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Review

Cystatin C: current position and future prospects

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Abstract

Cystatin C is a low-molecular-weight protein which has been proposed as a marker of renal function that could replace creatinine. Indeed, the concentration of cystatin C is mainly determined by glomerular filtration and is particularly of interest in clinical settings where the relationship between creatinine production and muscle mass impairs the clinical performance of creatinine. Since the last decade, numerous studies have evaluated its potential use in measuring renal function in various populations. More recently, other potential developments for its clinical use have emerged. This review summarises current knowledge about the physiology of cystatin C and about its use as a renal marker, either alone or in equations developed to estimate the glomerular filtration rate. This paper also reviews recent data about the other applications of cystatin C, particularly in cardiology, oncology and clinical pharmacology.
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Keywords: cancer; cardiovascular risk; chronic renal failure; creatinine; cystatin C; glomerular filtration rate.

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History

In 1961, three different studies independently described a new protein found on immunoelectrophoresis. Clausen, and Macpherson and Cosgrove found the protein in cerebrospinal fluid (CSF) of healthy patients but not in their blood (1, 2). Butler and Flynn found the protein in 79% of urines of 31 patients with tubular disease (3) and advanced the hypothesis that the protein originated from plasma but could simply not be measured because of a lack of methodological sensitivity. This alkaline low-molecular-weight protein appears in an electrophoresis after the γ globulin band, hence the first names given to it, such as ''post-γ protein'' or ''γ trace''. Slightly later, different authors confirmed that it was present in serum and other body fluids (colostrum, saliva, seminal fluid and ascites) (4–6). In 1979, Lofberg and Grubb from the Lund University (Malmö, Sweden) described the assay of γ trace protein by radial immunodiffusion, with a limit of detection of 300 μg/L and confirmed its presence in blood, saliva and CSF but in different amounts: its concentration in CSF was 5 times higher than in plasma, explaining why it was initially discovered in CSF (7). The same authors found far higher serum concentrations in three dialysed patients than in healthy people that, combined with the rise in urinary concentrations observed in tubulopathy, suggested to them that although the physiology of the protein was completely unknown, it underwent glomerular filtration and was catabolised in the renal tubule. It was only after its amino acid sequence and molecular weight (13260 Da) were described in 1982 (8) that Brzin and colleagues noted the similarity between the protein and a cysteine proteinase inhibitor protein belonging to the cystatin family (9). This was subsequently confirmed by Barret and co-workers who renamed γ trace protein ''cystatin C'' (10).

Cystatin C (CysC) is one of the family of cysteine proteinase inhibitor proteins described for the first time in chick egg white in 1968 (11). Cysteine proteinases (such as the cathepsins B, H and L and the calpains) play a major role in the intracellular catabolism of peptides and proteins through a process of prohormone and proenzyme proteolysis, destruction of collagen and in cancer cells crossing basal membranes. These proteinases can also be produced by micro-organisms (12).

The clinical history of CysC continued in 1984 when Grubb and colleagues suggested that its measurement in CSF may contribute to the diagnosis of hereditary cerebral haemorrhage with amyloidosis, where CSF levels are being abnormally low (13). It was, however, above all as a biological marker of glomerular

filtration rate (GFR) that CysC raised real interest from 1985 and after two other articles by Grubb and colleagues (14, 15). Although these two preliminary articles were not methodologically perfect, and the physiological bases supporting the use of CysC as a marker of GFR were weak and the authors did not show CysC to be superior to creatinine, the interest in this new marker had been raised.

Twenty years later this article proposes to review knowledge about CysC around four areas:

- Analytical aspects.
- Physiological bases of its use as a marker of glomerular filtration.
- Nephrology applications.
- Future perspectives for its application beyond estimation of GFR: cardiovascular diseases, cancer and clinical pharmacology.

Analytical aspects

After its initial determination by radial immunodiffusion and numerous tracer immunoassay methods (RIA, EIA), it was only in 1994 that rapid and entirely automated methods, all based on liquid agglutination of latex particles coated with polyclonal antibodies against CysC, were developed. Depending on the nature of the signal measured, these involved PETIA (particle-enhanced turbidimetric immuno-assay: measurement of transmitted light) or PENIA (particle-enhanced nephelometric immuno-assay: measurement of diffused light). The main difference between these two methods is that PETIA can be performed on a multi-analyte automated biochemistry analyser (wavelength approximately 340–650 nm depending on applications), whereas PENIA requires an infra-red wavelength and can only be performed on a dedicated automated immunonephelometer. Currently, only the PENIA and PETIA methods are used in clinical studies and we shall therefore focus on these methods.

PENIA and PETIA applications available in 2008

The antibodies have few sources and whilst the Siemens PENIA method (ex Dade-Behring, Siemens, Deerfield, IL, USA) uses its own polyclonal antibody, the great majority of other methods use the same reagents marketed by DakoCytomation (Glostrup, Denmark), consisting of latex particles coated with polyclonal rabbit antibodies. The DakoCytomation reagents can be used in PETIA or PENIA. Avian antibodies marked by Gentian AS (Moss, Norway) have recently been developed and assessed for use in PETIA (16).

It is essential to stress that for a long period of time Siemens was the only company to offer PENIA and its acronym must not be associated exclusively with it, as the Siemens kit can be used in PETIA (17) and the DakoCytomation reagents are sold to be used in PETIA or PENIA.

Human recombinant CysC is available, although there is at present no reference material to act as a primary standard. Two types of calibration material are used: i) the DakoCytomation and Gentian AS applications use human CysC – stripped serum spiked with recombinant CysC, and ii) the Siemens application used purified urinary CysC.

Immunonephelometric applications are only available on immunonephelometers belonging to the Siemens gamma BN® range and the Beckman-Coulter IMMAGE range (Beckman-Coulter, Fullerton, CA, USA). Since the initial assessment performed on a Cobas Fara® (Roche SA, Basel, Switzerland), the DakoCytomation kit is currently being used or is being evaluated in PETIA on numerous automated biochemistry analysers as the installation procedures are available on the DakoCytomation internet website (www.dako.com).

Performances and comparison of methods

Since initially described (18), the Siemens PENIA method has been the most widely evaluated and is currently the reference method. The PETIA methods using DakoCytomation antibodies have been developed on numerous different automated instruments and have not been subject to an inter-method assessment. The only published data are those from a Swedish external quality assessment reported by Flodin et al. which, although not providing much detail, reported a range of results in a control sample from 0.66 to 1.09 mg/L for 17 laboratories using the DakoCytomation kit (19). The Gentian AS method has been introduced too recently to have sufficient analytical experience (16).

The main results obtained from the initial evaluations of the three antibody systems are shown in Table 1. A review of the evaluations published in 2002 concluded that the Siemens PENIA method was slightly superior to the DakoCytomation method in terms of the limit of detection, sensitivity to interferences, and intra- and inter-batch precision (21). In the only evaluation published, the Gentian AS method performed excellently. It should be noted that compared to the Siemens PENIA method, it produced very similar results for approximately 80 human sera between 0.5 and 6 mg/L, whether on the P Modular (Roche Diagnostics, Basel, Switzerland) or Architect ci8200 (Abbott, Abbott Park, IL, USA), both methods being calibrated with calibrants provided by the manufacturers (16).

The DakoCytomation PETIA and Siemens PENIA (ex Dade-Behring) methods were directly compared in two studies, which produced inconsistent results. In the older study on 120 samples containing between 0.5 and 9 mg/L by PENIA (18), the two methods correlated excellently ($r = 0.97$), although when each was calibrated with the calibrator provided by the manufacturer, the PETIA (used on a Monarch 2000 automated centrifugal analyser) produced far higher values ($PENIA = 0.76 \times PETIA + 0.15$). Conversely, when a common calibrator was used the slope of the Passing-Bablock line was not significantly different from 1. The recent work by Flodin on samples containing between 0.5 and 8 mg/L by PENIA reported very dif-

Table 1 The main analytical features of the three methods when they were initially described.

