Kidney International, Vol. 47 (1995), pp. 312–318

Serum cystatin C measured by automated immunoassay: A more sensitive marker of changes in GFR than serum creatinine

DAVID J. NEWMAN, HANSA THAKKAR, ROBERT G. EDWARDS, MARTIN WILKIE, THOMAS WHITE, ANDERS O. GRUBB, and CHRISTOPHER P. PRICE

Departments of Clinical Biochemistry and Nephrology, London Hospital Medical College, London, England, United Kingdom, and Departments of Clinical Physiology and Clinical Chemistry, University of Lund, Sweden

Serum cystatin C measured by automated immunoassay: A more sensitive marker of changes in GFR than serum creatinine. Serum cystatin C has been suggested as a new marker of GFR. For the introduction of this marker into clinical use a rapid and automated method is required. We have developed and validated an assay for serum cystatin C using latex particle-enhanced immunoturbidimetry. Intra- and inter-assay precision were $< 3\%$ and $< 5\%$ across the assay range. Analytical recovery was $93 \pm 3.8\%$ and no lack of parallelism was demonstrated. Regression analysis of a method comparison with an enzyme-enhanced radial-immunodiffusion method, gave PETIA = 0.074 + 0.93 × SRID, $r = 0.98$, $N = 100$. Inter-assay precision profiles showed cystatin C was measured with two-fold better precision than creatinine on the same analyzer. Cystatin C measurement was neither interfered with by icterus nor by hemolysis. 1/cystatin C versus 1/creatinine concentrations gave $r = 0.67$, $N = 469$. Comparison of Cr EDTA GFR with 1/cystatin C and 1/creatinine gave $r = 0.81$ and 0.50, respectively, $N = 206$. Calculating diagnostic sensitivity for abnormal GFR showed cystatin C to be significantly ($P < 0.05$) more sensitive than creatinine (71.4 vs. 52.4%). Cystatin C measurement using PETIA technology can be automated on the same instruments used routinely for the measurement of creatinine and offers better analytical performance and probably improved clinical sensitivity as a screening test for early renal damage.

Cystatin C is a non-glycosylated 13 kD basic protein that is a member of the cystatin superfamily of cysteine protease inhibitors [1–3]. It is produced by all nucleated cells and its production rate is unaltered in inflammatory conditions [4, 5]. The structure of the cystatin C gene and its promoter has been determined and the gene seems to be of the housekeeping type, which is compatible with a stable production rate of cystatin C by most cells [6]. The low molecular weight of cystatin C in combination with its stable production rate strongly indicates that the blood serum concentration of this protein is mainly determined by the glomerular filtration rate of the individual. There have been several papers during recent years suggesting that cystatin C measurement in serum correlates with glomerular filtration rate (GFR) [4, 5, 7].

Several other low molecular weight proteins, β_2-microglobulin, retinol binding protein and α_1-microglobulin (protein HC), have been investigated for their utility in monitoring GFR [5, 8]. None of these have proven useful, due mainly to the influence of non-renal factors on their circulating concentrations. The hypothesis is that low molecular weight proteins are freely filtered at the glomerulus and then either reabsorbed and metabolized or excreted into the urine. The end result is that they are cleared from the circulation and the circulating serum concentration will reflect GFR if the production rate remains constant. The difficulty with the aforementioned low molecular weight proteins is that the production rate may vary due to infection, dietary factors and liver disease [5, 7]. Creatinine and urea, which are more commonly used for the clinical assessment of GFR, also have a range of non-renal factors influencing their production, for example, muscle mass and protein intake, and for creatinine there are several well-reported difficulties concerning the analytical measurement [9, 10]. There is thus a need to provide an alternative to creatinine that is analytically more reliable and as or more clinically reliable.

Recent investigations have confirmed that the serum concentration of cystatin C is at least as good an indicator of glomerular filtration rate as the serum concentration of creatinine [4, 5, 7]. Although the numbers of samples have been small, there has also been evidence that intercurrent infection and malignancy do not affect circulating cystatin C concentrations. However, in these studies the serum concentrations of cystatin C were determined by enzyme amplified single radial immunodiffusion (SRID). Although this method is analytically accurate and reasonably precise, it is slow, and does not permit automation and is therefore far from ideal for clinical use. Other groups have developed methods for cystatin C using ELISA [11–14], which although enabling a greater output is still not amenable to routine analysis on the scale of routine creatinine measurement. The present work was undertaken in an effort to produce a rapid and automated procedure for the quantitation of serum cystatin C based upon the latex particle enhanced immunoturbidimetric assay (PETIA) technique [15–17].

Methods

All reagents were analar grade (BDH, Poole, UK) unless otherwise stated. Antiserum (Code No: A451) was a gift from Dakopatts, Denmark; purified recombinant cystatin C for calibration was prepared according to Abrahamson et al [18]. Latex particles, 77 nm in diameter (Bangs Laboratories, Indianapolis, IN, USA) were covalently coupled to rabbit anti-human cystatin C immunoglobulin fraction using a chloro-methyl styrene coupling

Received for publication November 1, 1993
and in revised form July 28, 1994
Accepted for publication July 28, 1994

chemistry [16]. Antibody was dialyzed into 15 mM phosphate buffer pH 7.4 and reacted overnight at 37°C in 15 mM phosphate buffer pH 7.4 containing 0.05% GAFAC RE610 (GAFco, Wythenshaw, Manchester, UK). After coupling, particles were centrifuged at 50,000 g, the supernatant removed, followed by washing with 50 mM glycine buffer pH 7.4 containing 0.05% GAFAC. This procedure was repeated four times and then the washed particles were resuspended in half the coupling volume of 500 mM glycine pH 7.4 containing 0.05% GAFAC. The reagent was then treated on ice with 2 × 60 seconds of ultrasonication at 20 KHz (MSE Soniprep, Crawley, Sussex, UK) to disrupt any aggregates and stored at 4°C.

