# Cystatin C improves cardiovascular risk prediction in cardiometabolic patients in addition to estimated glomerular filtration rate

**Inna Pavlivna Dunaieva**

*Department of Clinical Pharmacology and Internal Medicine, Kharkiv National Medical University, Kharkiv, Ukraine*

## Abstract

**Background:** Cystatin C (Cys C), deemed as a glomerular renal function biomarker with unique properties to be an independent predictor of cardiovascular disease (CVD), remains a matter of investigation among different categories of patients. The aim of the study is a determining the diagnostic and prognostic value of Cys C in the development of cardiovascular complications in patients with arterial hypertension (AH) and different comorbidities, such as type 2 diabetes mellitus (T2DM) and obesity, as a supplement to estimated glomerular filtration rate (eGFR).

**Material and methods:** 111 patients with AH (men/women — 50/61) and 20 control subjects were examined. All patients with AH at the age of 54.37 ± 1.18. During a thorough examination and follow-up of patients, they were classified into 4 groups depending on the comorbidities they had: patients with AH — group 1 (22 people); patients with AH in combination with obesity — group 2 (30 people); AH in combination with T2DM — group 3 (31 people); patients with AH, T2DM — group 4 (28 people). Cys C content and insulin levels in blood serum were measured by enzyme-linked immunosorbent assay on a Labline-90 analyzer (Austria) with commercial test systems manufactured by Elabscience (ELISA, China) and Monobind Inc. (ELISA, USA), according to the instructions included in the kits. Results: It has been proven that an increase in Cys C levels are associated with a decrease in eGFR in comorbid patients with T2DM ($r = –0.676$; $p = 0.038$) and without it ($r = –0.589$; $p = 0.016$). A significant increase in Cys C levels and cardiovascular accidents in comorbid patients was found.

**Conclusions:** Cys C is a significant marker for predicting cardiovascular risk in comorbid patients with AH.

**Key words:** cystatin C; cardiovascular disease; glomerular filtration rate

*Arterial Hypertens. 2024, vol. 28, no. 1, pages: 1–7*
*DOI: 10.5603/ah.97394*

## Introduction

Cystatin C (Cys C), deemed as a glomerular renal function biomarker with unique properties to be an independent predictor of cardiovascular disease (CVD), remains a matter of investigation among different categories of patients [1, 2].

Cys C refers to the family of papain-like cysteine protease inhibitors with the biological function of inhibiting cathepsins. Cys C in humans is constantly secreted by all nuclear cells and is present in large quantities in all biological fluids. Cys C is freely excreted by glomerular filtration through the glomerular membrane and then undergoes complete

**Address for correspondence:** Inna Pavlivna Dunaieva, Kharkiv National Medical University, 4 Nauky Ave, 61022 Kharkiv, Ukraine; e-mail: pavel.kravchun@gmail.com

tubular reabsorption through proximal tubular cells and catabolism. Cys C concentration in the blood serum is inversely associated with glomerular filtration rate (GFR), whereby Cys C indicates glomerular dysfunction even in the case of still intact creatinine level [3].

Cys C synthesis rate in the body is constant, and the excretion rate depends on renal pathology. The more acute the renal pathology, the worse Cys C is filtered in the kidneys and the higher its blood level. It is secreted by all nucleated cells in the body. When estimating the GFR (eGFR) with Cys C, researchers suggest considering gender, age, adipose tissue percentage, smoking, and alcohol consumption [4].

In 2004, Cys C was officially recognized by the Food and Drug Administration (FDA) as a marker for an alternative estimation of GFR. So far, several studies have demonstrated a direct relationship between Cys C and functional and predictive indicators of cardiovascular disease [5]. There is evidence that the Cys C level is more likely than creatinine to predict the mortality rate in a variety of cardiovascular diseases, peripheral arterial disease, metabolic syndrome, and diabetes mellitus (T2DM), regardless of the deterioration of renal function [6, 7].

