water allows the specimen to be flushed from the dispenser with no precipitation in the line.

There are potential benefits to the addition of SPE. Ion suppression can be further eliminated. SPE also allows extraction of larger specimen volumes. One purported advantage of MS/MS is the ability to monitor mass transitions for multiple drugs within the same analytical run. However, as the number of compounds being monitored increases, instrument dwell time or number of data points acquired for each mass transition may decrease, which could negatively affect the limit of quantification or precision. The use of a larger sample size in conjunction with SPE can help restore the observed response for the desired mass transitions.

**References**


Serum creatinine, mg/L

Log serum creatinine, y

Serum cystatin C, mg/L

Log serum cystatin C, y

1/Serum cystatin C, mg/L

Serum creatinine, μmol/L

Log serum creatinine, y

1/Serum creatinine, μmol/L

Clearance, mL·min⁻¹·(1.73 m²)⁻¹

Creatinine clearance

Log creatinine clearance

C&G-calculated clearance

Log C&G-calculated clearance

MDRD-calculated clearance

Log MDRD-calculated clearance

Simplified MDRD clearance

Log simplified MDRD clearance

<table>
<thead>
<tr>
<th>GFR test marker (y)</th>
<th>Regression equation</th>
<th>R²</th>
<th>Mean (95% CI) difference, mL·min⁻¹·(1.73 m²)⁻¹</th>
<th>95% limits of agreement, mL·min⁻¹·(1.73 m²)⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cystatin C, mg/L</td>
<td>y = −3.1512x + 6.965</td>
<td>0.77</td>
<td>6.7 (2.4–10.9)¹</td>
<td>−18.8 to 32.1</td>
</tr>
<tr>
<td>Log serum cystatin C, mg/L</td>
<td>y = −0.7121x + 1.365</td>
<td>0.73</td>
<td>4.5 (0.3–8.7)¹</td>
<td>−20.7 to 29.7</td>
</tr>
<tr>
<td>1/Serum cystatin C, mg/L</td>
<td>y = 1.0308x − 0.978</td>
<td>0.55</td>
<td>6.3 (2.4–10.3)¹</td>
<td>−17.3 to 30.0</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>y = −356.07x + 740</td>
<td>0.75</td>
<td>5.7 (2.1–9.3)¹</td>
<td>−16.1 to 27.4</td>
</tr>
<tr>
<td>Log serum creatinine, μmol/L</td>
<td>y = −0.8211x + 3.452</td>
<td>0.86</td>
<td>10.3</td>
<td>−18.8 to 32.1</td>
</tr>
<tr>
<td>1/Serum creatinine, μmol/L</td>
<td>y = 0.0132x − 0.013</td>
<td>0.75</td>
<td>16.1</td>
<td>−16.1 to 27.4</td>
</tr>
</tbody>
</table>

For all regression analyses, log-transformed values for ⁵¹Cr-EDTA were used because they gave a closer approximation to a gaussian distribution for that variable. All clearance estimates have been adjusted to a body surface area of 1.73 m².

¹ CI, confidence interval; C&G, Cockcroft and Gault.
² P < 0.05 compared with ⁵¹Cr-EDTA.

GFR was estimated from a single ⁵¹Cr-EDTA injection and three blood samples by mono-exponential analysis with the Brochner-Mortenson correction (27). GFR measurements were undertaken by technologists/scientists and reported by accredited specialist physicians working in a nuclear medicine department accredited for training purposes by the Institute of Physical Sciences in Medicine. Serum and urinary creatinine were measured by a kinetic Jaffe method (Integra 800 analyzer, Roche Diagnostics) calibrated with the manufacturer’s recommended human-serum-based material: between-day imprecision was <3% at concentrations of 113 and 333 μmol/L. Serum cystatin C was measured by a particle-enhanced nephelometric immunoassay (Dako Ltd.) on an automated rate nephelometer (Immage™ analyzer; Beckman-Coulter) (28): between-day imprecision was 6.6% at 0.97 mg/L and 2.6% at 5.53 mg/L. Absence of interference from paraprotein concentrations was determined by densitometric scanning. Analyses were undertaken in an accredited (CPA UK Ltd.) laboratory by state-registered biomedical scientists blinded to the ⁵¹Cr-EDTA results.

Data were analyzed by Analyze-it™ software (Analyze-it Software Ltd.). ⁵¹Cr-EDTA was the reference method: log-transformed values were used because this gave a closer approximation to a gaussian distribution for this variable. The analysis was complicated by the known nonlinear and inverse relationship between serum markers of GFR (creatinine and cystatin C) and GFR itself, compared with the other clearance estimates, which are expressed in the same units as GFR (creatinine clearance and formulaic estimates of clearance). To enable comparison, reciprocal and log-transformed analyses were also calculated. For creatinine clearance and the formulaic estimates of clearance, comparison with ⁵¹Cr-EDTA clearance was also undertaken by use of difference plots, and the bias and imprecision of the estimates were compared by the paired t-test and F-test, respectively.

Using the relevant reference interval for the kidney function tests (see file 1 in the online Data Supplement), we assessed sensitivity for the detection of moderate kidney disease against a recently proposed threshold of 60 mL·min⁻¹·(1.73 m²)⁻¹ (12). Differences in the sensitivities of the test markers for renal dysfunction were tested by the Fisher exact probability test (2-tailed P values are given). The existence of a relationship between cystatin C and tumor burden was tested by comparing cystatin C concentrations against (a) serum β₂-micro-
globulin and (b) paraprotein concentration and (c) by comparing cystatin C concentrations in patients with stage I, II, and III disease.

