Serum cystatin C: a practical alternative for renal function evaluation?

Cistatina C sérica: uma alternativa prática para avaliação de função renal?

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Abstract

Glomerular filtration rate is the main marker of renal function in healthy individuals and patients. Despite incontestable advances in medicine, it is still difficult to define precisely this test in clinical practice. Early markers of renal lesion are important, because glomerular filtration rate usually decreases before the first chronic renal failure symptoms or signs appear. Cystatin C has been pointed as an alternative, but it was not tested in many diseases. Advantages and disadvantages of this marker are discussed. Although serum cystatin C determination is increasingly being used in clinical practice worldwide, its limitations as well as the conditions its use is in fact indicated are not adequately established; on the other hand serum creatinine (and creatinine clearance) is an easily available and low cost laboratory marker with well-known limitations that can be used routinely in the assessment of renal function.

Keywords: cystatin C, creatinine, glomerular filtration rate, Chronic renal failure.

Resumo

A taxa de filtração glomerular é o principal indicador de função renal em indivíduos saudáveis e doentes. Apesar de todo o desenvolvimento da medicina em nossos dias, ainda há dificuldade para definir-se essa taxa com precisão na prática diária. Marcadores precoces de lesão renal são importantes, porque a taxa de filtração glomerular se reduz antes do aparecimento dos sintomas ou sinais de insuficiência renal. A cistatina C tem sido apontada como uma alternativa, mas ainda não foi testada em muitas condições. Vantagens e desvantagens desse marcador foram aqui discutidas. Embora a determinação sérica da cistatina C comece a ser usada na prática clínica em todo o mundo, ainda não foram completamente esclarecidas suas limitações ou as situações em que está de fato indicada sua aplicação; por outro lado, a creatinina sérica (e sua depuração) é um marcador laboratorial facilmente acessível, de baixo custo, cujas limitações são bem conhecidas, que pode ser usado de forma rotineira para avaliação de função renal.

Palavras-chave: cistatina C, creatinina, taxa de filtração glomerular, insuficiência renal crônica.

Introduction

The precise evaluation of renal function level is the key for diagnosis, monitoring and management of renal diseases, as well as for the proper calculation of drug dosage that is excreted from the kidneys. It is a known fact that renal function declines progressively in most diseases that affect the kidneys, resulting in complications such as hypertension, malnutrition, anemia, osteodystrophy, neuropathy and low quality of life.[1-3]

Glomerular filtration rate (GFR), defined as the clearance of a substance in the plasma which is exclusively metabolized by the kidneys and freely filtered by the glomeruli,[4] is the main indicator of renal function. It is also known that GFR is reduced before the symptoms of early renal failure.

The ideal substance to determine GFR must have the following characteristics: stable rhythm of production, constant maintenance of circulating level, which must not be influenced by other

disorders, free filtration by glomeruli without tubular interference, like secretion or reabsorption.[2,4,5]

How to determine GFR

Up until now, only exogenous substances have been considered ideal GFR markers: inulin, ethylenediaminetetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), iothalamate and, recently, iohexol. However, the use of such substances is limited to some protocols or situations in which the accurate information about renal function is obligatory, once the techniques are expensive, require a long time to be performed and are not practical for daily use.[2-5]

Endogenous markers are less complex and offer faster results. In clinical practice, serum creatinine is the most used endogenous marker by serum determination alone or combined with the 24-hour urine test to determine creatinine clearance. The plasma creatinine concentration, which derives from muscle creatine, with molecular mass of 113 Da[1], is inversely related to GFR. However, there are many factors that limit its accuracy, once serum concentration is a reflex of its production, which is proportional to muscle mass and influenced by age and sex.[1-4] This situation favors a considerable intra and inter-individual variation, which justifies a plasma level that is higher in adults than in children, and also higher in men than in women; there is also a significant linear growth in children.[6,7] On the contrary, subjects who lose muscle mass reduce creatinine production and, consequently, plasma level. There are some external factors that interfere in its analytical determination: endogenous substances (glucose, bilirubins, uric acid, triglycerides, ketones and plasma proteins) and some drugs (cephalosporins, sulfa and cimetidine) that inhibit its tubular secretion, increasing serum level without affecting GFR.[1,2,6]

