CYP3A5 Genotype Does Not Influence the Blood Concentration of Tacrolimus Measured with the Abbott Immunoassay

To the Editor:
Therapeutic drug monitoring of immunosuppressants is well established as an aid to optimizing patient management after allograft transplantation. Most laboratories measure cyclosporin and tacrolimus by immunoassays. However, measured values may be affected by the presence of circulating metabolites because of the limited specificity of the antibodies used in these assays.

Cyclosporin is metabolized by the cytochrome P450 3A4 (CYP3A4) and cytochrome P450 3A5 (CYP3A5) enzymes. Studies on the biotransformation of cyclosporin by CYP3A4 and CYP3A5 have demonstrated disparate patterns of metabolite profiles between the 2 enzymes for this substrate. CYP3A4 catalyzes the formation of 3 primary cyclosporin metabolites; 2 are monohydroxylated (AM1 and AM9), and the third is demethylated (AM4N). CYP3A5 produces only the AM9 metabolite (1). This may have important implications for immunoassay measurements of cyclosporin, as polymorphic expression of the CYP3A5 gene leads to some individuals not producing the CYP3A5 enzyme (2). In addition, the CYP3A5 enzyme has a lower catalytic activity for cyclosporin compared with CYP3A4. However, the preferential catalysis exhibited by these enzymes is not the same for all substrates.

Preferential production of particular metabolites could occur with tacrolimus. Genetic studies have shown that expressors of CYP3A5 require significantly larger doses of tacrolimus to attain therapeutic blood concentrations of this drug (3). These studies have been based on blood concentrations determined with an immunoassay (MEIA tacrolimus II immunoassay for the IMx analyzer; Abbott Diagnostics). If CYP3A4 and CYP3A5 were to produce different metabolite profiles from tacrolimus, then the degree of interference in the immunoassay would differ for expressors and nonexpressors of CYP3A5. To investigate this possibility, we compared tacrolimus results, obtained with the Abbott immunoassay, with those of an in-house HPLC–tandem mass spectrometry (MS/MS) assay, using whole blood samples taken from expressors (homozygous CYP3A5*1*1 and heterozygous CYP3A5*1*3) and nonexpressors (homozygous CYP3A5*3*3) of the CYP3A5 gene.

Predose blood samples collected from kidney transplant patients receiving tacrolimus were analyzed. The samples were collected at least 3 months posttransplantation. A total of 136 samples taken from 105 patients were grouped into expressors (n = 62 samples from 32 patients) and nonexpressors (n = 74 samples from 73 patients) of CYP3A5. The samples were analyzed by the Abbott immunoassay on the day of receipt, then stored frozen at −20 °C until analyzed by HPLC-MS/MS. Tacrolimus analysis was performed with the Abbott immunoassay and by an in-house HPLC-MS/MS method. The HPLC-MS/MS assay was used as the comparison method. For direct comparison, the Abbott calibrators and controls were used for both methods to minimize any calibration bias. The DNA was ex-
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 Prospect 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 (7). 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 intrapatient 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 intrapatient 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 intrapatient 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