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Serum Free Light Chains: An Alternative Test to Urine Bence Jones Proteins When Screening for Monoclonal Gammopathies

Peter G. Hill,[1] Julia M. Forsyth,[1]* Baldeep Rai,[1] and Stewart Mayne[2]

Background: **Retrospective analyses have established the role of quantitative serum free light chains (FLCs) in the diagnosis of monoclonal light chain disorders. The aims of this study were to assess (*a*) whether the addition of serum FLCs to serum protein electrophoresis (SPEP) identified additional patients with monoclonal gammopathies; (*b*) whether serum FLC measurements could replace urinalysis for Bence Jones protein (BJP); and (*c*) the cost/quality implications of routinely measuring serum FLCs.**

Methods: **Serum FLCs were added to consecutive requests for SPEP from August to November 2004 and measured by automated immunoassay.**

Results: **Seventy-one of 923 patients had abnormal serum FLC ratios. Seven patients with monoclonal gammopathies and 1 patient with malignant lymphoma (but no monoclonal band) were detected among 43 patients with negative SPEP but positive serum FLC ratios. Thirty-five patients with negative SPEP had false-positive serum FLC ratios. The false-positive rate for a ratio >1.65 was higher than previously described and associated with polyclonal increases in immunoglobulins and renal impairment. Serum FLC ratios were normal in 2 of 13 patients with low-level persistent urine BJP. However, no significant pathology would have been missed by replacing BJP with serum FLCs. Revenue and manpower savings offset 60% of the costs of serum FLCs.**

Conclusions: **Additional diagnostic information is gained by adding serum FLCs to SPEP as first-line tests for investigating possible B-cell disorders. The quality of the diagnostic service is enhanced by more confident exclusion of light chain disorders and improved interpretive assessment of SPEP and immunofixation electrophoresis.**

Initial laboratory investigations for possible disorders of B-cell lineage currently require both urine and serum samples *(1)*. B-cell disorders cannot be confidently excluded without urine samples by existing methods because light chain–only and nonsecretory multiple myelomas account for up to 15–20% of new diagnoses. In many of these, the serum light chain concentrations are below the detection limits of conventional immunofixation methods. However, most laboratories find it difficult to obtain both serum and urine samples from patients. In Derbyshire Royal Infirmary, despite publicity from the laboratory, concurrent urine samples are received from <40% of patients.

Automated immunoassays measuring serum free light chains (FLCs)[3] have been developed *(2)*, and their role in detecting and monitoring patients with light chain multiple myeloma, nonsecretory multiple myeloma and primary amyloidosis has recently been reviewed *(3)*. Retrospective studies have shown that serum FLC assays are more sensitive than serum protein electrophoresis (SPEP) or urine protein electrophoresis (UPEP) for the detection of these 3 diseases. Serum FLCs cannot replace SPEP in a screening protocol for monoclonal gammopathies, as they are slightly less sensitive than SPEP for intact immunoglobulin multiple myeloma *(4)*. However, their higher limit of detection for FLC, together with the problem of achieving a high percentage of concurrent urine samples, suggests that serum FLCs, as an alternative to urinalysis

Departments of [1] Chemical Pathology and [2] Clinical Haematology, Derbyshire Royal Infirmary, Derby Hospitals NHS Foundation Trust, Derby, United Kingdom.

*Address correspondence to this author at: Chemical Pathology Department, Derbyshire Royal Infirmary, London Rd., Derby DE1 2QY, United Kingdom. Fax 01332-254864; julia.forsyth@derbyhospitals.nhs.uk.

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[3] Nonstandard abbreviations: FLC, free light chain; SPEP, serum protein electrophoresis; UPEP, urine protein electrophoresis; BJP, Bence Jones protein; IFE, immunofixation electrophoresis; rGlob, raised globulins; pIgs, polyclonal increase in the gamma-globulin region; MGUS, monoclonal gammopathy of undetermined significance; PPV, positive predictive value.

and in addition to SPEP, might improve the detection of monoclonal gammopathies.

