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Developments in the assessment of glomerular filtration rate

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Abstract

The assessment of the glomerular filtration rate (GFR) is the most commonly used test of renal function. The accepted reference procedure employs an exogenous clearance marker whilst the most popular test is that of serum or plasma creatinine. All of these tests have limitations, although the surrogate endogenous markers are the most practical. Cystatin C, a low molecular weight protein which can be measured by light scattering immunoassay, possesses many of the attributes required of the ideal GFR marker. Data on reference ranges indicate that circulating cystatin C levels reflect the variation in GFR throughout life and the marker demonstrates a better correlation with the reference procedure than serum creatinine. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Whilst the kidney performs many vital functions in the body, two of its key roles are the removal of waste products, together with the maintenance of water

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balance. This is achieved by glomerular filtration and by tubular absorption or secretion. The measurement of the clearance of a substance is regarded as the most convenient method of assessing the ability of the kidneys to control the concentration of the substance in the extracellular fluid [1]. This function is used routinely as a means of assessing the overall function of the kidneys and specifically when excretory function has a bearing on a pharmacological intervention, e.g. optimising drug therapy or avoidance of toxicity [2]. It is the most valuable test of renal function in the elderly [3].

2. Measurement of glomerular filtration rate

The clearance of a substance is measured by the quotient UV/P , where U is the urine concentration of the substance, V the urine volume per minute and P the plasma concentration of the substance assuming that the filtered substance is not subsequently reabsorbed and/or catabolised.

The methods using exogenous markers for measuring GFR depend either upon a constant infusion or bolus injection of a substance whose concentration is measured in the plasma and/or urine compartments. There has been much discussion on the choice of methodology, particularly with respect to sampling techniques and the reader is referred to Refs. [4,5] for further details. One of the first exogenous markers was inulin [6] which was subsequently supplanted by more conveniently measured labelled compounds; the recent introduction of an enzymatic assay for inulin means that it has returned to popularity [7]. Isotopically labelled compounds that have been employed include iothalamate [8], iodothalamate [9], chromium ethylenediamine tetracetic acid ($^{51}\text{Cr-EDTA}$) [10] and diethylenetriamine pentacetic acid ($^{99\text{m}}\text{Tc-DTPA}$) [11]. The use of the contrast medium iohexol [12] has become popular more recently with measurement of the marker made by high pressure liquid chromatography.

A variety of endogenous markers have also been used for the measurement of GFR either as a clearance, in particular creatinine, urea or urate, or alternatively the direct plasma concentration (or its reciprocal). In the latter case the markers have included creatinine [13], urea [14], urate [15] and the low molecular weight proteins β_2 -microglobulin [16], α_1 -microglobulin [17] and retinol binding protein [18]. When seeking a marker substance with which to assess clearance the marker should (i) display a stable production rate; (ii) have a stable circulating level, i.e. not be affected by other pathological changes; (iii) not be protein bound; (iv) be freely filtered at the glomerulus; and (v) not be reabsorbed or secreted. These characteristics will ensure that an accurate assessment of the glomerular filtration rate (GFR) can be achieved. In the case of the low molecular weight proteins tubular reabsorption and catabolism of the analyte

Table 1
Limitations of some GFR markers

Unstable production rate	
Acute phase response:	β_2 Microglobulin (\uparrow) Retinol binding protein (\downarrow)
Malignancy:	Urate
Cell proliferation:	β_2 Microglobulin
Variation in muscle mass:	Creatinine
Variation in catabolic rate:	Creatinine Urea
Diet:	Creatinine Retinol binding protein
Sample collection	
24 h urine collection:	Creatinine clearance
Plasma sampling:	Exogenous markers
Analytical method:	Creatinine (serum/plasma in particular)

means that the assessment of GFR can only be made by the serum/plasma measurement of the protein.

The gold standard method is considered to be one using an exogenous marker, typically $^{51}\text{Cr-EDTA}$, $^{99\text{m}}\text{Tc-DTPA}$ or iothalamate [19], although as indicated earlier there are variations in sampling times quoted. There are also particular limitations in the case of the endogenous markers as indicated in Table 1. The lack of stability in the circulating level of the marker is a problem with several markers due to the coexistence of other pathologies, differences in body mass and in variations of the diet.