GFR estimation in elderly subjects is extremely difficult, because renal function declines with aging. Some studies have shown that the number of glomeruli decreases from approximately 1 million to 600,000 per kidney or less around the eighth decade of life, which causes the filtration area and the permeability of the glomerular basement membrane to decrease. However, this is not a reflex of creatinine increase in response to the muscle mass reduction in the elderly.[8-10]

Another factor that interferes in the use of creatinine as the ideal GFR marker is that it is secreted by the renal tubules, thus overestimating GFR.[4,5] Under normal conditions, the tubular clearance of creatinine corresponds to approximately 10 to 20% of the renal clearance of this substance.[2] Since the percentage of plasma creatinine clearance depends on its plasma level and on the mass of functional tubular tissue, tubular clearance may reach 50 to 70% of the renal clearance in some situations.

In order to surpass such limitations, many formulas have been developed to estimate creatinine clearance by means of serum creatinine concentration and anthropometric data.[11,12] In 1973, Cockcroft and Gault came up with a formula regarding age and weight, but not ethnicity. Such equation was firstly performed on male individuals, and for females an arbitrary adjustment of 85% was performed.[12] Results were not corrected to the body surface, being expressed in mL/min. Cockcroft-Gault formula was fastly spread and accepted due to its clinical applicability and simple calculations;[7,12] however, this formula overestimates GFR because of the tubular secretion of creatinine, especially when proteinuria is present.[7,12]

In 1999, a more complex mathematical equation was published considering the analysis of information obtained in the Modification of Diet in Renal Disease study (MDRD).[7,12-14] GFR estimation based on MDRD considered age, sex, ethnicity, serum creatinine, blood urea nitrogen and serum albumin. One year later, a simplified version of the MDRD equation was presented with less information: age, sex, ethnicity and serum creatinine.[7,12,14] This equation was included as a GFR marker in the Practice Guidelines for Chronic Kidney Disease, published in 2002 by the Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation (K/DOQI).[7]

In the past few years, many low molecular weight plasma proteins have been studied aiming at identifying a better GFR marker. In 1985, cystatin C proved to be at least similar to serum creatinine as a renal function marker.[5]

Cystatin C

Cystatin C is a non-glycated cationic protein, and its isoelectric point is 9.3.[15] Its molecular mass is 13,359 Da, being a part of the cystatin superfamily, which consists of 12 proteins (Table 1).[15,16] It is a powerful cysteine protease inhibitor (such as human cathepsins B, H and L)[17] compounded of 120 amino acids distributed in a simple polypeptide chain, whose sequence was determined in 1981.[6,7,15,17] It is synthesized as a pre-protein.[17] Subsequent studies showed that cystatin C is produced at a constant rate by all nucleated body cells and is present in biological

Table 1 Cystatin superfamily

Family 1	Family 2	Family 3
Intracellular cystatin	Extracellular cystatin	Intravascular and/or transcellular cystatin
Cystatin A	Cystatin C, D, E, F, G	LMW-kininogen
Cystatin B	Cystatin S	HMW- kininogen
	Cystatin SA	
	Cystatin SN	

fluids.[1-6,18] It is freely filtered by the glomeruli (due to its low molecular weight combined to positive electric charge),[4,5,19] and, according to initial studies, its serum concentration does not depend on age, sex, muscle mass and body weight.[5,16-20] No relevant difference between reference values for males and females has been reported. For healthy children, cystatin C concentration is stable in the second year of life, and the reference value is identical to that of adults.[7,15]

Cystatin C is almost completely catabolized in the proximal tubule, just like other low molecular weight proteins. However, unlike the latter (such as ß2-microglobulin, with 11,800 Da),[21] its serum level seems not to be affected by other extra-renal conditions, such as inflammation and neoplasms.[3,7,15,22] Cystatin C does not return intact to the blood flow, and its concentration in urine is practically undetectable,[3,24] because it is reabsorbed and metabolized at tubular level.[22,23]

The gene sequence that codifies cystatin C is located on the chromosome 20.[21,24] It seems to be a housekeeping gene, which matches the stable production rate of most nuclear cells.[7,15,22,24,25]