	Siemens (Dade-Behring)	DakoCytomation	Gentian AS
Reference	Finney et al., 1997 (18)	Kyhse-Andersen et al., 1994 (20)	Sunde et al., 2007 (16)
Principle	PENIA	PETIA	PETIA
Instrument	BNA 100	Cobas Fara®	Architect ci8200 (A) Modular P (MP)
Calibrating	Polyclonal, rabbit	Polyclonal, rabbit	Polyclonal, chick
Antibody	Purified human urinary CysC	Recombinant human CysC (*Escherichia coli*)	Recombinant human CysC (*Escherichia coli*)
Analytical time	6 min	7 min	≈10 min on both instruments
Limit of detection	0.23 mg/L	0.15 mg/L	A: 0.33 mg/L MP: 0.28 mg/L
Intra-batch CV	Between 2% and 3.2%	<2%	A: not performed MP: between 1.7% and 2.2%
Inter-batch CV	Between 3.2% and 4.4%	<2.2%	A: not performed MP: between 0.3% and 3.5%
Interferences			
Bilirubin	None up to 488 μmol/L	None up to 150 μmol/L Over-estimate <10% between 150 and 300 μmol/L	A: none up to 420 mg/L MP: none up to 800 mg/L
Haemoglobin	None up to 8 g/L	None up to 1.2 g/L	A: none up to 8 g/L MP: none up to 7 g/L Present on both instruments at 10 g/L
Triglycerides	None up to 23 g/L	None up to 9.4 g/L	A: none up to 11 g/L MP: none up to 16 g/L
Rheumatoid factor	None up to 2000 kUI/L	None up to 323 kUI/L	None (no cross-reactions with mammal Ig)
Passing-Bablock equation vs. Siemens PENIA (r)	Not applicable	PENIA not available in 1994	A: Gentian=0.9693× Siemens–0.0527 MP: Gentian=1.0141× Siemens–0.0157
Percentage recovery	95±2.2% (1 FE) for 0.52 mg/L 109±0.03% (1 FE) for 0.93 mg/L	≈100% for concentrations between 1.5 and 6.5 mg/L	≈100% for concentrations between 1.5 and 6.5 mg/L

A, Architect ci8200; MP, Modular P; CV, coefficient of variation.

ferent results (19). Linearity of both methods was lost above 2 mg/L for serum samples (but not for control samples): above this threshold the DakoCytomation method on an Architect ci8200 produced far lower results. In addition and in contrast to what was observed for control and calibration fluids provided by DakoCytomation, linearity was lost after dilution in serum samples at concentrations of >7 mg/L, suggesting a zone effect. This appears to indicate a difference in antibody reactivity against control/calibration fluids and serum samples. This effect did not exist in the same study either to the Siemens PENIA method or for the Gentian AS method on the Architect ci8200.

A final study compared the Siemens N-latex CysC kit (including calibrants) either in PENIA on a BN ProSpec (Siemens) or in PETIA on an Architect ci8200. The two methods displayed an excellent correlation and very low bias on 202 samples (PETIA= 1.0072x+0.0042; $r^2=0.987$) (17).

The results currently available to compare the different applications to measure serum CysC do not provide a precise outline of the transferability of results. The few studies which are available, however, particularly the one conducted by Flodin et al. on CysC (17) and the study by Thuillier et al. for other specific proteins (22) tend to suggest that it is the nature of the antibodies which is most important in inter-method variability rather than the type of detection (nephelometry or turbidimetry).

This situation therefore argues in support of greater between-method comparison, which is becoming increasingly necessary as the parameter appears to be increasing in clinical use.

Stability of CysC

The stability of CysC in serum has been examined in three main studies. These suggested that CysC was stable for 7 days at ambient temperature, for

1–2 months at –20°C and for at least 6 months at –80°C (18, 23, 24). In our personal experience, the length of stability at –80°C can be extended to several years. Freeze/thaw cycles have also been shown to have no effect on CysC.

Physiological bases for the use of CysC as a marker of glomerular filtration

Since the founding definition, mandatory properties that should characterise an ideal endogenous GFR marker have been clarified:

- Constant production and constant plasma concentration in the absence or variation of GFR.
- Low intra-individual variability.
- No plasma protein binding, allowing complete glomerular filtration.
- No secretion, reabsorption or tubular metabolism.
- No extra-renal clearance.

We shall confirm in the next section that these properties partially apply to CysC.

Is the production of CysC constant?

CysC is produced by all nucleated cells in the human body. Studies performed on sections of human tissue or cell lines have shown that when visualised by immunohistochemical labelling or messenger RNA detected by Northern blot, the protein is present in all types of cells studied (25–27). CysC is coded by a housekeeping gene, i.e., a gene expressed both constitutively and in an unregulated manner, the classical argument supporting constant production (25, 28).

CysC has long been considered dogmatically to be produced constantly, as this has been confirmed by work on large cohorts, which was unable to link the production of the protein to any pathophysiological situation other than impaired glomerular filtration (29). This certainty is now being questioned by numerous in vitro and clinical findings.

Physiological determinants of CysC production

Amongst the extra renal factors which may influence CysC values in healthy people, the most recent work has shown that in adults under 60 years old, CysC concentrations are lower in women than in men, the difference disappearing over the age of 60 years old (30–32). These results contradict the older studies which did not recommend establishing sex-related reference values (24, 33–37), except for results found by Pergande and Jung (38).

Age is also a factor involved in CysC variability. Higher values are found in neonates regardless of sex, weight or the child's height (39–41), including premature infants (35): falling after birth to return to identical values to those in adults by the age of 4 years old (30). Caution is however required in very young children and premature infants in whom high CysC values may reflect low GFR as part of the renal maturation process (35, 42). Most studies in adults show that age has a significant impact on CysC concentrations, implying different reference values for people over 50–60 years old (30, 33, 34, 37). It is important to note that reference values in both adults and children are systematically lower when measured by the Dade-Behring Siemens PENIA method (vs. the various PETIA applications of the DakoCytomation kit) (Table 2).

Intra-individual variability

In 1998, Keevil et al., using the PETIA method with DAKO reagents, described very considerable intra-individual variability of blood cystatin concentrations, suggesting that it could not be used for longitudinal assessment of glomerular filtration (46). This initial study has recently been refuted by works which have shown that the intra-individual variability of CysC, measured by the PENIA method with Siemens reagents is equivalent to that of creatinine (47, 48).

Influence of muscle mass

The major limitation of creatinine is its dependency for production on muscle mass (49). For the same GFR, an anorexic patient and weightlifter would have very different serum creatinine concentrations. Initially, Vinge et al. described blood cystatin concentrations as being independent from muscle mass (50). This study has recently been criticised in terms of its statistical and clinical methodology. Recently, MacDonald et al. showed more convincingly that serum cystatin is indeed partly dependent on muscle mass (51) (GFR determined by inulin clearance and lean mass by densitometry). In doing this, these authors confirmed the hypothesis put forward by Knight et al., who found that serum cystatin was dependent on height and weight in their cohort involving measurement of creatinine clearance (32). The influence of muscle mass on CysC production is explained by the fact that muscle cells constitute the largest number of nucleated cells in the body (51). Nevertheless, the variability of CysC due to muscle mass is far less than for creatinine. The advantage of CysC over creatinine in a patient with reduced muscle mass is therefore still considerable (52–55). In particular, malnutrition has been shown in children not to affect equations based on CysC concentrations in contrast to the serum creatinine-based Schwartz equation (53).

