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ORIGINAL RESEARCH

The Diagnostic Value of Bile Acids and Amino Acids in Differentiating Acute Coronary Syndromes

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Purpose: Acute coronary syndrome (ACS), comprising unstable angina and acute myocardial infarction, is the most dangerous and fatal form of coronary heart disease. This study evaluates serum bile acids (BAs) and amino acids (AAs) as potential predictors of AMI in UA patients.

Patients and Methods: A total of 72 Non-Coronary Artery Disease (NCAD) patients, 157 UA patients, and 79 AMI patients were analyzed. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) measured 15 bile acids and 19 amino acids. The data was split into training and validation sets (7:3). Univariate and multivariate analyses were performed. Diagnostic value and clinical benefits were assessed using receiver operating characteristic (ROC) curves, decision curve analysis, and metrics such as the area under the curve (AUC), integrated discrimination improvement (IDI), and net reclassification improvement (NRI).

Results: Orthogonal partial least squares discriminant analysis (OPLS-DA) of serum BAs and AAs effectively differentiated NCAD, UA, and AMI groups. The differences in serum BA and AA profiles between UA and AMI patients were primarily driven by four metabolites: deoxycholic acid (DCA), histidine (His), lysine (Lys), and phenylalanine (Phe). Together, they had an AUC of 0.830 (0.768 in the validation cohort) for predicting AMI in UA patients. After adjusting for multiple confounding factors, DCA, His, Lys, and Phe were independent predictors distinguishing UA from AMI. The results of AUC, IDI, and NRI showed that adding these four biomarkers to a model with clinical variables significantly improved predictive value, which was confirmed in the validation cohort.

Conclusion: These findings highlight the association of DCA, His, Lys, and Phe with AMI, suggesting their potential role in AMI pathogenesis.

Keywords: bile acid, amino acid, unstable angina, acute myocardial infarction, metabolomics

Introduction

Cardiovascular diseases (CVD)[1] are among the leading causes of death worldwide and a significant contributor to the global disease burden. Among CVDs, acute coronary syndromes (ACS), including unstable angina and acute myocardial infarction, are particularly critical in clinical diagnosis and treatment.[2] Unstable angina (UA) represents an acute exacerbation of angina, where patients experience frequent and prolonged chest pain at rest or with minimal exertion, indicating worsening myocardial ischemia and a high risk of myocardial infarction. Acute myocardial infarction often presents insidiously but progresses rapidly, potentially leading to severe complications such as cardiac arrest, acute heart failure,[3] and cardiogenic shock,[4] with a poor prognosis. Studies have shown that AMI induces cell necrosis and triggers complex inflammatory responses,[5–7] which affect infarct size, cardiac function,

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and clinical outcomes. Current biomarkers for diagnosing AMI, such as myocardial enzymes and cardiac troponin, are detectable only after myocardial damage, offering limited insight into its underlying mechanisms. While myoglobin is an early marker, its specificity is limited due to rapid clearance and its presence in skeletal muscle injuries. Therefore, it is crucial to identify novel biomarkers for early AMI detection and elucidate its underlying mechanisms.[8–10]

During the progression of coronary artery disease, there is a decrease in phospholipid catabolism and the tricarboxylic acid cycle. In contrast, amino acid metabolism and short-chain carnitine levels increase, and the biosynthesis of primary bile acids decreases.[11] Thus, serum metabolomics is highly valuable in describing metabolic disorders. The differences in small molecule metabolites may reflect the underlying presence of coronary artery disease and serve as biomarkers during its progression. Bile acids[12,13] and amino acids[14] are associated with a high inflammatory burden and regulate the immune system through various mechanisms, either enhancing or suppressing inflammatory responses. Additionally, bile acids play a critical role in regulating lipid, glucose, and amino acid metabolic homeostasis by targeting the Farnesoid X receptor (FXR) and other nuclear receptors. Therefore, studying the bile acid and amino acid profiles in the serum of patients with UA and AMI is significant.

Bile acids are endogenous metabolites synthesized from cholesterol in the liver and can be further modified by gut microbiota. As important metabolic and signaling molecules in the body, bile acids are crucial in regulating metabolic pathways, such as lipid, glucose, and amino acid metabolism, and maintaining gut microbiota homeostasis.[15] Previous studies have emphasized the potential harmful effects of bile acids in cardiovascular diseases. Li et al[16–18] found that lower fasting serum total bile acid levels were closely associated with coronary artery disease, myocardial infarction, and the severity of coronary artery lesions. However, the variation in bile acid composition and its role in UA and AMI in humans remains unclear.

Epidemiological studies have shown that various AAs are associated with CVDs. Amino acid metabolism plays a critical role in regulating and maintaining vascular functions, including vascular tone, coagulation and fibrinolysis, cell growth and differentiation, redox homeostasis, and immune and inflammatory responses.[19] Observational studies have found that certain AAs, such as glycine, arginine, and tryptophan,[20–22] may be linked to a lower risk of cardiovascular disease. However, higher levels of other amino acids, including branched-chain amino acids (BCAAs, composed of valine, leucine, and isoleucine), are associated with an increased risk of cardiovascular diseases.[23,24] Nevertheless, the changes in amino acids and their relationship in patients with UA and AMI remain unclear.

In this study, we conducted a targeted metabolomics analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify the levels of 15 BAs and 19 AAs in the serum of patients with UA and AMI. We characterized the absolute concentrations of these metabolites in serum and analyzed their correlations with clinical indicators and disease risk. These findings may offer new insights and targets for the early diagnosis and precise treatment of UA and AMI.

Materials and Methods

Study Population

Based on the inclusion and exclusion criteria, a total of 308 participants were included in the study. Patients were divided into training and validation cohorts using a 7:3 random sampling ratio, ensuring no significant differences in age, gender, or BMI. The training cohort consisted of 72 Non-Coronary Artery Disease (NCAD), 113 unstable angina (UA) patients, and 55 acute myocardial infarction (AMI) patients. The validation cohort included 44 UA patients and 24 AMI patients. All patients were enrolled at Huludao Central Hospital between August 2023 and June 2024.

This study was conducted by the Helsinki Declaration. Informed consent was obtained from all participants, and the study was approved by the Ethics Committee of Huludao Central Hospital (Approval No: 202319).

