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CASE REPORT

Serum separation abnormality in a multiple myeloma patient

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Abstract

Introduction: M-protein secreted by myeloma cells do not only contribute to myeloma-related complications but is also a well-recognised source of interference in laboratory assays. We describe a case of a 62-year-old woman whose blood sample showed improper serum separation even after resampling. *Case report*: Centrifugation of a biochemistry specimen in a serum-separator tube received by the laboratory failed to separate any serum, nor did repeating the process at a longer duration. Repeat sampling only yielded a small volume of serum from which highly elevated total protein was noted upon analysis. Additional history from the treating clinician unveiled a diagnosis of multiple myeloma in this patient. *Discussion*: This case represents one of the rare, but significant pre-analytical interferences caused by M-proteins.

Keywords: Multiple myeloma, pre-analytical interference, barrier gel

INTRODUCTION

Multiple myeloma is a plasma cell malignancy that remains incurable.[1] It is characterised by the growth of neoplastic plasma cells in the bone marrow, leading to disease onset and manifestation of symptoms. In addition, the malignant plasma cells synthesise M-protein and release it into the circulation. This M-protein does not only contribute to myeloma-related complications, but is also a well-recognised source of interference in laboratory assays.[2] Commonly reported interferences involve those that directly interfere with the analytical process of various analytes, giving rise to erroneous results.[2] One important mechanism of interference by M-proteins is precipitation where chemical tests that are measured via turbidimetry or absorbance are most susceptible.[3] Precipitates can change the specimen's turbidity and if sufficiently large, may significantly alter the amount of transmitted or scattered light.[4] Other mechanisms include volume displacement effect, which underlies the pseudohyponatraemia phenomenon, and hook effect that affects direct measurement of the monoclonal immunoglobulin.[2] Occasionally, M-protein may also interfere with the gel positioning of the serum separator tube (SST). While the exact mechanism remains unclear, increased density and/or viscosity of the M-protein rich serum is believed to play a significant role.[5] Here, we present a case of a patient with newly diagnosed multiple myeloma of IgA lambda paraproteinaemia, in whom biochemistry analysis was complicated by improper separation of the serum in a gel separator tube.

CASE REPORT

The Chemical Pathology Unit, Pathology Department of a tertiary hospital received a blood sample of a 62-year-old Malay woman for biochemistry analysis. The specimen, however, was noted to be unsuitable for analysis as it did not separate properly after centrifugation. The gel in the SST failed to constitute a separating barrier and instead remained at the bottom of the tube (Figure 1). Centrifugation was repeated for a longer duration, yet the outcome was the same. A request for a repeat sample was made, but this time a viscous material formed within the separated serum, with only little serum extracted from the topmost layer (Figure 2). The analyses

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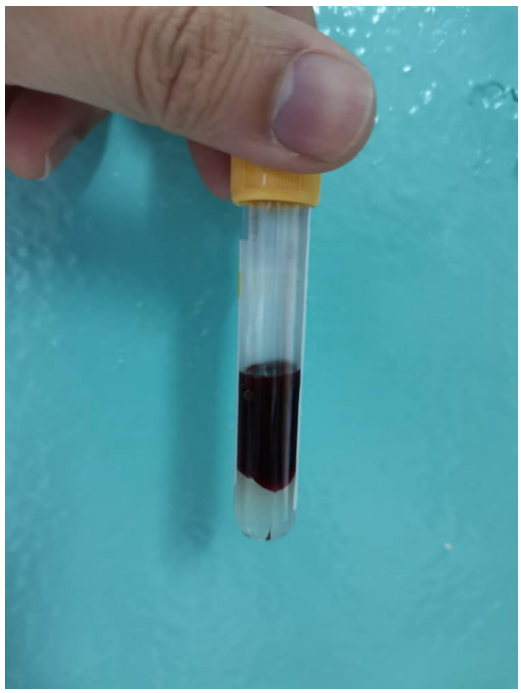


FIG. 1: Abnormal gel placement post-centrifugation

FIG. 2: Incomplete separation of blood components in repeat blood sampling

revealed an increased total protein concentration of 145 g/L (reference interval 64–83 g/L) with reversed albumin globulin ratio of 0.2 (albumin = 25 g/L and globulin = 120 g/L). Other significant findings included hyponatraemia and raised serum creatinine while albumin-adjusted calcium level was normal. Given the high total protein in the sample, the possibility of pseudohyponatraemia was considered and confirmed by a repeat testing using direct ion selective electrode (ISE) method, which revealed a serum sodium concentration of 135 mmol/L. At the same time, since very little clinical history was provided in the request form, the laboratory requested additional information regarding the patient and noted that her underlying diagnosis was multiple myeloma. The biochemistry results are summarised in Table 1.

