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REVIEW ARTICLE

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”^{1 2}. 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^{1 2 3 4}. 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^{1 2 3 5}. Cystatin C has an isoelectric point of 9.3 and it is positively charged in all human biological fluids examined^{1 6}.

There are three major types of inhibitors in the Cystatin protein superfamily. Stefins or human type 1 Cystatins which are mainly intracellular inhibitors includes Cystatins A and B; although low levels of these inhibitors are also found in some body fluids^{7 8}. The human type 2 are extracellular Cystatins; these constitutes the largest group of inhibitors, Cystatins C, D, E/M, F, G, S, SN and SA; they are synthesized with signal peptides for secretion^{7 8}. The human type 3 group Cystatins comprise L- and H-kininogen, which together with Cystatin C are the most important plasma inhibitors of cysteine proteases⁷.

Among the Cystatin human type 2 superfamily, Cystatin C is the most studied⁸. 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^{1 3 9}.

Cystatin C shows the highest affinity and the fastest inhibition of all Cystatins toward lysosomal cysteine proteases⁷.

The main functions of Cystatins are related to the inhibition of cysteine proteases, cell proliferation, cell migration and cell differentiation⁸. Cystatin C inhibits family C1 cysteine proteases through tight and reversible binding in a substrate-competing mechanism, resulting in an equimolar complex with the protease⁷. 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 cathepsins². Exogenous Cystatin C downregulates the proteolytic activities of family C1 (papain-like) cysteine proteases such as human cathepsins B, H, K, L and S². 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^{3 7}.

The synthesis of Cystatin C does not seem to be tissue-specific and all nucleated cells constitutively express and constantly secrete it³. 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^{7 8 9}.

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 placenta⁵. Increased Cystatin C level has been found in the serum of patients with several inflammatory diseases, such as tumors with metastases⁸. Cystatin C could play a protective and regulatory role under inflammatory conditions³. Previous data suggest that Cystatin C has also been involved in neurodegenerative and repair processes after post-ischemic and mechanical injury⁹. Cystatin C was shown to play a role in cancer development, protein catabolism, regulation of hormone processing and bone resorption, modulating inflammation⁸.

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 functions¹⁹.

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^{3,8}.

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 se-

quence 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 earlier¹¹. Additional N-terminal amino acids resulting from alternative signal sequence cleavage may, in turn, affect the protease inhibition function of Cystatin C¹¹. 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 CA¹². Secretory proteins are first synthesized in the cytosol and co-translationally translocated across the endoplasmic reticulum membrane to enter the secretory system¹¹. 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 receptor¹¹. The nascent polypeptide is extruded into the endoplasmic reticulum through the Sec61 translocon, and in the process, a signal peptidase complex cleaves the signal sequence¹¹. Consistent with its extracellular presence, Cystatin C has been demonstrated to have a 26-residue signal sequence¹¹. The proteases that Cystatin C binds to are typically localized to the lysosome¹¹.

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 2¹³.

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 level¹³.

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 Disease¹⁴. This BB genotype of the Cystatin C encoding gene could also be associated with dementia in Lewy body disease¹⁴.

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 Disease².

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 C¹².

3. Prognostic tool

Cysteine proteinase inhibitors, Cystatins, are involved in mechanisms regulating intracellular and extracellular protein degradation¹⁵.

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 response¹⁵.

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 dysfunction¹⁶.

The aim of this study was to prospectively evaluate, for the first time in the literature, the chronic kidney disease-epidemiology-serum creatinine-Cystatin C formula in newly diagnosed patients with symptomatic multiple myeloma¹⁶.

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 creatinine¹⁶.

Furthermore, the chronic kidney disease-epidemiology-Cystatin C formula independently predicted for survival¹⁶.

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 tool¹⁶.

As a member of cysteine protease inhibitors, Cystatin C also inhibits tumor invasion and metastasis¹⁷.

Cystatin C has also been proposed to have a role associated to the modification of the proteolytic system in cancer¹⁵.

Cystatin C level has been proposed as a prognostic tool proposed for follow up of diseases, efficacy of chemotherapy, and prediction of disease outcome¹⁵.