Inclusion and Exclusion Criteria

In this study, we included patients admitted for chest discomfort or pain with cardiovascular risk factors, ischemic electrocardiogram changes, or elevated cardiac enzymes, all of whom required coronary CT or angiography for confirmation. Patients with autoimmune diseases, malignancies, vascular, liver, kidney, thyroid, or infectious diseases were excluded, as were those who had undergone recent surgery or trauma or those with severe heart failure (left ventricular ejection fraction

<20%). Unstable angina was diagnosed based on recent angina episodes, absence of elevated troponin, and irregular angina at rest or during minimal exertion. AMI was defined by ischemic chest pain, elevated cardiac enzymes and troponin, and ST-T changes on the electrocardiogram.[25,26] NCAD was defined as coronary stenosis of less than 50%, confirmed by imaging techniques such as coronary angiography or computed tomography angiography.[27]

Physical and Laboratory Examination

The height, weight, right upper limb blood pressure in the supine position, and body mass index (BMI, kg/m²) of eligible patients were measured. After admission, Blood samples for UA and NCAD patients were collected after an 8-hour fast to ensure consistency in metabolic measurements. For AMI patients, samples were obtained as soon as clinically feasible following stabilization, given the urgent nature of their condition. Biochemical indicators measured included fasting glucose (GLU), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein (VLDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), urea, creatinine (CREA), creatine kinase (CK), creatine kinase-MB (CK-MB) , and total bile acids (TBA).

BAs and AAs Measurement and Classification

All participants had peripheral blood samples collected under fasting conditions upon admission. After centrifugation, the serum was stored at −80°C until analysis. Serum BAs and AAs were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS, AB SCIEX Jasper™ HPLC-Triple Quad™ 4500MD).

For BAs, serum samples were pretreated with organic solvent to remove proteins, followed by nitrogen evaporation and reconstitution. Fifteen bile acid subtypes were quantified using multiple reaction monitoring (MRM) in ESI negative mode, with separation on an Acquity UPLC BEH C18 column (1.7 μm, 2.1 mm × 100 mm; Waters). For AAs, serum samples were pretreated with organic solvent to remove proteins, and amino acids were derivatized with dansyl chloride. The supernatant after derivatization was analyzed, and 19 amino acids were quantified in MRM ESI positive mode, using a CORTECS T3 column (2.1 mm × 100 mm, 2.7 μm; Waters) for separation. The specific mass spectrometry (MS) conditions and liquid chromatography (LC) parameters are provided in the supplementary materials (Table S1–S5).

Bile acids (BAs) were divided into four groups based on their conjugation: (1) Unconjugated primary BAs, including cholic acid (CA) and chenodeoxycholic acid (CDCA); (2) Conjugated primary BAs, including glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), taurocholic acid (TCA), and taurochenodeoxycholic acid (TCDCA); (3) Unconjugated secondary BAs, including deoxycholic acid (DCA), ursodeoxycholic acid (UDCA), and lithocholic acid (LCA); (4) Conjugated secondary BAs, including glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), glycolithocholic acid (GLCA), taurolithocholic acid (TLCA), glycoursodeoxycholic acid (GUDCA), and tauroursodeoxycholic acid (TUDCA). Additionally, the following 19 amino acids were analyzed: aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), taurine (Tau), alanine (Ala), proline (Pro), cysteine (Cys), tyrosine (Tyr), valine (Val), methionine (Met), lysine (Lys), isoleucine (Ile), leucine (Leu), and phenylalanine (Phe). The absolute concentrations of each amino acid were expressed in μmol/L.

Statistical Analysis

All analyses were performed using R (version 4.3.2), SIMCA (version 14.1.0), and SPSS (version 25.0). The sample size was determined using PASS software (version 22.0) to ensure sufficient statistical power for the diagnostic model. Continuous variables with a normal distribution were expressed as mean ± standard deviation (SD), while non-normally distributed variables were presented as median (M) and interquartile range (IQR). Categorical variables were displayed as counts and percentages. The *t*-test was used to analyze normally distributed continuous variables, and the Kruskal–Wallis test (for three groups) or Mann–Whitney *U*-test (for two groups) was applied for non-normally distributed variables. Categorical variables were compared using the chi-square test. Univariate and multivariate analyses were performed to identify differential metabolites. We used the *t*-test or Mann–Whitney *U*-test for univariate analysis, depending on the distribution. Orthogonal partial least squares discriminant analysis (OPLS-DA) was applied for multivariate analysis, with potential biomarkers selected based on two criteria: 1) adjusted p-value (p.adj) < 0.05

using the false discovery rate method, and 2) variable importance in projection (VIP) ≥ 1. The performance of the model in OPLS-DA was assessed using a 200-permutation test, R2X, R2Y, and Q2.

Spearman correlations were used to examine the relationships between biomarkers, clinical indicators, and other metabolites. Logistic regression identified and validated the predictive roles of bile acids (BA) and amino acids (AA) in acute myocardial infarction (AMI), with three models developed: biomarkers alone, clinical indicators alone, and a combined model. Adding biomarkers improved predictive performance, as assessed through ROC curve analysis, where AUC, IDI, and NRI showed significant improvements. IDI > 0 and NRI > 0 indicated better predictive validity. Decision curve analysis further evaluated the clinical utility of the models.

Results

Clinical and Metabolic Characteristics of UA and AMI Patients

In both the training and validation sets, there were no statistically significant differences in age, gender, BMI, height, and weight between UA and AMI patients (Table 1) ($p>0.05$). Moreover, Table 1 indicates that levels of ALT, AST, CK, and CK-MB were higher in AMI patients compared to UA patients in both the training and validation sets ($p<0.05$). These findings confirm the comparability of UA and AMI patients across the training and validation cohorts.

Univariate Analysis of BAs and AAs Profiles in UA and AMI Patients

The concentrations of 15 serum BAs and 19 AAs in patients with UA and AMI are shown in Table S6. Significant differences were observed between the UA and AMI groups for several BAs and AAs, including DCA, TDCA, UDCA, His, Lys, Thr, TLCA, Met, CDCA, Trp, CA, GDCA, and Phe ($P <0.05$) (Table S6). After FDR correction, DCA, TDCA, His, Lys, and Phe remained statistically significant (p.adj <0.05) (Table S7). Compared to the UA group, the AMI group exhibited decreased levels of DCA, TDCA, His, and Lys (p.adj <0.05) and increased levels of Phe (p.adj <0.05) (Table S6).

