CYSTATIN C – MORE THAN THE MARKER OF THE GLOMERULAR FILTRATION RATE

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Introduction

The prevalence of kidney diseases, especially chronic kidney diseases (CKD), is constantly rising as a consequence of the increasing incidence of diabetes mellitus (DM) and hypertension, which represent the most common causes of CKD and end-stage renal disease (ESRD). A significantly higher number of patients are in the early stages of CKD [1]. Therefore, an accurate assessment of the renal function is crucial for the early recognition and management of kidney diseases.

Cystatin C as a Marker of Glomerular Filtration Rate

Cystatin C is superior to creatinine as a marker of kidney function, especially in the early stages of chronic kidney disease. Several formulas are available for calculating the glomerular filtration rate from serum cystatin C. Cystatin C in Various Physiological/Pathophysiological Conditions.

The level of cystatin C should be interpreted carefully because there are factors that can affect its level regardless of the renal function (thyroid dysfunction, glucocorticoids use, malignancies etc.). Higher cystatin C concentrations in the general population are associated with an increased cardiovascular risk, as well as with preeclampsia in pregnant women.

Conclusion

One of the significant advantages of cystatin C as a kidney function marker is its use in the creatinine “blind” area, in pediatric and the elderly population. In addition, cystatin C could be used as a marker for cardiovascular risk assessment, in predicting and detecting preeclampsia, in patients with malignant diseases, etc.

Key words: Cystatin C; Glomerular Filtration Rate; Biological Markers; Immunoassay; Kidney Function Tests; Renal Insufficiency, Chronic
Cystatin C is a protein of low molecular weight (about 13 kDa) which consists of about 120 amino acids. It belongs to the group of cysteine proteinase (lysosomal) inhibitors and it is probably one of the most potent inhibitors of those extracellular proteolytic enzymes [4]. Cystatin C also plays a role in the modulation of the immune system and has an antibacterial and antiviral effect (inhibition of viral replication) [5].

Cystatin C is produced in all of the nucleated cells in a constant amount. The isoelectric point of cysC is 9.3 which is why the protein is positively charged in the body fluids.

Unlike creatinine, serum concentration of cysC does not depend on muscle mass and protein intake, and the influence of gender is of little significance [6].

However, the level of cysC increases with age and in parallel with the decline in the GFR beginning in the third decade of life (average \( \sim 0.75 \text{ - 1 ml/min/year} \)) [7], due to changes in the renal structure and the reduction in the number of the functioning nephrons.

The catabolism of cysC is mostly done in the kidneys, because after passing through the glomerular filter, which is facilitated due to the positive charge of the cysC molecule at a physiological pH and its low molecular weight, it is taken over by the proximal tubule cells and is almost completely metabolized (~ 99%). Therefore, the urinary concentration of cysC is extremely low [8]. The extrarenal excretion of cysC is negligible.

**Determination of Cystatin C Level**

**Determination of Cystatin C Blood Level**

Cystatin C may be determined in the serum or plasma taken with anticoagulants such as lithium-heparin, sodium-heparin or potassium-ethylenediaminetetraacetic acid. As recommended by the majority of the manufacturers, cysC stability in serum/plasma is 7 days at 2-8°C, 3 months at -20°C if the sample is frozen up to 24 hours after the blood sampling, or at least 6 months at -80°C [9].

The laboratory methods which are mainly used to determine the level of cysC are PENIA (particle enhanced nephelometric immunoassay) and PETIA (particle enhanced turbidimetric immunoassay), whereby PENIA is more sensitive and is considered the method of choice [5]. However, high concentrations of the rheumatoid factor (RF) (values over 320 IU/mL) can lead to falsely elevated values of cysC in the blood. Besides, hypertriglyceridemia (and it is already on the rise) can affect the serum concentration of cysC. To overcome the impact of hypertriglyceridemia on cysC levels, a formula was derived for the correction of the cysC level:

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\text{CysC (mg/L)} = 0.963 \times \text{CysC (mg/L) measured} + 0.043 \times \text{triglycerides (mmol/L)} \]

In addition to the abovementioned methods, the enzyme-linked immunosorbent assay (ELISA test) has been developed as well, but it is not generally used in everyday clinical practice.

