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Screening for M-proteinemia: serum protein electrophoresis and free light chains compared

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Abstract

Background: The objective of this study was to evaluate the efficiency of free light chain (FLC) analysis in comparison to serum protein electrophoresis (SPE) for detecting M-proteinemia.

Methods: A total of 553 consecutive patients for whom evaluation of M-proteinemia was requested were included in this study. For all patients, serum FLC analysis and SPE followed by pentavalent immunofixation analysis was performed. Identification of monoclonal bands was performed using specific antisera. FLC analysis was performed using the Modular P analyzer in accordance with the manufacturer's recommendations. Local reference ranges for FLCs on this platform were established based on samples from patients with a normal electrophoretic pattern [no monoclonal bands, no hypo- or hypergammaglobulinemia, no acute phase pattern and normal kidney function, i.e., estimated glomerular filtration rate (eGFR) >60 mL/min].

Results: Local reference ranges (95%) were established ($n=243$): κ: 8.01–28.26 mg/L; λ: 8.07–23.58 mg/L and κ/λ ratio: 0.74–1.66. Negative and positive predictive values were 98.6% and 49.5%, respectively, for screening for M-proteinemia by SPE alone, 94.3% and 21.7% for FLC concentration and 95.1% and 21.4% for FLC with the κ/λ-ratio included. Combining protein electrophoresis and FLCs resulted in a negative predictive value of 99.0% and a positive predictive value of 23.4%.

Conclusions: Serum FLC analysis alone is not suitable for screening for M-proteinemia.

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Keywords: free light chain assay; immunofixation electrophoresis; monoclonal gammopathy; M-proteinemia; multiple myeloma; serum protein electrophoresis.

Introduction

Monoclonal gammopathies are characterized by clonal expansion of B cells that secrete intact monoclonal immunoglobulins, monoclonal light or heavy chains, or both. Detection of the monoclonal immunoglobulin or its fragments plays a central role in the diagnosis of monoclonal gammopathies (1–3). Because of their large size which precludes glomerular filtration, unless there is renal damage, intact immunoglobulins are generally found in the blood. In contrast, light chains are readily filtered as monomers or dimers in the glomerulus, absorbed in the proximal tubule and catabolized within the tubular cells (4). Therefore, light chains will appear in the urine in amounts sufficient to be detected by conventional methods once the tubular absorption mechanism is saturated. In patients with normal renal function, light chains might not be detected in the blood by conventional methods (5).

For the investigation of patients suspected of having monoclonal gammopathy, current recommendations advise serum protein electrophoresis (SPE) followed by immunofixation electrophoresis (IFE) using both serum and urine (6, 7). However, such a sequalae of investigations is laborious and time-consuming, especially when analysis is performed in batch mode. The development of turbidimetric or nephelometric assays for free light chains (FLCs) makes daily processing with use of automated chemistry analyzers possible (8). Excessive synthesis of one type of FLC may occur in monoclonal gammopathy, not only in FLC gammopathy (9) but often in intact immunoglobulin gammopathy also (10–12). In addition, the FLC assay is not only more sensitive than SPE and IFE for the detection of light chain disease, but also in revealing amyloidosis (13) and non-secretory myeloma (10). Most published studies have used the serum FLC assays in addition to SPE and IFE (14–20), or in combination with clinical information (21). Interestingly, after reading the paper by Abadie and Bankson (22), one might conclude that the FLC assay also could be used for screening of monoclonal gammopathy since the FLC assay had better positive and negative predictive values compared to SPE. Therefore, we investigated whether the FLC assay is, in addition to being a more rapid screening tool, a more sensitive screening tool for the detection of M-proteinemia compared with SPE alone. If correct, the FLC assay would allow a more rapid initial screening than the present procedure with the combination of SPE and IFE.