Elevated serum levels of Cystatin C are found to be related to poor outcome and metastatic potential of some malignant disorders. Two

examples of this are with lung and colorectal cancer¹⁵.

In patients with melanoma, increased Cystatin C serum levels correlated with the highest metastatic stage¹⁵.

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 up¹⁷.

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

The results of multivariate analysis demonstrated that preoperative serum Cystatin C was an independent factor for predicting overall survival of renal cell carcinoma patients¹⁷.

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 resection¹⁷.

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 assay¹⁸.

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 patients¹⁸.

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 hormones⁷.

NE differentiation (NED) occurs in most prostatic adenocarcinomas, and has been correlated with tumor progression, poor prognosis and resistance to hormonal therapy⁷.

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 grades⁷.

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 disease⁷.

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^{19 20 21 22}. Its filtration is unchanged in kidney tubular diseases where its urinary excretion is increased²⁰. 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 tubule²³. 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 C²³. Serum Cystatin C measurement has been described as a means to evaluate glomerular filtration rate²⁴. 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 factors²⁵.

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 status²¹.

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 creatinine²⁴. Cystatin C may substitute, in the clinical laboratory, creatinine clearance measurement given its practicality²⁴.

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 department²⁸. 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 well²⁸.

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 values¹⁹.

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 formula¹⁹.

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 populations¹⁹.

Since Cystatin C is not as influenced as creatinine by endogenous or exogenous agents, it is a better glomerular filtration marker for ≤ 1500 gr preterm infants²⁹. But when in sepsis it was no longer a useful marker of kidney function in neonates⁵.

Children with non-renal solid organ transplants are surviving longer, but outcome is sometimes complicated by Chronic Kidney Disease³⁰. 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 children³⁰

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 injury³¹. It showed best performance characteristics as a marker of acute kidney injury on post-operative days³¹.

In pediatric and adult sickle cell disease patients serum Cystatin C was found to be insufficient for the evaluation of sickle cell disease nephropathy³².

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 HbA1c²². Serum Cystatin C is useful in detecting individuals with chronic kidney disease having mild decrease in glomerular filtration rate compared to Serum Creatinine³³. Serum Cystatin C may be used to screen patients with poorly controlled diabetes mellitus or hypertension when Serum Creatinine level is inconclusive³³. Renal lesions initially occur in the renal tubule and then form in the renal glomerulus of patients with type 2 Diabetes Mellitus³⁴. 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 nephropathy³⁵. Serum Cystatin C was higher in microalbuminuric patients than those who had normoalbuminuria in patients of type 2 diabetes to detect early kidney injury³⁶. Microalbuminuria is the earliest marker for diabetic nephropathy; however, a large proportion of renal impairment occurs in non-albuminuric state³⁶. 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 Mellitus³⁴.

Renal lesions may initially occur in the renal tubule and then form in the renal glomerulus of patients with type 2 Diabetes Mellitus³⁴. 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 Mellitus³⁴. 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 disease³⁷.

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 well³⁸.

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 level²⁵.

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 cisplatin²⁵.

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 creatinine²⁵.

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 formulations³⁹. Previous studies have also used serum Cystatin C levels to evaluate that agma-

tine has a protective effect against cisplatin-induced nephrotoxicity⁴⁰.

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 chemotherapy³⁹.

Acute kidney injury is a frequent complication in critically ill patients and is associated with a high mortality⁴¹. 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 patients⁴¹.

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 individuals⁴². 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 individuals⁴².

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 normal⁴³.

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 marker⁴⁴.

5. Cardiovascular

Menon et al. reported that Cystatin C provided prognostic information beyond information about renal function⁴⁵. Cystatin C was correlated with body mass index (BMI), proteinuria, systolic arterial pressure, and inversely correlated with HDL cholesterol levels⁴⁶. 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 disease⁴⁷. High Cystatin C and retinol-binding protein 4 may contribute significantly to cardiovascular risk burden in addition to traditional cardiovascular markers⁴⁸. 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 group⁴⁸.

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 patients⁵¹.

Arterial stiffness is important in the evaluation of the cardiovascular risk in all patients⁵². Multivariate analysis revealed that serum Cystatin C but not albuminuria was significantly associated with pulse wave velocity in hypertensive patients⁵².

