Serum Cystatin C as a Biomarker

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Contents

Abstract

Cystatin C is a low molecular weight cationic protein produced by all nucleated cells which is a potent cysteine protease inhibitor. Its plasma concentration is proportional with glomerular filtration as it is synthesized at a constant rate, freely filtered through the glomerulus, and largely reabsorbed and catabolized in the proximal renal tubule with no tubular secretion which makes it ideal for GFR estimation. This protein has a

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V.B. Patel, V.R. Preedy (eds.), *Biomarkers in Kidney Disease*, Biomarkers in Disease: Methods, Discoveries and Applications, DOI 10.1007/978-94-007-7699-9_20

capability to detect early renal failure as it gives reliable GFR estimation at the critical level of 60 ml/min/1.73 m^2. Though cystatin C is superior to serum creatinine, non-standardization and several clinical situations such as thyroid dysfunction and the use of high doses of glucocorticoid limit its acceptance as a GFR marker to replace creatinine, the current endogenous gold standard biomarker.

Keywords

Creatinine • Cystatin C • Cystatins • Cysteine proteases • Glomerular filtration rate • Kidney diseases • Renal functions

Abbreviations

Ahsg	Alpha 2HS glycoprotein
CRES	Cystatin-related epididymal spermatogenic
CRPs	Androgen-regulated cystatin-related proteins
CSF	Cerebrospinal fluid
Cys C	Cystatin C
HCHWA-1	Hereditary cerebral hemorrhage with amyloidosis of Icelandic type
HMWK	High molecular weight kininogen
HRG	Histidine-rich glycoprotein
LMWK	Low molecular weight kininogen
MDRD	Modification of diet in renal disease
SCr	Serum creatinine

Key Facts of Renal Biomarker Cystatin C

- Kidney filtration function is tested with serum creatinine-based equations named as estimated glomerular filtration rate (eGFR).
- Creatinine is affected by nonrenal factors: age, gender, muscle mass, volume status, and analytical interferences such as bilirubin, ketone, and protein.
- Cystatin C is an endogenous protein synthesized in all nucleated cells that its plasma level is proportional with renal filtration capacity.
- Cystatin C is catabolized in the tubules of the kidney, that its high concentration in urine reflects tubular injury.
- In contrary to serum creatinine, cystatin C is less effected from volume status and not affected from hepatic dysfunctions which make it more reliable in the state of cirrhosis.

Definitions

Cathepsin Lysosomal enzyme responsible for terminal protein degradation

Cystatin A low molecular weight protein that inhibits reversibly the lysosomal enzyme cysteine proteases

GFR A test to determine the filtration function of the kidney

Housekeeping gene A type of gene that indicates a stable production rate

Introduction

Cystatin C (Cys C), originally called as gamma-trace and post-gamma-globulin, is a non-glycosylated low molecular weight protein found in many body fluids and secretions. In 1961, it was isolated as a cerebrospinal fluid-specific protein in human and named as gamma-trace protein (γ-CSF) (Clausen 1961). In 1981, the complete amino acid sequence of human cystatin C was determined by Grubb and Löfberg (Grubb and Löfberg 1982). The amino acid sequence of Cys C was the first sequence to be determined among cystatins. Later on the sequence similarity of the isolated human protein and chicken egg white cystatin was shown, and Cys C was identified as an inhibitor of cysteine proteases (Turk et al. 1983).

The cystatins inhibit the cysteine peptidases, the papain-like proteases (cathepsins), classified as clan C1, and some also inhibit the asparaginyl endopeptidase/legumain as clan C13 enzymes, and parasite proteases like cruzipain, where they appear to provide protective functions. Also cystatins have a role in the defense mechanism against microbial infections (Magister and Kos 2013; Turk et al. 2000; Abrahamson et al. 2003). Known as the tightest binding inhibitor of lysosomal and extracellular cysteine proteases, Cys C inhibits papain, cathepsin L, and cathepsin S in pM range. Cys C neutralizes the protease activity strongly and rapidly (Turk and Turk 2008).