The patient initially presented to a peripheral hospital with a month's history of generalised weakness, reduced effort tolerance, dizziness as well as unintentional weight loss of 5 kg. Her past medical history included hypertension and diabetes mellitus. The systemic examination was unremarkable apart from pallor. Complete blood count revealed bicytopaenia with haemoglobin of 4.6 mg/dL and platelet count of 56 X 10^9/L, and her urgent peripheral blood film showed marked rouleaux formation. Bone marrow aspiration was subsequently performed and showed hypercellular marrow with increased plasma cells (52%), suggestive of plasma cell myeloma. She received transfusion of packed red blood cells and platelets, after which she was transferred to a tertiary hospital for further management.

Serum protein electrophoresis (SPE) by agarose gel showed the presence of a monoclonal band in the beta region with a quantitation of 62.9 g/L, with marked immuneparesis. The immunofixation electrophoresis (IFE) of the serum was reported as IgA lambda paraproteinaemia (Figure 3). The serum free light chain (FLC) assay showed high level of free lambda (1850 mg/L; reference, 8.30-27.00) and normal level of free kappa (15.00 mg/L; reference 6.70-22.40) with serum free kappa/lambda ratio of 0.008 (reference, 0.310-1.560). Serum beta-2 microglobulin was raised at 79.7 mg/L (reference, 1.09-2.53). The patient was subsequently started on induction chemotherapy with a triple-drug regimen.

DISCUSSION

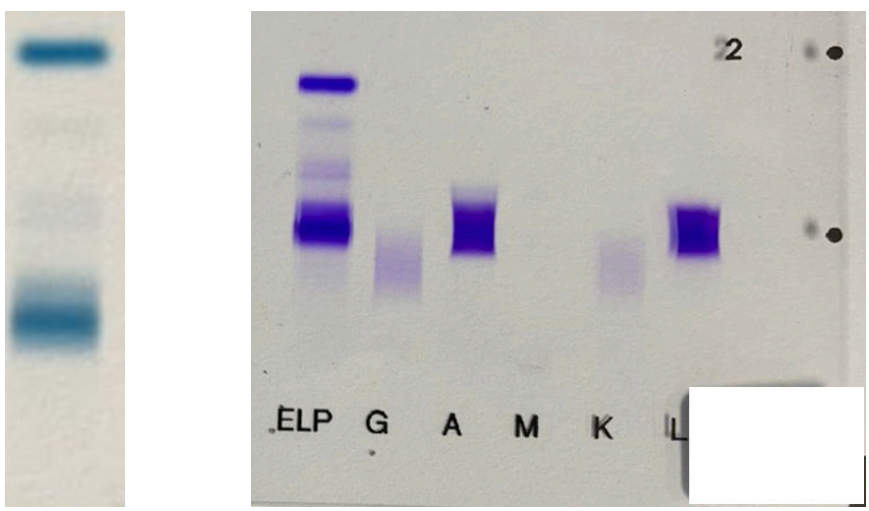
While paraprotein-related issues during sample analysis are commonly reported, reports documenting gross changes to the blood specimen are limited. Visual inspection of our

TABLE 1: Laboratory investigations

Parameter	Result	Reference Interval
Urea	10.1	(3.2-8.2) mmol/L
Sodium	129	(132-146) mmol/L
Potassium	3.5	(3.5-5.5) mmol/L
Chloride	89	(99-109) mmol/L
Creatinine	115.1	(44.2-97.2) μmol/L
Total protein	145	(57-82) g/L
Albumin	25	(34-50) g/L
Globulin	120	(25-39) g/L
Albumin/globulin ratio	0.2	(0.9-1.8)
Calcium	2.10	(2.18-2.60) mmol/L
Albumin-adjusted calcium	2.40	(2.18-2.60) mmol/L
Phosphate	0.94	(0.78-1.65) mmol/L
Magnesium	1.23	(0.53-1.11) mmol/L
Haemoglobin	9.5	(12.0-15.0) g/dL
White cell count	7.8	(4.0-10.0) X 10^9/L
Platelet	92	(150-410) X 10^9/L
MCV	98	(83-101) fL
MCH	33.1	(26.5-31.5) pg

