Predictive value of fecal sulfatide and neutrophil-to-lymphocyte ratio in coronary heart disease

KEFAN XUE[1,2*], YIHAN LI[1,2*], RUI HU[3*], XIAO HU[4,5], RAN GUO[6], HONGXIA GUO[7] and GANG LI[1]

[1]Division of Cardiology, Institute of Geriatric Diseases, Hebei General Hospital, Shijiazhuang, Hebei 050000, P.R. China; [2]Graduate School of Hebei Medical University, Shijiazhuang, Hebei 050000, P.R. China; [3]General Clinical Laboratory, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei 050000, P.R. China; [4]Department of Pathophysiology, Hebei Medical University, Shijiazhuang, Hebei 050000, P.R. China; [5]Hebei Key Laboratory of Critical Disease Mechanism and Intervention, Shijiazhuang, Hebei 050000, P.R. China; [6]The Third General Surgery Department, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei 050000, P.R. China; [7]Department of Anesthesiology, Hebei General Hospital, Shijiazhuang, Hebei 050000, P.R. China

Received November 7, 2024; Accepted March 20, 2025

DOI: 10.3892/etm.2025.12876

Abstract. The incidence of coronary atherosclerotic heart disease is on the rise, posing a serious threat to public health. Emerging evidence highlights the interplay between systemic inflammation and cardiovascular pathophysiology, suggesting novel diagnostic avenues. The aim of the present study was to evaluate the predictive value of fecal sulfatide and neutrophil-to-lymphocyte ratio (NLR), both individually and in combination, for coronary heart disease (CHD). A total of 523 patients diagnosed with CHD at the Cardiovascular Department of Hebei General Hospital (Shijiazhuang, China) from August 2022 to September 2023 were included in the experimental group, along with 198 healthy controls. The CHD group was further subdivided into stable angina pectoris (n=194), unstable angina pectoris (n=134), and acute myocardial infarction (AMI) groups (n=195). Fecal sulfatide and serum NLR levels were measured in both the experimental and control groups, as well as within each CHD subgroup. Multivariate logistic regression was utilized to assess whether these biomarkers serve as independent risk factors for CHD. The predictive value of fecal sulfatide and serum NLR was evaluated using receiver operating characteristic curves. Fecal sulfatide and serum NLR levels were distinctly higher in the CHD group compared with the control group (2.40±0.48 vs. 1.64±0.39 μmol/l and 2.92 vs. 1.65; P<0.05). Patients with AMI had higher NLR levels than those with stable and unstable angina pectoris (5.55 vs. 2.65 and 2.68; P<0.05). Fecal sulfatide levels were also elevated in patients with AMI (2.50±0.44 μmol/l) compared with patients with stable angina pectoris (2.32±0.48 μmol/l). Both fecal sulfatide (AUC=0.899) and NLR (AUC=0.811) exhibited strong predictive accuracy for CHD. When combined, the predictive value (AUC=0.945) was further improved. Elevated levels of fecal sulfatide and serum NLR in patients with CHD revealed that these biomarkers may serve as valuable adjuncts in the diagnosis of CHD. The combined use of these biomarkers enhances the accuracy and reliability of CHD prediction.

Introduction

Coronary heart disease (CHD) is a prevalent condition with a high incidence and mortality rate. The global incidence of CHD continues to rise annually, with an increasing trend toward a younger age of onset (1). According to the American Heart Association (2), an estimated 254.2 million people worldwide are affected by CHD, resulting in ~9.21 million deaths each year. CHD is the leading cause of heart failure, characterized by its long duration, challenging management, and poor prognosis. It not only severely affects the quality of life of patients, but also poses a significant threat to their longevity and overall health (3). The pathogenesis of CHD is multifactorial, with lipid deposition on the vessel walls, smooth muscle cell proliferation, and fibrous matrix formation being the primary contributors to atherosclerotic plaque development. Inflammation plays a critical role throughout the stages of atherosclerosis, involving various immune cells that contribute to plaque instability or rupture, arterial narrowing, and obstruction. These processes can lead to myocardial ischemia, hypoxia, necrosis, and ultimately, acute cardiovascular events (4). Several inflammatory biomarkers, such as C-reactive protein, interleukin-2 (IL-2), and tumor necrosis factor-α (TNF-α), have been implicated in the progression of atherosclerosis (5), and their detection is valuable for early

Correspondence to: Dr Gang Li, Division of Cardiology, Institute of Geriatric Diseases, Hebei General Hospital, 348 West Peace Road, Shijiazhuang, Hebei 050000, P.R. China
E-mail: 2252096555@qq.com

*Contributed equally

Key words: coronary artery disease, sulfatide, neutrophil-to-lymphocyte ratio

diagnosis of CHD. Despite advances in diagnostic techniques and treatments, there remains a significant need for simple, highly specific, and reproducible methods to diagnose CHD effectively.