Multivariate Analysis of BAs and AAs Profiles in UA and AMI Patients

Initially, a multivariate analysis was conducted using the OPLS-DA model on 15 BAs and 19 AAs. This analysis demonstrated that BAs and AAs significantly contributed to distinguishing different groups, as shown in the OPLS-DA 3D plots (Figure 1). The OPLS-DA score plot clearly showed differences among the three groups, with the model's goodness of fit and predictability indicated by R2X=0.425, R2Y=0.287, and Q2Y=0.196 in the training cohort (Figure 1A). To confirm the model was not overfitted, 200 permutation tests were conducted, showing Q2 values of less than 0.05 [Q2=(0.0, −0.128)] for the three-group analysis (Figure 1B). Similar separations were observed for the UA and AMI groups (Figure 1C), with further permutation tests confirming Q2 values of less than 0.05 [Q2=(0.0, −0.266)] (Figure 1D). Potential differential metabolites were identified through statistical analysis, followed by both univariate and multivariate analyses (Table S8, Figure 1, and Figure S1). Our findings indicated that DCA (p.adj =0.034, VIP=1.24), TDCA (p.adj =0.041, VIP=1.039), His (p.adj =0.034, VIP=1.39), Lys (p.adj =0.043, VIP=1.40), and Phe (p.adj =0.034, VIP=1.45) are potential biomarkers for distinguishing UA from AMI (Figure 2 and Table S8).

Associations Between Metabolites and Clinical Indicators or Metabolites in Three Groups of Patients

Using Spearman correlation analysis, we identified statistically significant correlations between various metabolites in the training cohort ($p<0.05$) (Figure 3A). Weak to moderate correlations were observed between Phe and TCDCA ($r = 0.220$, $p < 0.01$), Phe and TUDCA ($r = 0.176$, $p < 0.01$), Phe and TCA ($r = 0.202$, $p < 0.01$), and DCA and Pro ($r = 0.224$, $p < 0.01$). Strong correlations were found between Phe and Lys ($r = 0.472$, $p < 0.01$), His and Asn ($r = 0.541$, $p < 0.01$), and Lys and Leu ($r = 0.570$, $p < 0.01$). Additional correlations included DCA and Ala ($r = 0.137$, $p < 0.05$), DCA and Gly ($r = 0.152$, $p < 0.05$), His and Met ($r = 0.442$, $p < 0.01$), Lys and TCDCA ($r = 0.132$, $p < 0.05$), and Lys and Ile ($r = 0.469$, $p < 0.01$).

Correlation analysis between differential bile acids and amino acids with clinical indicators revealed that TG, TC, VLDL-C, and ALT were positively correlated with several AAs (Trp, Phe, Lys). In contrast, HDL-C was negatively

Table 1 Baseline Characteristics of All Patients in the Training Cohort and Validation Cohort

	Training Cohort					Validation Cohort		
Variables	**NCAD**	**UA**	**AMI**	**p value1**	**p value2**	**UA**	**AMI**	**p-value**
	n=72	**n=113**	**n=55**	**(3Groups)**	**(UA vs AMI)**	**n=44**	**n=24**	
Age, year	60.83±10.00	63.08±9.59	62.96±12.14	0.185	0.692	64.25±9.82	61.17±11.78	0.253
Male, sex	29 (40.28%)	76(67.26%)	37 (67.27%)	<0.001*	0.998	23(52.27%)	17(70.83%)	0.198
BMI, kg/m2	25.11±3.11	25.32±3.44	24.44±3.80	0.185	0.094	24.60±3.69	25.84±4.74	0.237
Weight, kg	67.75(60.00,75.00)	70.00(60.00,80.00)	70.00(60.00,75.00)	0.391	0.273	67.90±12.71	69.50±12.14	0.616
Height, cm	165.00(160.00,170.00)	168.00(160.00,173.00)	170.00(160.00,174.50)	0.148	0.986	165.50(159.00,170.00)	165.00(160.00,170.00)	0.832
SBP, mmHg	137.50(128.50,150.50)	140.00(128.00,160.00)	131.00(117.00,141.50)	0.002*	0.001*	139.00(132.00,157.50)	134.50(127.50,159.00)	0.289
DBP, mmHg	80.64±10.16	79.69±12.20	79.02±14.80	0.559	0.755	82.00(75.50,88.00)	84.00(73.50,90.00)	0.676
Glu, mmol/l	5.33(4.94,5.95)	5.59(5.18,6.37)	5.64(5.02,6.22)	0.052	0.466	5.58±0.93	6.14±0.89	0.017*
TC, mmol/L	4.65(3.87,5.26)	4.02(3.54,4.96)	4.62(3.81,5.22)	0.044*	0.091	4.69±0.93	4.63±0.99	0.792
TG, mmol/L	1.46(1.02,2.18)	1.48(1.03,2.18)	1.69(1.01,2.16)	0.956	0.97	1.46(1.02,2.32)	1.72(1.13,2.51)	0.369
HDL-C, mmol/L	1.17(0.93,1.31)	1.04(0.88,1.25)	0.92(0.78,1.10)	<0.001*	0.005*	1.08(0.96,1.38)	0.97(0.82,1.14)	0.004*
LDL-C, mmol/L	2.89(2.26,3.34)	2.50(2.02,3.20)	3.12(2.33,3.77)	0.014*	0.009*	2.99±0.89	3.04±0.86	0.848
VLDL, mmol/l	0.30(0.21,0.44)	0.29(0.21,0.43)	0.35(0.23,0.44)	0.701	0.414	0.31(0.22,0.46)	0.35(0.23,0.54)	0.397
UA, mmol/L	299.00(250.15,349.50)	318.00(262.00,372.20)	321.00(268.45,380.25)	0.246	0.578	300.85±84.78	312.73±70.25	0.561
UREA, mmol/l	5.03(4.36,5.66)	5.12(4.34,6.55)	5.55(4.63,6.74)	0.094	0.231	4.88(4.08,5.51)	5.29(4.55,6.04)	0.158
CREA, mmol/l	67.10(60.00,76.50)	72.00(61.00,83.00)	77.00(61.75,88.60)	0.112	0.32	62.50(56.00,72.50)	75.95(59.70,88.00)	0.025*
TBA, μmol/l	2.15(1.30,3.65)	2.50(1.40,4.00)	2.00(1.00,3.05)	0.15	0.062	2.90(1.35,4.05)	1.70(1.05,3.90)	0.213
ALT, U/L	16.90(13.25,24.05)	19.10(13.30,25.30)	26.90(17.90,34.20)	<0.001*	<0.001*	18.25(13.15,25.70)	32.60(17.70,45.06)	0.007*
AST, U/L	18.70(16.30,21.40)	18.30(15.50,22.56)	42.00(27.05,89.55)	<0.001*	<0.001*	18.00(15.37,22.89)	72.70(27.55,182.45)	<0.001*
CK, U/L	77.59(51.80,115.45)	86.40(58.00,128.10)	213.00(83.35,633.85)	<0.001*	<0.001*	80.40(49.50,102.65)	517.40(177.65,1365.40)	<0.001*
CK-MB, ng/mL	14.25(11.10,19.40)	14.60(11.70,19.10)	26.90(16.30,70.75)	<0.001*	<0.001*	13.75(11.53,18.15)	44.60(28.25,153.55)	<0.001*

Notes: Values are expressed as n (%), mean ± SD, or median (IQR). The *t*-test was used for comparisons of normally distributed continuous variables, while the Kruskal–Wallis test (for three groups) or Mann–Whitney *U*-test (for two groups) was applied to non-normally distributed variables (*P < 0.05).

