Kappa free light chains presenting as an unusual fraction on serum immunofixation electrophoresis

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A 79-year old man presented at the emergency ward of our hospital with symptoms characteristic for multiple myeloma. Although serum capillary zone electrophoresis appeared normal, kappa free light chains were found in serum and in urine by immunofixation electrophoresis. The kappa free light chain fraction detected in the serum, albeit appeared as a polyclonal-like smear with an aberrant localisation in the alpha-2- and beta-regions. Subsequent high resolution gel electrophoresis of the serum suggested the presence of a monoclonal fraction in the alpha-2- and beta-regions, but quantification of the band by densitometry was still not possible. Quantification of free light chains in the patient's serum by immunonephelometry indicated the presence of an excessive amount of kappa free light chains. However, overestimation of the amount of kappa free light chains by the immunonephelometric method was suspected because of discrepancy with the intensity of the fractions on serum and urine immunofixation electrophoresis.
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Introduction

Serum capillary zone electrophoresis (CZE) is used for screening for monoclonal immunoglobulins when there is clinical suspicion of a proliferative plasma cell disorder.[1] Monoclonal immunoglobulins in plasma cell neoplasia can be constituted of the whole immunoglobulin or only the free light chain (FLC) part of the immunoglobulin. Since these FLCs are easily cleared from the plasma by the kidneys, serum CZE was found to be not sensitive enough for detecting monoclonal FLCs. Therefore, combining serum CZE with serum and urine immunofixation electrophoresis (IFE) is recommended when monoclonal immunoglobulin FLCs are suspected.[2] Immunoassays additionally allow to separately quantify kappa (κ) and lambda (λ) FLCs in serum and the ratio of κ over λ provides an indicator of clonality.[3]

Case report

A 79-year old male patient presented at the emergency service with lower back pain and anorexia. Analysis of the peripheral blood demonstrated macrocytic hyperchromic anaemia, mild thrombocytopenia and a normal leucocytosis with a population of 11% plasma cells (*Table 1*). Serum chemistry showed a normal total protein and albumin level, an impaired renal function, hypercalcemia, and a decreased folate status (*Table 1*). Serum CZE (Capillarys 2, Sebia, France) revealed a decreased albumin peak, a slight increase of the alpha-1 fraction and an increase of the alpha-2 fraction (*Figure 1A*), without, however, the presence of any abnormal morphology. These findings could be interpreted as the early phase of an acute inflammatory process.

Serum IFE (Hydragel 4 IF – Hydrasys, Sebia) displayed the abnormal presence of a smear in the alpha-2- and

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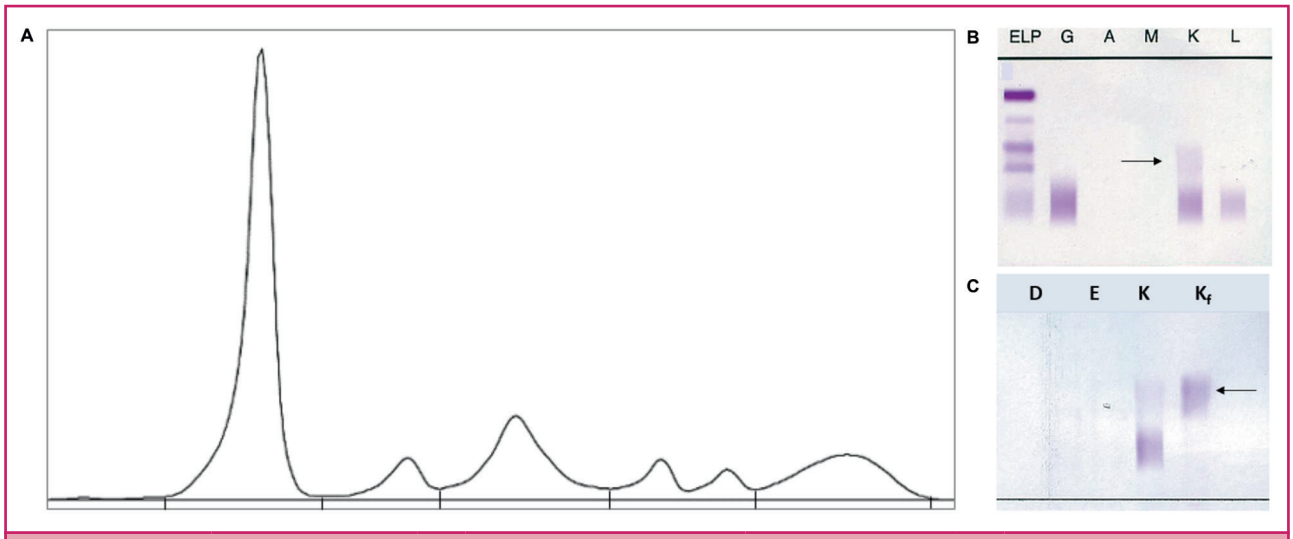
Table 1. Laboratory results at admission. Results outside the reference values are marked with an asterisk.

