functional CYP2D6*2 allelic variants, as described previously (7), and confirmed the proposed CYP2D6*35 haplotype (10). Phenotype-genotype correlation analysis subsequently showed that poor metabolizer status could effectively be ruled out by positive identification of a single functional −1584G-containing allelic variant in 43% of Caucasians but only 12% of African Americans. The −1584G assay can be performed directly on genomic DNA or on a long CYP2D6-specific PCR product encompassing upstream and coding regions of the CYP2D6 gene. The assay is useful for rapid and cost-effective identification of individuals with functional CYP2D6 activity and confirmation of an extensive metabolizer assignment when incorporated in a more intensive genotyping strategy. Although perfect linkage of −1584G to functional alleles was demonstrated in our population sample, it remains to be validated in populations of other ethnic backgrounds.

The significantly lower frequency of −1584G in African Americans compared with Caucasians further supports previous findings that CYP2D6 genotyping in African Americans presents unique challenges (6). The increased frequency of specific reduced-function and rare nonfunctional alleles (CYP2D6*17, *29, *40, and *42), and the potential existence of additional such alleles because of the residual genotype-phenotype discordance observed by us and others (6, 11, 12), make accurate prediction of phenotype from genotype data difficult in this heterogeneous population. Therefore, exclusion of poor metabolizer status through identification of −1584G carriers offers one solution to this problem.

Overrepresentation of CYP2D6*35 among duplication-negative ultra-rapid metabolizers may be attributable to −1584G and increased gene transcription rather than the Val11Met substitution (31G→A) (10), which had no effect on activity in vitro (13). Although DM phenotype does not appear to differentiate gene duplication-carrying ultra-rapid metabolizers from other groups, a correlation between CYP2D6*35 and rapid metabolism (DM/DX <0.003) was evident (data not shown), further supporting the association of the −1584G polymorphism with more rapid metabolism.

In conclusion, the −1584C→G polymorphism rules out poor metabolizer status, identifies additional important ethnic differences, and therefore is a valuable addition to CYP2D6 genotyping strategies applied to Caucasian and African-American populations.

We thank Roger Gaedigk, PhD, for support with sequencing; Stephen Simon, PhD, for statistical analyses; and Ivy Hurwitz, PhD, for CYP2D6*4 upstream sequence data. This study was supported by Children’s Mercy Hospital Research Vision Core Laboratory Project Grant 01.4888 (to A.G.).

References

Rerevaluation of Formulas for Predicting Creatinine Clearance in Adults and Children, Using Compensated Creatinine Methods, Birgitté Wuyts,1 Dirk Bernard,2 Nele Van Den Noortgate,3 Johan Van De Walle,4 Bruno Van Vlem,3 Rita De Smet,3 Frank De Geeter,5 Raymond Vanholder,3 and Joris R. Delanghe1 (1 Department of Clinical Chemistry, Nephrology Section, Department of Internal Medicine, and 2 Department of Pediatrics, Ghent University Hospital, De Pintelaan 185, B9000 Gent, Belgium; Departments of 3 Clinical Chemistry and 5 Nuclear Medicine, AZ St Jan, Ruddershove 10, B8000 Brugge, Belgium; * author for correspondence: fax 32-9240-4985, e-mail joris.delanghe@rug.ac.be)

In clinical practice, glomerular filtration rate (GFR) is the most important marker for evaluation of renal function (1). Dosages of drugs that are eliminated by glomerular filtration are often based on GFR. At present, the most reliable methods for accurate assessment of overall GFR require intravenous administration of exogenous compounds and are both cumbersome and expensive. In clinical practice, creatinine clearance (CrCl) is widely accepted as a simple measure of GFR. However, CrCl systematically overestimates GFR because creatinine is freely filtered by the glomerulus and is also secreted by
the proximal tubule. In the earliest methods, serum creatinine was assayed by the Jaffe reaction after deproteinization, eliminating the pseudo-chromogen effect of proteins (2). Similarly, the first automated methods used dialysis membranes to prevent interference from plasma proteins. Today, however, analyzers use undiluted serum and plasma, making them subject to the so-called “protein error” (3). This produces a positive difference of ~27 μmol/L creatinine compared with HPLC methods (4–7). Because urine contains relatively little or no protein, the protein error affects only creatinine determinations in serum. Therefore, CrCl is underestimated when creatinine methods affected by protein error are used. This underestimation has been stated to be compensated by the overestimation attributable to tubular secretion of creatinine. However, studies confirming this statement are lacking.

