Identifying early renal insufficiency has become increasingly important, as the number of patients with end-stage renal failure is growing worldwide. Recent evidence demonstrates that serum cystatin C is an excellent marker of renal function facilitating precise and accurate estimation of the glomerular filtration rate (GFR). Cystatin C is superior to creatinine as a marker of GFR, and the clinical utility of cystatin C has been shown for a number of different patient groups suffering from kidney disease.

Cystatin C - a future significant marker in clinical diagnosis

Estimation of the glomerular filtration rate (GFR) is the most widely used test of renal function and reflects the kidney’s ability to clear a particular substance from plasma. GFR is defined as the quantity of glomerular filtrate formed per unit time in all nephrons of both kidneys. The most precise and accurate methods for estimating GFR are based upon determinations of plasma clearance of substances like $^{51}$Cr-EDTA, iothalamate or iohexol. These so-called “gold standard” methods require injection of an exogenous radioactive or contrast agent, and are complex, laborious, expensive and impractical in the clinical setting and for larger research studies. Therefore, the measurement of endogenous blood substances to estimate GFR is common practice. For several decades clinicians have relied on measurements of serum creatinine as a rapid first-line test to determine GFR. This test is convenient and cheap, but results are affected by age, sex, muscle mass, diet, race and tubular creatinin secretion, particularly when GFR is reduced. Thus, there has been an ongoing search for suitable alternative endogenous markers of GFR.

Cystatin C

Cystatin C was first discovered in 1961 as an alkaline protein in normal cerebrospinal fluid. It is a 13-kDa, non-glycosylated basic protein belonging to the cystatin super-family of cysteine proteinase inhibitors. Unique among cystatins, it seems to be produced by all human nucleated cells. It is produced at a stable rate, which is unaffected by inflammatory processes, sex, age, diet, and nutritional status [1]. Only a few circumstances have been identified that have an impact on the production of cystatin C, such as very large doses of glucocorticoids and thyroid dysfunction. In the normal kidney, cystatin C is freely filtered through the glomerular membrane and then almost completely reabsorbed and degraded by the proximal tubular cells. Therefore, the plasma concentration of cystatin C is almost exclusively determined by the GFR, making cystatin C an excellent indicator of GFR. Studies of the serum level of cystatin C in large patient cohorts have failed to correlate the serum level to any pathophysiological states besides those affecting the GFR.

Cystatin C as a marker of GFR

The interest in cystatin C as a marker of renal function has increased tremendously over the last few years and the number of articles and reviews about cystatin C continues to grow. Numerous studies and a meta-analysis incorporating 4,492 subject samples, comparing the use of serum cystatin C and creatinine as markers of GFR have shown that serum cystatin C is clearly superior to serum creatinine as a marker of GFR [2]. Cystatin C responds more quickly to changes in the GFR than creatinine, which is not a sensitive marker for early decline in GFR. A substantial proportion of patients with reduced GFR display serum creatinine levels within the normal range and even a 50% reduction of GFR is not infrequently associated with a normal concentration of serum creatinine. Cystatin C is accurate in this “creatinine-blind area” helping the clinician to get an earlier indication of deteriorating renal function, and thus allowing the possibility of taking preventive action. Moreover, cystatin C does not have the previously mentioned limitations of creatinine, and its measurement is a much simpler way of assessing renal function than methods such as iohexol clearance. A particularly important advantage of cystatin C as a marker of GFR is that it can also be used to evaluate GFR in patient populations for whom it is difficult to obtain an accurate assessment of GFR based on the creatinine value.

Estimation of GFR from cystatin C

Clinicians need a fast estimate of a patient’s renal function to calculate the correct amount of antibiotics or cytotoxic drugs for the individual patient, for instance, before initiating treatment, and also for monitoring patient response during and after therapy. Cancer therapeutics, in particular, have the potential to inflict severe damage to the kidneys. An early indication of renal dysfunction would allow the oncologist to adjust the drug dosage before irreversible kidney damage had occurred.

Formulae for estimating the GFR have attracted considerable interest in recent years. Various formulae such as the Cockcroft-Gault and the Modification of Diet in Renal Disease (MDRD) have been suggested for calculating GFR from serum creatinine concentration. These formulae include anthropometric variables such as body weight, gender, age and race to compensate for the inadequacies of creatinine level as a marker of GFR. However, even including these many variables, the formulae have several limitations in estimating the GFR.

