

Samples were stored at -20°C for less than 1 week before analysis. Urinary α_1 -microglobulin was undetectable in 26 samples, in keeping with the observations of Jung et al. (5). The upper reference limit was 1.5 mg/mmol of creatinine, in close agreement with others (5–7), although somewhat higher than that derived by Tencer et al. (8). All patients and healthy volunteers had the study explained to them and gave informed consent. The study had full approval of the local research ethics committee.

Some differences were observed in measured α_1 -microglobulin concentrations in both untreated (Friedman statistic, 59.2; $P < 0.0001$) and neutralized urines (Friedman statistic, 81.4; $P < 0.0001$; Table 1). This was attributable to significant changes in treatment groups 1, 2, 4, 8, 9, 1A, 2A, 7A, and 9A as a consequence of between-assay variation (although the assay remained in control throughout the study). The median differences observed were <4 mg/L under all storage conditions. However, two specimens did show appreciable losses ($>40\%$) at -20°C irrespective of neutralization. The urinary α_1 -microglobulin concentration was stable at -80°C for up to 6 months irrespective of whether samples were neutralized.

Our data are consistent with those of Tencer et al. (2) in suggesting that α_1 -microglobulin is stable in urine for at least 24 h at room temperature and up to 1 month at 4°C . Ideally, however, we would recommend that longer-term storage (beyond 2 months) should be at -80°C . In our hands, α_1 -microglobulin demonstrated excellent stability in human urine ex vivo, and neutralization was not necessary to ensure sample integrity. Consequently, samples can be conveniently handled by diagnostic laboratories with reasonable confidence that any increased excretion of α_1 -microglobulin will be detected.

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Serum Cystatin C as a Marker of Kidney Dysfunction in an Elderly Population

To the Editor:

The prevalence of end-stage renal disease is increasing worldwide. Because nephropathy induced by type 2 diabetes accounts for most of the

increase, a growing proportion of the patients are elderly. As preventive and renoprotective interventions are available, early identification of nephropathy is crucial, and there is a growing demand for a clinically convenient and reliable marker of renal function. Serum creatinine is widely used as a marker of the glomerular filtration rate (GFR), but the influence of muscle mass and, hence, the considerable interindividual variability limit its usefulness, especially in elderly individuals (1). Screening for microalbuminuria is used in assessing incipient nephropathy in diabetic patients, but the intraindividual variation and the need for repetitive urine sampling make it impractical in a geriatric setting. Serum cystatin C has been claimed to be a more sensitive indicator of GFR than serum creatinine (2,3). It is unaffected by muscle mass, and it has been reported that cystatin C, unlike creatinine, might be able to mirror the involutional decrease in GFR that occurs with ageing (4,5). Separate reference intervals for the elderly have been proposed in a few studies (5–8), but the usefulness of cystatin C as a marker of renal function has not been extensively examined in large elderly populations.

In a cross-sectional epidemiologic study, we compared serum cystatin C, serum creatinine, and the urinary albumin/creatinine ratio (ACR) as markers of renal function in 1260 elderly residents (533 men and 727 women; mean age, 74 years; range, 64–100 years) in Lieto, Finland. After implementing strict criteria for exclusion, i.e., renal or urogenital disease (reported in the medical history or detected by urine dipstick tests or ACR >2 mg/mmol), diabetes, hypertension, or use of glucocorticoids or angiotensin-converting enzyme inhibitors, a reference sample group ($n = 315$; 143 men and 172 women; mean age, 72.2 years; range, 65–94 years) was identified, and regression-based age-dependent reference intervals were calculated for cystatin C.

Cystatin C was determined using a particle-enhanced nephelometric immunoassay (PENIA) method (N La-

tex Cystatin C on the BN II System; Dade Behring) (3). Serum and urinary creatinine were measured using the Jaffe reaction. Urinary albumin was analyzed by an immunoturbidimetric method (Optima Microalbuminuria Kit; Thermo Clinical Lab-system). The study was approved by the Joint Commission of Ethics for the Hospital District of Varsinais-Suomi, Finland.

Statistical analyses were performed using SPSS 10.0 software (SPSS Inc.), except for the regression-based estimation of reference limits (SAS 8.1 software; SAS Institute). *P* values for the between-gender differences were derived by the Kolmogorov-Smirnov two-sample test. Correlation coefficients were calculated using the nonparametric Spearman correlation. A gaussian distribution was accepted if the skewness and kurtosis coefficients were between -1 and 1. The age-related reference limits and their confidence intervals were calculated as described by Virtanen et al. (9) and used by Suominen et al. (10). The reference limits for serum creatinine were calculated by a parametric method using GraphROC for Windows software (11).