Obstructive sleep apnea syndrome is associated with systemic inflammation and increased risk of cardiovascular and chronic kidney disease⁵³. Serum Cystatin C serum levels are increased in obstructive sleep apnea syndrome patients without comorbidities,

suggesting an increased renal and cardiovascular disease risk⁵³.

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 factors⁵³. 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 hypertension⁵³.

Biomarkers are commonly used to estimate the presence of subclinical cardiovascular disease in patients with essential arterial hypertension⁴⁹.

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 microalbuminuria⁴⁶.

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 disease⁴⁹.

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 alterations⁴⁹.

The accumulation of epicardial adipose tissue is a cardiovascular risk factor independent from visceral adiposity, obesity, hypertension and diabetes⁵⁴. 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 patients⁵⁴. Association between high levels of Cystatin C and increased risk of future development of metabolic syndrome was found¹⁰. In metabolic syndrome previous studies have found, Cystatin C has been found to be associated with severity of coronary artery disease⁵⁵. Cystatin C levels progressively increased in association with the number of metabolic syndrome component disorders coexistent within an individual⁴⁷.

Over the last few years, studies have focused on the association of Cystatin C with cardiovascular events and mortality in patients with coronary artery disease⁵⁶. 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 women⁵⁷.

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 disease⁵⁸⁵⁹. 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 diseases⁵⁶. 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 overactivity⁵⁵⁵⁶.

Serum Cystatin C, but not neutrophil gelatinase-associated lipocalin levels, could predict the severity of coronary artery disease in di-

abetic patients⁵⁵. 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 collaterals⁵⁹.

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 function⁶⁰.

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 equations⁶¹. 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 disease⁶¹.

Cystatin C is an independent predictor for long-term mortality and major adverse cardiac events of acute coronary syndrome octogenarians with diabetes mellitus⁵⁸. The relationship between Cystatin C and cardiac prognosis appears to be either directly or indirectly due to hemodynamic effects⁶².

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 mellitus⁵⁸. Serum Cystatin C level was an accurate biomarker for predicting the long term prognosis for acute coronary syndrome octogenarians with diabetes mellitus⁵⁸.

Cystatin C was significantly lower in people with chronic exposure to noise⁶³. Serum Cystatin C level is affected in some diseases such as abdominal aortic aneurysm which involve

arterial wall extracellular matrix⁶³. Several previous studies demonstrated that serum Cystatin C level is decreased in abdominal aortic aneurysm⁶³.

Cystatin C is a known biomarker reflecting renal function and is considered a cardiac prognostic factor, particularly in ischemic heart disease and chronic heart failure⁶². This marker is well suited for acute heart failure in the emergency room and has been widely investigated⁶².

The prognosis of acute heart failure can be determined by cardio-renal function which is assessed by Cystatin C⁶². 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 peptide⁶².

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 years⁶². 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 failure⁶².

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 not⁵¹. 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 dysfunction⁵¹. 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 syndrome⁴⁵.

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^{13 64}.

Cystatin C is being considered as a marker, which can be used in the diagnosis of neurodegenerative diseases⁶⁵. Biochemical markers of neurodegenerative diseases may both contribute to the diagnostics and can be used for the monitoring of treatment effects⁶⁵.

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' syndrome⁶⁴.

In patients with cerebral amyloid angiopathy amyloid b protein and Cystatin C were co-deposited in the vessels of leptomeninges and cortical surface⁶⁴.

The only well-established human neurological disease in which involvement of Cystatin C is proven is hereditary cerebral hemorrhage with amyloidosis in Iceland⁶⁴. Abnormally low Cystatin C levels are described in the Icelandic variant of hereditary cerebellar hemorrhage with amyloidosis and progressive dementia⁶⁵. 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^{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) mutation¹¹.

Recently, the Cystatin C gene has been considered as a candidate gene for Alzheimer's Disease¹³. Cystatin C colocalizes with the A peptide in brain amyloid deposits in patients with Alzheimer's Disease, in senile plaque, and in vessel walls¹³. 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 hindered¹¹. Many case-control studies have been performed to assess the associations between Cystatin C gene G73A polymorphism and Alzheimer's Disease¹³. The G73A polymorphism was thought to be associated with an increased risk for Alzheimer's Disease susceptibility¹³. 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 Asians¹³.