The most commonly used test of renal function is the measurement of serum creatinine. Correction for differences in muscle mass can be undertaken by correction of the serum or clearance value to a constant body surface area using the height and weight measures. The measurement of serum creatinine is also fraught with methodological interferences as far as the routine Jaffé method is concerned [20]. The most common problem associated with clearance measurements is the inability to ensure a complete collection of urine.

3. Cystatin C

An alternative low molecular weight protein that has been proposed as a marker of GFR is cystatin C. It is a non-glycosylated basic protein ($pI = 9.2$) consisting of 120 amino acids with a molecular mass of 13.36 kD and a member of the cystatin superfamily of cysteine protease inhibitors [21]. Structural analysis of the gene and its promoter has shown that it is constitutively produced

by all nucleated cells and therefore exhibits a stable production rate even in the presence of an acute inflammatory response [22–24]. It has also been shown to be a protein that is freely filtered by the glomerulus [25].

3.1. Methods for measuring cystatin C

A total of four approaches have been described to date for the measurement of cystatin C, all based on immunoassay. The first approach described by Lofberg and Grubb [26] employed an enzyme amplified immunodiffusion assay; however, this was a rather cumbersome method not suitable for handling large numbers of samples. Radio, enzyme and fluorophore labelled heterogeneous immunoassays [27–29] have been described which have improved the throughput capability but still require about 3 h to complete a batch of tests. The main advances in quantitation of cystatin C have been made with the advent of light scattering immunoassay, Kyhse-Anderson et al. [30] and Newman et al. [31] describing turbidimetric immunoassays while Finney et al. [32] described a nephelometric immunoassay. These assays have enabled a shorter reaction time to be employed with an homogeneous immunoassay format, a result being available in about 15 min.

3.2. Performance of light scattering assays

The within-run imprecision has been reported to be from 1.1 to 6.2% (coefficient of variation, CV) across the concentration range 0.97–6.36 mg/l for the turbidimetric and nephelometric assays [30–35]. The between-run imprecision has been reported in these same studies to be within a range from 1.2 to 5.5%. A typical precision profile obtained from analysis of duplicates with the Dade Behring (Marburg GmbH) nephelometric assay is shown in Fig. 1; the imprecision has been shown to be better with the nephelometric assay at concentrations of cystatin C below 2 mg/l [32].

The correlation between turbidimetric and nephelometric methods has been shown to be good, albeit early reports indicated a significant bias. This was thought to be due to the differences in assignment of values to the calibrators, and a reduced bias has been reported more recently [32,35–37]. The two approaches that have been attempted for preparation of a primary calibrator have been purification from human urine and use of a recombinant protein; an internationally agreed reference preparation is required to ensure correlation between methods and transferability of clinical data.

3.3. Lowest detectable concentration

The detection limits of methods described for the quantitation of cystatin C vary between 0.2 and 300 $\mu\text{g/l}$ depending on the type of assay. The detection

