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ORIGINAL RESEARCH

Correlation Analysis and Diagnostic Value of Serum Homocysteine, Cystatin C and Uric Acid Levels with the Severity of Coronary Artery Stenosis in Patients with Coronary Heart Disease

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Background: Coronary angiography (CAG) is an invasive examination with high risks and costs and various complications may occur. It is necessary to find a diagnostic method, non-invasiveness, inexpensive with low risk. This study aims to analyze the correlation between the levels of serum homocysteine (Hcy), cystatin C (Cys C) and uric acid (UA) and Gensini score in patients with coronary heart disease (CHD) and assess their diagnostic value for CHD.

Methods: A retrospective analysis was conducted on 1412 patients underwent CAG from October 2019 to December 2021, and we conducted this study from January to July 2022. A total of 765 patients with CHD confirmed by CAG were selected as the research group, while 647 patients revealed as non-obstructive stenosis by CAG as the control group. The serum Hcy, Cys C and UA levels were detected and the correlation between Gensini score and variables was analyzed. The receiver-operating characteristic (ROC) curve was performed to assess the diagnostic value of the Hcy, Cys C and UA for CHD.

Results: The serum Hcy, Cys C and UA levels in the research group were higher as compared with the control group ($p<0.05$). Spearman correlation and multivariate linear regression analysis showed that there was a significantly positive correlation between Gensini score and serum Hcy, Cys C and UA levels ($p<0.05$). The ROC curve analysis presented the combined Hcy and Cys C with UA having the highest specificity of diagnostic value for CHD (area under the curve (AUC)=0.768, 95% CI 0.706–0.823, specificity = 72.34%, sensitivity = 67.88%, Youden Index = 0.4022).

Conclusion: The serum Hcy, Cys C and UA levels in patients with CHD were significantly increased, positive correlation with Gensini score. The combined Hcy and Cys C with UA could be used to assess the severity of coronary artery stenosis and provide predictive and early intervention treatment values for CHD and a new way of diagnosing CHD, which is cheap, safe, effective and deserving of clinical application.

Keywords: serum homocysteine, cystatin C, uric acid, gensini score, correlation analysis, diagnostic value

Introduction

With the development of an aging population, the incidence of coronary heart disease (CHD) is getting higher and higher and the mortality rate is also showing a trend of increasing year by year, which seriously affects human health.[1] Currently, in terms of the diagnosis of CHD, coronary angiography (CAG) is still the "gold standard." However, it is an invasive examination with high risks and costs, which is difficult for patients to accept and not available in all hospitals. Considering this condition, a non-invasive method is urgently needed to predict the severity of coronary artery disease with low risk, low cost and easy operations, especially for those hospitals that cannot perform CAG. In the past

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few decades, many studies have been analyzed in order to explore or find one or more widely available and inexpensive biochemical parameters to predict the severity of CHD, such as concentrations of N-terminal pro-brain natriuretic peptide (NT-proBNP), glycemic variability, metabolic syndrome (MS) score, glycated hemoglobin, angiogenin, epicardial adipose tissue, waist–hip ratio, d-dimers, eosinophils count in the peripheral circulation and serum vascular cell adhesion molecule-1 and even gut microbiota,[2–12] which mainly assessed the severity of coronary artery stenosis from AUC and sensitivity, with a lack of specificity. Moreover, for the non-invasive diagnosis of CHD, there are also some studies, based on the correlation between the two variables, which have some limitations; these have shown that serum Hcy, Cys C and UA levels are positively correlated with the severity of coronary artery disease, respectively.[13–15] While considering that Hcy, Cys C and UA are all involved in the formation of atherosclerosis by affecting endothelial function, implying that they are interconnected and have a close and mutual influence on pathophysiological mechanisms, there are some limitations.[16–18] Therefore, how to assess their clinically diagnostic value of CHD overall, such as the AUC, sensitivity, specificity and the Youden index, has not been reported and there is also no definite conclusion on combining the three to predict the diagnostic value of the severity of coronary artery stenosis.

In this paper, the correlation between serum homocysteine (Hcy), cystatin C (Cys C) and uric acid (UA) levels and the severity of coronary artery stenosis in patients with CHD was analyzed in order to determine the optimal combination with the strongest specificity to assess the diagnostic value for CHD, which might provide value for the prevention and intervention of the disease, especially for those hospitals that cannot perform CAG.

Clinical Material and Methods

Clinical Material

Based on the inclusion and exclusion criteria, a retrospective analysis was conducted on 1412 patients who were hospitalized with the chief complaint of "chest tightness and pain", clinically diagnosed as Acute Coronary Syndromes (ACS) and needed to undergo CAG further in the Heart Center of our hospital from October 2019 to December 2021; we conducted this study from January to July 2022. A total of 765 patients with CHD confirmed by CAG were selected as the research group, while 647 patients revealed as non-obstructive stenosis by CAG were selected as the control group. Gensini score was calculated to quantify the extent of coronary artery stenosis. The study was in accordance with the Declaration of Helsinki and approved by a local ethics committee, and all patients provided written informed consent. The authors had no access to any information that could identify individual participants during or after data collection.

Inclusion criteria were: (1) CAG was performed for all the involved patients with Judkins method; (2) New York Heart Association functional class from grade I to II; (3) with complete clinical information; (4) no major surgery or stroke in the past 6 months and no use of glucocorticoids or immunosuppressants; (5) not taking drugs inhibiting the formation of UA or increasing the elimination of UA, vitamin B nor folic acid tablets and folic acid antagonists; and (6) signed informed consent for the treatments.