Sulfatide, an acidic sphingolipid composed of ceramide, galactose, and sulfate, is widely distributed in the organs and serum of both animals and humans. A previous study has demonstrated a strong association between serum sulfatide levels and atherosclerosis, inflammation, and thrombosis (6). The present study proposes for the first time the measurement of fecal sulfatide levels and the investigation of their relationship with the onset and progression of CHD. The aim of the present study was to identify new stool biomarkers for CHD, providing a non-invasive, convenient, and accurate method for detecting cardiovascular disease (CVD) risk factors. This could pave the way for a new era of fecal biomarker detection in the prevention and treatment of CHD.

Both neutrophil and lymphocyte counts reflect the natural physiological response of the body to stress, trauma, surgery, and infection. The neutrophil-to-lymphocyte ratio (NLR), a combined inflammatory marker, integrates cellular and humoral immunity, offering a more comprehensive indication of dynamic changes in inflammatory and immune response pathways compared to single indicators. NLR is a simple, reliable, and effective parameter for assessing the intensity of neuroendocrine stress and immune-inflammatory responses (7). In recent years, NLR has gained significant attention in CHD research. A previous study showed a strong association between elevated NLR levels and the severity of coronary artery disease (8). Higher NLR levels have also been found to predict the prognosis of patients with CHD, recurrence of myocardial infarction, and long-term adverse events in patients with acute coronary syndrome (ACS) following percutaneous coronary intervention (PCI) and coronary artery bypass grafting (9). The present study explored the predictive value of fecal sulfatide and NLR, both individually and in combination, for CHD, offering valuable insights for early detection.

Materials and methods

Participants. A total of 523 patients diagnosed with CHD at the Division of Cardiology of Hebei General Hospital (Shijiazhuang, China) from August 2022 to September 2023 were included in the experimental group. The mean age of the participants was 64.79±0.41 years, with 353 males and 170 females. The experimental group was further subdivided into three categories: i) Stable angina pectoris (194 cases), ii) unstable angina pectoris (134 cases), and iii) acute myocardial infarction (AMI; 195 cases). The control group consisted of 198 healthy individuals who underwent routine physical examinations at the aforementioned hospital during the same period. Inclusion criteria for the experimental group required patients to meet the diagnostic criteria for CHD and provide informed consent. Exclusion criteria included the presence of malignant tumors, congenital heart disease, valvular disease, cardiomyopathy, thyroid disease, immune system and blood disorders, cerebrovascular diseases, acute and chronic infections, and mental disorders. The present study was approved by the Ethics Committee of Hebei General Hospital (approval no. 20220231) and was conducted in accordance with The Declaration of Helsinki. All participants provided informed consent before participation.

Clinical characteristics. Data on general characteristics were collected for all participants, including age, sex, body mass index (BMI), smoking history (defined as smoking ≥1 pack/day for at least one year), and alcohol consumption history (defined as regular alcohol consumption ≥100 ml/day of 50% alcohol content for at least one year).

Biochemical analysis. Professional nurses collected 10 ml of cubital venous blood from each participant after an 8-h overnight fast using a sodium citrate anticoagulant tube. The blood samples were then sent to the laboratory for analysis. Biochemical parameters and cell flow analysis were performed using a Beckman-Coulter automatic biochemical analyzer with the necessary reagents, all provided by the Laboratory Department of Hebei General Hospital. The biochemical markers assessed included albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and the NLR.

Measurement of fecal sulfatide. Approximately 1 g of fresh stool was harvested from each patient, rinsed three times with PBS, and then subjected to ultrasonic crushing. The sample was centrifuged at 11,180 x g for 10 min at 4˚C, and the supernatant was collected for further analysis. Fecal sulfatide levels were quantified using the ELISA method according to the instructions provided with the kit (human sulfatide ELISA kit; cat. no. MM-60644H2; Jiangsu Meimian Industrial Co., Ltd.).