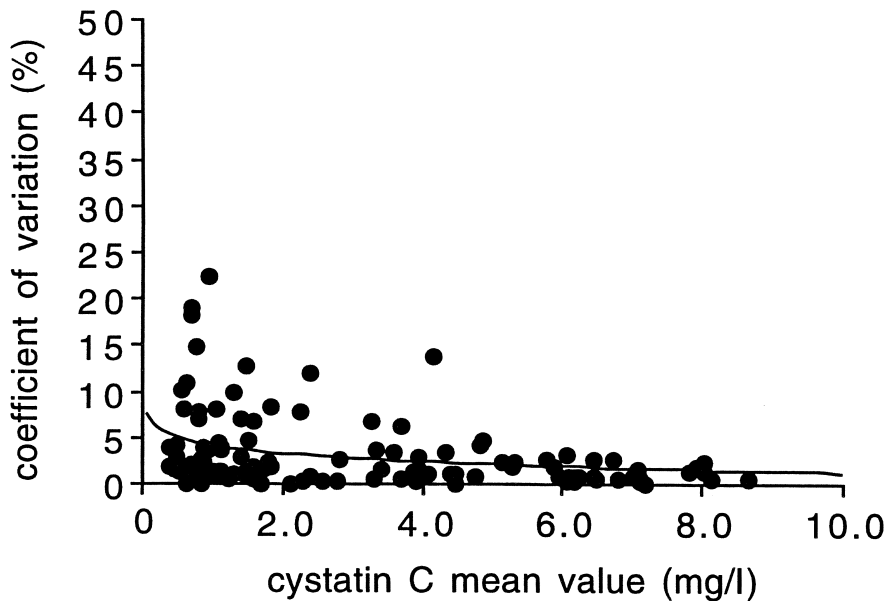


Fig. 1. A precision profile for cystatin C based on duplicate analysis of patient samples using the Dade Behring nephelometric immunoassay.

limits reported for the automated light scattering assays range between 27 and 170 $\mu\text{g/l}$ [30–32].

3.4. Stability of cystatin C

The cystatin C concentration in serum has been shown to be stable for at least 2 days when stored at room temperature, for up to 1 week at 4°C, at least 1 week at –20°C and up to 6 months at –80°C [17,32,35]. Mussap et al. [36] found that cystatin C was stable in serum when stored at room temperature for 48 h, at 4°C for 1 week and at least 1 month at –20°C. A series of 10 freeze/thaw cycles spread over a period of 57 days resulted in a decrease in apparent analyte concentration of 15% [32].

4. Correlation between cystatin C and reference methods

If serum or plasma cystatin C is to replace serum creatinine or creatinine clearance as the routine method of choice, it is important to determine the correlation of results with those using a reference method; as stated earlier the reference method for assessment of GFR is regarded as one employing an exogenous marker. There have been several studies in which the reference GFR method has been compared with serum or plasma cystatin C, creatinine and

Table 2

Correlation data reported for comparison between reciprocal of serum cystatin C and exogenous marker clearance technique

Author	Clearance technique	Correlation coefficient (<i>r</i>)			Ref.
		Cystatin C	Creatinine	Creatinine clearance	
Grubb et al.	⁵¹ Cr-EDTA	0.77	0.75	–	[23]
Newman et al.	⁵¹ Cr-EDTA	0.81	0.50	–	[31]
Bökenkamp et al.	Inulin	0.88	0.72	–	[33]
Nilsson-Ehle et al.	Iohexol	0.87	0.71	–	[38]
Simonsen et al.	⁵¹ Cr-EDTA	0.73	0.70	–	[39]
Stickle et al.	Inulin	0.77	0.84	–	[40]
		(4–12 y)	0.87	–	
Randers et al.	^{99m} Tc-DTPA	(12–19 y)	0.87	0.81	[41]
		0.87	0.81	0.80	
Risch et al.	Iothalamate	0.83	0.67	0.57	[42]
Seco et al.	Inulin	0.35	0.25	0.22	[43]
Helin et al.	⁵¹ Cr-EDTA	0.83	0.67	–	[44]

sometimes creatinine clearance. A summary of these studies is given in Table 2 [38–44].

Whilst the correlation coefficient is a fairly insensitive parameter to judge agreement between methods it can be seen that, in most of the studies reported, the value is higher for the reciprocal of the cystatin C value compared with the reciprocal of the creatinine value and also in the case of the creatinine clearance when it was also performed.