Since this problem is quite urgent, our study was aimed at determining the diagnostic and prognostic value of Cys C in the development of cardiovascular complications in patients with arterial hypertension (AH) and different comorbidities, such as T2DM and obesity, as a supplement to eGFR.

## Material and method

These studies were carried out under the ethical and moral requirements of the Ukrainian Association for Bioethics and following the GCP (1992) and GLP (2002) standards, the principles of the Declaration of Helsinki on Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, and approved by the Ethics and Bioethics Committee of Kharkiv National Medical University.

111 patients with AH (men/women — 50/61) and 20 control subjects were examined. All patients with AH at the age of 54.37 ± 1.18 were treated at the clinic of the Government Institution "L.T. Malaya Therapy National Institute" of the National Academy of Medical Sciences of Ukraine. During a thorough examination and follow-up of patients, they were classified into 4 groups depending on the comorbidities they had: patients with AH (group 1) — 22 people; patients with AH in combination with obesity (group 2) — 30 people; AH in combination with type 2 diabetes (group 3) — 31 people; patients with AH, type 2 diabetes and obesity (group 4) — 28 people.

Body weight and height were measured in all patients; body mass index (BMI) was calculated [BMI = body weight/height$^2$ (m$^2$)]; systolic and diastolic blood pressure was measured.

Cys C content and insulin levels in blood serum were measured by enzyme-linked immunosorbent assay on a Labline-90 analyzer (Austria) with commercial test systems manufactured by Elabscience (ELISA, China) and Monobind Inc. (ELISA, United States), according to the instructions included in the kits.

Biochemical studies (creatinine, urea, serum lipid spectrum, and glycated hemoglobin level) were performed on a Labline-90 analyzer (Austria). Serum urea levels were measured by the kinetic enzymatic method with urease/glutamate dehydrogenase using Liquick Cor-UREA 30 kits (Cormay, Poland) according to the manufacturer's instructions. The serum creatinine level was measured by the modification of the Jaffe method without deproteinization using Liquick Cor-CREATININ 30 reagent kits (Poland) according to the manufacturer's instructions.

The following were the exclusion criteria for the study: Type 1 diabetes mellitus, congenital heart and urinary tract defects, artificial pacemakers, artificial heart valves, stage II B and III heart failure, acute heart attack, infectious and severe inflammatory processes, and hematological diseases.

Statistical analysis was performed using Statistica 13.0 and Medcalc 19.2.6. The hypothesis of normal distribution was verified with the Shapiro-Wilk test. Quantitative features were given as M ± m (arithmetic mean ± standard error of the arithmetic mean), as well as the confidence interval depending on the type of distribution (normal or non-normal). Values with a normal distribution were presented as a 95% confidence interval (95% CI), while values with a significantly different distribution were presented as the 25% and 75% percentiles. Groups of variables were compared with the Mann-Whitney U test. Spearman's rank correlation coefficient was used to determine the relationship between the indicators. Hypotheses were tested at a significance level of 0.05.

To determine possible model predictors, a ROC analysis was performed by constructing receiver operating characteristic (ROC) curves, as well as area under curve (AUC), a numerical indicator of the area under the ROC curve. An area value from

0.9 to 1 corresponds to excellent model quality, 0.8–0.9 is very good, 0.7–0.8 is good, 0.6–0.7 is average, and 0.5–0.6 is unsatisfactory.

To assess the filtration function of the kidneys, the GFR was estimated by blood creatinine level according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (2021), as well as by Cys C level in blood according to the formula of Hoek et al. (2003): GFR [mL/min/1.73 $m^2$] = 80.35/Cystatin C [mg/mL]) — 4.32.

According to the Kidney Disease: Improving Global (KDIGO) and the Association of Nephrologists of Ukraine, GFR < 60 mL/min/1.73 $m^2$ indicates a decrease and, accordingly, GFR from 89 mL/min/1.73 $m^2$ to 60 mL/min/1.73 $m^2$ indicates a slight decrease in the filtration capacity of the kidneys.