Statistical analysis. Statistical analyses were performed utilizing SPSS v.26.0 (IBM Corp.) software. Normally distributed data were summarized as the means ± standard deviation (SD). For comparisons between two groups, an unpaired Student's t-test was used, while a one-way analysis of variance (ANOVA) was employed for comparisons among multiple groups and Bonferroni method was used for comparison among groups. When significant differences were found between groups, pairwise comparisons were conducted using LSD or Games-Howell tests. Non-normally distributed data were presented as median and interquartile range, with comparisons between two groups performed using the Mann-Whitney U test, and comparisons between multiple groups using the Kruskal-Wallis test. Frequency or percentage data were analyzed utilizing Pearson's chi-squared test for group comparisons. To assess the influencing factors of CHD, multiple logistic regression was applied. Receiver operating characteristic (ROC) curves were generated to evaluate the diagnostic value of fecal sulfatide and NLR for CHD, and Z-tests were conducted to compare differences in the area under the curve (AUC). Statistical significance was set at $P<0.05$.

Results

The baseline characteristics of the study participants are summarized in Table I. Comparison between the CHD and non-CHD groups revealed no statistically significant

Table I. Baseline and clinical data of individuals in the non-CHD and CHD groups.

Characteristics	Non-CHD group (n=198)	CHD group (n=523)	t/Z/χ^2	P-value
Age (years)	60 (55, 62)	66 (58, 72)	-8.333	<0.001
Male [n (%)]	139 (70.2)	353 (67.5)	0.486	0.486
BMI (kg/m2)	25.7±2.9	25.5±3.5	0.797	0.426
Smoking [n (%)]	70 (35.4)	190 (36.3)	0.059	0.808
Drinking [n (%)]	35 (17.7)	97 (18.5)	0.073	0.787
Albumin (g/l)	44.5±2.9	39.6±4.7	17.053	<0.001
ALT (U/l)	21.2 (15.8, 30.0)	23.6 (15.9, 41.1)	-2.713	0.007
AST(U/l)	23.1 (19.0, 27.6)	25.6 (19.0, 64.6)	-4.613	<0.001
HDL-C (mmol/l)	1.4±0.3	1.1±0.3	12.050	<0.001
LDL-C (mmol/l)	3.2±0.8	4.1±0.8	-14.567	<0.001
NLR	1.65 (1.33, 2.41)	2.92 (2.02, 4.92)	-12.895	<0.001
Fecal sulfatide (μmol/l)	1.64±0.39	2.40±0.48	-21.932	<0.001

Data are presented as the frequency (percentage) for categorical variables and as the mean ± standard deviation or median (interquartile range) for continuous variables. CHD, coronary heart disease; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NLR, neutrophil-to-lymphocyte ratio.

Table II. Sex differences in fecal sulfatide and NLR levels.

Parameter	Male (n=492)	Female (n=229)	t/Z	P-value
Fecal sulfatide (μmol/l)	2.17±0.58	2.24±0.54	-1.605	0.109
NLR	2.52 (1.75, 3.83)	2.33 (1.54, 3.75)	-1.450	0.147

Data are presented as mean ± standard deviation or mean (interquartile range). NLR, neutrophil-to-lymphocyte ratio.

differences in the proportion of males, BMI, smoking history, or alcohol consumption history (P>0.05), suggesting that these factors may not be primary contributors to the development of CHD in this cohort. However, the CHD group had notably higher levels of age, ALT, AST, and LDL-C compared with the non-CHD group (P<0.05). These findings suggest that older age, elevated liver enzymes (ALT and AST), and increased LDL-C levels may be associated with an elevated risk of CHD. By contrast, the levels of albumin and HDL-C were distinctly lower in the CHD group (P<0.05). Furthermore, it was observed that the NLR and fecal sulfatide levels were notably higher in the CHD group compared with the non-CHD group. These results highlight the potential role of inflammatory markers and lipid metabolism in the development of CHD, underscoring the need for further exploration of these parameters.

Additionally, as demonstrated in Table II, when examining the sex differences in fecal sulfatide and NLR levels across the entire cohort, it was observed that the average sulfatide level in males was 2.17±0.58 μmol/l, and in females, it was 2.24±0.54 μmol/l, with no statistically significant difference between the groups (P=0.109). Similarly, the NLR level in males was 2.52 (1.75,3.83), while in females, it was 2.33 (1.54,3.75), with no significant difference observed between the groups (P=0.147). When further analyzing the differences in fecal sulfatide and NLR levels by sex within the CHD and non-CHD groups (Table III), it was found that in male patients, the CHD group had higher sulfatide levels (2.40±0.48 μmol/l vs. 1.60±0.36 μmol/l; P<0.001) and NLR levels [2.96 (2.09,4.85) vs. 1.69 (1.35,2.16)] compared with the non-CHD group. Similarly, in female patients, the CHD group exhibited higher sulfatide levels (2.41±0.47 μmol/l vs. 1.75±0.43 μmol/l; P<0.001) and NLR levels [2.81 (1.83,5.32) vs. 1.60 (1.28,2.06)] compared with the non-CHD group. These findings revealed that the elevated levels of fecal sulfatide and NLR in the CHD population are not significantly influenced by sex.