In compensated Jaffe methods, the values assigned to the calibrator set point are adjusted to minimize the pseudo-creatinine contribution of proteins. The result is that compensated methods produce lower creatinine values. Alternatively, the protein error can be avoided by use of enzymatic creatinine methods. Collection of timed urine for CrCl is often a major source of error; therefore, simple formulas have been introduced to estimate GFR based on serum creatinine concentration, age, gender, body weight, and body length (8–13); these formulas thus do not require urine collections. However, it is not always clear which creatinine method was used when applying these formulas.

We examined 80 patients [33 males (age range, 51–74 years) and 47 females (age range, 56–81 years)] referred for nuclear medicine clearance (51Cr-EDTA clearance) before chemotherapy because of renal insufficiency or for nephrologic evaluation (geriatric patients). We also examined 27 pediatric patients [15 males (7–17 years) and 12 females (6–17 years)], in whom inulin clearance had been determined for nephrologic evaluation of a nephroblastoma or because they had received nephrotoxic drugs. Clearance determinations succeeded in 23 children. In 4 children, urine collections were inadequate.

The elimination rate of 51Cr-EDTA was measured according to the methods of Chattler and coworkers (14, 15) and Van de Wiele et al. (16). Inulin clearance was determined by an enzymatic assay (17). Serum creatinine was measured by a standard HPLC method (18). Serum and urinary creatinine were measured on a Modular P analyzer with commercial reagents (Roche): (a) a kinetic rate-blanked Jaffe assay based on the modified kinetic alkaline picrate method (19); (b) a kinetic rate-blanked Jaffe compensated assay for reactive proteins according to the manufacturer’s instructions; (c) an enzymatic assay using the Creatinine Plus method (20–22). Serum total protein, albumin, urea nitrogen, uric acid, and total bilirubin were measured with use of commercial reagents (Roche).

CrCls were calculated according to the formula: \(UV/PT\), where \(U\) represents the urinary creatinine concentration (μmol/L), \(V\) is urinary collection volume (mL), \(P\) is serum creatinine concentration (μmol/L), and \(T\) is urinary collection time (1440 min). In children, CrCl values were corrected for body surface. CrCl values were also calculated according to the Cockcroft–Gault method (8) and the abbreviated Modification of Diet in Renal Disease Study (MDRD) method (9, 10) in adults and the method of Schwartz and coworkers (11–13) in children.

Values are expressed as the median (interquartile range). Methods were compared using the Pearson correlation coefficient. Correlation studies were performed according to Bland and Altman (23).

Median serum creatinine concentrations in adults were 183.2 (84.8–204.5) μmol/L by HPLC, 173.8 (72.3–207.7) μmol/L by the enzymatic assay, 178.2 (89.1–213.7) μmol/L by the uncompensated Jaffe method, and 174.7 (71.6–207.3) μmol/L by the compensated Jaffe method. Linear regression equations for serum creatinine in adults were as follows:

- **Enzymatic method** \((y)\) vs HPLC \((x)\): \(y = 0.96x - 2.47\) (μmol/L) \((r = 0.98)\)
- **Compensated Jaffe** \((y)\) vs uncompensated Jaffe method \((x)\): \(y = 0.95x + 0.44\) (μmol/L) \((r = 0.98)\)
- **Uncompensated Jaffe method** \((y)\) vs HPLC \((x)\): \(y = 0.84x + 24.94\) (μmol/L) \((r = 0.98)\).

The equations demonstrate an overestimation of serum creatinine by the uncompensated Jaffe method in the range <155 μmol/L and an underestimation in the higher range compared with the HPLC serum creatinine results. (Additional tables and figures are available as a Data Supplement accompanying the online version of this Technical Brief at http://wwwclinchemorg/contentvol49issue6/).

Effects of patient variables (gender, age, and body mass index) and concentrations of uric acid, bilirubin, total protein, albumin, and creatinine on the differences between creatinine methods were studied in detail. In a multivariate regression model, we found a highly significant correlation \((P < 0.0001)\) only between the serum uncompensated Jaffe creatinine concentration and the difference between uncompensated Jaffe and enzymatic serum creatinine concentrations (Fig. 1A). This difference between the uncompensated Jaffe and enzymatic creatinine methods was age dependent when children and adults were examined together (Fig. 1B). In the overall group of adults, we observed no impact of patient variables, including age, or of the concentrations of other substances on the difference between the creatinine methods. However, in a subgroup of nephrotic patients \((n = 9)\) presenting with extremely low serum protein concentrations (<50 g/L), we observed a smaller positive difference attributable to pseudo-chromogens (median, 15 μmol/L; interquartile range, 12–19 μmol/L; \(P < 0.05)\).

