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ORIGINAL RESEARCH

Diagnostic Value of Homocysteine Metabolic Pathway in Coronary Atherosclerosis: A Study on Reference Intervals and Risk Prediction

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Purpose: To determine the reference intervals of homocysteine (Hcy) metabolic pathway in healthy adults using direct continuous sampling, and to explore their correlations with coronary atherosclerosis (CA) for improving the risk assessment and diagnostic accuracy of CA.

Patients and Methods: A total of 426 reference individuals were selected via direct continuous sampling of the two centers from healthy adults based on questionnaires and laboratory indicators. Additionally, 75 patients were diagnosed with CA by cardiologists. Standardized analysis of Hcy, cysteine (Cys) and methionine (Met) levels in serum were conducted using commercial assay kits compatible with LC-MS/MS.

Results: Although Met and Hcy showed significant differences between the sexes, they could not meet the criteria for setting reference intervals. After outlier removal and normality conversion, 95% reference intervals of Hcy, Cys and Met were calculated as 7–25 μmol/L, 227–410 μmol/L, and 21–39 μmol/L, respectively. In addition, the correlations among Hcy, Cys, and Met were influenced by CA, and each served as an independent risk factor. The area under the curve (AUC) for the combined three indicators increased to 0.86 (95% CI: 0.81–0.91). The specificity increased to 83%, while the sensitivity reached 78%.

Conclusion: The Hcy metabolic pathway significantly enhances risk assessment capability and may be helpful in providing reference values for the diagnosis and risk prediction of CA.

Keywords: homocysteine, cysteine, methionine, reference intervals, coronary atherosclerosis, risk assessment

Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid that cannot be synthesized de novo in humans and is produced exclusively through the metabolism methionine (Met).[1] Metabolically, Hcy can not only be remethylated to Met but also enter the transsulfuration pathway to produce cysteine (Cys).[2] The interplay among these amino acids leads to considerable variation in total Hcy levels in the body. Various physiological or pathological conditions may lead to abnormal accumulation of Hcy in the bloodstream. Clinically, elevated Hcy levels are recognized as a risk factor for neurodegenerative diseases, cardiovascular diseases, malignancies, and chronic kidney disease.[3–6] Previous studies have demonstrated a strong association between Hcy levels and both the severity of coronary heart disease and the extent of coronary artery stenosis in elderly patients.[7,8] Furthermore, elevated Hcy concentrations are also associated with an increased risk of coronary events.[9] High Hcy concentrations are considered an independent risk factor for coronary heart disease and coronary artery stenosis, particularly in elderly individuals.[8–10]

Cys is the most abundant thiol in plasma and serves as a precursor for the synthesis of the potent antioxidants taurine and glutathione.[2] Glutathione, in particular, has been extensively studied in various fields, including metabolic disorders, neurological diseases, and cancer.[11] Notably, similar to Hcy, elevated Cys levels have been observed in patients with coronary

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artery disease and are considered a significant contributing factor to coronary atherosclerosis (CA).[12] Fasting total Cys levels are positively associated with the risk of myocardial infarction.[13] Studies have shown that Cys is also independently linked to atherosclerotic lesions and cardiovascular risk scores.[14] Some researchers have suggested that elevated total Cys levels are as significant as elevated total Hcy levels and that Cys is a more reliable predictor of coronary heart disease than Hcy.[12,15]

Met is an essential amino acid whose intake significantly affects Hcy levels. Reduced activity of methionine synthase and 5,10-methylenetetrahydrofolate reductase (MTHFR) impairs the methylation of Hcy by 5-methyltetrahydrofolate, thereby leading to Hcy accumulation in the body. Beyond its influence on Hcy metabolism, Met plays multiple roles owing to its central involvement in one-carbon metabolism. Blood Met levels are routinely assessed for the clinical diagnosis of hypermethioninaemia.[16] Restricting Met in the diet extends life expectancy and delays the development of cancers.[17,18] In pregnant women, those carrying fetuses with congenital heart defects exhibited higher Hcy and lower Met levels compared to controls.[19] Recent evidence suggests that an elevated plasma Cys/Met ratio may reflect diastolic dysfunction in patients with heart disease.[20] Selhub et al reported significant atheromatous lesions in mice fed a high-Met diet, even in the presence of normal Hcy levels.[21] Moreover, a prospective cohort study in Finland found that a high-Met diet was associated with an increased relative risk of acute coronary events in men without preexisting coronary artery disease.[22] These findings underscore the potential clinical importance of monitoring Met levels.