A recent prospective study, on serum samples only, showed that additional patients with B-cell disorders were identified when serum FLC measurement was used with capillary zone electrophoresis *(5)*. Our objectives, in a prospective study with consecutive samples received for SPEP, were to evaluate (*a*) whether the addition of serum FLCs identified extra patients with monoclonal gammopathies at the time of a first request for SPEP; (*b*) whether serum FLCs could replace urinalysis for Bence Jones protein (BJP); and (*c*) the cost and quality implications of routinely measuring serum FLCs.

Materials and Methods

Serum FLC measurement was added to all requests for SPEP received from primary care or hospital sources from August 1 to November 30, 2004. Samples from patients previously diagnosed as myeloma, Waldenstrom's macroglobulinaemia, and lymphoma were excluded. The study received approval from the local ethics committee.

At report authorization, additional requests for SPEP were generated when serum globulins were >48 g/L (excluding chronic liver disease, rheumatoid arthritis, and cases in which SPEP had been carried out in the preceding 6 months) or when serum globulins were <27 g/L. Serum FLCs were also measured on these samples.

κ and λ serum FLC concentrations were measured quantitatively by immunoturbidimetry (The Binding Site) on a Roche modular analyzer (coefficient of variation for between batch precision: κ = 8%, λ = 3%). SPEP, UPEP, and immunofixation electrophoresis (IFE) were carried out with the Hydrasys system (Sebia). All gels were interpreted by experienced clinical scientists. Serum IFE was routinely performed when a monoclonal band (or suspicion of a band) was seen on SPEP and in all samples demonstrating hypogammaglobulinaemia. For such samples, the SPEP gels were interpreted without reference to the serum FLC results. Serum IFE was carried out in the absence of a band (or suspicion of a band) by SPEP when the serum FLC κ/λ ratios were outside the normal range of 0.26–1.65 *(6)*. When concurrent urine samples were available, urine total protein was measured (Benzethonium chloride, Roche modular analyzer, or 917 analyzer) followed by UPEP (Sebia Hydragel β1-β2 15/30 gels, HR3 program and acid violet stain); unconcentrated urine was used on the basis of recent recommendations *(7)*. This system has a detection limit of ~10 mg of albumin per liter; an albumin calibrator (12 mg/L) was included in each gel to confirm satisfactory detection limit. This was followed by IFE to confirm the presence of BJP when the urine sample was not clearly negative by UPEP or when a monoclonal band or hypogammaglobulinaemia were noted in the serum.

SPEP gels were interpreted visually and categorized as either (*a*) no abnormality detected; (*b*) raised globulins (rGlob) when total globulins were above the upper limit of the reference range (>42 g/L) but no specific increase in the gamma-region was evident; (*c*) polyclonal increase in the gamma-globulin region (pIgs); (*d*) probable monoclonal gammopathy, i.e. monoclonal band (or suspicion of a band) requiring IFE for confirmation; or (*e*) hypogammaglobulinaemia when suppression of the γ region was evident (hypogammaglobulinaemia).

Serum total protein (Roche; Biuret method catalog no. 11929917) and albumin (bromcresol purple) *(8)* were measured on a Roche modular or 917 analyzer.

The annual costs incurred by replacing UPEP and urine IFE with serum FLCs were based on the following assumptions: (*a*) a urine sample was received with every request for SPEP; (*b*) postanalytical costs were equivalent for urinalysis and serum FLC analysis; (*c*) no additional preanalytical costs were incurred by introducing serum FLCs on samples received for SPEP; (*d*) staff costs for the automated analysis of urine total protein were considered insignificant and not included; (*e*) no significant additional staff costs were incurred for serum FLC analysis, which was performed on the same analyzer as serum total protein and albumin; (*f*) estimates were based on costs and workload data (2800 samples for SPEP and serum FLC analysis) for the period spanning April 2004 to March 2005.