For cystatin C, no between-gender difference was found in the study population ($P = 0.776$), whereas the association with gender was highly significant for creatinine ($P < 0.0001$) and borderline for the ACR ($P = 0.048$). The correlation between cystatin C and creatinine was significant in both the study population ($r = 0.555$; $P < 0.001$) and the reference sample group ($r = 0.396$; $P < 0.001$), the lower coefficient of correlation probably reflecting the ability of creatinine to detect only fairly gross impairment of GFR. Cystatin C correlated significantly with age ($r = 0.453$; $P < 0.001$) in the study population, as did creatinine ($r = 0.123$; $P < 0.001$) and ACR ($r = 0.273$; $P < 0.001$), but in the reference group the correlation reached significance only for cystatin C ($r = 0.420$; $P < 0.001$; Fig. 1). In the study population, the correlation between cystatin C and ACR was fairly strong ($r = 0.174$; $P < 0.001$), whereas that be-

tween creatinine and ACR was less distinct ($r = 0.075$; $P = 0.009$).

Microalbuminuria was, for practical reasons, assessed by ACR measured from one early-morning urine sample. However, the more distinct association observed between cystatin C and an increased ACR possibly supports the notion that cystatin C, rather than creatinine, might be a useful marker for slight decreases in GFR.

Because a consistent age-dependent increase in cystatin C values was observed (Fig. 1), regression-based reference intervals were constructed. The 95% reference limits and their respective 95% confidence intervals were 0.60 (0.57–0.63) to 1.30 (1.26–1.33) mg/L for the age group 65–74 years ($n = 234$) and 0.70 (0.68–0.73) to 1.47 (1.41–1.53) mg/L for the age group 75–85 years ($n = 68$). The age group >85 years ($n = 13$) was too small to give separate reference limits. For serum creatinine, no significant age-related dependency was observed, and conventional reference limits were calculated for women (64–104 mmol; $n = 172$) and men (72–118 mmol; $n = 143$).

Because of the differing analytical techniques, calibration, antisera, measuring principles, and age distributions in the elderly populations examined, it is problematic to make exact comparisons between the results of our study and those of previous ones. Galteau et al. (5), who also used the PENIA method, pro-

posed a lower reference interval (0.63–1.03 mg/L) for individuals >60 years of age. Their study included 92 nondrinkers and nonsmokers 60–79 years of age, whereas the reference sample group in our study was notably older and their smoking and drinking habits were not taken into account. In a study of 398 individuals 65–101 years of age, Finney et al. (6), who also used the PENIA method, suggested considerably higher reference limits, 0.93–2.68 mg/L for the age group 60–79 years and 1.07–3.35 mg/L for the age group ≥ 80 years. Exact exclusion criteria were not described, and they also suggested higher upper reference limits for serum creatinine (149 $\mu\text{mol/L}$ for women, 204 $\mu\text{mol/L}$ for men), which might indicate the inclusion of individuals with undiagnosed renal disease. Because there is no exact generally accepted definition of pathologic vs involutational changes in the ageing kidney (12), the most appropriate criteria for a reference group are difficult to identify, and the results are prone to some variation.

In conclusion, compared with serum creatinine, serum cystatin C is a more reliable marker of glomerular function in the elderly and offers a simple screening assay for the detection of early renal impairment in the ageing kidney.

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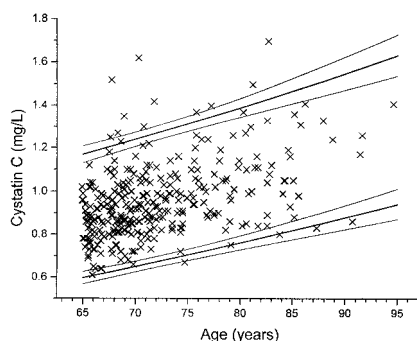


Fig. 1. Serum cystatin C concentrations as a function of age in the reference group.

The plot was produced using the regression-based method described by Virtanen et al. (9). The thick lines represent the upper and lower limits of the 95% reference interval, and the thin lines represent the 95% confidence intervals for these limits.

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