Summary:

- Normal renal tubular metabolism prevents urine excretion of significant amounts of monoclonal FLCs. This mechanism ensures that some patients have abnormal sFLCs but normal urine.
- Urine is not usually supplied at initial patient screening, whereas a serum sample is available.
- Methodology for urine electrophoresis varies considerably between laboratories, and interpretation can be challenging.
- Further benefits of sFLC analysis over urine tests include improved clinical sensitivity and a more rapid reporting turnaround time.

24.1. Introduction

Most of the issues relating to the use of serum versus urine for the measurement of free light chains (FLCs) have been detailed individually in preceding chapters. This chapter summarises these arguments and attempts to provide a coherent discussion of the relative merits of serum versus urine testing. Some laboratories continue to favour urine over serum measurements: this chapter aims to persuade them otherwise. An analogy with diabetes mellitus is helpful: 50 years ago, all patients were monitored using urine glucose tests, whereas now they are monitored using blood glucose due to its overwhelming clinical advantages. As glucose and FLCs are handled in a similar manner by the kidneys, similar benefits accrue from testing serum over urine for FLC analysis.

"If free light chains are in the urine they are always in the serum first".

24.2. Renal threshold for FLC excretion

As described in Chapter 3, serum FLCs (sFLCs) are cleared primarily through the renal glomeruli and then metabolised in the proximal tubules of the nephrons. Only when the tubular absorptive capacity is exceeded are significant amounts of FLCs observed in the urine as “overflow proteinuria”. Since normal production is about 500 mg/day and the renal absorptive capacity is 10 - 30 g/day, production must increase many times before urine contains measurable quantities of FLCs.

The clinical effect of renal tubular absorption on urine FLC concentrations is shown in Figure 24.1. Serum and urine FLC concentrations were compared in four patients undergoing treatment. Patients 1 and 2 had large amounts of serum and urine FLCs with good correlations between changes in concentrations. In patients 3 and 4, urine FLC excretion was minimal and unchanging over many months, while serum levels could be used to monitor the changing tumour burden. Despite similar sFLC concentrations, there were no urine FLCs in these latter patients because there was no renal impairment, and therefore, no overflow proteinuria.
Nowrousian et al. studied the concentrations of sFLCs necessary to cause overflow proteinuria and a positive urinary immunofixation electrophoresis (uIFE) test result, in a group of patients attending a myeloma clinic. Of 98 serum samples with monoclonal κ sFLCs, the corresponding urine was positive for monoclonal FLCs (urinary Bence Jones protein; uBJP) by uIFE in 51% of cases. The median κ sFLC concentration associated with positive uBJP was 113 mg/L (range 7 - 39,500), and 40 mg/L (range 6 - 710) for negative urine (Figure 24.2). Of the 107 serum samples with monoclonal λ sFLCs, the corresponding urine was positive for uBJP in 35% of cases. The median λ sFLC concentration associated with positive uBJP was 278 mg/L (range 5 - 7,060), and 44 mg/L (range 3 - 561) for negative urine. The large overlap of sFLC concentrations between patients with/without uBJP presumably reflects differences in renal reabsorptive capacity and the extent of renal damage between individuals.

Thus, median sFLC levels associated with uBJP were 5-fold above normal for κ-producing patients (upper limit of normal range: 19.4 mg/L) and 10-fold for λ patients (upper limit of normal range: 26.3 mg/L) when the urine contained uBJP. The higher serum levels necessary for λ overflow proteinuria can be explained by the dimerisation of λ molecules (Chapter 3). Therefore, when FLC production is below the renal reabsorptive capacity, serum tests are more sensitive than urinalysis for detecting monoclonal FLC.

24.3. Problems measuring urine samples

Urinary FLC measurements are normally based upon electrophoretic tests: urine protein electrophoresis (UPE) and uIFE (Chapter 4). The methodology employed for urine electrophoresis varies considerably between laboratories, and contributes to quantitative differences in the results reported. In addition, the visual interpretation of electrophoretic gels presents many challenges even to experienced users. These include “ladder banding” and high background staining in the presence of proteinuria.

Furthermore, difficulties of 24-hour urine collection include: 1) collections are cumbersome for patients with painful or fractured bones; 2) accurate timing of a 24-hour collection is important but may be difficult for ill patients; 3) large volumes of urine are produced in polyuric patients - perhaps larger than the bottle volume; 4) problems can occur handling and storing large volumes of urine at the laboratory; 5) collections may be embarrassing in front of friends or work colleagues.