Lysosomal cysteine proteases, generally known as cathepsins (clan C1), are the papain family that are responsible for terminal protein degradation in the lysosomes (Magister and Kos 2013; Turk et al. 2000). Also they may be secreted to degrade extracellular matrix components (Turk and Turk 2008). The increased cysteine protease activity has been shown to be related to a variety of pathopysiological conditions such as bone resorption, chronic inflammation (rheumatoid arthritis, bronchiectasis), cancer progression and metastasis, viral and parasitic infections, and septic shock (Turk and Turk 2008; Ni et al. 1997). Likewise, failure in the function of the protease inhibition results in neurodegeneration, cardiovascular diseases, osteoporosis, arthritis, and cancer. In atherosclerotic lesions, overexpression of cathepsins was found (Dinic et al. 2014).

Cystatin Superfamily

Cystatins, a superfamily of cysteine protease inhibitors, are comprised of 12 different inhibitors. They provide protective functions for uncontrolled proteolysis and tissue damage which are found in a variety of human fluids and secretions (Turk et al. 2002).

Table 1 The human cystatin superfamily classification

Family 1 (stefins)	Family 2 (cystatins)	Family 3 (kininogens)
Intracellular	Extracellular/transcellular	Intravascular
Stefin A	Cystatin C	LMW kininogen
Stefin B	Cystatin D	HMW kininogen
	Cystatin E/M	
	Cystatin F	
	Cystatin G	
	Cystatin S	
	Cystatin SA	
	Cystatin SN	

The cystatin superfamily consists of three types classified on the basis of the presence of one, two, or three copies of cystatin-like segments and the presence or absence of disulfide bond (Table 1). The members of the cystatin superfamily are reversible competitive inhibitors of cysteine proteases (Rashid et al. 2006). In addition to the family 1, 2, and 3 cystatins, the proteins containing cystatin domains but lacking inhibitory activities are also the members of the cystatin superfamily.

Family 1 cystatins (**stefins**): Stefin A (also named cystatin A, acid cysteine protease inhibitor, epidermal SH-protease inhibitor) and stefin B (also named cystatin B, neutral cysteine protease inhibitor) are intracellular cystatins (Magister and Kos 2013). These single-chain proteins lack disulfide bonds and carbohydrate side chains and are composed of 98 amino acid residues with 11,175 Da and 11,006 Da, respectively (Machleidt et al. 1983; Ochieng and Chaudhuri 2010).

The structure of stefin molecule consists of a five-stranded antiparallel β-sheet wrapped around a five-turn α-helix with an additional C-terminal strand (Stubbs et al. 1990).

Stefin A and B are potent inhibitors of papain and cathepsins L, S, and H. Their genes do not encode signal peptides (Ni et al. 1997). These intracellular cytoplasmic proteins of many types of cells have been detected in extracellular fluids as well (Kos and Schweiger 2002).

Family 2 cystatins (**cystatins**): Cys C, D, E/M, F, G, S, SA, and SN are type 2 extracellular and/or transcellular proteins distributed in body fluids at high concentrations with molecular masses of 13–14 kDa. Cys C, D, E/M, F, G, S, SA, and SN are encoded by genes located on the chromosome 20 (Abrahamson et al. 1990). Some members of the family 2 are glycosylated (Ochieng and Chaudhuri 2010). They all contain characteristic intrachain disulfide bonds toward the C-terminal unlike stefins. S type (S, SA, and SN) were first isolated from human saliva (Magister and Kos 2013; Isemura et al. 1984). Cys D, S, SN, and SA, mainly found in saliva, are poorer inhibitors of cysteine proteases than Cys C which is the

most abundant human cystatin that strongly inhibits clan C1 and clan C13 (Magister and Kos 2013). Human Cys D, present in tears and saliva, strongly inhibits cathepsin H and S and weakly neutralizes cathepsin L, but not cathepsin B (Balbín et al. 1994). The target proteases of cystatin E/M are the papain-like cysteine proteases including cathepsin B, L, and V (Cheng et al. 2006). Legumain, the asparaginyl endopeptidase, is mostly inhibited by its potent inhibitor cystatin E/M (Abrahamson et al. 2003).

Cys C is a major local regulator of extracellular proteolytic activity that inhibits cysteine proteases belonging to the papain (C1) and legumain (C13) families and especially inhibits the cathepsin B, H, L, and S. Cys C controls the activity of cathepsins which have elastolytic and collagenolytic activities that contributes to atherosclerotic process (Dinic et al. 2014). The high activity of legumain and cathepsins has been shown to ease the invasion of tumor cells (Briggs et al. 2010). It has been suggested that in breast cancer, the loss of cystatin E/M activity leaded an increase in tumor cell growth and metastasis (Ni et al. 1997).