Patients and methods

Analytical methods

SPE was performed using the SAS-3-SP-60 SB kit (prod no.: 300200, Helena Biosciences, Tyne and Wear, UK) with the SPIFE 2000 (Helena Biosciences). This method is a split-β method since due to the use of a high resolution gel, two

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β-bands are obtained. IFE was performed using the SAS-3 urine analysis kit (prod no.: 300400 and 321300, Helena Biosciences) for the immunofixation analysis with pentavalent antiserum and the SAS-IFE-9 kit (prod no.: 300300 en 300301, Helena Biosciences) for immunofixation analysis with the specific antisera. All types of IFE were performed with the SPIFE 2000. FLC analysis was performed using the Modular P (Roche Diagnostics, Mannheim, Germany) and the Freelite κ and λ kits (prod no.: LK016.H en LK018.H) supplied by The Binding Site (Birmingham, UK). All analyses were performed according to the manufacturer's recommendations.

Reference values

For the FLC assays, the reference ranges suggested by the supplier were derived from a study by Katzmann et al. (23). However, the reference ranges have been shown to be dependent on the platform used (24, 25). To establish local reference values for the FLC assays, we selected samples from patients with a normal pattern assessed with SPE, and the absence of monoclonal bands in the IFE with pentavalent antiserum. FLCs may be increased by increased production due to stimulation of the immune system by infection or autoimmune disease, or by reduced elimination due to renal disease (4). In contrast, suppression of the immune system might lead to decreased FLC concentrations. Therefore, in addition to samples suspicious for the presence of monoclonal bands following SPE and IFE, samples with hypo- or hypergammaglobulinemia (gammaglobulin <6 g/L or >16 g/L), an acute phase pattern [α_1-globulin band >3 g/L or a C-reactive protein (CRP) >10 mg/L] and renal dysfunction [estimated glomerular filtration rate (eGFR) <60 mL/min according to the isotope dilution mass spectrometry (IDMS) traceble 4-variable modification of diet in renal disease (MDRD) equation] (26, 27) were excluded for establishing reference values. According to International Federation of Clinical Chemistry (IFCC) recommendations, we made no assumptions about sample distribution and used a non-parametric method for establishing the 95% reference interval (28).

Patients and samples

Serum samples (SST-tubes, prod no.: 367955, Becton Dickinson, Rutherford, NJ, USA) from 553 successive patients with a request for exclusion/detection of M-proteinemia were collected. Serum for FLC analysis and protein electrophoresis/IFE was frozen at –20°C until analysis. Samples from patients known to have M-proteinemia according to the laboratory's records were excluded.

Procedures

The efficacy of the M-protein screening was considered for four procedures. The first M-protein screening procedure was based on SPE alone. Results were considered positive in cases with suspicion of monoclonal bands, deviations in the pattern of the β-bands and hypogammaglobulinemia (<6 g/L). In these cases, SPE was followed by immunofixation analysis. For M-protein screening based on FLCs, we considered the procedure based on the results for FLC concentrations alone, and that based on the combination of FLC concentrations with the κ/λ-ratio as separate procedures. For the fourth procedure, SPE was combined with FLC analysis (concentration+κ/λ-ratio). All these screening procedures were compared with the results of the (''gold'') standard procedure consisting of the combination of SPE and IFE with pentavalent antiserum.

Statistical methods

Sensitivity, specificity, positive predictive value and negative predictive value were calculated to determine the diagnostic significance of the four screening procedures. The result of the SPE and immunofixation analysis with the pentavalent antiserum was used as the reference indicator (gold standard).

Results

Reference ranges for FLCs were established from results obtained using patient samples with a normal pattern for SPE and IFE, as described in the Methods section. After excluding samples from patients with signs of infection (increased CRP/hypergammaglobulinemia/clinical data), renal insufficiency (eGFR <60 mL/min) or suspicion of M-proteinemia, 243 samples were eligible for evaluation of the reference range. $P_{0.5\%}$, $P_{2.5\%}$, $P_{97.5\%}$ and $P_{99.5\%}$ (95% and 99% range) were calculated for the concentrations of κ and λ FLCs as well as for the κ/λ-ratio. The results are shown in Table 1 together with the reference ranges suggested by the manufacturer.

