Clinical Role, Diagnostic and Prognostic Value of Human Cystatin C Throughout the Body

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Abstract

Cystatin C is a low-molecular-weight biomarker that meets the conditions necessary to be a marker of the glomerular filtration rate. This endogenous cysteine proteinase inhibitor belonging to the type 2 cystatin superfamily plays a key role in health and disease. Human cystatin C is encoded by the CST3 gene, ubiquitously expressed at moderate levels throughout the body, given it is produced in all nucleated cells in a constant amount. It is present in all human body fluids, and may be determined in the serum, plasma, capillary blood and urine. In this review, we present and discuss most of the available data from the literature for the use of cystatin C in clinical practice. In addition to a kidney function marker, studies suggest cystatin C could be used as a marker for cardiovascular risk assessment, in predicting and detecting preeclampsia, in patients with malignant neoformations, etc. Local cystatin C deficiency has also been demonstrated in atherosclerosis, aneurismal lesions, among others, suggesting a protective role of cystatin C. Far beyond its humble beginning, it promises uses from prognosis to treatments for everything from prostate cancer to periodontal disease. Cystatin C has begun its journey into a multitude of disciplines; we can only begin to imagine the many more purposes yet to find going forward.
1. Introduction

Cystatin C was first discovered in 1961 by Flynn and Butler as an electrophoretic band in urine; that same year Clausen found a similar band in cerebrospinal fluid, which he named “γ-trace”. In 1984 the name Cystatin C was proposed for γ-trace (Gamma trace) or post γ globulin; and this protein was formally identified as an endogenous cysteine protease inhibitor. Cystatin C, belongs to family 2 of the Cystatin superfamily, it is a single non-glycosylated cationic low molecular weight protein with 120 amino acid residues, which has a molecular weight of 13.359 kDa cysteine protease inhibitor produced by all nucleated cells at a constant rate. Cystatin C has an isoelectric point of 9.3 and it is positively charged in all human biological fluids examined.

There are three major types of inhibitors in the Cystatin protein superfamily. Stefins or human type 1 Cystatins which are mainly intracellular inhibitors include Cystatins A and B; although low levels of these inhibitors are also found in some body fluids. The human type 2 are extracellular Cystatins; these constitute the largest group of inhibitors, Cystatins C, D, E/M, F, G, S, SN and SA; they are synthesized with signal peptides for secretion. The human type 3 group Cystatins comprise L- and H-kininogen, which together with Cystatin C are the most important plasma inhibitors of cysteine proteases.

Among the Cystatin human type 2 superfamily, Cystatin C is the most studied. Cystatin C is a natural inhibitor of the enzymes involved in the processing of prohormones and proenzymes and also in the catabolism of different peptides and proteins; it has a generally protective function, to prevent connective tissue destruction by intracellular enzymes which are secreted or leaked from the lysosomes of dying or diseased cells.

Cystatin C shows the highest affinity and the fastest inhibition of all Cystatins toward lysosomal cysteine proteases.

The main functions of Cystatins are related to the inhibition of cysteine proteases, cell proliferation, cell migration and cell differentiation. Cystatin C inhibits family C1 cysteine proteases through tight and reversible binding in a substrate-competiting mechanism, resulting in an equimolar complex with the protease. Cystatin C has been suggested to be a protective protein and prevents brake down of the extracellular matrix by preventing enzymatic cleavage of connective tissues by cathepsins. Exogenous Cystatin C downregulates the proteolytic activities of family C1 (papain-like) cysteine proteases such as human cathepsins B, H, K, L and S. The balance between the Cystatins and the family C1-cathepsins is of major importance in the regulation of the proteolytic activity under normal physiological conditions, but also in pathological degradation of proteins in inflammatory and neoplastic diseases.

The synthesis of Cystatin C does not seem to be tissue-specific and all nucleated cells constitutively express and constantly secrete it. Cystatin C has a widespread distribution in human tissues and is produced at a constant rate by all types of cells and localized mainly in extracellular fluids; it was previously shown that the concentration of Cystatin C in seminal plasma is higher than in any other body fluid with high concentrations also found in cerebrospinal fluid, and lower concentrations in milk, synovial fluid, serum, urine, bile, saliva, blood and eye fluids.

Gender, body weight, diet, hydration and nutritional status have no influence on Cystatin C concentration. At birth, Cystatin C concentration is higher than in adults and decreases within 2 months; it is also known
that Cystatin C does not cross the placenta\(^5\). Increased Cystatin C level has been found in the serum of patients with several inflammatory diseases, such as tumors with metastases\(^8\). Cystatin C could play a protective and regulatory role under inflammatory conditions\(^3\). Previous data suggest that Cystatin C has also been involved in neurodegenerative and repair processes after post-ischemic and mechanical injury\(^9\). Cystatin C was shown to play a role in cancer development, protein catabolism, regulation of hormone processing and bone resorption, modulating inflammation\(^8\).

The members of the same enzyme family are responsible for the penetration of certain viruses, microorganisms and malignant cells into normal tissue and participate in local and systematic inflammation, this suggests it may also have antimicrobial and antiviral functions\(^1\)

Cystatin C is currently considered as a marker of renal function in both healthy and diseased patients and has also been associated with various diseases like chronic inflammation, tumors, atherosclerosis, hypertension, Alzheimer’s disease and thyroid dysfunction among others which are important in clinical and experimental medicine\(^3\)

### 2. Genes

Genome-wide association studies for genetic determinants of Cystatin C have located the strongest signal of the genome associating with variation of plasma concentration of Cystatin C at the Cystatin C locus on chromosome 20, represented by the single nucleotide polymorphism (SNP) rs13038305\(^2\)\(^10\). Genetic elevation of Cystatin C measured by a single nucleotide polymorphism at the Cystatin C locus on chromosome 20 robustly associated with plasma Cystatin C (rs13038305)\(^2\). Cystatin C has been shown to undergo signal sequence cleavage between residues Gly26 and Ser27. Under normal conditions, the signal sequence cleavage site of Cystatin C is after Gly26 (referred to as ‘site 2’ cleavage)\(^11\). However, in particular circumstances when the residues around site 2 are modified (such as by the presence of an N-terminal FLAG tag immediately after Gly26, or by a Gly26Lys (G26K) mutation), Cystatin C has an alternative signal sequence cleavage after Ala20 (‘site 1’) or even earlier\(^11\). Additional N-terminal amino acids resulting from alternative signal sequence cleavage may, in turn, affect the protease inhibition function of Cystatin C\(^11\).

The primer sequence of Cystatin C is: forward 3’-AGA TCT ACG CTG TGC CTT GG-5’ and reverse 3’-CAG AGC CTG TGG GGT AAA CA-12.

Secretory proteins are first synthesized in the cytosol and co-translationally translocated across the endoplasmic reticulum membrane to enter the secretory system\(^11\). Typical secreted proteins are directed into the endoplasmic reticulum by a short, hydrophobic N-terminal signal sequence of generally 10–20 residues. Signal recognition particles in the cytosol recognize this hydrophobic signal sequence as it emerges from the ribosome and target the nascent polypeptide to the endoplasmic reticulum through interactions with the signal recognition particles receptor\(^11\). The nascent polypeptide is extruded into the endoplasmic reticulum through the Sec61 translocon, and in the process, a signal peptidase complex cleaves the signal sequence\(^11\). Consistent with its extracellular presence, Cystatin C has been demonstrated to have a 26-residue signal sequence\(^11\). The proteases that Cystatin C binds to are typically localized to the lysosome\(^11\).

The Cystatin C gene CST3 has been considered as a candidate gene for Alzheimer Disease. At least four polymorphisms have been described: two in the 5-untranslated region and in exons 1 and 2\(^13\).
A polymorphism associated with Alzheimer Disease is located in exon 1: A G/A transition results in Ala/Thr as the penultimate amino acid of the signal peptide, which is believed to reduce secretion and constitutive extracellular level.

The BB genotype of the Cystatin C encoding gene CST3 leads to reduced Cystatin C secretion in vitro and has been shown to convey susceptibility to Alzheimer Disease. This BB genotype of the Cystatin C encoding gene could also be associated with dementia in Lewy body disease.

The function of rs13038305 and the causal relation between rs13038305 and Cystatin C are unknown. It is possible that rs13038305 or the causal variant or variants that it represents may influence more than one trait with opposite effects on Alzheimer Disease.

