Clinical implications of plasma Nogo-A levels in patients with coronary heart disease

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Abstract

Introduction: Nogo-A is an important neurite growth-regulatory protein in the adult and developing nervous system. Recently, increasing evidence has shown that Nogo-A plays important roles in cardiac development and may act as a potential indicator for heart failure. In addition, increased oxidative stress has been found in individuals with cardiovascular diseases. However, not much is known regarding the expression levels of Nogo-A and reactive oxygen species (ROS) in patients with coronary heart disease (CHD). Therefore, we sought to investigate the relationship between Nogo-A, ROS levels and CHD.
Material and methods: The plasma Nogo-A and ROS concentrations of 122 acute coronary syndrome (ACS), 101 unstable angina pectoris (UAP), and 21 acute myocardial infarction (AMI) patients and 56 healthy controls were measured by enzyme-linked immunosorbent assay (ELISA). We further generated a receiver operating characteristic (ROC) curve to assess the diagnostic accuracy of Nogo-A and ROS in CHD.
Results: The Nogo-A and ROS levels were significantly higher in patients with CHD than those in healthy controls. In addition, multivariate logistic regression analysis revealed that the level of Nogo-A (odds ratio (OR) = 1.624, 95% confidence interval: 1.125–2.293, $p = 0.009$) is a risk factor for prediction of CHD. Nogo-A has diagnostic value, with an optimal threshold of 5.466 ng/ml for maximized diagnostic performance (59% sensitivity and 78.6% specificity, area under curve, $p < 0.05$). However, ROS concentration is not a risk factor for prediction of CHD (OR = 0.999, 95% confidence interval: 0.997–1.001, $p = 0.320$).
Conclusions: Increased plasma Nogo-A level may be associated with CHD.

Key words: Nogo-A, reactive oxygen species, coronary heart disease, oxidative stress.

Introduction

Cardiovascular diseases are the number one cause of death globally. Among these, coronary heart disease (CHD) is responsible for more than half of all cardiovascular events in individuals less than 75 years age. The prevalence of CHD is estimated to be 6.4% in US adults aged 20 years or older, which represents approximately 15.4 million Americans [1]. Despite the numerous studies, the pathophysiology of CHD remains largely unknown. Most recently, a growing body of evidence has shown that oxidative stress and the inflammatory response play important roles in the development of CHD [2–4]. Higher levels of reactive oxygen species (ROS) are often observed with microvascular pathology in CHD.

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Excessive ROS production represents endothelial and smooth muscle dysfunction, resulting in oxidative stress-induced signaling activation and mitochondrial-mediated cell apoptosis, which leads to the progress of CHD [5, 6]. Therefore, therapeutic strategies are needed that target the oxidative damage and the downstream consequences such as apoptosis [7].

For this purpose, researchers from Jin's laboratory first identified a novel role for the amino-Nogo-A against oxidative stress and proposed a mechanism of the amino-Nogo-A-mediated signal-transduction pathway of scavenging ROS [8]. Nogo-A is a high-molecular-weight membrane protein mostly expressed in the central nervous system (CNS), oligodendrocytes and subsets of neurons [9]. Inhibition of Nogo-A can enhance axonal sprouting and help neurological recovery in a rodent model of ischemic injury [10]. In addition to being highly expressed in the brain and spinal cord, Nogo-A is clearly detectable in other tissues, including the heart, where its function remains largely unclear despite evidence that it may play a role in normal cardiac development [11, 12]. Knockdown of Nogo-A in cardiomyocytes is found to significantly inhibit hypoxia/reoxygenation-induced activation of the intrinsic pathway of apoptosis [13]. Moreover, Nogo-A has been reported to be a potential indicator of heart failure due to its evaluated expression in genetic models of dilated cardiomyopathy (DCM) and its increased mRNA expression in end-stage heart failure in humans [14]. However, to date, the correlation between Nogo-A and CHD is still uncharacterized.

Most recently, we investigated the plasma Nogo-B concentrations in patients with acute coronary syndrome (ACS) and unstable angina pectoris (UAP) [15]. We found that Nogo-B levels were significantly higher in patients with ACS and UAP when compared with healthy controls. Moreover, based on analysis of the receiver operating characteristic (ROC) curves, plasma Nogo-B may have diagnostic values for cardiovascular diseases. Therefore, we proposed that Nogo-B may serve as a risk factor for prediction of the development of severe coronary stenosis. In this study, to further understand the roles of Nogo-A and oxidative stress in CHD, we performed a case-control study to analyze the change of Nogo-A and ROS levels in CHD. We also assessed the relation between these parameters and CHD.