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 Disease².

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 formation⁶⁵.

ROC analysis showed at least one tie between Alzheimer's Disease and neurodegenerative diseases by cerebrospinal fluid Cystatin C⁶⁵.

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 function².

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 patients⁶⁵. 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 Disease⁶⁶. Decreases in Cystatin C concentration caused by the Cystatin C polymorphism or by specific presenilin 2 mutations can lead to the development of the disease⁶⁶.

Cystatin C also participates in the process of neuronal degeneration and reparation of the central nervous system⁶⁵.

There is also evidence that Cystatin C has anti-amyloidogenic properties as co-incubation of Cystatin C with monomeric A β 42 attenuates the formation of A β oligomers and protofibrils in vitro, and increased expression of Cystatin C has been shown to reduce parenchymal A β load in mouse models of Alzheimer Disease¹⁴. In addition, Alzheimer Disease patients may have reduced cerebro-spinal fluid Cystatin C levels, and it has been suggested that a deficient A β binding capacity in cerebro-spinal fluid may contribute to the amyloidogenic process in Alzheimer Disease¹⁴. Kaeser et 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 to this study, Cystatin C also binds amyloid-beta and inhibits its fibril formation⁶⁵. Cystatin C is able to reduce Amyloid- β 40 secretion in human brain microvascular endothelial cells and facilitates soluble amyloid protein precursor α secretion. The inhibition of A β 40 secretion is caused by the Cystatin C-induced degradation of β -site amyloid protein precursor cleaving enzyme-1 through the ubiquitin/proteasome pathway⁶⁷
⁶⁸.

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 metalloproteinase 10 mediated by silent information regulator 1^{67 68}.

Cystatin C is able to shift the amyloidogenic amyloid protein precursor processing to non-amyloidogenic pathway, causing reduced Amyloid- β 40 and increased amyloid protein precursor α secretion in brain endothelial cells⁶⁷.

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 localizes⁶⁸.

Cystatin C in amyloid protein precursor processing suggests a potential therapeutic application in Alzheimer Disease⁶⁸.

Parkinson's disease is a degenerative disorder of the central nervous system with slow progression, which often occurs in the elderly and is incurable⁶⁹.

The β -amyloid 1-42 (A β 1-42) 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 manner⁶⁹.

A study revealed that serum A β 1-42 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 controls⁶⁹. The serum A β 1-42, 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 severity⁶⁹.

Amyotrophic lateral sclerosis is a fatal neurologic disease characterized by progressive motor neuron degeneration⁷⁰. 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 sclerosis⁷¹.

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 utility⁷⁰.

Histopathologically Cystatin C is one of only two known proteins that localize to Bunina bodies⁷⁰. The epidermis of amyotrophic lateral sclerosis was immunohistochemically strongly positive for Cystatin C as compared with that of controls⁷¹. 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 sclerosis⁷¹. Correlations were also found between cerebrospinal fluid Cystatin C levels to both amyotrophic lateral sclerosis disease progression and patient survival⁷⁰.

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 degeneration⁶⁴.

Positive immunoreactivity of Cystatin C was found in all the cell types, such as neuron, astrocytes, oligodendrocytes and microglia in primary human neural cell cultures⁶⁴.

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 vasodilation⁷².

They are passively perfused at a high flow rate throughout systolic and diastolic periods, and by contrast, their smallest arteries have low resistance⁷². They are exposed to high shear stress and are susceptible to hypertensive insults and variations in blood pressure⁷². These effects might partially contribute to the occurrence of cerebral microbleeds in renal insufficiency⁷².

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 accurately⁷².

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 none⁷².

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 stroke⁷³. Given its association with the severity of cerebral microbleeds, Cystatin C levels could help stratify the risk for intracranial hemorrhage more accurately⁷².

Serum Cystatin C level in patients with acute ischemic stroke were significantly higher than that in controls; with its values decreasing after one-week therapy⁷³. This was consistent with Cystatin C expression after ischemia/reperfusion injury in a mouse focal ischemia/reperfusion injury model⁷³.

