To the Editor:

Cystatin C (CST3), which belongs to the type II cystatin family, is a nonglycosylated 13-kDa protein constitutively secreted shortly after its synthesis (1). Because its low molecular mass and its positive charge at physiologic pH allow it to be freely filtered by kidney glomeruli and because CST3 normally is reabsorbed and then almost completely catabolized by proximal tubular cells (2), CST3 that was not metabolized is eliminated in urine and may represent a useful marker of tubular injury (3) or renal tubular dysfunction (4).

The quantitative CST3 assay we developed is highly reliable and may be used as a part of the standard screening panel for renal failure. However, the use of 24-h urine samples is time-consuming and fails to offer clinically relevant data regarding tubular status in emergency cases. To evaluate whether a full 24-h collection is necessary, we analyzed, over a 24-h period, CST3 urinary release to see whether circadian variations were evident.

We recruited 11 healthy individuals and collected urine samples every 2 h during a 24-h period. These volunteers were active during this period and ate fixed amounts of nutrients at 7, 12, and 20 h. To avoid any CST3 proteolytic degradation, urine collection was performed with an inhibitory mixture containing either antiprotease or antimicrobial agents, which was added directly into the collection container to allow immediate action on freshly voided urine. This inhibitory cocktail, originally described by Tencer et al. (5), contained 0.2 mol/L benzamidinium chloride, 0.9 mol/L disodium EDTA, 0.5 mol/L sodium azide, and 1.7 mol/L tris-(hydroxymethyl)-aminomethane (all reagents from Sigma). Each sample was then frozen at −20 °C until CST3 quantification.

Urine CST3 was measured by the N latex Cystatin C assay (Dade Behring) with slight modifications to improve its sensitivity. The assay was originally designed for CST3 serum analysis and has a limit of detection of 0.17 mg/L. In plasma or serum, two subsequent dilutions of the sample (first 1:5 and then 1:20) were used to give a final dilution of 1:100 at the time of CST3/anti-CST3 reaction. The analyzer program was modified to allow the quantification of urinary CST3, which typically is present in concentrations 10-fold lower than in plasma. To increase sensitivity, we suppressed the second dilution, which allowed CST3 detection at concentrations close to 0.03 mg/L. That was the only modification we made on the nephelometer, using the same calibrators and controls as for plasma determinations. All CST3 quantifications were performed the same day on the same BNII instrument with the same batch of anti-CST3 reagent.

Urine CST3 values during the 24-h period were within the reference interval for all 11 healthy volunteers [reference interval, 0.03–0.18 mg/L, determined from 69 healthy patients; similar to the range described by Löfberg and Grubb (6), who developed the first CST3 assay]. As shown in Fig. 1A, no apparent circadian variation was evident in our cohort. Either the nonparametric Kruskal–Wallis test or one-way ANOVA was performed. Although we observed a peak tendency and smaller increases at 8, 14, and 22 h, respectively, suggesting the existence of a postprandial effect on tubular catabolism of CST3, these differences did not appear to be statistically significant (Kruskal–Wallis, P = 0.9558; one-way ANOVA, P = 0.9875). The absence of circadian rhythm was further analyzed by the population mean Cosinor method (7), which showed no large fluctuations in the concentrations of CST3 excreted in urine (P = 0.54; Fig. 1B). Altogether, the nonsignificance in both the ANOVA and Cosinor statistical tests confirmed that urinary CST3 is excreted without a circadian rhythm in our cohort of patients.

Because no circadian rhythm is evident in healthy individuals, we can assume that if renal tubules are freely functional and catabolize the majority of freely filtered CST3, only small amounts of CST3 would be found in urine. Hence, the presence of increased CST3 concentrations in single-void urine samples would directly reflect tubular damage.

CST3 turns out to be a particularly interesting marker for the nephrologist because (a) its plasma concentration reflects, with excellent sensitivity and specificity, the glomerular filtration rate, and (b) its urinary quantification seems to be a good reflection of proximal tubular function.
As a marker of tubular disruption, urine CST3 concentrations can be measured in hospitalized patients as well as in emergency situations in which acute renal failure is suspected. In the latter cases, collection of 24-h urine is impossible because physicians need rapid laboratory results for immediate patient staging.

The absence of a circadian rhythm for CST3 allows its quantification in a single urine sample. As its automatic measurement allows a result in less than 1 h, both plasma/serum and urine CST3 could be used as standard markers for assessment of renal function.

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References


Pseudoparaproteinemia after Iopamidol Infusion for Coronary Angiography

To the Editor:

Automated capillary zone electrophoresis (CZE) is increasingly used for separation of serum proteins in routine clinical laboratories (1). Detection of proteins is based on the absorbance of peptide bonds at 214 nm, in contrast to older, gel-based methods, which use dye binding.

A 62-year-old man with ischemic heart disease, type II diabetes, and chronic renal failure underwent coronary angiography and received 62 g (100 mL) of iopamidol (Jopamiron®; Bracco s.p.a) intraarterially during the procedure. Blood was taken ~6 h after angiography for serum protein electrophoresis (SPE). Analysis by CZE (Paragon CZE® 2000; Beckman Coulter®) showed a defined peak of “5.2 g/L” in the cathodal end of the α2-globulin region. Immunofixation on the same instrument failed to confirm this peak as an immunoglobulin, and assays for apoprotein-B100, haptoglobin, and ceruloplasmin were within reference values. Repeat CZE after 2 and 5 days showed a progressive decrease in peak size. This relatively rapid clearance suggested a nonprotein compound, which was confirmed by the absence of an abnormal band on agarose gel electrophoresis (AGE; Paragon® SPE Kit; Beckman Coulter).

To confirm the interference of iopamidol in the CZE analysis, we added iopamidol to serum aliquots to give concentrations comparable to