A persistent additional fraction on capillary zone electrophoresis with negative immunofixation electrophoresis : paraproteinemia or pseudoparaproteinemia?

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Case description

A 53-year-old man, diagnosed with type 1 diabetes mellitus since 30 years and suffering from various diabetes complications, such as nephropathy, mild retinopathy and moderate sensory-motor neuropathy, presented at the endocrinology department of our hospital for his yearly check-up. The patient had no complaints. As standard procedure, a venous blood sample was sent to the laboratory for routine biochemistry and serum protein electrophoresis.

Routine laboratory investigation of the patient's blood revealed a renal insufficiency, with secondary hyperparathyroidism and an increased haemoglobin A_1C of 7.9 %, indicating insufficient metabolic control of his type 1 diabetes mellitus. The serum protein concentration was normal (70 g/L, reference interval 60 – 80 g/L), but capillary zone electrophoresis (CZE) of serum proteins (Protein 6, Capillarys 2, Sebia, France) revealed a marked additional fraction between the β_2 and γ zone (Figure 1).

Routine immunofixation (IF) electrophoresis (Hydragel 4 IF, Hydrasys, Sebia, France) with antisera against G, A and M heavy chains and κ and λ light chains was negative (Figure 1). As extended IF electrophoresis with antisera against D and E heavy chains and κ and λ free light chains was also negative, we strongly believed that this was a case of pseudoparaproteinemia. However, IF electrophoresis with an anti-fibrinogen antiserum was negative, agarose gel electrophoresis (AGE) (Hydrasys, Sebia, France) was completely normal and previously described common non-protein interferences were ruled out or considered very unlikely.

At his next yearly check-up, CZE electrophoresis of the serum proteins revealed again a marked additional fraction between the β_2 and γ zone (Figure 2).

During the search for an explanation in the patient's medical record, however, it was noticed that the patient's most recent immunoglobulin measurement dated from 10

years ago. At that time the immunoglobulin concentrations were at the lower limit of the reference interval (IgG 5.6 g/L, reference interval 7.0 – 16.0 g/L; IgA 0.7 g/L, reference interval 0.7 – 4.0 g/L; IgM 1.8 g/L, reference interval 0.4 – 2.3 g/L). Analysis of the immunoglobulins on the current serum sample (nephelometry, BN II, Siemens Healthineers, The Netherlands) revealed a markedly increased IgM of 10.4 g/L with a decreased IgG of 5.1 g/L and a decreased IgA of 0.4 g/L. Review of literature revealed an interesting publication of Bossuyt et al. (1) that suggested treatment of the serum sample with mercapto-ethanol before performing IF electrophoresis. This revealed a monoclonal IgM κ (Figure 2).

Following this finding, further investigations were performed. Bone marrow biopsy showed a monoclonal B-cell population of 16.4%. Flow cytometry of the peripheral blood showed the same monoclonal, mature B-cell population (17.9%). Molecular tests demonstrated the presence of a MYD88 L265P mutation. With all these components, the diagnosis of lymphoplasmacytic lymphoma was made.

Every 6 months, the patient visits our hospital for a check-up. Routine lab investigations with CZE show a slow progression of the monoclonal peak (additional fraction of 15.6% or 9.7 g/L). This evolution is confirmed by flow cytometry of the peripheral blood, where a monoclonal B-cell population of 43.4% is observed. Since the patient has no symptoms or cytopenia, a watch and wait approach is chosen.

Case Discussion

CZE has become a routine laboratory test for the evaluation of serum protein profiles and the detection of monoclonal proteins. Abnormal CZE should be followed by IF or immunosubtraction (IS) electrophoresis to confirm and identify the monoclonal protein (2). These techniques are also important to discriminate between true paraproteinemia

and pseudoparaproteinemia. An abnormal peak in the CZE pattern does not always equal the presence of a monoclonal protein since CZE is influenced by many endogenous and exogenous interferences (3,4).

In our case, after the first finding of the additional fraction between the β_2 and γ zone and since routine IF electrophoresis did not reveal a monoclonal protein, additional tests were performed. Extended IF electrophoresis with additional antisera was negative. Therefore, pseudoparaproteinemia was considered very likely.