## Results

Differences in the Cys C level in the examined patients with AH and various comorbidities were evaluated compared with those in the control group (Tab. 1).

The obtained data reveal a significant increase in Cys C in all examined patients compared to healthy subjects ($p < 0.05$).

The examined patients were redistributed into 2 groups depending on the presence or absence of diabetes mellitus to compare Cys C with creatinine levels in such patients (Tab. 2).

During the examination, a correlation between creatinine level and Cys C ($r = 0.77$; $p < 0.01$) was found, and it was found to be significant at normal creatinine values.

We compared the eGFR measured by creatinine and Cys C concentrations in comorbid patients with T2DM (Tab. 3).

The criterion for renal dysfunction was an eGFR level below 90 mL/min/1.73 $m^2$, standardized by body surface area.

A mathematical model of the relationship between Cys C and eGFR was developed (Fig. 1).

The data obtained show that a significant decrease in GFR was observed at high levels of Cys C compared with the control group ($p < 0.01$).

In patients with primary damage to renal tubular cells, Cys C was significantly higher compared with the control group.

**Table 1.** Assessing the differences between groups when distributed by the cystatin C (Cys C) level

| Group | AH | AH + obesity | AH + T2DM | AH + obesity + T2DM | Control group |
|---|---|---|---|---|---|
| $\bar{X} \pm S_{\bar{X}}$ | 135.62 ± 22.10 | 141.65 ± 20.93 | 136.31 ± 24.62 | 121.55 ± 22.14 | 83.51 ± 12.32 |
| 95% CI | [125.82; 145.42] | [134.16; 149.14] | [127.64; 144.98] | [113.76; 129.34] | [78.73; 88.29] |
| p | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |

AH — arterial hypertension; T2DM — type 2 diabetes mellitus; CI — confidence interval

**Table 2.** Cystatin C (Cys C) and creatinine levels in the examined patients

| Examined group | Creatinine [μmol/l] | Cys C [ng/ml] | r | p |
|---|---|---|---|---|
| Group of patients without T2DM (n = 51) | 93.34 | 138.90 | 0.77 | < 0.01 |
| Group of patients with T2DM (n = 60) | 94.45 | 129.63 | 0.67 | < 0.01 |
| Control group (n = 20) | 76.50 | 83.51 | 0.93 | < 0.05 |

T2DM — type 2 diabetes mellitus

**Table 3.** Glomerular filtration rate (GFR) estimated by creatinine and cystatine C (Cys C) concentration in the group of patients with type 2 diabetes mellitus (T2DM) (ml/min/1.73 $m^2$)

| Value | GFR | |
|---|---|---|
| | By creatinine | By Cys C |
| Me | 64.30 | 57.66* |
| Q25–Q75 | [62.17; 66.43] | [57.11; 58.21] |

*Significance of the relationship $p < 0.05$

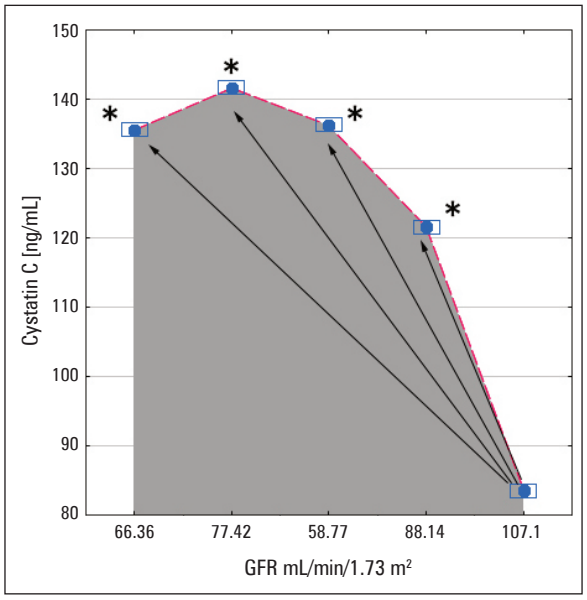


**Figure 1.** A mathematical model of the relationship between cystatin C (Cys C) and estimated glomerular filtration rate (eGFR)

During this examination, we performed ROC analysis of Cys C with the development of cardiovascular accidents separately for all groups of patients (Fig 2–5).