A comparison of clinical data across different subgroups of CHD is presented in Table IV. Significant differences were noted in age, albumin, ALT, AST, HDL-C, LDL-C, NLR and fecal sulfatide levels among the three subgroups (P<0.05). Pairwise comparisons revealed that patients in the stable angina pectoris group were younger than those in the AMI group. Additionally, albumin levels were notably higher in the stable angina and unstable angina pectoris groups compared with the AMI group. Furthermore, ALT, AST, LDL-C, and NLR levels were elevated in the AMI group relative to both the stable and unstable angina groups. The fecal sulfatide levels were also higher in the AMI group than in the stable angina pectoris group.

For the multivariate analysis, CHD was set as the dependent variable, and age, albumin, ALT, AST, HDL-C, LDL-C, NLR, and fecal sulfatide levels were considered as independent

Table III. Sex differences in fecal sulfatide and NLR levels within the CHD and non-CHD groups.

Parameter	Sex	Non-CHD group	CHD group	t/Z	P-value
Fecal sulfatide (μmol/l)	Male	1.60±0.36	2.40±0.48	-20.075	<0.001
	Female	1.75±0.43	2.41±0.47	-9.444	<0.001
NLR	Male	1.69 (1.35, 2.16)	2.96 (2.09, 4.85)	-11.049	<0.001
	Female	1.60 (1.28, 2.06)	2.81 (1.83, 5.32)	-6.765	<0.001

Data are presented as frequency (percentage) for categorical variables and as mean ± standard deviation or median (interquartile range) for continuous variables. CHD, coronary heart disease; NLR, neutrophil-to-lymphocyte ratio.

Table IV. Comparison of clinical data between different coronary heart disease subgroups.

Characteristics	Stable angina pectoris (n=194)	Unstable angina pectoris (n=134)	Acute myocardial infarction (n=195)	F/H	P-value
Age (years)	64 (57, 69)	66 (57, 73)	68 (59, 73)[a]	13.763	0.001
Albumin (g/l)	41.51±3.52	40.79±3.39	36.77±5.12[a,b]	59.997	<0.001
ALT (U/l)	18.7 (14.4, 29.2)	20.2 (14.8, 29.1)	42.4 (21.8, 105.3)[a,b]	107.270	<0.001
AST (U/l)	21.0 (17.8, 25.6)	20.4 (17.4, 26.8)	106.0 (50.9, 367.4)[a,b]	271.031	<0.001
HDL-C (mmol/l)	1.15±0.29[b]	1.06±0.24[a]	1.11±0.28	3.725	0.025
LDL-C (mmol/l)	3.87±0.71	3.94±0.73	4.57±0.64[a,b]	59.160	<0.001
NLR	2.25 (1.67, 2.93)[b]	2.68 (1.99, 3.43)[a]	5.55 (3.37, 11.83)[a,b]	180.217	<0.001
Fecal sulfatide (μmol/l)	2.32±0.48	2.39±0.50	2.50±0.44[a]	7.040	0.001

[a]Indicates P<0.05, compared with the stable angina group; [b]indicates P<0.05, compared with the unstable angina group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NLR, neutrophil-to-lymphocyte ratio.

Table V. Logistic regression analysis of the factors influencing CHD.

Characteristics	B	SE	Wald	OR	95% CI	P-value
Age (years)	0.007	0.025	9.344	1.080	1.028-1.135	0.002
Albumin (g/l)	-0.383	0.079	23.418	0.682	0.584-0.796	<0.001
ALT (U/l)	0.009	0.020	0.190	1.009	0.970-1.049	0.663
AST (U/l)	0.028	0.026	1.170	1.028	0.978-1.081	0.279
HDL-C (mmol/l)	-6.041	0.847	50.879	0.002	0.000-0.013	<0.001
LDL-C (mmol/l)	-0.352	0.396	0.791	0.703	0.324-1.528	0.374
NLR	1.365	0.265	26.463	3.917	2.328-6.589	<0.001
Fecal sulfatide (μmol/l	7.243	0.883	67.311	1,397.627	247.72-7, 885.122	<0.001

CHD, coronary heart disease; B, regression coefficient; SE, standard error; Wald, Wald χ^2 test statistic; OR, odds ratio; 95% CI, 95% confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NLR, neutrophil-to-lymphocyte ratio.

variables. As demonstrated in Table V, age, albumin, HDL-C, NLR, and fecal sulfatide were identified as significant factors in the development of CHD. Age, NLR, and fecal sulfatide were found to be risk factors, while albumin and HDL-C were protective factors.