Other genes have been associated with the regulation of the Cystatin C gene. Fluorescence RT-qPCR was employed to detect the expression of the miR-92a gene in vivo, in order to determine whether miR-92a expression inhibit the Cystatin C gene. Transfection of miR-92a into endothelial cells demonstrated that miR-92a is able to regulate the expression of Cystatin C.

3. Prognostic tool

Cysteine proteinase inhibitors, Cystatins, are involved in mechanisms regulating intracellular and extracellular protein degradation.

A wide-ranging scale of biological significance has been advocated for Cystatin C, as well as for controlling inflammation, hormone processing, protein catabolism, bone resorption, antigen presentation and T-cell dependent immune response.

The estimation of glomerular filtration rate in multiple myeloma is based on equations that use serum creatinine, such as the Modification of Diet in Renal Disease. However, the Chronic Kidney Disease Epidemiology Collaboration group has suggested that estimation of glomerular filtration rate based on both serum creatinine and Cystatin C is more accurate than other formulae for the estimation of kidney dysfunction.

The aim of this study was to prospectively evaluate, for the first time in the literature, the chronic kidney disease-epidemiology-creatinine-Cystatin C formula in newly diagnosed patients with symptomatic multiple myeloma.

Results suggest that equations based on Cystatin C reveal higher number of multiple myeloma patients with renal injury compared with equations based only in serum creatinine.

Furthermore, the chronic kidney disease-epidemiology-Cystatin C formula independently predicted for survival.

Based on the data, chronic kidney disease-epidemiology equations based on Cystatin C should substitute Modification of Diet in Renal Disease, as it has been suggested for patients with several other renal disorders as a renal injury prognostic tool.

As a member of cysteine protease inhibitors, Cystatin C also inhibits tumor invasion and metastasis.

Cystatin C has also been proposed to have a role associated to the modification of the proteolytic system in cancer.

Cystatin C level has been proposed as a prognostic tool proposed for follow up of diseases, efficacy of chemotherapy, and prediction of disease outcome.

Elevated serum levels of Cystatin C are found to be related to poor outcome and metastatic potential of some malignant disorders.
examples of this are with lung and colorectal cancer\textsuperscript{15}.

In patients with melanoma, increased Cystatin C serum levels correlated with the highest metastatic stage\textsuperscript{15}.

An elevated preoperative Cystatin C level was demonstrated to be related with worse survival in patients with renal cell carcinoma. Measuring preoperative serum Cystatin C seems to be an effortless way for finding poor prognostic patients and patients with elevated preoperative Cystatin C level should be more closely followed up\textsuperscript{17}.

Patients with preoperative high serum Cystatin C (\textgreater{}1.09 mg/L) had shorter overall survival and disease free survival than patients with low serum Cystatin C (1.09 mg/L)\textsuperscript{17}.

The results of multivariate analysis demonstrated that preoperative serum Cystatin C was an independent factor for predicting overall survival of renal cell carcinoma patients\textsuperscript{17}.

In addition, preoperative serum Cystatin C was also an independent factor for predicting disease free survival of non-metastatic renal cell carcinoma patients treated with complete surgical resection\textsuperscript{17}.

Most patients with esophageal cancer are unresectable in time due to lack of effective diagnostic approaches. Serum markers are of significant importance for early diagnosis and prognosis in esophageal cancer patients. The serum levels of cathepsin B and Cystatin C from patients with esophageal carcinoma and healthy donors were determined preoperatively by using enzyme-linked immunosorbent assay\textsuperscript{18}.

The correlation and survival rates were compared between cathepsin B and Cystatin C in diagnostic significance in esophageal cancer and it was determined than the ratio of serum Cystatin C / cathepsin B proved to be a good prognostic tool for the survival of esophageal carcinoma patients\textsuperscript{18}.

It was previously shown that neuroendocrine (NE) cells in a variety of human tissues, including the prostate, have Cystatin C immunoreactivity, and it was suggested that the inhibitor might be involved in the intracellular regulation of the peptide hormones\textsuperscript{7}.

NE differentiation (NED) occurs in most prostatic adenocarcinomas, and has been correlated with tumor progression, poor prognosis and resistance to hormonal therapy\textsuperscript{7}.

The intensity of Cystatin C immunostaining in Gleason grade 2 and 3 prostate cancer was significantly higher than in benign prostatic tissues but decreased significantly with increasing Gleason grades. There was strong expression of Cystatin C in neuroendocrine-like cells, which increased significantly with increasing Gleason grades\textsuperscript{7}.

There were more strongly Cystatin C-positive neuroendocrine-like cells in prostate cancer than in benign prostatic tissue suggesting a connection between Cystatin C and neuroendocrine differentiation in prostate cancer prognosis given neuroendocrine-like cells in prostate cancer has a worst outcome in the disease\textsuperscript{7}.

### 4. Renal

Serum Cystatin C is a relatively new endogenous marker that offers the advantages that its produced at a constant rate by all nucleated cells, it is freely filtered by the renal glomerulus, and entirely catabolized in the proximal tubule\textsuperscript{19, 20, 21, 22}. Its filtration is unchanged in kidney tubular diseases where its urinary excretion is increased\textsuperscript{20}. The multi-ligand endocytic receptors megalin and cubilin are heavily expressed in the proximal tubule brush border membrane and essential for the reabsorption
of a large number of filtered proteins in the proximal tubule \(^{23}\). The increase in urinary Cystatin C excretion after ischemia/reperfusion injury is associated with decreased tubular uptake but not with reduced megalin expression, which is essential for the normal tubular recovery of endogenous Cystatin C \(^{23}\). Serum Cystatin C measurement has been described as a means to evaluate glomerular filtration rate \(^{24}\). Regarding the fact that this marker is not secreted by the kidney and is not reabsorbed to the blood stream after complete glomerular filtration, this marker is close to the ideal endogenous marker and it is not changed by external factors \(^{25}\).

Recently, as an alternative marker of kidney function, either alone or in combination with serum creatinine several studies have claimed that the estimation of serum Cystatin C could be a better marker of kidney excretory function and a better predictor of adverse outcomes than serum creatinine \(^{5,26}\). Serum creatinine concentration is the most commonly used diagnostic marker for the clinical assessment of glomerular filtration rate; however, it has a range of nonrenal factors influencing their production, for example: muscle mass and protein intake, and for creatinine there are several well reported difficulties concerning its analysis \(^{20,27}\). Serum creatinine levels are confounded by muscle mass and variable tubular secretion, whereas serum Cystatin C has a different volume of distribution and may vary with the volume status \(^{21}\).

When comparing results of measured creatinine clearance in 24 h urine, with obtained for serum Cystatin C from 100 patients; it was found that Cystatin C concentration correlated well with measured creatinine clearance, these results were comparable statistically with calculated clearance creatinine \(^{24}\). Cystatin C may substitute, in the clinical laboratory, creatinine clearance measurement given its practicality \(^{24}\).

Many clinical studies have demonstrated the ability of neutrophil gelatinase associated lipocalin neutrophil gelatinase associated lipocalin and Cystatin C to allow early identification of acute kidney injury, 48 hours earlier than plasma creatinine level in cardiac surgery, contrast agent use, critically ill patients and in the emergency department \(^{28}\). Plasma and urinary Cystatin C and urinary neutrophil gelatinase associated lipocalin are useful markers in predicting acute kidney injury in septic critically ill patients as well \(^{28}\).

The Chronic Kidney Disease Epidemiology Collaboration formula Cystatin C-derived equations (CDK EPI Cystatin C and CDK EPI Cystatin C-creatinine) outperform the Chronic Kidney Disease Epidemiology Collaboration formula (CDK EPI creatinine) in approximating the residual renal function values \(^{19}\).

Hoek and colleagues using serum concentrations of Cystatin C showed that a simple linear equation can be used to estimate glomerular filtration rate values surpassing the accuracy of the modification of diet in renal disease creatinine formula \(^{19}\).

It has been shown that Cystatin C is superior or at least equivalent to creatinine for estimation of glomerular filtration rate in normal pediatric and adult populations \(^{19}\).

Since Cystatin C is not as influenced as creatinine by endogenous or exogenous agents, it is a better glomerular filtration marker for \(\leq 1500\) gr preterm infants \(^{29}\). But when in sepsis it was no longer a useful marker of kidney function in neonates \(^{5}\).