The median (interquartile range) CrCl values in adults \((n = 80)\) were 43.4 (15.3–74.2) μmol/L for the HPLC, 49.6 (15.3–76.7) μmol/L for the enzymatic, 37.5 (14.9–56.8) μmol/L for the uncompensated Jaffe, and 48.0 (15.8–74.6)
μmol/L for the compensated Jaffe creatinine methods. The linear regression statistics are shown in Table 1.

Cockcroft–Gault estimates of clearance in adults (n = 80) produced median (interquartile range) values of 48.3 (24.5–69.5) μmol/L for the HPLC, 55.0 (24.4–78.7) μmol/L for the enzymatic, 46.0 (23.2–64.5) μmol/L for the uncompensated Jaffe, and 46.0 (23.2–64.5) μmol/L for the compensated Jaffe methods. The linear regression statistics are shown in Table 1. Abbreviated MDRD estimated clearance in adults (n = 80) produced median (interquartile range) values of 50.3 (25.9–74.8) μmol/L for the HPLC, 58.7 (26.1–92.4) μmol/L for the enzymatic, 47.1 (26.0–71.8) μmol/L for the uncompensated Jaffe, and 56.3 (25.7–88.7) μmol/L for the compensated Jaffe methods. The linear regression statistics are shown in Table 1.

Schwartz estimated clearance in children (n = 23) produced median (interquartile range) values of 173.8 (127.8–193.7) μmol/L for the enzymatic, 108.4 (87.1–114.8) μmol/L for the uncompensated Jaffe, and 169.5 (116.3–179.9) μmol/L for the compensated Jaffe methods. For the inulin clearance, the median (interquartile range) was 123.1 (97.3–152.8) mL/min (n = 23). Linear regression equations are shown in Table 1 (n = 23).

Median GFRs estimated by the Cockcroft–Gault equation varied by as much as 18%, depending on the creatinine method used. Similarly, median GFRs estimated by the MDRD equation varied as much as 20%. In adults, the use of the enzymatic method produced the highest estimated GFR and the uncompensated Jaffe the lowest regardless of the equation used.

We observed marked differences among the various methods for serum creatinine. Because they were affected by the protein error, uncompensated Jaffe methods produced higher serum creatinine results, whereas the results obtained with the compensated Jaffe, enzymatic, and HPLC creatinine methods were comparable. The difference between the uncompensated Jaffe and the enzymatic method depended mainly on the underlying concentration of serum creatinine. We observed no impact of patient variables or other substances. Because of their lower serum creatinine concentrations, we observed a relatively higher difference between the uncompensated Jaffe and enzymatic serum creatinine methods in children. In infants, who generally present with a higher protein error and even lower serum creatinine concentrations, a larger difference between these two creatinine methods is to be expected.

In adults, the uncompensated Jaffe CrCl was lower than the $^{51}$Cr-EDTA clearance. In contrast, enzymatic and compensated Jaffe CrCl values were slightly higher,