ROC analysis was implemented to evaluate the predictive value of NLR and fecal sulfatide, both individually and in combination, for the diagnosis of CHD. As illustrated in Table VI the AUC for NLR was 0.811, with an NLR threshold of >2.529 providing strong predictive power for CHD, showing a sensitivity of 0.618 and specificity of 0.879. The AUC for fecal sulfatide was 0.899, and a fecal sulfatide level >1.825 μmol/l was highly predictive of CHD. The AUC for the combined use of NLR and fecal sulfatide was 0.945 (95% CI, 0.929-0.960;

Table VI. Comparison of the areas under ROC curves of different indexes.

Predictors	AUC	95% CI	Sensitivity	Specificity	P-value
NLR	0.811	0.779-0.843	0.618	0.879	<0.001
Fecal sulfatide	0.899	0.874-0.924	0.920	0.702	<0.001
NLR + fecal sulfatide	0.945	0.929-0.960	0.864	0.879	<0.001

ROC, receiver operating characteristic; AUC, area under the curve; 95% CI, 95% confidence interval; NLR, neutrophil-to-lymphocyte ratio.

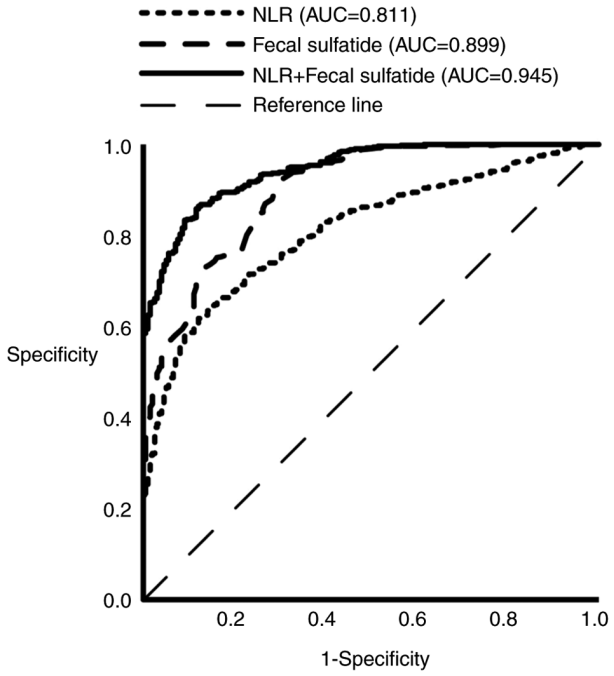

Figure 1. Receiver operating characteristic curve analysis of the predictive power of NLR and fecal sulfatide alone or in combination in patients with coronary heart disease. AUC, area under the curve; NLR, neutrophil-to-lymphocyte ratio.

P<0.05). Based on Fig. 1 and Table VI, it was concluded that the combination of NLR and fecal sulfatide demonstrates superior predictive ability for the occurrence of CHD compared with either marker alone.

Discussion

CHD is a common and increasingly prevalent clinical condition, posing a significant threat to public health worldwide (1). The development and progression of CHD involve the formation of coronary atherosclerotic plaques, plaque rupture, and subsequent intravascular thrombosis. Traditional risk factors for CHD include age, male sex, obesity, elevated total cholesterol, high LDL-C levels, smoking, hypertension, and a strong family history of CHD, among others (5). Inflammation is pivotal in the pathogenesis of atherosclerosis. During plaque formation, endothelial cells are activated by various stimuli, triggering the aggregation of leukocytes. Monocytes continuously migrate and accumulate within the developing plaques, where they differentiate into macrophages, proliferate, and form lipid-laden foam cells that accelerate plaque progression. Persistent pathological inflammation further exacerbates this process by reducing collagen production in the extracellular matrix and increasing the activity of collagenolytic enzymes. This leads to thinning of the fibrous cap, making it more vulnerable to rupture and thrombosis (10).