Consistent with the various issues described above, Siegel et al. reported that urine electrophoresis results can be highly susceptible to error. Analysis of laboratory results from 207 monoclonal gammopathy patients, monitored during treatment and follow-up, revealed fluctuating urine electrophoresis results and 19% of urine samples were reported to have
unexpectedly high monoclonal protein levels. The samples with these elevated results had correspondingly high creatinine clearance data suggesting that errors in the measurement of 24-hour urine volumes had occurred. No such fluctuations were observed for sFLC values. An example is shown in Figure 24.3.

Figure 24.4 compares serum and urine results in a patient with relapsing light chain multiple myeloma (LCMM). The FLC concentrations in both fluids are considerably elevated, indicating that the renal threshold is exceeded (compare with patients 3 and 4 in Figure 24.1). However, the urine measurements are variable and do not show a definitive rise until day 160. By contrast, the steady rise in sFLC concentrations from day 40 indicates relapse of the tumour 3 to 4 months earlier.

In addition to the analytical issues described above, biological variation of FLCs may contribute to variations in both serum and urine measurements (Section 7.2.6).

24.4. Urine compliance

Urine provision remains an important issue in the serum versus urine debate; lack of urine with diagnostic serum samples has repeatedly been reported (Section 23.5). Even once a diagnosis of monoclonal gammapathy has been confirmed, problems with urine provision continue. In a study of 496 newly diagnosed monoclonal gammapathy patients by Holding et al. [6], only 30% of serum samples had a matched urine sample provided within 7 days; this increased to 57% within 90 days. Foster et al. [7] reviewed laboratory test usage to monitor 4591 MM patients followed at community clinics across the United States. At baseline, 58.1% received a sFLC test and 79.2% received an SPE test but only 27.7% received a UPE test. In the subgroup of patients with LCMM, 84.8% were followed with sFLC while only 15.2% were followed by UPE. The authors conclude that despite 24-hour urine tests being included in IMWG guidelines, they are often not performed in routine clinical practice, which may reflect the impracticalities of urine collection and storage.

Overall, the annual UK National Pathology Benchmarking Review for 2007/2008 reported that the number of UPE tests performed was much lower than serum protein electrophoresis (SPE), comprising only 14% of SPE requests (298,392) [6]. Recent National guidelines omit the use of urine BJP testing to screen for a monoclonal protein because of poor compliance, and the potential to miss diagnoses (Section 25.7). The authors of a screening study carried out at New Cross Hospital, Wolverhampton, UK, reported an extremely poor provision of matched urine samples (<5%) and concluded that “the debate over the relative merits of the sFLC assay versus uBJP analysis borders on the irrelevant” [8].

24.5. Urine FLC immunoassays

As an alternative to urine electrophoretic tests, FLC immunoassays can be used on urine samples (Section 4.5.3). Although Van Hoeven et al. [9] reported that the sensitivity of urine FLC assays was greater than UPE for FLC measurement (75% vs. 44%; n=73), all data indicate that uIFE remains the most sensitive assay for the identification of monoclonal proteins in urine [10][11]. However, urinary FLC immunoassays do not solve any of the problems relating to renal threshold, urine collection and urine measurement indicated above. Furthermore, the diagnostic sensitivity of sFLC ratios is superior to urine FLC measurements [12]; this is a function of the proximal tubule absorption of FLCs and the much wider normal ranges for urinary FLC concentrations and the $\kappa/\lambda$ FLC ratio (Section 6.4) [10].

The International Myeloma Working Group (IMWG) guidelines recommend sFLC assays in combination with SPE and serum IFE (sIFE) to screen for pathological monoclonal plasma cell proliferative disorders other than AL amyloidosis, which also requires a 24-hour uIFE [13]. The guidelines also state that “Measurement of urine FLC levels is not recommended” (Chapter 25).

24.6. Clinical benefits of sFLC analysis

The improved sensitivity of serum over urine FLC measurements has had a major impact on the ease of diagnosis, monitoring and assessing risk of progression for many patients with the following diseases:
1. Light chain multiple myeloma (LCMM) (Chapter 15).
2. Nonsecretory multiple myeloma (NSMM) (Chapter 16).
3. Intact immunoglobulin multiple myeloma (IIMM) (Chapters 17 and 18).
4. Smouldering (asymptomatic) multiple myeloma (SMM) (Chapter 14).
5. Myeloma kidney (Chapter 26).
6. Plasmacytoma (Chapter 21).
7. AL amyloidosis (Chapter 28).
8. Light chain deposition disease (LCDD) (Chapter 29).
9. Monoclonal gammopathy of undetermined significance (MGUS) (Chapter 13).

