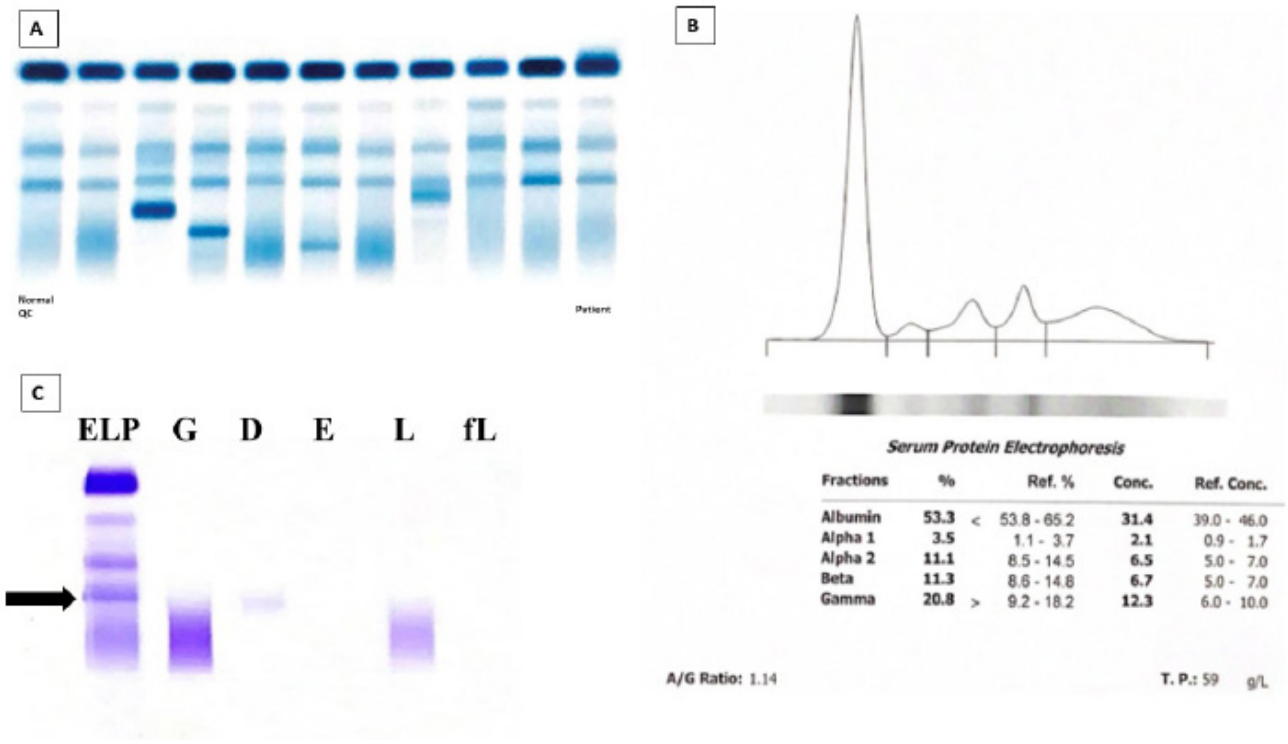

Fig.2 Serum protein electrophoresis and immunofixation during follow up (Hydrasys 2 scan, Sebia).
A: The patient's serum protein electrophoresis did not show presence of obvious monoclonal bands.
B: Densitogram showed the total concentration of gamma globulins at 12.3 g/L with no obvious peaks. C: Serum immunofixation using the anti-Ig G, IgD, IgE, total λ and free λ antisera showed one discrete band at the fast γ region (denoted by the thick arrow) corresponding to IgD HC and λ LC, as evidenced by a sharp, well-demarcated and dense band at the IgD and λ lane. The intensity of the IgD band was greater than the λ LC band. The previous band located at the mid γ region was no longer detected.

DISCUSSION

MM, a disorder characterised by clonal proliferation of plasma cells in bone marrow, accounts for up to 15% of all haematological malignancies (1). Being a rare subtype, IgD MM constitutes only 1-2% of all MM cases and has a male preponderance (1), similar to our patient. Mechanisms behind the disproportionate sex distribution in MM incidence is still not well understood, however research in tumour genetics have revealed sex-dependent features in these patients. For example, hyperdiploidy is more commonly found in males, possibly influenced by male hormonal difference or genetic variation on the sex chromosome. Compared to other myeloma subtypes, IgD MM arise in a B cell before Ig class switching (1). This may be one of the reasons why IgD MM has a different clinical behaviour and typically presents in a younger age group (1).