Sulfatide, a major glycolipid found in the serum lipoproteins of various mammals and humans, is widely distributed throughout the body and plays a significant physiological role in the metabolism of several tissues and organs. A previous study showed that in animal models with atherosclerotic lesions, serum sulfatide concentrations are distinctly elevated, leading to its accumulation in atherosclerotic plaques and thickened arterial walls (11). This suggests that sulfatide may play a critical role in the development of atherosclerosis and CVDs. Previous studies from the authors have demonstrated a strong association between sulfatide and atherosclerosis. Specifically, serum sulfatide levels were found to be positively associated with carotid intima-media thickness, serving as an independent risk factor for atherosclerosis in hypertensive patients (12). Additionally, high serum sulfatide concentrations were revealed to be associated with an increased risk of restenosis in patients with CHD following PCI (6). Moreover, serum sulfatide levels have been shown to reflect the severity of heart injury, with significant correlations observed in patients with ST-elevation myocardial infarction (13). Other studies have also established a close relationship between serum sulfatide and inflammation, thrombosis (14), and intimal hyperplasia (15). Traditional methods for measuring blood lipids and serum sulfatide, which typically require fasting venous blood samples, may not provide timely and accurate results, leading to potential inconvenience and risks for patients. By contrast, fecal sulfatide detection is simple, non-invasive, and more adaptable. Therefore, the aim of the present study was to explore the association between fecal sulfatide levels and the occurrence of CHD.

The NLR has emerged as a promising biomarker for CHD risk stratification, reflecting the complex interactions between systemic inflammation, immune dysregulation, and vascular pathology. Several mechanisms help explain its prognostic value. Neutrophils, key players in innate immunity, contribute to vascular damage through the release of pro-inflammatory cytokines such as IL-6 and TNF-α, as well as reactive oxygen species (16,17). These factors impair the bioavailability of endothelial nitric oxide and promote the oxidative modification of LDL, which accelerates foam cell formation and the progression of atherosclerotic plaques. Therefore, the NLR serves as a marker of the inflammatory environment that

drives atherosclerosis. Moreover, previous studies have found that neutrophils can promote plaque vulnerability through releasing neutrophil extracellular traps (NETs), which expose pro-coagulant molecules (such as tissue factor) and degrade collagen in the fibrous cap (18,19). Elevated NLR has been associated with unstable plaque features, such as a thin fibrous cap and a large necrotic core. Additionally, NETs activate platelets and trigger the coagulation cascade, linking inflammation to atherosclerotic thrombosis, particularly in acute coronary syndrome. NLR may also reflect sympathetic-adrenal activation and stress responses (20). Research indicates that during acute myocardial ischemia, a surge in catecholamines stimulates the bone marrow, leading to neutrophilia and lymphocyte apoptosis, thereby temporarily elevating NLR. These stress-induced immune changes may exacerbate endothelial injury and predict adverse outcomes following myocardial infarction (21).

The use of NLR as a biomarker for cardiovascular diseases is well-established, with numerous studies (22-26) confirming its diagnostic, evaluative, and prognostic value across various conditions, including cardiovascular diseases. This raises concerns about the novelty of using NLR alone as a biomarker for CHD. A meta-analysis has demonstrated that NLR can predict hospitalization and long-term outcomes in patients with ST-segment elevation myocardial infarction following PCI (22). In a study involving 364 consecutive patients undergoing PCI, the high NLR group exhibited a notably higher incidence of major adverse cardiovascular events compared with the low NLR group (23). Additionally, Xu *et al* (24) found that NLR could predict the long-term prognosis of patients with myocardial infarction involving left main and/or three-vessel lesions. In a cohort of 2,967 patients with ACS and 571 patients without ACS, NLR was identified as a strong predictor of a high Gensini score, with an NLR >2.04 indicating the presence of ACS (25). Another study suggested that NLR is effective in monitoring vascular inflammation and platelet stability, making it a timely predictor for atherosclerotic cardiovascular and cerebrovascular diseases (26). The novel aspect of the present study lies in the addition of fecal sulfatide as a complementary biomarker to NLR. The reported increase in AUC from 0.811 (NLR alone) and 0.899 (fecal sulfatide alone) to 0.945 (NLR + fecal sulfatide) suggests a potential clinical benefit in combining these markers for enhanced prediction of CHD.

