CASE REPORT

Cinderella in Serum Protein Electrophoresis

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Abstract Paraproteinemia is characterised by clonal proliferation of plasma cells. A common laboratory finding in paraproteinemia being a monoclonal peak in serum protein electrophoresis (M band). But there are factors which produce a peak similar to M spike in serum protein electrophoresis and these factors are known as pseudoparaproteins. This case report discusses a rare cause of pseudo M spike in a known case of autoimmune hemolytic anaemia due to administration of drug-Rituximab, a monoclonal antibody by itself.

Keywords Paraproteins · M spike · Rituximab

Abbreviations

AIHA	Autoimmune hemolytic anemia
CZE	Capillary zone electrophoresis
Ig	Immunoglobulin
k	Kappa
MAT	Monoclonal antibody therapy
CD	Cluster of differentiation
LOS	Length of stay

Case Description

An African male aged 17 years, presented with chronic anaemia and splenomegaly since one year. A comprehensive diagnostic workup was done at the time of admission including basic haematological and biochemical investigations. Complete blood count (Sysmex XN 1000) showed a low haemoglobin (9 g/dL), low RBC count (3.5 million/cumm) with high reticulocyte count (5.5%). The blood indices including MCV, MCH, MCHC were within normal limits. Peripheral blood smear showed presence of schistocytes and tear drop cells. These findings were indicative of haemolysis. Routine biochemical investigations (Vitros 5600) did not show any significant abnormality except elevated LDH (1002 U/L) and unconjugated Bilirubin (3.2 mg/dL). Direct coomb's test was strongly positive and the patient was diagnosed to have autoimmune haemolytic anaemia (AIHA). The patient was admitted after consultation with haemato oncologist and started on treatment with weekly Rituximab infusion (4 weeks regimen) for AIHA. During the course of management, the patient was followed with diagnostic re-evaluation after 2 weeks of initiation of Rituximab infusion to assess the treatment outcome. Complete blood count and peripheral smear study showed a decrease in haemolysis indicating a response to the management. The haematological evidence was supported by serum LDH (420 U/L) and unconjugated bilirubin (0.9 mg/dL) returning back to normal levels. All other biochemical results were within the biological reference intervals except for a serum total protein which showed a significant elevation (from 6.7 g/dL at the time of admission to 9.3 g/dL within a short span of 2 weeks) along with a reversal of albumin globulin ratio. Based on this incidental observation, a reflex testing of serum protein electrophoresis (Sebia Minicap flexpiercing) and

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Fig. 1 Serum protein electrophoresis picture of the patient by CZE with SEBIA MINICAP showing monoclonal peak in gamma region 2 weeks after initiation of Rituximab therapy

immunofixation electrophoresis was performed during the same time. The electrophoretogram showed a distinct tall peak with narrow base in the gamma region (Fig. 1). Immunofixation electrophoresis (Sebia Minicap flexpiercing) revealed an elevated IgG fraction and free kappa chains (Fig. 2) following which a confirmatory testing of serum immunoglobulin (immunoturbidimetry, Vitros 5600) quantification and free light chain assay (Binding Site-immunoturbidimetry, Vitros 5600) was performed. This showed an elevated serum immunoglobulin G (2200 mg/dL) and kappa light chain (102.5 mg/L). The laboratory findings supported a diagnosis of plasma cell disorder but this did not correlate with clinical picture and other ancillary investigations.

Discussion

In serum protein electrophoresis (SPE), a sharp peak with a narrow base and pointed apex if evident, is commonly indicative of presence of paraproteins [1] (M spike). Paraproteins are monoclonal immunoglobulins produced by a clonal population of mature B cells, most commonly plasma cells. According to Greipp PR, Sam Miguel JF, paraproteins are pathognomonic of plasma cell disorders which include a spectrum of conditions ranging from monoclonal gammopathy of undetermined significance,

through asymptomatic, to symptomatic myeloma [2]. Other common causes of a distinct peak resembling the M spike in capillary zone serum protein electrophoresis include transferrin, fibrinogen, beta lipoproteins, radio contrast dyes etc. These are known as pseudo paraproteins [3].

The serum protein electrophoresis report of this patient showed a tall pointed spike in gamma region, corresponding to a gamma globulin level of around 4.5 g/dL and his serum total protein was 9.3 g/dL. Both these findings arouse a high index of diagnostic suspicion of plasma cell dyscrasia. But an increment in serum total protein of 2.6 g/dL from the base line value of 6.7 g/dL (at the time of admission) in a short span of 2 weeks (after initiation of Rituximab infusion) was contradictory to the diagnosis of plasma cell disorder, since its least likely for these disorders to develop and progress in such a short span. Consultation with the treating physician revealed that the patient had been administered weekly Rituximab infusion—375 mg/m² (4 weeks regimen) for AIHA. Rituximab belongs to a group of drugs known as monoclonal antibody therapy (MAT). Rituximab is a chimeric murine/human monoclonal antibody. It contains the complementarity determining regions of the murine anti-CD20 antibody 2B8 in conjunction with human kappa and IgG1 heavy-chain constant region sequences [4]. Being a monoclonal antibody (IgG Kappa), Rituximab produces a monoclonal spike in gamma region in SPE [5, 6]. Rituximab has a mean

Fig. 2 Serum immunosubtraction capillary electrophoresis picture of the patient by CZE with SEBIA MINICAP showing a subtraction of IgG and kappa fractions

serum half life of 59.8 h after first infusion and 174 h after fourth infusion. Since, in SPE, a false M spike is bound to persist in gamma region till the drug is eliminated from the circulation, a confirmatory assessment of drug interference in form of pseudo M spike was done by repeating the SPE, serum immunoglobulin G quantification and serum free kappa light chain assay after 3 months [7]. SPE showed absence of M spike (Fig. 3) and IgG, kappa values returned to normal limits (IgG—1350 mg/dL and kappa—36 mg/L). This revealed that the M spike in this patient was due to interference from MAT.

Based on these laboratory findings, there is a high possibility of misdiagnosing such patients on MAT, as plasma cell dyscrasias which may lead to undue laboratory and radiological investigations to follow. This ultimately culminates in mental and physical agony of patients and

their families and extended LOS at the hospital. Hence a sufficient knowledge about the factors which can produce pseudo paraproteins including MAT, is one of the essential requirements for every laboratory medicine specialist to ‘how to interpret serum protein electrophoretograms’. In clinical practice, as there will be such false positive SPEs, due to various factors including drugs having monoclonal antibodies, it is advisable to add a disclaimer in SPE report whenever a M spike is seen suggesting interference due to MAT.

Pseudo paraprotein is rather a ‘not so uncommon’ entity which may be encountered in clinical laboratory practice [3]. An insight into the pseudo paraprotein, its causative factors and its diagnostic significance is essential to differentiate them from ‘True’ paraprotein. For this to happen, every laboratory test request shall be accompanied by

Fig. 3 Serum protein electrophoresis picture of the patient by CZE with SEBIA MINICAP repeated 3 months after cessation of Rituximab therapy

Methodology : Capillary Electrophoresis Total Protein : **7** g/dL

relevant clinical information and every laboratorian shall have an consultation with clinician when needed. This would form the ideal ‘good clinical laboratory practice’.

Compliance with Ethical Standards

Conflict of interest None of the authors of this study have any conflict of interest.

Ethical Approval This article contains study with a human participant performed by the authors. Informed consent was obtained from the individual participant included in the study.

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