Comparison studies

A total of 553 successive serum samples with requests for detection/exclusion of serum M-proteinemia were studied. The results for free κ and λ concentrations in samples from patients with renal insufficiency, signs of infection and M-proteinemia are shown separately in Figure 1. As expected, patients with renal insufficiency or with signs of infection often did show increased concentrations of free κ and λ, with essentially normal κ/λ-ratios. In patients with monoclonal immunoglobulins one would expect abnormal free κ and λ concentrations or abnormal κ/λ-ratios in the majority of samples. However, Figure 1C shows that a substantial amount of the cases had values within the reference range. The results for the four screening procedures are displayed in Table 2. Since SPE and immunofixation analysis do not reveal all the bands as being unambiguous, we divided the samples into groups with obvious bands that can be quantified by electrophoresis together with the faint bands that proved to come from light chains or immunoglobulin M, and groups with faint bands that

Table 1 Locally established reference ranges and manufacturer's reference ranges for free κ and free λ chains and the κ/λ ratio.

Limit	κ light chain, mg/L	λ light chain, mg/L	κ/λ ratio
Local ranges			
$P_{0.5\%}$	7.36	7.24	0.64
$P_{2.5\%}$	8.01	8.07	0.74
$P_{97.5\%}$	28.26	23.58	1.66
$P_{99.5\%}$	34.66	32.18	1.94
Supplier's ranges			
$P_{2.5\%}$	3.3	5.7	0.31
$P_{97.5\%}$	19.4	26.3	1.20

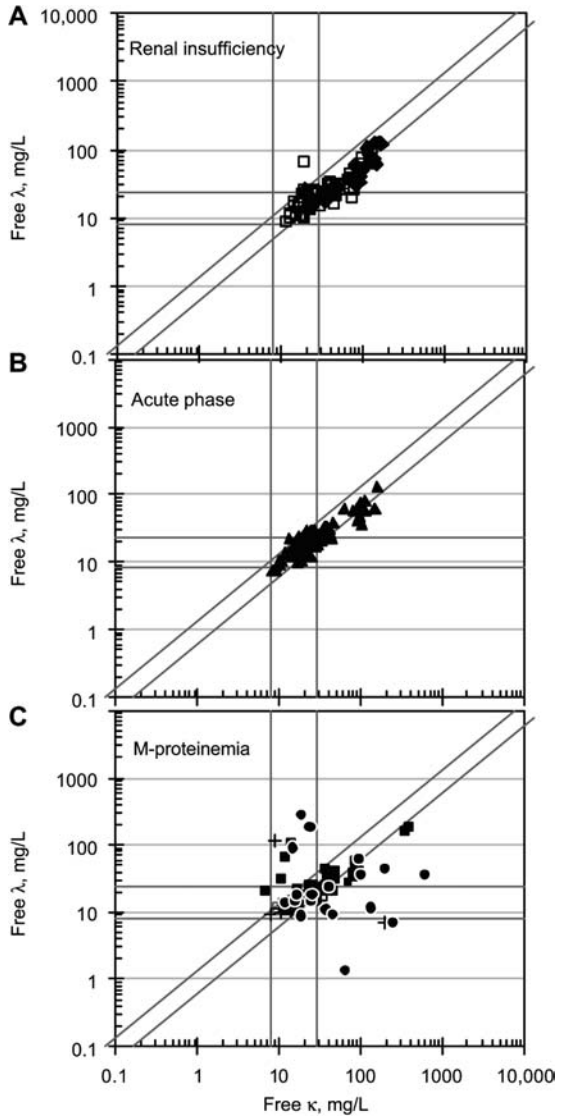

Figure 1 Free κ and λ light chain concentrations.
In sera from patients with (A) different grades of renal insufficiency (defined by eGFR according to the MDRD calculation; ♦, <30 mL/min and □, 30–60 mL/min), (B) signs of infections (acute phase pattern or hypergammaglobulinemia in serum protein electrophoresis or an elevated CRP), and (C) various grades of M-proteinemia (♦, M-proteinemia with concentration <3 g/L and ●, M-proteinemia with concentration >3 g/L) or high suspicion of M-proteinemia (+, hypogammaglobulinemia).

cannot be quantified with protein electrophoresis. From the results presented in Table 2, sensitivity, specificity and positive and negative predictive values were calculated for the four screening procedures (Table 3). The difference between clear and faint bands was used for separate calculations.