Homocysteine arises solely from dietary methionine released in the gut, is channeled into the one-carbon cycle to generate S-adenosylmethionine, and is subsequently converted to S-adenosylhomocysteine, the direct precursor of homocysteine in every organ. Steady-state levels of homocysteine are governed by two competing enzymatic routes: (i) re-methylation back to methionine—dependent on methionine synthase, methyl-tetrahydrofolate supplied by the MTHFR enzyme, and the vitamin B12/B9 cofactor axis—and (ii) transsulfuration to cysteine, catalyzed by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) with vitamin B6 as co-factor.[23] Genetic or nutritional impairment of either pathway leads to hyperhomocysteinemia (HHcy), an established, independent risk factor for coronary atherosclerosis. Elevated circulating methionine itself, even when homocysteine remains within the normal range, promotes atheromatous lesions, whereas increased cysteine levels correlate positively with myocardial infarction risk and cardiovascular risk scores. Thus, the integrated methionine–homocysteine–cysteine metabolic axis, rather than any single metabolite, dictates atherosclerotic burden, underscoring the need for simultaneous monitoring of Hcy, Met, and Cys to refine risk stratification and therapeutic targeting in coronary artery disease.[24]

The enzymatic cycling method is commonly used for Hcy detection in clinical practice but is limited by single-analyte detection, complex reaction steps, enzyme instability, and low specificity. In contrast, liquid chromatography–tandem mass spectrometry (LC-MS/MS) offers superior specificity, stability, and the capability for simultaneous multi-analyte detection.

Although several hospitals in China offer triple-indicator testing for Hcy, Cys, and Met, they continue to rely on single-indicator RIs derived from Mayo Clinic data and other international sources, which overlook the integrative nature of the metabolic pathway and population-specific differences. To date, only one study has reported triple-indicator RIs for Hcy metabolism, and it employed an indirect approach.[25] However, the indirect method is limited by the absence of detailed clinical background data.[26]

This study is the first to directly establish RIs for Hcy, Cys, and Met in adults based on the Hcy metabolic pathway. It further explores the predictive potential of this pathway for coronary heart disease risk and aims to provide a reference for clinical diagnosis and risk assessment.

Materials and Methods

Subjects

Fresh residual serum samples from 426 apparently healthy individuals were obtained from patients undergoing physical examination at Central Hospital of Dalian University of Technology and First Affiliated Hospital of Jinzhou Medical University. Before sample collection and testing, questionnaires were used to determine the eligibility of these individuals for inclusion. Laboratory test results were used to confirm eligibility and exclude individuals who did not meet the criteria. According to CLSI EP28-A3c, the inclusion and exclusion criteria are detailed in Supplemental Table 1.[27] The flowchart illustrating the inclusion and exclusion process is presented in Figure 1.