Experimental procedures

All analyses were performed on a Monarch 2000 centrifugal analyzer (Instrumentation Laboratory, Warrington UK) operating at 37°C and monitoring at 340 nm in a disposable cuvette rotor with a path length of 0.74 cm.

Assay optimization

All experiments were performed using solutions of purified recombinant cystatin C prepared in horse serum (Sigma, Poole, Dorset, UK) over a concentration range of 0 to 10 mg/liter. The effect of reaction conditions on serum based nonspecific aggregation reactions was assessed at all stages. Antibody coated particles were prepared with different protein loadings (0.5 to 3 mg of antibody/ml particles) and at different particle concentrations (1 to 3% solids) and their functional immunoreactivity assessed at a range of pH (pH 6 to 8), polyethylene glycol (molecular wt 6 to 8000, 0 to 2%), and salt concentrations [15]. The influence of sample volume on the reaction kinetics and calibration curve was also assessed. Horse serum (Sigma) and cystatin C free human serum (prepared by affinity adsorption using an anti-cystatin C antibody coupled solid-phase) were both evaluated for use as a calibration matrix.

Assay validation

Analytical recovery and parallelism. Using the optimized assay protocol analytical recovery was assessed in ten different serum samples at two cystatin C concentrations (2.5 and 5.0 mg/liter). Ten serum samples with high creatinine concentrations were diluted in horse serum to assess parallelism.

Imprecision. The intra-assay precision was assessed using 20 replicate analyses of three serum pools at approximately 1, 3 and 8 mg/liter. Inter-assay precision was assessed in two ways: by analyzing the aforementioned serum pools across twenty working days, and by establishing a precision profile from analyzing 206 samples on two separate occasions. The same samples were used to develop a precision profile for serum creatinine measurement using a Jaffe method (Instrumentation Laboratories).

Method comparison and interferences. A total of 100 patient samples was assayed for cystatin C using the method described and the enzyme-enhanced radial immunodiffusion procedure [5]; a common calibrator was employed in both methods. Potential interferences by rheumatoid factor was assessed by assay of 20 samples with elevated RF titers in both the proposed and SRID methods. The potential interference of bilirubin, hemoglobin and lipemia were assessed by performing analytical recovery experiments in increasing concentrations of interferents; bilirubin (Sigma) up to 700 μM, hemoglobin (human hemolysate) up to 1.0 g/liter, and triglyceride (Intralipid, Kabi Vitrum Ltd., Uxbridge, UK) up to 40 mM equivalent triglyceride concentration.

Plasma versus serum. The effect of anticoagulants and clotting was assessed by collecting blood ($N = 10$) into plain, heparin and EDTA vacutainer tubes. The appropriate serum and plasma fractions were assayed for cystatin C.

Sample stability. The effects of incomplete clotting and storage of serum samples were evaluated by collecting five samples from normal individuals and handling them in the following ways: (a) allowed to clot for one hour, separated and analyzed immediately; (b) allowed to clot for one hour then separated and stored at 4°C overnight before analysis, or (c) at −20°C before analysis; (d) stored on the clot overnight at 4°C before separation and analysis.

Clinical evaluation

Serum samples were obtained from 206 patients mainly from the Departments of Nephrology, Rheumatology and Urology at the University Hospital of Lund, Sweden. These included cases of chronic glomerulonephritis, chronic pyelonephritis, amyloidosis and post-operative assessments following surgery of the kidney and urinary tract. GFR measurements had been made on these patients according to the single injection technique using chromium-EDTA complex (Cr^{51}; Behringwerke AG, Marburg, Germany) following the method of Brochner-Mortensen [19] as modified by Brauner and Westling [20]. All GFR measurements were performed at 7.30 a.m. with the subjects fasting. These patients studied were selected on the basis of having a serum creatinine of less than 300 μmol/liter in order to study more intensively the moderate changes in GFR. Over the period of this study 263 random samples were also obtained from the Clinical Biochemistry Laboratory at the Royal London Hospital. All these samples were analyzed for serum cystatin C (PETIA) and creatinine (Jaffe method; Instrumentation Laboratories).

Statistical procedures

Regression analyses were performed using the Minitab Statistical Package (Minitab Inc. PA, USA) and the method of Deming [21]. Additional statistical analyses, Mann-Whitney U test, paired and unpaired *t*-tests were performed using Statview® Abacus Concepts Inc. (Berkeley, CA, USA) for Macintosh computers. Clinical sensitivity and specificity was calculated according to Galen and Gambino [22], with statistical comparison using an unpaired *t*-test.