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; GLU, glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL, very low-density lipoprotein; UA, uric acid; CREA, creatinine; TBA, total bile acids; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CK-MB, creatine kinase-MB.

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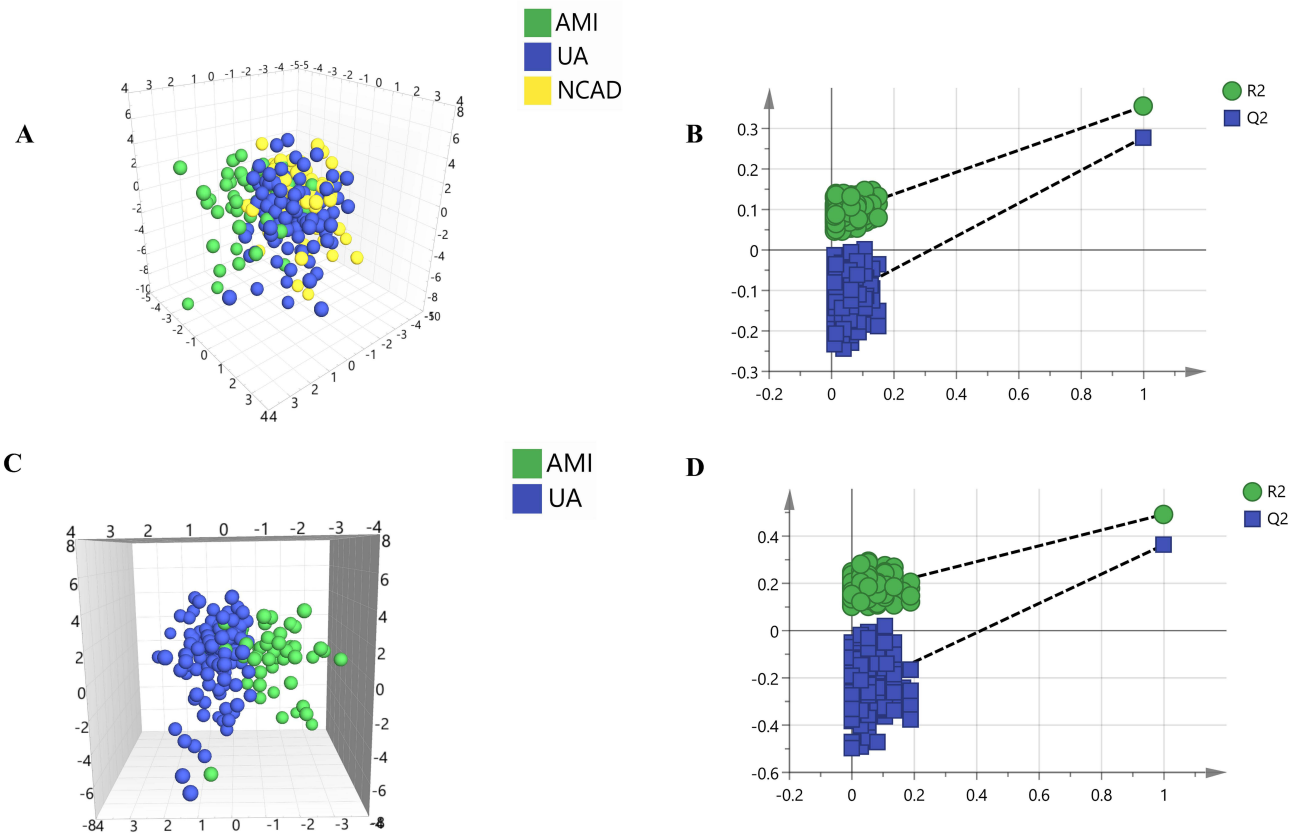

Figure 1 Metabolomic profiling by targeted metabolomics in the training cohort. (**A**) OPLS−DA 3D models among NCAD, UA, and AMI groups. R2X = 0.425, R2Y = 0.287, and Q2Y = 0.196. (**B**) The 200−permutation test demonstrated no overfitting in the OPLS-DA model [Q2 = (0.0, −0.128)]. (**C**) OPLS−DA 3D models between UA and AMI groups. R2X = 0.416, R2Y = 0.492, and Q2Y = 0.263. (**D**) The 200−permutation test demonstrated no overfitting in the OPLS-DA model [Q2 = (0.0, −0.266)].