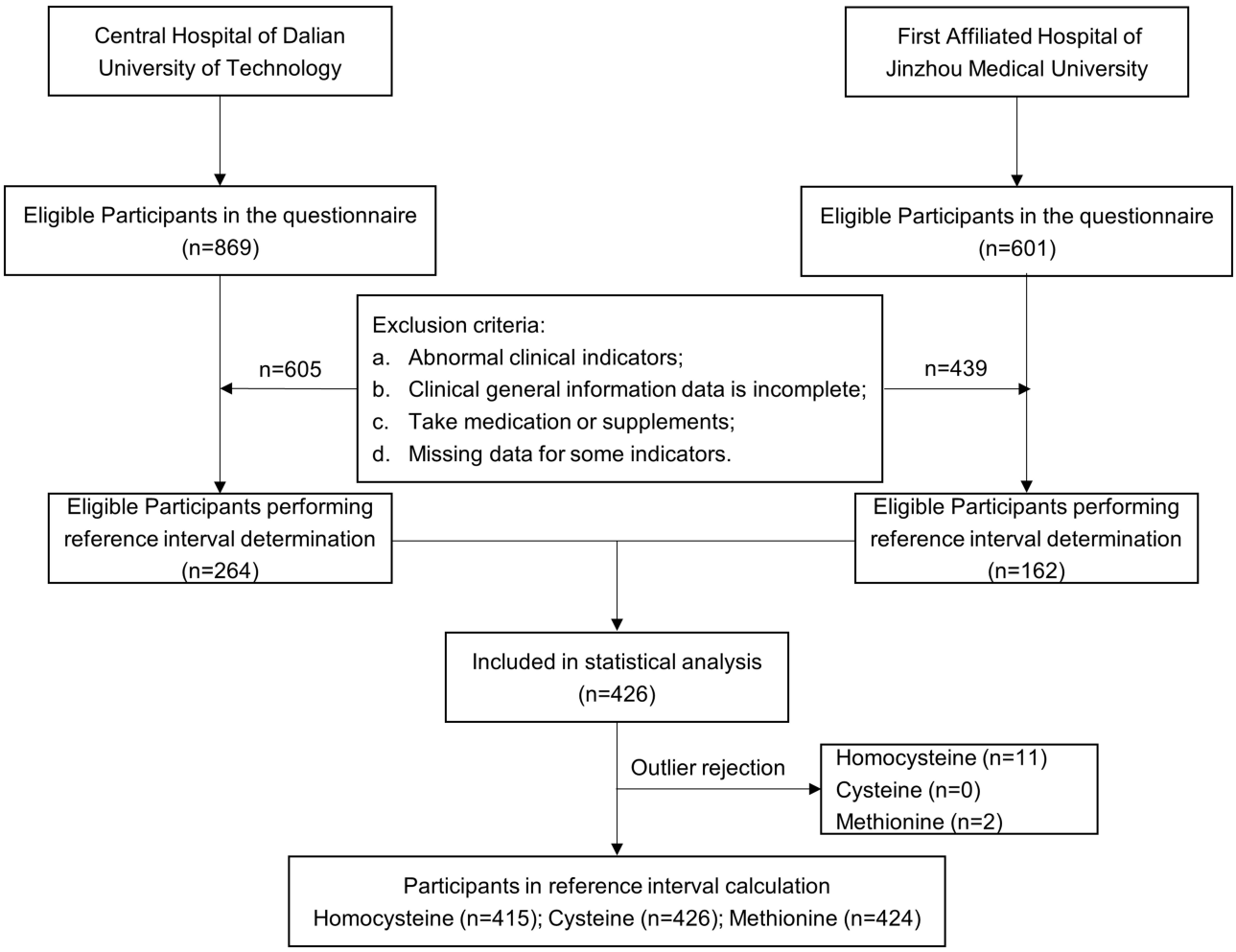

Figure 1 Flow chart of reference individual inclusion and exclusion.

Residual serum was collected from 75 in-patients with coronary artery disease (CA) diagnosed by cardiologists at the Fourth Hospital of Harbin Medical University (n = 10) and Huludao Central Hospital (n = 65). CA was defined as ≥ 50% stenosis in ≥ 1 major coronary artery on angiography and subclassified as stable angina, unstable angina, acute, or old myocardial infarction per ESC/AHA criteria; patients with cerebral infarction were excluded.

This study follows the Helsinki Declaration and has been approved by four ethics committees: Central Hospital of Dalian University of Technology ethics committee (YN2022-042-10), the First Affiliated Hospital of Jinzhou Medical University ethics committee (2023111), the Fourth Hospital of Harbin Medical University ethics committee (2024 - Ethical Review - 03) and Huludao Central Hospital ethics committee (Approval No: 202319). Written informed consent was obtained from every patient prior to their inclusion in the study.

Sample Collection and Disposal

All participants were requested to fast before blood collection and to avoid unusually strenuous exercise. Samples were collected between 7:00 a.m. and 10:00 a.m. Venous blood was collected using yellow vacuum tubes containing an inert separating gel procoagulant. The samples were centrifuged at 3500 rpm for 10 minutes within 30 minutes of collection. Serum samples were stored at −4 °C and transported to the laboratory via cold chain for testing by qualified personnel. If immediate testing was not possible, serum samples were stored at −70°C and tested within 1 week. The stability of the storage and transport conditions has been verified by the laboratory.[28]

LC-MS/MS Measurement

All samples were tested using Hcy, Met, and Cys kits (suitable for LC-MS/MS; lot no. B0123022701 and B0123030101; 100 tests/box), which were developed by Dalian Boyuan Medical Testing Laboratory and had completed pilot production. The method was consistent with the one described in a previous study.[28] Serum levels of Hcy, Met and Cys were determined by high-performance liquid chromatography-tandem mass spectrometry, with an AB SCIEX TRIPLE QUAD 4500MD mass spectrometer equipped with a Jasper HPLC system (ABsciex, Toronto, Canada). To verify the validity of the method, the validation was conducted in accordance with industry standard YY/T 1870–2023 (General Requirements for Liquid Chromatography-Mass Spectrometry Testing Kits). The analytical performance of the kit met the requirements. The liquid was clear and free of impurities, and the reductant was a white solid. The inner and outer packages were complete and undamaged, with clear and legible labels. The linear correlation coefficient (r) was ≥0.9900 within within the linear range of 1–100 μmol/L (Hcy), 2–200 μmol/L (Met), and 5–500 μmol/L (Cys); the relative deviation was within ±15.0%. The intra-batch and inter-batch precision were in the ranges of 1.9–7.8% and 2.4–7.5%, respectively.