Discussion

Measurement of FLCs in serum has been shown to be useful for the diagnosis and monitoring of light chain myeloma, non-secretory myeloma and amyloidosis. In addition to the possible use of FLC analysis for the diagnosis of M-proteinemia, its use is recommended by the International Myeloma Working Group for the monitoring of serum M-proteins (29). Since we were intrigued by the potential use of the FLC analysis alone as a sensitive and rapid screening tool for M-proteinemia, as suggested in the paper from Abadie and Bankson (22), we studied the efficiency of the FLCs assay as a screening tool. It has been reported previously that reference values might be platform dependent (24, 25). Thus, as a first step, we established local reference values using our Modular P analyzer. The reference ranges suggested by the supplier were based on healthy donors in whom the presence of an M-spike was excluded by SPE and immunofixation analysis (23). Since healthy donors usually are not comparable to the diseased patients our measurements are used for (e.g., with respect to age and disease state), we, like Pattenden et al. (25), established our reference ranges for FLCs using samples from local patients without M-proteinemia. Conceptually, FLC concentrations in blood are determined by the equilibrium between production and elimination. FLC concentrations are increased by increased production as a consequence B-cell dyscrasias as well as by stimulation of the immune system due to infection or autoimmune disease (illustrated in Figure 1B). Also, decreased elimination due to renal disease results in increased FLC concentrations; the degree of renal failure determines the extent of increase in FLCs (Figure 1A). Therefore, samples from patients with renal insufficiency, as established using an eGFR <60 mL/min, and signs of infection as estimated by the acute phase pattern in protein electrophoresis or an increased CRP were excluded for the determination of the reference range for FLCs. Our local reference ranges for FLC concentrations are a little higher than those published by Katzmann et al. (23). The major difference is that we used selected patients while Katzmann used healthy donors. Since Katzmann also established slightly higher values in patients with greater age, age might be the reason for the slightly higher ranges we found. On the contrary, Pattenden et al. (25) used patients for establishing reference values on two different platforms. They excluded patients with M-proteinemia and renal insufficiency and found different reference values using the two platforms they used. Thus, the platform used may be a reason for differences in reference values too. Since our concentration range for the free light concentrations starts at a higher level than that described in the studies described earlier, we conclude that platform differences probably play a major role in the observed differences. However, because our reference range has a tighter range than that reported by Pattenden, we conclude that for the establishment of reference values for FLCs, patients as well as apparently healthy donors with increased production or diminished excretion must be excluded carefully.

The main issue examined in this study was whether the FLC assay allows for more sensitive and rapid screening of M-proteinemia compared with SPE. Since SPE is usually performed in batch mode, it is time consuming. This effect is intensified by the

Table 2 Results of four procedures for screening for M-proteinemia using serum protein electrophoresis and the free light chains assay (κ/λ-ratio included as well as excluded) compared to the results according to the reference procedure (gray colored boxes; serum protein electrophoresis combined with immunofixation analysis).

Procedure	Number (%)		Number (%)	
Serum protein electrophoresis alone	Neg.: 442 (80)		Pos.: 111 (20)	
	Neg.: 436 (79)	Pos.: 6 (1.1) Clear bands[a]: 2 Faint bands[a]: 4	Neg.: 56 (10)	Pos.: 55 (9.9) Clear bands[a]: 36 Faint bands[a]: 19
Free light chains (concentration) alone	Neg.: 369 (68)		Pos.: 184 (32)	
	Neg.: 348 (63)	Pos.: 21 (3.8) Clear bands[a]: 11 Faint bands[a]: 10	Neg: 144 (26)	Pos: 40 (7.2) Clear bands[a]: 27 Faint bands[a]: 13
Free light chains (concentration + ratio)	Neg.: 348 (63)		Pos.: 205 (37)	
	Neg.: 331 (60)	Pos.: 17 (3.1) Clear bands[a]: 7 Faint bands[a]: 10	Neg.: 161 (29)	Pos.: 44 (8.0) Clear bands[a]: 31 Faint bands[a]: 13
Serum protein electrophoresis + free light chains (concentration + ratio)	Neg.: 306 (55)		Pos.: 247 (45)	
	Neg.: 303 (55)	Pos.: 3 (0.5) Clear bands[a]: 0 Faint bands[a]: 3	Neg.: 189 (34)	Pos.: 58 (10.5) Clear bands[a]: 38 Faint bands[a]: 20