Family 3 cystatins (**kininogens**): Intravascular low molecular weight kininogen (LMWK; MW 50e80 kDa) and high molecular weight kininogen (HMWK; MW 120 kDa) are large extracellular proteins comprised of about 335 amino acid residues that contain three family 2-like domains. These glycosylated forms of human kininogens have additional disulfide bonds and differ in length of the C-terminal regions. Structurally both HMWK and LMWK are composed of a light chain, a heavy chain connected by disulfide bridges, and the kinin segment. The light chains of both kininogens are different; however, the heavy chain and the kinin segment have identical amino acid sequences (Kellermann et al. 1986). LMWK binds papain and cathepsin L and S; HMWK binds papain, cruzipain, and cathepsin S.

CRES (cystatin-related epididymal spermatogenic) proteins: cystatin-like proteins named as cystatin-related epididymal-specific proteins were firstly found in mouse epididymis. Though structural homology was shown with cystatins, CRES proteins have no inhibitory effect on cysteine proteases papain and cathepsin B (Cornwall and Hsia 2003).

Other cystatin-like proteins, which lack cysteine protease inhibitory properties, are fetuin A, alpha 2HS glycoprotein (ahsg), histidine-rich glycoprotein (HRG), and androgen-regulated cystatin-related proteins (CRPs), testatin, and cystatin T. Testatin and cystatin T, specifically expressed in the testis, have similar sequence with family 2 cystatins (Ochieng and Chaudhuri 2010; Eriksson et al. 2002).

Structure of Cys C

The structure of the Cys C is a composition of five antiparallel β-sheets wrapped around a central helix with the disulfide bonds of between residues 73 and 83 and between residues 97 and 117. The molecular mass of Cys C is 13,343 Da

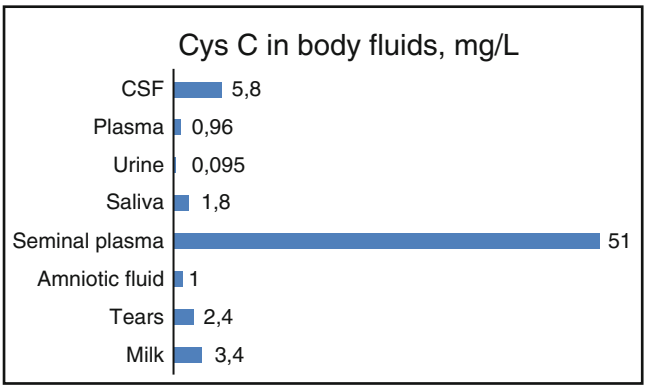

Fig. 1 Cystatin C concentrations (mg/L) in the body fluids

(nonhydroxylated) and 13,359 Da (hydroxylated proline residue at position 3). The isoelectric point of Cys C is 9.3 and thus positively charged in all body fluids (Filler et al. 2005).

Cys C is composed of 120 aa residues encoded by the "housekeeping type" CST3 gene located in the short arm of chromosome 20 at p.11.2 (Grubb and Löfberg 1982; Abrahamson et al. 1990). The protein is synthesized as a 146 aa pro-protein with a 26-residue hydrophobic signal peptide by all nucleated cells (Abrahamson et al. 1987). With the cleavage of the signal peptide, mature Cys C is released into the bloodstream in a short time. The N-terminal amino acid residue is the part of the Cys C molecule that has a high binding affinity to papain.

The amino acid sequence of the single polypeptide chain of human Cys C is SSPGK PPRLV GGPMD ASVEE EGVRR ALDFA VGEYN KASND MYHSR ALQVV RARKQ IVAGV NYFLD VELGR TTCTK TQPNL DNCPF HDQPH LKRKA FCSFQ IYAVP WQGTM TLSKS TCQDA (Grubb and Löfberg 1982).

The concentrations of Cys C in human biological fluids are figured (Fig. 1). Cystatin C is found at high concentrations in the body fluids, particularly in cerebrospinal fluid and seminal plasma (Filler et al. 2005; Magister and Kos 2013). The CSF concentration is five times more than in blood plasma which is the dominant cysteine proteinase inhibitor supplied mainly by the choroid plexus (Cole et al. 1989).