Traceability

The Hcy calibrator was traced to the Hcy reference material in frozen human serum (Beijing Institute of Medical Devices Inspection, Product no.: GBW(E) 091011). The Met calibrator was referenced to a Met solution reference material (Beijing Coastal Hongmeng Reference Material Technology Co. Ltd.; catalog no. GBW(E) 100670). The Cys calibrator was directly prepared using a certified reference material from Supelco (catalog no. 95437).

Statistical Analysis

All data were analyzed using SPSS 22.0 software. Figures were drawn using R software 4.3.1 and GraphPad Prism 8.0. The Shapiro–Wilk test was used to determine the distribution of the experimental data. The data with a normal distribution were described as mean ± SD, while non-normally distributed data were described as median (25th, 75th percentiles). Comparisons between groups were made using Student's *t*-test or Mann–Whitney *U*-test. Outliers were removed using Dixon and Tukey's tests. Groupings were determined using the Z-test or the Harris and Boyd test. Correlations were assessed using Pearson or Spearman correlation coefficients, as appropriate. According to the recommendations of the CLSI EP28-A3c document, after the measured values were converted to normal distribution by Box-Cox, the two-sided reference interval was determined by mean ± 1.96SD.[27] Sample size was calculated with G*Power 3.1 ($\alpha = 0.05$, power = 0.80, effect size d = 0.50), indicating a minimum of 64 CA patients were required; 75 evaluable samples were ultimately enrolled to ensure sufficient statistical power and allow for 15% attrition.

Results

Characteristics

A total of 426 healthy individuals who met the criteria (Supplemental Table 1) were included in subsequent analyses. The baseline information of the reference population is shown in Table 1. The reference sample group consisted of 41.78% males and 58.22% females, with a median age of 40 years and a median BMI of 22.28 kg/m^2. Student *t* test or Mann–Whitney *U*-test showed that the clinical general examination results of females were generally significantly lower than those of males ($P < 0.01$), as shown in Table 1. However, triglycerides and white blood cell count were not statistically different between males and females.

The 75 CA patients consisted of non-obstructive CA (n=9), stable angina (n=1), unstable angina (n=43), acute myocardial infarction (n=15) and old myocardial infarction (n=7). There were 41 males and 34 females, the age ranged from 36 to 82 years, with a mean age of 63.0 ± 10.0 years.

Age

In Figure 2A, serum Cys concentration in healthy adults showed a significant positive correlation with age (male: r=0.2152, P =0.0039; female: r = 0.1256, P = 0.0483). In males, a weak correlation was observed between Hcy

Table 1 Baseline characteristics of the Reference Population

Indicator	Total (n=426)	Male (n=178)	Female (n=248)	P-value
Age, years	40 (35, 47)	43 (37, 51)	38 (33, 44)	< 0.001
BMI, kg/m^2	22.28 (20.79, 24.3)	23.18 (21.35, 24.61)	21.66 (20.31, 23.92)	< 0.001
SBP, mmHg	121 (113, 128)	123 (116, 129)	119 (111, 126)	< 0.001
DBP, mmHg	76 (70, 81)	79 (75, 83)	73 (67, 79)	< 0.001
ALT, U L^{-1}	17 (12, 23)	22 (17, 28)	14 (11, 18)	< 0.001
AST, U L^{-1}	19 (16, 22)	21 (18, 24)	17 (15, 20)	< 0.001
GGT, U L^{-1}	17 (13, 24)	23 (18, 31)	14 (12, 17)	< 0.001
ALB, g L^{-1}	45.33 ± 1.98	45.96 ± 1.96	44.88 ± 1.87	< 0.001
TC, mmol L^{-1}	4.72 ± 0.68	4.73 ± 0.64	4.71 ± 0.71	0.82
TG, mmol L^{-1}	0.94 (0.72, 1.27)	1.13 (0.85, 1.49)	0.85 (0.61, 1.13)	< 0.001
Glu, mmol L^{-1}	4.97 (4.73, 5.23)	5.09 (4.84, 5.34)	4.93 (4.68, 5.15)	< 0.001
Cr, μmol L^{-1}	60.35 (52.3, 71.6)	74 (67.9, 80.6)	53.95 (49.45, 58.55)	< 0.001
Ua, μmol L^{-1}	299 (248.96, 344.25)	356.98 (319.45, 386)	263.15 (232, 298.1)	< 0.001
Urea, mmol L^{-1}	4.98 (4.33, 5.86)	5.64 (4.8, 6.2)	4.61 (4.1, 5.3)	< 0.001
WBC,×10^9/L	5.56 (4.83, 6.45)	5.56 (4.86, 6.49)	5.54 (4.78, 6.39)	0.51
Hgb, g L^{-1}	139 (130, 152)	153 (147, 159.4)	132 (126, 138)	< 0.001