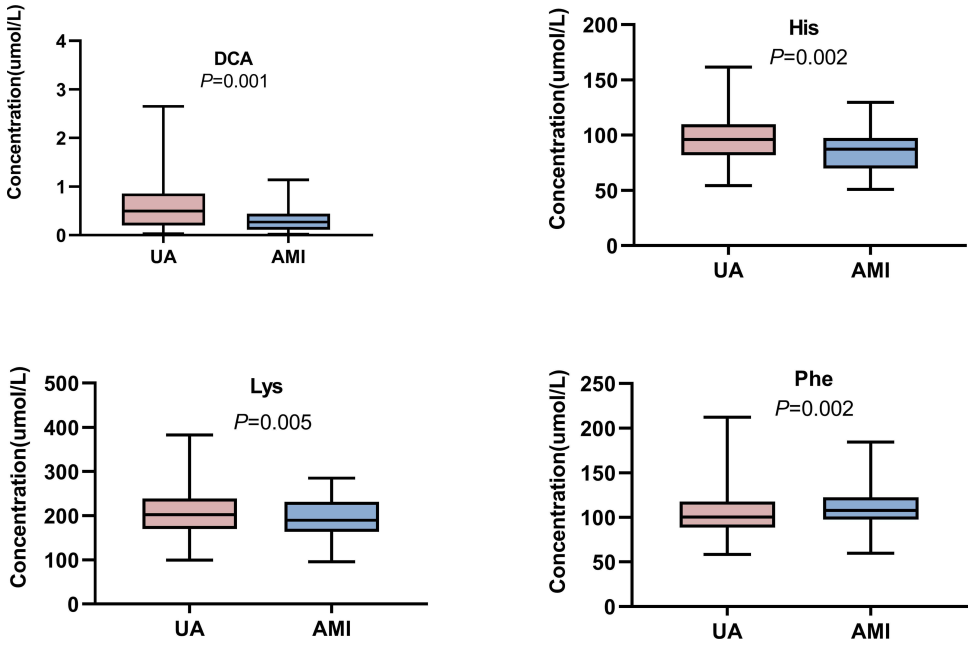

Figure 2 Markers of bile acid and amino acid differences between the UA and AMI.

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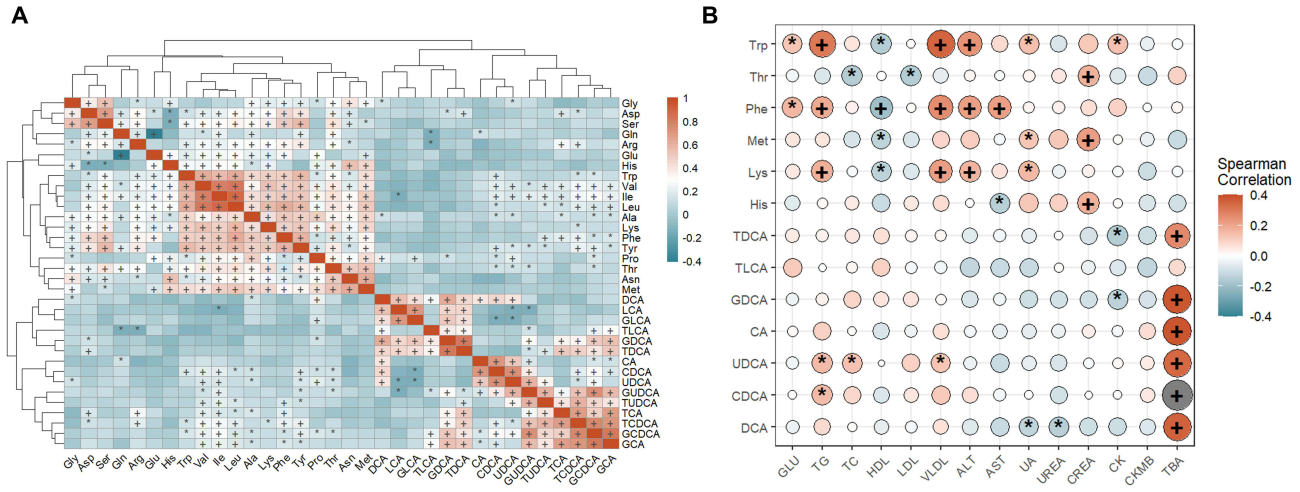

Figure 3 Clustering Analysis and Correlation of Serum Metabolites and Clinical Indicators. (**A**) Clustering analysis of serum bile acids and amino acids levels in patients with non-coronary artery disease (NCAD), unstable angina (UA), and acute myocardial infarction (AMI). (**B**) Heatmap of Spearman correlation analysis between differential bile acids, amino acids, and clinical indicators. (**A** and **B**) *p<0.05, +p<0.01.

Notes: The circles' size and transparency indicate the correlation's strength; larger circles with deeper colors represent stronger correlations between the corresponding bile acid and clinical indicators. Orange indicates a positive correlation, while blue indicates a negative correlation.

correlated with these AAs (Trp, Phe, Lys) (Figure 3B and Table S8). Additionally, TBA was positively correlated with DCA (r=0.33, p<0.01) (Table S8 and Figure S2).

Predictive Ability of Serum BAs and AAs in UA and AMI Patients

In this study, we conducted univariate and multivariate logistic regression analyses on differential metabolites to determine the potential of serum BAs and AAs in distinguishing between UA and AMI patients (Table 2). Initially, we performed univariate logistic regression analysis on DCA, TDCA, His, Lys, and Phe and selected the indicators with p<0.05 for multivariate logistic regression analysis. After adjusting for multiple confounding factors such as gender, age, BMI, ALT, AST, HDL-C, LDL-C, and TBA, DCA, His, Lys, and Phe were identified as independent predictors for differentiating UA from AMI (Figure S3).

In Table 3, the AUCs for metabolites DCA, His, Lys, and Phe were 0.655 (p<0.05), 0.649 (p<0.05), 0.632 (p<0.05), and 0.647 (p<0.05), respectively, indicating their potential as biomarkers for predicting AMI. The prediction model 1, based on DCA, His, Lys, and Phe, had an AUC of 0.830 (95% CI: 0.761, 0.899, p<0.05) (Figure S3A). Subsequently, we constructed prediction model 2 based on clinical factors, including HDL-C, LDL-C, ALT, AST, CK, and CK-MB.

Table 2 Univariate and Multivariate Analysis of BAs and AAs for Differentiating UA or AMI

Variable	Univariate Analyst		Multivariate Analyst	
	OR (95%)	p-value	OR (95%)	p-value
DCA	0.233(0.086,0.538)	0.002	0.193(0.058,0.525)	0.003
TDCA	0.000(0.000,1.91)	0.116	–	–
His	0.974(0.957,0.990)	0.002	0.974(0.953,0.994)	0.015
Lys	0.989(0.981,0.997)	0.006	0.976(0.963,0.987)	<0.001
Phe	1.021(1.007,1.035)	0.003	1.053(1.031,1.078)	<0.001

Notes: After adjusting for multiple confounding factors such as gender, age, BMI, ALT, AST, HDL-C, LDL-C, and TBA, DCA, His, Lys, and Phe were identified as independent predictors for differentiating UA from AMI.

Abbreviations: AUC, area under the curve; DCA, deoxycholic acid; TDCA, taurodeoxycholic acid; His, histidine; Lys, lysine; Phe, phenylalanine.