Parameter		Result		Reference range
Haematology	Haemoglobin	10.5 g/dL	*	14.0-18.0 g/dL
	Red blood cells	3.1 • 10^{12} cells/L	*	4.50-6.00 • 10^{12} cells/L
	MCV	102.9 fL	*	76.0-96.0 fL
	MCH	33.9 pg	*	27.0-32.0 pg
	MCHC	32.9 g/dL		3.0.-35.0 g/dL
	White blood cells	6.99 • 10^{9} cells/L		4.00-10.00 • 10^{9} cells/L
	Neutrophils	44.0%		35.0-77.0%
	Eosinophils	7.0%	*	≤ 6.0%
	Lymphocytes	31.0%		20.0-50.0%
	Monocytes	7.0%		2.0-10.0%
	Plasma cells	11.0%	*	≤ 0.0%
	Platelets	111 • 10^{9} cells/L	*	150-450 • 10^{9} cells/L
Chemistry	Total protein	68 g/L		66-87 g/L
	Albumin	36.8 g/L		35.0-52.0 g/L
	Creatinine	1.58 mg/dL	*	0.67-1.17 mg/dL
	eGFR	41 mL/min/1.73m^2		
	Calcium	3.04 mmol/L	*	2.15-2.55 mmol/L
	Phosphate	0.93 mmol/L		0.74-1.52 mmol/L
	Folic acid	4.3 µg/L	*	4.6-18.7 µg/L
	Vitamin B12	193 ng/L		191-663 ng/L
Immunology	IgG	10.90 g/L		7.51-15.60 g/L
	IgA	< 0.07 g/L	*	0.82-4.53 g/L
	IgM	0.17 g/L	*	0.46-3.04 g/L
	Kappa (κ) FLC	11,300.00 mg/L	*	3.30-19.40 mg/L
	Lambda (λ) FLC	10.50 mg/L		5.70-26.30 mg/L
	κ/λ ratio	1,076.19	*	0.26-1.65



	Patient (%)		Reference interval (%)	Patient (g/L)		Reference interval (g/L)
Albumin	51.4	*	55.8 - 66.1	35.2	*	40.2 - 47.6
α 1	6.1	*	2.9 - 4.9	4.2	*	2.1 - 3.5
α 2	18.3	*	7.1 - 11.8	12.5	*	5.1 - 8.5
β	9.4		8.4 - 13.1	6.4		6.0 - 9.4
γ	14.8		11.1 - 18.8	10.1		8.0 - 13.5

Figure 1. A. Capillary zone electrophoresis (CZE) of the patient's serum, demonstrating a decreased albumin peak, a slight increase of the alpha-1 fraction and an increase of the alpha-2 fraction. The table below the graph shows the quantitative data of the serum CZE and the reference intervals; the relative quantities of the five fractions are shown at the left side of the table, the absolute quantities (recalculated from the relative quantities and the total protein content of the serum, *cfr.* table 1) are shown at the right side of the table. Results outside the reference values are marked with an asterisk. **B.** Immunofixation electrophoresis (IFE) of the patient's serum. The first lane (ELP) shows the electrophoretic profile of the serum proteins, the second, third and fourth lanes show the IgG, IgA and IgM immunoglobulins, respectively, and the fifth and sixth lanes are stained for κ (K) and λ (L) light chains, respectively. The black arrow indicates a smear in the alpha-2- and beta-regions of the κ chains. **C.** Serum IFE with antibodies directed towards IgD, IgE, κ light chains and κ free light chains (FLCs) (D, E, K and Kf, respectively). The smear in the alpha-2- and beta-regions is composed of κ FLCs (black arrow).