5. Diagnostic performance of cystatin C

The diagnostic accuracy of the reciprocal of cystatin C has been assessed in several papers using receiver operator curve (ROC) analysis. Randers et al. [41] using comparison of non-parametric ROC curves in 76 patients with various types of renal disease found no significant differences between the reciprocal of cystatin C, of creatinine, and creatinine clearance (AUC 0.9665, 0.9554 and 0.9731, respectively). Bökenkamp et al. [33] studied 101 children and found no significant differences between the three ROC curves (AUC 0.970, 0.956 and 0.894 for cystatin C, creatinine clearance and creatinine, respectively). With a sensitivity of 90% for detecting an abnormal clearance (<84 ml/min/1.73 m²) a cystatin C cut-off of 1.39 mg/l detected renal impairment with a specificity of 86%, a figure of 87.1 ml/min/1.73 m² and a specificity of 89% being found for creatinine clearance. A figure for serum creatinine was not considered because

of the age relationship of the reference range. Plebani et al. [45] measured serum cystatin C and creatinine in 38 renal transplant patients and compared results with inulin clearance measurement of GFR. These authors used ROC curve analysis and found the area under the curve for cystatin C (0.859) to be significantly greater than that for creatinine (0.743, Z-score 18.1). Risch et al. [42], when studying 29 renal transplant patients, also found a significantly higher diagnostic accuracy for the reciprocal of cystatin C compared to creatinine (using a cutoff of 60 ml/min; $P = 0.024$); these authors found no significant difference with creatinine clearance.

Newman et al. [31] in studying 206 patients with various types of renal disease demonstrated that the increase in cystatin C occurred earlier than with creatinine as the GFR value fell, using a ^{51}Cr -EDTA single injection technique as the reference method.

Tian et al. [46] similarly found that a mild reduction in GFR was more readily detected by a change in cystatin C than creatinine.

6. Biological variation

In order to determine whether cystatin C could be used to detect early changes in GFR it is important to determine the biological variability of the marker. Keevil et al. [47] studied the variability of serum cystatin C and creatinine in 12 healthy subjects. Inter-individual variation accounted for 93 and 25% for creatinine and cystatin C, respectively, with intra-individual variation being 7 and 75% for creatinine and cystatin C, respectively. The critical difference for sequential values (significant at $P < 0.05$) was 37% for serum cystatin C and 14% for serum creatinine. The authors concluded that creatinine may be better than cystatin C for detecting temporal changes in GFR in patients with established renal disease, but that cystatin C might be a better marker for screening a population for reduced GFR. These authors noted, however, that the cystatin C assay used demonstrated precision that was worse than the creatinine method in use which may have influenced the findings.

7. Reference ranges

One of the criticisms of serum creatinine as a reflection of GFR is the dependence of the circulating level on muscle mass and consequently the variation of the reference range with age. This has made the use of creatinine measurements particularly difficult in children and elderly patients. There have been several studies of the cystatin C reference range from birth up to 101 years

of age. Clearly the numerical values will depend on the method used and in particular the procedure for value assignment to the calibrator [37]. However, the data reported for cystatin C in children indicate that, at birth, the values are elevated, falling to a constant level after 1 year of life, a value that is maintained throughout adulthood to about 50 years. The mean value at birth is typically double that found in adulthood, an observation consistent with the maturation of kidney function in the newborn [48,49]. By contrast, the circulating creatinine level approximates to the adult values at birth (presumably reflecting maternal levels) falling to a nadir of about 40 $\mu\text{mol/l}$ at 1 year (reflecting low muscle mass) gradually rising to reach adult levels at about 18–20 years of age.

There have been several studies on the adult reference range for serum cystatin C, albeit most have been on small numbers of samples [37]. Mussap et al. [36] determined the reference range to be 0.37–1.22 mg/l with a mean value of 0.80 mg/l in 52 subjects; these authors found a small difference between males and females. Finney et al. [50] have reported one of the largest studies, analysing a total of 309 blood donor samples, in which they found a mean value of 0.70 mg/l with a range (± 1.96 S.D.) of 0.53–0.92 mg/l. These authors found no significant difference between males and females. Norlund et al. studied a population of 259 people between the ages of 20 and 90 years of age [51]. These authors proposed a reference range of 0.70–1.21 mg/l for 20–50 years and 0.84–1.55 mg/l for 50 years and above; these authors found no difference in the distribution of results between the sexes. Erlandsen et al. [52] studied a total of 270 healthy blood donors between 20 and 65 years of age; they found a reference interval of 0.54–1.21 mg/l with no significant difference between the sexes.