Children with non-renal solid organ transplants are surviving longer, but outcome is sometimes complicated by Chronic Kidney Disease \(^{30}\). Thus, glomerular filtration rate estimated by serum Cystatin C, creatinine, urea, and height followed by Filler formula is
an adequate method to monitor renal function closely and frequently in these children.\textsuperscript{30}

A small cohort of infants undergoing cardiopulmonary bypass, found that serum Cystatin C is an early and specific biomarker for identification of neutrophil gelatinase-associated Lipocalin-positive acute kidney injury.\textsuperscript{31} It showed best performance characteristics as a marker of acute kidney injury on post-operative days.\textsuperscript{31}

In pediatric and adult sickle cell disease patients serum Cystatin C was found to be insufficient for the evaluation of sickle cell disease nephropathy.\textsuperscript{32}

Measurement of serum Cystatin C concentrations is a useful marker for screening of diabetic nephropathy in diabetic patients; but, it cannot be used for monitoring of these patients as a substitute of HbA1c.\textsuperscript{22} Serum Cystatin C is useful in detecting individuals with chronic kidney disease having mild decrease in glomerular filtration rate compared to Serum Creatinine.\textsuperscript{33} Serum Cystatin C may be used to screen patients with poorly controlled diabetes mellitus or hypertension when Serum Creatinine level is inconclusive.\textsuperscript{33} Renal lesions initially occur in the renal tubule and then form in the renal glomerulus of patients with type 2 Diabetes Mellitus.\textsuperscript{34} There is group of patients with decreased estimated glomerular filtration rate but without increased urinary albumin excretion, in which serum Cystatin C level was indicated to be used as an early biomarker of diabetic nephropathy.\textsuperscript{35} Serum Cystatin C was higher in microalbuminuric patients than those who had normoalbuminuria in patients of type 2 diabetes to detect early kidney injury.\textsuperscript{36} Microalbuminuria is the earliest marker for diabetic nephropathy; however, a large proportion of renal impairment occurs in non-albuminuric state.\textsuperscript{36} Meanwhile urinary Cystatin C levels was a more efficient indicator than serum Cystatin C for the evaluation of early renal function impairment among patients with type 2 Diabetes Mellitus.\textsuperscript{34}

Renal lesions may initially occur in the renal tubule and then form in the renal glomerulus of patients with type 2 Diabetes Mellitus.\textsuperscript{34} Urinary Cystatin C levels may be used as an efficient indicator for the evaluation of early renal function impairment among patients with type 2 Diabetes Mellitus.\textsuperscript{34} Estimated glomerular filtration rate calculated by CKD EPI Cystatin C is a better option for assessing the renal status in patients of early diabetic kidney disease.\textsuperscript{37}

Combined detection of plasma homocysteine and Cystatin C can be used to predict the impacts of plasma homocysteine on glomerular filtration function and degree of renal damage of diabetic nephropathy as well.\textsuperscript{38}

Renal function can be impaired in cancer patients treated with cisplatin and one of the laboratory markers proposed for early diagnosis of renal dysfunction is serum Cystatin C level.\textsuperscript{25}

Serum Cystatin C concentration had better sensitivity, specificity, positive predicted value, and negative predicted value compared to creatinine in detection of early stages of renal dysfunction in cancer patients under treatment with cisplatin.\textsuperscript{25}

Stabuc et al. reported Cystatin C and its application in patients who were treated by cisplatin and it was reported that it has a higher efficacy compared to serum creatinine.\textsuperscript{25}

There was no correlation among the level of Cystatin C after the cisplatin infusion and serum creatinine level following the third course and modification of diet in renal disease and creatinine clearance–Cockcroft–Gault formulations.\textsuperscript{39} Previous studies have also used serum Cystatin C levels to evaluate that agma-
tine has a protective effect against cisplatin-induced nephrotoxicity \(^{40}\).

Even though the serum Cystatin C levels were correlated with the serum creatinine levels, it was concluded that it was not an appropriate parameter to predict the potential impairments in the renal function during the chemotherapy \(^{39}\).

Acute kidney injury is a frequent complication in critically ill patients and is associated with a high mortality \(^{41}\). Urinary Cystatin C level and the urinary Cystatin C / urinary creatinine ratio were useful biomarkers of intrinsic acute kidney injury, and the urinary Cystatin C / urinary creatinine ratio was predictive of in-hospital death in acute kidney injury patients \(^{41}\).

The accuracy and precision of glomerular filtration rate estimating equations based on plasma creatinine, Cystatin C and the combination of these markers have recently been assessed in HIV-infected individuals \(^{42}\). Of the three Chronic Kidney Disease Epidemiology Collaboration glomerular filtration rate equations (CKD EPI Cystatin C, CKD EPI Cystatin C-creatinine and CKD EPI creatinine), CKD EPI Cystatin C had the strongest associations with mortality, cardiovascular events and opportunistic disease in HIV-infected individuals \(^{42}\).

Cystatin-C-estimated glomerular filtration rate correlated with height-adjusted total kidney volume in autosomal dominant polycystic kidney disease patients in early stages of the disease; even when renal function remains normal \(^{43}\).

In the future, Cystatin C may also be a good marker of renal function in patients with lupus nephritis, since factors such as disease activity or use of drugs such as glucocorticoids do not modify the renal levels of this marker \(^{44}\).

5. Cardiovascular

Menon et al. reported that Cystatin C provided prognostic information beyond information about renal function \(^{45}\). Cystatin C was correlated with body mass index (BMI), proteinuria, systolic arterial pressure, and inversely correlated with HDL cholesterol levels \(^{46}\). Increased Cystatin C is associated with an enhanced cardiovascular risk factor profile in Aboriginal youth and may be an early event in the natural history of vascular disease \(^{47}\). High Cystatin C and retinol-binding protein 4 may contribute significantly to cardiovascular risk burden in addition to traditional cardiovascular markers \(^{48}\). Significantly higher levels of both proteins, Cystatin C and retinol-binding protein 4 were found in the Framingham Risk Score higher risk groups compared to low risk Framingham Risk Score group \(^{48}\).

Epidemiologic studies have shown that Cystatin C is an independent predictor in older adults, more sensitive than creatinine and glomerular filtration rate for detecting a high risk of with an increased risk of renal disease progression, death, or cardiovascular events, death and cardiovascular events in a population with chronic kidney disease \(^{49}\) \(^{50}\). It also provided significant prognostic information, in terms of morbidity and mortality, among hypertensive, coronary, acute and chronic heart failure patients \(^{51}\).

Arterial stiffness is important in the evaluation of the cardiovascular risk in all patients \(^{52}\). Multivariate analysis revealed that serum Cystatin C but not albuminuria was significantly associated with pulse wave velocity in hypertensive patients \(^{52}\).

Obstructive sleep apnea syndrome is associated with systemic inflammation and increased risk of cardiovascular and chronic kidney disease \(^{53}\). Serum Cystatin C serum levels are increased in obstructive sleep apnea syndrome patients without comorbidities,
suggesting an increased renal and cardiovascular disease risk.\(^5\)

In a large study that evaluated the role of early kidney dysfunction as a risk factor for hypertension that included 2,767 individuals with a median follow-up of 3 years, Cystatin C levels were elevated and associated with older age and traditional cardiovascular risk factors.\(^5\) After adjustment for established arterial hypertension risk factors, each increase in serum Cystatin C levels of 15nmol/L was associated with a 15% greater incidence of hypertension.\(^5\)

Biomarkers are commonly used to estimate the presence of subclinical cardiovascular disease in patients with essential arterial hypertension.\(^4\)

In patients with essential arterial hypertension, Cystatin C is an early marker for lesions on target organs related with hypertension, such as carotid artery intima-media thickness, left ventricular mass, and microalbuminuria.\(^4\)

Serum Cystatin C concentration is positively and independently associated with intima-media thickness of common carotid arteries in patient with unresponsive hypertensive phenotype and subclinical cardiovascular disease.\(^4\)

Recent studies suggested that higher serum Cystatin C concentration is found in hypertensive patients, in association with stiffness of large arteries in older adults, and with cardiac structural and functional alterations.\(^4\)