### Table 1. Linear regression statistics for the various creatinine methods.

<table>
<thead>
<tr>
<th>Creatinine¹</th>
<th>$x$</th>
<th>Slope</th>
<th>Intercept, mL/min</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>$^{51}$Cr-EDTA</td>
<td>0.88</td>
<td>9.77</td>
<td>0.87</td>
</tr>
<tr>
<td>Enzymatic</td>
<td>$^{51}$Cr-EDTA</td>
<td>1.06</td>
<td>−1.99</td>
<td>0.87</td>
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<tr>
<td>Compensated Jaffe</td>
<td>$^{51}$Cr-EDTA</td>
<td>1.00</td>
<td>−0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>Uncompensated Jaffe</td>
<td>$^{51}$Cr-EDTA</td>
<td>0.69</td>
<td>3.96</td>
<td>0.82</td>
</tr>
<tr>
<td>Cockcroft–Gault algorithm²</td>
<td>HPLC</td>
<td>$^{51}$Cr-EDTA</td>
<td>0.96</td>
<td>1.93</td>
</tr>
<tr>
<td>Enzymatic</td>
<td>$^{51}$Cr-EDTA</td>
<td>1.06</td>
<td>2.77</td>
<td>0.90</td>
</tr>
<tr>
<td>Compensated Jaffe</td>
<td>$^{51}$Cr-EDTA</td>
<td>0.99</td>
<td>4.61</td>
<td>0.90</td>
</tr>
<tr>
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<td>$^{51}$Cr-EDTA</td>
<td>0.74</td>
<td>9.75</td>
<td>0.87</td>
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<tr>
<td>Abbreviated MDRD³</td>
<td>HPLC</td>
<td>$^{51}$Cr-EDTA</td>
<td>0.92</td>
<td>5.32</td>
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<tr>
<td>Enzymatic</td>
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<td>1.21</td>
<td>−0.45</td>
<td>0.86</td>
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<tr>
<td>Compensated Jaffe</td>
<td>$^{51}$Cr-EDTA</td>
<td>1.12</td>
<td>1.67</td>
<td>0.86</td>
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<tr>
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<td>$^{51}$Cr-EDTA</td>
<td>0.80</td>
<td>7.99</td>
<td>0.85</td>
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<tr>
<td>Schwartz algorithm⁴</td>
<td>Inulin</td>
<td>1.14</td>
<td>18.89</td>
<td>0.76</td>
</tr>
<tr>
<td>Enzymatic</td>
<td>Inulin</td>
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<td>18.73</td>
<td>0.65</td>
</tr>
<tr>
<td>Compensated Jaffe</td>
<td>Inulin</td>
<td>0.46</td>
<td>46.62</td>
<td>0.64</td>
</tr>
</tbody>
</table>

¹ $y = $ clearance (mL/min) based on the method specified.
² In adults (n = 80).
³ In children (n = 23).
which is attributable to a relatively small tubular secretion of creatinine (24, 25). The Cockcroft–Gault and abbreviated MDRD algorithms for calculating GFR correlated closely with $^{51}$Cr-EDTA clearance when calculated with the creatinine results from the HPLC, enzymatic, and rate-blanked compensated Jaffe methods. However, the results obtained with the same algorithms were lower than the $^{51}$Cr-EDTA clearance when based on uncompensated Jaffe test results. The abbreviated MDRD and Cockcroft–Gault equations correlated well.

The median GFRs obtained with the Schwartz equation varied by 39%, depending on the creatinine method used. In children, the use of the enzymatic method produced the highest estimated GFR and the uncompensated Jaffe the lowest.

In children, practical problems in timed urine collection have contributed largely to the widespread use of calculated CrCl values based on serum or plasma creatinine concentration and body length. In contrast to the results for adults, the results obtained with Schwartz CrCl values in children and infants are significantly higher (in our series up to twofold higher in 4 of 23 cases) than inulin clearances when the compensated Jaffe or enzymatic creatinine method is used. If the uncompensated Jaffe test is used, the negative analytical effect of the protein error on CrCl is countered by the positive physiologic effect of the relatively more important tubular secretion of creatinine. Because serum creatinine values are lower in children, especially between ages 1 and 3 years, relative differences between compensated and uncompensated creatinine methods are very important.

Care should be taken when using estimated GFRs based on CrCl algorithms for drug administration, in particular for drugs such as cis-platinum and aminoglycoside antibiotics. In the example of the cytostatic drug cis-platinum, it is recommended to administer one-half the dose when CrCl decreases to <60 mL/min.

In conclusion, because collection of timed urine is cumbersome and susceptible to errors, calculated GFRs (Cockcroft–Gault and MDRD algorithms in adults and Schwartz algorithm in children) are often used. However, care should be taken in the choice of the serum creatinine method when applying these formulas.

We wish to thank Dr. G. Klein, Prof. W. Hoelzel, and Dr. Engel (Roche) for kindly providing the diagnostic reagent sets for this study.

References

Multiple Lipoprotein Abnormalities Associated with Insulin Resistance in Healthy Volunteers Are Identified by the Vertical Auto Profile-II Methodology, James W. Chu,1 Fahim Abbasi,1 Krishnaji R. Kulkarni,2 Cynthia Lamentola,1 Tracey L. McLaughlin,1 Janet N. Scalisi,2 and Gerald M. Reaven3 (1 Stanford University School of Medicine, Department of Medicine, Stanford, CA 94305; 2 Atherotech, Birmingham, AL 35211; * address correspondence to this author at: Falk Cardiovascular Research Center, Stanford University Medical Center, Stanford, CA 94305; fax 650-725-1599, e-mail greaven@cvmed.stanford.edu)

The lipoprotein abnormalities first associated with insulin resistance (IR) and compensatory hyperinsulinemia were high plasma triglyceride (TG) and low HDL-cholesterol