estimate of costs of urine electrophoresis and urine immunofixation

1. Staff costs in sample reception: a factor of 10 was applied to the number of urine samples to reflect the complexity of handling with transfer to secondary tubes relative to blood samples. Preanalytical costs for UPEP and urine IFE were then estimated as a proportion of all sample reception staff costs.
2. Staff costs (biomedical scientist grade 1) for sample analysis for upep and urine IFE.
3. Consumables costs for urine total protein analysis, UPEP, and urine IFE gels.

estimate of costs of serum immunofixation

1. Staff costs (biomedical scientist grade 1) for serum IFE.
2. Consumables costs for serum IFE.

estimate of costs of serum flcs

1. Costs of serum FLC reagent sets, assuming that 80 patient samples could be analyzed from a 100-test reagent set, with the remainder required for sample dilutions and quality control.

The additional costs for introducing serum FLC analysis were then calculated as follows: (Costs of serum FLC reagent sets + Staff and consumables costs for additional serum IFE generated as a consequence of serum FLC analysis) – (Staff costs in sample reception for UPEP and urine IFE + Staff costs for sample analysis for UPEP and

urine IFE + Consumables costs for urine total protein and UPEP and urine IFE gels).

Results

After exclusions (Fig. 1), matched SPEP and serum FLC results were available on samples received during the 4 months of the study from 923 consecutive patients (female: $n = 523$, ages 17–99 years, median 72 years; male: $n = 400$, ages 18–99 years, median 69 years). One hundred thirty-three were laboratory-generated requests because of high ($n = 117$) or low ($n = 16$) serum globulin concentrations. Paired urine samples were received (within ±3 weeks) with 370 (40%) of the serum samples.

SPEP and FLC κ/λ ratios

Table 1 shows the distribution of serum FLC κ/λ ratios by SPEP category for the 923 samples. Seventy-one (7.7%) samples had κ/λ ratios outside the typical range of 0.26–1.65. Five hundred fifty-one (97%) of 568 with no abnormality detectable by SPEP had κ/λ ratios within the usual range. By our standard protocol, all samples in the probable monoclonal band ($n = 79$) and hypogammaglobulinaemia ($n = 19$) categories would proceed to serum IFE. Of the 825 with "negative" SPEP (i.e., categories rGlob, pIgs, and no abnormality detected), 43 (5.2%) had abnormal κ/λ ratios and required serum IFE for the purpose of this study, representing an increase in serum IFE of 44%. Those with high κ/λ ratios have been subdivided into 3 groups (κ/λ ratios of 1.66–3.0, >3–5.0, and >5).

Serum immunofixation and FLC κ/λ ratios

Table 1 also shows the percentage of samples with abnormal κ/λ ratios in which either a monoclonal band was confirmed by serum IFE or a diagnosis of B-cell disorder was made. As can be seen, the percentages of positives increase as κ/λ ratios rise. When expressed as positive predictive values (PPVs), for a κ/λ ratio cutoff of 1.65, the PPV was 34%, increasing to 58 and 78% when κ/λ ratios of >3.0 or >5.0, respectively, were used as cutoff points. Of particular interest for this study were the 43 patients with abnormal κ/λ ratios (low ratio: $n = 1$; high ratios: $n = 42$), but no evidence of monoclonal bands or hypogammaglobulinaemia by SPEP. The results in these 43 are discussed below.

Negative SPEP but monoclonal bands by serum IFE ($n = 4$)

Two of these 4 had very small monoclonal bands (<1 g/L); these had rGlob by SPEP. The other 2 had IgA-κ monoclonal bands comigrating with the β-globulins; no abnormality was detected by SPEP. These results are summarized in Table 2 (patients 1–3 and 7).