The accumulation of epicardial adipose tissue is a cardiovascular risk factor independent from visceral adiposity, obesity, hypertension and diabetes.\(^5\) A strong association between epicardial adipose tissue and Cystatin C was previously found, indicating that epicardial adipose tissue accumulation may play an important role in Cystatin C secretion, possibly contributing to cardiometabolic risk in type 2 diabetes patients.\(^4\) Association between high levels of Cystatin C and increased risk of future development of metabolic syndrome was found.\(^1\) In metabolic syndrome previous studies have found, Cystatin C has been found to be associated with severity of coronary artery disease.\(^5\) Cystatin C levels progressively increased in association with the number of metabolic syndrome component disorders coexistent within an individual.\(^7\)

Over the last few years, studies have focused on the association of Cystatin C with cardiovascular events and mortality in patients with coronary artery disease.\(^5\) Some formulas have been tried for example the CKD EPI Cystatin C and CKD EPI Cystatin C-creatinine equations did not improve the accuracy or predictive ability for cardiovascular disease mortality compared to CKD EPI creatinine equation in a cohort of elderly women.\(^5\)

Cystatin C is an endogenous anti-angiogenic factor recently considered as an emerging biomarker in cardiovascular disease and an important predictor for adverse outcomes among patients with coronary heart disease.\(^5\) Ix et al. have shown that increased Cystatin C levels are associated with all-cause mortality, cardiovascular events, and incident heart failure in patients with stable coronary artery diseases.\(^5\) It has been suggested that high Cystatin C concentrations are directly related to both inflammation and atherosclerosis, and that inflammation may be one of the mechanisms associated with Cystatin C and cardiovascular risk given that inflammatory cytokines stimulate the production of lysosomal cathepsins, which degrade extracellular matrix proteins; and concentration of Cystatin C, a cathepsin inhibitor, is elevated to counterbalance this elastolytic overactivity.\(^5\)

Serum Cystatin C, but not neutrophil gelatinase-associated lipocalin levels, could predict the severity of coronary artery disease in di-
abetic patients. It reflects angiographic coronary collateralization in patients with stable coronary artery disease, and 0.97 mg/L of Cystatin C indicates a great risk of poor coronary collaterals.

Carotid intima-media thickening has been widely used as a biomarker for early detection of cardiovascular disease; Cystatin C was more strongly associated with carotid thickening and plaque than other measures of kidney function.

A significant association was found between the state of presence of coronary artery disease and serum Cystatin C values, age, gender, glomerular filtration rate CKD-EPI creatinine, CKD-EPI Cystatin C and CKD-EPI Cystatin C-creatinine equations. After the adjustment of covariates, patients with male gender, older age, and higher CKD-EPI Cystatin C, measurements were more likely to be associated with the diagnosis of coronary artery disease.

Cystatin C is an independent predictor for long-term mortality and major adverse cardiac events of acute coronary syndrome octogenarians with diabetes mellitus. The relationship between Cystatin C and cardiac prognosis appears to be either directly or indirectly due to hemodynamic effects.

Mortality and major adverse cardiac events of acute coronary syndrome octogenarians with diabetes mellitus were higher than those of acute coronary syndrome octogenarians without diabetes mellitus. Serum Cystatin C level was an accurate biomarker for predicting the long term prognosis for acute coronary syndrome octogenarians with diabetes mellitus.

Cystatin C was significantly lower in people with chronic exposure to noise. Serum Cystatin C level is affected in some diseases such as abdominal aortic aneurysm which involve arterial wall extracellular matrix. Several previous studies demonstrated that serum Cystatin C level is decreased in abdominal aortic aneurysm.

Cystatin C is a known biomarker reflecting renal function and is considered a cardiac prognostic factor, particularly in ischemic heart disease and chronic heart failure. This marker is well suited for acute heart failure in the emergency room and has been widely investigated.

The prognosis of acute heart failure can be determined by cardio-renal function which is assessed by Cystatin C. Cystatin C was a better prognostic biomarker for cardiac events two years after acute heart failure, compared with uric acid and N-terminal pro-B-type natriuretic peptide.

Cystatin C was an independent risk factor for cardio-renal prognosis and its hazard ratio was persistently steady throughout the follow-up duration of two years. Cystatin C could provide more useful information for risk-stratification than uric acid and N-terminal pro-B-type natriuretic peptide particularly in acute heart failure.

In patients with chronic respiratory diseases N-terminal pro-B-type natriuretic peptide has predictive value in terms of mortality whereas Cystatin C alone does not. When used together, Cystatin C adds complementary information to the data provided by N-terminal pro-B-type natriuretic peptide alone; contributing valuable information to the early identification of patients at risk of developing clinical ventricular dysfunction. This combined biomarker analysis could be an alternative for identifying patients at increased risk, even though they only have slightly impaired cardiac or renal function but may be said to have cardio-renal syndrome.
6. Neurodegenerative disease

Growing evidence shows that cerebrospinal fluid Cystatin C levels are one of the indicators for the evaluation of the condition of cysteine protease system in various neurologic diseases, as it has wide distribution and is localized to both neurons and glia\textsuperscript{13,64}.

Cystatin C is being considered as a marker, which can be used in the diagnosis of neurodegenerative diseases\textsuperscript{65}. Biochemical markers of neurodegenerative diseases may both contribute to the diagnostics and can be used for the monitoring of treatment effects\textsuperscript{65}.

Previous studies, we have demonstrated that Cystatin C levels in the cerebrospinal fluid were decreased in patients with sporadic cerebral amyloid angiopathy, leptomeningeal metastasis and neurologic inflammatory diseases, such as multiple sclerosis and Guillain–Barre’ syndrome\textsuperscript{64}.

In patients with cerebral amyloid angiopathy amyloid b protein and Cystatin C were co-deposited in the vessels of leptomeninges and cortical surface\textsuperscript{64}.

The only well-established human neurological disease in which involvement of Cystatin C is proven is hereditary cerebral hemorrhage with amyloidosis in Iceland\textsuperscript{64}. Abnormally low Cystatin C levels are described in the Icelandic variant of hereditary cerebellar hemorrhage with amyloidosis and progressive dementia\textsuperscript{65}. A truncated form of Cystatin C, with a deletion of the first 10 amino-terminal residues, this leads to amyloid deposition in the cerebral microvessels and most patients die from recurrent brain hemorrhage before 40 years of age\textsuperscript{64,65}. Two variations in the Cystatin C coding sequence have been associated with this disease. A Leu68Gln (L68Q, also identified as L94Q according to amino acid numbering which includes the typical signal sequence) mutation\textsuperscript{11}.

Recently, the Cystatin C gene has been considered as a candidate gene for Alzheimer’s Disease\textsuperscript{13}. Cystatin C colocalizes with the A peptide in brain amyloid deposits in patients with Alzheimer’s Disease, in senile plaque, and in vessel walls\textsuperscript{13}. Ala25Thr (A25T) polymorphism has been associated with both autosomal recessive late-onset Alzheimer’s disease as well as exudative age-related macular degeneration. Previous studies suggest the A25T polymorphism does not cause a significant reduction in Cystatin C secretion, but instead predisposes the protein to be cleaved at an alternative signal sequence cleavage site if site 2 is hindered\textsuperscript{11}. Many case–control studies have been performed to assess the associations between Cystatin C gene G73A polymorphism and Alzheimer’s Disease\textsuperscript{13}. The G73A polymorphism was thought to be associated with an increased risk for Alzheimer’s Disease susceptibility\textsuperscript{13}. The results suggested that Cystatin C G73A polymorphism might contribute to individual susceptibility to Alzheimer’s Disease in Caucasians. But evidence was not enough to confirm this association in Asians\textsuperscript{13}.

Genetic elevation of serum Cystatin C is not related to altered risk of Alzheimer Disease, suggesting that there is no causal relationship between plasma Cystatin C and altered risk of Alzheimer Disease\textsuperscript{2}.

It seems that very low concentrations of Cystatin C, both in the cerebrospinal fluid and serum of Alzheimer’s Disease patients, are caused by its accumulation in the reactive astrocytes before amyloid formation\textsuperscript{65}.

ROC analysis showed at least one tie between Alzheimer’s Disease and neurodegenerative diseases by cerebrospinal fluid Cystatin C\textsuperscript{65}.