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Table 3 Performance of Different Models for the Prediction of AMI Patients

Model Number of Features		Training Cohort		Validation Cohort	
		AUC (95% CI)	p-value	AUC (95% CI)	p-value
Model1	4	0.830(0.761,0.899)	<0.001	0.768(0.652,0.884)	<0.001
Model2	7	0.903(0.853,0.953)	<0.001	0.991(0.979,1)	<0.001
Model3	11	0.961(0.933,0.989)	<0.001	1.000(1.000,1.000)	<0.001
DCA	1	0.655(0.470,0.570)	0.001	0.680(0.543,0.817)	0.015
His	1	0.649(0.561,0.737)	0.002	0.646(0.503,0.790)	0.047
Lys	1	0.632(0.538,0.725)	0.006	0.553(0.398,0.708)	0.472
Phe	1	0.647(0.588,0.737)	0.002	0.578(0.428,0.728)	0.29

Abbreviation: AUC, area under the curve.

Table 4 Discriminatory Capacity of Models with Clinical Variables Alone or with Biomarkers

Comparison	Index	Data Set	Estimate	95% CI low	95% CI up	p-value
Model3 vs Model2	Difference in AUC	Training	0.058	0.014	0.102	0.009
		Validation	0.021	0.004	0.021	0.195
	NRI	Training	0.994	0.717	0.271	<0.001
		Validation	1.542	1.342	1.741	0.038
	IDI	Training	0.192	0.122	0.263	<0.001
		Validation	0.161	0.056	0.266	0.003

Notes: Prediction Model 1 includes the biomarkers deoxycholic acid (DCA), histidine (His), lysine (Lys), and phenylalanine (Phe). Prediction Model 2 is based on clinical factors, including HDL-C, LDL-C, ALT, AST, CK, and CK-MB. Prediction Model 3 combines the elements of both Model 1 and Model 2.
Abbreviations: CI, confidence interval; AUC, area under the ROC curve; IDI, integrated discrimination improvement; NRI, net reclassification improvement.

Prediction Model 3 combined elements from Model 1 and Model 2, showing a significantly higher AUC compared to Model 2 (0.961 vs 0.903, P<0.05) (Table 3 and Figure S3A).

Table 4 demonstrates that incorporating the identified biomarkers with clinical indicators in the training set significantly enhanced the performance of the prediction model. Decision curve analysis further supports the clinical relevance of these biomarkers, showing that their combination with clinical indicators substantially increased net benefit (Figure S3B).

The Study of Four Potential Biomarkers in the Validation Cohort

In the validation cohort, the differences in BAs and AAs profiles between UA and AMI patients are shown in Table S5. The OPLS-DA 3D score plot demonstrates good separation between the two groups, with R2X=0.328, R2Y=0.564, and Q2Y=0.263 (Figure S4A). No overfitting was detected in the 200-time permutation test [Q2 = (0.0, −0.502)] (Figure S4B).

The AUCs for model 1, model 2, and model 3 were 0.768, 0.991, and 1, respectively. Similarly, model 3 showed better predictive performance in the validation cohort than model 2 (Table S9 and Figure S3C). However, the AUCs for models 1 and 2 did not reach statistical significance (P>0.05). These results indicate that, despite the lack of statistical significance in the validation cohort, the potential biomarkers still show promise in predicting AMI. When combined with clinical indicators, the models' predictive performance and clinical net benefit significantly improved (Figure S3D).

Discussion

In this study, we conducted a comprehensive analysis of serum bile acid and amino acid profiles in patients with UA and AMI, identifying four key biomarkers: DCA, His, Lys, and Phe. These biomarkers showed significant potential in distinguishing UA from AMI, highlighting distinct metabolic changes associated with the progression of these conditions. Notably, when integrated with traditional clinical indicators, these biomarkers not only enhanced the early diagnostic accuracy of AMI but

also offered a more personalized approach to cardiovascular disease management, underscoring the clinical importance of bile acid and amino acid metabolism in acute coronary syndromes. In the multivariate regression analysis, Phe, along with DCA, His, and Lys, was identified as an independent predictor for differentiating UA from AMI. In the training set, the combined predictive model incorporating these four biomarkers achieved an AUC of 0.830, which increased to 0.961 with the addition of clinical indicators. In the validation set, the predictive AUC improved from 0.768 to 1 when clinical indicators were included. Decision curve analysis further supported the clinical value of these biomarkers, demonstrating that their combination with clinical indicators significantly increased net benefit.

This study found that DCA levels were significantly lower in AMI patients compared to UA patients, demonstrating a protective effect in AMI patients. Research indicates that DCA is one of the potent activators of TGR5, and its protective effect following myocardial infarction is primarily achieved through its anti-inflammatory mechanisms. Activation of the DCA-TGR5 signaling pathway can alleviate inflammation and improve post-infarction cardiac function, thus exerting protective effects.[28] Wang et al[29] found that TGR5 regulates the function and distribution of CD4+ T cells in the heart, playing a protective role in myocardial infarction. The pathogenesis of acute myocardial infarction involves various metabolic disorders, among which the inflammatory response during post-infarction cardiac remodeling is crucial for cardiac repair. These metabolic changes can affect the overall inflammatory activation state, and increasingly, studies suggest that bile acid (BA) metabolites act as signaling molecules influencing various cardiovascular functions.[30] FXR agonists can improve post-myocardial infarction cardiac dysfunction and prevent post-infarction cardiac remodeling and dysfunction by stimulating adiponectin secretion.[31] Moreover, FXR knockout maintains cardiac function after myocardial infarction by reducing fibrosis and chronic apoptosis.[32] Deoxycholic acid (DCA), a secondary bile acid produced by gut bacteria,[33] plays a crucial role in improving inflammatory responses by regulating bile acid metabolism and associated signaling pathways, which has significant clinical implications for myocardial infarction patients.

