

Plasma Cystatin C Is Superior to 24-h Creatinine Clearance and Plasma Creatinine for Estimation of Glomerular Filtration Rate 3 Months after Kidney Transplantation

To the Editor:

In a recent report (1), we provided data on the early follow-up of renal function by plasma cystatin C in transplanted adults. Cystatin C was found to be an alternative and more sensitive marker of acute changes in glomerular filtration rate (GFR) than plasma creatinine, especially during acute rejection episodes. We present here some additional data on the same group of patients 3 months after surgery ($n = 25$; 5 patients moved to another institution). We measured GFR by the reference ^{51}Cr -labeled EDTA clearance performed over five 30-min periods starting 1 h after injection of 50–100 μCi of ^{51}Cr -labeled EDTA, with all individual results normalized for body surface area. Plasma cystatin C was determined by a latex particle-enhanced immunonephelometric assay (BN100; Dade-Behring) as described in detail previously (1). Plasma and urinary creatinine were measured by the Jaffé reaction on an Hitachi 747 analyzer (Boehringer); 24-h creatinine clearance was calculated from plasma and 24-h urinary creatinine. The accuracy of plasma markers to estimate GFR was calculated from the relative increases in their plasma concentrations vs their upper reference limits (creatinine, females, 100 $\mu\text{mol/L}$; males, 109 $\mu\text{mol/L}$; cystatin C, 0.94 mg/L) (1) and the relative decrease in ^{51}Cr -labeled EDTA-measured GFR vs the lower limit of 80 $\text{mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$. This widely used threshold value of GFR (2) was preferred to age- and sex-matched reference values because of the relatively limited number of patients. Results are presented as the median and range after they were checked for non-gaussian distribution. Statistical analysis was performed with SigmaStat (Jandel Scientific), using linear regression analysis and the Kruskal–Wallis test; $P < 0.05$ was considered significant.

Three months after surgery, GFR

measured by the ^{51}Cr -labeled EDTA clearance was 31–97 $\text{mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$ (median, 49 $\cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$). Renal function was classified as severely impaired ($< 40 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$; $n = 5$), impaired ($40 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2} < \text{GFR} < 50 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$; $n = 8$), moderately impaired ($50 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2} < \text{GFR} < 80 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$; $n = 11$), or normal ($> 80 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$; $n = 1$). Results of 24-h creatinine clearance significantly correlated with those of ^{51}Cr -labeled EDTA clearance ($r = 0.874$; $P < 0.0001$) but overestimated GFR by $\sim 40\%$ ($P < 0.0001$), with six (24%) false-negative (misleadingly normal) results in patients in the 60–80 $\text{mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$ range by EDTA clearance. The reciprocal of plasma creatinine significantly correlated with ^{51}Cr -labeled EDTA clearance ($r = 0.784$; $P < 0.0001$), but plasma creatinine overestimated GFR by 30% ($P < 0.0001$) with seven (28%) false negatives (48, 54, and $> 60 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$; Fig. 1). The reciprocal of plasma cystatin sig-

nificantly correlated with ^{51}Cr -labeled EDTA clearance ($r = 0.879$; $P < 0.0001$). GFR could be estimated from plasma cystatin C according to the following formula: $\text{GFR} (\text{mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}) = 78 \times (1/\text{cystatin C, in mg/L}) + 4$. Overall, plasma cystatin C underestimated GFR by 14% (-21% to $+47\%$; $P < 0.001$) with no false negatives (Fig. 1).

In our institution, GFR is measured 3 months after transplantation by the ^{51}Cr -labeled EDTA clearance. This method is time-consuming and expensive, but it provides an accurate measurement of GFR at the beginning of the long-term follow-up period of the transplant recipient (> 3 months post surgery). Despite their strong correlation with ^{51}Cr -labeled EDTA clearance, we as others (2), confirm the poor abilities of 24-h creatinine clearance and plasma creatinine to evaluate GFR, a phenomenon that has been attributed to creatinine tubular secretion (2). In the present study, the use of creatinine to monitor GFR led to gross overestimation of GFR (30–40%). The diag-

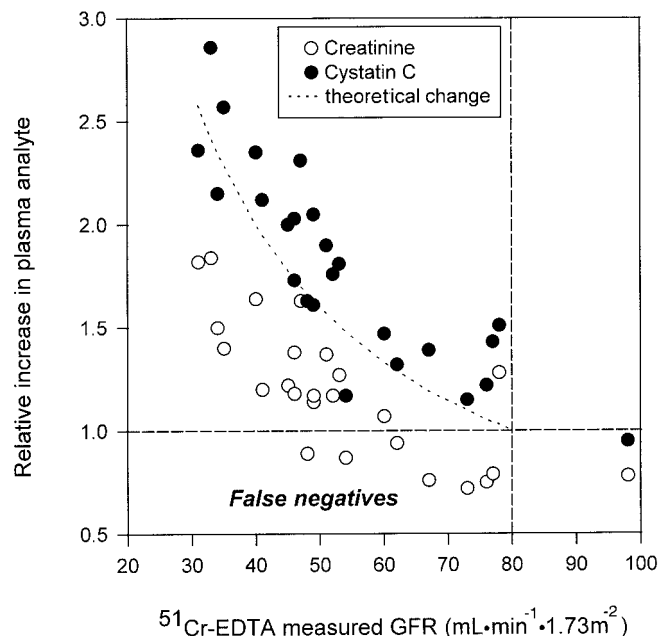


Fig. 1. Accuracy of plasma markers cystatin C and creatinine to estimate GFR in transplanted patients.