beta-regions of the κ chains (*Figure 1B*, indicated by the black arrow). This smear was also detected when antibodies against κ free light chains (FLCs) were applied, suggesting the specific presence of an abnormal kappa FLC fraction in the alpha-2- and beta-regions (*Figure 1C*). Staining of the gel with antibodies directed towards IgD and IgE immunoglobulins was negative (*Figure 1C*). Quantification of the serum immunoglobulins indicated a normal IgG level but low IgA and IgM (*Table 1*). Urine IFE suggested the presence of monoclonal κ FLCs (*Figure 2*, indicated by the black arrow). The 24-h urine collection contained a total protein amount of 0.16 g (reference ≤ 0.15 g). The total amount of κ chains in the urine was 34 mg/L (reference ≤ 5 mg/L, Immage® Beckman Coulter Inc., United States of America) and the total amount of λ chains was < 15 mg/L (reference < 15 mg/L, Immage® Beckman Coulter Inc.). Quantification of the κ FLCs in the patient's serum with a kinetic immunonephelometric method (FLC reagent of The Binding Site, United Kingdom), by means of a BNII nephelometer (Siemens, Germany) indicated an excessive amount of κ FLCs and an increased κ/λ ratio (*Table 1*). In addition, antigen excess was reported for the quantification of the κ FLCs. In the end, only serum high resolution gel electrophoresis (HRE, Hydragel 7 HR - Hydrasys Focusing, Sebia) suggested a monoclonal

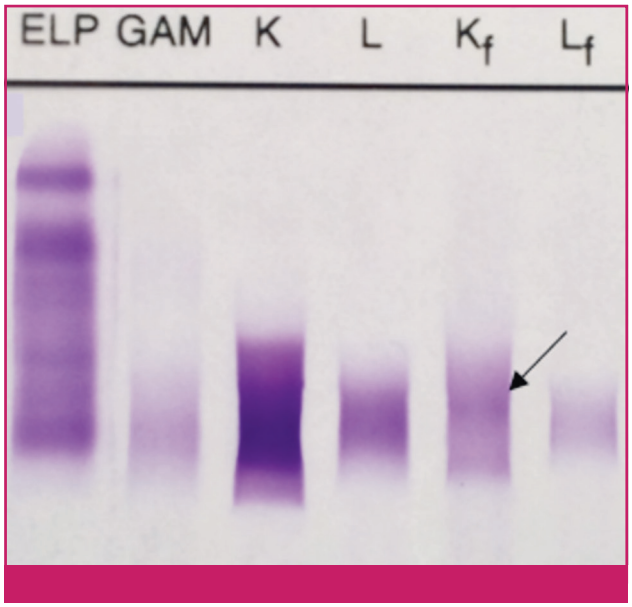


Figure 2. Urine immunofixation electrophoresis (IFE): The first lane shows the electrophoretic profile of the urine proteins (ELP), the second lane detects the γ, α and μ heavy chains (GAM), the third and fourth lane show the κ (K) and λ (L) chains, and the fifth and sixth lanes show the κ and λ free light chains (FLCs), respectively (Kf and Lf). Urine IFE suggests the presence of κ FLCs (black arrow).

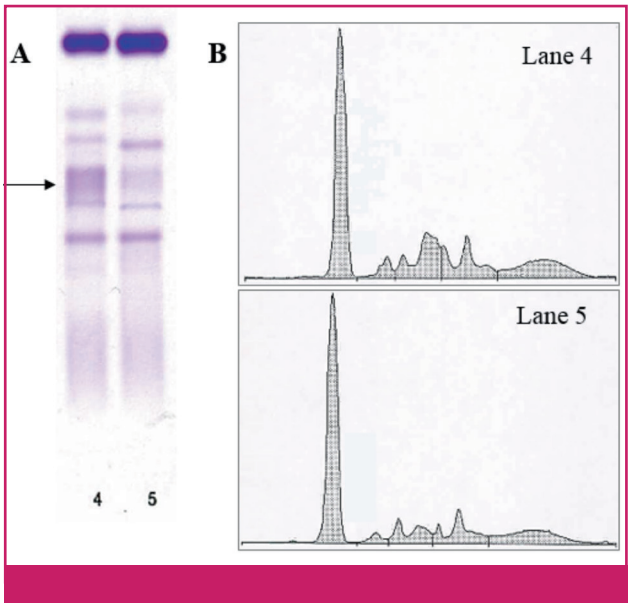


Figure 3. A. High resolution gel electrophoresis (HRE) of the patient's serum (lane 4) and a normal control (lane 5). A presumable monoclonal band was observed in the alpha-2- and beta-regions of lane 4 (black arrow). **B.** Densitogram of the HRE lanes shown in panel A of this figure. Lane 4; patient's serum, Lane 5; Normal control. The presumable monoclonal band is indicated with a black arrow.

fraction as evidenced from visual interpretation of the gel (*Figure 3*, indicated by the black arrow in *Figure 3A*). However, quantification of this monoclonal fraction by densitometry did not succeed because of overlay with another peak (*Figure 3B*).