Exclusion criteria were: (1) New York Heart Association functional class from grade III to IV; (2) complications of serious arrhythmia, hyperthyroid cardiopathy, congenital and idiopathic cardiomyopathy, valvular heart disease, cardiomyopathy, chronic obstructive pulmonary disease, cor pulmonale; (3) serious diseases of the liver, kidney, brain or other important organ;[19] (4) infectious diseases; (5) tuberculosis, tumors, autoimmune and hematological diseases; (5) allergies, pregnancy or lactation. (6) angina with an established precipitating cause (eg, anemia or tachydysrhythmia).

Methods

The Severity of Coronary Artery Stenosis

CAG was performed by the Judkins method, and the results of angiography were determined by 2–3 senior cardiac catheterization specialists. In the research group, CHD was defined as the rate of one coronary artery stenosis at least of 50%, while in the control group, non-obstructive stenosis by CAG was defined as the rate of one coronary artery stenosis less than 50% or even normal. Meanwhile, the extent of coronary artery stenosis was quantified by Gensini score, which

was calculated for every patient in the research group as the sum of the integral of each lesion location multiplying its coefficient as described in Figure 1 of the previous literature by Rampidis et al.[20]

Determinations of Lipid Profile, Serum Creatinine, NT-ProBNP, Fasting Plasma Glucose, Hcy, Cys C and UA Levels

A quantity of 5 mL fasting venous blood was taken from all the patients in tubes with separating gel in the early morning of the next day after admission and centrifuged for 10 min at 3000 rpm. NT-ProBNP was detected by electrochemiluminescence, with a NT-ProBNP detector (Cobas e 601, Roche Diagnostic GmbH, Mannheim, Germany), which was operated in accordance with the Elecsys proBNP II kit instructions (Roche Diagnostics GmbH, Mannheim, Germany). Fasting plasma glucose, total cholesterol (TC) and triglyceride (TG) were determined by enzymatic method, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined by direct measurement, operated by automatic biochemical analyzer (AU5800, Beckman Kurt Co., Ltd., USA) in accordance with the relevant kit instructions (Beckman Kurt experimental systems (Suzhou) Co., Ltd., Suzhou, China). Serum creatinine (Scr) was also determined by enzymatic method, operated by automatic biochemical analyzer (AU5800, Beckman Kurt Co., Ltd., USA) in accordance with the kit instructions (Kyowa Medex Co., Ltd., Tokyo, Japan). The concentration of Hcy was determined by chemical luminescence with a Hcy detector (ADVIA Centaur XP, Siemens Medical Diagnostic Co., Ltd., USA), which was operated in accordance with the kit instructions (Siemens Medical Diagnostic Products (Shanghai) Co., Ltd., Shanghai, China). While the concentration of Cys C determined by latex particle enhanced immunoturbidimetry and the concentration of UA in blood determined by uricase-peroxidase method were detected by automatic biochemical analyzer (AU5800, Beckman Kurt Co., Ltd., USA), which was operated in accordance with the relevant kit instructions (Jiuqiang Biotechnology Co., Ltd., Beijing, China).

Calculating the Estimated Glomerular Filtration Rate (eGFR)

All the patients' eGFR was calculated by using the Chinese modified Modification of Diet in Renal Disease (MDRD) study equation:[21]

$$\text{eGFR (mL/min/1.73m}^2\text{)} = 175 \times \text{standardized creatinine (mg/dL)}[0.795\times(\text{enzymatic method Scr, μmol/L})+0.29]^{-1.234}\times\text{age (year)}^{-0.179}\times 0.79 \text{ (if female)}$$

Calculating the Left Ventricular Ejection Fraction (LVEF)

LVEF was calculated by using cardiac color Doppler ultrasound diagnostic system (CX50, Philips, Netherlands) and corrected body surface area of all patients.

Statistical Analysis

Measurement data were presented as means ± SDs, a Student's *t*-test was used to analyze the differences between the research and control groups. Enumeration data were presented as ratios, and the chi-square test was used to analyze the differences between the two groups. The receiver-operating characteristic (ROC) curve was performed to evaluate the diagnostic and predictive value of the Hcy, Cys C and UA for CHD. The Spearman correlation analysis was conducted to assess the relationship between Gensini Score and variables. To select the variables independently associated with Gensini Score, variables with a $p<0.2$ in the Spearman correlation analysis were retained to evaluate for further multivariate linear regression analysis (method: Enter). All the data were analyzed by using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS, Inc., Chicago, USA); $p<0.05$ was considered statistically significant.