GFR was measured by the ^{51}Cr -labeled EDTA clearance (lower reference limit, 80 $\text{mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$) in 25 kidney transplant patients 3 months after surgery. Changes in plasma cystatin C and creatinine concentrations are presented as the relative increase vs the upper reference limit (creatinine, females, 100 $\mu\text{mol/L}$; males, 109 $\mu\text{mol/L}$; cystatin C, 0.94 mg/L) (1). Horizontal and vertical dashed lines represent the upper and lower reference limits, respectively.

nostic value was also poor, with ~25% false negatives. By contrast, plasma cystatin C better reflected GFR with the strongest correlation with ^{51}Cr -labeled EDTA clearance and no false negatives.

In a group of adult transplant recipients at steady-state renal function (average time since transplantation, 6 years), cystatin C was shown to have a better diagnostic value than serum creatinine for a GFR cutoff set at 60 mL/min (3). Despite a similar diagnostic value, 24-h creatinine clearance overestimated GFR by >20% in two-third of patients (3). The underestimation of GFR by plasma cystatin C we report here might be attributable to an inappropriate cutoff for cystatin C (too low), renal function not at steady state, or other unknown factors. Underestimation of GFR by serum cystatin C recently has been reported in pediatric transplantation (4). Serum cystatin C concentrations were 25% higher (range, 19–31%) in transplanted children vs non-transplanted children having the same GFR determined by inulin clearance (4). Interference with the cystatin C assay, tubular back-leakage of intact cystatin C into the circulation, or impaired filtration of antibody-cystatin C complexes have been suggested without definitive proof (4).

Despite the underestimation of GFR, plasma cystatin C appears superior to creatinine and 24-h creatinine clearance for evaluation of GFR in the postoperative follow-up of adult kidney transplant recipients. Moreover, GFR can be rapidly estimated from the reciprocal of the plasma cystatin C concentration, using a simple formula independent of age, body surface, and sex of the recipient.

References

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Falsely Increased Values for Rabbit Immunoglobulin-based Nephelometric Immunoassays Attributable to Human Anti-Rabbit Antibodies

To the Editor:

A large number of serum proteins are routinely measured by automated immunonephelometric assays utilizing antisera derived from rabbits, goats, or sheep. Because this type of immunoassay uses polyclonal reagents derived from animals other than mice, it is free from interference by the human anti-mouse antibodies that commonly plague monoclonal antibody-based sandwich assays and competitive immunoassays (1). Nonspecific antibodies against murine immunoglobulins can be detected in the serum of a significant proportion of patients, with a prevalence estimate as high as

80% (2). The incidence of anti-rabbit antibodies in serum samples is considerably lower, with estimates between 0.1% and 5% (1, 3–6). Interference from human anti-rabbit antibodies has been documented for two-site immunoassays and radioimmunoassays for several hormone assays as well as for creatine kinase MB (3–8). It recently was reported that anti-rabbit antibodies produced falsely increased C-reactive protein values when measured by nephelometric methods utilizing rabbit reagents (9). In this study, two patients developed anti-rabbit antibodies during treatment with rabbit anti-lymphocyte globulin for immunosuppression. Interestingly, other nephelometric assays utilizing rabbit antisera such as IgG and albumin were not affected, indicating that the interference was unique to the C-reactive protein assay (9).

Our clinical laboratory recently identified a sample with an abnormally high serum transthyretin value of 1406 mg/L (reference interval, 200–400 mg/L) that was being requested for assessment of nutritional status. The serum sample was analyzed by nephelometry using the automated BN II analyzer (Dade Behring), which uses rabbit anti-transthyretin antibodies. Other samples that were subsequently submitted for testing had transthyretin values of 1038–1107 mg/L (Table 1, specimens A–D). The increased transthyretin values could not be explained by the patient's clinical history or medications. The patient was a 56-year-old female, status post chemotherapy for malignant B-cell lymphoma with an IgM monoclonal protein of 15 g/L. She was taking erythropoietin and furosemide. To investigate the presence of an interfering substance, transthyretin was remeasured using the Array automated nephelometer (Beckman Coulter). Results were considerably lower on reanalysis and were slightly increased or within the reference interval (Table 1). Because the Array uses antisera derived from goats whereas the BN II uses antisera from rabbits, the interference may have been attributable to anti-rabbit antibodies.