patient's blood sample following centrifugation revealed the formation of viscous material that appeared to be composed of clotted blood suspended in a viscous serum, while the gel remained at the bottom of the tube. SSTs are the primary tubes used in our laboratory for serum biochemistry analysis. It contains an inert gel with a specific density that falls between the densities of the serum and the blood cells.[6] The thixotropic property of the gel causes its viscosity to decrease during centrifugation, thus allows it to move before returning to the original semi-solid state when the force is stopped. Because of this, the gel can flow and position itself between the two blood components when centrifugation force is applied.[6]

Several factors may influence the final position of the gel, including density and viscosity of the serum, as well as centrifugal force.[5] Occasionally, low centrifugal force may lead to incomplete



A B

FIG. 3: The SPE (A) shows a monoclonal band in the beta region. The IFE (B) confirms the monoclonal band as IgA lambda.

separation of the serum. Quality of specimen separated by centrifugation depends on two main variables, relative centrifugal force (RCF) and centrifugation time.[7] While the maximum RCF is limited by factors such as tube resistance, the centrifugation time can readily be varied to improve barrier formation, serum clarity and yield.[7] The manufacturer of the SST gel tubes (Becton Dickinson, NY, USA) used by our laboratory recommends that these tubes be spun for 10 minutes at 1000 to 1300 RCF.[8] To minimise turnaround time to match the high volume workload, our laboratory practices spinning samples at 2000 RCF for 5 minutes. Repeating centrifugation for 10 minutes however, did not solve our patient's blood sample abnormality hence low centrifugal force deemed unlikely.

An attempt to find a case similar to ours through a literature search was unsuccessful. Interestingly, many cases described the findings of floating gel, which is the exact opposite of what was observed in our case.[9-12] A study by Fatas *et al*. (2008) demonstrated that increased density of plasma caused by the high protein concentration, rather than its viscosity causes the gel to move higher than the plasma.[10] However, viscosity may have been a contributing factor in our patient as the total protein concentration was highly elevated. Serum viscosity correlates with immunoglobulin levels, and although more commonly seen in IgM paraproteinaemia, a patient with IgA concentration greater than 60 g/L is also at risk of hyperviscosity related event.[13] At such high concentrations, immunoglobulins may aggregate with each other and may also interact with blood cells, causing red cell aggregation.[6] As the gel must move around the clot and along the wall of the SST tube,[6] the viscous serum and blood clot mixture might have prevented this movement in our patient. Serum viscosity, however, was not measured in our case. A somewhat similar case was described by Zhang *et al*. (2018) in which improper serum separation resulted in low serum yield with too much haematocyte in SST, while the opposite was observed in sodium citrate and plain tube.[15] The difference between their case and ours was that the gel in their SST was still able to move upwards.

A study by Chakraborty *et al*. (2014) showed that incomplete serum separation gel tubes is a relatively unusual phenomenon with a prevalence of 0.05%, but interestingly all were associated with M-protein.[5] The phenomenon was seen to occur across a wide range of total protein and M-protein concentrations and is therefore difficult to predict. This fact, together with the many variables affecting gel barrier formation, makes it difficult to draw a conclusive explanation on the exact mechanism for this atypical occurrence.[5] Chakraborty group also attested that the event may be intermittent, as some patients did not have the same issue on redraw or centrifugation at higher centrifugal force, hence these measures should be attempted. Another potential solution as highlighted by Ricorius *et al*. (2018) and Zhang *et al*. (2018) is to use non-gel tubes for subsequent sampling.[15,16]

CONCLUSION

This case presents one of the rare, but important pre-analytical interferences caused by M-proteins. Although the exact mechanism is unclear, laboratorians should be aware of such abnormalities, and the practice of visual inspection of samples should be emphasised. If undetected, occlusion of probes may occur from abnormal gel positioning, and falsely low results may be produced from aspiration of an insufficient sample. More importantly, it can also be the first clue of an underlying monoclonal gammopathy.

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