Fibrinogen, which plays an important role in the coagulation cascade, may be seen in serum of patients with disorders of coagulation or receiving anticoagulation therapy, or when a plasma sample instead of a serum sample is send to the laboratory. When CZE is performed on these samples, fibrinogen migrates to the β/γ- region and it may be misinterpreted as a monoclonal immunoglobulin. In these samples, IF electrophoresis is negative (4). To exclude the presence of fibrinogen interference in our patient, an IF electrophoresis with anti-fibrinogen antibodies was performed. This test was also negative.

As all additional tests, especially AGE, in our patient were negative, we assumed a non-protein origin of the additional fraction between the β_2 and γ zone. Since AGE quantifies the protein fractions based on protein specific dye binding, it is not interfered by the presence of non-protein substances such as iodinated contrast. In our case AGE was normal, making the presence of a non-protein interference very likely. Therefore, we were surprised when the next CZE of the patient (approximately one year later) revealed the same additional fraction between the β_2 and γ zone.

Thorough revision of the patient's medical record did not reveal any possible medical, therapeutic, social, occupational or environmental reason that could explain a pseudoparaproteinemia. The patient did not receive any antimicrobial therapy, nor any

radiological examination with administration of contrast media. The sample was not haemolytic and CRP was normal (0.03 mg/dL). Since the patient had no complaints, no tumour markers were analysed. Numerous serum protein electrophoreses (AGE) (Hydrasys, Sebia, France) in the patient's history were normal.

Meanwhile it became clear that the last analysis of the immunoglobulins dated from years ago. Therefore, the immunoglobulins were measured in the current serum sample. This showed a markedly increased IgM with a decreased IgG and IgA. This finding together with the additional fraction on CZE suggested a false negative IF electrophoresis. Therefore, a review of the literature was performed revealing an article from Bossuyt et al. (1) about false-negative IF electrophoresis results. This article described a rare serum sample that contained an IgM monoclonal protein, which was negative with the semi-automated gel electrophoresis system Hydrasys from Sebia. They attributed the failure of the Sebia system to detect the IgM monoclonal protein to the use of plastic applicators to apply the serum, and hypothesized that the (polymerized) IgM monoclonal protein remained adhered to the microporous membrane of the applicator and did not diffuse into the gel (1). This article suggested to treat the serum sample with mercapto-ethanol, which should cleave the disulfide bonds in the IgM pentamer. This pre-treatment of the sample should reveal the monoclonal protein with the Sebia system. In another article Zetterberg et al. suggested, as an alternative, treatment of the sample with D/L-penicillamine to cleave the disulfide bounds in the IgM pentamer trough the thiol activity of penicillamine (5).We opted for treating the serum sample with mercapto-ethanol before performing IF electrophoresis. This revealed a monoclonal IgM κ. Thereafter the patient underwent further investigations which showed the presence of a monoclonal B-cell population leading to the diagnosis of a lymphoplasmacytic lymphoma wherefore the patient is regularly checked-up. Molecular tests revealed a

MYD88 L265P mutation. Zanwar et al described in a recent paper the importance of mutational analysis in current hematopathology since the MYD88 L265P mutation is an independent predictor of transformation in Waldenström macroglobulinemia and is associated with a shorter time-to-transformation and inferior overall survival (6).

IF electrophoresis missing a monoclonal IgM κ was surprising, since IF electrophoresis is often used as gold standard for the detection of monoclonal proteins and for the evaluation of the performance of different CZE systems (7).

On the contrary, Szymanowicz et al described a case about a false negative CZE and IS electrophoresis with a Paragon CZE 2000 (Beckman Coulter, USA). In this case the Sebia Hydrasis system detected the monoclonal protein. But most of all, the alertness of the lab for rare, false negative CZE and/or IF electrophoresis was punctuated in literature (8, 9).

In conclusion, we demonstrated with this case that a negative IF electrophoresis and/or AGE does not exclude the presence of a monoclonal protein. The semi-automated electrophoresis system from Sebia may give problems with some rare serum samples containing an IgM monoclonal protein. Therefore, there is need for additional techniques in the evaluation of possible monoclonal proteins such as CZE, nephelometry and pretreatment of the sample with mercapto-ethanol. As a general recommendation, laboratory specialists that interpret CZE should be aware of potential interferences.