Results

The antibody particle reagent synthesis was optimized at 0.5 mg antibody/ml of 1% particles. Nonspecific aggregation was minimal with an appropriate specific absorbance change at a reaction pH of 7.5, in a 340 mM sodium phosphate buffer containing 10 g/liter BSA and 1 g/liter sodium azide. The final assay protocol is shown in Table 1, and typical calibration curves are shown in Figure 1 for both calibration matrices.

Analytical recovery and parallelism were demonstrated; mean serum recovery was 93 ± 3.8%. Intra- and inter-assay precision was less than 3 and 5%, respectively, and for the serum assay between 1.0 and 10 mg/liter, whether measured using quality assessment pools or using the precision profile (Table 2 and Fig. 2). The precision profile for the Jaffe creatinine method is also shown in Figure 2. The detection limit (2.5 SD from the zero calibrator) for the serum cystatin C assay was 0.027 mg/liter.

Table 1. Assay protocol

Parameters on the IL Monarch	
Sample μ *liter*	5
Diluent	5
Buffer	74
Antibody/particle	165
Temperature *°C*	37
Wavelength *nm*	340
Initial read *seconds*	5
Read time *seconds*	300

The assay buffer was 340 mM sodium phosphate pH 7.5, containing 10 g/liter BSA and 1 g/liter sodium azide.

Antibody/particle was 0.5 mg antibody/ml 1% particles diluted to a starting absorbance of approximately 0.7 (0.74 cm path length).

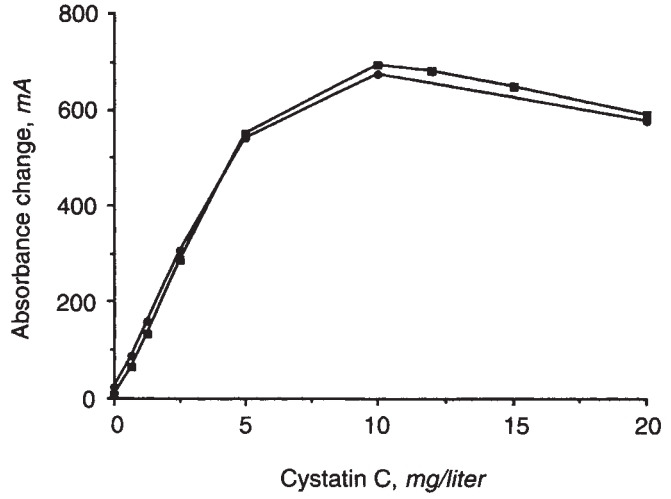

Fig. 1. *Calibration curves for the cystatin C PETIA in both horse serum (●) and cystatin C free human serum (□).* This figure shows a hook effect at about 10 mg/liter cystatin C, and equivalent standard curves between the two matrices.

Table 2. Assay precision for the PETIA method

Intra-assay				Inter-assay			
	mean	SD	CV		mean	SD	CV
N	*mg/liter*		%	*N*	*mg/liter*		%
20	1.34	0.034	2.56	12	1.29	0.05	3.5
20	2.85	0.062	2.20	12	2.89	0.11	3.8
20	8.13	0.073	0.90	12	8.14	0.37	4.5

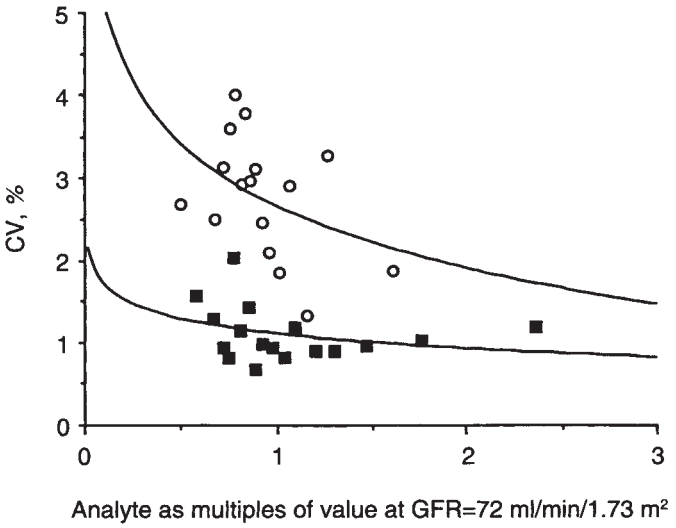

Fig. 2. *Inter-assay precision profiles for serum cystatin C (■) and creatinine (○), both assayed on the Monarch 2000 automated analyzer,* N = *206.* Analyte concentrations on the abscissa have been normalized against their respective values at a GFR of 72 ml/min/1.73 m^2.

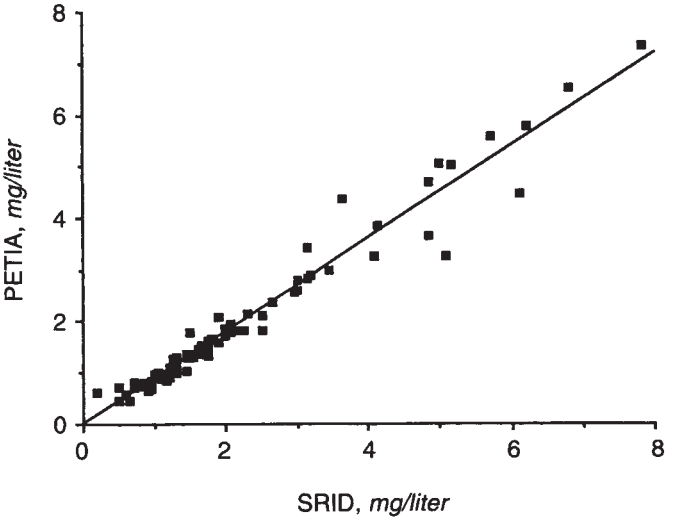

Fig. 3. *Methods comparison between the cystatin C PETIA and an enzyme-enhanced SRID assay.* Deming regression analysis gave PETIA = 0.074 + 0.93 × SRID, N = 100, r = 0.98.