Results

The Outcome of Clinical Material of the Patients

As shown in Table 1, the clinical data of patients in the research and control groups, including gender, age, body mass index (BMI), smoking or not, whether complicated with hypertension, Type II diabetes or hyperlipidemia and lipid profile, renal function test and eGFR calculated were compared, and there was no significant difference ($p>0.05$). When compared with the control group, the levels of NT-ProBNP, fasting plasma glucose and the incidence of atrial fibrillation

Table 1 Comparison of the Clinical Data of Patients in Two Groups

Variables	Research Group (n=765)	Control Group (n=647)	χ^2/t	*p*
Age, yr	60.17±10.13	63.70±10.22	−1.775	0.184
Male, n(%)	402 (52.55)	361 (55.80)	0.817	0.366
BMI, kg/m^2	24.07±1.87	23.64±1.42	1.450	0.149
Smoking, n(%)	392 (51.24)	301 (46.52)	3.124	0.077
Hypertension, n(%)	450 (58.82)	407 (62.91)	2.449	0.118
Type II diabetes, n(%)	181 (23.66)	151 (23.34)	0.020	0.887
LVEF, %	42.35±7.14	45.32±8.58	−2.172	0.034
NT-ProBNP, pg/mL	3938.31±2225.03	2052.49±1153.55	7.76	0.000
Atrial fibrillation, n(%)	199 (26.06)	138 (21.32)	4.232	0.040
Hyperlipidemia, n(%)	348 (45.49)	280 (43.28)	0.696	0.404
TC, mmol/L	4.97±0.25	4.90±0.28	1.730	0.085
TG, mmol/L	1.70±0.44	1.59±0.42	1.519	0.130
LDL-C, mmol/L	3.52±0.400	3.50±0.39	1.113	0.267
HDL-C, mmol/L	1.01±0.20	1.07±0.20	−1.903	0.058
Fasting plasma glucose, mmol/L	7.04±1.26	5.93±0.98	5.593	0.000
Scr, μmol/L	99.52±13.36	96.43±13.71	1.393	0.165
eGFR, mL/min/1.73m^2	65.24±14.02	65.96±13.48	−0.314	0.754

Notes: There were no statistically significant differences on baseline data of the patients in the 2 groups except LVEF, NT-ProBNP, atrial fibrillation, fasting plasma glucose ($p < 0.05$).

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; NT-ProBNP, N-terminal pro-brain natriuretic peptide; Scr, serum creatinine; TC, total cholesterol; triglyceride; TG, triglyceride.

in the research group were higher and LVEF was lower, with significant difference ($p<0.05$). Meanwhile, the mean Gensini score was 56.01±39.24 in the research group.

The Serum Levels and Clinical Value of Hcy, Cys C and UA in Diagnosis CHD

As shown in Table 2, the serum Hcy, Cys C and UA levels in the research group were 16.89±6.26μmmol/L, 1.10 ±0.34mg/L and 347.88±71.68μmmol/L, respectively, which were noticeably higher as compared with the control group, which had levels 12.75±2.74 μmmol/L, 0.96±0.15mg/L and 310.87±61.34μmmol/L, respectively, indicating a statistically significant difference ($p<0.05$). The ROC curve analysis of Hcy, Cys C and UA showed that the AUC were 0.761 (95% confidence interval (CI), 0.697–0.816), 0.612 (95% CI, 0.543–0.678) and 0.639 (95% CI, 0.570–0.704) for CHD, respectively. For Hcy, Cys C and UA, respectively, the optimal cut-off value, the sensitivity and specificity in diagnosing CHD are shown in Table 3 and Figure 1a-c. In addition, the area under the curve (AUC) of Hcy was the highest (0.761) among the three, and there was notably significant difference when compared with the other two (p=0.0003 and 0.0193), shown in Figure 1d, while the Youden index showing the authenticity of the test was 0.3804, 0.2423 and 0.2290, respectively.

Spearman Correlation Analysis and Multivariate Linear Regression Analysis of the Correlation Between Gensini Score and Variables

For the patients with CHD, Spearman correlation analysis showed that Gensini score was positively correlated with age, BMI, hypertension, NT-ProBNP, atrial fibrillation, type II diabetes, fasting plasma glucose, serum creatinine, eGFR, serum Hcy, Cys C and UA levels (r=0.227, 0.231, 0.148, 0.294, 0.153, 0.248, 0.252, 0.411, −0.318, 0.314, 0.295 and 0.307, respectively; p=0.003, 0.003, 0.045, 0.000, 0.049, 0.001, 0.001, 0.000, 0.000, 0.000, 0.000 and 0.000, respectively). While multivariate linear regression analysis displayed that there was a significantly positive correlation between Gensini score and some variables including age, BMI, NT-ProBNP, Type II diabetes, fasting plasma glucose, Hcy, Cys C and UA (β=0.178, 0.138, 0.180, 0.154, 0.134, 0.278, 0.147 and 0.419, respectively; p=0.029, 0.046, 0.011, 0.034,

Table 2 The Serum Levels and Clinical Diagnostic Value of Cys C, Hcy and UA

Biomarkers	Research Group (n=765)	Control Group (n=647)	t^a	p^a	AUC	Z	*p*	95% CI		Cut Off value	Sensitivity (%)	Specificity (%)	Youden Index
								Lower	Upper				
Hcy (μmol/L)	16.89±6.26	12.75±2.74	3.022	0.000	0.761	6.782	0.0001	0.697	0.816	>12.62	84.85	53.19	0.3804
Cys C(mg/L)	1.10±0.34	0.96±0.15	2.436	0.016	0.612	2.634	0.0084	0.543	0.678	>1.17	28.48	95.74	0.2423
UA (μmol/L)	347.88±71.68	310.87±61.34	2.137	0.034	0.639	3.145	0.0017	0.570	0.704	>315	65.45	57.45	0.2290

Notes: [a]Stands for the Student's t-test between the research group and control group.