Takeaways

- A negative IF electrophoresis does not exclude the presence of a monoclonal protein.
- False-negative IF electrophoresis can cause failed detection of monoclonal proteins.
- Beside IF electrophoresis, additional techniques are needed in the evaluation of possible monoclonal proteins such as capillary zone electrophoresis and nephelometry.

Disclosure statement/ Declaration of interest

No potential conflict of interest was reported by the authors.

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Figure captions

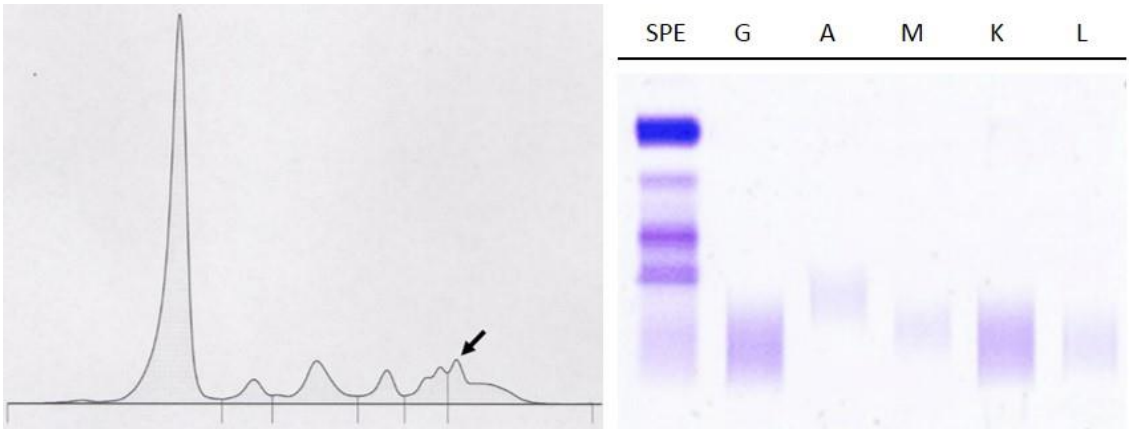

Figure 1. Left: Initial CZE of the patient's serum. The arrow indicates a marked additional fraction present between the β_2 and γ zone. Right: Routine IF electrophoresis of the patient's serum.

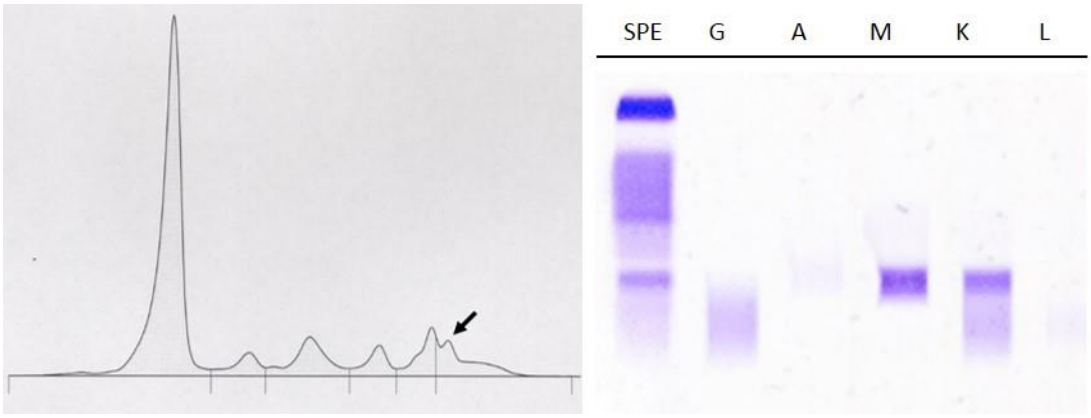

Figure 2. Left: CZE of the patient's serum approximately one year later with his next diabetes check-up. The arrow indicates a marked additional fraction present between the β_2 and γ zone. Right: Pretreatment of the serum with mercapto-ethanol before IF electrophoresis, revealing a monoclonal IgM κ.