Regression analysis (Deming) of the method comparison gave PETIA = 0.074 + 0.93 × SRID, r = 0.98 (Fig. 3). There was no interference from rheumatoid factor, bilirubin or hemolysis, but triglycerides at concentrations greater than 10 mmol/liter caused a reduced recovery of cystatin C. Both heparin and EDTA caused a significant under-recovery of cystatin C (mean 27.3 and 52.6% of serum concentrations, respectively). There was no significant difference (paired t-test, $P > 0.05$) between samples analyzed immediately versus those separated and stored at −20°C or 4°C, or left unseparated on the clot overnight.

The relationship between reciprocal serum concentrations of creatinine and cystatin C gave a regression analysis of 1/cystatin C = 0.14 + 73.4 × 1/creatinine, r = 0.67, N = 469 (Fig. 4B). The relationship between cystatin C concentrations and GFR is shown in Figure 5, showing the classical curvilinear relationship demonstrated by serum creatinine. Both cystatin C and creatinine relationships with GFR are linearized by plotting their reciprocals as shown in Figure 6 A and B; Deming regression analyses gave 1/cystatin C = 0.265 + 0.008 × GFR, r = 0.81, and 1/creatinine = 0.0062 + 0.000062 × GFR, r = 0.50, N = 206 in both cases. Taking a lower limit of the GFR reference range to be 72 ml/min/1.73 m^2 [23], the sensitivity, specificity, predictive value of positive and negative plus diagnostic efficiency were calculated for both cystatin C and creatinine. The results are shown in Table 3.

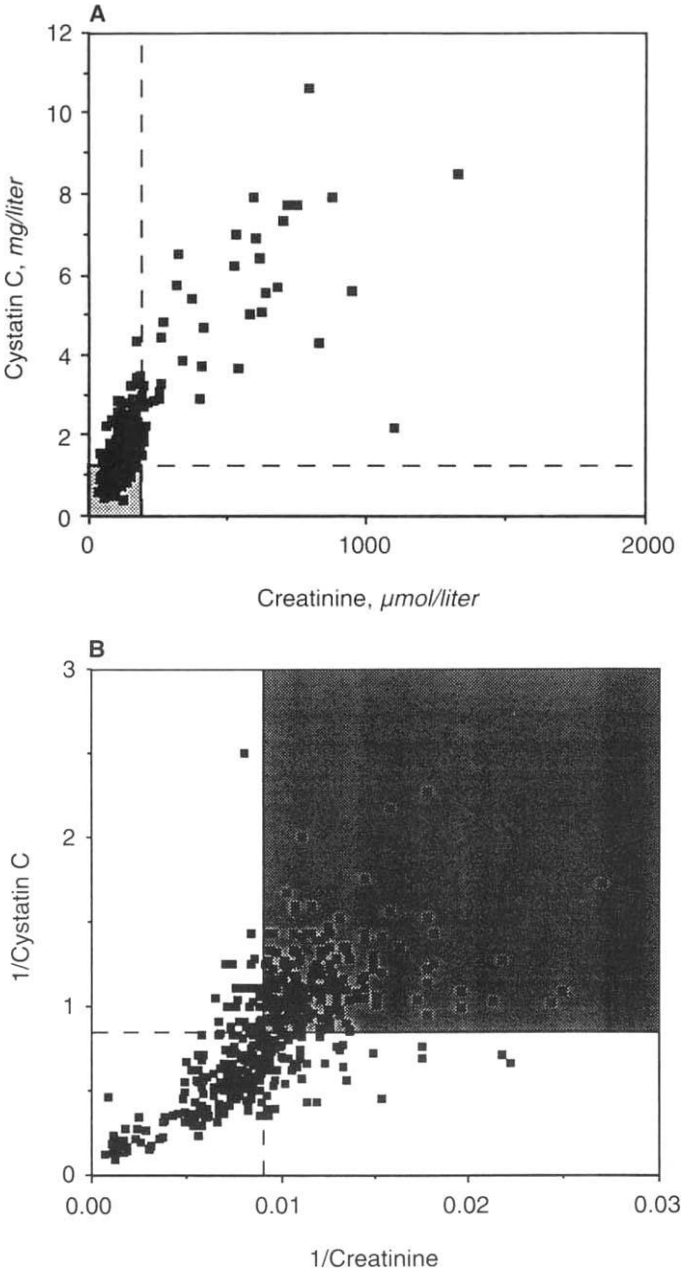

Fig. 4. *Relationship between serum creatinine and cystatin C concentrations,* N = *469.* Shaded area shows where both parameters are within their respective reference limits; dotted lines show upper or lower reference limits. (a) direct relationship and (b) reciprocal relationship. Deming regression analysis gave 1/cystatin C = 0.14 + 73.4 × 1/creatinine, r = 0.67.