**Figure 24.5** illustrates sFLC concentrations in patients with low monoclonal immunoglobulin production rates at the time of clinical diagnosis. Samples from patients with NSMM are shown as white circles which, by definition, have no detectable monoclonal proteins by both serum and urine electrophoretic tests. Hence, other patients with monoclonal sFLCs at or below these concentrations but with other types of plasma cell dyscrasias are difficult to identify by conventional tests. The figure also includes samples from many patients with AL amyloidosis and IIMM who were in remission by IFE.

### 24.7. Elimination of urine studies when screening for monoclonal gammopathies

A number of studies have now been published identifying the benefit of including sFLC analysis in screening protocols for lymphoproliferative disorders. In addition, two large diagnostic studies from the Mayo clinic led to the conclusion that urine analysis could be eliminated, unless AL amyloidosis was suspected. These data are presented and discussed in Chapter 23 and the conclusions are now reflected in the IMWG guidelines (Chapter 25).

### 24.8. Comparison of sFLCs and urinalysis for monitoring patients

Apart from the practical advantages of using serum samples rather than urine, the principal advantages of monitoring patients with serum tests derives from their greater clinical sensitivity and prognostic utility. The main studies are reported in detail in the relevant chapters, listed in Section 24.6, and monitoring examples are shown in Figure 24.1 and Section 15.2.

The relative sensitivity of the serum and urine tests was first highlighted more than a decade ago in a retrospective analysis of monitoring samples from 82 LCMM patients. After treatment, 32% of patients achieved complete remission (CR) according to their urine results while only 11% achieved normalisation of their sFLC ratios; a percentage closely aligned to the 9.8% CR rate seen in patients monitored by their monoclonal intact immunoglobulin (and receiving the same treatment). Similar results have now been reported in a number of studies by the French IFM group.

![Figure 24.6. Normalisation of serum and urine...](image)

![Figure 24.7. Survival outcomes according to s...](image)
In a large retrospective analysis of the IFM 2007-02 trial, Dejoie et al. [16] compared sFLC and urine electrophoresis measurements for monitoring 157 IIMM and 25 LCMM patients. At each time point, the κ/λ sFLC ratio was more sensitive than uIFE for the detection of monoclonal FLCs and showed a better correlation with sIFE results (Figure 24.6). A separate analysis of 111 LCMM patients enrolled into the IFM-2009 trial [17] concluded that in keeping with previous reports, sFLC analysis was a more sensitive indicator of disease than urinalysis. The study also demonstrated that normalisation of sFLC parameters (iFLC or sFLC ratio) translated into an improved outcome, whereas UPE or urine IFE status was not prognostic (Figure 24.7). The prognostic value of urine electrophoresis and sFLCs was further compared when patients who were negative by UPE or uIFE (after three treatment cycles) were grouped according to their iFLC concentrations or sFLC ratio: those with abnormal FLC parameters had a significantly worse outcome compared with those with normal values (Figure 24.8). By contrast, when patients with elevated iFLC concentrations were separated according to positive/negative UPE results at the same time point, there was no significant difference in PFS between the two groups (Figure 24.8). In a further study of IFM-2009 trial data, Caillon et al. [20] demonstrated that the presence of an abnormal sFLC ratio showed better agreement with the presence of minimal residual disease (by 7-colour flow cytometry) than serum or urine electrophoresis. Dejoie et al. [16] concluded that the “improved sensitivity and prognostic value of serum over urine measurements provide a strong basis for recommending the former for monitoring LCMM patients”.

Katzmann et al. [21] assessed the monitoring potential of sFLCs and urinary monoclonal proteins (utilising UPE) in patients with clinically stable monoclonal gammopathy. The authors concluded that sFLCs were measurable in more patients than were urinary monoclonal proteins, and that sFLC measurements exhibited a lower total coefficient of variation (see also Section 7.2.6).