Abbreviations: AUC, Area Under The Curve; CI, confidence interval; Cys C, cystatin C; Hcy, homocysteine; UA, uric acid.

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Table 3 The Relationship Between Gensini Score and Variables

Variables	Spearman Correlation Analysis		Multivariate Linear Regression Analysis	
	r	*p*	*β*	*p*
Age	0.227	0.003	0.178	0.029
Gender	−0.009	0.910	–	–
BMI	0.231	0.003	0.138	0.046
Smoking	0.095	0.225	–	–
Hypertension	0.148	0.045	0.039	0.580
LVEF	0.127	0.104	0.116	0.089
NT-ProBNP	0.294	0.000	0.180	0.011
Atrial fibrillation	0.153	0.049	0.025	0.716
Type II diabetes	0.248	0.001	0.154	0.034
Hyperlipidemia	0.087	0.267	–	–
TC	0.001	0.994	–	–
TG	0.031	0.694	–	–
LDL-C	0.100	0.202	–	–
HDL-C	−0.101	0.196	−0.025	0.715
Fasting plasma glucose	0.252	0.001	0.134	0.047
Scr	0.411	0.000	–	–
eGFR	−0.318	0.000	−0.182	0.024
Hcy	0.314	0.000	0.278	0.017
Cys C	0.295	0.000	0.417	0.006
UA	0.307	0.000	0.149	0.044

Notes: For the patients with CHD, Spearman correlation analysis showed that Gensini score was positively correlated with age, BMI, hypertension, NT-ProBNP, atrial fibrillation, type II diabetes, fasting plasma glucose, serum creatinine, eGFR, serum Hcy, Cys C and UA levels ($r > 0$, $p < 0.05$). While multivariate linear regression analysis displayed that there was a significantly positive correlation between Gensini score and some variables including age, BMI, NT-ProBNP, Type II diabetes, fasting plasma glucose, Hcy, Cys C and UA ($\beta > 0$, $p < 0.05$), and a significantly negative correlation between Gensini score and eGFR ($\beta = -0.182$, $p = 0.024$).

Abbreviations: BMI, body mass index; CHD, coronary heart disease; Cys C, cystatin C; eGFR, estimated glomerular filtration rate; Hcy, homocysteine; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; NT-ProBNP, N-terminal pro-brain natriuretic peptide; Scr, serum creatinine; TC, total cholesterol; triglyceride; TG, triglyceride; UA, uric acid.

0.047, 0.017, 0.006 and 0.044, respectively), and a significantly negative correlation between Gensini score and eGFR (β=−0.182, p=0.024). All are presented in Table 3.

The Clinical Value of Combined Detection of Hcy, Cys C and UA in Diagnosis of CHD

The ROC curve analysis of combined Hcy with Cys C, combined Hcy with UA, combined Cys C with UA and combined Hcy and Cys C with UA are drawn in Figure 2a-d, showing that AUC were 0.762 (95% CI, 0.698–0.817), 0.769 (95% CI, 0.706–0.824), 0.657 (95% CI, 0.588–0.720) and 0.768 (95% CI, 0.706–0.823) for CHD, as well as the sensitivity and specificity in diagnosing CHD are shown in Table 4. The AUC of combined Hcy with Cys C, combined Hcy with UA and combined Hcy and Cys C with UA having no statistically significant difference (p>0.05), was higher as compared with combined Cys C with UA, and the difference was statistically significant (p=0.014, 0.0024 and 0.0027). The Youden index presented that the authenticity of combined Cys C with UA was 0.2753, which is the lowest in Table 4.

The Comparison of the AUC Among the Hcy, Combined Hcy with Cys C, Combined Hcy with UA and Combined Hcy and Cys C with UA

In Table 5, the AUC of Hcy, combined Hcy with Cys C, combined Hcy with UA and combined Hcy and Cys C with UA was the same amount 0.761–0.769, and the difference among them was not statistically significant (p>0.05). Meanwhile, we compared the sensitivity and specificity of the bio-markers above, finding that the combined Hcy and Cys C with UA