Detection method: PETIA (particle-enhanced turbidimetric immunoassay) and PENIA (particle-enhanced nephelometric immunoassay). Both methods make possible to measure routinely and rapidly, but the higher cost compared with SCr constraints its use routinely.

Specimens: Serum, plasma (EDTA, lithium heparin).

Assay Interferent Factors

Hemoglobin≤8 g/L, triglycerides ≤23 mmol/L, bilirubin ≤488 μL, and rheumatoid factor ≤2,000 kIU/L did not show any significant interference analyzing with nephelometric and turbidimetric methods (Finney et al. 1997; Delanaye et al. 2008).

Stability of cystatin C: Cys C is stable up to 48 h in the whole blood. Stored plasma samples are stable up to 4 years at −80 °C (Hoek et al. 2003).

Reference Range

Cys C production rate is constant throughout the ages of 1–50 years and increases significantly above the age of 50–60 in both gender which might be due to the physiological aging of the renal function (Finney et al. 2000; Galteau et al. 2001).

Plasma
<1 year: 0.59–1.97 mg/L (Finney et al. 2000)
1–50 years: 0.53–0.92 mg/L
>50 years: 0.58–1.02 mg/L

In premature infants, reference ranges are higher than adults (1.10–2.06 mg/L) (Schwartz and Work 2009).

Urine: 0.03–0.18 mg/L (Conti et al. 2005).

Cys C cannot be detected in urine under physiological conditions; however, in state of tubular injury, urine Cys C may become a measurable level, a potential biomarker for AKI (Slocum et al. 2012).

Cys C and Children

In pediatric population, Cys C is more advantageous than serum creatinine, especially under 4 years of age due to their lower muscle mass. Cys C levels are higher in the first weeks of life until 1 year of age, and the production rate is constant till 50–60 years unlike serum creatinine which increases until the early years of adolescence, due to muscle mass gain (Finney et al. 2000).

Cys C and Pregnancy

During the pregnancy, the serum Cys C concentrations were found as differed significantly with gestational age. In the first trimester, Cys C values were detected as higher than the second trimester and were increased in the third trimester which is

at the highest value after delivery. It was considered as a reliable GFR marker in pregnancy instead of serum creatinine that is unreliable (Babay et al. 2005).

Factors Affecting Cys C

Cystatins are involved in a number of immunomodulatory functions; it was shown to be associated in the pathophysiology of multiple sclerosis and Alzheimer' s disease (Bollengier 1987; Levy et al. 2001) During the inflammatory processes, Cys C release has been found as downregulated, contributing to increased cysteine protease activities in the macrophage microenvironment (Chapman et al. 1990) However, contrary to earlier suggestions, no significant effect of systemic inflammation on plasma Cys C concentrations was detected in a recent study by Grubb et al. (2011).

Cys C levels were found as affected by visceral obesity and insulin resistance (Ognibene et al. 2006). In fact, visceral obesity and insulin resistance are frequently associated with GFR increase and lower Cys C concentrations. Thyroid hormones increase Cys C levels; thus, hypothyroidism has been associated with lower Cys C concentrations and hyperthyroidism with higher Cys C concentrations (Schmid et al. 2012; Fricker et al. 2003).

It has been shown that moderate or high doses of glucocorticoids increase the Cys C production (Risch et al. 2001).

Advantages of Cys C

Cys C is a promising marker due to its proven satisfactory criteria, thus gaining popularity. Cys C has a short half-life (1.5 h) compared with serum creatinine (4 h with normal GFR) (Sjostrom et al. 2004). Serum creatinine is distributed in whole body water, whereas Cys C is distributed in extracellular part. Therefore Cys C rises more rapidly than serum creatinine and advantageous for early contrast-induced renal injury detection (Briguori et al. 2010). Less than 10 % increase in Cys C at 24 h can be a reliable marker for ruling out contrast-induced acute renal injury, and more than $\geq$10 % increase in serum Cys C at 24 h is an independent predictor of 1-year major adverse events such as death and dialysis (Briguori et al. 2010).

Although there are some conflicting data regarding greater intraindividual variability of Cys C than serum creatinine, in a recent study intraindividual variability of Cys C has been confirmed as similar to serum creatinine that Cys C seems as accurate as SCr for longitudinal patient follow-up (Delanaye et al. 2008). Interindividual variations of Cys C and creatinine were found as similar about 15.1 % and 14.4 %, respectively (Reinhard et al. 2009).