This ROC analysis revealed a valid model for comparing the likelihood of cardiovascular accidents and an increase in the Cys C level in the groups of patients with AH + obesity and AH + T2DM (AH + obesity: AUC = 0.833, 95% CI: 0.810–0.860; AH + T2DM: AUC = 0.750, 95% CI: 0.671–0.8344).

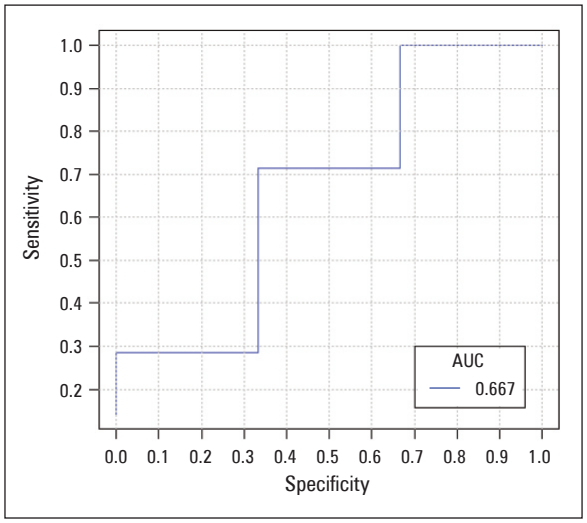


**Figure 2.** Receiver operating characteristic (ROC) curve of the relationship between cystatin C (Cys C) and the development of cardiovascular accidents in patients with arterial hypertension (AH). AUC — area under the curve

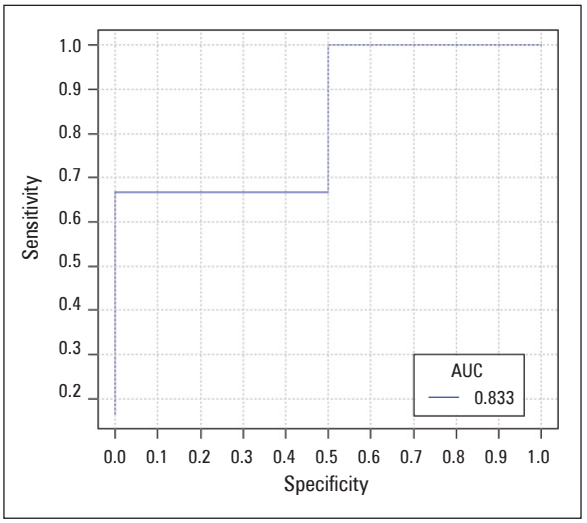


**Figure 3.** Receiver operating characteristic (ROC) curve of the relationship between cystatin C (Cys C) and the development of cardiovascular accidents in patients with arterial hypertension (AH) and obesity. AUC — area under the curve

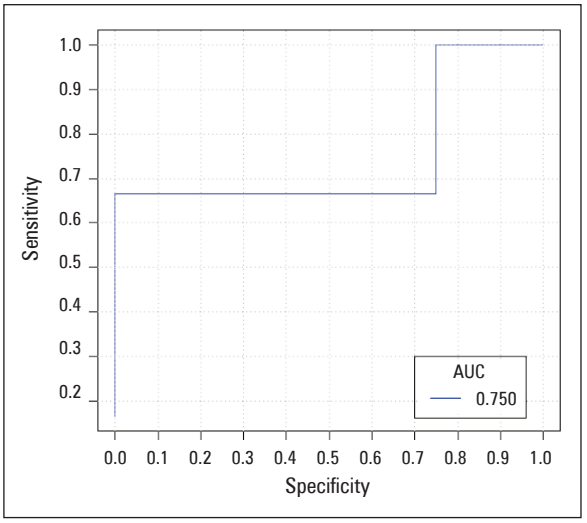


**Figure 4.** Receiver operating characteristic (ROC) curve of the relationship between cystatin C (Cys C) and the development of cardiovascular accidents in patients with arterial hypertension (AH) and type 2 diabetes mellitus (T2DM). AUC — area under the curve