Hormonal influences

In vitro, CysC production by cultured HeLa cells was described as early as 1995 as being transcriptionally stimulated by corticosteroids (56). Although no rise in serum CysC concentrations was found in children suffering from nephrotic syndrome treated with high dose corticosteroids (57), contradictory results were found in other studies. An increase in CysC concentrations, dependent on corticosteroid doses was demonstrated in asthmatics (58) and in studies including adult renal transplant patients (59, 60) and in children suffering from cancerous or renal disease (61). But it

Table 2 Reference values for children and adults.

References	Method	Sample, n	Age, years	Reference values, mg/L
Filler et al. (43)	PETIA[b]	216	0.8–18	0.18–1.38
Bokenkamp et al. (39)	PETIA[b]	200	1–18	0.7–1.38
Randers et al. (41)	PENIA[a]	96	1–14.1	0.51–0.95
Finney et al. (35)	PENIA[a]	30 79 182	Premature 1 day to 1 year 1–17	0.43–2.77 0.59–1.97 0.5–1.27
Harmoinen et al. (44)	PENIA[a]	58 50 65 72 162	Premature Neonates 8 days to 1 1–3 3–16	1.34–2.57 1.34–2.23 0.75–1.87 0.68–1.60 0.51–1.31
Galteau et al. (30)	PENIA[a]	246	4–19	0.58–0.92
Fischbach et al. (45)	PENIA[a]	51 47	1 month to 18 months 18 months to 18	0.7–1.18 0.44–0.94
Bahar et al. (42)	PENIA[a]	98	3 days	0.72–1.98
Norlund et al. (37)	PETIA[b]	249 (124 men, 125 women)	M <50 M >50 F <50 F >50	0.79–1.05 0.88–1.34 0.75–0.99 0.85–1.35
Sunde et al. (16)	PETIA[c]	138	Not stated	0.57–1.09
Galteau et al. (30)	PENIA[a]	1223 (530 men, 693 women)	H <60 F <60 >60 (M and F)	0.64–0.84 0.565–0.735 0.727–0.933

[a]Siemens reagent; [b]DakoCytomation reagent; [c]Gentian AS reagent.

appears that the corticosteroid dose-dependent elevation of CysC concentration has little impact on the estimation of GFR in patients with low or moderately high glucocorticosteroid doses.

Hyperthyroidism increases serum CysC concentrations (62–66). As CysC production and GFR move in opposite directions in response to thyroid hormones, the use of CysC would appear inappropriate in dysthyroid states; in addition, this suggests that thyroid function should be measured in any study designed to validate diagnostic instruments using serum cystatin concentrations.

Influence of inflammation

Whilst it was previously believed that CysC production was independent of inflammation (67), it now appears that interleukin-6 causes a fall in CysC expression at least in dendritic cells (68). Knight et al. also showed in a large cohort (n=8058) that C-reactive protein (CRP) was an independent determinant of CysC concentration in univariate analysis. CRP values in this study, however, were more a reflection of microinflammation (and associated cardiovascular risk) than acute inflammation as seen in infection or inflammatory disease. It should also be noted that in study by Knight et al. GFR was measured by creatinine clearance, which is open to criticism (32). Regardless, whilst the influence of inflammation on plasma CysC concentrations remains somewhat contentious, it appears to be far less than for other medium molecular weight proteins in severe inflammation (such as β_2 microglobulin).

Influence of neoplasia

Tumours have been suggested to influence CysC production, although this is still widely debated, as discussed further on in this article. GFR was not measured using a reference method in any of the available studies.

Others

Some studies found that smoking (30–32) and alcohol consumption (31) influence CysC concentrations. These should be assessed as possible factors contributing to CysC variability.

What is the renal fate of CysC?

There are relatively few specific physiological studies on CysC, the main one of which was conducted in the rat (69). After being filtered without restriction by the glomeruli because of its low molecular mass and absence of protein binding, CysC is entirely reabsorbed by the proximal tubules, where it is almost entirely catabolised (26, 27, 69). Tubular reabsorption occurs through a receptor, megalin (common to many proteins including albumin) by endocytosis (70–72). It is widely accepted that no tubular secretion of CysC

occurs, although one study in human beings published data which may suggest the opposite (73). The methodology in this study was widely criticised and its conclusions must, however, be interpreted with caution (74–76).

Physiological urinary CysC concentrations are therefore extremely low in the region of a tenth of 1 mg/L and can be measured by immunonephelometry (77, 78). In addition, the absence of circadian variation allows a measurement to be performed rapidly on a random sample (79). Raised urinary CysC concentrations are believed to indicate a tubular abnormality (77, 80–82).

Whilst the features of the urinary CysC excretion open future perspectives for its use as a marker of tubular dysfunction, they preclude the use of its urinary clearance as a measurement of GFR. The use of serum cystatin concentration alone corrected for production variation factors should, however, theoretically enable satisfactory GFR estimation.

In conclusion, CysC therefore appears to be an interesting marker for the estimation of GFR. It does offer several advantages over creatinine or other similar molecular weight proteins. The inability to measure urinary clearance is not a major problem for an endogenous marker of GFR, such as CysC. Although measurable, creatinine clearance is progressively being abandoned in international recommendations in favour of formulae to estimate the GFR. This choice is guided in particular by the great difficulty in obtaining reliable urinary collections.

CysC is not, however, a perfect marker for GFR in the strict sense of the term. Whilst its renal fate is consistent with that of an ideal endogenous marker of GFR, its production appears to depend on physiological determinants and hormonal, humeral or anthropometric factors. These factors should be taken into account when serum cystatin concentrations are interpreted and when any equation to estimate GFR based on CysC is constructed and validated. In general terms, more rigorous studies could still improve our physiological knowledge, particularly the renal fate, of the protein.

Nephrological use of CysC as a marker of low GFR

The use of serum cystatin as an endogenous marker of GFR in general populations of renal failure patients has been widely assessed. Two meta-analyses are available (83, 84). Although the patients included in the studies were clinically heterogeneous, the two analyses reached almost identical conclusions (Table 3) and agree that serum cystatin is superior to serum creatinine to rule in renal impairment in the cut-off range of GFR between 60 and 79 mL/min/1.73 m^2 (83).

In this article, we shall describe the most recent studies using GFR measurement algorithms based on serum cystatin and focus on knowledge obtained in certain specific populations, in whom measurement of GFR is both essential and unsatisfactory using the serum creatinine.

GFR measurement algorithms incorporating CysC

Whilst CysC was firstly studied as an early detection marker for reduced GFR, several authors quickly introduced the concept of estimating GFR more precisely and more accurately from equations based on CysC, analogous to the equations based on serum creatinine [such as the Cockcroft and the ''Modification of Diet in Renal Disease'' (MDRD) equations] (21, 85). Since then we have seen a real ''epidemic'' of equations based on CysC (21, 110–115), particularly as simultaneously with the discovery of extra renal effects on serum cystatin, some authors have logically developed different equations depending on patient type or equations expressing a corrective factor based on age, sex or disease (115–117) (Table 4). Some authors have also recently advanced the hypothesis that an equation combining creatinine and CysC may be useful (111, 115–117, 125, 126). Moreover, the performance of a CysC-based equation in predicting GFR may differ from one study to another. Amongst other factors, the techniques of GFR measurement used as a reference method are quite heterogeneous across studies and may have contributed to this variability (Table 4).

Some equations, however, have been constructed from sample sizes which have been too small and/or populations which are too specific. Others are complex as they use additional non-biological parameters which do not provide any apparent advantage. In general, it can also be stated that these equations have been subject to very limited validation in populations other than those in which they were constructed (110–115). At present, these equations appear to offer very limited advantage compared to the MDRD equation, which is based on serum creatinine, age, sex and race, at least for the general population (110–115, 127, 128). These equations also appear to offer limited precision (52, 110, 113, 116, 124, 129, 130). As we shall see below, they could be more useful in certain subpopulations in which creatinine-based equations are particularly inaccurate, as in paediatrics (116, 117, 124), transplantation (113, 115, 131–133) or oncology (134). Validation studies on large independent populations, however, would appear to be needed.