Cys C plasma concentration is inversely correlated with GFR as it is only catabolized in the kidney. Its renal clearance cannot be measured. Urine Cys C can

Table 2 Concentrations of human cystatin c in body fluids of healthy adults (mg/L; mean and range) (Filler et al. 2005)

Blood plasma 0.96, 0.57–1.79
Cerebrospinal fluid 5.8, 3.2–12.5
Urine 0.095, 0.033–0.29
Saliva 1.8, 0.36–4.8
Seminal plasma 51.0, 41.2–61.8
Amniotic fluid 1.0, 0.8–1.4
Tears 2.4, 1.3–7.4
Milk 3.4, 2.2–3.9

only be detected in case of renal proximal tubular impairment, which is more specific than serum Cys C (Koyner et al. 2008). Rapid testing is available with commercial automated assay procedures.

Disadvantages of Cys C

The test is relatively expensive; in daily routine, a large volume of testing requires more evidences clinically and careful consideration.

Cys C levels are lower in the hypothyroid and higher in hyperthyroid state. Very high doses of glucocorticoids increase the Cys C production, whereas low or medium doses of glucocorticoids decrease the Cys C production. Some evidence suggests that inflammation, osteoporosis, and diabetes affect the cystatin levels. In a study authors found 8.5 % higher levels of Cys C in patients with diabetes mellitus. However, that effect might be due to the interaction with proteinuria and Cys C. As in a study with type 1 diabetic patients with normal renal function without proteinuria, no influence of diabetes was found on the relationship between Cys C and GFR (Hofstra et al. 2009). The advantages and disadvantages are summarized in Table 2.

Cys C and GFR

GFR estimation is a widely used measurement protocol instead of the invasive methods based on exogenously injection of substances such as inulin, ^{125}-Iothalamate, iohexol, ^{51}Cr-EDTA which are traumatic for patients, especially for pediatric population. Moreover some techniques imply exposure to radiation.

Creatinine is produced by the muscle from creatine; thus, the muscle mass affects its concentration. Besides serum creatinine differs by age, gender, rhabdomyolysis, and dietary meat (Ochieng and Chaudhuri 2010). Moreover creatinine is secreted by renal tubules in a varied amount, and drugs may influence the tubular secretion of creatinine (Grubb et al. 2012). Therefore a new endogenous biomarker is being searched for GFR estimation that Cys C seems ideal in this aspect (Table 3).

Table 3 Advantages and disadvantages of cystatin C measurement

Advantages of Cys C
Less effected from nonrenal factors: gender, ethnicity (Caucasian, Afro-American and Asian), and muscle mass
Cys C production rate is constant from 1 year of age to 50 years
Cys C secretion does not have a circadian rhythm (Larsson et al. 2008)
The absence of a circadian rhythm for Cys C allows its quantification in a single urine sample (Conti et al. 2005)
Interindividual variation of Cyc C is about 15.1 % (Reinhard et al. 2009), intraindividual variation 4.5 % (Delanaye et al. 2008; Bandaranayake et al. 2007)
Short half-life (1.5 h) and extracellular distribution cause a rapid rise in serum concentration that is an advantage over serum creatinine
Less effected from volume status
Rapid and precise testing is available with automated methods
Sensitive to small changes in GFR
Reliable in liver disease
More convenient in the pediatric population
Specific to proximal renal tubule injury; only small amounts of Cys C can be found in urine (tenfold lower than in plasma), and increased concentrations in single-void urine samples directly reflect tubular damage (Conti et al. 2005)
Disadvantages of Cys C
Non-standardized (lack of international standard)
Expensive
Levels altered by thyroid dysfunctions
High doses of corticosteroids increase Cys C production
Malignancy increases serum concentrations

To overcome the creatinine-based limitations, several formulas have been developed and some parameters have been added to the GFR prediction equations (Cockcroft and Gault 1976; Levey et al. 1999). However, neither Cockcroft and Gault equation that produces GFR values in ml/min nor the modification of diet in renal disease (MDRD) equation that gives results as ml/min $(1.73\ m^2)^{-1}$ is suitable for children or for adults especially at eGFR >90 ml/min $(1.73\ m^2)^{-1}$ level. Instead, Schwartz equation or Counahan-Barratt equation is preferred for children (Schwartz et al. 1976; Counahan et al. 1976; Grubb et al. 2005).