A new study has been carried out to investigate the possibility of introducing formulae for the estimation of GFR from the plasma (or serum) cystatin C concentration without the use of anthropometric variables. Plasma cystatin C concentrations were measured with the DakoCytomation Cystatin C Immunoassay for 451 patients [Figure 1]. An equation for the conversion of the cystatin C concentration in mg/L to GFR in mL/min (determined by iohexol clearance) was established [3]:
The study showed that the formula based on cystatin C has lower bias and higher accuracy in predicting GFR than the Cockcroft-Gault formula. Thus, cystatin C provides a more precise and accurate estimation of GFR, calculated from a single measurement of plasma (or serum) cystatin C.

**Clinical utility in different patient groups**

In contrast to serum creatinine, serum cystatin C is unaffected by muscle mass. This means that selected patient groups, whose muscle mass is either reduced or undergoes rapid change, may particularly benefit from the use of cystatin C for estimating the GFR. This is true for children and the elderly.

The reference range for serum creatinine increases with age up to the end of puberty and has to be adjusted for gender from puberty onwards. In contrast, the reference range for serum cystatin C is identical for men, women and children as the cystatin C level is constant after the age of one and virtually identical to the reference range for adults. In the first years of life, renal function matures physiologically. Accordingly high cystatin C concentrations have been found at birth, followed by a rapid decline after birth reflecting maturation of kidney function. Unlike serum creatinine, cystatin C can thus be used to assess the GFR of newborns and even of the foetus [1].

GFR decreases with age as the nephrons start to decrease from about the age of 50. At approximately the same age, muscle mass also begins to decline. In the elderly, serum creatinine is notoriously unreliable as an indicator of GFR because the daily production of creatinine is diminished as a result of the reduced muscle mass. Several studies have also shown cystatin C to be a superior marker for early detection of renal impairment in elderly people.

In a clinical situation, the influence of muscle mass can be essential, for instance when diagnosing reduced GFR in paralysed patients. This has been investigated for patients with spinal cord injury who have varying degrees of muscle atrophy. The results show that cystatin C is much more reliable as an indicator of GFR because the daily production of creatinine is diminished as a result of the reduced muscle mass. Several studies have also shown cystatin C to be a superior marker for early detection of renal impairment in elderly people.

Diabetes is a highly complex disorder with many ramifications and is the commonest cause of kidney failure in younger people globally. Treatment comprises dialysis or kidney transplantation. If early damage to the kidneys can be detected, preventive action can then be taken. Cystatin C has been reported to be advantageous compared with serum creatinine for the detection of mild diabetic nephropathy, whereas the two markers were equally efficient in detecting advanced diabetic nephropathy [5].

Cystatin C has been measured before and after chemotherapy in cancer patients. The results show that serum cystatin C is a superior marker to serum creatinine for the estimation of GFR, independent of the presence of metastases, and independent of chemotherapy [6]. It has also been shown that cystatin C can be used to characterise glomerular function in children with cancer [7]. In multiple myeloma, a study has demonstrated no correlation between cystatin C and tumour burden.

It has recently been suggested that cystatin C could be used as a valuable parameter in the monitoring of pregnancies complicated by pre-eclampsia. Pre-eclampsia is a pregnancy-specific disorder associated with increased foetal and maternal risk. The cause is unknown and delivery is the only definitive cure for this condition. There is a real need for sensitive and specific diagnostic tests for pre-eclampsia. During uncomplicated pregnancy, the renal-flow progressively increases, leading to about 40% higher GFR than in a non-pregnant woman. Pre-eclampsia is characterised by hypertension and renal structural changes, and the kidney function, in particular, is of major concern. Because pre-eclampsia is characterised by a decrease in GFR, kidney function needs to be monitored closely to ensure timely delivery before the development of toxaemia and serious kidney tissue injury. Cystatin C has been shown to provide superior diagnostic accuracy for pre-eclampsia compared to serum urate and creatinine, and cannot only be used as a marker for impaired renal function, but also for the degree of glomerular endotheliosis (the only consistently found pathological lesion in pre-eclampsia) [8]. Thus, cystatin C seems to be useful for optimising the timing of neonatal delivery.