Notes: Data are mean ± SD or median (25th, 75th). Student's *t*-test or Mann–Whitney *U*-test was used to compare between sexes. P < 0.05 indicates statistical significance. The unit of WBC is expressed as ×10^9/L, and × denotes multiplication by 10^9.
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, γ-glutamyl transferase; ALB, albumin; TC, total cholesterol; TG, triglycerides; Glu, glucose; Cr, creatinine; Ua, uric acid; WBC, white blood cell count; Hgb, hemoglobin.

concentration and age (r = −0.0489, P = 0.5203), but no correlation was found between Met and age after multivariate analysis (P > 0.05). The overall degree of correlation was evaluated comprehensively, and the age-specific influence was not considered when determining the reference intervals.

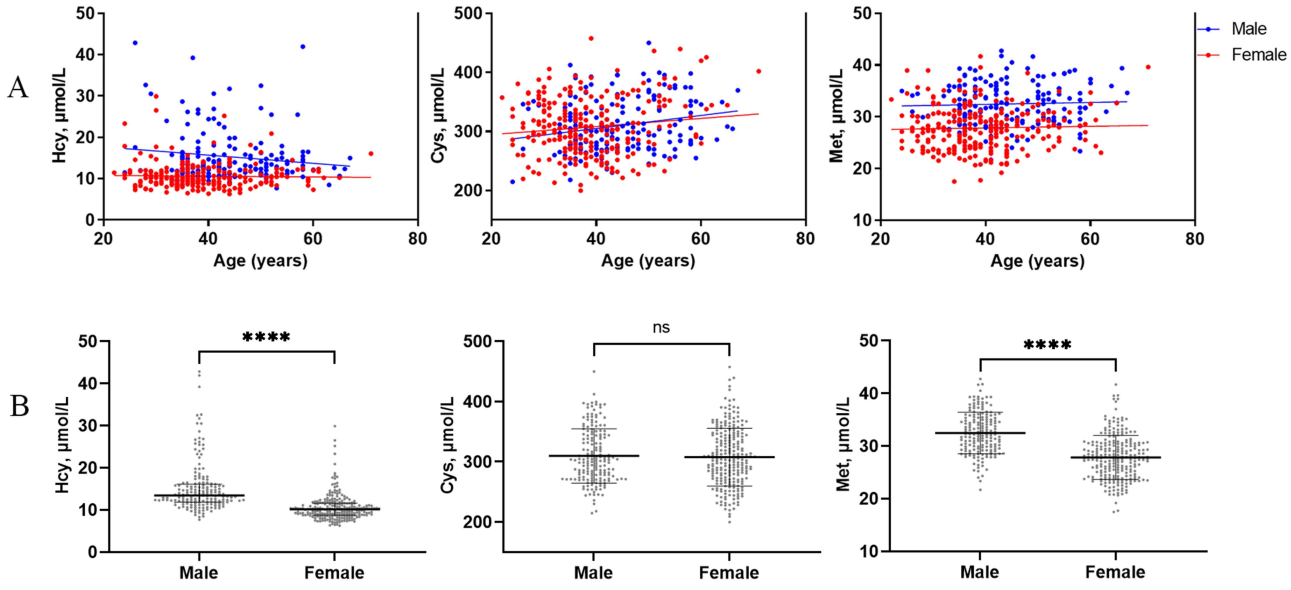

Figure 2 The effect of age (**A**) and sex (**B**) on the distribution of serum homocysteine, cysteine and methionine.
Notes: (**A**) This line represents simple linear regression across sexes; (**B**) The center lines represent the mean or median. Student's *t*-test or Mann–Whitney *U*-test was used to compare between sexes. ****, P < 0.0001, ns, not significant (P ≥ 0.05).

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Table 2 The Central 95% Reference Intervals

Analyte	N	Mean ± SD	95% CI	RIs
Hcy	415	0.980 ± 0.032	0.917–1.043	7–25
Cys	426	4.988 ± 0.114	4.765–5.211	227–410
Met	424	29.76 ± 4.68	20.59–38.93	21–39

Notes: Hcy and Cys were transformed into normal distribution by Box-Cox, and the mean ± 1.96SD was used to calculate the reference interval; Units is μmol/L;
Abbreviations: N, number; CI, confidence interval.