[a]Monoclonal bands were divided into clear bands, where concentration can usually be quantitated from the serum protein electrophoresis (exceptions are light chain bands and part of the bands from the immunoglobulin M-type), and faint bands, where concentrations can never be quantitated from serum protein electrophoresis using densitometry.

Table 3 Sensitivity, specificity and negative and positive predictive values for the 4 screening procedures for the detection/exclusion of M-proteinemia (numbers between parentheses are the results for the clear bands only).

Procedure	Sensitivity, %	Specificity, %	Negative predictive value, %	Positive predictive value, %
Serum protein electrophoresis alone	90.1 (94.7)	88.6 (85.4)	98.6 (99.5)	49.5 (32.4)
Free light chains (concentration) alone	65.6 (71.1)	70.7 (69.5)	94.3 (97.0)	21.7 (14.7)
Free light chains (concentration + ratio)	72.8 (81.5)	67.3 (66.2)	95.1 (97.1)	21.4 (15.1)
Serum protein electrophoresis + free light chains (concentration + ratio)	95.0 (100)	61.6 (59.4)	99.0 (100)	23.5 (15.3)

sequential analysis of protein electrophoresis and IFE. However, FLC analysis enables for more rapid analysis since it can be automated easily using routine chemistry analyzers. In addition, the FLC analysis is much more sensitive than SPE for FLC M-proteinemia as well as intact M-proteinemia. These two characteristics favor FLC analysis over SPE as a screening tool for the detection/exclusion of M-proteinemia. According to Abadie and Bankson (22), the positive and negative predictive value of the FLC assay (negative and positive predictive values: 98% and 88%) was better than that of SPE alone (negative and positive predictive values: 94% and 35%). However, in our study, the results for SPE alone (negative and positive predictive values: 98.6% and 49.5%) are considerably better than those for the serum FLC concentrations alone (negative and positive predictive values: 94.3% and 21.7%) as well as for the κ/λ-ratio included (negative and positive predictive values: 95.11% and 21.4%). Combination of the FLC assay and SPE resulted in a negative and positive predictive value of 99.0% and 23.5% (Abadie and Bankson: 100% and 89%). Thus, in our hands, FLC analysis proved to be less suitable as a screening tool for the detection/exclusion of M-proteinemia compared with SPE. Exclusion of the small faint bands does not change these results. One can only speculate on the reasons for these discordant results. According to the method description, Abadie and Bankson used an electrophoretic method with a limited resolution. This resulted in only one β-band, whereas we used high resolution separation with two β-bands. Another difference might be due to differences in data interpretation. Abadie and Bankson (22) reported a considerable number of patients as being false positives or MGUS based on protein electrophoresis. Apparently, they used the clinical diagnosis as the gold standard. In our study, we considered the presence of a monoclonal band as determined by protein electrophoresis and immunofixation analysis with pentavalent antiserum to be the gold standard. In our procedure, small monoclonal bands were included in the true positive group. However, Abadie and Bankson considered such samples to be false positives. Combining SPE and the FLC assay did exclude monoclonal bands of any significance. This result is in accordance with the results of Abadie and Bankson. Unfortunately, this excellent negative predictive value was obtained at the expense of a significant number of false positive results.

It was our objective to examine the usefulness of the FLC assay as a screening procedure for the detection/exclusion of monoclonal bands. We conclude

that the FLC assay could not replace SPE in the screening for monoclonal proteins.

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