Tschautscher et al. [22] examined whether serial dFLC measurements could be used in place of 24-hour urinary M-protein levels to monitor for progressive disease (PD). The retrospective study included 122 patients with a measurable urinary M-protein at baseline, who subsequently developed PD (based on 24-hour urine electrophoresis, Section 25.3.5). At PD, the corresponding median dFLC increase was 740 mg/L (interquartile range 340 – 1680 mg/L), and the median percentage increase was 110% (interquartile range 55 – 312%). The authors conclude that serial dFLC measurements can be used in place of 24-hour urine collections to monitor for PD, and that a dFLC increase >100 mg/L from nadir best defines PD by dFLC. Once patients have reached this dFLC threshold, a 24-hour urine M-protein measurement can be performed to confirm progression based on current IMWG criteria. This strategy was likely to result in “better patient compliance, ease of testing, and reduced financial burden”.

The correlation between serial measurements of sFLCs and urine monoclonal protein (by UPE) in both LCMM or IIMM patients is insufficient to consider the tests interchangeable [16][23]. Consequently, international guidelines from 2009 [13] recommended monitoring MM patients with sFLCs in the following situations: 1) in all MM patients who do not have sufficient concentrations of measurable serum or urine intact immunoglobulin monoclonal proteins (serum monoclonal protein <10 g/L or a urine monoclonal protein <200 mg/24 hours); and 2) in all MM patients to look for a “stringent complete response” (Chapter 25). However, British guidelines state that there is a “clear rationale” for using sFLC assays “to assess response in light chain only disease, irrespective of the extent of light chain excretion in the urine” (Section 25.6). It seems probable that, as experience and confidence in the sFLC assays grows, they will increasingly replace urine assays for monitoring all patients.

### 24.9. Discrepant serum and urine results

As demonstrated above, sFLC analysis is generally more sensitive than urine electrophoresis for indicating the presence of monoclonal FLCs. However, this advantage is dependent upon efficient renal reabsorption of FLCs (Section 3.5). The renal metabolism of filtered FLCs combined with the analytical sensitivity and specificity of sFLC analysis over conventional urine tests makes sFLC analysis superior for the diagnosis and monitoring of monoclonal gammopathies associated with FLC production.

Occasionally, patients are reported to have “positive” urine when sFLC results are normal. This may be due to a number of reasons: 1) serum and urine samples collected at different time points; 2) false positive urine results (caused by the staining of
intact immunoglobulin in urine); or 3) true positive urine results (when proximal tubule reabsorption is impaired). Such discrepant results and other scenarios are further discussed in Section 7.7.

24.10. Organisational cost savings and other benefits of sFLC analysis

As well as improved clinical diagnosis, there are organisational cost savings and other benefits to introducing sFLC assays. The laboratory issues and costs were analysed in 2004 at the Christie Hospital in Manchester, UK [24]. Superior analytical performance of the serum assays, faster reporting times and reduced laboratory costs were identified (Table 24.1). Cost benefits in relation to clinical outcomes were not analysed in this study but they may accrue from earlier diagnosis and by treatments that reduce morbidity. It is well known that a delay in the diagnosis of MM is associated with more complications and reduced disease-free survival [25].

<table>
<thead>
<tr>
<th></th>
<th>sFLCs</th>
<th>Urine electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>1.5 mg/L</td>
<td>50 mg/L</td>
</tr>
<tr>
<td>Precision</td>
<td>5%</td>
<td>&gt;15%</td>
</tr>
<tr>
<td>Analysis time</td>
<td>15 minutes</td>
<td>1 hour</td>
</tr>
<tr>
<td>Reporting turnaround</td>
<td>1 hour</td>
<td>1 week</td>
</tr>
<tr>
<td>Cost per year (700 requests)</td>
<td>£6,500</td>
<td>£4,500</td>
</tr>
<tr>
<td>Extra staff costs per year</td>
<td>£0</td>
<td>£1,000</td>
</tr>
<tr>
<td>24-hour urine bottle usage</td>
<td>Not relevant</td>
<td>£1,000</td>
</tr>
<tr>
<td>Storage needs</td>
<td>30 cm³</td>
<td>10 m³</td>
</tr>
</tbody>
</table>

Table 24.1. Analytical and cost/benefit study of sFLC and urine electrophoresis tests [24].

Most comparisons of the relative cost/benefits of serum versus urine FLC analysis have been made in the context of screening studies and these are presented in Section 23.6. In addition to the financial benefits, Tschautscher et al. [22] comment that monitoring with sFLC analysis in place of 24-hour urine analysis is likely to result in better patient compliance, because urine tests are both bothersome and time consuming.

24.11. Conclusions

When both serum and urine tests are available, it is clinically reassuring to have two separate tests. Clearly, samples do occasionally get incorrectly analysed or mislabelled, so any discrepancy between sFLC and urine BJP tests will direct the laboratory to further investigate [26]. In addition, supporting evidence from either FLC test can be helpful when making a diagnosis or changing treatment, and it should be remembered that, in the context of a stem cell transplant in MM patients, for example, the additional cost of performing both serum and urine tests is inconsequential.