Immunophenotypic analysis of the bone marrow indicated the presence of a large population of plasma cells (66.7%), and in the peripheral blood a population of 21% of plasma cells was found. The diagnosis of multiple myeloma, escalating to plasma cell leukaemia was accepted. Treatment with bortezomib, melphalan and prednisone resulted in a fast decline of the amount of plasma cells in the peripheral blood. Currently, the plasma cell count of the peripheral blood is negative and the amount of κ FLCs decreased to 29.90 mg/L (normal range: 3.30-19.40 mg/L) without the reporting of antigen excess and with a normalised κ/λ ratio of 1.38 (normal range: 0.26-1.65). The renal function improved [creatinine: 1.11 mg/dL (normal range: 0.67-1.17 g/dL)], the hypercalcemia decreased [calcium: 2.63 mmol/L (normal range: 2.15-2.55 mmol/L)], but the haematological parameters did not improve [red blood cell count: 3.19 • 10^{12} cells/L (normal range: 4.50-6.00 • 10^{12} cells/L), haemoglobin: 10.1 g/dL (normal range: 14.0-18.0 g/dL), and platelet count: 58 • 10^{9} cells/L (normal range: 150-450 • 10^{9} cells/L)].

Discussion

In the case described here, the authors present the diagnosis of a plasma cell neoplasia in which serum CZE did not display a monoclonal immunoglobulin fraction. Based on clinical findings and peripheral blood analysis a proliferative plasma cell disorder was suspected. According to the guidelines, serum and urine IFE were performed.[1] Urine IFE showed a monoclonal fraction of κ FLCs (*Figure 2*) but on serum IFE the fraction appeared as a polyclonal-like smear (*Figure 1B* and *C*). Only on serum HRE this smear could be interpreted as being a monoclonal fraction (*Figure 3*). Quantification of the κ FLCs in the patient's serum additionally showed the presence of a large amount of κ FLCs. The diagnosis of a plasma cell neoplasia was confirmed by bone marrow investigation and immunophenotypic analysis of the peripheral blood and bone marrow. Therapy was effective.

Remarkable is that the excessive amount of κ FLCs did not cause abnormalities on the serum CZE (*Figure 1A*). With urine IFE, however, a monoclonal fraction was found (*Figure 2*), confirming the higher sensitivity of

Key messages for clinical practice

1. When a proliferative plasma cell disorder is suspected on clinical grounds and serum capillary zone electrophoresis is normal, additional serum and urine immunofixation electrophoresis are necessary investigations.

2. One should be aware of the possibility of overestimation of the free light chain quantity when assayed by an immunonephelometric technique. In this case, the free light chain quantity cannot be used to predict prognosis or to follow-up the therapeutic response.

urine IFE for detecting monoclonal FLCs.[2,4] On serum IFE the aberrant fraction appeared as a smear instead of a monoclonal band (*Figure 1B* and *C*). This could be explained by polymerisation of the FLCs, although this hypothesis was not confirmed here.[3] Additional serum HRE suggested the presence of a monoclonal fraction (*Figure 3*), which indicates the added value of serum HRE for the detection of monoclonal immunoglobulin FLCs in serum.

Despite the rather small abnormalities in serum IFE, quantification of the κ FLCs by immunonephelometry indicated an excessive amount with antigen excess being reported. The discrepancy might result from a possible overestimation of the amount of κ FLCs by the immunonephelometric method, because of the suspected polymerisation of the FLCs.[5]
Another remark is the different staining intensity of the κ FLC-fraction on serum IFE when stained with antibodies directed towards κ FLCs (Lane Kf in *Figure 1C*) as compared to the staining intensity with antibodies directed towards the total κ light chains (Lane K in *Figure 1C*). This observation can be explained by an increased immunoreactivity of the 'detection antibody' towards polymerised FLCs.[5,6]

Serum and urine IFE are necessary investigations when plasma cell neoplasia is suspected, even when serum CZE does not show abnormalities. This is in accordance with the guidelines of the International Myeloma Working Group.[1] Moreover, the phenomenon of polymerisation might explain the 'normal' result of the serum CZE, the smear on the serum IFE and the possible overestimation of the amount of κ FLCs by immunonephelometry.

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