A high level of Cystatin C just before Alzheimer Disease onset occurs as result of co-variation with cerebro-vascular disease risk factors and impaired renal function\textsuperscript{2}.
Polymorphism of Cystatin C gene (a polymorphism of a gene for Cystatin C is associated with higher risk of Alzheimer’s Disease) and decreased serum Cystatin C level were found in some of the Alzheimer’s Disease patients. A role for Cystatin C in the pathogenesis of Alzheimer’s disease has been suggested by the genetic linkage of a Cystatin C gene polymorphism with late-onset Alzheimer’s Disease. Decreases in Cystatin C concentration caused by the Cystatin C polymorphism or by specific presenilin 2 mutations can lead to the development of the disease.

Cystatin C also participates in the process of neuronal degeneration and repairation of the central nervous system.

There is also evidence that Cystatin C has anti-amyloidogenic properties as co-incubation of Cystatin C with monomeric Aβ 42 attenuates the formation of Aβ oligomers and protofibrils in vitro, and increased expression of Cystatin C has been shown to reduce parenchymal Aβ load in mouse models of Alzheimer Disease. In addition, Alzheimer Disease patients may have reduced cerebro-spinal fluid Cystatin C levels, and it has been suggested that a deficient Aβ binding capacity in cerebro-spinal fluid may contribute to the amyloidogenic process in Alzheimer Disease. Kaeser at al. also found overexpression of human Cystatin C in the brains of amyloid beta precursor protein transgenic mice reduced the cerebral amyloid-beta deposition. According this study, Cystatin C also binds amyloid-beta and inhibits its fibril formation. Cystatin C is able to reduce Amyloid-β40 secretion in human brain microvascular endothelial cells and facilitates soluble amyloid protein precursor α secretion. The inhibition of Aβ40 secretion is caused by the Cystatin C-induced degradation of β-site amyloid protein precursor cleaving enzyme-1 through the ubiquitin/proteasome pathway. Exogenously applied Cystatin C can direct amyloidogenic amyloid protein precursor processing to non-amyloidogenic pathway in brain endothelial cells, due to increased expression of a disintegrin and metalloprotease 10 mediated by silent information regulator 1.

Cystatin C is able to shift the amyloidogenic amyloid protein precursor processing to non-amyloidogenic pathway, causing reduced Amyloid-β40 and increased amyloid protein precursor α secretion in brain endothelial cells.

Upregulation of Cystatin C is possibly one of the self-defense responses during neuroinflammation or neurodegeneration to inhibit protease release from lysosomes, where Cystatin C localizes.

Cystatin C in amyloid protein precursor processing suggests a potential therapeutic application in Alzheimer Disease.

Parkinson’s disease is a degenerative disorder of the central nervous system with slow progression, which often occurs in the elderly and is incurable. The β-amyloid 1-42 (Aβ1-42) is a biomarker for Parkinson’s disease, which induces the degeneration of the dopamine neurons and changes the morphology of DA neurons in a dose-dependent manner. A study revealed that serum Aβ1-42 and Uric Acid levels were significantly lower while the serum Cystatin C level was significantly higher in Parkinson’s disease patients than in the healthy controls. The serum Aβ1-42, Cystatin C and uric acid may be related to the pathogenesis of Parkinson’s disease and be potential markers for Parkinson’s disease diagnosis, particularly, serum Cystatin C level may be valuable for assessing Parkinson’s disease severity.
Amyotrophic lateral sclerosis is a fatal neurologic disease characterized by progressive motor neuron degeneration \(^70\). Several abnormalities of skin have been described in these patients, called Bunina bodies, which are small eosinophilic intraneuronal inclusions in the remaining lower motor neurons; they are the only pathologically specific hallmark of amyotrophic lateral sclerosis \(^71\).

Cystatin C has recently gained interest as a candidate diagnostic biomarker for amyotrophic lateral sclerosis, but further studies are required to fully characterize its biomarker utility \(^70\).

Histopathologically Cystatin C is one of only two known proteins that localize to Bunina bodies \(^70\). The epidermis of amyotrophic lateral sclerosis was immunohistochemically strongly positive for Cystatin C as compared with that of controls \(^71\). Data suggest that a metabolic alteration of Cystatin C may take place in the skin of amyotrophic lateral sclerosis and the increased Cystatin C in skin is likely to be related to the disease process in amyotrophic lateral sclerosis \(^71\). Correlations were also found between cerebrospinal fluid Cystatin C levels to both amyotrophic lateral sclerosis disease progression and patient survival \(^70\).

7. Cerebrovascular disease

Cystatin C concentration in the cerebrospinal fluid is 5.5-fold of that in serum. In the healthy brain, Cystatin C is stably localized in the neurons, leptomeningeal cells, glial cells and choroid plexus. It has been hypothesized that Cystatin C has multiple functions and contributes to the neurogenesis and neuronal degeneration \(^64\).

Positive immunoreactivity of Cystatin C was found in all the cell types, such as neuron, astrocytes, oligodendrocytes and microglia in primary human neural cell cultures \(^64\).

Chronic renal insufficiency, diagnosed using CKD EPI creatinine or microalbuminuria, has been associated with the presence of cerebral microbleeds. Both of these organs have vascular beds are exposed to high pulsatile pressure because of upstream vasodilation \(^72\).

They are passively perfused at a high flow rate throughout systolic and diastolic periods, and by contrast, their smallest arteries have low resistance \(^72\). They are exposed to high shear stress and are susceptible to hypertensive insults and variations in blood pressure \(^72\). These effects might partially contribute to the occurrence of cerebral microbleeds in renal insufficiency \(^72\).

Considering the similar pathomechanisms in small vessel diseases of the brain and kidney, Cystatin C, a more reliable marker of renal function, might represent the degree of severity of cerebral microbleeds more accurately \(^72\).

Patients with a proteinuria grade of one or more had at least a twofold increased risk of having cerebral microbleeds compared to patients with trace proteinuria or none \(^72\).

Cystatin C has been shown to be a more sensitive renal function indicator and a strong predictor of cardiovascular events than conventional renal markers \(^72\) \(^73\).

Serum Cystatin C is highly associated with acute ischemic stroke and is an independent prediction marker for acute ischemic stroke \(^73\). Given its association with the severity of cerebral microbleeds, Cystatin C levels could help stratify the risk for intracranial hemorrhage more accurately \(^72\).

Serum Cystatin C level in patients with acute ischemic stroke were significantly higher than that in controls; with its values decreasing after one-week therapy \(^73\). This was consistent with Cystatin C expression after ischemia/reperfusion injury in a mouse focal ischemia/reperfusion injury model \(^73\).
Multivariate logistic regression analyses showed that elevated Cystatin C is an independent risk factor of acute ischemic stroke \textsuperscript{73}. Serum Cystatin C was not associated with either neurological deficits or the location of ischemic area \textsuperscript{73}.

Exogenous Cystatin C exerted neuroprotective effects by reducing infarct volume in an animal stroke model \textsuperscript{73}.

In white matter lesions brain sections, Cystatin C expression was only detected in astrocytes \textsuperscript{64}. Astrocytes seem to be the main source of secreted Cystatin C in the Central Nervous System and fewer astrocytes in white matter lesions leads to low concentrations of Cystatin C in the cerebrospinal fluid \textsuperscript{64}.

In the diseased state of animal brains, Cystatin C protein expression increases in the hippocampal neurons after the events of stroke / ischemia, epileptic episodes and rat facial nerve axotomy \textsuperscript{64}.

Cystatin C levels in the cerebrospinal fluid were significantly decreased in the patients with white matter lesions \textsuperscript{64}. Immunohistochemical studies showed that the Cystatin C immunoreactivity was found in astrocytes, and the number of astrocytes in the white matter in the severe white matter lesions group was decreased when compared with that in controls and in the mild white matter lesions group \textsuperscript{64}. There was decrease a in the number of astrocytes in the severe white matter lesions, although rarefaction of white matter is common \textsuperscript{64}. Low levels of cerebrospinal fluid Cystatin C in ischemic white matter lesions might be due to the decreased number of astrocytes that secrete Cystatin C in response to the stimuli of proteases and inflammatory cytokines \textsuperscript{64}.

A study in transient forebrain ischemia in rats indicated that Cystatin C immunoreactivity was localized in morphologically degenerative neurons in the hippocampus suggesting that Cystatin C is involved in cell survival in vivo \textsuperscript{64}. Thrombin treatment dramatically increased the expression and secretion of Cystatin C in astrocyte cultures dose dependently and that thrombin in high doses induced cell death \textsuperscript{64}.