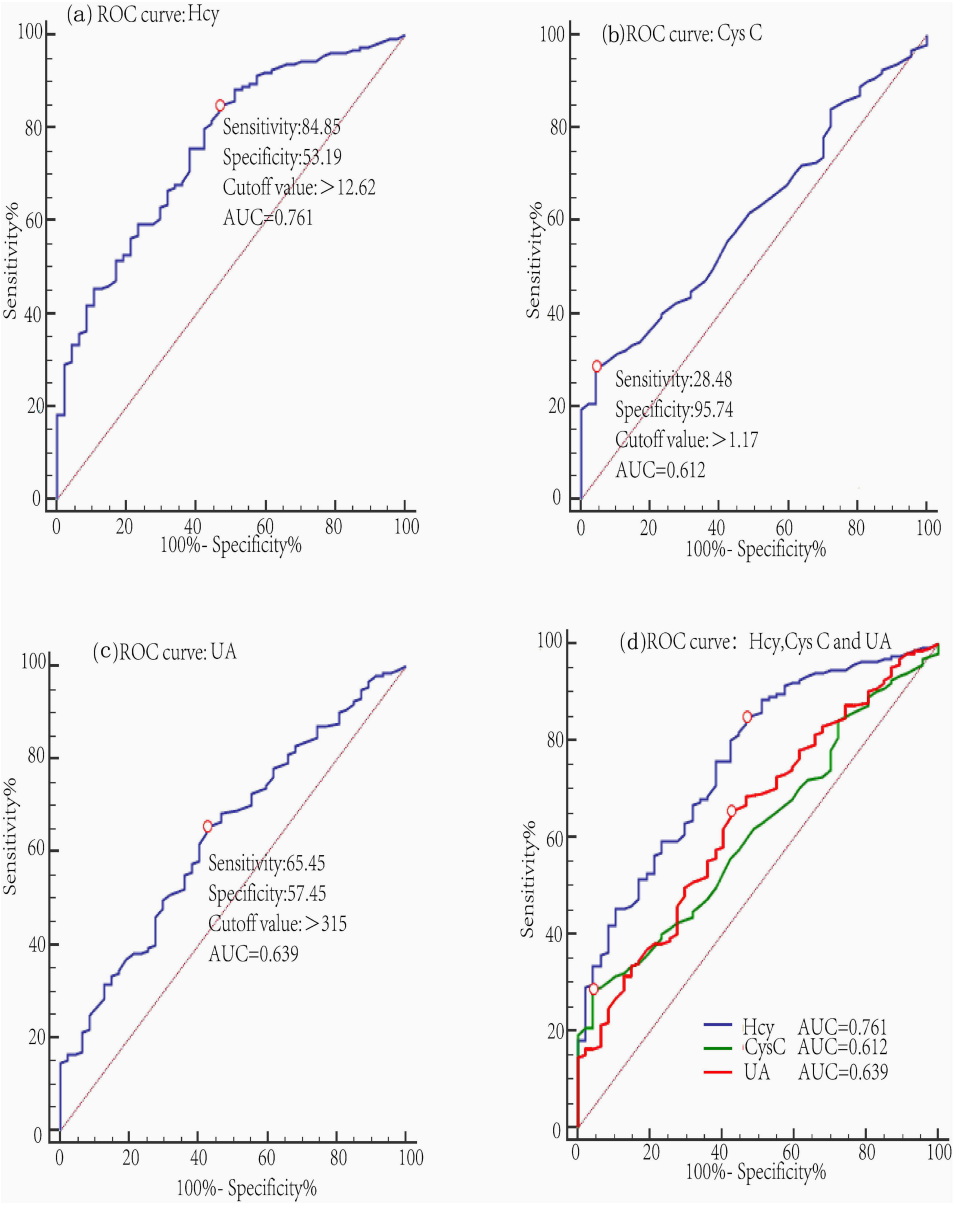

Figure 1 The ROC curve of Hcy, Cys C and UA for diagnostic value of CHD. The ROC curve analysis of Hcy, Cys C and UA showed that the AUC were 0.761 (95% CI, 0.697–0.816), 0.612 (95% CI, 0.543–0.678) and 0.639 (95% CI, 0.570–0.704) for CHD, respectively. For Hcy, Cys C and UA, respectively, the optimal cut-off value, the sensitivity and specificity in diagnosing CHD were shown in (**a-c**). In addition, the AUC of Hcy was the highest (0.761) among the three, and there was notably significant difference when compared with the other two (p = 0.0003 and 0.0193), shown in (**d**). While the Youden index showing the authenticity of the test was 0.3804, 0.2423 and 0.2290, respectively.

Abbreviations: AUC, area under the curve; CHD, coronary heart disease; CI, confidence interval; Cys C, cystatin C; Hcy, homocysteine; ROC, receiver-operating characteristic; UA, uric acid.

had the lowest sensitivity (p<0.001) but the highest specificity (p<0.05), whose Youden index was 0.4022, showing a high authenticity of the test.

Discussion

At present, although CAG is still the "gold standard" for the diagnosis of CHD, various complications may occur during and after operation because of its invasion, such as allergic reaction, the induction of acute myocardial infarction, severe arrhythmia, acute heart failure and shock, contrast-induced nephropathy, etc. All of these could cause great physical and psychological issues for patients and seriously affect their life. It is necessary to find a diagnostic method, which is non-