As applies to equations based on the serum creatinine (135, 136), problems of methodological difference and calibration problems in CysC measurement can have important consequences. It is unlikely therefore that an equation constructed with serum cystatin measured by the Siemens PENIA method would offer a precise measurement of GFR if it incorporated a serum cystatin measurement using different antibodies and/or calibrants and/or reading method and vice versa (18, 19, 127, 137, 138). As the relationship between GFR and serum CysC is exponential, the impact of the precision of the equation would, as for the MDRD equations, be less with lower CysC values. This problem has been clearly emphasised by Lars-

Table 3 Comparison of cystatin C and creatinine as markers of kidney function: results of two meta-analysis.

References	Roos et al. (83)	Dharnidharka et al. (84)
Publication date	2007	2002
Criteria for selecting studies		
Publication date between	January 1984 and February 2006	Until December 2001
Use of a reference method for GFR	Yes	Yes
Use of PENIA or PETIA methods	Yes	Not described
Study of sensitivity/specificity	Yes	Yes
Cut-off values for the reference test to discriminate normal from abnormal renal function	60 to 79 mL/min/1.73 m^2	Not described
Statistical analysis	Sensitivity/specificity Diagnostic odd ratios (DOR)	Combined ROC curves
Sample size	2007 (23 studies)	997 (14 studies)
References	(20, 26, 54, 85–104)	(85, 87, 88, 93, 95, 105–109)
Results	DOR: Cystatin C: 54.001 (95% CI: 30.115–96.641) Creatinine: 16.297 (95% CI: 8.348–31.785)	Combined AUC: Cystatin C: 0.926 (95% CI: 0.892–0.960) Creatinine: 0.837 (95% CI: 0.796–0.878)
Limitations	No differentiation in the analysis of results obtained with different cystatin C methods of measurement Very heterogeneous populations (paediatrics, cirrhosis, diabetics, etc.)	Number of studies and data limited No cut-off value for GFR in the inclusion criteria Very heterogeneous populations (paediatrics, cirrhosis, diabetics, etc.)
Conclusion	Cystatin C is a better parameter than creatinine for the detection of a true renal impairment, although the 95% CI DOR overlap	Cystatin C is better than creatinine as a marker of kidney function

AUC, area under the curve; CI, confidence interval.

Table 4 GFR predicting equations based on cystatin C alone or in combination with creatinine.

References	Sample, n	GFR measurement	Cystatin C	Population	Equations
Bokenkamp et al. (85)	83	Inulin	PETIA	Paediatrics	(162/CC)–30
Tan et al. (118)	40	Iohexol	PENIA	Diabetics and health	(87.1/CC)–6.87
Hoek et al. (119)	47	Iothalamate	PENIA	Various	(80.35/CC)–4.32
Larsson et al. (120)	100	Iohexol	PENIA PETIA	Various	$77.24\times CC^{-1.2623}$ $99.43\times CC^{-1.5837}$
Filler et al. (121)	536	^{99}Tc-DTPA	PENIA	Paediatrics	$91.62\times(1/CC)^{1.123}$
Le Bricon et al. (122)	25	^{51}Cr-EDTA	PENIA	Transplant	$[(78\times(1/CC)]+4$
Sjostrom et al. (123)	381	Iohexol	PETIA	Various	(124/CC)–22.3
Grubb et al. (124)	536	Iohexol	PETIA	Various + paediatrics (n = 85)	$84.69\times CC^{-1.68}\times 1.384$ if less than 14 years old
Rule et al. (115)	204	Iothalamate	PENIA	Various excluding transplant	1) $66.8\times CC^{-1.3}$ 2) $[(66.8\times CC-^{-1.3})\times(273\times SCr^{-1.22}\times age^{-0.299}\times 0.738$ if female$)]^{0.5}$
Rule et al. (115)	206			Transplant	$76.6\times CC^{-1.16}$
MacIsaac et al. (112)	125	^{99}Tc-DTPA	PENIA	Diabetics	(84.6/CC)–3.2
Bouvet et al. (116)	67	^{51}Cr-EDTA	PENIA	Paediatrics	$63.2\times(SCr/96)^{-0.35}\times(CC/1.2)^{-0.56}\times(weight/45)^{0.3}\times(age/14)^{0.4}$
Zappitelli et al. (117)	103	Iothalamate	PENIA		1) $75.94/(CC^{1.17})\times 1.2$ if renal transplant 2) $(43.82\times e^{0.003\times height})/(CC^{0.635}\times SCr^{0.547})$
Ma et al. (111)	376	^{99}TcDTPA	PENIA	Various, Chinese	$\{(87\times CC^{-1.132})\times(175\times SCr^{-1.234}\times age^{-0.179}\times 0.79$ if female$)\}^{0.5}$
Stevens et al. (125)	3418	Iothalamate	PENIA	Chronic kidney disease	1) $127.7\times(CC)^{-1.17}\times(age)^{-0.13}\times(0.91$ if female$)\times(1.06$ if black$)$ 2) $177.6\times(SCr)^{-0.65}\times(CC)^{-0.57}\times(age)^{-0.20}\times(0.82$ if female$)$ $\times(1.11$ if black$)$

In all cases, serum cystatin is expressed in mg/L, serum creatinine in mg/dL (to convert to μmol/L multiply by 88.4), age in years, weight in kg. CC, cystatin C; SCr, serum creatinine. In this Table, all of the PENIA methods are Dade-Behring Siemens methods, and all of the PETIA methods used the DakoCytomation kit adapted for various automated instruments.

son et al. who has published two different method-specific equations for measurement of CysC (120).

Paediatric populations

New biological markers of GFR are perhaps even more difficult to study in paediatrics than in adults. In addition to the methodological constraints which may be seen in adults (use of a reference method for GFR, robust statistics, sufficient representative sample, etc.), some more paediatric-specific difficulties are often encountered. It is, for example, difficult to justify performing GFR measurements using a reference method in healthy people. The control populations in these studies are usually therefore children with normal GFR but who also have underlying renal or urological disease (vesico-ureteric reflux, nephrotic syndrome) and cannot strictly be considered to be a *true* healthy control population (86). Even more problematic in measuring markers of GFR is the lack of a clear consensus on the very definition of normal GRF values themselves in children. Some believe that age-related reference values should be reported, which makes analysis of the sensitivity and specificity of new markers difficult (139). The lack of simple data on normal GFR values in paediatric practice explains why the values considered to be ''normal'' for GFR in receiver operator characteristic (ROC) curve analyses vary depending on the author from 60 to 100 mL/min/1.73 m^2 (124, 140).

The fact that CysC does not depend much, if at all, on muscle mass is an important theoretical advantage over creatinine in paediatric practice (50, 51). Creatinine reference values must therefore be interpreted as a function of patient age (141–143). Several authors have demonstrated that CysC reference values are identical (or very similar) in adults and children over 1 year old (Table 2). Several studies have examined the ability of CysC in paediatrics to detect renal failure earlier than the serum creatinine or creatinine-based estimated GFR equations [the best known being the Schwartz equation which includes patient height (143)]. Results are contradictory, some being in favour of CysC (55, 85–87, 144–146), whereas others find that it has no added value (87–89, 104, 140). This may be explained by the inherent limitations of specifically studying children, discussed above, and also by the fact that many authors did not separate out children who were or were not receiving corticosteroid therapy (144, 147, 148). The use of different creatinine assay methods (Jaffé vs. enzymatic) and more or less appropriate correct use of the Schwartz equation (with or without a laboratory-specific correction factor) could also explain some discrepancies between the results (117, 124).