The present study identified age as a significant risk factor for CHD, while albumin and HDL-C were found to be protective factors, which is consistent with previous research. It was observed that fecal sulfatide and serum NLR levels were notably higher in the CHD group compared with the control group. Furthermore, NLR levels were elevated in patients with AMI compared to those with stable and unstable angina pectoris. Similarly, fecal sulfatide levels were higher in patients with AMI than in those with stable angina pectoris. Multivariate logistic regression analysis revealed that both fecal sulfatide and NLR were independent risk factors for CHD. Notably, fecal sulfatide (AUC=0.899) and NLR (AUC=0.811) demonstrated strong predictive ability for CHD, and the combination of both biomarkers notably enhanced predictive accuracy (AUC=0.945).

Despite these promising findings, the present study has some limitations. The sample size was relatively small and derived from a single center. Moreover, factors such as diet and medication may have influenced the levels of the biomarkers studied. Future research will expand the sample size and include a multi-center approach to better assess the predictive value of these biomarkers for CHD.

The present study is the first, to the best of our knowledge, to measure fecal sulfatide levels in patients diagnosed with CHD using the ELISA method. The results suggest that elevated fecal sulfatide levels are closely associated with the onset and progression of CHD. Additionally, the integration of fecal sulfatide as a complementary biomarker to NLR is a novel aspect of the present study. In order to strengthen the theoretical basis for the early diagnosis and treatment of CHD, a larger clinical study will be performed by the authors, to explore the mechanisms related to the influence of NLR and fecal sulfatide on CHD.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 82103475), the Natural Science Foundation of Hebei Province (grant no. H2023307028; and H2024206056), the Medical Science Research Program of Health Commission of Hebei Province (grant no. 20240370), the Hebei Province Government-Funded Clinical Medicine Excellent Talent Training Program (grant no. ZF2025013) and the Hebei Province Medical Adaptation Technology Tracking Project (grant no. GZ2024002).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

KX, YL and RH jointly participated in the preliminary design of the study, and completed the data extraction and collation and the first draft of the paper. XH participated in data sorting and data analysis. RG and HG participated in the design of the experimental method. GL participated in the preliminary design of the study, funding acquisition and reviewed and revised the first draft of the paper. KX and YL confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Hebei General Hospital (approval no. 20220231). The methods were carried out in accordance with The Declaration of Helsinki. Written informed consent was obtained from all participants after they were fully informed of the study's purpose, procedures, and potential risks.

Patient consent for publication

All the patients have been informed and signed informed consent before the experiments.

Competing interests

The authors declare that they have no competing interests.

