IgG Anti-Transglutaminase Autoantibodies in Systemic Lupus Erythematosus and Sjögren Syndrome

To the Editor:

In a recent issue of this journal, van der Sluijs Veer and Vermes (1) reported a high prevalence and concentration of IgG anti-tissue transglutaminase (anti-tTG) antibodies in patients with systemic lupus erythematosus (SLE) and increased anti-dsDNA antibodies, as well as in anti-SSA/SSB-positive patients. No increase was observed in patients with other autoantibodies, such as anti-Sm/RNP, anti-nucleolar, anti-histidyl-tRNA synthetase, anti-centromere protein B, anti-topoisomerase I, anti-proteinase 3, and IgM rheumatoid factor, or in patients with chronic inflammation. The authors proposed that in situations involving an imbalance between the supply and clearance of apoptotic bodies (as demonstrated in SLE), the immune system may detect intracellular tTG-substrate protein complexes, leading to an autoimmune response against the substrate and/or the tTG. Moreover, they argued that the difference in IgG anti-tTG concentrations between the SLE or anti-SSA/SSB-positive groups and the other autoimmune disease groups could probably depend on a different pathogenetic mechanism, and they concluded that IgG anti-tTG determination in anti-dsDNA-positive SLE or anti-SSA/SSB-positive patients might provide additional clinical information and have clinical value in the monitoring of these individuals.

In response to a subsequent comment by Di Tola et al. (2), these same authors reported that when they used a recombinant tTG prepared in a baculovirus system as antigen, no positivity for IgG anti-tTG was observed, whereas these autoantibodies were detected when they used a guinea pig extract. Our findings lend further support to this point, which may be diagnostically relevant.

In a large study designed to assess anti-tTG prevalence in autoimmune diseases other than celiac disease, we performed IgA and IgG anti-tTG autoantibody assays in 750 individuals, including 100 SLE and 100 Sjögren patients (85% anti-SSA positive, 53% anti-SSB positive), using a human recombinant antigen (rhEu-tTG; Eurospital). Only one SLE patient was positive for IgA anti-tTG (celiac disease was subsequently confirmed by intestinal biopsy), and only two SLE and one Sjögren patient had IgG anti-tTG antibody concentrations above the cutoff (42, 46, and 61 kilounits/L, respectively; reference value, <30 kilounits/L). These three patients are currently under investigation to determine whether their original autoimmune disease is associated with celiac disease. The mean IgG anti-tTG antibody values in patients with SLE (20.9 ± 6.8 kilounits/L) or Sjögren syndrome (11.9 ± 7.0 kilounits/L) were not significantly different from those observed in healthy individuals (16.8 ± 3.6 kilounits/L; P, not significant, Mann-Whitney test).

The results of our study confirm those obtained (and not yet published) by van der Sluijs Veer and Vermes when they used a recombinant antigen rather than the native antigen extracted from guinea pig liver (3). Many positive IgG anti-tTG cases detected by use of extractive substrates are most likely false positives directed against contaminating antigens or tTG-substrate proteins (e.g., actin, keratin, histones, myosin, troponin, or tubulin). Indeed, antibodies to these proteins are also detectable in other autoimmune diseases in which, like SLE and Sjögren syndrome, marked B-polyclonal activation is present (4).

Thus, our data and those of van der Sluijs Veer and Vermes clearly show that the use of antigen extracted from guinea pig liver may be the cause of false-positive reactions, especially in samples from patients with autoimmune disease; indeed, the more recent diagnostic methods that use human recombinant antigens not only are equally sensitive, but also guarantee greater specificity.

References

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Practical Method for Estimating the Frequency of Specimen Mix-Up in Clinical Chemistry Laboratories

To the Editor:

Reporting the result of one patient for another as a consequence of specimen mix-up, mislabeled specimens, or misidentification of patients is a serious laboratory mistake with potentially catastrophic consequences. For brevity, all mistakes in specimen identification are hereafter referred to as “specimen mix-up”. Because it is assumed to be relatively frequent, specimen mix-up is usually considered one of the most important labo-
ratory blunders. Therefore, much has been done to try to prevent it, and several result verification methods, such as delta check (1) and multivariate delta check (2), have been developed to detect it.