These data are expressed in a different way in Figure 7; the measured cystatin C and creatinine concentrations are binned into different GFR ranges, and the mean concentrations in each bin are divided by the concentration of the analyte at a GFR of 72 ml/min/1.73 m^2, thus expressing the data as multiples of their respective concentrations at the lower limit of the GFR reference range (mean ± SD). Serum cystatin C rose significantly earlier and to a greater extent than serum creatinine as the GFR fell (unpaired t-test).

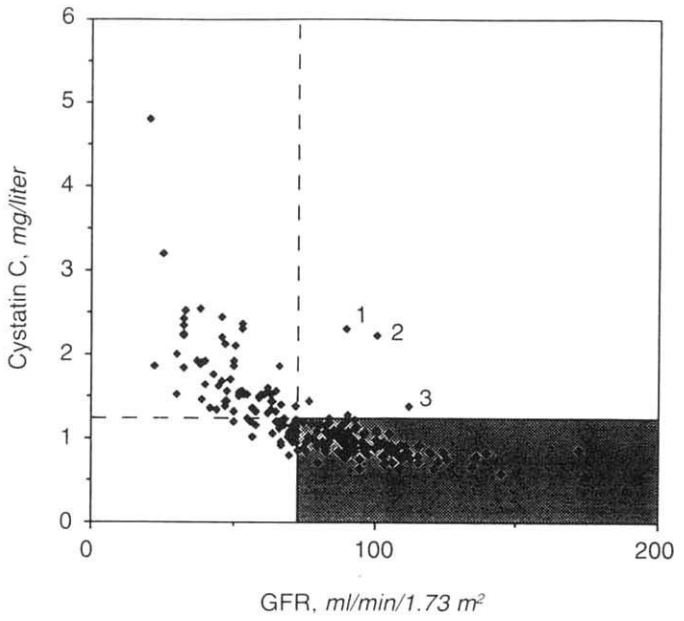

Fig. 5. *Relationship between serum cystatin C and Cr EDTA GFR measurement,* N = *206.* Shaded area shows where both parameters are within their respective reference limits; dotted lines show upper or lower reference limits. Sample 1 is from a renal transplant recipient, following several rejection episodes, and on steroid and full immunosuppression therapy, plus antihypertensive therapy. Sample 2 is from a patient with systemic sclerosis and muscle wasting whose serum creatinine was 65 μmol/liter. Sample 3 is from a patient with severe rheumatoid arthritis and muscle wasting whose serum creatinine was 67 μmol/liter.

Discussion

The PETIA assay was optimized with an antibody loading of 0.5 mg/ml of 1% particles; this does not represent a saturated particle surface with regards to total protein loading, but represents an optimal immunoreactivity. Using the conventional validation procedures for analytical recovery and parallelism the accuracy of the PETIA was demonstrated in serum. The SRID assay was deemed to be a more reliable reference method than the EIA, both published by one of us (AOG). A good correlation was observed between the two methods further confirming the accuracy of the PETIA even in the presence of rheumatoid factor. Figure 1 shows a maximum signal change at about 10 mg/liter, and any sample greater than this should be assayed after dilution with horse serum. The extended calibration curve is included to demonstrate that the highest concentrations of cystatin C found pathologically (12 to 15 mg/liter) will give values of greater than 8 mg/liter when assayed without pre-dilution. The security range for this assay is thus very good and no false-low (that is, normal) concentrations will be reported in end-stage renal failure.

No significant interferences were observed, although drug interferences have not yet been assessed. Heparin and EDTA plasma showed significantly different cystatin C concentrations to serum. We would thus recommend that serum is the matrix of choice for cystatin C analysis. The nature of these differences is not clear, but may be charge-related in that the EDTA will complex divalent cations which can play a role in promoting