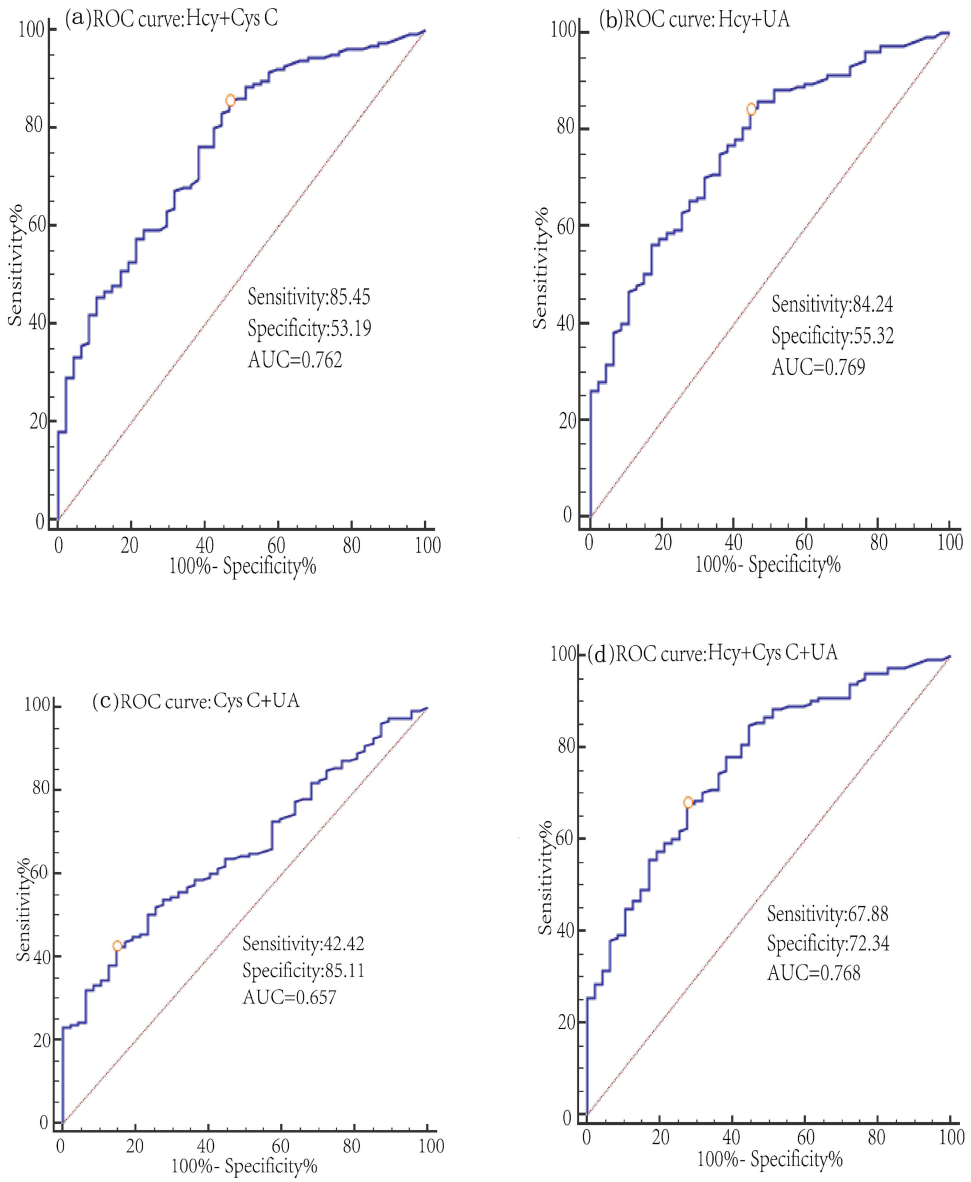

Figure 2 The ROC curve of combined detection of Hcy, Cys C and UA for diagnosing CHD. The ROC curve analysis of combined Hcy with Cys C, combined Hcy with UA, combined Cys C with UA and combined Hcy and Cys C with UA were drawn in (**a-d**), showing that AUC were 0.762 (95% CI, 0.698–0.817), 0.769 (95% CI, 0.706–0.824), 0.657 (95% CI, 0.588–0.720) and 0.768 (95% CI, 0.706–0.823) for CHD. The AUC of combined Hcy with Cys C, combined Hcy with UA and combined Hcy and Cys C with UA having no statistically significant difference ($p > 0.05$), was higher as compared with combined Cys C with UA, and the difference was statistically significant ($p = 0.014$, 0.0024 and 0.0027). The Youden index presented that the authenticity of combined Cys C with UA was 0.2753. While the combined Hcy and Cys C with UA had the lowest sensitivity ($p<0.001$) but the highest specificity ($p < 0.05$).

Abbreviations: AUC, area under the curve; CHD, coronary heart disease; CI, confidence interval; Cys C, cystatin C; Hcy, homocysteine; ROC, receiver-operating characteristic; UA, uric acid.

invasive and inexpensive with low risk and can not only reduce the possible procedure-associated complications, accidents and economic burden brought about by CAG, but by which the patients with atypical symptoms of CHD should be discovered, diagnosed and treated.

Up to now, many biochemical parameters have been shown to be related to the severity of CHD, such as concentrations of NT-proBNP, glycated hemoglobin, etc, the same results were found in our study. More importantly, in this study, we retrospectively analyzed the levels of Hcy, Cys C and UA and their correlation with the degree of coronary artery stenosis in patients with CHD diagnosed by CAG. Also, we conducted the ROC curve to analyze the AUC, specificity, sensitivity and Youden index; it was found that the levels of Hcy, Cys C and UA in the research group were higher than those in the control group, with a difference that was significant. Also, multivariate linear regression analysis showed that

Table 4 The Clinical Diagnostic Value of Combined Detection of Hcy, Cys C and UA

Biomarkers	AUC	*p*	95% CI		Sensitivity%	Specificity%	Youden Index
			Lower	Upper			
Hcy+Cys C	0.762	<0.0001	0.698	0.817	85.45	53.19	0.3865
Hcy+UA	0.769	<0.0001	0.706	0.824	84.24	55.32	0.3956
Cys C+UA	0.657	0.0001	0.588	0.720	42.42	85.11	0.2753
Hcy+Cys C+UA	0.768	<0.0001	0.706	0.823	67.88	72.34	0.4022

Notes: The ROC curve analysis of combined Hcy with Cys C, combined Hcy with UA, combined Cys C with UA and combined Hcy and Cys C with UA showed that AUC were 0.762 (95% CI, 0.698–0.817), 0.769 (95% CI, 0.706–0.824), 0.657 (95% CI, 0.588–0.720) and 0.768 (95% CI, 0.706–0.823) for CHD, as well as the sensitivity and specificity in diagnosing CHD. The AUC of combined Hcy with Cys C, combined Hcy with UA and combined Hcy and Cys C with UA having no statistically significant difference ($p > 0.05$), was higher as compared with combined Cys C with UA, and the difference was statistically significant ($p = 0.014$, 0.0024 and 0.0027). The Youden index presented that the authenticity of combined Cys C with UA was 0.2753.
Abbreviations: AUC, Area Under The Curve; CI, confidence interval; Cys C, cystatin C; Hcy, homocysteine; ROC curve, receiver operator characteristic curve; UA, uric acid.