Such aspects have introduced the idea that serum cystatin C levels are better GFR markers than creatinine, fact that has been confirmed by different studies.[3,5,8-10,15,18,26-29] On the other hand, some of them do not show a significant difference between blood determination of cystatin C and creatinine for such purpose.[5,30,31]

In the pediatric population, cystatin C has an advantage over serum creatinine, mainly to detect small and early changes in GFR, once this population has reduced muscle mass, especially children under the age of 4 years, that have particularly low serum creatinine values.[32] Serum cystatin C concentration is increased in the first day of life and rapidly reduces within the weeks, but serum creatinine concentration increases until the early years of adolescence, due to muscle mass gain.[26]

Bokenkamp *et al.*[33] have recently reported that serum cystatin C is higher in children who are subjected to kidney transplant than in those who have renal diseases, although GFR is similar in both. This finding brought up the hypothesis that immunosuppression would have the potential to influence such results, once all the patients of the first group received prednisone and cyclosporine A.

In an attempt to unveil the contribution of one of these drugs over cystatin C levels, Bjarnadóttir *et al.*[34] conducted an *in vitro* study in which HeLa cells were exposed to different concentrations of dexamethasone. Then, a dose-dependent increase in the production of cystatin C by cells exposed to corticoid was observed.

In 2001, Risch *et al.*[35] published a prospective study with the objective to clarify the influence of immunosuppresion with glucocorticoids over the serum cystatin C concentration in patients submitted to kidney transplant. In this study, 20 patients who received low doses of corticosteroid were compared to 20 patients under cyclosporine and 20 patients under cyclosporine associated with azathioprine. Besides, 13 patients received high doses of methylprednisolone. This study showed that patients under corticosteroid presented higher serum cystatin C levels than those of the groups of patients who did not take such immunosuppressive medication. Also, cystatin C levels were significantly higher in the group that received high doses of methylprednisolone compared to the group treated with a low-dose prednisone. Such finding demonstrates a dose-dependent relation, even though the increase was transitory. Approximately 8 days after the suspension of methylprednisolone, serum cystatin C concentration had returned to basal levels.

Other studies showed an increase in serum cystatin C levels related to high doses of corticosteroid in patients with bronchial asthma, subarachnoid hemorrhage and severe ophthalmopathy secondary to Graves' disease.[36-39] However, the mechanisms involved in these changes are not well known yet.

On the other hand, in 2002, a study conducted with children who had steroid-sensitive idiopathic nephrotic syndrome, treated according to the *German Working Group for Pediatric Nephrology* protocol, showed that serum cystatin C concentration was not affected by high doses of corticosteroid.[40]

It is a known fact that the thyroid function may interfere in serum creatinine levels. It has been demonstrated that patients with hypothyroidism have higher creatinine levels, while those with

hyperthyroidism have lower levels. After reaching a state of euthyroidism with introduction of treatment, creatinine levels decreased and increased, respectively. As a consequence, studies were conducted to identify a possible interference of thyroid hormones on cystatin C levels. The result is that, in opposition to what happens with creatinine, serum cystatin C concentration levels are lower in hypothyroidism and higher in hyperthyroidism, when compared to that observed in euthyroidism.

Possible explanations for these findings are based on the effects of thyroid hormones over renal hemodynamic, salt and water homeostasis in the kidney and active tubular transport of sodium, potassium and hydrogen ions. Regarding creatinine, it is possible that is tubular secretion be decreased in hypothyroidism and increased in the opposite state. As to cystatin C, since the state of the thyroid influences the metabolism, it may also influence its production.[41,42]

Another characteristic that requires attention is the interference of protein ingestion and nutritional status in renal function analysis. A recent study conducted with a great number of patients with moderate to severe chronic kidney disease showed that serum cystatin C, unlike serum creatinine, was not affected by proteins ingested, regardless of the changes in GFR, which indicates that cystatin may provide more accurate estimates on GFR than creatinine in patients with reduced protein ingestion.[43] There is evidence that cystatin C levels are not affected by malnutrition, whilst creatinine levels decrease; thus, GFR is overestimated by this last marker.[44]