However, most of the available information on the frequency of this problem is anecdotal. To our knowledge, no study has quantitatively measured the frequency of all mistakes in specimen identification in a large clinical chemistry laboratory. Furthermore, the results could probably not be generalized to other laboratories. To determine how often a specific specimen mix-up occurs in the core laboratory of Helsinki University Central Hospital, we developed a simple but accurate method to estimate the frequency of specimen mix-up and to test the method in practice. Although the results of our study may not be generalizable, our method can be used by anyone who is interested in checking the frequency of specimen mix-ups in a large clinical chemistry laboratory.

Our method consists of determining the ABO and RhD blood groups, hereafter referred to as “blood group”, of blood samples taken for other purposes and comparing the results with the previously known blood groups of each patient. The method assumes that the blood groups are known before the study, but this is not a major limitation because in many hospitals these groups are routinely determined in all patients, or at least in all patients in surgical wards. The blood groups of the patients to be studied could also be ascertained in the laboratory, independent of the second part of the study, the day before the study.

Not all cases of specimen mix-up will produce a discrepancy between the previously known blood group and the result of the new blood group determination, but the proportion of mix-ups that will produce a discrepancy can be estimated without a significant bias if the study population is homogeneous and the frequencies of blood groups in the study population are known. Specimen mix-up can be assumed to be a random process, or at least a process that is independent of the blood groups of the mixed-up specimens. Hence, the probability of detecting a mix-up is equal to the probability that two randomly selected samples from the study population will be of a different blood group.

We collected all blood samples taken for complete blood counts from selected wards during periods of 1 to 4 weeks. From this set of samples, we excluded the samples from those patients whose blood groups were not known beforehand. A total of 504 samples were included in the study. A routine blood group determination was then performed on these samples by laboratory technicians unaware of the previously known blood group. In addition, all other laboratory technicians and other persons who handled the samples were kept unaware of this study to prevent any bias. The ethics committee of our hospital approved the study.

The distribution of blood groups in our sample was very similar to that in the general Finnish population. The distributions did not differ in a statistically significant way (chi-squared test, \( P = 0.283 \)). Hence, we used in our calculations the frequencies of blood groups in the Finnish population. The frequencies of blood groups A pos, A neg, B pos, B neg, O pos, O neg, AB pos, and AB neg (pos, RhD-positive; neg, RhD-negative) are 38%, 6%, 15%, 2%, 27%, 4%, 7%, and 1%, respectively. For a specimen in each group, the probability that the blood group of another specimen will be different is 1 minus the frequency of that group. Therefore, the probability of discrepancy for all groups combined is the sum of the products of each frequency and 1 minus that frequency. On the basis of the frequencies of blood groups in the general Finnish population, the probability of discrepancy is 0.7496; for the frequencies of blood groups in our sample, the result was nearly the same, 0.7493. The observed frequency of mix-ups was the product of the probability of discrepancy and the true frequency of mix-ups. Hence, an unbiased estimate of the true frequency of mix-ups would be the observed frequency divided by this probability.

We did not detect any cases of discrepancy in blood group results. Therefore, we could not get a point estimate of the frequency of mix-ups in our hospital. We could, however, calculate the upper limit of a 95% confidence interval for the frequency of mix-ups. We first corrected \( n \) (\( n = \text{number of patients} \)) for the fact that our approach would reveal a mix-up with a probability of 0.7496. Therefore, our sample of 504 was equivalent to 504 \( \times 0.7496 = 378 \).

The probability of not detecting a mix-up in \( n \) cases is \( (1 - P_{\text{mix}})^n \), where \( P_{\text{mix}} \) is the probability of a mix-up. To obtain the upper limit of the confidence interval, we set this probability to \( 1 - 95\% = 0.05 \) and obtained the following equation:

\[
(1 - P_{\text{mix}})^n = 0.05
\]

This can be rewritten, by taking the \( n \)th root of each side, as:

\[
1 - P_{\text{mix}} = 0.05^{1/n}
\]

which is equivalent to:

\[
P_{\text{mix}} = 1 - 0.05^{1/n} \quad (3)
\]

For \( n = 378 \), \( P_{\text{mix}} = 0.00789 \approx 0.79\%. \) Hence, the 95% confidence interval for the frequency of specimen mix-ups in our hospital is 0.00–0.79%.