This suggests that increased Cys C levels in patients with AH + obesity and AH + T2DM may be associated with more severe endothelial damage and vascular wall inflammation, resulting in stimulation of lysosomal cathepsins with a subsequent increase in Cys C in blood plasma.

We also developed mathematical models of Spearman's rank correlation coefficients between GFR and Cys C levels in the group of patients without T2DM ($r = -0.589$; $p = 0.016$) (Fig. 6) and with T2DM ($r = -0.676$; $p = 0.038$) (Fig. 7).

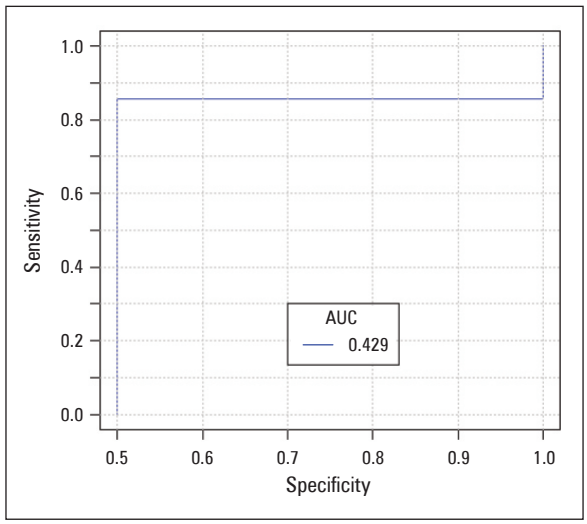


**Figure 5.** Receiver operating characteristic (ROC) curve of the relationship between cystatin C (Cys C) and the development of cardiovascular accidents in patients with arterial hypertension (AH), obesity, and type 2 diabetes mellitus (T2DM). AUC — area under the curve

An increase in the Cys C level was found to be associated with a decrease in GFR. A negative correlation was found with GFR (group of patients without T2DM r = –0.589; p = 0.016; group of patients with T2DM r = –0.676; p = 0.038).

## Discussions

This study examined the correlation between Cys C and the early development of cardiovascular accidents in patients with AH and concomitant comorbidities: T2DM and obesity. Since Cys C is a cysteine protease inhibitor, it is constantly secreted by all nucleated cells, including adipose tissue cells, is completely filtered, and is not secreted by the proximal tubules [7]. On the one hand, an increase in Cys C levels in adipose tissue is protective in nature by blocking cathepsin proteinases. On the other hand, it reduces the proliferation of adipose tissue in the same way as in atherosclerosis by inhibiting cysteine proteinases and prevents the development of atherosclerotic lesions in the vascular wall in patients with AH, T2DM, and obesity [8, 9]. Moreover, an increase in the Cys C concentration in the blood serum is followed by a decrease in its concentration in the arterial wall, which may raise the risk of adverse cardiovascular accidents. Scientists from Japan have confirmed the role of Cys C as an indicator of adverse cardiovascular outcomes [10].

A systematic review of current data on Cys C has shown that adverse outcomes and risk stratification across the entire range of cardiovascular diseases are associated with high Cys C levels in plasma [7]. There is evidence that elevated Cys C concentrations are associated with CVD risk and the causal role of Cys C in the etiology of CVD has been confirmed [5, 11, 12].

Therefore, our study shows the latest evidence of the role of Cys C in the development of cardiovascular complications in comorbid patients with AH and agrees with other studies on the use of Cys C for cardiovascular risk stratification.