Cys C is present in many biological fluids at higher concentrations, and its low molecular weight and positive net charge facilitate its glomerular filtration. Its plasma concentration is proportional with glomerular filtration as it is produced constantly by all nucleated cells. Cys C is later largely reabsorbed and catabolized in the proximal renal tubule with no tubular secretion which makes it ideal for GFR estimation (Grubb et al. 1985, 1992). Cys C has been proposed as an alternative marker of renal function because of its possible advantages over serum creatinine (Dharnidharka et al. 2002; Grubb et al. 2005). This protein has a capability to detect early renal failure as it gives reliable GFR estimation at the critical level of 60 ml/min/1.73 m^2 (Bargnoux et al. 2012). Grubb et al. concluded that instead of

Table 4 Ideal GFR marker characteristics (Ochieng and Chaudhuri 2010; Swan 1997)

Endogenously produced at a constant rate
Filtered and excreted only by the kidney
Unaffected by nonrenal factors such as age, gender, weight, nutrition, disease state, etc.
Freely filtered through the glomerulus
Neither reabsorbed nor secreted by the renal tubules
Undergo no extrarenal elimination
Stable in urine for further analysis

creatinine, Cys C-based prediction equation for GFR estimation that uses only serum concentration and a prepubertal factor (GFR $[\text{ml/min}^{-1}.(1.73\ \text{m}^2)^{-1}] = 84.69 \times \text{cys c}\ (\text{mg/L})^{-1.680} \times 1.384$ (if <14 years)) might replace both the MDRD prediction equation for adults and the Schwartz and Counahan-Barratt prediction equations for children. In that formula, a prepubertal factor added to compensate the prepubertal concentrations that is higher than the older individuals where 13–14 years of age limit represents the start of the puberty (Grubb et al. 2005). Ideal GFR marker characteristics are listed in Table 4.

The gold standard exogenous marker ^{51}Cr-EDTA has been compared with Cys C to estimate GFR; this endogenous marker gave excellent correlation with GFR (Simonsen et al. 1985). Cys C is more sensitive to small changes in the so-called creatinine-blind GFR (40–70 ml/min) (Schwartz and Work 2009). It was reported that in children, the serum Cys C is better correlated with GFR than serum creatinine (Grubb et al. 2005).

There are equations recommended to estimate GFR from Cys C concentration:

(A) Grubb's equation : $\text{GFR}_{\text{Grubb}} = 84.69 \times \text{Cystatin C}^{-1.680} (\times 1.384 \text{ if child} < 14 \text{ years})$ (Grubb et al. 2005).

(B) Larsson's equation: $\text{GFR}_{\text{Larsson}} = 99.43 \times \text{Cystatin C}^{-1.5837}$; cystatin C is measured in mg/L (Larsson et al. 2004).

The problem that restricts the widespread use of Cys C is the lack of standardization. Though recently a certified reference material (ERM-DA471/IFCC) has been released, the different assay systems, differences in the established reference intervals for different populations are the factors that affect the reliability of Cys C-based equations inevitably (Grubb et al. 2005). Besides the use of large doses of corticosteroids, thyroid dysfunction reduces the performance of Cys C-based equations. In those cases the diagnostic performance of creatinine-based equation is more reliable.

The use of Cys C in combination with SCr to calculate eGFR strengthens the risk classification of chronic kidney disease. The risk of death was found as increased below the threshold of ~85 ml/min/1.73 m^2 when using both Cys C and creatinine-based eGFR (Shlipak et al. 2013). It has been concluded that Cys C is more sensitive to small changes in GFR than serum creatinine in contrast-induced acute kidney injury (Briguori et al. 2010).

Although Cys C-based equations offer significant advantages over creatinine-based equations, Cys C cannot replace creatinine as it also has some limitations mentioned above and not perfectly tested in some clinical situations. Consequently Cys C-based equations cannot replace gold standard methods (Grubb et al. 2005; Andersen et al. 2009).

Cys C and Cirrhosis

In patients with liver cirrhosis, renal dysfunction is associated with poor prognosis. In these patients, SCr may be influenced directly by nonrenal factors such as protein-calorie malnutrition, muscle wasting, and increased tubular secretion, and impaired liver function caused reduced creatinine production. And indirectly, hemodynamic changes will affect serum creatinine concentrations. In cirrhosis, GFR estimation with creatinine has been shown to overestimate the true GFR by up to 200 % (Sherman et al. 2003); consequently the renal failure is greatly underestimated.