Table 5 The Comparison of the AUC Among the Hcy, Combined Hcy with Cys C, Combined Hcy with UA and Combined Hcy and Cys C with UA

Biomarkers	AUC	*p*	95% CI		Sensitivity%	Specificity%	Youden Index
			Lower	Upper			
Hcy	0.761	<0.0001	0.697	0.816	84.85	53.19	0.3804
Hcy+Cys C	0.762	<0.0001	0.698	0.817	85.45	53.19	0.3865
Hcy+UA	0.769	<0.0001	0.706	0.824	84.24	55.32	0.3956
Hcy+Cys C+UA	0.768	<0.0001	0.706	0.823	67.88	72.34	0.4022

Notes: The AUC of Hcy, combined Hcy with Cys C, combined Hcy with UA and combined Hcy and Cys C with UA was the same amount 0.761–0.769, and the difference among them was not statistically significant ($p > 0.05$). The sensitivity and specificity of the biomarkers above displayed that the combined Hcy and Cys C with UA had the lowest sensitivity ($p < 0.001$) but the highest specificity ($p < 0.05$), whose Youden index was 0.4022, showing a high authenticity of the test.
Abbreviations: AUC, Area Under The Curve; CI, confidence interval; Cys C, cystatin C; Hcy, homocysteine; UA, uric acid.

there was a significantly positive correlation between Hcy, Cys C and UA and Gensini score ($\beta>0$ and $p<0.05$), which indicated that they are independent risk factors for CHD. One possible reason for this change might be that Hcy, Cys C and UA were involved in the occurrence and development of coronary heart disease.

Hcy is an intermediate product of methionine metabolism in vivo, and it is a sulfur-containing amino acid, which can cause vascular injury. Normally, it is metabolized by decomposition, so its level in the human body is very low. However, for a number of reasons, the metabolism of Hcy can be abnormal in the body, resulting in an increased concentration in the blood and hyperhomocysteinemia.[13] It has been reported that the risk of ischemic heart disease increases by 11–16% and mortality increases by 33.6% for every increase of 5 μmol/L in the level of Hcy.[22,23] Through direct or indirect oxidative stress, Hcy could cause endothelial vascular injury, promote the proliferation of vascular smooth muscle cells, activate platelets and promote platelet aggregation and adhesion, inflammation and immunoreaction, which could cause or accelerate the process of atherosclerosis and then promote the occurrence and development of CHD.[13,24–28] Some studies have reported that Hcy is an independent risk factor for atherosclerosis, leading to the incidence of coronary, stroke, carotid, peripheral artery disease and even claudication.[29–31] Smith et al reported that there are over 100 diseases associated with Hcy and the most common associations were with cardiovascular disease (CVD) and diseases of the central nervous system, as well as a large number of developmental and age-related diseases. They also pointed out that Hcy is not only a disease biomarker but also a guide for the prevention of disease,[22] while other studies showed that Hcy levels were less relevant to the prevalence of CHD and not a risk factor for CHD in patients with DM.[32,33] The results of our study showed that the levels of Hcy in CHD are obviously elevated and positively correlated with the degree of

coronary artery stenosis. The ROC curve analysis showed that the AUC of Hcy had the largest area and highest sensitivity but the lowest specificity of the three biological indicators which predict CHD.

Cys C is an important part of the cysteine protease inhibitor family and is produced by cells in vivo, which can inhibit the production of cysteine protease and regulate the activity of cysteine protease. By affecting the phagocytosis and chemotaxis of neutrophils, it can also participate in the process of inflammation.[34,35] In addition, it can participate in the occurrence and development of atherosclerosis by affecting the remodeling process of the matrix of the vascular wall, regulate the regression, formation and stability of atherosclerotic plaques; and reflect the degree of atherosclerotic plaque burden.[36] It can also affect the process of myocardial remodeling by inhibiting the activity of endogenous cysteine protease in patients with CHD[37,38] and predict major cardiovascular events.[39] Reports showed that Cys C is mainly used as a reliable biomarker of kidney function and would help to identify patients at risk of cardiac dysfunction, thus predicting further cardiovascular events.[36,40,41] Fabjan et al revealed that a high level of Cys C was positively associated with the increased risk of cardiovascular morbidity and mortality, which could be used for the detection of new onset or deteriorating CVD.[42] In our study, consistent with literature reports, it was shown that the level of Cys C in the patients of CHD was obviously higher than in the control group and was positively correlated with the degree of coronary artery stenosis, more specific but less sensitive, and the smallest AUC.