Of the studies supporting CysC, those conducted by Filler et al. are based on a large database of GFR measurements (86). Apart from an advantage found in an overall population (86) and in a ''sub-population'' of transplant patients (144), Filler et al. demonstrated the utility of CysC in patients with spina bifida who very often had greatly reduced muscle mass (55).

Several authors have developed equations to calculate GFR based on CysC, some combined with creatinine (Table 4). The equations by Filler et al. (constructed using the Siemens PENIA method) (121) and Grubb et al. (DakoCytomation PETIA on P Modular) (124) were constructed based on a study on a large number of patients (both n=536), although these have not been validated in paediatric populations other than those of which they were constructed. Zappitelli et al. have been alone in validating a few equations and obtained good results provided that they were corrected in order to be applicable to their own methodology (regression factor). Uncorrected, the results were far less useful. In addition to this validation work, Zappitelli et al. also developed two GFR estimation equations, one using only CysC and the other using CysC and creatinine. It is interesting to note that Zappitelli et al. used correction factors in these equations depending on the clinical context (presence of a renal transplant, spina bifida) (117). Bouvet et al. also developed an equation combining creatinine and CysC in a smaller number of patients (n=67) also incorporating height and weight and again highlighting the importance of non-renal factors. This equation was validated by the same authors in an independent population of 33 children (116).

In conclusion, because its reference values are independent of age and although not all studies agree, serum cystatin is undoubtedly a tool of choice to screen for and monitor renal failure in paediatric patients. In contrast to many studies in adults, its clinical performance has been evaluated against a reference method for GFR measurement which makes the good results obtained particularly robust. The equations for estimating GFR based on CysC require prospective validation studies before they can be recommended in everyday clinical practice (116, 117, 120, 124) and particularly before they can replace GFR measurement by a reference method when this is required in children (117, 124, 119).

Utility of CysC in transplantation

CysC is of significant theoretic use in transplantation, as there is a high risk that renal function will deteriorate in transplant patients because, amongst other things, of the very widespread use of nephrotoxic calcineurin inhibitors (149). In addition, creatinine can be very inappropriate in these patients as they often have important co-morbidities and are treated with steroids, which have a negative effect on muscle mass (150); furthermore, cyclosporine can also influence tubular creatinine secretion (151). In this context, several groups have tried to establish whether CysC could be a more sensitive marker than creatinine for the early detection of deterioration in GFR in renal transplant patients. Results are inconsistent, some authors finding CysC to offer improved sensitivity (90, 91, 122, 152), whereas according to others the diagnostic performance (assessed by ROC curve methods) does not differ significantly between the two markers, in particular for the critical GFR threshold of 60 mL/min (52, 92, 153).

Despite these contradictory results about the utility of serum cystatin in isolation, it is now seeing a return in interest for equations incorporating CysC designed to estimate GFR. This is partly explained by the fact that equations based on creatinine considerably overestimate GFR in renal transplantation (154–156). Overall, equations using CysC appear to offer better predictive performance, although it remains to be shown that this improvement in prediction is clinically significant (52, 131, 133, 157, 158). They provide a more accurate estimate of GFR than the MDRD equation (133) and improve classification of renal transplant patients into the different stages of chronic renal disease (158). It should be noted, however, that in a recently published study, the superiority of GFR estimation based on CysC compared to serum creatinine was not confirmed in renal transplantation (130). This study, however, had a number of methodological limitations which could have influenced its results (137).

In heart transplantation, the Rule equation (115) incorporating CysC significantly increases the accuracy of GFR prediction compared to the MDRD equation (52). Equations based on CysC have also been reported to offer better predictive performance in liver transplantation (131).

Of the different equations using CysC, which have been tested in transplantation, the equation providing the best estimate of GFR is not always consistent between studies. It is possible that equations specific for transplant patients may be needed. Rule et al. confirmed previous results which had already suggested that CysC may underestimate GFR and found that GFR was 19% higher in transplanted patients (148), compared to renal failure patients with their own kidneys (115). The most widely proposed explanation for this is that CysC production is increased by immunosuppressant treatments, particularly steroids (59). This had led some authors to construct specifically developed equations for adult (115, 122) or child (117) transplant patients. The Rule and Le Bricon equations are often found to be amongst the best performing equations in transplantation (115, 122). It remains to be demonstrated, however, that any equation developed specifically for transplantation offers a significantly better estimate of GFR.

Diabetic patients

In view of the increasing incidence and high prevalence of diabetic nephropathy (159), it is not surprising that CysC has been specifically studied in diabetic patients. It has a potentially important use in early screening for diabetic nephropathy, early management of which is undoubtedly beneficial. In this section, we shall consider the studies which have specifically examined either type 1 or type 2 diabetic populations. We will highlight the studies which have been best constructed methodologically (reference measurement for the GFR, adequate statistical analysis, sufficient population in terms of patient number and range of GFR studied). CysC (or the reciprocal of CysC) has correlated as well and occasionally better than creatinine with GFR in all of the studies which have compared the utility of CysC to that of creatinine in the early detection of renal failure in diabetic patients (GFR >60 mL/min/1.73 m^2) (93–96, 160–162). The only exception is the study by Oddoze et al. (95) in which the performance of creatinine can be considered to be abnormally good. Perlemoine et al. did not find CysC to offer any advantage in detecting GFR <80 mL/min/1.73 m^2, except in the sub-group of patients with a creatinine of less than 1 mg/dL (88 μmol/L) (96). Of these different studies, the study by Pucci et al. which examined 288 diabetic patients (both types) with GFR measurement by plasma iohexol clearance and a wide range of GFR is undoubtedly one of the most important studies (162). The authors found a significantly better correlation between CysC and GFR than between creatinine and GFR. CysC had a higher product (sensitivity$\times$ specificity) for detecting GFR of less than 90 and 75 mL/min/1.73 m^2, although its diagnostic value was no greater than that of creatinine to detect a GFR of less than 60 mL/min/1.73 m^2. This was predictable given the good performance of creatinine at this level of renal failure (163). The best threshold (positive predictive value of 93% and negative predictive value of 87%) to detect a GFR <90 mL/min/1.73 m^2 was 0.98 mg/L, i.e., a value very close to the upper end of the reference interval in a general population (30) (Table 2).

The diabetic population lends itself relatively well to longitudinal follow-up studies of renal function. This type of study is extremely important in order to compare the performance of biological markers in early diagnosis. Three authors have conducted this type of study on CysC in diabetic patients, all of which reported the marker to be useful (119). The most convincing study both methodologically and in terms of its results was undoubtedly the study by Perkins et al. (164). Perkins et al. followed 30 diabetic type 2 obese hyperfiltrating diabetic Pima Indians (GFR >120 mL/min) longitudinally for 4 years with at least one measurement of GFR per year (urinary iothalamate clearance). Of these 30 patients at risk of developing nephropathy because of their hyperfiltrating state (165), 20 subsequently did. The fall in GFR was better reflected by change in serum cystatin in these 20 patients (although this remained within reference values) than by changes in serum creatinine or derived equations, all of which under-estimated the fall in GFR (164). In a study on 20 subjects with reduced GFR, Beauvieux et al. showed that GFR estimation equations based on CysC better reflected changes in measured GFR at 2 years (urinary ^{51}Cr-EDTA clearance) than creatinine-based equations (110).

CysC therefore appears to be a useful detection marker in the diabetic population (transverse use or in longitudinal follow-up) for early nephropathy. The use of equations based on CysC (alone or in association with creatinine) to estimate GFR has not been greatly studied and results of the few published studies on the subject are contradictory and difficult to compare (110, 112, 126). It should be noted that, with two exceptions, none of the equations based on CysC

have been constructed from a strictly diabetic population (112, 118). This could be important in terms of the influence of extra renal determinants of CysC.