The amino acid profile in UA and AMI is significantly influenced by inflammatory responses. Certain amino acids, such as arginine and tryptophan, are associated with a lower cardiovascular disease (CVD) risk, while elevated branched-chain amino acids (BCAAs) indicate higher risk.[24] Würtz et al reported that increased phenylalanine (Phe) levels and metabolic disruptions are linked to poor outcomes in critically ill patients.[34] Aromatic amino acids (AAAs), including Phe, tryptophan, and tyrosine, stimulate bile acid (BA) synthesis through classical and alternative pathways, exacerbating liver injury, promoting fat deposition, and contributing to lipid metabolism disorders.[35] Dysregulated Phe metabolism may disrupt amino acid pathways by interfering with the balance of other essential amino acids. Specifically, elevated Phe levels can competitively inhibit the transport and metabolism of tyrosine and tryptophan, critical precursors for neurotransmitters and metabolic intermediates. This imbalance impairs protein synthesis, oxidative stress response, and energy metabolism.[36,37] Furthermore, Phe dysregulation along the liver-heart axis has been linked to cardiac aging, providing a mechanistic explanation for its association with adverse cardiovascular outcomes.[38]

In this study, lysine (Lys) showed significant correlations with bile acids such as TCDCA and amino acids like leucine and isoleucine, suggesting that changes in Lys levels are associated with lipid metabolism disorders and inflammatory responses in UA and AMI patients.[39] The correlation between Lys and TCDCA further indicates that Lys may regulate lipid metabolism and inflammation via bile acid metabolism pathways, influencing coronary artery disease progression.[40] Similarly, Phe, a precursor of tyrosine involved in catecholamine synthesis, was significantly correlated with bile acids (such as TCDCA, TUDCA, and TCA) and amino acids (such as Lys). These findings suggest that Phe may contribute to cardiovascular disease progression by modulating tyrosine metabolism and bile acid pathways.[36] Additionally, histidine (His), an essential amino acid, has a dual role in the inflammatory response in cardiovascular diseases. Its metabolite histamine can exacerbate inflammation via H1 receptors or exert anti-inflammatory effects through H2 receptors.[41,42] Variations in His levels may reflect the patient's inflammatory state and oxidative stress levels, which are critical factors in the progression of AMI.[43]

This study has several limitations that need to be addressed in future research. First, potential confounding factors that could affect bile acid (BA) and amino acid (AA) concentrations, such as statin use and dietary factors, were not considered. Future studies should take these variables into account. Second, the small sample size limits the generalizability of the results, necessitating validation in larger populations. Finally, the study did not explore the specific mechanisms of action of BAs and AAs in-depth, and further research using animal models and cell experiments is recommended. Addressing these issues will help more accurately assess the clinical value of BAs and AAs in cardiovascular diseases.

Conclusion

In conclusion, Phenylalanine, Histidine, Lysine, and Deoxycholic Acid are Beneficial in Differentiating Unstable Angina from Acute Myocardial Infarction. The findings suggest a strong association between the progression from unstable angina to acute myocardial infarction and disruptions in BAs and AAs metabolism. Emerging serum biomarkers provide additional evidence for predicting these conditions and serve as a complement to existing diagnostic methods.

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.

References

1. Roth GA, Mensah GA, Johnson CO, et al. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. *J Ame College Cardiol*. 2020;76(25):2982–3021. doi:10.1016/j.jacc.2020.11.010
2. Barstow C, Rice M, McDivitt JD. Acute coronary syndrome: diagnostic evaluation. *Am Family Phys*. 2017;95(3):170–177.
3. Akhtar KH, Khan MS, Baron SJ, et al. The spectrum of post-myocardial infarction care: from acute ischemia to heart failure. *Prog Cardiovasc Dis*. 2024;82:15–25. doi:10.1016/j.pcad.2024.01.017
4. Kapur NK, Thayer KL, Zweck E. Cardiogenic SHOCK IN THE SETTING OF ACUTE MYOCARDIAL INFARction. *Methodist DeBakey Cardiovasc J*. 2020;16(1):16–21. doi:10.14797/mdcj-16-1-16
5. Matter MA, Paneni F, Libby P, et al. Inflammation in acute myocardial infarction: the good, the bad and the ugly. *Eur Heart J*. 2024;45(2):89–103. doi:10.1093/eurheartj/ehad486
6. Clerc OF, Haaf P, Buechel RR, Gaemperli O, Zellweger MJ. New therapies to modulate post-infarction inflammatory alterations in the myocardium: state of the art and forthcoming applications. *Curr Radiopharm*. 2021;14(3):273–299. doi:10.2174/1874471013666201210140743
7. Zuo W, Sun R, Ji Z, Ma G. Macrophage-driven cardiac inflammation and healing: insights from homeostasis and myocardial infarction. *Cell Mol Biol Lett*. 2023;28(1):81. doi:10.1186/s11658-023-00491-4
8. Kristjansson RP, Oddsson A, Helgason H, et al. Common and rare variants associating with serum levels of creatine kinase and lactate dehydrogenase. *Nat Commun*. 2016;7:10572. doi:10.1038/ncomms10572
9. Jaffe AS, Ravkilde J, Roberts R, et al. It's time for a change to a troponin standard. *Circulation*. 2000;102(11):1216–1220. doi:10.1161/01.CIR.102.11.1216
10. Jaffe AS, Vasile VC, Milone M, Saenger AK, Olson KN, Apple FS. Diseased skeletal muscle: a noncardiac source of increased circulating concentrations of cardiac troponin T. *J Ame College Cardiol*. 2011;58(17):1819–1824. doi:10.1016/j.jacc.2011.08.026
11. Fan Y, Li Y, Chen Y, et al. Comprehensive metabolomic characterization of coronary artery diseases. *J Ame College Cardiol*. 2016;68(12):1281–1293. doi:10.1016/j.jacc.2016.06.044
12. Guan B, Tong J, Hao H, et al. Bile acid coordinates microbiota homeostasis and systemic immunometabolism in cardiometabolic diseases. *Acta pharmaceutica Sinica B*. 2022;12(5):2129–2149. doi:10.1016/j.apsb.2021.12.011
13. Qaradakhi T, Gadanec LK, McSweeney KR, Abraham JR, Apostolopoulos V, Zulli A. The anti-inflammatory effect of taurine on cardiovascular disease. *Nutrients*. 2020;12(9):2847. doi:10.3390/nu12092847
14. Borghi M, Puccetti M, Pariano M, et al. Tryptophan as a central hub for host/microbial symbiosis. *Int J Tryptophan Res*. 2020;13:1178646920919755. doi:10.1177/1178646920919755
15. Zhang S, Zhou J, Wu W, Zhu Y, Liu X. The role of bile acids in cardiovascular diseases: from mechanisms to clinical implications. *Aging Dis*. 2023;14(2):261–282. doi:10.14336/AD.2022.0817
16. Li W, Shu S, Cheng L, et al. Fasting serum total bile acid level is associated with coronary artery disease, myocardial infarction and severity of coronary lesions. *Atherosclerosis*. 2020;292:193–200. doi:10.1016/j.atherosclerosis.2019.11.026

