extracted from whole blood by use of Qiagen Mini Prep Kits. Genotyping was performed with a LightCycler® (Roche) with the FastStart DNA Master Hybridization Probe PCR method.

The tacrolimus concentrations, as measured by both methods in CYP3A5 nonexpressors and expressors, are shown in panels A and B, respectively, of Fig. 1.

The ratio immunoassay/chromatographic assay for each tacrolimus concentration was calculated, and the ratio for expressors and nonexpressors was compared by ANOVA. The mean ratios were 0.965 in the expressors and 1.01 in the nonexpressors. The mean difference in the ratios was 0.045 (95% confidence interval, −0.026 to 0.118; *P = 0.22, not significant). The study had a power of 80% to detect a difference in ratios of 0.11 and a power of 95% to detect a difference of 0.14 at a significance level of 5%.

Our results demonstrate that CYP3A5 genotype does not affect tacrolimus measurements by the Abbott immunoassay. Thus, the genetic influence on the pharmacokinetics of tacrolimus is most likely related to a genotype-phenotype association, rather than an artifact resulting from the specificity of the immunoassay. Although the presence of metabolites has the potential to interfere with the Abbott immunoassay, we found a high degree of concordance between the 2 assays for expressors and nonexpressors of the CYP3A5 gene.

Cystatin C Intrapatient Variability in Children with Chronic Kidney Disease Is Less than Serum Creatinine

To the Editor:

Serum Cystatin C (CysC) (1) is a promising new marker for glomerular filtration rate (GFR) in children (2) because of its independence from height and sex (3). Although the superiority of CysC over serum creatinine (Scr) for the detection of impaired GFR has been demonstrated in a metaanalysis (4), widespread clinical use of the marker remains limited because of previously reported substantial intrapatient variability of CysC in healthy volunteers (5).

After obtaining approval from the Institutional Review Board, we analyzed the analytical imprecision of CysC (nephelometric Dade Behring assay; BN Prospec platform) and Scr (enzymatic assay; Ortho Clinical Diagnostics) as well as interpatient variability in 38 children [19 males; mean (SD) age, 10.1 (4.95) years] who underwent 99mTc diethyleneetriaminepentaacetic acid GFR renal scans and had a normal (i.e., within reference values) GFR between 90 and 135 mL·min⁻¹·(1.73 m²)⁻¹ and intrapatient variability in 54 children [14 females; mean (SD) age, 9.6 (5.4) years] with a GFR <60 mL·min⁻¹·(1.73 m²)⁻¹ (8 patients were on hemodialysis and 3 on peritoneal dialysis). GFR estimates were calculated by use of the Schwartz formula (6) with validated constants of 38 for children above 1 year of age and of 48 for adolescent males (7) and by a novel CysC-based formula (8). For statistical analysis of the CV, we used the standard components of variation as described by Fraser and Harris (8). The Wilcoxon matched-pairs test was used to compare intrapatient variability.

The analytical imprecision for both analytes is given in Table 1. Despite the higher analytical variance for CysC, the interpatient CV in the 38 children with a normal GFR was lower for CysC (20% vs 36% for Scr; Table 1).

The interpatient CV was determined from 494 simultaneous Scr and CysC measurements (median of 9 per patient) over an 18-month study period. The mean (SD) GFR in the nondialyzed patients was 30 (14) mL·min⁻¹·(1.73 m²)⁻¹, and in the dialysis population was 11 (5) mL·min⁻¹·(1.73 m²)⁻¹. The combined analytical and interpatient CV for the entire patient group was significantly lower for CysC (12%) than for Scr (13%; *P = 0.0012; Table 1).

When we analyzed the nondialyzed group only, the combined analytical and interpatient CV for the nondialyzed patient group was significantly lower for CysC (12%) than for Scr (13%; *P = 0.0144; Table 1); however, the CV of the GFR estimated by Schwartz formula (median, 11%) was not significantly different from the GFR calculated from CysC (median, 11%; *P = 0.38). There was a trend toward lower intrapatient variability of the CysC-derived GFR in the hemodialysis group compared

References


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The analytical imprecision for both analytes is given in Table 1. Despite the higher analytical variance for CysC, the interpatient CV in the 38 children with a normal GFR was lower for CysC (20% vs 36% for Scr; Table 1).