Multivariate logistic regression analyses showed that elevated Cystatin C is an independent risk factor of acute ischemic stroke⁷³. Serum Cystatin C was not associated with either neurological deficits or the location of ischemic area⁷³.

Exogenous Cystatin C exerted neuroprotective effects by reducing infarct volume in an animal stroke model⁷³.

In white matter lesions brain sections, Cystatin C expression was only detected in astrocytes⁶⁴. 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 fluid⁶⁴.

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 axotomy⁶⁴.

Cystatin C levels in the cerebrospinal fluid were significantly decreased in the patients with white matter lesions⁶⁴. 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 group⁶⁴. There was decrease a in the number of astrocytes in the severe white matter lesions, although rarefaction of white matter is common⁶⁴. 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 cytokines⁶⁴.

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 vivo⁶⁴. Thrombin treatment dramatically increased the expression and secretion of Cystatin C in astrocyte cultures dose dependently and that thrombin in high doses induced cell death⁶⁴.

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 severity⁷⁴.

Serum Cystatin C level is independently associated with the long-term progression rate of the cerebral white matter hyperintensities volume⁷⁴.

Serum Cystatin C level might predict the progression of cerebral white matter hyperintensities⁷⁴.

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 degeneration⁷⁴.

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 hyperintensities⁷⁴.

Cystatin C demonstrated protection on ischemic brain injury making it a novel and promising therapeutic target for acute ischemic stroke⁷³.

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^{8 11 75}.

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 tumors⁷⁵.

Enhanced cathepsin expression and activity in Cystatin C-deficient mice contributed to the progression of dysplasia by altering premalignant tissue epithelial proliferation, apoptosis, and neovascularization⁷⁵.

Cathepsin to Cystatin ratio increases in most tumor types, compared to normal tissue, particularly for advanced cancers⁷⁵.

Nearly 50% of cancers show decreased expression of Cystatin C; however, tumor Cystatin levels vary widely⁷⁵.

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^{8 75}.

According to recent studies, some Cysteine and aspartate proteases are directly involved in the progression and metastasis of colon and ovarian cancer⁷⁶.

Increased Cystatin C level has been found in the serum of patients with several inflammatory diseases, such as colorectal tumors with metastases⁸. During colorectal cancer progression, Cystatin C remained unchanged between stages⁷⁵.

Serum Cystatin C levels are significantly higher in patients with ovarian cancers than in benign ovarian tumors or in healthy women⁷⁵.

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 cancers¹⁷. Cystatin C was suggested as a diagnostics tool for of early-stage and inflammatory breast cancer⁸. Mean IL-6 and serum Cystatin C concentrations were significantly increased in breast cancer patients compared to healthy subjects.⁷⁷

In breast cancer patients no correlation was highlighted between IL-6 and Cystatin C or between these molecules and some clinicobiological parameters of malignant progression⁷⁷.

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 cancer⁷⁸.

Cystatin C resulted significantly higher in prostate cancer but not in benign prostatic hyperplasia patients as compared to healthy subjects⁷⁷. Cystatin C levels remained high at early stages and decreased at late stages⁷⁵.

Cystatin C level is rarely detected or reduced in tumor tissues of renal carcinoma patients comparing that in normal tissues¹⁷.

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 metastasis¹⁷.

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 ⁷⁹.

Serum Cystatin C may reflect tumor burden in diffuse large B-cell lymphoma ¹⁵.

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 ¹⁵.

High serum Cystatin C levels were correlated with age over 60 years, extra-nodal involvement and with high serum lactate dehydrogenase ¹⁵.

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 ¹⁵.

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 ⁷⁵.

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 ⁸.

In patients with head and neck squamous-cell carcinoma; serum Cystatin C levels in these patients are higher than those from control groups ⁷⁵.

When melanoma cells were injected intravenously, Cystatin C deficient lungs showed re-

duced metastasis, but subcutaneous growth of melanoma cells was not different from that in control mice ⁷⁵.

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 ⁷⁵.

Eye tear Cystatin C and lactoferrin level, increased in malignant and benign eye tumors, seems to be a perspective for diagnostics in these disorders ⁸.