The elderly

Epidemiological studies have highlighted the high prevalence of nephropathy in the elderly. American registers report the prevalence of microalbuminuria to be 18% in people between 60 and 69 years old and 30% in people over 70 years old (166). Similarly, the prevalence of stage 3 renal insufficiency in people over 70 years old (estimated GFR <60 mL/min/1.73 m^2) is estimated to be around 35% (167). French data confirm the increase in the prevalence of renal failure with age. The REIN register in France reported a prevalence of 2042 dialysed patients per one million over 75 years old, with a clear male preponderance [(REIN) Réseau Epidémiologie et Information en Néphrologie register www.soc-nephrologie.org, nephro/register space)]. The presence of pre-dialysis chronic renal failure (CRF) is far less clearly documented. In practice, renal function estimation in the elderly is based on measurement of the creatinine and predictive equations based on it. Age-related sarcopaenia, however, causes a fall in creatinine production. Predictive equations, including age and sex, partially take this factor into account. The Cockcroft-Gault equation, however, systematically underestimates GFR in the elderly (168). The more reliable MDRD equation, however, can only take into account the mean fall in muscle mass and creatinine associated with age (169). Inflammation, malnutrition and loss of muscle bulk (often associated with chronic diseases, such as heart failure and bronchopneumonia) can further accentuate the muscle metabolic abnormalities and influence the value of creatinine-based predictive equations (170–172).

CysC therefore emerges as an alternative marker. Serum cystatin values in the population increase with age, particularly over 70 years old (30, 31, 97, 173). An increase of 0.045 mg/L every 10 years has recently been reported (31). This increase may theoretically be due to renal factors (age-related deterioration in renal function) (30, 173) or extra renal factors (174) raising the question of specific reference values in the elderly. In the elderly diabetic, for example (64–100 years old), the prevalence of nephropathy estimated from CysC is 64.7% compared to only 21.4% if age-adjusted reference values are used (175). Amongst the extra renal factors most often found are inflammation (but which may however be a consequence of the CRF itself) (176–178) and corticosteroid treatments (174). Finally, despite contradictory results, a relationship between the CysC gene polymorphism (CST3 on exon 1) and Alzheimer's disease has been strongly suggested (179–181). A very recent Taiwanese study has shown that circulating CysC concentrations are negatively associated with the presence of CST3 polymorphism and were significantly lower in Alzheimer subjects (180). Overall, CysC appears to be less sensitive to metabolic and extra renal factors than creatinine in the elderly (174). Potential sources of bias between these two markers may explain the discrepancies seen in the elderly between GFR estimation by CysC, measured clearance and predictive equations (182, 183). These discrepancies are seen above all in people with co-morbidities (183) and may result in differences in the reported prevalence of CRF. There are still too few studies which have compared CysC concentrations with a reference measurement. Hojs et al. have recently reported a better correlation between the reciprocal of CysC and ^{51}Cr-EDTA clearance compared to the reciprocal of creatinine or measured creatinine clearance in elderly patients with renal failure (184). A simple comparison of correlations is not, however, statistically sufficient to confirm that CysC is superior to creatinine. CysC could be a more sensitive marker than creatinine to investigate for moderate reductions in GFR in the elderly (69–92 years old) (185, 186), although the results reported are inconclusive (98, 99).

In conclusion, CysC appears to be a promising marker for the early diagnosis of renal dysfunction in the elderly. However, the interactions between potential confounding variables, such as inflammation or the presence of concomitant diseases (such as, neurological), need to be better defined.

CysC and acquired immunodeficiency syndrome (AIDS)

Many studies have examined the utility of CysC measurement in populations with reduced muscle mass. Few studies, however, have been conducted in people infected with the human immunodeficiency virus (HIV) who may, however, differ from the general population as a result of malnutrition and common changes in body morphology.

End stage CRF is no longer particularly rare in this population and the number of HIV-infected patients dialysed is increasing in the United States and Europe (187). A prevalence of CRF in different populations of HIV-infected people (whether or not treated, controlled or otherwise) may be as high as 5% to 25% (188–190). Highly active anti-retroviral therapy (HAART) treatment has not eliminated HIV-specific renal disease, the HIV-associated nephropathy (HIVAN), which is responsible for 40% to 60% of the histological renal disease (191), or the need for transplantation in HIV-infected patients (192). Apart from the specific role of the virus, people infected with HIV have a large number of risk factors for non-specific CRF, including age, hypertension, non-insulin-dependent diabetes and exposure to multiple long-term drug treatments (193).

The American Society for Infectious Diseases published the initial recommendations on the management of renal function in HIV-infected people in 2005 and recommended creatinine measurement if muscle mass was normal and GFR estimation equations in other situations (194). The Cockcroft-Gault equation is frequently used to adjust dosages for renal function, as most clinical studies consulted to produce the recommendations used this equation (195, 196). No

equation, however, can be formally recommended as none has been validated in the group of people infected with HIV. This population also has significantly lower muscle mass than seronegative patients (197) and it should be noted that this is one of the clinical situations in which the experts of the Kidney Disease Improving Global Outcome (KDIGO) recommended GFR measurement using a reference method and not simply by estimation (166).

Recent studies have shown that CysC concentrations are higher in HIV+ subjects than seronegative patients, even if creatinine concentrations are normal (198, 199). Serum cystatin concentrations correlate positively with viral load and negatively with duration of anti-retroviral treatments (which delay the progression of the renal disease). This suggests that serum cystatin may be a good marker of progression, either deterioration or improvement, of the viral disease. The authors also propose that CysC be used as an early marker of improvement in renal function on HAART (198).

Measurement of CysC may therefore be a useful alternative for estimating GFR in HIV+ patients, although this proposal, however, needs to be confirmed in studies in which GFR is measured by a reference method.

CysC and hepatocellular failure

When examined in populations of patients with cirrhosis, CysC has been shown to be equivalent or even superior (100, 101, 113) than creatinine (200) in assessing renal function. In a recent study, CysC was the only marker to correlate with measured GFR in all stages of hepatocellular failure (201). In addition, serum cystatin concentrations also appear to be a better marker than creatinine and the Cockcroft equation for the earlier diagnosis of renal disease in end stage liver failure (202). It has also been recommended for the follow-up of renal function after liver transplantation (203). The Hoek et al. (119) and Larsson et al. (120) equations perform at least as well as the MDRD equation in these populations (131). CysC appears to be a better predictor of acute renal failure after liver transplantation (204), including children (146), and to provide better follow-up for moderate changes in renal function (152).

As the model for end stage liver disease (MELD) score, which measures the extent of end stage hepatocellular failure, includes measurement of serum creatinine to assess the impact of renal function on patient prognosis and is used to prioritise liver transplantation candidates (205), the use of CysC appears to be promising in these cirrhotic patients, particularly as creatinine measurement is subject to interferences with high bilirubin levels (which does not apply to CysC) (206).

Future perspectives for CysC applications

CysC as a cardiovascular risk marker

End stage CRF (207) or stage 3 of the Kidney Disease Outcome Quality Initiative (KDOQI) of the National Kidney Foundation (208) is currently recognised to be an independent risk factor for cardiovascular diseases. The appearance of a biological marker enabling potentially earlier deterioration in renal function and one which is less dependent on extra renal factors than creatinine has led several groups, particularly the Shlipak group, to examine the relationships between cardiovascular diseases, mortality and circulating CysC (209).