Establishment of RIs

Although Figure 2B shows that Hcy and Met levels are significantly lower in women than in men, with significant differences, results calculated by Z-score and s2/s1 grouping (Supplementary Table 2) do not support sex-specific reference interval establishment. After excluding outliers from the 426 reference individuals, 415, 426, and 424 individuals were included in the establishment of RIs for Hcy, Cys, and Met, respectively. Met data followed a normal distribution, and thus its reference interval was calculated directly using the raw data. In contrast, the original data for Hcy and Cys did not conform to a normal distribution. Therefore, a Box-Cox transformation was applied to normalize the data, with the transformation parameter (λ) set at −0.908 for Hcy and −0.049 for Cys. Following the transformation, the mean and standard deviation of the transformed Hcy and Cys values were calculated. Parametric methods were then used to compute the two-sided 95% reference intervals for Met, as well as for the transformed Hcy and Cys data. Through this approach, the reference intervals for the normalized Hcy and Cys values were established. Finally, the upper and lower limits of the transformed Hcy and Cys reference intervals were back-transformed to their original scales to determine the reference intervals for the raw data. Detailed results are presented in Table 2.

Healthy Individuals Versus CA Patients

The interrelationships among the three indicators in the Hcy metabolic pathway appear to be altered in disease states. As shown in Figure 3A, Hcy, Cys and Met were significantly correlated in the healthy control group ($r > 0.21$, $P < 0.001$). However, in the CA patient group (Figure 3B), these correlations were attenuated, with only a weak correlation observed between Hcy and Cys ($r = 0.27$, $P = 0.018$). In addition, we further analyzed the differences between healthy individuals and CA patients. As shown in Figure 4, Hcy levels were significantly higher in CA patients (12.48 [9.86, 16.00] μmol/L) than in the healthy control group (11.18 [9.21, 13.10] μmol/L; $P = 0.0012$). In contrast, Cys and Met levels were lower in CA patients than in the healthy control group (Cys: 262.40 ± 49.08 μmol/L vs 284.00 ± 33.95 μmol/L, $P < 0.0001$; Met: 24.49 [21.48, 26.94] μmol/L vs 29.14 [26.27, 32.40] μmol/L, $P < 0.0001$).

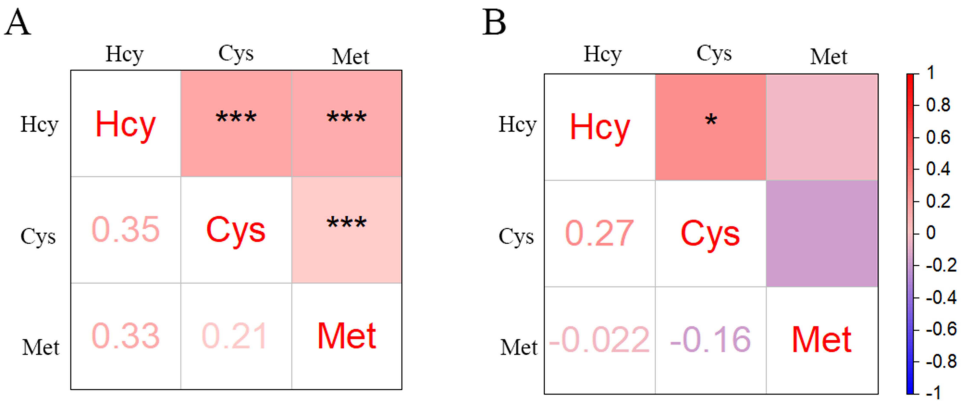

Figure 3 Correlation assessment of serum homocysteine, cysteine and methionine.
Notes: (**A**) Healthy individuals; (**B**) Patients with coronary atherosclerosis. The purple and red numbers represent the correlation coefficient, *, $P < 0.05$; ***, $P < 0.001$.