It is reasonable to assume that mix-ups do occur in our laboratory and that our sample size was too small to detect any. Whether it is worthwhile to collect a larger sample depends on how exactly we want to measure the frequency of mix-ups. If specimen mix-ups are observed, the 95% confidence interval for their frequency is the 95% confidence interval of the binomial distribution corresponding to the observed number of events (mix-ups) and the corrected sample size. The sample size is corrected by multiplying it by the probability of discrepancy obtained from the frequencies of blood groups in the patient population. The probability of discrepancy can be easily calculated, using our method, for any population and for any combination of mutually exclusive blood groups, not necessarily just the ABO and RhD.
blood groups. Two factors contribute to the probability of discrepancy and thus to the power of this method: the power will be higher if the number of blood groups is higher or if the frequencies are more similar.

References


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Guidelines and Recommendations in Laboratory Medicine

To the Editor:

Dr. Keffer (1) has provided a thought-provoking report on the noncompliance of physicians with most clinical practice guidelines (CPGs). He also refers to the Laboratory Medicine Practice Guidelines (LMPGs) produced by the National Academy of Clinical Biochemistry (NACB). As contributors to several LMPGs (2–4) and active participants in the NACB LMPG program, we offer some insights into the rationale of NACB and how the NACB LMPG process differs from that of CPGs authored by other professional societies. We discuss some reasons for the difficulty in assessing impact and suggest unrecognized impacts of these guidelines.

Most CPGs are aimed at physicians and have focused on clinical practices for a disease (e.g., asthma) or symptom (e.g., chest pain). By contrast, the intended audience for LMPGs includes not only physicians, but also clinical laboratorians and manufacturers of clinical assays. These guidelines include recommendations on the appropriateness of offering certain tests for particular clinical situations and for denying or limiting the availability of other assays. These changes and their effects may be difficult to document, but consensus recommendations can prompt manufacturers to construct new assays, such as the urine immunoassay for methylenedioxymethamphetamine (Ecstasy) as recommended (3).

Because of their potential impact, all NACB LMPGs are presented and thoroughly discussed in sessions at national meetings, and an estimation of the degree of consensus is sought. The proposed guidelines are also presented at other meetings where pertinent disciplines are represented (e.g., cardiologists, emergency medicine physicians, clinical toxicologists, and endocrinologists). This may be unlike some CPGs that are prepared and published by experts, but without open presentation.

Although traditional “evidence-based” documentation is noted for many NACB recommendations [e.g., randomized control trials (5,6)], other recommendations do not lend themselves to support by outcome studies. The NACB guideline for testing of newborns (7), for example, recommended that testing for alkaline phosphatase isoenzymes not be performed. It was impractical to perform a randomized trial to support this recommendation. In the NACB guideline for emergency toxicology testing (3), a 1-h turnaround time was recommended for reporting of test results. There are no outcome studies to show that this improves the clinical management of intoxicated patients or reduces length of stay in an emergency department. It is expected, nonetheless, that external expert opinion (in the consensus guidelines) will lend credence to the opinions of the local community of laboratory practitioners.

Dr. Keffer (1) attempted to show through a Medline search and other means that there are few, if any, published evaluations of the NACB guidelines. We suggest that the impact of guidelines can be better measured by citations in laboratory procedure manuals (in hospitals and commercial laboratories), manufacturers’ literature and product labeling, and internal documents used by industry to set performance requirements for their products. These occurrences are difficult to monitor, but we believe they are more representative of the value of NACB LMPGs.

Clinical and laboratory guidelines are reached by consensus-building and may not alter practice in most settings because they are already based, at least in part, on what most practitioners feel should be the standard of practice. That standard is largely established based on the collective current practical experiences of those practitioners. This concept has recently been addressed in the area of clinical practice in an editorial by van Walraven (8). Guidelines of this nature thus are likely to change practices more drastically at the fringes than they are to move the central tendencies of practitioners. Indeed, it is our opinion that they may not be accepted as guidelines in the mainstream of clinical practice unless they represent the practices already in use by the mainstream and that the mainstream often has little to change to be in compliance with these guidelines.

