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Serum free light chain immunoassay as an adjunct to serum protein electrophoresis and immunofixation electrophoresis in the detection of multiple myeloma and other B-cell malignancies

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Abstract

Background: Protein and immunofixation electrophoresis of serum and urine are established as diagnostic aids for identifying monoclonal gammopathies. However, many patient sera sent to laboratories are not accompanied by urine samples and recent reports suggest the use of serum free light chain (sFLC) analysis in combination with serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE) could eliminate the need for urinalysis. The aim of the study was to assess the utility of sFLC measurement in addition to serum protein electrophoresis in the identification of patients with B-cell malignancies.
Methods: A total of 952 serum samples were analysed by serum protein electrophoresis and those with abnormal bands were analysed by immunofixation. sFLCs were measured in a retrospective manner by automated assay.
Results: In our study of 952 patient sera, it was found that FLC analysis identified 23 additional cases of B-cell malignancies which were missed by SPE.
Conclusions: The additional malignancies identified by sFLC analysis add support for its inclusion in the routine screening protocol for B-cell malignancies.
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Keywords: immunofixation electrophoresis (IFE); monoclonal gammopathy; multiple myeloma; serum free light chain assay; serum protein electrophoresis (SPE).

Introduction

Multiple myeloma comprises approximately 10% of all haematological neoplasms and is characterised by the clonal expansion of plasma cells expressing monoclonal immunoglobulins. Tumours producing only monoclonal free light chain (FLC) account for approximately 15%–20% of all multiple myeloma diagnoses. Screening of the monoclonal proteins, using protein electrophoresis plus immunofixation electrophoresis (IFE), is an established aid in the diagnosis of multiple myeloma. Serum protein electrophoresis (SPE) has a low sensitivity (500–2000 mg/L) for the detection of FLC and urine electrophoresis immunofixation [sensitivity of approximately 10 mg/L (1)] is preferred. However, the majority of sera sent to laboratories for the investigation of potential B-cell malignancies are not accompanied by urine samples (2); therefore, it is not possible to complete the diagnosis. Furthermore, co-migration of FLC and other proteins in concentrated urine samples can make accurate diagnosis difficult. Nephelometric and immunoturbidimetric assays for the measurement of FLC in serum have been developed (3) and have been shown to be useful in the identification of nonsecretory myeloma, light chain only myeloma, AL amyloidosis and other lymphoproliferative disorders (3–7). Recent reports have indicated that use of serum FLC assays in addition to SPE and immunofixation can eliminate the requirement for urinalysis at diagnosis (2, 8). The objective of this study was to assess whether the use of serum FLC measurement, in addition to SPE, would help to identify additional B-cell malignancies amongst patients whose serum samples had been sent for investigation.

Materials and methods

Serum samples from 952 patients (mean age: 60 years) referred to the Laboratory of Biochemistry (Hôpital Haut-Lévêque, Centre Hospitalier Universitaire de Bordeaux) for investigation of potential B-cell malignancies were studied. SPE and IFE (γ, α, μ, κ and λ) were performed using an agarose electrophoresis system (Spife 3000; Helena; Hydrasys, SEBIA, Norcross, USA) in accordance with the manufacturer's instructions. Serum FLC measurements were made retrospectively on all samples (Modular P; Roche, Basel, Switzerland) using previously described assays (3) and the ratio of free κ to free λ light chains was calculated. Serum immunofixation was routinely performed when there was evidence of a monoclonal band, bands exhibited restricted migration or samples demonstrated unexplained hypogammaglobulinaemia. Immunofixation was also carried out in the absence of these three criteria if the measured κ/λ ratio was outside the published normal range of 0.26–1.65 (9). Analysis of the serum FLC measurements was made approximately 24 months after the initial investigations of the sera. Further clinical data were requested when FLC results iden-

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tified a potential B-cell malignancy which had not been indicated by the initial SPE analysis.

Results

In practice, it was not possible to separate samples from previously diagnosed patients and those from new referrals and results from both groups were included in the analysis. From SPE analysis, 111/952 sera were identified as having abnormal patterns, potentially indicating a B-cell malignancy. Out of these 111 sera, 83 were subsequently found to have monoclonal bands by immunofixation and 75/111 had abnormal FLC ratios. Out of 952 samples, 841 had a normal appearance by SPE of which 57/841 had abnormal FLC ratios (Figure 1). Immunofixation confirmed the presence of monoclonal proteins in 23/57 sera. Examination of the clinical records for the SPE-normal/FLC-abnormal patients revealed that 10/23 of the IFE-positive patients had a relevant clinical diagnosis (Table 1). Of the 34 samples with abnormal FLC ratios but normal by IFE, 13/34 had a relevant clinical diagnosis (Table 2). A total of 33 out of 57 patients with normal SPE but abnormal FLC results were lost to follow-up and the presence or absence of a clinically relevant diagnosis could not be determined, and 1/57 patients had no relevant clinical diagnosis.