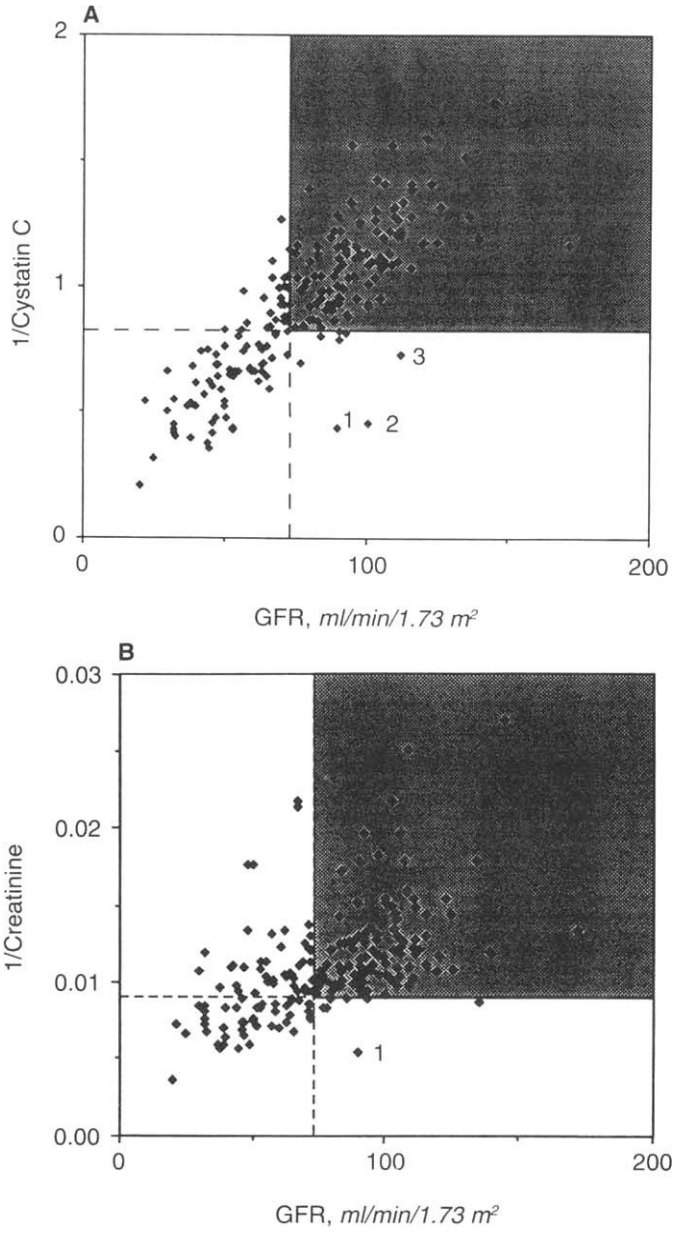

Fig. 6. *Relationship between reciprocal concentrations of cystatin C* (**A**) *and creatinine* (**B**) *with Cr EDTA GFR,* N = *206.* Shaded area shows where both parameters are within their respective reference limits, dotted lines show upper or lower reference limits. Deming regression analyses gave 1/cystatin C = 0.265 + 0.008 × GFR, r = 0.81, and 1/creatinine = 0.0062 + 0.000062 × GFR, r = 0.50.

Table 3. Biochemical markers of glomerular filtration rate

	Sensitivity	Specificity	PV + ve	PV − ve	Diagnostic efficiency
			%		
Cystatin C					
URL = 1.25 mg/liter	71.4[a]	95.1	90.9	82.9	85.4
URL = 1.50 mg/liter	50.0	98.4	95.5	74.1	78.6
Creatinine (Jaffe)					
URL = 110 *μmol/liter*	52.4[a]	91.8	81.5	73.4	75.3
URL = 120 *μmol/liter*	39.3	97.5	91.7	70.0	73.8

Assuming the lower reference limit for GFR = 72 ml/min/1.73 m². Healthy = GFR >72, N = 122. Disease = GFR <72, N = 84. URL = upper reference limit of analyte; two concentrations have been used for the URL of each analyte in order to compare sensitivities and specificities at different cut off levels.

[a] Significantly different (unpaired t-test), $P < 0.05$

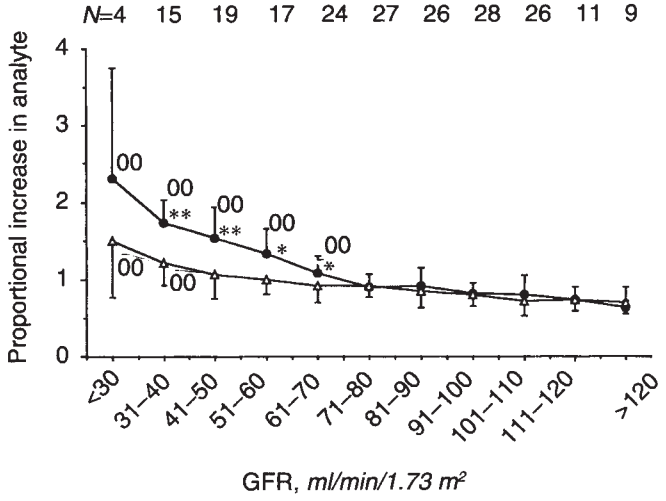

Fig. 7. *Proportional changes in cystatin C* (●) *and creatinine* (△) *normalized using their respective concentrations at 72 ml/min/1.73 m² GFR.* The error bars show the standard deviations for the distribution of results in each GFR grouping. Statistical analyses used an unpaired t-test, cystatin C vs. creatinine $^{*}P < 0.05$, $^{**}P < 0.01$; cystatin C or creatinine vs. values at 72 ml/min/1.73 m² GFR, $^{0}P < 0.05$, $^{00}P < 0.01$. This illustrates the earlier and greater proportional rise in cystatin C concentrations as GFR decreases.

immunoaggreagation reactions; the effect of the negatively-charged heparin molecules is more difficult to explain as the latex particles carry a net negative charge themselves.

The precision profile for the cystatin C PETIA shows a two- to threefold better precision than that achieved for the creatinine assay. This is particularly important at the upper limits of their respective reference ranges (1.25 mg/liter for cystatin C and 110 μmol/liter for creatinine). PETIA technology is not influenced by icterus or hemolysis, both of which cause interference in creatinine assays [9, 10]. Thus a PETIA for cystatin C offers better analytical performance than serum creatinine measurement on a routine automated clinical chemistry analyzer. The cost of a PETIA assay is currently about the same as an enzymatic creatinine measurement, that is, about two to three times as expensive as using the Jaffe based methodologies, as used here. The cost is therefore not unreasonable and could possibly be reduced further by replacement of the polyclonal antibody with a monoclonal antibody whereby the economies of scale, in reagent production, might be increased. The technology has been shown to be extremely robust with calibration stabilities in excess of one year [15–17]. PETIA technology can be applied to all clinical analyzers including those in use in a doctor's office situation, and thus it

offers an appropriate technological approach if the clinical applicability of cystatin C measurement can be confirmed.