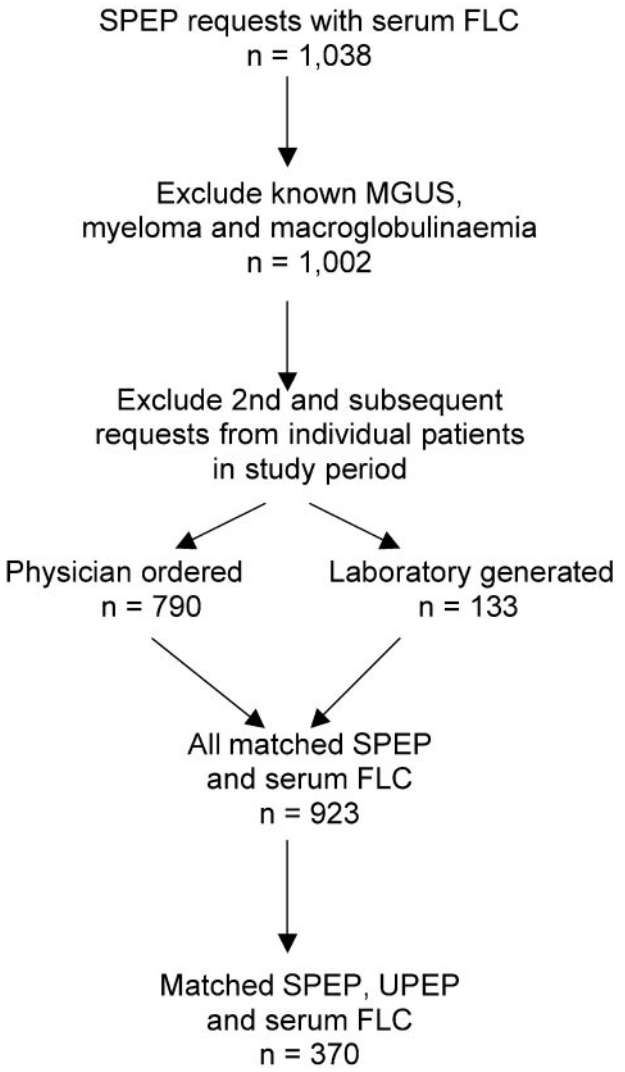

Fig. 1. Serum samples and exclusions for this study.

Negative SPEP and polyclonal immunoglobulins by serum IFE ($n = 22$)

Twenty-two of the 43 patients showed either a pIgs ($n = 16$) or rGlob ($n = 6$) by SPEP. Twenty of these 22 were not followed up; in 18, the reason for the initial SPEP was rGlob, and IFE excluded a monoclonal protein as the cause of the rGlob concentration; in 2, infections or inflammation explained the increase in immunoglobulins. The κ/λ ratios in these 20 patients were 1.68–4.55 (mean, 2.46). In one patient, IFE revealed an oligoclonal pattern and bone marrow biopsy showed increased plasma cells and lymphocytes; the patient was subsequently found to be positive to the human immunodeficiency virus (κ = 107, λ = 34.7 mg/L; κ/λ ratio = 3.09). The remaining patient had Sjogren's syndrome, with initially greatly increased total globulins (120 g/L; IgA, 11.5 g/L; IgG, 78.3 g/L), high serum FLC concentrations (κ = 547 mg/L, λ = 126 mg/L; κ/λ ratio = 4.35) and low glomerular filtration rate (31 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$). Six months after the initial

Table 1. Distribution of serum FLC κ/λ ratios by SPEP category.

	Serum FLC κ/λ ratio											
	All		Low ratio (<0.26)		Normal ratio (0.26–1.65)		High ratio (>1.65)					
							1.66–3		>3–5		>5	
SPEP	n	(%Pos)	n	(%Pos)	n	(%Pos)	n	(%Pos)	n	(%Pos)	n	(%Pos)
prMG[a]	79	(72)	9	(89)	57	(70)	7	(57)	4	(100)	2	(50)
Hypo	19	(16)	5	(20)	13	(8)	0		0		1	(100)
rGlob	144		0		134		10	(20)	0		0	
plgs	113		0		97		11	(0)	5	(0)	0	
NAD	568		1	(100)	551		9	(11)	1	(100)	6	(83)
Total	923		15	(67)	852		37	(19)	10	(50)	9	(78)