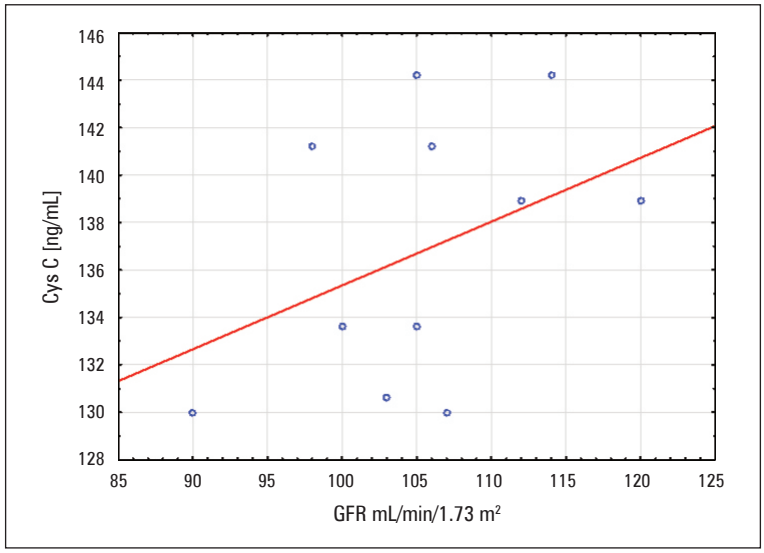


**Figure 6.** Mathematical model of Spearman's rank correlation coefficients between glomerular filtration rate (GFR) and cystatin C (Cys C) levels in the group of patients without type 2 diabetes mellitus (T2DM) (r = –0.589; p = 0.016)

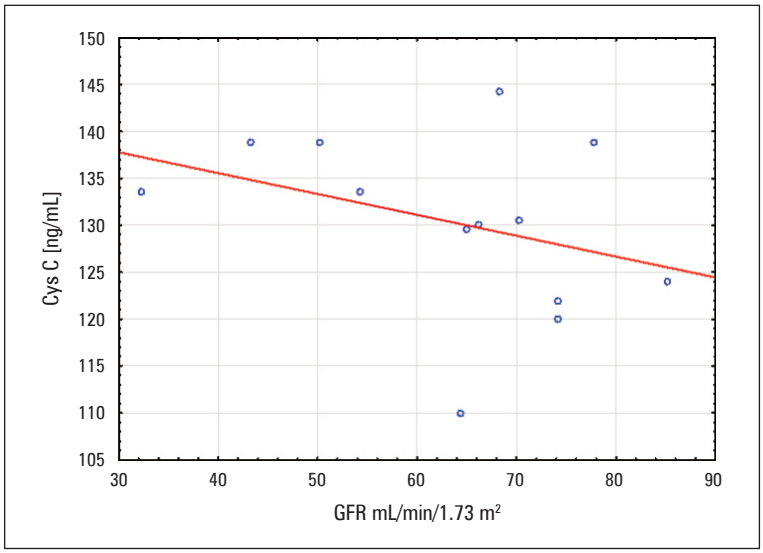


**Figure 7.** Mathematical model of Spearman's rank correlation coefficients between glomerular filtration rate (GFR) and cystatin C (Cys C) levels in the group of patients with type 2 diabetes mellitus (T2DM) (r = 0.676; p = 0.038).

## Conclusions

It has been proven that an increase in Cys C levels is associated with a decrease in glomerular filtration rate in comorbid patients with type 2 diabetes mellitus (r = –0.676; p = 0.038) and without it (r = –0.589; p = 0.016).

A significant increase in Cys C levels and cardiovascular accidents in comorbid patients was found.

Cys C is a significant marker for predicting cardiovascular risk in comorbid patients with hypertension.

### Data availability statement

The data used to support the findings of this study are included within the article.

### Ethics statement

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and GCP statement.