In addition to determining cysC levels in plasma and serum samples, studies done on healthy adult population have shown that the cysC concentration can be determined in samples of capillary blood by the immunonephelometric method.


**Determination of Urinary Cystatin C Level**

Determination of the urinary cysC level is associated with certain difficulties. In fact, cysC concentrations are extremely low in the urine of healthy individuals and ranges from 0.03 to 0.3 mg/L, i.e. 5.2 to 13.3 mg/mmol of creatinine [8], which significantly limits the use of this test in general everyday practice. Urinary cystatin C is stable (at pH≥5) for 48 hours at room temperature and for 7 days at -20°C to +4°C [11].

Urinary Cystatin C may appear in increased concentrations (up to 200 times) in case of tubular lesions independent from glomerular damage, then due to overcoming the threshold for reabsorption in tubulocytes or due to competitive inhibition in the case of massive proteinuria [12].

**Cystatin C as a Marker of the GFR**

Cystatin C is almost an ideal marker of the GFR because of its features such as: easy passage through the glomerular filter, absent tubular secretion and negligible/absent extrarenal excretion. A significant number of studies indicate that cysC is superior to creatinine as a marker of the GFR [13], especially in the early stages of CKD, i.e. in the creatinine “blind” area (in GFR between 60-90 ml/min/1.73 m²) [14].

It is considered that the GFR calculated by the derived predictive equations using serum concentrations of cysC (cysCequ) is due to the existence of different unsynchronized methods which are used to determine the blood level of cysC, thus applying different mathematical models, as well as the variations present in the calibrators that are in use [16]. The equations which have been developed for the PENIA method cannot be used to calculate the GFR from a level of cystatin C estimated by the PETIA method because the difference in the calculated GFR range up to 10 ml/min/1.73 m² [17].

In most studies, the GFR calculated using cysCequ is slightly more correlated with the GFR values measured by nuclear-medical methods which are the referent methods, rather than the GFR calculated using CrCequ [17]. Therefore, the cysCequ are more sensitive than the CrCequ in detecting patients with CKD in which the GFR is 60-90 ml/min/1.73 m², also the application of cysCequ is very important in patients with GFR <60 ml/min/1.73 m² who are without other signs of kidney damage [18, 19]. In fact, if the GFR estimated using cysCequ is below 60 ml/min/1.73 m² it may be considered that the person has kidney dysfunction [15].

However, despite this, it is recommended not to use only one endogenous marker for the assessment of the renal function in clinical practice [15], that is both formulas, the cysCequ and Crequ should be used to calculate the GFR.

If the calculated GFR values are in accordance, then we calculate their middle value [20], but if not, it is necessary to estimate the impact of other factors on the calculated GFR, i.e. the level of creatinine and cysC in the serum [13]. In that case, it is more appropriate to use a formula derived from a substance which is not subjected to the impact of the identified interfering factors (e.g., thyroid dysfunction, the use of glucocorticoids on cysC or the muscle mass on creatinine level, etc.). If we cannot find an objective reason for the discrepancy in the calculated GFR values, it is preferable to measure the GFR by one of the reference methods [13] (Table 1).

**Cystatin C in Various Physiological and Pathophysiological Conditions**

There are factors in the general population contributing to an increased production or increased degradation of cysC that can affect its level, regardless of the renal function. These factors are age, elevated C-reactive protein levels, smoking, use of nephrotoxic drugs [24], as well as a variety of other physiological and pathophysiological conditions in particular. Therefore, the level of cysC should be interpreted carefully taking into consideration the impact of these so-called non-renal factors on the serum concentration of cysC.

**Thyroid Dysfunction and Cystatin C**

Cystatin C is in a significant positive correlation with the blood levels of the thyroid hormones, i.e. it is negatively correlated with the level of the thyroid stimulating hormone, which is why the calculated GFR using the serum concentrations of cysC is significantly lower in patients with thyroid gland hyperfunction than in the healthy population (when paired by gender and age), and vice versa in patients with thyroid gland hypofunction [25]. An increased level of cysC in patients with hyperthyroidism is most likely due to the increased metabolic activity in the organism, and vice versa in patients with hypothyroidism. For assessing the renal function in patients with thyroid dysfunction it is necessary to avoid the use of cysC until euthyroid state is achieved.