Cerebral white matter hyperintensities are central Magnetic Resonance Imaging (MRI) markers of the brain aging process, but the mechanisms for its progression remain unclear. Serum Cystatin C level correlates with white matter hyperintensities severity \textsuperscript{74}.

Serum Cystatin C level is independently associated with the long-term progression rate of the cerebral white matter hyperintensities volume \textsuperscript{74}.

Serum Cystatin C level might predict the progression of cerebral white matter hyperintensities \textsuperscript{74}.

Serum Cystatin C level might also reflect the functional status of cerebral penetrating arterioles and the activity of neuronal degeneration process, as Cystatin C is highly secreted from neurons and glial cells, deposited in brain parenchyma and the walls of micro vessels, and its accumulation might induce further neuronal and vascular degeneration \textsuperscript{74}.

Serum Cystatin C level is independently associated with the long-term progression rate of the cerebral white matter hyperintensities volume and might predict the progression of cerebral white matter hyperintensities \textsuperscript{74}.

Cystatin C demonstrated protection on ischemic brain injury making it a novel and promising therapeutic target for acute ischemic stroke \textsuperscript{73}.

8. Neoplasia

Cysteine protease cathepsins are important in extracellular matrix protein degradation, cell
migration, cell differentiation, cell proliferation, cell apoptosis, angiogenesis, inflammation and cancer; and as well as a number of anti-bacterial, anti-viral and anti-amyloid processes.  

Mice lacking cathepsins are protected from tumor progression in several animal models, suggesting that the regulation of cathepsin activities controls the growth of various malignant tumors.  

Enhanced cathepsin expression and activity in Cystatin C-deficient mice contributed to the progression of dysplasia by altering premalignant tissue epithelial proliferation, apoptosis, and neovascularization.  

Cathepsin to Cystatin ratio increases in most tumor types, compared to normal tissue, particularly for advanced cancers.  

Nearly 50% of cancers show decreased expression of Cystatin C; however, tumor Cystatin levels vary widely.  

The relative ratio of Cystatin C to cathepsins in tumor tissues in situ, and not solely the proteases or inhibitors or those in the circulation, are essential in regulating tumor growth; disturbances in the balance of proteases/inhibitors play the important role in tumor growth and metastasizing processes.  

According to recent studies, some Cysteine and aspartate proteases are directly involved in the progression and metastasis of colon and ovarian cancer.  

Increased Cystatin C level has been found in the serum of patients with several inflammatory diseases, such as colorectal tumors with metastases. During colorectal cancer progression, Cystatin C remained unchanged between stages.  

Serum Cystatin C levels are significantly higher in patients with ovarian cancers than in benign ovarian tumors or in healthy women.  

Downregulation of Cystatin C is frequently reported in breast, prostate, stomach, uterus, and non-small cell lung cancer tissues, but reports are controversial in other cancers. Cystatin C was suggested as a diagnostics tool for of early-stage and inflammatory breast cancer. Mean IL-6 and serum Cystatin C concentrations were significantly increased in breast cancer patients compared to healthy subjects.  

In breast cancer patients no correlation was highlighted between IL-6 and Cystatin C or between these molecules and some clinicobiological parameters of malignant progression.  

Serum Cystatin C levels predict renal function in patients with prostate neoplasia but were not a biomarker for the development of prostate neoplasia and were not correlated with the clinicopathological characteristics of prostate cancer.  

Cystatin C resulted significantly higher in prostate cancer but not in benign prostatic hyperplasia patients as compared to healthy subjects. Cystatin C levels remained high at early stages and decreased at late stages.  

Cystatin C level is rarely detected or reduced in tumor tissues of renal carcinoma patients comparing that in normal tissues.  

Cystatin C expression in cancer tissues indicates that Cystatin C expression is primarily suppressed by tumorigenesis and decreased Cystatin C level may induce tumor formation, invasion and metastasis.  

Peritoneal macrophages from mice with experimental HA-1 hepatoma secreted ascitic fluid compared to those from intact mice secreted more Cystatin C with maximum polysaccha-
ride-stimulated secretion after 30 min of incubation. LDL and HDL induced Cystatin C secretion by tumor macrophages also. Serum Cystatin C may reflect tumor burden in diffuse large B-cell lymphoma.

Serum Cystatin C levels were significantly more elevated in diffuse large B-cell lymphoma patients than in controls. They were significantly reduced to normal range after the remission.

High serum Cystatin C levels were correlated with age over 60 years, extra-nodal involvement and with high serum lactate dehydrogenase.

It was detected that patients with non-Hodgkin lymphoma had significantly greater Cystatin C levels compared to healthy controls; the same was detected in patients with relapse of the disease when compared to patients without relapse.

Cystatin C, the most important, abundant, and widely expressed cathepsin inhibitor in humans and animals, decreased with concomitant increase of cathepsin B in high-grade gliomas.

Cystatin C was shown to be implicated in the invasiveness of human glioblastoma cells and as a result, sense transcripts of Cystatin C may prove useful in cancer therapy. However, in some hemoblastoses (lymphoma, lymphogranulomatosis) opposite data were obtained: increased serum Cystatin C level was revealed, and decreased Cystatin C concentration in acute leukosis.

In patients with head and neck squamous-cell carcinoma; serum Cystatin C levels in these patients are higher than those from control groups.

When melanoma cells were injected intravenously, Cystatin C deficient lungs showed reduced metastasis, but subcutaneous growth of melanoma cells was not different from that in control mice.

Cystatin C overexpression was associated with decreased glioblastoma cell invasion in vitro and tumor growth in vivo; caused by higher apoptosis in vivo for metastatic melanoma cells, and dramatically blocked lung metastasis of human fibrosarcoma cells in mice.

Eye tear Cystatin C and lactoferrin level, increased in malignant and benign eye tumors, seems to be a perspective for diagnostics in these disorders.

However, it is impossible to differentiate choroidal melanoma and benign eye tumors according to the level of Cystatin C and lactoferrin in eye tear fluids.

9. Pregnancy

When plasma concentration of Cystatin C was determined in the last trimester of uncomplicated pregnancy, it was noted that the level was increased in contrast to the creatinine level, which was decreased. The uteroplacental unit does not contribute significantly to the maternal levels of Cystatin C in normal pregnancy, and the proteins are not likely to be transferred across the placental barrier.

High activity of cathepsin B and increased level of Cystatin C are typical for women in late pregnancy. Those levels significantly decrease after delivery which can be associated with potential role of those markers in placental separation. Serum Cystatin C levels were not found to be decreased in term pregnancy.

The insignificant changes of Cystatin C level in the peripartum period seemed to exclude the possibility of using Cystatin C as a marker for renal insufficiency in the peripartum period but nevertheless, serum Cystatin C seems to
reflect glomerular filtration rate reliably in both non-pregnant and pregnant, healthy and hypertensive women. Both serum Cystatin C and creatinine levels were significantly related to glomerular filtration rate for both pregnant and non-pregnant women. However, the correlation between Cystatin C and glomerular filtration rate was set at different levels for pregnant and nonpregnant women. This indicates a physiological difference between the filtration processes in kidneys of pregnant and non-pregnant women, whether it is size-dependent, configuration-dependent or charge-dependent.

Prior studies have shown increased Cystatin C levels in preeclampsia. Pregnancy increases plasma Cystatin C, but levels are much higher in preeclampsia. Preeclampsia manifests by proteinuria, hypertension, and impaired renal function. Assessment of renal function is important in the evaluation of the pregnant hypertensive patient. Serum Cystatin C seems to reflect glomerular filtration rate reliably in hypertensive pregnant women and avoids the inaccuracy associated with the 24-hour urine collection, which is time consuming and subject to improper collection. To determine the cutoff point of Cystatin C for the detection of renal impairment in hypertensive pregnancies. The cutoff point in detecting renal impairment in hypertensive pregnancies showed to be better for second trimester than for third trimester since it maximizes the value of sensitivity and specificity.

A case-control study of 100 preeclampsia cases and 100 random pregnancies uncomplicated by hypertension were analyzed. At delivery plasma Cystatin C was measured. Adjusted odds ratios and 95% confidence intervals of preeclampsia by quartiles of maternal plasma Cystatin C were estimated using multivariable logistic regression models. Mean Cystatin C levels were elevated in preeclampsia cases compared with controls.