References

1. Hu SS; Writing Committee of the Report on Cardiovascular Health and Diseases in China: Epidemiology and current management of cardiovascular disease in China. J Geriatr Cardiol 21: 387-406, 2024.
2. Martin SS, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, Baker-Smith CM, Barone Gibbs B, Beaton AZ, Boehme AK, *et al*: 2024 heart disease and stroke statistics: A report of US and global data from the American heart association. Circulation 149: e347-e913, 2024.
3. Pastena P, Frye JT, Ho C, Goldschmidt ME and Kalogeropoulos AP: Ischemic cardiomyopathy: Epidemiology, pathophysiology, outcomes, and therapeutic options. Heart Fail Rev 29: 287-299, 2024.
4. Kong P, Cui ZY, Huang XF, Zhang DD, Guo RJ and Han M: Inflammation and atherosclerosis: Signaling pathways and therapeutic intervention. Signal Transduct Target Ther 7: 131, 2022.
5. Gupta L, Thomas J, Ravichandran R, Singh M, Nag A and Panjiyar BK: Inflammation in cardiovascular disease: A comprehensive review of biomarkers and therapeutic targets. Cureus 15: e45483, 2023.
6. Li G, Hu R, Wu HZ and Chen SX: High serum concentration of sulfatide is a risk factor for restenosis in patients with coronary heart disease after percutaneous coronary intervention. Metabolomics (Los Angel) 5: 1, 2015.
7. Zahorec R: Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy 122: 474-488, 2021.
8. Li S, Chen H, Zhou L, Cui H, Liang S and Li H: Neutrophil-to-lymphocyte ratio predicts coronary artery lesion severity and long-term cardiovascular mortality in patients with unstable angina pectoris. Acta Cardiol 77: 708-715, 2022.
9. Agarwal R, Aurora RG, Siswanto BB and Muliawan HS: The prognostic value of neutrophil-to-lymphocyte ratio across all stages of coronary artery disease. Coron Artery Dis 33: 137-143, 2022.
10. Conti CR: Epidemiology, pathophysiology, and therapeutic targets in stable ischemic heart disease. Cardiovasc Innov App 3: 279-283, 2019.
11. Hara A and Taketomi T: Characterization and changes of glycosphingolipids in the aorta of the Watanabe hereditable hyperlipidemic rabbit. J Biochem 109: 904-908, 1991.
12. Li G, Hu R, Gu J and Wu HZ: Relationship between carotid artery atherosclerosis and sulfatide in hypertensive patients. Genet Mol Res 14: 4840-4846, 2015.
13. Li G, Hu R, Guo Y, He L, Zuo Q and Wang Y: Circulating sulfatide, a novel biomarker for ST-segment elevation myocardial infarction. J Atheroscler Thromb 26: 84-92, 2019.
14. Inoue T, Taguchi I, Abe S, Abe S, Li G, Hu R, Nakajima T, Hara A, Aoyama T, Kannagi R, *et al*: Sulfatides are associated with neointimal thickening after vascular injury. Atherosclerosis 211: 291-296, 2010.
15. Shimazawa M, Kondo K, Hara H, Nakashima M and Umemura K: Sulfatides, L- and P-selectin ligands, exacerbate the intimal hyperplasia occurring after endothelial injury. Eur J Pharmacol 520: 118-126, 2005.
16. Libby P, Ridker PM and Hansson GK; Leducq Transatlantic Network on Atherothrombosis: Inflammation in atherosclerosis: From pathophysiology to practice. J Am Coll Cardiol 54: 2129-2138, 2009.
17. Silvestre-Roig C, Braster Q, Ortega-Gomez A and Soehnlein O: Neutrophils as regulators of cardiovascular inflammation. Nat Rev Cardiol 17: 327-340, 2020.
18. Hofbauer TM, Ondracek AS and Lang IM: Neutrophil extracellular traps in atherosclerosis and thrombosis. Handb Exp Pharmacol 270: 405-425, 2022.
19. Adamstein NH, MacFadyen JG, Rose LM, Glynn RJ, Dey AK, Libby P, Tabas IA, Mehta NN and Ridker PM: The neutrophil-lymphocyte ratio and incident atherosclerotic events: Analyses from five contemporary randomized trials. Eur Heart J 42: 896-903, 2021.
20. Tamhane UU, Aneja S, Montgomery D, Rogers EK, Eagle KA and Gurm HS: Association between admission neutrophil to lymphocyte ratio and outcomes in patients with acute coronary syndrome. Am J Cardiol 102: 653-657, 2008.
21. Buonacera A, Stancanelli B, Colaci M and Malatino L: Neutrophil to lymphocyte ratio: An emerging marker of the relationships between the immune system and diseases. Int J Mol Sci 23: 3636, 2022.
22. Zhang S, Diao J, Qi C, Jin J, Li L, Gao X, Gong L and Wu W: Predictive value of neutrophil to lymphocyte ratio in patients with acute ST segment elevation myocardial infarction after percutaneous coronary intervention: A meta-analysis. BMC Cardiovasc Disord 18: 75, 2018.
23. Choi DH, Kobayashi Y, Nishi T, Kim HK, Ki YJ, Kim SS, Park KH, Song H and Fearon WF: Combination of mean platelet volume and neutrophil to lymphocyte ratio predicts long-term major adverse cardiovascular events after percutaneous coronary intervention. Angiology 70: 345-351, 2019.
24. Xu N, Tang XF, Yao Y, Zhao X, Chen J, Gao Z, Yang Y, Gao RL, Xu B and Yuan JQ: Predictive value of neutrophil to lymphocyte ratio in long-term outcomes of left main and/or three-vessel disease in patients with acute myocardial infarction. Catheter Cardiovasc Interv 91: 551-557, 2018.
25. Chen J, Chen MH, Li S, Guo YL, Zhu CG, Xu RX, Zhang Y, Sun J, Qing P, Liu G and Li JJ: Usefulness of the neutrophil-to-lymphocyte ratio in predicting the severity of coronary artery disease: A Gensini score assessment. J Atheroscler Thromb 21: 1271-1282, 2014.
26. Dziedzic EA, Gąsior JS, Tuzimek A, Dąbrowski M and Jankowski P: Neutrophil-to-lymphocyte ratio is not associated with severity of coronary artery disease and is not correlated with vitamin D level in patients with a history of an acute coronary syndrome. Biology (Basel) 11: 1001, 2022.