If a choice has to be made between serum or urine tests, then the use of serum is clearly preferable for the many reasons given above and summarised in Table 24.2 [27]. For example, Dejoie et al. [17] state, "We believe there is now sufficient evidence and experience to propose that sFLC analysis is the method of choice for response evaluation in LCMM patients, whereas urine exploration may remain a rational practice to assess total proteinuria and, together with serum creatinine measurements, monitor renal function in these patients." A recent editorial by Mollee and Tate [26] concludes that "...the time has come for the [sFLC] assay to be the preferred tool to monitor myeloma not measurable by [SPE]. This will greatly facilitate the monitoring of myeloma, enable diagnosis of light chain escape, and leave only the occasional patient who requires [24-hour UPE] monitoring."
Serum versus urine measurements

<table>
<thead>
<tr>
<th>sFLC</th>
<th>Urine electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy to collect</td>
<td>Difficult to collect</td>
</tr>
<tr>
<td>$\kappa/\lambda$ ratio less affected by renal function</td>
<td>Renal function affects levels</td>
</tr>
<tr>
<td>Easily analysed</td>
<td>Samples may need concentrating</td>
</tr>
<tr>
<td>Easily stored</td>
<td>More difficult to store</td>
</tr>
<tr>
<td>More frequently abnormal in NSMM and AL amyloidosis</td>
<td>Less frequently abnormal</td>
</tr>
<tr>
<td>More sensitive for monitoring patients</td>
<td>Less sensitive for monitoring patients</td>
</tr>
</tbody>
</table>

Table 24.2. Summary of clinical and analytical comparisons of sFLC and urine electrophoresis tests [27].

Figure 24.1. Serum and urine FLCs in four patients with light chain multiple myeloma (LCMM) or AL amyloidosis.

Serum tests (blue) were useful even when urine FLC excretion (pink) was minimal in patients 3 and 4. HDC ASCT: high-dose chemotherapy autologous stem cell transplant. (Reproduced with permission from the American Journal of Hematology and John Wiley and Sons)
24.2. Renal threshold for FLC excretion

24.3. Problems measuring urine samples

24.8. Comparison of sFLCs and urinalysis for monitoring patients

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**Figure 24.2. Amounts of sFLCs required to produce light chain proteinuria for κ and λ myelomas.**

The median (line), 95% ranges (boxes) and 100% ranges (whiskers) are shown. (Courtesy of MR Nowrousian).

**View source:**

- 24.2. Renal threshold for FLC excretion

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**Figure 24.3. Example of a clinically unexpected increase in 24-hour urine protein.**

Graph based on data supplied in[5].

**View source:**

- 24.3. Problems measuring urine samples

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**Figure 24.4. Serum and urine FLCs in a patient with LCMM.**
Disease relapse can be identified 3 months earlier using serum rather than urine samples.

**Figure 24.5. sFLCs in patients with low monoclonal immunoglobulin production rates.**

By electrophoretic tests, sFLCs are usually undetectable or unquantifiable in most of these patients. (See relevant chapters for details of the patient data).

**Figure 24.6. Normalisation of serum and urine tests during follow-up.**

IIMM. Cycle 2, n=44; Cycle 4, n=48; ASCT, n=38. (Chart generated using published data).

View source:
- 24.8. Comparison of sFLCs and urinalysis for monitoring patients

Figure 24.7. Survival outcomes according to serum and urine FLC characteristics at the end of induction therapy.

PFS for patients with (A) negative vs. positive UPE; (B) normal vs. elevated iFLC; (C) negative vs. positive uIFE; and (D) normal vs. abnormal κ/λ sFLC ratio. (This research was originally published in Blood © the American Society of Hematology).

View source:
- 24.8. Comparison of sFLCs and urinalysis for monitoring patients

Figure 24.8. Abnormal sFLC measurements stratify patients with normal urine results after induction.
PFS according to (A) normal vs. elevated iFLC in patients with a negative UPE; (B) negative vs. positive UPE in patients with elevated iFLC; and (C) normal vs. abnormal κ/λ sFLC ratio in patients with negative uIFE. (D) Overall survival for patients with normal vs. abnormal κ/λ sFLC ratio in individuals with negative uIFE. (This research was originally published in Blood © the American Society of Hematology).

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