Table 1 Serum protein electrophoresis negative, immunofixation positive patient samples with abnormal serum free light chain ratios.

	IFE positive	κ, mg/L	λ, mg/L	Ratio
AL amyloidosis	1	3.24	23.75	0.136
LC multiple myeloma	4	19.65	1.86	10.565
		1.5	350.88	0.004
		9.59	324.96	0.030
		1.5	32.58	0.046
Multiple myeloma	4	21.43	6.08	3.525
		2297.75	7.73	297.251
		13.03	2.75	4.738
		5.65	173.46	0.033
MGUS	1	27.48	11.21	2.451

AL, amyloid light chain; LC, light chain; MGUS, monoclonal gammopathy of undetermined significance.

Table 2 Serum protein electrophoresis negative, immunofixation negative patient samples with abnormal serum free light chain ratios.

	IFE negative	κ, mg/L	λ, mg/L	Ratio
Acute lymphoblastic	2	5.54	2.78	1.993
leukaemia		1.71	8.11	0.211
Chronic lymphocytic	4	3.19	15.62	0.204
leukaemia		8.34	1.5	5.560
		3.11	16.37	0.190
		17.45	9.37	1.862
LC multiple myeloma	3	40.83	3.18	12.840
		9.15	2.89	3.166
		178.09	6.64	26.821
Multiple myeloma	1	6.04	31.68	0.191
MGUS	1	2.49	1.5	1.660
Lymphoma	2	7	2.83	2.473
		1.5	7.44	0.202

LC, light chain; MGUS, monoclonal gammopathy of undetermined significance.

Discussion

When sera are referred for investigation of potential lymphoproliferative disorders, accompanying urine samples may be provided in less than 40% of cases (2). The standard laboratory protocol for multiple myeloma diagnosis is therefore limited to SPE and subsequent serum immunofixation. In this study, utilising SPE alone, 83/952 patients were found to have serum abnormalities which were confirmed by IFE. However, an additional 23 patients, subsequently found to have B-cell malignancies, would have been identified by serum FLC analysis of their initial serum samples. A new reference range to account for increased κ/λ ratios in response to deteriorating renal function has been proposed (10). Applying this range

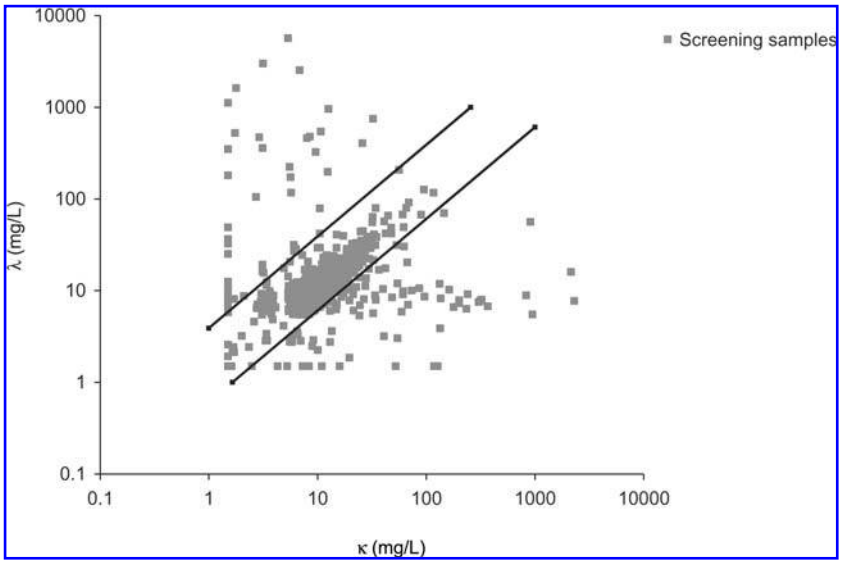

Figure 1 Serum free light chain measurements in 952 patient samples compared to the published κ/λ ratio normal range (9) shown by the parallel lines.

to the 57 patients negative by SPE with abnormal serum FLC ratios, all patients with significant monoclonal disease would have been identified (data not shown). In total, 33 patients with an abnormal FLC result were lost to follow-up, of which 13 had a monoclonal band by IFE. It is proposed that in the absence of symptoms requiring intervention it is unlikely that there was an underlying malignancy for these patients. The data in Tables 1 and 2 show that light chain only multiple myeloma was the monoclonal gammopathy most frequently missed by SPE, which is in agreement with previous reports (11). While monoclonal immunoglobulin secretion is most commonly associated with plasma cell dyscrasias, it is also known to occur in other B-cell malignancies (12, 13). Of the 952 patients studied here, nine SPE-negative/FLC abnormal patients were diagnosed with tumours of immature B lymphocytes.

In conclusion, the additional abnormalities identified by serum FLC analysis add support for the inclusion of FLC measurement in the routine screening protocol for B-cell malignancies.

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