

CORRESPONDENCE

Cross reactivity between fibrinogen and epsilon heavy chain antiserum in immunofixation electrophoresis

Sir,

Pseudoparaproteins are benign proteins that are not normally present, or present at low concentrations, in serum and could be misinterpreted upon protein electrophoresis as a monoclonal paraprotein. Strobel estimated that pseudoparaproteins were observed in 10.5% of specimens sent for protein electrophoresis.¹ Multiple myeloma is a common cause of chronic renal failure (CRF). Analysis of samples from patients with CRF is sometimes difficult because paraproteins as well as pseudoparaproteins are frequently encountered in this group of patients. Fibrinogen is an important source of pseudoparaproteins in CRF arising from ineffective clotting of blood due to anticoagulation during haemodialysis, or excessive citrate content in transfused blood.

The patient was a 66-year-old woman with no significant history except hysterectomy 20 years previously. She was not on any medication or herbal medicine and was admitted to the casualty department because she had been feeling generally unwell for 2 months. She also complained of nausea, decreased urinary output and frothy urine. Physical examination revealed an anxious patient with a blood pressure of 180/80; no significant chest and abdominal finding was noted. Initial investigation on blood samples saved in the casualty department revealed high blood/plasma creatinine (1207 µmol/L), haemoglobin 6.1 g/L, erythrocyte sedimentation rate (ESR) 113 mm/h, random glucose 10.7 mmol/L, and normal clotting profile (prothrombin time 11.6 s, activated partial thromboplastin time 29.5 s). Liver function test results were normal except for a high alkaline phosphatase (ALP) of 168 U/L. Adjusted calcium and phosphate were 1.70 mmol/L and 3.19 mmol/L, respectively. Venous blood gas analysis showed compensated metabolic acidosis (pH 7.28, pCO₂ 4.2 kPa, HCO₃ 14 mmol/L). CRF was diagnosed and the

patient was subsequently admitted to the renal ward and treated with haemodialysis and transfusion of packed cells.

In the casualty department, a clotted blood sample was saved for serum protein electrophoresis (SPE) before the commencement of haemodialysis and blood transfusion. A protein band, measuring 5.8 g/L by densitometry, was detected at the β/γ region (Fig. 1). No immune paresis was observed. Immunofixation electrophoresis (IFE) was subsequently performed. No visible band was seen by antibodies against γ , α , μ heavy chains or κ/λ light chains (Beckman Coulter, USA). IFE was repeated with various dilutions of the serum to rule out prozone effect but the findings were consistent. IFE with δ and ϵ heavy chain antisera (Dako, Denmark) was also performed. Prominent ϵ and barely visible δ heavy chain bands were observed at the corresponding position. Serum total IgE concentration by chemiluminescence immunoassay was 15 kIU/L (Immulite 1000 analyser; Diagnostic Products Corp., USA; reference interval of authors' laboratory 10–180 kIU/L, 1 IU=2.4 ng). Urine protein electrophoresis demonstrated glomerular proteinuria without Bence-Jones protein. Repeated SPE performed on another specimen obtained later could not detect the abnormal protein band. Analytical interference was suspected, and IFE with the use of fibrinogen antiserum revealed a strong positive band at the same position.

The preliminary results of electrophoretic studies suggested a case of IgE paraproteinaemia which may be caused by IgE myeloma. Since IgE myeloma is extremely rare, analytical interference was investigated before making the diagnosis. The possibility of IgE myeloma was subsequently excluded by the normal serum total IgE concentration. In the case of paraproteinaemia, high dose hook effect of our serum total IgE assay was unlikely because of the wide dynamic range (1–2000 kIU/L, corresponding to 2.4 mg/L–4.8 g/L). If total IgE concentration is found grossly elevated and no definite light chain band is identified by IFE, immunoselection may be indicated for the diagnosis of ϵ heavy chain disease.²