[a] The %Pos columns indicate the percentage in which a monoclonal band was detected by serum IFE or a subsequent diagnosis of a B-cell disorder was made. Abbreviations: prMG, probable monoclonal band; Hypo, hypogammaglobulinaemia; NAD, no abnormality detected.

sample, there was a decreasein serum FLC concentrations (κ = 48 mg/L, λ = 38 mg/L; κ/λ ratio = 1.25), as total globulins (58 g/L) and immunoglobulin concentrations (IgA 6.1 g/L, IgG 28.7 g/L) fell with treatment and glomerular filtration rate improved (66 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$).

NEGATIVE SPEP AND NO ABNORMALITY BY SERUM IFE ($n = 17$)

Of these patients, 15 showed no abnormality by SPEP, and 2 showed rGlob. One patient with no abnormality detected by SPEP and a low serum FLC κ/λ ratio (0.18, Table 1) was found to have a malignant lymphoma. Ten patients had κ/λ ratios of 1.66–2.18; urine samples from 5 were negative for BJP. Two patients had κ/λ ratios of 3.5 and 5.6; repeat samples after 4–6 months showed normal serum FLC concentrations and κ/λ ratios. Results for the remaining 4 patients are summarized in Table 2 (patients 4–6 and 8). A diagnosis of FLC monoclonal gammopathy of undetermined significance (MGUS) was made in a patient with a κ/λ ratio of 14.6. Light chain multiple myeloma was confirmed in the 2 patients with the highest κ/λ ratios (51.1 and 193). The κ/λ ratio in patient 5 (κ/λ ratio = 29.7) had not changed significantly after 9 months, but the patient declined further investigation at this time. A small κ band was detected in the urine, and it is likely that this patient has a FLC MGUS.

MATCHED SERUM AND URINE SAMPLES

Of the 370 urine samples, 141 (38%) were indeterminate by UPEP; subsequent testing by urine IFE revealed monoclonal κ or λ bands in 15. Eleven of these had abnormal κ/λ ratios (5 with λ-BJP: κ/λ ratios of 0.004–0.13; 6 with κ-BJP: κ/λ ratios of 3.2–855). Four of the patients with positive urine IFE had normal κ/λ ratios (0.31–1.21) and were therefore potential "false negatives" for the serum FLC assays. However, in 1, SPEP detected a band identified by serum IFE as an IgG-λ paraprotein (7 g/L, κ/λ ratio = 0.31); follow-up samples after 4 months showed similar results (κ/λ ratio = 0.35). No excess plasma cells were seen in a bone marrow biopsy. There were thus 3 patients (out of 370) who would not have been followed up if only SPEP and serum FLCs had been done. In all 3, the BJP concentration was ~50 mg/L. In one, the BJP resolved when high serum immmunoglobulins, because of a chest infection, normalized; in another, a follow-up urine was negative. Review of the initial urine IFE gel in this patient indicated a pattern more consistent with

Table 2. Results in 8 patients with abnormal serum FLC κ /λ ratios and negative SPEP.

Patient			Serum results						Urine results			
No.	Age, years	Sex	SPEP	IFE	M band,[a] g/L	κ, mg/L	λ, mg/L	κ/λ	UPEP	IFE	GFR	Diagnosis/other details
1	71	M	rGlob	IgA-κ	8	21.3	12	1.72	Neg	Neg		IgA MGUS, skeletal survey shows no abnormality
2	84	F	rGlob	IgG-κ	<1	52.1	25	2.11	Neg	Neg	50	Progressed to polyclonal IgG; declined further investigations
3	54	F	NAD	IgA-κ	5	17.1	7.1	2.52	Neg	Neg	88	IgA MGUS, no change in IgA in 6 months
4	70	M	NAD	NAD		293	20	14.6	Pos	κ	24	FLC MGUS; bone marrow biopsy: normal
5	72	F	NAD	NAD		310	10.4	29.7	Pos	κ	59	Probable FLC MGUS; declined further investigations
6	82	F	NAD	NAD		537	10.5	51.1	Pos	κ	53	LCMM confirmed
7	72	F	NAD	IgA-κ	<1	778	12	63.7	Pos	κ	55	IgA MGUS; bone marrow biopsy: normal
8	42	M	NAD	NAD		2601	13.5	193	Pos	κ	24	LCMM confirmed