The interpatient CV was determined from 494 simultaneous Scr and CysC measurements (median of 9 per patient) over an 18-month study period. The mean (SD) GFR in the nondialyzed patients was 30 (14) mL·min⁻¹·(1.73 m²)⁻¹, and in the dialysis population was 11 (5) mL·min⁻¹·(1.73 m²)⁻¹. The combined analytical and interpatient CV for the entire patient group was significantly lower for CysC (12%) than for Scr (13%; *P = 0.0012; Table 1).

When we analyzed the nondialyzed group only, the combined analytical and interpatient CV for the nondialyzed patient group was significantly lower for CysC (12%) than for Scr (13%; *P = 0.0144; Table 1); however, the CV of the GFR estimated by Schwartz formula (median, 11%) was not significantly different from the GFR calculated from CysC (median, 11%; *P = 0.38). There was a trend toward lower intrapatient variability of the CysC-derived GFR in the hemodialysis group compared
with the Schwartz formula (12% vs 19%; \( P = 0.054 \); Table 1).

This study is the first to evaluate intrapatient variability of CysC in children with stage 3 to 5 chronic kidney disease (CKD) (9). In contrast to the study in healthy adults, the intrapatient CV of CysC was significantly lower than that of SCr in children with CKD. The most probable explanation for this difference relates to the fact that muscle mass is changing in our pediatric patients. The mean (SD) height gain over the study period was 6.7 (4.4) cm. When we used the Schwartz formula, which accounts for the variability in height, the median intrapatient CV for the Schwartz formula (11%) was not significantly different from the CV for CysC-derived GFR (11%).

We propose that CysC is a better tool for longitudinally monitoring patients with advanced CKD, as no additional recording of height is required. Recently, we showed that the CV for CysC in pediatric renal transplant patients also was not higher than that for SCr (10). Our data suggest that the higher intrapatient variability of CysC, and thus the inferior performance for longitudinal follow-up, is not an issue. Given the additional possibility of error with calculating ratios and the need for accurate determination of height for the Schwartz formula, measurement of GFR with CysC appears beneficial. Because of the demonstrated benefit of CysC in children (11), CysC could be used for the longitudinal follow-up of patients with CKD, with improved intrapatient variability compared with SCr.

### Table 1. Variability analysis for CysC and creatinine.

<table>
<thead>
<tr>
<th></th>
<th>CysC</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical CV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low concentration</td>
<td>3.1%</td>
<td>1.8%</td>
</tr>
<tr>
<td>Medium concentration</td>
<td>3.5%</td>
<td></td>
</tr>
<tr>
<td>High concentration</td>
<td>6.7%</td>
<td>1.8%</td>
</tr>
<tr>
<td><strong>Controls [GFR, 90–135 mL·min(^{-1})·(1.73 m(^2))(^{-1})]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 38</td>
<td>52.4 (\mu)mol/L</td>
<td></td>
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<tr>
<td><strong>Interpatient CV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 38</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td><strong>CKD patients</strong></td>
<td></td>
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<tr>
<td>Stages 3–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean concentration</td>
<td>2.81 mg/L</td>
<td>188.9 (\mu)mol/L</td>
</tr>
<tr>
<td>Intrapatient CV</td>
<td>12%</td>
<td>13%</td>
</tr>
<tr>
<td>Stages 3 and 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean concentration</td>
<td>2.37 mg/L</td>
<td>155 (\mu)mol/L</td>
</tr>
<tr>
<td>Intrapatient CV</td>
<td>12%</td>
<td>13%</td>
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<tr>
<td>GFR estimate intrapatient CV</td>
<td>11%</td>
<td>11%</td>
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<tr>
<td>Hemodialysis patients</td>
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<td></td>
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<tr>
<td>Intrapatient CV</td>
<td>12%</td>
<td>19%</td>
</tr>
<tr>
<td>GFR estimate intrapatient CV</td>
<td>17%</td>
<td>24%</td>
</tr>
</tbody>
</table>

\( ^{a} \) Wilcoxon matched-pairs test: \( ^{a} P = 0.0012; ^{b} P = 0.0144; ^{c} P = 0.38; ^{d} P = 0.0234; ^{e} P = 0.0547. \)

### References


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### Autosampler Programming for Improved Sample Throughput in Liquid Chromatography/Mass Spectrometry

To the Editor:

Liquid chromatography/mass spectrometry (LC/MS) has rapidly assumed an important role in the clinical laboratory because of its speed and inherently superior sensitivity and selectivity. It has been applied extensively in clinical research in areas such as therapeutic drug monitoring, drug metabolism, pharmacokinetics, and clinical toxicology (1–