The $R^2$ of linear regression analyses comparing the test marker ($y$) with $^{51}$Cr-EDTA ($x$) were 0.91 for Cockcroft and Gault and simplified MDRD clearances, 0.90 for MDRD clearance and creatinine clearance, 0.86 for serum creatinine, and 0.73 for cystatin C (Table 1).

On average, creatinine clearance and the three formulaic estimates of clearance all showed a slight positive bias compared with $^{51}$Cr-EDTA ($P < 0.05$ in all cases; Table 1). In terms of the precision of the estimate of GFR, the 95% limits of agreement [mL·min$^{-1}$·(1.73 m$^2$)$^{-1}$ in all cases] did not differ ($P > 0.05$) between the simplified MDRD (43.5), MDRD (47.3), Cockcroft and Gault (50.4), and measured creatinine clearance (50.9) estimates (Table 1).

There was no relationship ($P > 0.05$) between serum paraprotein concentration and either log-transformed GFR ($R^2 = 0.02$) or cystatin C ($R^2 = 0.03$). We observed a significant positive relationship between serum cystatin C and $\beta_2$-microglobulin concentrations [$R^2 = 0.66$ ($P < 0.0001$); $\beta_2$-microglobulin = 3.704(cystatin C) - 0.44]; however, when $\beta_2$-microglobulin concentration was adjusted for glomerular dysfunction (by expressing as a ratio to serum creatinine), this relationship was abolished ($R^2 = 0.00$). There were no significant differences ($P > 0.05$) between the cystatin C/creatinine ratios in patients with stage I, II, or III disease. There was a significant relationship between serum cystatin C and serum creatinine [$R^2 = 0.77$ ($P < 0.0001$); cystatin C = 0.0077(creatinine) + 0.56].

GFR was <60 mL·min$^{-1}$·(1.73 m$^2$)$^{-1}$ in 20 of 39 patients, but serum creatinine was increased above the reference interval (i.e., true positive for moderate kidney disease) in only 8 of 20, compared with 20 of 20 for cystatin C ($P < 0.0001$; Fig. 1). Cockcroft and Gault- and MDRD-calculated clearances were <60 mL·min$^{-1}$·(1.73 m$^2$)$^{-1}$ in 15 of 20 patients, both of which appeared to be inferior to cystatin C for detecting moderate kidney disease ($P = 0.0471$). The simplified MDRD and measured creatinine clearances detected 16 of 20 of these patients ($P > 0.05$ compared with cystatin C).

Among 19 patients with GFR $\geq$60 mL·min$^{-1}$·(1.73 m$^2$)$^{-1}$, 10 had serum cystatin C concentrations exceeding the reference interval (i.e., false positive for moderate kidney disease) compared with none for serum creatinine ($P = 0.0004$). All 19 had MDRD and simplified MDRD clearance estimates >60 mL·min$^{-1}$·(1.73 m$^2$)$^{-1}$ ($P = 0.0030$); one measured creatinine clearance and one Cockcroft and Gault clearance estimate was <60 mL·min$^{-1}$·(1.73 m$^2$)$^{-1}$ ($P = 0.0004$). (P values are compared with cystatin C in all cases). There were no particular distinguishing clinical features (drug history, presence of free light chains, skeletal lesions, or comorbid conditions) in the patients with increased serum cystatin C concentration and GFR $\geq$60 mL·min$^{-1}$·(1.73 m$^2$)$^{-1}$.

Finney et al. (31) also demonstrated a strong correlation between serum cystatin C and creatinine in myeloma patients ($r = 0.89$ compared with $r = 0.88$ in the present study), but an estimate of GFR was not reported by these authors. They also observed no relationship between serum cystatin C and disease burden. Tumor burden probably does not exert a strong influence on serum cystatin C concentration independently of the effect of myeloma itself on kidney function. Given this, does cystatin C measurement confer any advantages in the assessment of kidney function in these patients compared with conventional markers? Cystatin C was more sensitive than serum creatinine at detecting moderate reductions in GFR, and we observed a good correlation with GFR, although the strength of this relationship was lower than that observed with all other markers tested, including creatinine clearance. The good performance of creatinine clearance in this setting is unusual: in nearly all other studies, the performance of creatinine clearance was inferior to that of serum creatinine and calculated estimates of GFR (12, 32) and serum cystatin C (17, 21, 33). Many of our patients had been receiving medical care of their disease for some time: they may have been more familiar, and consequently competent, with the 24-h urine collection procedure. All calculated clearance estimates gave reasonable $R^2$ coefficients against $^{51}$Cr-EDTA and showed reasonable sensitivity and specificity for the detection of moderate kidney disease.

In summary, cystatin C appears to reflect GFR in patients with multiple myeloma, as observed in other
clinical settings, but calculated estimates of GFR appear to provide equally good information at much lower cost. In keeping with current best practice guidelines (12), we recommend that calculated estimates of GFR should supplement serum creatinine measurement alone and replace measured creatinine clearance.

We thank Wendy Van Der Steen of the Nuclear Medicine Department for the $^{51}$Cr-EDTA measurements. We are grateful to Drs. Y. Williams, A. Borg, R. Gale, and C. Pocock, from the Department of Hematology, for assistance with patient recruitment. We would also like to thank the staff of the Clinical Biochemistry Department, Kent and Canterbury Hospitals, for their cooperation and help. We are grateful to Dr. J. Sheldon from the Protein Reference Unit, St. George’s Hospital, London, for the $\beta_2$-microglobulin measurements. This work was funded by the South East Regional NHS Project Grant Scheme (Grant Reference SEO 150).

References