The immediate interest of CysC in cardiovascular diseases is, however, related to its role as a protease inhibitor and not as a marker of glomerular filtration. The proteinases, particularly cathepsins K and S, were implicated very early on in the rupture of the tunica elastica of the arterial wall and a hypothesis of in situ imbalance between arterial wall cathepsins and inhibitors was proposed. Reduced tissue CysC concentrations have been found in atheromatous plaques, aneurysms (210) and angioplasty lesions in animal models (211) and implicated in the pathophysiology of aneurysms. CysC has been confirmed to play a protective role in situ in genetic models of arterial disease. Apolipoprotein E (ApoE)–/– mice in which the CysC gene has been incapacitated (Cyst–/–) develop aneurysmal lesions and rupture of the limiting internal elastic lamina compared to ApoE–/–, Cyst+/+ mice (212, 213). These studies on animal models are supported by occasional human genetic data (214, 215). Patients with mutations in the CysC promoter gene have low circulating CysC concentrations (214, 215). The same mutations are associated with a higher number of coronary artery stenoses in a sub-group of patients (n=237) undergoing coronary angiography during an infarction, although they had no influence on the severity of the stenoses (214). These genotypes also do not influence patient survival at 3 years (215). These experimental and clinical findings suggest that CysC may have a role in vascular remodelling.

Epidemiological studies based on large patient cohorts have clearly demonstrated increased CysC values (above 1.30 mg/L) to be an independent risk factor for cardiovascular disease. However, most of these studies do not use a reference method to determine GFR which could hamper interpretations of results.

The initial studies established the prognostic importance of CysC in the follow-up of heart disease. In a cohort of 1033 people who were suffering from coronary artery disease, increased CysC concentration (1.24 mg/L) was a significant predictive factor for a second cardiovascular accident even after adjusting for classical risk factors, CRP and converting enzyme inhibitor treatment. Conversely, neither creatinine nor creatinine clearance displayed the same association (216). At the same time, Shlipak et al. (217) showed that CysC was a better predictive factor for death in patients with heart failure. Retrospective studies on existing cohorts very rapidly extended these findings to the entire population and in particular to the elderly. CysC was significantly associated with all cause mortality (209, 217–223), cardiovascular mortality (209, 217–219, 222, 223), myocardial infarction (209,

224), cerebrovascular accident (209, 225) and peripheral arterial disease (226). Whilst the association with all cause or cardiovascular mortality was found systematically, other authors failed to find relationships between CysC concentrations and non-coronary vascular accidents (218) mostly in middle-aged men (227). These relationships between cardiovascular disease and increased CysC concentrations have been described both in cohorts or sub-groups of patients selected on the basis of a past history of cardiovascular disease (216, 217, 219, 221, 225, 226) and in patients without cardiovascular history (220, 222–224, 228, 229). Some also consider that increased CysC values may be associated with morphological cardiac abnormalities, such as left ventricular hypertrophy or left ventricular dysfunction on echocardiography (228), functional abnormalities, such as heart failure (230), or poor exercise tolerance (231). In all of these studies, the association between cardiovascular disease and CysC appeared to be stronger than with creatinine or GFR estimation algorithms based on creatinine. Furthermore, the association between cardiovascular disease and CysC appears to be linear, increasing with the CysC level. In particular, in the MDRD cohort, a linear progression in risk was observed between values of 1.45 and 3.17 mg/L (222). Establishing a significance threshold obviously depends on the population selected and whether or not patients with renal failure are excluded. The significance threshold generally found without stratification by renal failure is around values of 1.30 mg/L. The significance threshold in populations without CRF detected by predictive equations, however, can be reduced to 1 mg/L (219, 223).

Following these epidemiological studies and after adjusting for the major classical risk factors, CysC has been proposed as an independent population marker of vascular risk, which is superior to creatinine-based GFR estimation. However, the association between CysC and cardiovascular disease appears somewhat complex and this connection should be carefully discussed taking into account at least three factors: i) recognition of moderate alteration in GFR with CysC unmasking an early association between cardiovascular disease and renal injury, ii) the interaction between CysC and non-traditional risk factors present in renal failure, such as inflammation, and iii) a direct action of CysC on the arterial wall.

CysC is more sensitive than creatinine to screen for early renal failure

The limitations of creatinine as a marker of glomerular filtration are partly due to extra renal factors, such as age, diet, physical activity, and, above all, muscle mass. These limitations must be taken into account particularly in the elderly, in whom many studies have been conducted on the predictive value of CysC for vascular disease. Estimation of glomerular filtration using creatinine is also imprecise for stages 1 and 2 KDOQI with GFR >60 mL/min. In these situations, CysC may therefore be better at identifying vascular risk due to a moderate decline in renal function (232).

The vascular risk associated with nephropathy may be revealed by other early markers of injury, such as microalbuminuria. At present, however, there are only a few studies comparing the predictive value of CysC and microalbuminuria (227). Finally, very few studies have compared the predictive value of CysC, creatinine and a reference method for measuring GFR in cardiovascular diseases. The study by Menon et al. (222) found CysC to have the same or even closer association with cardiovascular mortality [RR = 1.64 for a reduction of 1 SD (1.28–2.08)] than GFR measurement from iothalamate clearance [RR = 1.28 (1.04–1.59)] or creatinine clearance [RR = 1.32 (1.05–1.64)] from data from the MDRD cohort. However, the generalisability is limited because the MDRD study cohort consists of patients recruited with stage 3 and 4 renal failure, leading to an artificial restriction of GFR range, and because most of them (66%) reached end stage renal failure during a median follow-up of approximately 6 years.

High CysC concentrations may reflect the existence of a low noise inflammatory process

A link between CysC and inflammation has previously been found in large population studies (32, 174). This association between inflammatory markers (CRP, interleukin-6 and tumour necrosis factor) and serum cystatin concentrations has been reported in most of the studies which showed a relationship between CysC and cardiovascular disease (218, 224, 229, 233) or in populations at high renal or cardiac risk, such as diabetics (234). Except for the PRIME study (224), however, the association of CysC/cardiovascular event remains when inflammatory markers are included in the multivariate analysis (218, 229). Interestingly, the association between inflammatory markers and CysC appeared to be linear in the ''Cardiovascular Health Study'' cohort, whereas it produces a U-shaped curve with creatinine (177). These associations between inflammation, CysC and cardiovascular disease suggest that inflammation may be the unifying link (235). Alternatively, the association between inflammation and CysC may partly explain the predictive value of CysC for non-cardiovascular mortality including neoplasia (236).

Direct role of CysC in the arterial wall

Finally, it is not possible to exclude CysC having a specific role in the arterial wall, which is strongly suggested from in vitro studies and on animal models. Recent work by Niccoli et al. describes a possible relationship between fundamental and epidemiological findings. Niccoli et al. studied coronary lesions in 70 consecutive patients with acute coronary syndrome and normal renal function (defined as estimated GFR >90 mL/min/1.73 m^2) and found a positive association between serum cystatin concentrations and number of stenoses in this small sample, although increased CysC values appeared to be associated with a stable fibro-muscular plaque phenotype as determined by the angiographic index. This result, which needs to be confirmed on larger studies, may confirm

the role of CysC in vascular remodelling, including that which occurs in coronary disease. The possible consequences of changes in arterial wall CysC expression on circulating concentrations remain to be defined (237).

In conclusion, CysC therefore appears to be an independent risk marker for cardiovascular diseases and could reflect complex interactions between the detection of early renal abnormalities, cystatin relationship with inflammation and its role in vascular remodelling. Further studies, including GFR determination by reference methods, are needed to better determine the relative contribution of each factor.

CysC and cancer

Cystatin is of interest to the oncology community at two levels: its tissue expression is being studied as a prognostic indicator, whereas serum concentrations may represent a useful alternative to serum creatinine, which performs poorly in a group of patients many of whom have reduced muscle mass, for assessment of renal function.