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 ⁸.

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 ⁸⁰. 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 ⁸¹.

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 ⁷⁶. Serum Cystatin C levels were not found to be decreased in term pregnancy ⁸².

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^{76 82}. Both serum Cystatin C and creatinine levels were significantly related to glomerular filtration rate for both pregnant and non-pregnant women⁸². However, the correlation between Cystatin C and glomerular filtration rate was set at different levels for pregnant and nonpregnant women⁸². 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-dependent⁸².

Prior studies have shown increased Cystatin C levels in preeclampsia. Pregnancy increases plasma Cystatin C, but levels are much higher in preeclampsia^{76 83}. Preeclampsia manifests by proteinuria, hypertension, and impaired renal function⁸³. Assessment of renal function is important in the evaluation of the pregnant hypertensive patient¹. 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 collection¹. 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 specificity⁸⁴.

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 controls⁸³.

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 postpartum⁸⁵.

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 well⁸⁶. Cystatin C has recently been shown to be associated with the incidence of type 2 diabetes mellitus and the progression to the pre-diabetic state⁸⁶. Several studies have also found the Cystatin C level also to be correlated with different components of the metabolic syndrome⁸⁶. 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 mellitus⁸⁶. It is considered as a useful index in the screening of diabetic micro- and macro-vascular complications⁸⁶.

Yousefzadeh et al. reported that Cystatin C could be a reliable, useful and promising marker of gestational diabetes mellitus⁸⁶. 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 women⁸⁶. 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 women⁸⁶.

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 ⁷⁶.

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 ⁸⁷. The assessment of renal function is of vital importance in management of patients with cirrhosis ⁸⁸.

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 ⁸⁸. By contrast, Cystatin C is mostly unrelated to muscle volume and liver function giving a better reflection of renal function ⁸⁸.

Some studies have shown that malignant conditions and liver disorders can bring about alterations in serum Cystatin C level ³³. 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 ⁷⁹.

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 ^{87 89}.

Serum Cystatin C and Cystatin C -based 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 ⁸⁷.

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 ⁸⁸. 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 ⁸⁸.

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 ⁸⁸.

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 ⁸⁹. 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 ⁸⁹.

Hepatitis B virus-related acute-on-chronic liver failure is a severe liver disease which results in a high mortality in China ⁹⁰. To early predict the prognosis of the patients may prevent the complications and improve the survival ⁹⁰. A new prognostic index combining serum Cystatin C with total bilirubin early predicted the short-term mortality of these patients ⁹⁰.

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 ⁹⁰. 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 patients⁹⁰.

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 prostatectomy⁹¹. Hydroxyethyl starch is used for repletion of acute intravasal volume loss in surgical patients⁹¹. However, in critically ill patients, it's associated with acute kidney injury⁹¹. The results indicated that the administration of a median dose was not associated with a post-operative deterioration of renal function in patients with normal to near-normal baseline renal function undergoing radical prostatectomy⁹¹.

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 intra-epithelial neoplasia, and prostate cancer patients, whose disease was newly diagnosed and histologically confirmed, were retrospectively collected⁷⁸.

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 group⁷⁸. There were no significant differences in serum Cystatin C levels among the three groups⁷⁸. Age and serum creatinine contributed in part to the variations in serum Cystatin C levels of prostate cancer patients⁷⁸. 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 cancer⁷⁸.

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 stages⁷⁵. Cystatins are endogenous inhibitors of cysteinyl cathepsins⁷⁵. Enhanced cathepsin expression and activity in Cystatin C deficient mice contributed to the progression of dysplasia by altering premalignant tissue epithelial proliferation, apoptosis, and neovascularization⁷⁵. Cysteine protease cathepsins are important in extracellular matrix protein degradation, cell apoptosis, and angiogenesis⁷⁵. 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 tumors⁷⁵.

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 tract⁷. 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 hormones⁷. Neuroendocrine differentiation occurs in most prostatic adenocarcinomas, and has been correlated with poor prognosis, tumor progression and resistance to hormonal therapy⁷.

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 neuroen-

doctrine differentiation in prostate cancer progression⁷.