The alternative view is that just because everyone is adhering to a particular practice does not make it optimal. Eventually, widely used but antiquated tests and methods must be replaced with new ones. Ultimately, recommendations must be a balance between consensus and an evidence-based approach. However recommendations are derived, codification can document and firmly establish a standard from which to build.

Dissemination of guideline information requires improvement. The
NACB recently conducted an informal e-mail survey of 2596 foreign and domestic doctoral members of AACC and NACB concerning familiarity with and use of the NACB guidelines. Overall, only 148 replies were received. This low response rate alerted the NACB leadership to a potential need for wider dissemination of these guidelines, and efforts are underway to use the Internet and other means to facilitate this.

In the NACB survey, although the numbers were small, there was an indication that when the guidelines were used, they were used in ways that met the original objectives of NACB. Among 57 clinical laboratorians who reported that they had used the guidelines, 60% indicated that they specifically selected tests, reagent sets, or products that followed the guidelines; 21% indicated they told their vendor representatives what their companies had to do to be in compliance; and 14% indicated that they had modified a vendor’s procedure to be in compliance with the guidelines. Among 16 industry laboratorians who responded to industry-focused questions, 6 indicated that their companies used the guidelines in product design (such as sensitivity, specificity, and choice of analytes), and 8 indicated that their companies use the guidelines in customer education.

One of the reasons for these somewhat less than ideal survey results is that the first four NACB guidelines were presented during satellite meetings of the AACC annual meetings, whereas more recently, the guidelines have been presented at EduTrak sessions at the AACC meeting in addition to meetings of co-sponsoring medical societies. Moreover, the first four guidelines were published in monograph form only, whereas the latter guidelines have also been published in peer-reviewed journals (2, 5, 6). With the exception of the thyroid guidelines, of which >50 000 copies were distributed, <5000 copies of the monographs were usually printed. More recently, beginning with the guideline for cardiac markers (2), the NACB has posted preliminary versions of the guidelines on the NACB web page and invited e-mail commentaries. This combined approach has led to more widespread recognition of their existence and broader participation in formulating the recommendations made therein.

We applaud Dr. Keffer’s efforts in bringing this important issue to the forefront. The process of creating new guidelines warrants discussion to improve the products and assess their impact.

References

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α2-Microglobulin Is Stable in Human Urine ex Vivo

To the Editor:
Increased concentrations of urinary α2-microglobulin may imply proximal tubular damage (1). α2-Microglobulin has generally been considered to be stable in human urine (1, 2). Tencer et al. (2) observed good stability in 10 urine samples stored at room temperature for 7 days, at 4 °C for 30 days, and at −20 °C for 6 months. In contrast, Donaldson et al. (3) noted significant losses of α2-microglobulin in urine stored at −20 °C and that this problem was exacerbated in more acidic (pH <6.0) urines; they recommend that urine should be neutralized on receipt. The manufacturers of our assay recommend that urines be assayed fresh or stored at 4 °C for a period of less than 1 week and warn against freezing samples. Urine samples are often stored before batch analysis. To clarify the appropriate storage conditions for urinary α2-microglobulin, we studied stability under standardized conditions.

Random unpreserved urine samples were collected from 19 patients at a single nephrology clinic, and urinary pH was determined (mean pH 5.87; range, 5.08–6.85). Samples were then divided into two aliquots, one of which was neutralized (mean pH 7.58; range, 7.23–7.94) by dropwise addition of 5 mol/L NaOH. Within 6 h of collection, α2-microglobulin and creatinine were measured in both aliquots. Ten (~1 mL each) aliquots of both the untreated and neutralized urines were stored in capped polystyrene tubes at room temperature, 4 °C, −20 °C, or −80 °C.
for periods of time from 24 h to 6 months (Table 1). Before analysis, samples were equilibrated to room temperature, mixed by inversion, and left to settle under gravity for 5 min (i.e., not centrifuged). Each aliquot was subjected to only a single freeze-thaw cycle.