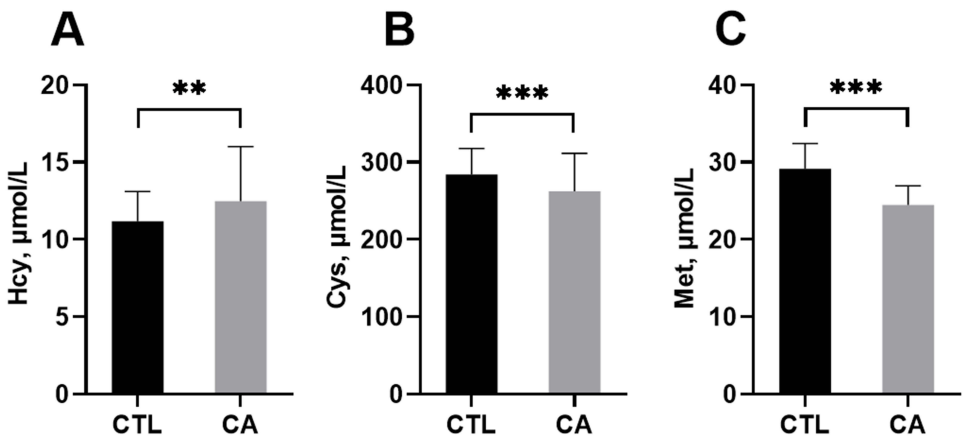

Figure 4 Comparison of homocysteine (**A**), cysteine (**B**) and methionine (**C**) levels between healthy individuals and coronary atherosclerosis patients.
Notes: Student's *t*-test or Mann–Whitney *U*-test was used to compare between groups. **: P < 0.01, ***: P < 0.001.
Abbreviations: CTL, healthy individuals; CA, coronary atherosclerosis.

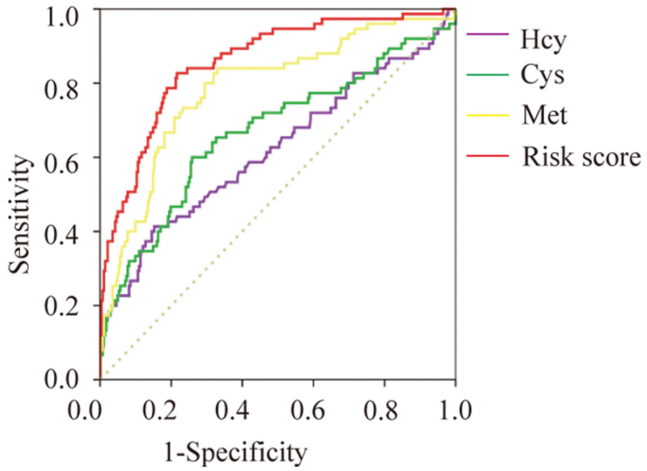

Figure 5 Receiver operating characteristic (ROC) curve of the risk score based on homocysteine (Hcy), cysteine (Cys) and methionine (Met) for predicting coronary atherosclerosis.

Risk Assessment of CA

The metabolites involved in the Hcy metabolic pathway may serve as potential biomarkers for assessing the risk of CA. As shown in Figure 4, significant differences were observed in all three indicators between the healthy control and CA patient groups. Higher Hcy levels and lower Cys and Met levels may indicate an increased risk of CA. Multinomial logistics regression analysis analysis (Supplemental Table 3) showed that Hcy, Cys, and Met were independent factors associated with CA. Receiver operating characteristic (ROC) curve analysis revealed that the area under the curve (AUC) for Hcy (0.62; 95% CI: 0.54–0.70, cutoff=14.33 μmol/L), Cys (0.67; 95% CI: 0.59–0.74, cutoff=261.88 μmol/L), and Met (0.79; 95% CI: 0.73–0.85, cutoff=27.64 μmol/L) indicated their predictive value for assessing CA risk. When the three indicators were combined, the composite AUC increased to 0.86 (95% CI: 0.81–0.91), with a specificity of 83% and a sensitivity of 78% (Figure 5).

Discussion

A comprehensive analysis of the Hcy metabolic pathway can substantially enhance the diagnostic, predictive, and prognostic utility of traditional single biomarkers, offering a more integrated framework for evaluating human health risk. Therefore, this study established RIs for Hcy, Cys, and Met in Chinese adults (Table 2) and preliminarily

investigated changes in Hcy, Cys, and Met between healthy individuals and CA patients, as well as their potential for disease risk assessment (Figure 5).

Consistent with previous reports, our findings revealed sex-related differences for Hcy and Met, with higher concentrations observed in males than in females.[25,29,30] Beyond the influence of folic acid and vitamin B12, sex differences in Hcy levels may be attributed to various contributing factors, including lifestyle habits such as diet, physical activity, sleep duration, smoking, and alcohol consumption.[31] Furthermore, Hcy remethylation status,[32] creatinine levels, lean body mass, and sex hormones (eg, testosterone and estradiol)[33] also contribute to these sex-specific differences. In line with previous studies,[25,33] our results demonstrated a positive correlation between Hcy and Met levels and blood creatinine, with higher concentrations observed in males compared to females. Estrogen levels, rather than nutritional status or lean body mass, were identified as the primary determinants of Hcy levels, after adjusting for smoking, creatinine, and folic acid.[34] Furthermore, the study reported a significant increase in plasma Hcy levels following androgen therapy in female-to-male transsexuals, whereas Hcy levels were markedly reduced after the administration of estrogen combined with anti-androgen therapy in male-to-female transsexuals.[35] In our study, Hcy and Met levels also showed a positive correlation with hemoglobin and were higher in males, as illustrated in Supplemental Figure 1. This may be attributed to differences in erythropoiesis and hemoglobin synthesis between estrogen and androgen exposure. These findings underscore the critical role of sex hormone levels in modulating the expression of Hcy and Met. As sex hormone quantification was not performed in this study, the specific contribution of sex hormones to these findings could not be assessed.