Cystatin C is also seen as an alternative to evaluate renal function in subjects with great muscle mass when there is suspicion of discrete renal function deficit. It has been shown that body weight and lean mass are not correlated with serum cystatin levels.[45] In obese subjects, it is difficult to analyze renal function, and there is no consensus in studies. In the evaluation of obese patients, adiposity is associated with serum cystatin levels. Against the expectation of being a proper marker, in this group, formulas based on cystatin C overestimate GFR in individuals with higher body mass index (BMI).[46]

It is important to emphasize that some formulas have been developed in the past few years with the objective to better evaluate renal function with cystatin C, just as with serum creatinine, to estimate GFR,[20,22,27,47-51] as demonstrated in Table 2.

Some of the formulas involving cystatin C presented better[20,47,48] or similar[27,49] performances in relation to the equations with creatinine, according to researchers. For some, the combination of serum creatinine and cystatin C in formulas is the best option when considering demographic data.[51-54] However, there is no consensus as to the superiority of formulas that involve cystatin C, nor to the combination of both markers; so, it is possible that the available formulas are not adequate for different populations, as tested by Urbaniak et al.[55]

Table 2 Equations to estimate glomerular filtration rate based on serum cystatin C (mg/L) alone or combined with serum creatinine (mg/dL)

Authors	Formulas
Hoek *et al.*[20]	GFR = -4.32 + 80.35 x 1/ cystatin
Tan *et al.*[22]	GFR = 87.1 / cystatin – 6.87
Rule *et al.*[27]	GFR = 66.8 x cystatin $^{-1,30}$
Grubb *et al.*[47]	GFR = 99.19 x cystatin $^{-1,713}$ x 0.823 (if female)
Grubb *et al.*[48]	GFR = 87.62 x cystatin $^{-1,693}$ x 0.94 (if female)
MacIsaac *et al.*[49]	GFR = 86.7 / cystatin – 4.2
Larsson *et al.*[50]	GFR = 77.239 x cystatin $^{-1,2623}$
Stevens *et al.*[51]	GFR = 177.6 x creatinine$^{-0,65}$x cystatin $^{-0,57}$x age$^{-0,20}$x 0.82 (if female) x 1,11 (if black)

It must be considered that many studies were conducted or are being performed to define the role of cystatin C. One of these has recently demonstrated that cystatin C identified more clearly than serum creatinine which subjects would develop vascular complications in a population with chronic kidney disease, proving that cystatin C can be an important risk marker in this group of subjects.[56,57] However, based on their results, Eriksen et al. analyzed a very representative group of subjects, and showed that GFR estimates based on cystatin C are not superior to those based on creatinine in the general population. They also reported that the best performance previously described in the assessment of vascular risk may have been due to other factors than GFR.[58]

Nowadays, the elderly are subject for concern as to the determination of GFR. In a recent systematic literature review,[59] the conclusion was that there is no precise method to analyze renal function in this group of subjects, but cystatin C, as well as Cockcroft-Gault and MDRD formulas, are useful parameters – although favorable evidence to one marker or another is still insufficient.

Finally, the use of cystatin C seems to be promising for acute kidney injury, as it has been shown to be an accurate biomarker for early detection of this condition and superior to creatinine in selected populations of certain studies; however, results are still inconsistent. Researchers also question whether it is cost-effective in relation to creatinine and if both tests would have complementary roles.[60]

From a practical point of view, it is worth remembering that cystatin C determination as a direct marker of the renal function may be performed in serum or plasma, under the same conditions of creatinine collection. Reference values vary according to the kit, and each laboratory must check them. In Brazil, this test is not available in most services, and the cost is still very high; in some high-quality laboratories that perform the examination, its cost is approximately eight times higher than that of the serum creatinine test.

Conclusion

Serum cystatin C determination is being introduced in clinical practice around the world, but the limitations or situations in which it may be indicated are not completely clear. The intention of this study was to show the known advantages and disadvantages of this laboratory test, which has been considered promising for GFR determination.

It is worth to emphasize that it is still a high cost determination in our country, which is another reason to be aware of its real contribution in the analysis of different renal diseases/disorders. While such aspects are not well established, the recommendation is not to despise other tests such as serum creatinine , whose limitations are well known, and consequently they can at least in part be solved.

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