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 cancer⁷⁷.

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 subjects⁷⁷.

The administration of zoledronic acid, an aminobisphosphonate 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 metastasis⁷⁷. 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 metastases⁷⁷.

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 population⁹². Studies have reported that renal function decline is associated with loss of bone mass and increased fracture risks in chronic kidney disease patients⁹³¹⁶. It's suggested that a decline of renal function assessed by the CKD EPI Cystatin C equation might be a useful tool to detect osteopenia¹⁶⁹²⁹³. Furthermore, a recent study also proposed Cystatin C as a predictor of hip fracture risk in elderly female patients⁹³. Osteoporosis and osteoporotic fractures

are serious public health threats resulting in high morbidity and mortality⁹². Osteoporosis is diagnosed by measuring bone mineral density, which is the best predictor of primary osteoporotic fractures⁹². 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 fractures⁹².

Cystatin C is also a significant predictor of osteoprotegerin (OPG) independently of age, free testosterone index and total 17 β -estradiol⁹⁴. 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 lineage⁹⁴. When RANKL binds to RANK, osteoclastogenesis and bone resorption are induced⁹⁴. Thus, bone resorption may be prevented by OPG binding to RANKL⁹⁴.

Osteoclastic bone resorption and differentiation depends on the activity of various proteolytic enzymes, in particular those belonging to the group of cysteine proteinases⁹⁵. Cystatin C is a natural cysteine proteinase inhibitor⁹⁶. 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 differentiation⁹⁴⁹⁵. 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%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 matrix⁹⁵. 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 degradation⁹⁵.

Bone marrow, calvaria and ex vivo calvarial in vitro cultures were examined for the effects of Cystatin C on mouse osteoblastic cells⁹⁶. Cystatin C-stimulated cells showed increased alkaline phosphatase activity, mineralization of the new bone matrix, and calvarial bone formation⁹⁶. 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 mineralization⁹⁶. 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 cells⁹⁶. These results suggested that Cystatin C affects the BMP signaling cascades in osteoblastic cells and then promotes osteoblast differentiation, mineralization, and bone formation⁹⁶.

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 women⁹⁷.

Periodontitis is a disease of the periodontium characterized by loss of attachment and supporting alveolar bone^{3 98}. 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)^{3 98}. Degradative enzymes, such as the lysosomal cysteine proteinases, cathepsins, and their inhibitors, Cystatins^{3 98}.

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 alone³. 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 matrix⁹⁹. The increased ability of protein accumulation by Cystatin C is one of several factors mediating cyclosporine A-induced gingival overgrowth⁹⁹. 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 cells⁹⁹. The addition of periodontal pathogens and proinflammatory cytokines significantly increased the expression of Cystatin C compared with cyclosporine A alone⁹⁹.

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^{3 100}. 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^{3 100}.

Concentration of Cystatin C in gingival crevicular fluid seem to regularly increase from healthy to periodontitis sites³. Graziani et al. reported substantial reduction of Cystatin C in serum concentration after non-surgical periodontal therapy³. 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 treatment³. The mean Cystatin C concentration in gin-

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 group³.

This suggests that Cystatin C increases with disease progression to prevent further periodontal degeneration and decreases after treatment due to bone metabolic homeostasis³.

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 higher¹⁰⁰. The origin of Cystatin activity was investigated by collecting submandibular, sublingual, and parotid saliva from seven subjects with mild gingivitis¹⁰⁰. Total Cystatin activity was about five times higher in submandibular saliva than in parotid saliva¹⁰⁰. In submandibular and sublingual saliva, both Cystatins S and C were demonstrated¹⁰⁰. In parotid samples, solely Cystatin C was detectable¹⁰⁰. 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 saliva¹⁰⁰.

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 groups³. 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 detectable³. A few of the samples of periodontally healthy group showed values nearing those of gingivitis group which could be because of subclinical levels of inflammation in the clinically healthy tissues³.

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 periodontitis³. This suggests that Cystatin C acts as anti-inflammatory marker in gingival crevicular fluid and serum³. Thus, in future Cystatin C and recombinant Cystatin C may have important implications in the design of novel therapies for periodontal disease³.

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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