To test utility of Cystatin-C as a marker of glomerular filtration rate during pregnancy, Saxena et al. performed serial correlations with inulin clearance during pregnancy and postpartum with serum Cystatin-C but it did not correlate with inulin clearance during pregnancy or postpartum.

Cystatin C is considered a more sensitive measure for estimating the glomerular filtration rate than serum creatinine, it is superior to creatinine as a marker of kidney function, risk factor of cardiovascular disease and diabetes retinopathy as well. Cystatin C has recently been shown to be associated with the incidence of type 2 diabetes mellitus and the progression to the pre-diabetic state. Several studies have also found the Cystatin C level also to be correlated with different components of the metabolic syndrome. Previous studies have demonstrated that serum Cystatin C is a strong marker for lower limb ischemia, diabetic retinopathy and diabetic peripheral neuropathy in Chinese patients with type 2 diabetes mellitus. It is considered as a useful index in the screening of diabetic micro- and macro-vascular complications.

Yousefzadeh et al. reported that Cystatin C could be a reliable, useful and promising marker of gestational diabetes mellitus. Hong Liu et al. also revealed that Cystatin C was correlated with gestational diabetes mellitus but not with the fetal outcome in gestational diabetes mellitus women. Serum Cystatin C is significantly and independently associated with insulin resistance and gestational diabetes mellitus. It may be a helpful biomarker to identify the risk of gestational diabetes mellitus in pregnant women.

Cystatin C is considered to be a high-sensitivity marker of renal insufficiency and
can also be useful in pregnancy complicated by gestational diabetes mellitus or preeclampsia. 

10. Liver

Serum Cystatin C has been proposed as a novel biomarker of the renal function. Several studies have reported its value in different sets of patients. The assessment of renal function is of vital importance in management of patients with cirrhosis.

Some studies have postulated that serum creatinine based estimated glomerular filtration rate does not reflect true renal function because of muscle wasting and impaired liver function. By contrast, Cystatin C is mostly unrelated to muscle volume and liver function giving a better reflection of renal function.

Some studies have shown that malignant conditions and liver disorders can bring about alterations in serum Cystatin C level. Mice that have hepatocellular carcinoma induced produce ascitic fluid rich in peritoneal macrophages that secrete Cystatin C even more intensely than those of intact mice.

In patients with cirrhosis, Cystatin C based equations are more accurate indicators of glomerular filtration rate, showing the highest significant correlation to glomerular filtration rate than creatinine based equations.

Serum Cystatin C and Cystatin C formulae were not only the best measures that reflected the actual renal performance in cirrhotic patients, but also the most accurate ones in detecting early stages of renal impairment in these patients.

Accumulating evidence has suggested that Cystatin C-based estimation of glomerular filtration rate may play a promising role in assessing the renal function in patients with cirrhosis or predicting the outcome in cirrhotic patients with ascites or hepatorenal syndrome. These results suggest that estimated glomerular filtration rate by Cystatin C could estimate renal function and predict outcome more accurately compared with creatinine-based estimated glomerular filtration rate in cirrhotic patients.

In Japanese population, Cystatin C-based glomerular filtration rate estimating formulas, are the best available option for estimating glomerular filtration rate and predicting survival in Japanese cirrhotic patients.

In liver transplant assessing pre-transplant renal function with CKD-EPI Cystatin C-creatinine equations for their predictive value on long-term survival after liver transplantation turned up that renal function before liver transplantation impacts survival after liver transplantation. But this estimated pre-liver transplantation renal function is predictive of post- liver transplantation survival only when assessed using the chronic kidney disease-epidemiology Cystatin C equation.

Hepatitis B virus-related acute-on-chronic liver failure is a severe liver disease which results in a high mortality in China. To early predict the prognosis of the patients may prevent the complications and improve the survival. A new prognostic index combining serum Cystatin C with total bilirubin early predicted the short-term mortality of these patients.

Previous studies have shown that Cystatin C levels significantly correlated with the model for end-stage liver disease scoring system, was commonly applied as a prognostic indicator in patients with acute-on-chronic liver failure; suggesting that it should be a good marker for assessing the prognosis in these patients. Results showed that the prognostic index combining serum Cystatin C with total bilirubin was a good indicator for early predicting the short-term mortality in patients.
with hepatitis B virus-related acute-on-chronic liver failure patients.

**11. Prostate**

In prostate, Cystatin C has been used to evaluate the influence of perioperatively administered hydroxyethyl starch on glomerular filtration rate determined by sequential Cystatin C measurements in a general patient population undergoing open radical prostatectomy or robot-assisted radical prostatectomy. Hydroxyethyl starch is used for repletion of acute intravasal volume loss in surgical patients. However, in critically ill patients, it’s associated with acute kidney injury. The results indicated that the administration of a median dose was not associated with a postoperative deterioration of renal function in patients with normal to near-normal baseline renal function undergoing radical prostatectomy.

To evaluate the role of Cystatin C in tumorigenesis and progression of prostate cancer, the clinical information from the records of benign prostatic hyperplasia, prostatic intraepithelial neoplasia, and prostate cancer patients, whose disease was newly diagnosed and histologically confirmed, were retrospectively collected.

Pretreatment serum Cystatin C levels were compared across the various groups and then analyzed to identify relationships, if any, with clinical and pathological characteristics of the prostate cancer patient group. There were no significant differences in serum Cystatin C levels among the three groups. Age and serum creatinine contributed in part to the variations in serum Cystatin C levels of prostate cancer patients. Serum Cystatin C levels predicted renal function in patients with prostate neoplasia but were not a biomarker for the development of prostate neoplasia and were not correlated with the clinicopathological characteristics of prostate cancer.

Nearly 50% of cancers show decreased expression of Cystatin C. In prostate cancers, Cystatin C levels remained high at early stages and decreased at late stages. Cystatins are endogenous inhibitors of cysteine cathepsins. Enhanced cathepsin expression and activity in Cystatin C deficient mice contributed to the progression of dysplasia by altering premalignant tissue epithelial proliferation, apoptosis, and neovascularization. Cysteine protease cathepsins are important in extracellular matrix protein degradation, cell apoptosis, and angiogenesis. Mice lacking cathepsins are protected from tumor progression in several animal models, suggesting that the regulation of cathepsin activities controls the growth of various malignant tumors.

It was previously shown that the concentration of Cystatin C in seminal plasma is higher than in any other body fluid, and that Cystatin C is widely expressed in the male reproductive system, suggesting that the presence of this potent protease inhibitor might be significantly important in the regulation of normal, physiological proteolysis, as well as in inflammatory diseases and malignancies in the male genital tract. Neuroendocrine cells in a variety of human tissues, including the prostate, have Cystatin C immunoreactivity, suggesting that the inhibitor might be involved in the intracellular regulation of the peptide hormones. Neuroendocrine differentiation occurs in most prostatic adenocarcinomas, and has been correlated with poor prognosis, tumor progression and resistance to hormonal therapy.

There were more strongly Cystatin C-positive neuroendocrine-like cells in prostate cancer than in benign prostatic tissue suggesting a connection between Cystatin C and neuroendocrine...
Endocrine differentiation in prostate cancer progression\textsuperscript{7}.

The clinical significance of serum interleukin-6 (IL-6) and its correlation with Cystatin C, was investigated by immunoassays in patients with bone metastasis from breast cancer or prostate cancer\textsuperscript{77}. Mean IL-6 levels were higher in prostate cancer patients and in patients with benign prostatic hyperplasia than in healthy subjects while Cystatin C resulted significantly higher in prostate cancer but not in benign prostatic hyperplasia patients as compared to healthy subjects\textsuperscript{77}.

Mean IL-6 levels were higher in prostate cancer patients and in patients with benign prostatic hyperplasia than in healthy subjects while Cystatin C resulted significantly higher in prostate cancer but not in benign prostatic hyperplasia patients as compared to healthy subjects\textsuperscript{77}.

The administration of zoledronic acid, an amniobisphosphonate derivative endowed with anti-osteoclastic and analgesic activity, to patients with bone metastases induced a statistically significant increase of serum IL-6 and Cystatin C only prostate cancer patients with bone metastasis\textsuperscript{77}. These data indicate that IL-6 and Cystatin C may be regarded as novel targets for cancer treatment and as markers of increased osteoblastic activity associated to bisphosphonate treatments in prostate cancer patients with bone metastases\textsuperscript{77}.