CysC is a major inhibitor of the cathepsins, enzymes able to proteolyse the extracellular matrix and therefore facilitate degradation of basal membranes by tumour cells and by the metastatic process itself. Cathepsin B in particular has been widely shown to have a role through a correlation between expression of cathepsin B in tumour tissues, disease progression and adverse clinical prognosis in several types of tumour: gastric (238), pulmonary (239), breast (240), and head and neck carcinoma (241). Conversely, expression correlates with poorer in vivo and in vitro invasive potential for glioblastomas (242), improved survival of patients suffering from upper respiratory tract tumours (243) and a lower Gleason score in prostate tumours (244). The anti-tumour effect of CysC may also be due to a ''cytokine-like'' role independent of its protease inhibitor function. CysC and a mutant devoid of inhibitory activity on cathepsin B are both antagonists of the tumour growth factor-β (TGF-β) receptor and inhibitors of the TGF-β signalling pathway in fibrosarcoma cells (245). The promoter events for the metastatic process caused by TGF-β in mammary tumour cells (reduced cell polarisation, loss of inter-cellular adhesion, induction of invasive and migratory abilities) are inhibited by CysC expression induced by retroviral infection (246).

Curiously, other studies indicate that CysC may have a potential promoting effect on the metastatic process. Specifically, seven times fewer pulmonary metastatic colonies developed following intravenous injection of the highly metastatic B16-F10 murine melanoma line in mice inactivated for the CysC gene, compared to wild type animals (247). Our understanding of the relationships between CysC expression and oncogenesis appears therefore only to be in its infancy.

As in many other clinical situations, serum CysC concentrations have been measured in oncology as a marker of glomerular filtration. The major question which arises is that of the influence of tumour presence on circulating CysC which could make CysC lose its relevance as a glomerular marker. As we have observed, the expression of CysC is probably involved in oncogenesis. Serum cystatin concentrations may also be a marker of tumour mass as CysC is expressed by all nucleated cells. All of the studies which have endeavoured to answer this question have conflicted with the major difficulty of using a reference method to measure GFR in patients whose management is already complex.

Some studies describe an increase in serum CysC in patients with neoplasia compared to healthy individuals, although renal function was either not assessed (248–250) or an assessment was based on the serum creatinine alone (243, 248, 251–253) in these studies, greatly limiting their relevance, as acknowledged later by some of the authors themselves (254).

Four studies have compared serum CysC concentrations in patients with malignancy to those in healthy volunteers, all of whom have been shown to have normal equivalent renal function. Their results are inconsistent. Two studies showed no difference between the groups: the Al Tonbary et al. study (255) (34 children mostly with malignant blood dyscrasias vs. 13 controls, GFR assessed by measured creatinine clearance adjusted for body surface area) and a study by Mojiminiyi et al. (256) (29 adults with malignant blood dyscrasias vs. 27 controls, GFR measured using the Cockcroft-Gault equation). On the other hand, Demirtas et al. showed that mean cystatin concentration was 5 times higher in 19 patients with blood dyscrasias before bone marrow transplantation compared to 20 controls (GFR assessed by measured creatinine clearance adjusted for body surface area). It should be noted that values as high as those reported by Demirtas et al. are very rare in the literature, including patients with end stage renal failure (257). A fourth study, which assessed GFR from carboplatin elimination clearance (equal to GFR+25 mL/min) in 40 patients with malignancy and 40 healthy volunteers, also concluded that serum cystatin concentrations were significantly higher in cancer patients (258).

The only study to date to have used a reference method to measure GFR in individuals suffering from cancer did not compare the serum cystatin concentrations of patients to those of individuals without cancer and with equivalent renal function, which could have identified the contribution of tumour presence to the increase in CysC concentrations. In addition, as this study included renal failure patients (inulin clearance between 43.6 and 115.1 mL/min) it is impossible to compare the serum cystatin concentrations found with reference values in the literature (134).

The question on the impact of tumour presence on CysC therefore remains. Without a study which formally measures GFR in a sufficient number of patients, it remains impossible to attribute higher CysC values to the influence of tumour load or to low level deterioration in renal function.

The difficulty raised by interpretation of isolated serum cystatin concentrations in oncology is less critical when follow-up is being considered. Several studies have examined the use of serum CysC in assessing nephrotoxicity from drugs used to treat cancer, particularly cisplatin (134, 254, 255, 259). Most of these have been performed in children, in whom measurement of urinary creatinine clearance is particularly difficult and all agree that serum cystatin is a far more sensitive marker than serum creatinine to detect reduced GFR or creatinine clearance after cisplatin administration. The most convincing is the Benohr study which showed a 21% increase in serum cystatin on day 5 of cisplatin administration in parallel with a 23% fall in inulin clearance (134). Serum cystatin also appears to be an effective tool to predict a clinically significant fall in urinary creatinine clearance (254, 255). Combined with its use in predicting the development of some cytotoxic agents (see below), CysC appears therefore to be a promising tool in patient chemotherapy management. Other studies on this subject, however, would be useful, including more patients, homogeneous chemotherapy protocols and measurement of GFR using a reference method.

It should be noted that at the time when this text was written no GFR estimation equation based on serum cystatin had been assessed in an oncological context (Table 3).

CysC and drug monitoring

Many drugs require drug monitoring in renal failure. This monitoring is usually necessary because the clearance of the drug is mostly renal but also occasionally because of nephrotoxicity of the drug which must therefore be used with caution in pre-existing renal disease. Both difficulties occasionally co-exist as, for example, with the aminoglycosides or cisplatin.

In most cases, the dosage adjustment recommended in the Summaries of Product Characteristics (SPC) refers to GFR ''range'' or usually a Cockcroft-Gault clearance range. Sometimes, and this applies to drugs with a very narrow therapeutic margin, such as the cardiac glycosides or cisplatin, adaptation is individual and is based on measurement or usually estimation of the clearance of the drug. This calculation is made from equations combining demographic (age, sex), morphometric (height, weight) and biological (serum creatinine, Cockcroft-Gault clearance) details.

The first publication which examined the use of CysC in this area referred to the dosage adjustment for digoxin in the elderly (260). It concluded that this new parameter was not superior to creatinine in predicting drug clearance. However, these results were rapidly refuted (261) and two studies based on population pharmacokinetics methodology, the most robust in this field, definitively demonstrated the utility of CysC in predicting the clearance of drugs which were eliminated either exclusively or only partially by the kidneys, i.e., two cytotoxic agents, topotecan (262) and carboplatin (263). Interestingly, both of these studies showed an advantage of combining CysC with creatinine rather than using either individually. This suggests that the two parameters are not entirely redundant and that serum cystatin does not only depend on GFR. Since these two studies, others also conducted using population pharmacokinetics have published equivalent conclusions for cefuroxime (264) and vancomycin (265).

Conclusions

This review of recent information on CysC raises several issues:

- There is an urgent need to assess the transferability of automated methods for the measurement of serum CysC. In order to avoid repeating the same difficulties observed with creatinine, estimated GFR equations based on serum cystatin must be able to rely on low inter-method variability in order to maximise their application in the populations concerned. A project is currently being set up in collaboration with AFSSAPS, the Société de Néphrologie, the Société Francophone d'Hémodialyse and the SFBC.
- In order to use this marker optimally, there appears to be a need to complete our knowledge about its physiological variability and factors contributing to variability of production, the major of which are inflammation and cancer.
- Of the sub-populations in which CysC has been assessed as a marker of GFR, it is undoubtedly the paediatric populations which will benefit most from this new marker of glomerular filtration.
- Since 2005 and the KDIGO recommendations (266), we have moved into the era of estimator GFR equations. Given the increasingly numerous demonstrations that non-renal factors may influence serum cystatin, it is likely that CysC will need to be associated with other non-biological co-variables in these algorithms. A major study on 3418 American and European CRF patients shows that an equation combining CysC, serum creatinine, sex, age and race produces a better estimate of GFR than the MDRD equation. This equation was developed in a sample of 1935 subjects and was validated internally (in the USA) in 1045 subjects and externally (France) in 438 subjects (125).
- Many equations for measuring GFR based on serum cystatin have been proposed. There is, however, a serious lack of validation studies for these instruments against a GFR reference measurement, particularly in sub-populations in which serum creatinine-based equations are used by default (the elderly, AIDS, cancer, etc.).

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