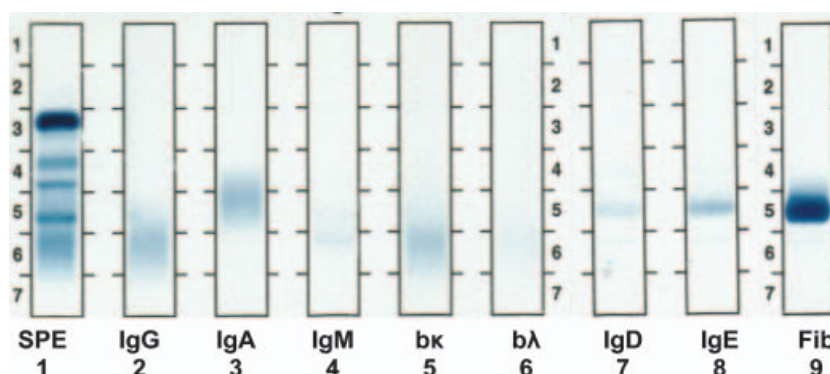


Fig. 1 Immunofixation electrophoresis of serum from the patient. The anode is at the top of the gel. From left to right: serum protein electrophoresis, immunofixation electrophoresis with the use of antisera against γ , α , μ , bound κ , bound λ , δ , ϵ , and fibrinogen. The positions of the anti- δ , - ϵ , and -fibrinogen bands were slightly different to that of serum protein electrophoresis because they were performed on different occasions, resulting in slight variation of sample application, hence the positions of the cut-and-paste aligned bands could not be directly compared.

Subsequent IFE with fibrinogen antiserum confirmed that this was a case of pseudoparaproteinaemia caused by fibrinogen in an incompletely clotted blood sample. Fibrinogen interference with SPE is a well-known problem, especially in CRF patients. In this case interference by fibrinogen was not suspected initially because (1) the intensity and appearance of the protein band were not characteristic of fibrinogen band, (2) the sample was collected before commencement of any medical treatment, and (3) there was a positive reaction with antisera against ϵ heavy chains. Fibrinogen is normally present in plasma at concentrations of 1.5–4.0 g/L with a typical intra-individual variation of 1.0 g/L.³ Increased fibrinogen concentration has been reported in individuals with acute phase reactions and various genetic and environmental conditions.³ Frequently the fibrinogen band is faint because some fibrinogen has been used up during the incomplete clotting process; therefore the strong fibrinogen band observed in this case is quite exceptional unless a wrong specimen type (i.e., plasma) or a heavily contaminated specimen was submitted. Fibrinogen band on gel-based electrophoresis has been described as irregular, curved or asymmetrical.⁴ In contrast, in the reported case the protein band was well-defined and regular in appearance.

Interference of SPE by fibrinogen is usually obvious as IFE will be negative with antibodies against immunoglobulin heavy chains and light chains. The most definitive proof is to repeat IFE with the use of fibrinogen antiserum. Other methods such as visual inspection of serum for clots⁵ and pre-treatment of samples with thrombin,⁵ ethanol⁶ and reptilase⁷ have been suggested. Ethanol pre-treatment of specimens is inexpensive and straightforward. However, residual irregularity in the γ -globulin fraction and a change in electrophoretic mobilities of other proteins have been observed, possibly as a result of denaturation by ethanol. Reptilase pre-treatment is superior to ethanol if quantitation of various proteins/paraproteins is necessary. Cross-reactivity between fibrinogen and antisera against α and μ heavy chains in IFE has been reported before.⁸ It has been recommended to evaluate new lots of immunoglobulin heavy/light chains antisera for IFE with plasma for minor cross-reactivity. This is the first report of fibrinogen interference with anti- ϵ chain antibodies. It is not known why the fibrinogen would affect IFE since fibrinogen shared no structural similarity with IgD/IgE. A possibility is that the IgE antigen for raising the antiserum was contaminated with fibrinogen.

The source of anticoagulant contamination remains obscure. The clotting profile on the sample taken simultaneously was normal. Although the low calcium level was compatible with EDTA contamination, it was appropriate in the context of CRF as the concomitant phosphate was high. The absence of EDTA contamination was further supported by normal potassium and high ALP. It is possible that the specimen had been contaminated by the heparin syringe used for blood gas analysis. The clinician had not replaced the needle with a clean one after rinsing the syringe with heparin. The heparinised syringe was filled after the citrate bottle (for clotting profile) and before the clotted blood bottle (for protein electrophoresis). This resulted in the contamination, and hence observation of a pseudoparaprotein band in the serum, but the clotting profile was unaffected.

This case illustrates that fibrinogen is a possible trap in electrophoresis interpretation. The proper blood collection protocol must not be overlooked and fibrinogen must be considered when an abnormal protein band is detected at the β/γ region.

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