There are only a few reported studies of renal function in the elderly, which indicate a reduction of the GFR in people over 60 years of age. The studies of cystatin C in the elderly clearly show that the circulating cystatin C level rises gradually above the age of 50 years; this is consistent with our knowledge of renal function in this age group. The largest study was reported by Finney et al. [53] who also studied reference populations from birth to 50 years using the same method; a summary of the accumulated data from birth to 101 years is shown in Fig. 2.

8. Clinical application of cystatin C measurement

Whilst several authors, as already discussed, have demonstrated a strong correlation of the reciprocal of the serum cystatin C value with a reference technique estimation of GFR—in most instances superior to that achieved with creatinine—there is currently little evidence of the clinical benefit; this is because the studies have not yet been done. Several authors have also

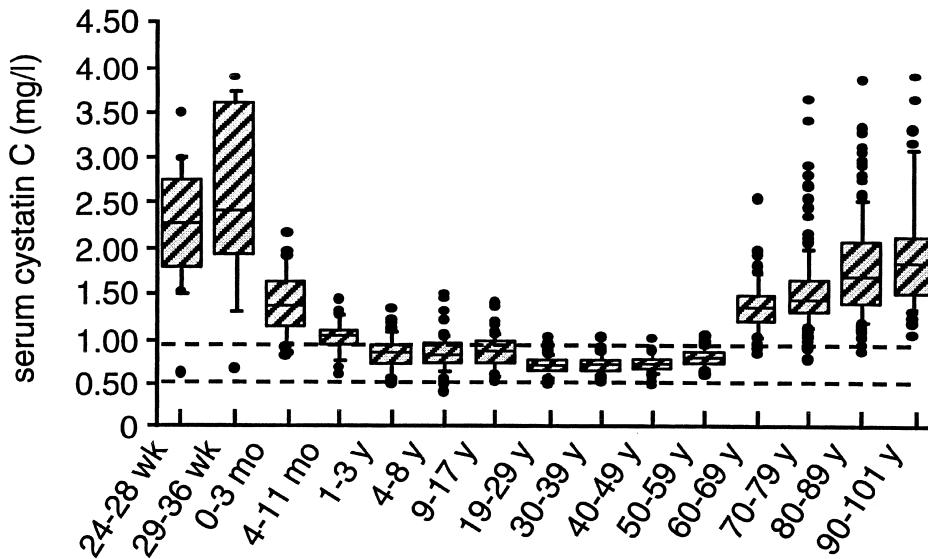


Fig. 2. Reference range data from birth to 101 years of age (10, 25, 50, 75, 90 percentiles) obtained using the Dade Behring nephelometric immunoassay.

demonstrated that the circulating cystatin C level rises prior to that of creatinine as the GFR falls, adding further weight to the argument that it is a more sensitive marker of deteriorating function, a view that gains further support when appreciating the smaller impact of non-renal influences on the circulating marker level. In this respect, however, it is important to extend the work of Keevil et al. [47] on biological variation, taking into account the limitations that they identified in their study.

Some suggested clinical scenarios in which serum cystatin C may add value to current means of assessing renal function are set out in Table 3. The measurement of serum (or plasma) cystatin C will be of undoubted value when repeated observations are required, such as in monitoring patients with chronic renal disease or in patients treated with toxic drugs where a knowledge of the clearance rate is critical to the optimisation of therapy.

There have been isolated reports that serum cystatin C levels may be increased in patients with certain malignancies either as a consequence of

Table 3
Clinical uses of GFR measurement

Early detection of renal impairment
Monitoring of progression to end stage renal disease
Monitoring adequacy of renal replacement therapy
Adjunct to optimisation of drug therapy

enhanced expression in the tumour or as a result of the tumour burden [54,55]. However, further studies are required before these observations represent a limitation to the use of cystatin C as a renal function test.

9. Conclusions

There is now a practical alternative to the performance of clearance studies for the assessment of glomerular filtration rate. Cystatin C meets many of the criteria for an endogenous surrogate marker that can be measured rapidly and precisely. The distribution of cystatin C levels in reference populations from birth to advancing years reflects what is known of the changes in glomerular filtration rate over a lifespan.

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