**Pregnancy and Cystatin C**

The level of cysC during the first and second trimester of pregnancy does not differ significantly from the level of cysC in non-pregnant women [26]. In the third trimester, an increase occurs in the cysC levels, even up to 29-39% when compared to the previous two trimesters [26].

A significant increase in the serum concentrations of cysC in pregnancy can be found in preclampsia (PE). Moreover, the level of cysC is not only significantly higher in the pregnant women who develop PE when compared to the women with normal pregnancies, but the increased levels of cysC are also present a few weeks and/or months before the development of PE [27]. This increase is a re-
flection of the impaired renal function, and the increased placental production of cysC [28]. Therefore, cysC may be used as a marker of PE independently from other parameters, but it is recommended to use it in association with other markers in order to achieve better detection of PE [27, 29].

In addition, the level of cysC in the amniotic fluid is an indicator of the development and maturation of the fetal kidneys. It is especially suitable for testing and detecting the existence of a fetal obstructive uropathy when the level of cysC is significantly higher than in fetuses with normal renal function (cut-off: 0.97 mg/L with a diagnostic accuracy of 96%) [30].

**Children, the Elderly and Cystatin C**

Cystatin C is a more sensitive marker of GFR reduction in children when compared with the serum creatinine level, particularly in the early stages of CKD [31], then in children with low muscle mass [32], as well as in the detection of an acute kidney injury [33]. In fact, the serum creatinine level is in a significant positive correlation with the body height and mass in children [34], while the serum concentration of cysC after the first year of life and during the childhood is stable and is not subjected to these factors. The serum concentration of cysC is significantly higher in the first year of life than in older age [32]. Besides, cysC has advantages in comparison with the serum creatinine level because of its analytical performance, as there is lesser influence of hemolysis and hyperbilirubinemia on its level measuring, which is of particular importance in the pediatric population.

With the aging process there is a “physiological” decline in the GFR, and there is a reduction in the muscle mass with the consequent reduced creatinine production, which ultimately can give a false picture of a preserved functional status of the kidneys, that is if Cre_equ are used for estimating the GFR. Given that the serum level of cysC is not affected by the muscle mass, a reduction of the GFR in the elderly can be detected earlier by using the cysC_equ than by using Cre_equ [35].

**Malignant Disease and Cystatin C**

Using cysC to estimate the GFR is limited in patients with certain malignancies because in malignant cells an overproduction of cysC could be found. Besides, in malignant diseases, due to the increased activity of the cysteine protease enzymes which contribute to the invasiveness of the tumor, there is probably a reactive increase in the production of cysC which is an inhibitor of these enzymes. This consequently leads to an increase in cysC circulating levels [36]. A dysfunction or a decreased activity of cysC is associated with higher metastatic ability of the tumor cells.

Studies indicate that there is an increase in the level of cysC in the following malignant diseases (in the absence of a renal impairment): leukemia, colorectal cancer, melanoma, hepatocellular carcinoma, prostate cancer, breast cancer, non-Hodgkin’s lymphoma, particularly in its aggressive forms. In fact, some studies indicate that cysC could be a marker of progression or relapse in certain malignancies [37]. Therefore, cysC could be a therapeutic goal when applying the anticancer therapy.

**Liver Disease and Cystatin C**

In patients with chronic liver diseases, cysC should be measured in parallel with the serum creatinine as it is believed to contribute to a more accurate assessment of the renal function, which is very important, especially in the patients with liver cirrhosis who are being prepared for transplant. In addition, one of the serious complications of liver cirrhosis is the hepatorenal syndrome whose main characteristics are renal vasoconstriction, renal hypoperfusion and reduction of the GFR, so in these patients an early detection of the GFR reduction is of extreme importance.

In liver diseases, serum creatinine as a marker of the GFR has significant shortcomings. First of all, there is a negative analytical interference between hyperbilirubinemia and creatinine [38], and a reduction in the serum creatinine is associated with a characteristic reduction in muscle mass in chronic liver diseases. In a significant number of patients in advanced stages of liver cirrhosis, an increased level of cysC indicates a renal dysfunction, despite the fact that the serum creatinine concentration is within the reference range [39].

In addition, cysC could be used for monitoring the progression of liver dysfunction as studies indicate that chronic liver diseases, such as chronic hepatitis B and C, hepatocellular carcinoma and liver cirrhosis in particular are associated with higher levels of cysC when compared to healthy subjects [40].