Inulin clearance was considered as the gold standard in cirrhosis (Caregaro et al. 1994). However, this method is very cumbersome to perform in clinical practice. Moreover, urine collection is difficult to execute in clinical practice because of urinary losses and incomplete urinary bladder emptying. ^{51}CR-EDTA and ^{99}TC-DPTA are other measurement techniques that imply exposure to radiation. Conversely, Cys C is independent of gender, age, and muscle mass, and not influenced by serum bilirubin, inflammation, or malignancy. Accordingly, Cys C has been proposed as a specific marker of GFR and an early indicator of impaired renal function in patients with cirrhosis (Ćulafić et al. 2014; Ustundag et al. 2007).

Cys C and Malignancy

Cys C is shown to be increased in malignancies in some studies irrespective of kidney function. High concentrations of cathepsin B and H are detected in the sera of patients with colorectal cancer and malign melanoma which are associated with increased serum Cys C (Kos et al. 1998). Imbalance between cysteine proteinases and cystatins is associated with tumoral cell metastasis that is considered to facilitate tumor cell invasion and metastasis. Though high levels of Cys C may inhibit cathepsin activities, increased levels of Cys C was found to be associated with poor prognosis (Kos et al. 2000). Increase in the inhibitory functions of cystatin could lead to a harmful impairment of the antitumor response of cysteine cathepsins (Magister and Kos 2013).

Cysteine proteases take part in tumoral metastasis that involves local invasion and angiogenesis. Cathepsin B, detected to be present on the surface of the tumor cell (Mai et al. 2000), plays a key role in tumoral cell invasion (Ochieng and Chaudhuri 2010) which is particularly inhibited by Cys C. Though increases in Cys C concentration were determined in malignant diseases such as colorectal cancer or melanoma, in those studies, GFR measurement was not performed with a reference

technique (Kos et al. 1997, 1998). In myeloma, serum Cys C was found as more sensitive than SCr for GFR estimation that detected moderate GFR reductions. Additionally, Cys C also correlated with advanced disease and provided important information for prognosis (Terpos et al. 2009; Nückel et al. 2012).

Amyloid Angiopathy

Cys C is a protein with amyloidogenic properties that aggregates in the brain arteries of elderly people with amyloid angiopathy. A more severe disease massive amyloidosis is associated with the L68Q mutant of human Cys C that leads to death in young adults with massive cerebral hemorrhage. This autosomal dominant disorder, associated with mutation in the Cys C gene CST3, is known as Icelandic type. A point mutation that replaces leucine amino acid with glutamine at position codon 68 of the Cys C gene is a disorder known as hereditary cerebral hemorrhage with amyloidosis of Icelandic type (HCHWA-1) (Ghiso et al. 1986; Levy et al. 1989; Revesz et al. 2009). This less stable deposit of mutant Cys C (ACys) aggregates mainly in the brain arteries to form plaques called amyloid deposits that impairs the elasticity of the arterial wall by replacing the muscle fibers and elastic fibers (Pezzini et al. 2009).

Summary Points

- This chapter reviews cystatin C and its significance for renal function.
- Cystatins are cysteine protease inhibitors where they appear to provide protective functions.
- Cystatin C is a protein synthesized by all nucleated cells and secreted shortly after its synthesis and not affected by nonrenal factors.
- Cystatin C is freely filtered through the glomerulus and almost completely catabolized by tubules that are an advantage for GFR estimation.
- Low molecular weight and positive net charge of Cys C facilitate its glomerular filtration. The plasma concentration is proportional with glomerular filtration as it is produced constantly.
- Cystatin C is an endogenous GFR marker that can be easily detected in serum with automated methods.
- Urine concentration is very low (tenfold lower than plasma concentration), and increased concentrations in urine samples directly reflect tubular damage.
- Cys C is a protein with amyloidogenic properties that aggregates in the brain arteries of elderly people with amyloid angiopathy.
- Lysosomal cysteine proteases, generally known as cathepsins (clan C1), are the papain family that are responsible for terminal protein degradation in the lysosomes.

- The cystatin superfamily consists of three types classified on the basis of the presence of one, two, or three copies of cystatin-like segments and the presence or absence of disulfide bond.
- Cystatin C is found at high concentrations in the body fluids, particularly in the cerebrospinal fluid and seminal plasma.

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