**Cystatin C Immunoassay**

The DakoCytomation Cystatin C Immunoassay is intended for the quantitative determination of cystatin C in human serum and plasma by turbidimetry and nephelometry. The components of the assay are CE-marked for in vitro diagnostic use in Europe and have obtained a 510(k) marketing clearance from the FDA in the US (K041627) [Table 1]. The assay is based on particle-enhanced immunoturbidimetry [Figure 2]. The measuring range is optimised to 0.4 – 7.5 mg/L, which covers the concentration range in normal and diseased states. The assay can be performed on most clinical chemistry analysers available on the market and the total assay time is approximately 10 minutes. An expanding list of detailed application notes is available for a range of instruments [Table 1]. This enables cystatin C to be measured 24 hours/day in most clinical chemistry laboratories.

**Assay performance**

The Cystatin C Immunoassay shows good analytical performance. A Hitachi 917 chemistry analyser was used to obtain the performance data shown in Table 2, unless otherwise stated. The assay demonstrated very good precision with total coefficients of variation (CVs) below 3.0% within the measured range. The linearity of the assay was found to be acceptable and the detection limit was estimated to be 0.034 mg/L. The assay provides highly accurate results and no excess antigen was detected up to at least 28.3 mg/L. No interference was observed up to 10 g/L haemoglobin, 600 mg/L bilirubin, 10 g/L intralipid, 1200 IU/mL rheumatoid factor and 15 g/L triglyceride. In addition, no interference was observed when tested with a range of drugs. The reagents on board the instrument and the calibration curve stored in the instrument remain stable for up to 90 days.

Reference intervals were estimated on a Modular P chemistry analyser from a population of 69 subjects ≤50 years and 94 subjects >50 years of age, all with a normal glomerular filtration rate. The following reference intervals were established:

\[
\text{GFR (mL/min) = 89.12 \times \text{cystatin C (mg/L)}^{-1.675}}
\]
Renal Function

When human serum or plasma from a patient is mixed with the antibody reagent (the immunoparticles), a specific reaction between the immunoparticles and cystatin C in the patient specimen results in the formation of immune complexes, and the solution becomes turbid. If light is passed through the reaction cuvette, the incident light is scattered due to the formation of the immune complexes. The degree of turbidity can be quantified by turbidimetry or nephelometry on an automated analyser. Turbidimetry is based on the principle of measuring the intensity of the transmitted light and nephelometry on measuring the intensity of the scattered light [10]. The instrument calculates the cystatin C concentration of a patient specimen by interpolation of the obtained signal on a 6-point calibration curve.

**Figure 2. Principle of the Cystatin C immunoassay.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (mg/L)</th>
<th>Total CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C Control 1</td>
<td>3.95</td>
<td>2.1</td>
<td>80</td>
</tr>
<tr>
<td>Cystatin C Control 2</td>
<td>0.96</td>
<td>2.6</td>
<td>80</td>
</tr>
<tr>
<td>Low serum pool</td>
<td>1.71</td>
<td>2.0</td>
<td>80</td>
</tr>
<tr>
<td>High serum pool</td>
<td>5.37</td>
<td>2.3</td>
<td>80</td>
</tr>
</tbody>
</table>

**Table 2. Precision levels of the Cystatin C immunoassay measured on a Hitachi 917 clinical analyser.**

**Conclusion**

Rapid determination of renal function followed by timely therapy could improve patient care. Cystatin C seems to be an excellent marker of renal function, and available evidence demonstrates that serum cystatin C is superior to serum creatinine as a marker of GFR, particularly in identifying small decreases in GFR. The use of cystatin C provides the best possible information on GFR following initial examination of patients whose GFR is of interest, and in situations where gold standard clearance measurements cannot be performed for biomedical or economical reasons. A great deal has already been accomplished in establishing a role for routine cystatin C determination in many clinical situations. It is essential that the clinical chemists work closely together with the nephrologists, the oncologists and the obstetricians in conducting more studies with larger patient groups. This should provide the necessary data to fully convince clinicians of the usefulness of cystatin C as a routine marker.

**References**


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