Serum cystatin C concentrations measured by PETIA agree well with those previously reported [4, 5, 7], and show a similar relationship with creatinine concentration and ^{51}Cr EDTA GFR. The high creatinine sample (sample 1) in Figure 4A had a repeat estimation of creatinine and a confirmation of the cystatin C concentration on dilution and by SRID analysis; no explanation for this discrepancy has been identified as yet. The proportional rise in cystatin C concentrations is much greater than that for creatinine, around the lower limit of the GFR reference range, and the rate of relative increase appears to rise as the GFR falls well below normal. The relationship with creatinine concentration was not described in the previous studies [4, 5].

The reciprocal relationships between cystatin C and creatinine with GFR show that the slope of the regression line is much steeper with cystatin C (approximately twice as steep if the concentration units are normalized) than for creatinine. There is a much tighter distribution of results around the regression line for cystatin C as indicated by the better correlation coefficient r = 0.81 versus 0.50 for creatinine. This compares with previously published correlation coefficients for cystatin C of 0.77 and 0.75 [4, 5]. These studies involved 135 and 106 Cr EDTA GFR comparisons. Therefore, in nearly 500 GFR studies serum cystatin C measurement has been shown overall to be a reliable reflector of GFR.

The clinical sensitivity of serum cystatin C measurement as a predictor of GFR has not been previously calculated. Our data show that, with either of the two upper reference concentrations for the two analytes, cystatin C offers the greater sensitivity in detecting an abnormal GFR, with equivalent specificity and overall better diagnostic efficiency. This greater sensitivity is also reflected by the expression of the data in Figure 7, where cystatin C concentrations can be seen to rise more rapidly than those of creatinine. Both cystatin C and creatinine show good specificities for detecting an abnormal GFR. The poor sensitivity of creatinine may be due to analytical or pathophysiological factors, and in the former case analytical imprecision and interference could be important. In the second instance the variety of non-renal influences on the circulating creatinine concentration result in a wide reference range for creatinine; thus, GFR can change considerably before creatinine becomes abnormal. The non-renal influences upon the circulating cystatin C concentration are less well studied. It is known that there are age-related changes that parallel known age-related changes in GFR [24].

Until very recently the available data suggested that serum cystatin C concentrations were independent of gender [4, 5, 24]; however, the recent work of Pergande and Jung suggested that the serum cystatin C concentrations were lower in women than men [14]. Analysis of the data described in our study on the basis of sex shows that, in two populations with a roughly equal spread in age and GFR (Fig. 8), the mean female cystatin C concentration was 1.17 mg/liter and that of the male population 1.24 mg/liter. The mean male concentration was 6% higher than the mean female for cystatin C (not statistically significantly different, Mann-Whitney U test), but for creatinine the difference was 17% ($P <$ 0.05), suggesting that male/female differences are significantly greater for creatinine. The difference between our studies and those of Pergande and Jung are as yet unexplained. Their ELISA used the same commercial source of antibody as this work but a different calibrator material, and the latter is important as they report significantly higher serum concentrations than other reports (including our own). Additionally, there is no confirmatory evidence that their reference populations had normal GFRs. A full reference range study using a reference GFR procedure to confirm a normal GFR needs to be performed; this could explore the influences of sex, age, body mass. Further work is also necessary to evaluate the response of cystatin C concentration to the different renal replacement modalities. Further prospective studies are required to monitor individual patients with different renal pathologies, for example, diabetic nephropathy.

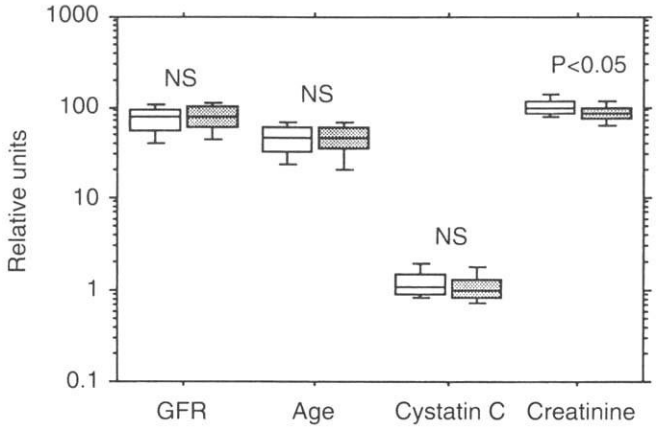

Fig. 8. *Distribution of age, GFR, cystatin C and creatinine in male (N = 115) and female (N = 91) subjects.* Statistical analyses with the Mann-Whitney U-test, $P > 0.05$ considered not significant. Symbols are: (□) male; ( ) female.

In summary, we have developed a fully automated assay for serum cystatin C allowing the extensive clinical evaluation of this new biochemical marker of GFR. Using PETIA technology, we have produced an assay that is applicable to all clinical analyzers used for the measurement of creatinine. Serum cystatin C has been shown to be in all likelihood a more sensitive marker of early deterioration in GFR than serum creatinine.