Serum UA is an end product of purine catabolism in the human body. The relationship between uric acid and cardiovascular disease is complex and difficult because of its pathophysiological mechanisms in the cardiovascular system. When myocardial ischemia occurs, the activity of xanthine oxidase affects the production of UA by causing a compensatory increase, leading to an increase in the concentration of UA.[43] An excessive concentration of UA in the blood could induce platelet synthesis and release more vasoactive substances, which can aggravate endothelial cell damage and lipid deposition. Meanwhile, as UA precipitates and deposits in blood vessels, subcutaneous regions, the joints, kidneys, etc. in the form of crystals phagocytosed by leukocytes, the damage to the heart and vascular intima appears. In this process, a large number of cytokines and inflammatory mediators will be released from leukocytes, causing a severe acute inflammatory response[44] and further exacerbating the damage to the heart and vascular intima.[45] Moreover, the increased concentration of UA can also accelerate the apoptosis of vascular smooth muscle cells and lipid peroxidation and reduce the stability of plaques,[46,47] resulting in further injury to the heart and vascular intima. Several studies showed that the risk of Coronary Artery Calcium increased by 31% and mortality increased by 9% for every increase of 1 mg/dl in the level of serum UA,[48,49] and a high-serum UA level was associated with higher coronary plaque vulnerability.[50] Studies have shown a strong correlation between the level of serum UA and CVD, such as hypertension, heart failure, CHD and even atrial fibrillation, which in turn increases heart failure and coronary artery disease in patients with hyperuricaemia.[51–54] Mengozzi A et al reported that serum UA levels can predict mortality (all-cause and cardiovascular), while the results of our study showed the cutoff value of UA for the diagnosis CHD was 315umol/L, as in Mengozzi A et al's study, suggesting that the serum UA level was lower than the traditional threshold for defining hyperuricemia and gout.[55] There are many convincing studies supporting UA as a proactive approach to early cardiovascular risk and discrimination and reclassification in predicting mortality risk in patients without established CVD.[56,57] Meanwhile, the results of our study showed that the specificity and sensitivity of UA in the diagnosis of CHD were unsatisfactory, compared with the Hcy and Cys C.

Considering the common pathophysiology mechanisms of Hcy, Cys C and UA in CHD and in order to obtain the optimal specificity of the diagnostic value for CHD clinically, we tried to analyze the ROC curve of combined Hcy with Cys C, combined Hcy with UA, combined Cys C with UA and combined Hcy and Cys C with UA. We further found that the AUC of combined Hcy with Cys C, combined Hcy with UA and combined Hcy and Cys C with UA was higher than that of combined Cys C with UA ($p<0.05$). The Youden index of combined Cys C with UA was the lowest (0.2753). After that, we continued to analyze the AUC of Hcy, combined Hcy with Cys C, combined Hcy with UA and combined Hcy and Cys C with UA, and found that combined Hcy and Cys C with UA had the lowest sensitivity but the highest specificity. Therefore, in terms of the specificity of the clinical diagnosis, combined Hcy and Cys C with UA showed the highest diagnostic value. We found that the detection of combined Hcy and Cys C with UA could be used to assess the severity of coronary artery stenosis and might provide diagnostic and predictive value for CHD in terms of clinical specificity for the first time.

Overall, the results indicated that the combined Hcy and Cys C with UA had clinical diagnostic value and could be used to predict the severity of coronary artery stenosis in patients with CHD. To some extent, because of its non-invasiveness, low cost with low risk and easy operation, it not only reduces the unnecessary CAG and risk, economizes iatrical resources and lessens the economic burden of patients and society, but also increases the diagnosis of patients with atypical symptoms of CHD to improve the prognosis and quality of life. Furthermore, it will be particularly meaningful especially for those hospitals in which CAG is not available; the allocation of medical resources and social resources can be further optimized through targeted screening, which would benefit humankind.

Limitations

The limitations of this study include the fact that the results might be biased, as there was no significant difference in the prevalence of classical risk factors for CHD according to the presence or absence of CHD, because it was a retrospective analysis and single race, which might limit the popularization and promotion. It would be more persuasive if prospective studies could be conducted. Also, no long-term follow-up was conducted and data on the incidence of atrial fibrillation were especially lacking because of the influence of UA; also, we cannot evaluate the effects of the combined Hcy and Cys C with UA on the long-term prognosis of patients, leading to biased results in this study. There were also some patients with severe renal dysfunction and severe hepatic dysfunction who were not taken into account clinically. Besides, this study analyzed data from non-randomized patients and focused on the specificity rather than sensitivity to the clinically diagnostic value of CHD, which may have affected the results of the study. Studies with a large sample analysis, multicenter studies, longer duration, widespread geographical areas and evidence-based medicine will still need further discussion in the future to support the current findings.

Conclusions

The serum Hcy, Cys C and UA levels in patients with CHD were significantly increased, which showed a significantly positive correlation with the Gensini score. The detection of combined Hcy and Cys C with UA could be used to assess the severity of coronary artery stenosis and might provide predictive and early intervention treatment values for CHD and a new way of diagnosing CHD, which is cheap, safe, effective and deserving of clinical application. We hope through this study clinicians could take the above factors into consideration to make comprehensive prediction and evaluation of CHD in the future, especially for those hospitals in which CAG is not available.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of the People's Hospital of Liaoning Province, China. Written informed consent was obtained from each patient.

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Disclosure

The authors declare no competing interests in this work.

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