The current studies on Hcy RIs are comprehensive and include studies on sex, different age groups and specific populations such as young adults, pregnant females and the MTHFR C677T genotype.[29,36,37] Hcy RI varies considerably between two races such as Indians and Chinese.[38] The reference interval for Hcy in this study was relatively higher compared to other studies, potentially due to regional variations. Qu et al reported higher Hcy levels in northern China.[39] Coincidentally, Yang et al reported a significant increase in the frequency of the MTHFR C677T gene mutation in northern populations compared to those in the south,[40] indirectly explaining the geographic influence. At present, there is only one indirect study focusing on the RIs of three indicators in the Hcy metabolic pathway. In this study, no significant difference was observed between the RIs for Cys and Met established through the direct method.[25] RIs for Cys have been rarely reported, and the primary population for Met is children, which complicates the comparison of RIs. Furthermore, most studies have reported sex differences for Hcy and Met, but not for Cys. Previous reports on the RIs for Hcy, Cys, and Met are summarized in Supplemental Table 4.

Several studies have shown that both Hcy and Cys are independent predictors of coronary artery disease, and that Met is also associated with the risk of acute coronary events.[9,15,22] In this study, Hcy levels were found to be elevated in CA patients, consistent with the findings of Wu et al.[41] Surprisingly, both Cys and Met levels decreased, and the presence of CA disrupted the correlation between these three indicators, as shown in Figure 3A and B. In the investigation of CA risk assessment, it was found that the three indicators were independent risk factors for CA, and the predictive ability was greatest when they were combined, as shown in Figure 5. However, due to the small sample size in this study and the inclusion of healthy adults in the control group, the results require further validation. The methionine–homocysteine–cysteine axis balances methylation and redox tone. When folate/B12 or B6 is lacking, re-methylation or transsulfuration falters.[42] Hcy and thiolactone accumulate and injure endothelium; excess methionine and compensatory cysteine further oxidize LDL and deplete H_2S.[43] Our finding of low Cys and Met despite high Hcy suggests dual-pathway blockade that accelerates atherosclerosis beyond lipids.

This study has the following advantages. (1) Unlike the indirect method, we screened the target medical examination population using a questionnaire, which allowed for the immediate collection of clinical information about the reference population. This approach enabled the direct exclusion of factors such as diseases or medications known to affect Hcy metabolism, thus compensating for the limitations of the retrospective nature and incomplete baseline information of the indirect method. As a result, the reference intervals (RIs) obtained in this study are more accurate, reliable, and reflective of the target population compared to those derived from the indirect method. (2) Unlike the study of Hcy alone, we have also established RIs for Cys and Met, other key indicators of the Hcy pathway. First, this enables clinicians to better identify the underlying causes of changes in Hcy levels within the pathway and more effectively target treatment.

Second, increasing evidence suggests that Cys and Met are closely associated with cardiovascular disease, although there are fewer reports on the reference intervals for these indicators. (3) This study utilizes in vitro diagnostics rather than laboratory-developed tests, which involve more steps in a standardized process. This approach offers clear advantages in terms of standardization, reliability, and reproducibility. Nevertheless, the establishment of reference measurement procedures, reference laboratories, and standardized testing methods remains essential.

The present study has the following limitations: (1) Only preliminary differences and trends in CA patients could be explored due to the limited sample size, and the findings need to be validated in a larger cohort study. (2) The overall age of CA patients was high, and medication use as well as related complications were not considered. (3) The effect of age on the Hcy, Cys, and Met RIs was not assessed due to limitations in human and financial resources. (4) The individuals included in the RIs were all from a single medical center.

Conclusion

We established RIs of the Hcy metabolic pathway in the Chinese population using direct continuous sampling at two centers. These RIs are suitable for LC-MS/MS analysis using kit products with standardized and complete traceability chains. Additionally, the preliminary exploration of CA disease risk assessment revealed that Hcy, Cys, and Met are independent risk factors, and the combination of these three indicators significantly enhances the ability to assess risk. This approach provides valuable references for disease diagnosis and risk prediction.

Data Sharing Statement

The datasets utilized and/or analyzed in this study can be obtained from the corresponding author upon reasonable request.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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