17. Charach G, Grosskopf I, Rabinovich A, Shochat M, Weintraub M, Rabinovich P. The association of bile acid excretion and atherosclerotic coronary artery disease. *Ther Adv Gastroenterol*. 2011;4(2):95–101. doi:10.1177/1756283X10388682
18. Feng X, Zhai G, Yang J, Liu Y, Zhou Y, Guo Q. Myocardial infarction and coronary artery disease in menopausal women with type 2 diabetes mellitus negatively correlate with total serum bile acids. *Front Endocrinol*. 2021;12:754006. doi:10.3389/fendo.2021.754006
19. Oberkersch RE, Santoro MM. Role of amino acid metabolism in angiogenesis. *Vasc Pharmacol*. 2019;112:17–23. doi:10.1016/j.vph.2018.11.001
20. Yu E, Ruiz-Canela M, Hu FB, et al. Plasma arginine/asymmetric dimethylarginine ratio and incidence of cardiovascular events: a case-cohort study. *J Clin Endocrinol Metab*. 2017;102(6):1879–1888. doi:10.1210/jc.2016-3569
21. Yu E, Ruiz-Canela M, Guasch-Ferré M, et al. Increases in plasma tryptophan are inversely associated with incident cardiovascular disease in the prevención con dieta mediterránea (PREDIMED) study. *J Nutr*. 2017;147(3):314–322. doi:10.3945/jn.116.241711
22. Ding Y, Svingen GF, Pedersen ER, et al. Plasma glycine and risk of acute myocardial infarction in patients with suspected stable angina pectoris. *J Ame Heart Ass*. 2015;5(1). doi:10.1161/JAHA.115.002621.
23. Wang Y, Huang K, Liu F, Lu X, Huang J, Gu D. Association of circulating branched-chain amino acids with risk of cardiovascular disease: a systematic review and meta-analysis. *Atherosclerosis*. 2022;350:90–96. doi:10.1016/j.atherosclerosis.2022.04.026
24. Du X, Li Y, Wang Y, et al. Increased branched-chain amino acid levels are associated with long-term adverse cardiovascular events in patients with STEMI and acute heart failure. *Life Sci*. 2018;209:167–172. doi:10.1016/j.lfs.2018.08.011
25. Collet JP, Thiele H, Barbato E, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Revista espanola de cardiologia*. 2021;74(6):544. doi:10.1016/j.rec.2021.05.002
26. Alsagaff MY, Putra TS, Khrisna BPD, Nugraha RA. Case report: wellens syndrome in acute critical coronary occlusion saved by collateral. *F1000Research*. 2022;11:1486. doi:10.12688/f1000research.125820.1
27. Knuuti J, Wijns W, Saraste A, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J*. 2020;41 (3):407–477. doi:10.1093/eurheartj/ehz425
28. Wang J, Zhang J, Lin X, et al. DCA-TGR5 signaling activation alleviates inflammatory response and improves cardiac function in myocardial infarction. *J Mol Cell Cardiol*. 2021;151:3–14. doi:10.1016/j.yjmcc.2020.10.014
29. Wang J, Xu T, Xu M. Roles and mechanisms of TGR5 in the modulation of CD4(+) T cell functions in myocardial infarction. *J Cardiovasc Transl Res*. 2022;15(2):350–359. doi:10.1007/s12265-021-10164-2
30. Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. *Circul res*. 2016;119(1):91–112. doi:10.1161/CIRCRESAHA.116.303577
31. Xia Y, Zhang F, Zhao S, et al. Adiponectin determines farnesoid X receptor agonism-mediated cardioprotection against post-infarction remodelling and dysfunction. *Cardiovasc Res*. 2018;114(10):1335–1349. doi:10.1093/cvr/cvy093
32. Li C, Yang J, Wang Y, Qi Y, Yang W, Li Y. Farnesoid X receptor agonists as therapeutic target for cardiometabolic diseases. *Front Pharmacol*. 2020;11:1247. doi:10.3389/fphar.2020.01247
33. Nowiński A, Chabowski D, Giebułtowicz J, Aleksandrowicz M, Ufnal M. Deoxycholic acid, a secondary bile acid, increases cardiac output and blood pressure in rats. *Nutrients*. 2023;16(1):32. doi:10.3390/nu16010032
34. Würtz P, Havulinna AS, Soininen P, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation*. 2015;131(9):774–785. doi:10.1161/CIRCULATIONAHA.114.013116
35. Chen J, Qin Y, Li Z, Shan A, Ma Q. Aromatic amino acids promote lipid metabolism disorders by increasing hepatic bile acid synthesis. *J Nutr*. 2024;154(4):1321–1332. doi:10.1016/j.tjnut.2023.12.047
36. Murr C, Grammer TB, Meinitzer A, Kleber ME, März W, Fuchs D. Immune activation and inflammation in patients with cardiovascular disease are associated with higher phenylalanine to tyrosine ratios: the ludwigshafen risk and cardiovascular health study. *J Amino Acids*. 2014;2014:783730. doi:10.1155/2014/783730
37. Zhang W, Ames BD, Walsh CT. Identification of phenylalanine 3-hydroxylase for meta-tyrosine biosynthesis. *Biochemistry*. 2011;50 (24):5401–5403. doi:10.1021/bi200733c
38. Czibik G, Mezdari Z, Murat Altintas D, et al. Dysregulated phenylalanine catabolism plays a key role in the trajectory of cardiac aging. *Circulation*. 2021;144(7):559–574. doi:10.1161/CIRCULATIONAHA.121.054204
39. Wang Y, Wen H, Fu J, et al. Hepatocyte TNF receptor-associated factor 6 aggravates hepatic inflammation and fibrosis by promoting lysine 6-linked polyubiquitination of apoptosis signal-regulating kinase 1. *Hepatology*. 2020;71(1):93–111. doi:10.1002/hep.30822
40. Tan Y, Chrysopoulou M, Rinschen MM. Integrative physiology of lysine metabolites. *Physiol Genom*. 2023;55(12):579–586. doi:10.1152/physiolgenomics.00061.2023
41. Norrby K. Evidence of a dual role of endogenous histamine in angiogenesis. *Int J Exp Pathol*. 1995;76(2):87–92.
42. Sörbo J, Jakobsson A, Norrby K. Mast-cell histamine is angiogenic through receptors for histamine1 and histamine2. *Int J Exp Pathol*. 1994;75(1):43–50.
43. Tabrez S, Shait Mohammed MR, Jabir NR, Khan MI. Identification of novel cardiovascular disease associated metabolites using untargeted metabolomics. *Biol Chem*. 2021;402(6):749–757. doi:10.1515/hsz-2020-0331