IgD MM is an aggressive subtype commonly associated with high lactate dehydrogenase (LDH) and renal impairment at diagnosis (1), as demonstrated in our patient. Elevated LDH reflects high proliferative activity and tumour burden (1), while renal impairment is due to overproduction and deposition of excess free LC damaging the proximal tubules (1). Our patient also demonstrated other biochemical changes in renal impairment such as hyponatraemia, from decreased water excretion; hyperkalaemia, due to acidosis-induced extracellular potassium shift; metabolic acidosis, as a result of decreased hydrogen exchange; and hypercalcaemia from activation of osteoclast-activating cytokines by tumour cells (2).

The main diagnostic challenge in our case was the identification of a monoclonal protein versus fibrinogen band. Migrating at the β / γ junction (3), a fibrinogen band is commonly observed when electrophoresis is performed on plasma sample or poorly-clotted serum sample (such as patients with disorders of coagulation or on anticoagulation therapy) (4). It is difficult for laboratories like ours to detect the type of sample because these samples (commonly referred tests) are often received as secondary aliquots. An alternative in this case is the measurement of lithium concentration in the received secondary aliquot. When blood from a subject without lithium exposure is collected in a lithium heparin tube (plasma sample) instead of a plain tube (serum sample), a raised lithium heparin level is expected. Unfortunately, lithium analysis was unable to be performed in this case as the received secondary aliquot was insufficient. Other recognised methods of identifying the presence of fibrinogen are via its removal with ethanol or thrombin before repeating the SPE, or utilising an antibody against fibrinogen on IF (4). While these methods are proven to be useful, they are often not available in many centres including ours. Lastly, correlation with IF also helps to establish the

monoclonality of an abnormal band visualised on SPE. IF will be negative for a suspected fibrinogen band whereas a discrete corresponding HC and/or LC band will be detected for a monoclonal protein (4). In our patient, the atypical appearance of a broad, diffuse smear rather than a characteristic discrete, well-demarcated band of a monoclonal paraprotein was observed at the IgD and λ lanes in IF. These isolated, atypical IF findings, coupled with the epidemiological rarity of an IgD MM, resulted in the initial impression of a fibrinogen band rather than a paraprotein. This unusual phenomenon is hypothesised to be due to aggregation of high concentration IgD monoclonal proteins with other plasma components or complex formation (4), and remains to be confirmed with future studies. Despite being time-consuming and costly, IgD quantification and IF on a repeat serum sample, should ideally be performed in this scenario to confirm the presence of IgD paraprotein. Other substances that may cause analytical interference in SPE particularly by mimicking a paraprotein band at the β / γ junction includes gelatin-based plasma substitutes and contrast dyes such as Iomeprol and Loversol (3). Unlike our case which uses AG electrophoresis, these interferences are typically observed in capillary zone electrophoresis whereby radio-opaque substances interfere with analysis by absorbing the same wavelength as a monoclonal component (3).

One of the other diagnostic challenges in IgD MM is the presence of low concentration IgD in the serum due to its slow synthesis and short half-life (1). This results in a subtle or non-visible monoclonal protein band on gel electrophoresis which may go undetected or wrongly diagnosed as light chain myeloma (1). In our patient, another interesting point to note in the follow up IF is the difference in intensity of the IgD and λ free LC bands. Although having identical electrophoretic positions at the mid γ region, the intensity of the IgD band was greater than the λ LC band. This weak staining of λ has been reported in the literature (5), and may be explained by the difference in sensitivity of the antisera whereby IgD antisera has a superior sensitivity, allowing for it to bind to IgD at a lower concentration compared to λ antisera. Thus, this sensitivity difference between antisera adds further challenge to the laboratory diagnosis of the already rare IgD subtype.

CONCLUSION

This case reports the diagnostic challenges in an epidemiologically rare IgD MM, complicated with atypical IF bands mistaken as fibrinogen interference. The correct identification of fibrinogen interference, either via a repeat serum sampling, or selective elimination of fibrinogen is crucial to prevent delayed or misdiagnosis. It is also worth mentioning that the absence of paraprotein bands on SPE does not rule out MM and in cases where clinical suspicion for MM is high, reliance on other laboratory investigations is paramount. Although revision to the diagnosis was eventually made and the chemotherapy regime remained uninterrupted in our patient, correct identification of MM subtype is vital to ensure optimal patient disease monitoring and prognostication.

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