Urinary $\alpha_1$-microglobulin was measured by rate immunonephelometry on an Immage™ analyzer (Beckman Coulter Ltd.) using a goat anti-human $\alpha_1$-microglobulin antibody. The assay was standardized using the manufacturer’s calibrant (target value, 40.2 mg/L). The limit of detection of the assay was 4 mg/L. The within-day imprecision (CV) determined using 18 replicates of both a patient’s urine (mean value, 7.4 mg/L) and the manufacturer’s control material (mean value, 14.7 mg/L) was <2.0%. Between-day imprecision during the study period, determined with the manufacturer’s control material, was 3.7% ($n = 22$).

Urinary creatinine concentrations (mean, quartiles, as demonstrated by lower urinary creatinine concentrations (mean, 10.3 vs 9.9 mmol/L; $P < 0.01$). Results were therefore analyzed for differences between storage conditions, using two separate Friedman analyses of variance for the untreated and neutralized urine data sets. The Wilcoxon matched-pairs signed-rank test was used to test for differences within data sets by comparing each treated sample against its respective baseline sample (i.e., against either F or FA in Table 1). $P < 0.01$ was considered significant.

In the absence of a recognized reference preparation for urinary $\alpha_1$-microglobulin, assay commutability was demonstrated by estimating an upper reference limit (0.95 percentile) for the urinary $\alpha_1$-microglobulin/creatinine ratio, using a nonparametric approach (4). Random urine samples were collected from 80 healthy volunteers with no history of renal disease (52 females and 28 males; mean age, 39.1 years; median age, 40 years; range, 20–65 years).

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>n</th>
<th>Mean (95% CI), mg/L</th>
<th>Median (98.1% CI), mg/L</th>
<th>Range, mg/L</th>
<th>Mean difference vs F or FA (95% CI mean difference), mg/L</th>
<th>Median difference vs F or FA (98.1% CI mean difference), mg/L</th>
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<tr>
<td>F</td>
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<td>37.1 (20.0–54.2)</td>
<td>20.1 (11.8–56.5)</td>
<td>7.1–137</td>
<td>3.4 (1.4 to 5.5)</td>
<td>2.1 (0.7 to 5.9)</td>
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<td>24 h</td>
<td>19</td>
<td>40.5 (22.0–59.1)</td>
<td>21.3 (12.5–59.4)</td>
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<td>1 week</td>
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<td>37.0 (19.8–54.2)</td>
<td>19.4 (11.8–54.0)</td>
<td>6.5–134</td>
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<td>20.5 (11.7–66.5)</td>
<td>8.1–146</td>
<td>4.1 (1.4 to 6.8)</td>
<td>1.7 (0.8 to 6.9)</td>
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<td>8.4–145</td>
<td>3.0 (1.1 to 4.8)</td>
<td>2.2 (0.6 to 5.0)</td>
</tr>
<tr>
<td>10A 6 months</td>
<td>18</td>
<td>35.1 (18.6–51.7)</td>
<td>20.4 (11.0–49.7)</td>
<td>7.3–138</td>
<td>–0.5 (–2.7 to 1.6)</td>
<td>0.7 (0.0 to 1.3)</td>
</tr>
</tbody>
</table>

* Samples were initially assayed within 6 h of collection, both before (F) and after (FA) neutralization. They were reanalyzed after storage at room temperature, 4 °C, –20 °C, and –80 °C for periods of time from 24 h to 6 months, and each treatment was compared against its respective baseline sample (i.e., against F or FA).

* CI, confidence interval; RT, room temperature.

* $P < 0.01$ compared with treatment F.

* $P < 0.01$ compared with treatment FA.
Samples were stored at −20 °C for less than 1 week before analysis. Urinary α1-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 α1-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-assembly 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 α1-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 α1-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, α1-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 α1-microglobulin will be detected.

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References

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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 as assessing incipient nephropathy in diabetic patients, but the intrindividual 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-
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 Laboratory). 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 between 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, proposed a lower reference interval (0.63–1.03 mg/L) for individuals >60 years of age. Their study included 92 nondrinkers and smokers 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 μmol/L for women, 204 μmol/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 involutional 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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References

![Graph](image-url)

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