[a] M band, monoclonal band; GFR, glomerular filtration rate (mL/min/1.73m^2); rGlob, raised globulins; Neg, negative; NAD, no abnormality detected; Pos, positive; LCMM, light chain multiple myeloma.

polyclonal κ light chains, and we consider the initial "positive" report to have been an interpretive error. Only 1 of the 3 has a persisting low concentration of BJP in follow-up urine samples. Bone marrow biopsy and skeletal survey do not indicate a B-cell disorder in this patient.

ADDITIONAL ANNUAL COSTS INCURRED BY REPLACING URINE ELECTROPHORESIS AND URINE IMMUNOFIXATION WITH SERUM FLCS

Table 3 shows the estimate of costs and the additional annual costs incurred by replacing UPEP and urine IFE with serum FLCs. Sixty percent of the costs of introducing serum FLC analysis could be offset by savings from UPEP and urine IFE.

Discussion

This prospective study evaluated serum FLCs in 923 consecutive serum samples for which SPEP was requested. Of these sera, 370 had matched urine samples. Together they provide an appropriate large clinical sample with which to evaluate the effectiveness of adding serum FLCs to SPEP and UPEP as first-line tests for B-cell proliferative disorders.

Most samples (92.3%) had normal serum FLC κ/λ ratios. Of those samples for which serum IFE would not normally have been indicated by SPEP, 94.8% had normal κ/λ ratios. In the 4 months of the study, 98 samples required serum IFE because of possible monoclonal bands or hypogammaglobulinaemia by SPEP, and an additional 43 samples required serum IFE on the basis of abnormal κ/λ ratios alone (Table 1).

A recent report *(9)* evaluating the performance of serum FLCs in clinical practice found no patients with false-positive κ/λ ratios among 121 patients without monoclonal gammopathies. Unlike that study, in which the majority of requests were from patients in clinical hematology treatment, our samples were from primary care patients and from a wide range of clinical specialties within the hospital. Seventy-one (7.7%) of our patients had abnormal κ/λ ratios; 23 of these were found to have monoclonal bands by serum IFE. In an additional 3 patients, either light chain multiple myeloma or light chain MGUS was diagnosed; 1 of those patients died from lymphoma, and light chain MGUS seems likely in 1 patient, who has declined further investigation. On the basis of the currently accepted reference interval of 0.26–1.65 for the κ/λ ratio, we found 43 false positives and 28 true positives (i.e., an overall PPV for B-cell disorders for an abnormal κ/λ ratio of 39%). The PPV for κ/λ ratios of <0.26 was 67%. For increased κ/λ ratios, the PPV was 34, 58, and 78% at κ/λ ratios of >1.65, >3.0, and >5.0, respectively. Our study, in a patient sample population typical of many diagnostic laboratories, shows higher numbers of false positives than those described from a specialist centre *(9)*. Similarly, as a prospective screening study that used a stepwise investigation procedure in which urine IFE was not used as a first line screening tool, a patient with BJP below the limit of detection of the UPEP assay (10 mg/L) and no serum abnormality would not have been identified.

Table 3. Estimate of additional annual costs and additional cost/patient incurred by replacing urine electrophoresis and urine immunofixation with serum FLCs for a workload of 2800 patient samples.