12. Bone

Osteoporosis, a skeletal condition causing an increased risk of fractures, is becoming more prevalent due to the recent global expansion of the aging population\textsuperscript{92}. Studies have reported that renal function decline is associated with loss of bone mass and increased fracture risks in chronic kidney disease patients\textsuperscript{93 16}. It’s suggested that a decline of renal function assessed by the CKD EPI Cystatin C equation might be a useful tool to detect osteopenia\textsuperscript{16 92 93}. Furthermore, a recent study also proposed Cystatin C as a predictor of hip fracture risk in elderly female patients\textsuperscript{93}. Osteoporosis and osteoporotic fractures are serious public health threats resulting in high morbidity and mortality\textsuperscript{92}. Osteoporosis is diagnosed by measuring bone mineral density, which is the best predictor of primary osteoporotic fractures\textsuperscript{92}. The bone mineral density measurements of 865 Korean adults (325 men and 540 women) who participated in a comprehensive medical examination program found that serum Cystatin C levels might help identify women with osteoporosis who are susceptible to fractures\textsuperscript{92}.

Cystatin C is also a significant predictor of osteoprotegerin (OPG) independently of age, free testosterone index and total 17β-estradiol\textsuperscript{94}. OPG is a soluble decoy receptor, secreted by osteoblasts and other cell types, it competitively binds RANKL. RANKL is expressed primarily by osteoblasts and its receptor RANK is expressed in preosteoclasts and other cells of this lineage\textsuperscript{94}. When RANKL binds to RANK, osteoclastogenesis and bone resorption are induced\textsuperscript{94}. Thus, bone resorption may be prevented by OPG binding to RANKL\textsuperscript{94}.

Osteoclastic bone resorption and differentiation depends on the activity of various proteolytic enzymes, in particular those belonging to the group of cysteine proteinases\textsuperscript{95}. Cystatin C is a natural cysteine proteinase inhibitor\textsuperscript{96}. Biochemical studies have shown that Cystatins inhibit bone matrix degradation by decreasing the formation of osteoclasts by interfering at a late stage of pre-osteoclast differentiation\textsuperscript{94 95}. By measuring the Ca-release in calvarial bone explants following a 24 hour incubation in the presence of both egg white Cystatin and human Cystatin C decreased calcium release into the medium significantly, 41% of the cells were adjacent to areas of demineralized non-degraded bone matrix, whereas in controls, this was only 6%\textsuperscript{95}. Microscopic analyses of the bone explants demonstrated that in the presence of Cystatin C inhibitor, a high percentage of osteoclasts was
associated with demineralized non-degraded bone matrix. This provides evidence that Cystatins reversibly inhibit bone matrix degradation in the resorption lacunae adjacent to osteoclasts; suggesting the involvement of Cystatins in the modulation of osteoclastic bone degradation.

Bone marrow, calvaria and ex vivo calvarial in vitro cultures were examined for the effects of Cystatin C on mouse osteoblastic cells. Cystatin C-stimulated cells showed increased alkaline phosphatase activity, mineralization of the new bone matrix, and calvarial bone formation. The cells treated with Cystatin C immunodepleted by anti-Cystatin C antibody and E-64, a chemical papain-like cysteine proteinase inhibitor, did not induce mineralization. Elevated mRNA levels of the differentiation marker osteocalcin, bone morphogenetic protein (BMP)-2, and a master osteogenic transcription factor, Runx2, were observed in Cystatin C-treated cells. These results suggested that Cystatin C affects the BMP signaling cascades in osteoblastic cells and then promotes osteoblast differentiation, mineralization, and bone formation.

13. Teeth

Recent studies have suggested that the use of CKD EPI Cystatin C had a stronger and more linear association with periodontitis than CKD EPI creatinine in 502 elderly Japanese women.

Periodontitis is a disease of the periodontium characterized by loss of attachment and supporting alveolar bone. Destructive process in periodontitis is caused in part by an imbalance of the homeostasis between degradative enzymes such as MMPs and their inhibitors, the Tissue Inhibitors of Metalloproteinases (TIMPs). Degradative enzymes, such as the lysosomal cysteine proteinases, cathepsins, and their inhibitors, Cystatins.

Periodontal pathogens, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans with proinflammatory cytokines when added to gingival fibroblast induced by cyclosporine A, significantly increases the expression of Cystatin C compared with cyclosporine A alone. Cyclosporine A, is an immunosuppressant used to treat transplant patients as well as for rheumatoid arthritis and psoriasis, has gingival overgrowth as a common side-effect because of a fibroblast proliferation and deposition of excess extracellular matrix. The increased ability of protein accumulation by Cystatin C is one of several factors mediating cyclosporine A-induced gingival overgrowth. The Cystatin C staining in gingival tissue was stronger in the cyclosporine A-induced gingival overgrowth group than in the normal gingival group; in the cytoplasm of fibroblasts, epithelial cells and inflammatory cells. The addition of periodontal pathogens and proinflammatory cytokines significantly increased the expression of Cystatin C compared with cyclosporine A alone.

Henskens et al. found significantly increased cysteine proteinase inhibitory activity in whole saliva of 67 gingivitis and 60 periodontitis patients in comparison with 19 healthy subjects. No Cystatin C levels were found in gingival crevicular fluid samples in healthy subjects while increased concentrations of Cystatin C were detected in whole saliva in both gingivitis and periodontitis subjects. Concentration of Cystatin C in gingival crevicular fluid seem to regularly increase from healthy to periodontitis sites. Graziani et al. reported substantial reduction of Cystatin C in serum concentration after non-surgical periodontal therapy. Further there was significant overlap between gingivitis group and the after periodontal therapy group, which can be due to the individual variation in the resolution of periodontitis after treatment. The mean Cystatin C concentration in gin-
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gival crevicular fluid and serum was observed to be the highest in periodontitis group and lowest in periodontally healthy group with intermediate concentration in gingivitis group and after periodontal therapy group. This suggests that Cystatin C increases with disease progression to prevent further periodontal degeneration and decreases after treatment due to bone metabolic homeostasis.

In health clarified human whole saliva contained Cystatin S, whereas Cystatin C was barely detectable. In clarified human whole saliva of gingivitis and periodontitis patients, both Cystatin C and S levels were higher. The origin of Cystatin activity was investigated by collecting submandibular, sublingual, and parotid saliva from seven subjects with mild gingivitis. Total Cystatin activity was about five times higher in submandibular saliva than in parotid saliva. In submandibular and sublingual saliva, both Cystatins S and C were demonstrated. In parotid samples, solely Cystatin C was detectable. The introduction of experimental gingivitis in one periodontally healthy subject resulted in the appearance of a Cystatin C band in parotid saliva and in an increase of Cystatins S and C in submandibular saliva.

Further, when the concentration of Cystatin C in gingival crevicular fluid was compared to that of serum, the concentration of Cystatin C in gingival crevicular fluid was significantly higher than corresponding serum concentration in all the groups. The few samples of gingivitis group showed values nearing that of chronic periodontitis group especially in gingival crevicular fluid, which could be attributed to near conversion of gingivitis lesions to chronic periodontitis lesions that is not clinically detectable. A few of the samples of periodontally healthy group showed values nearing those of gingivitis group which could be due to subclinical levels of inflammation in the clinically healthy tissues.

Studies show increased Cystatin C level in gingival crevicular fluid indicates amplification of osteoclastic activity signals and so Cystatin C increase for counterbalance these pathways in the inflamed periodontium as in gingivitis and periodontitis. This suggests that Cystatin C acts as anti-inflammatory marker in gingival crevicular fluid and serum. Thus, in future Cystatin C and recombinant Cystatin C may have important implications in the design of novel therapies for periodontal disease.

14. Conclusion

A growing body of evidence demonstrates the clinical relevance of Cystatin C in today's world. The selected groups of patients and diseases in which the measurement of Cystatin C is recommended for their diagnosis and future potential treatment is ever-growing.

From renal function to odontological applications, from its use in pediatric and elderly population, from pharmacological to prognostic applications, the evaluation and definition of the role of Cystatin C in clinical practice as a biochemical marker is still has a long way to go.
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