Acknowledgments

DJN, and HT are supported by a grant from Du Pont de Nemours International SA, Geneva, Switzerland.

Reprint requests to Dr. David J. Newman, The London Hospital Medical College, Department of Clinical Biochemistry, Turner St., E1 2AD London, England, United Kingdom.

References

1. Barrett AJ, Davies ME, Grubb A: The place of human γ-trace (cystatin C) amongst the cysteine proteinase inhibitors. *Biochem Biophys Res Commun* 120:631–636, 1984
2. Grubb A, Lofberg H: Human γ-trace, a basic microprotein: Amino acid sequence and presence in the adenohypophysis. *Proc Natl Acad Sci USA* 79:3024–3027, 1982
3. Barrett AJ, Fritz H, Grubb A, Isemura S, Jarvinen M, Katunuma N, Machleidt W, Sasaki M, Turk V: Nomenclature and classification of the proteins homologous with chicken cystatin. (abstract) *Biochem J* 236:312, 1986
4. Simonsen O, Grubb A, Thysell H: The blood serum concentration of cystatin C (γ-trace) as a measure of the glomerular filtration rate. *Scand J Clin Lab Invest* 45:97–101, 1985
5. Grubb A, Simonsen O, Sturtfelt G, Truedsson L, Thysell H:

Serum concentration of cystatin C, Factor D and β_2-microglobulin as a measure of glomerular filtration rate. *Acta Med Scand* 218:499–503, 1985
6. ABRAHAMSON M, OLAFSSON I, PALSDOTTIR A, ULVSBACK M, LUNDWALL A, JESSON O, GRUBB A: Structure and expression of the human cystatin C gene. *Biochem J* 268:287–294, 1990
7. GRUBB A: Diagnostic value of analysis of cystatin C and protein HC in biological fluids. *Clin Nephrol* 38:S20–S27, 1992
8. AYATSE JO, KWAN JTC: Relative sensitivity of serum and urine retinol binding protein and alpha 1 microglobulin in the assessment of renal function. *Ann Clin Biochem* 28:514–516, 1991
9. PERRONE RD, MADIAS NE, LEVEY AS: Serum creatinine as an index of renal function: New insights into old concepts. *Clin Chem* 38:1933–1953, 1992
10. SPENCER K: Analytical reviews in clinical biochemistry: The estimation of creatinine. *Ann Clin Biochem* 23:1–25, 1986
11. COLLE A, TAVERA C, PREVOT D, LEUNG-TACK J, THOMAS Y, MANUEL Y, BENVENISTE J, LEIBOWITCH J: Cystatin C levels in sera of patients with human immunodeficiency virus infection. *J Immunoassay* 13:47–60, 1992
12. ISHIGURO H, OHKUBO I, MIZOKAMI M, TITANI K, SASAKI M: The use of monoclonal antibodies to define levels of cystatin C in normal human serum. *Hybridoma* 8:303–313, 1989
13. OLAFSSON I, LOFBERG H, ABRAHAMSON M, GRUBB A: Production, characterisation and use of monoclonal antibodies against the major extracellular human cysteine proteinase inhibitors cystatin C and kininogen. *Scand J Clin Lab Invest* 48:573–582, 1988
14. PERGANDE M, JUNG K: Sandwich enzyme immunoassay of cystatin C in serum with commercially available antibodies. *Clin Chem* 39:1885–1890, 1993
15. NEWMAN DJ, HENNEBERRY H, PRICE CP: Particle enhanced light scattering immunoassay. *Ann Clin Biochem* 29:22–42, 1992
16. PRICE CP, TRULL AK, BERRY D, GORMAN EG: Development and validation of a particle-enhanced turbidimetric immunoassay for C-reactive protein. *J Immunol Meth* 99:205–211, 1987
17. MEDCALF EA, NEWMAN DJ, GILBOA A, GORMAN EG, PRICE CP: A rapid and robust particle-enhanced turbidimetric immunoassay for serum β_2 microglobulin. *J Immunol Meth* 28:407–411, 1990
18. ABRAHAMSON M, DALBEGE H, OLAFSSON I, CARLEN S, GRUBB A: Efficient production of native, biologically active human cystatin C by *Escherichia coli. FEBS Lett* 236:14–18, 1988
19. BROCHNER-MORTENSEN J: A simple method for the determination of glomerular filtration rate. *Scand J Clin Lab Invest* 30:271–274, 1982
20. BRAUNER L, WESTLING H: On the necessity of strict bed rest during determination of ^{51}Cr-EDTA clearance. *Clin Physiol* 1:175–180, 1981
21. DEMING WE: *Statistical Adjustment of Data.* New York, John Wiley and Sons, 1943, p 184
22. GALEN RS, GAMBINO SR: *Beyond Normality: The Predictive Value and Efficiency of Medical Diagnoses.* New York, John Wiley and Sons, 1975
23. MORRISON RBI: Urinalysis and assessment of renal function (chapt 11), in *Renal Disease,* edited by BLACK D, JONES NF, Oxford, Blackwell Scientific (4th ed) 1979, p 314
24. LOFBERG H, GRUBB AO, SVEGER T, OLSSON J-E: The cerebrospinal fluid and plasma concentrations of γ-trace and β_2-microglobulin at various ages and in neurological disorders. *J Neurol* 223:159–170, 1980