### Author contributions

I.D. carried out the experiment, analysed literature, wrote the manuscript.

### Funding

No funding to declare.

### Acknowledgments

Acknowledgements to Kharkiv national Medical University administration.

### Conflict of interest

Author declare no conflict of interest.

## References

1. Ferguson TW, Komenda P, Tangri N. Cystatin C as a biomarker for estimating glomerular filtration rate. Curr Opin Nephrol Hypertens. 2015; 24(3): 295–300, doi: 10.1097/MNH.0000000000000115, indexed in Pubmed: 26066476.
2. Krstic D, Tomic N, Radosavljevic B, et al. Biochemical Markers of Renal Function. Curr Med Chem. 2016; 23(19): 2018–2040, doi: 10.2174/0929867323666160115130241, indexed in Pubmed: 26769095.
3. Levey AS, Fan Li, Eckfeldt JH, et al. Cystatin C for glomerular filtration rate estimation: coming of age. Clin Chem. 2014; 60(7): 916–919, doi: 10.1373/clinchem.2014.225383, indexed in Pubmed: 24871681.
4. Onopiuk A, Tokarzewicz A, Gorodkiewicz E. Cystatin C: a kidney function biomarker. Adv Clin Chem. 2015; 68: 57–69, doi: 10.1016/bs.acc.2014.11.007, indexed in Pubmed: 25858868.
5. Carvalho LS, Silva TQ, Coelho-Filho OR. Cystatin C as a Candidate Biomarker of Cardiovascular Outcomes: Too Near, but too Far from Reality. Arq Bras Cardiol. 2018; 111(6): 808–809, doi: 10.5935/abc.20180226, indexed in Pubmed: 30517376.
6. Magnusson M, Molvin J, Engström G, et al. Cystatin C and Risk of Diabetes and the Metabolic Syndrome — Biomarker and Genotype Association Analyses. PLoS One. 2016; 11(5): e0155735, doi: 10.1371/journal.pone.0155735, indexed in Pubmed: 27218257.
7. van der Laan SW, Fall T, Soumaré A, et al. Cystatin C and Cardiovascular Disease: A Mendelian Randomization Study. J Am Coll Cardiol. 2016; 68(9): 934–945, doi: 10.1016/j.jacc.2016.05.092, indexed in Pubmed: 27561768.
8. Luo J, Wang LP, Hu HF, et al. Cystatin C and cardiovascular or all-cause mortality risk in the general population: A meta-analysis. Clin Chim Acta. 2015; 450: 39–45, doi: 10.1016/j.cca.2015.07.016, indexed in Pubmed: 26192218.
9. Xu Y, Ding Y, Li X, et al. Cystatin C is a disease-associated protein subject to multiple regulation. Immunol Cell Biol.

2015; 93(5): 442–451, doi: 10.1038/icb.2014.121, indexed in Pubmed: 25643616.
10. Satoh-Asahara N, Suganami T, Majima T, et al. Japan Obesity and Metabolic Syndrome Study (JOMS) Group. Urinary cystatin C as a potential risk marker for cardiovascular disease and chronic kidney disease in patients with obesity and metabolic syndrome. Clin J Am Soc Nephrol. 2011; 6(2): 265–273, doi: 10.2215/CJN.04830610, indexed in Pubmed: 21051748.
11. Dejenie TA, Abebe EC, Mengstie MA, et al. Dyslipidemia and serum cystatin C levels as biomarker of diabetic nephropathy in patients with type 2 diabetes mellitus. Front Endocrinol (Lausanne). 2023; 14: 1124367, doi: 10.3389/fendo.2023.1124367, indexed in Pubmed: 37082121.
12. Dedual MA, Wueest S, Challa TD, et al. Obesity-Induced Increase in Cystatin C Alleviates Tissue Inflammation. Diabetes. 2020; 69(9): 1927–1935, doi: 10.2337/db19-1206, indexed in Pubmed: 32616516.