**Diabetes Mellitus and Cystatin C**

Diabetic nephropathy (DN) is a chronic complication of DM, a disease that is characterized by a high rate of morbidity and mortality, as well as being the leading cause of ESRD. Although the results of the studies are contradictory, the majority of them indicate that cysC is a better marker of the GFR than creatinine in the patients with both type 1 and type 2 DM [41], especially in the early stages of DN [42, 43].

Although the glomerular damage is considered to be the main factor in the development and progression of DN, tubular damage may contribute to the progression of DN as well. Given the fact that elevated urinary levels of cysC indicate tubular kidney damage, this marker could be used in the prediction of renal dysfunction in patients with DM [44].

**Cystatin C in Patients with Transplanted Kidneys**

Monitoring renal function in kidney transplant patients is of crucial importance after transplantation. Some studies point to the advantages of cysC compared to creatinine [45], while others indicate similar diagnostic characteristics between cysC_equ and Cre_equ.
The use of cysC in these patients is limited largely due to the administration of corticosteroids. In fact, corticosteroids cause a dose-dependent increase in serum concentrations of cysC [46] since they lead to an increased expression of the genes that encode the synthesis of cysC. This increase in the cysC level happens 48-72 hours after a non-complicated transplantation, unlike the creatinine levels, which decrease after transplantation [47]. Therefore, in order to use the level of cysC as a marker of the GFR in patients with transplanted kidneys, it is necessary to establish new reference values for this marker.

**Cardiovascular Diseases and Cystatin C**

The serum concentrations of cysC are a predictor of CV disease, and all cause mortality, independent from the functional status of the kidneys [48]. In addition to being associated with an increased risk for progression of the renal dysfunction, the serum concentration of cysC ≥1.0 mg/L is also associated with an increased CV risk [49]. In fact, some studies indicate an association between the elevated serum concentrations of cysC and the thickness of the carotid intimo-medial complex of the common carotid artery (CCIMT) which is a surrogate marker for subclinical atherosclerosis [50]. When the serum concentration of cysC is ≥1.0 mg/L, the prevalence of patients with CCIMT >0.9 mm is increased. In addition, elevated levels of cysC, independent from the measured GFR, are associated with an increased stiffness of the arteries [51].

In addition, some studies indicate that cysC is a more sensitive predictor of CV morbidity than the measured GFR, as well as the calculated GFR using the MDRD formula. In fact, in a significant number of patients with preserved GFR estimated by the MDRD formula, the level of cysC was elevated [52]. This feature of cysC recognizing a subtle renal dysfunction that is not shown by the serum creatinine level and the GFR estimated by the Cr_{eq} may be an explanation for the greater sensitivity of cysC when compared with other markers of the renal function in the prediction of CV morbidity.

Although some studies indicate that inflammation has no impact on the level of cysC [24], other studies point to a significant correlation between the level of cysC and the level of C-reactive protein [53]. This fact suggests that inflammation, which is one of the unavoidable contributing factors for the development of early atherosclerosis, is a possible connection between cysC and the elevated CV risk. It is possible that the increase in serum concentrations of cysC during the development of atherosclerosis reflects the established new balance between the increased levels of the lysosomal cathepsins in different locations and the levels of cysC, which is an inhibitor of those enzymes, whose harmful effect is to stimulate the atherosclerotic plaque rupture [54].

**Conclusions**

Since it is still unreliable to assess the renal function on the basis of an endogenous marker, cystatin C cannot completely replace creatinine. Therefore, in order to assess the functional status of the kidneys it is preferable to determine the levels of both markers simultaneously, and then to estimate the glomerular filtration rate using predictive formulas. In doing so, attention should be paid to the impact of non-renal factors on these markers. In the detection of chronic kidney disease it is especially of great importance to estimate one or both of the above markers repeatedly, and to follow their concentration changes in the serum over time.
One of the significant advantages of cystatin C is its use in the pediatric and elderly population, while a significant disadvantage is its markedly higher price for its determination (up to 50 times higher) than the price for determining creatinine.

In addition, cystatin C could be used as a marker for cardiovascular risk assessment, in predicting and detecting pre-eclampsia, and it could also be used as a marker in patients with malignant diseases and other pathophysiological conditions, which still requires additional testing.

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