	£
1. Costs of urine electrophoresis and urine immunofixation	
Staff costs in sample reception	3855
Staff costs for sample analysis	7320
Consumables costs	9490
2. Costs of additional serum immunofixation	
Staff costs for additional serum IFE	1221
Consumables costs for additional serum IFE	951
3. Costs of serum FLCs	
Consumables costs	31 734
4. Additional annual costs incurred = £(line 2 + line 3) − line 1	13 241
Additional cost per patient	4.73

Our data validate the κ/λ ratio reference interval; 551 (97%) of 568 patients in whom no abnormality was detected by SPEP had κ/λ ratios within the reference interval of 0.26–1.65. In the study that defined this reference interval *(6)*, all 25 patients with polyclonal increases in immunoglobulins (defined by SPEP and serum IFE) had normal κ/λ ratios, although κ and λ serum FLC concentrations were increased in 52 and 58%, respectively. Fourteen percent of our 113 patients with polyclonal increases in immunoglobulins had abnormal κ/λ ratios (median, 0.99; range, 0.43–4.55), with increased κ and λ FLC concentrations in 87 and 70%, respectively. Polyclonal increases in immunoglobulins were found in 22 of the 43 samples in which raised κ/λ ratios were the only indication for proceeding to serum IFE; the increases in immunoglobulins were the commonest cause of "false-positive" κ/λ ratios. In 14 of these samples, the glomerular filtration rate was <60 mL/min, indicating renal impairment *(10)*; in only 1 of the 22 patients was glomerular filtration rate above the mean for age and sex. Our data support the use of the κ/λ ratio reference interval of 0.26–1.65 when SPEP shows no abnormality. However, κ/λ ratios >1.65 have a PPV for a monoclonal gammopathy or B-cell disorder of ~34%, and the broader clinical picture and laboratory data must be taken into account when interpreting serum FLC results.

As with many diagnostic tests, greater experience leads to a better understanding, so interpretation is based on more than just the reference interval. Inevitably, in a general hospital population, there will be patients with various degrees of renal impairment and/or acute phase responses, leading to more false positives. However, our study demonstrated that substantial additional clinical information was gained by adding serum FLCs to SPEP. From the 43 samples with raised κ/λ ratios and "nega-

tive" (nondiagnostic) SPEP that would not normally have proceeded to serum IFE, κ light chain multiple myeloma was diagnosed in 2 patients and MGUS in 4 (Table 2). An additional patient is likely to have FLC MGUS (κ/λ ratio = 29.7) but has declined further investigation. One patient, initially found to have 2 small monoclonal IgG-κ bands (IgG = 20.4 g/L; κ/λ ratio = 2.1), has progressed to a typical polyclonal increase in IgG with no further increase in IgG, although the κ/λ ratio has increased over 12 months to 3.5; urine BJP is negative, and she has declined further investigation.

In light of this study, we have now adopted SPEP and serum FLCs as our first-line tests for the investigation of possible B-cell disorders. Because no substantial pathology would have been missed by replacing urine BJP with serum FLCs, we no longer require a urine sample as part of the initial screen.

Laboratory protocols vary considerably, making it difficult to estimate the proportional increase in costs through adding serum FLCs as a first-line test. As Table 3 shows, the consumables for serum FLCs are expensive; in addition, laboratory costs were higher because the introduction of serum FLCs increased the indication for serum IFE from 10.6% to 15.3% of samples. We estimate (Table 3) that replacing urinalysis with serum FLCs releases revenue and manpower savings, which offset ~60% of the additional costs of serum FLC analysis. However, the quality of the diagnostic service we offer has improved by adding serum FLC analysis; our previous practice was suboptimal, as urine samples were received from only 40% of patients being screened. An additional benefit is that serum FLC measurements are available on our main analyzer within the day for most patients, whereas urine BJP analysis was performed in batches with a turnaround time of ~1 week when urine IFE was also indicated.

We also find that the availability of the serum FLC and κ/λ ratio results at the time of viewing the SPEP gel improves the interpretive process by helping to identify those samples that require serum IFE to detect small monoclonal bands, particularly those hidden in the α_2- or β-globulin regions, and to detect light chain monoclonal bands that may be too small for reliable detection by SPEP.

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