Material and methods

Study population

Between June 2013 and June 2015, a total 122 patients with ACS, 101 patients with UAP and 21 patients with acute myocardial infarction (AMI) were admitted to the Department of Cardiology, Hangzhou First People's Hospital. Exclusion criteria were ongoing maintenance dialysis, a grave acute infectious disease, neoplastic disease, severe liver dysfunction, major surgery, a chronic inflammatory disease, and systemic lupus erythematosus (SLE). Patients who suffered severe life-threatening injury to other organs were also excluded. Moreover, 56 healthy age- and gender-matched controls were recruited in this study. The study protocol and informed consent approach were approved by the Ethics Committee of Hangzhou First People's Hospital before implementation. The study individuals or their relatives provided written informed consent to participate in this trial.

Clinical and laboratory assessment

Detailed demographics, anthropometrics, vital parameters, medical history, drug administration, medication and pedigree information were recorded for each participant through personal interviews. Blood pressure (BP) was measured by a sphygmomanometer. Body mass index (BMI) was calculated as the body weight (kg) divided by the square of the height (m^2).

Serum triacylglycerol (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and kidney function parameters were determined using routine automated assay methods following an overnight fast (Hitachi, Tokyo, Japan). Glycated hemoglobin A_{1c} (HbA_{1c}) was detected by high-pressure liquid chromatography (HPLC) (Variant II; Bio-Rad, Hercules, CA, USA). Serum C-reactive protein (CRP) was measured by particle-enhanced immunonephelometry assay (Dade Behring Inc., NJ, USA). In addition, we measured the estimated glomerular filtration rate (eGFR); eGFR = 175 × $(Scr)^{-1.154}$ × $(age)^{-0.203}$ [14].

Diagnosis of ACS, UAP and AMI

Coronary arteriography was carried out using the standard Judkins technique [16], and all major coronary arteries were examined in at least two orthogonal views. The arteriographic analysis was conducted by two experienced interventional cardiologists, who were blinded to the study's objective and design. Acute coronary syndrome was diagnosed according to the Guidelines of the American College of Cardiology (ACC) and American Heart Association (AHA) [17]; it was defined as either unstable angina or acute myocardial infarction, confirmed by electrocardiographic changes (ST-segment and T-wave changes) and cardiac enzyme. Unstable angina pectoris (UAP) was diagnosed in 101 patients, based on clinical symptoms, namely typical precordial chest pain of class IIB or IIIB in the Braunwald classification, angio-

graphic evidence (documented severe stenosis of 75% according to the AHA classification in one or more major coronary artery), and changes in serum creatine kinase (CK) concentrations (no significant evidence of increase) [18]. The diagnosis of AMI was based on clinical symptoms, ST segment elevation (STEMI) on the electrocardiogram with non-ST segment elevation acute myocardial infarction (NSTEMI), coronary angiographic findings (occlusion of main coronary artery branch with thrombolysis in myocardial infarction (TIMI) grade flow of 0, 1, or 2), and changes in serum CK concentrations (more than two-fold increase from the upper limit of the normal range) [19].

Determination of Nogo-A and ROS levels in plasma

The blood samples were immediately placed into sterile ethylenediaminetetraacetic acid test tubes and centrifuged at 1500 g for 20 min at 4°C to collect plasma. Plasma was stored at –70°C until assayed. The concentrations of Nogo-A and ROS in plasma were analyzed by ELISA using commercial kits (Nova Biomedical, MA, USA), in accordance with the manufacturer's instructions.

Statistical analysis

The SPSS software suite, version 19.0, was used for all statistical analyses (SPSS Inc., Chicago, IL). Normally distributed data were expressed as mean ± standard deviation. For comparison of the continuous variables among patients with CHD and the control group, a standard one-way analysis of variance (ANOVA) test and Student's t-test were performed. The differences in the distribution of categorical variables were compared using the χ^2 test. Initially, baseline variables were evaluated for univariate association with clinical outcome. Variables that were significant in univariate analyses were included in a multivariate logistic regression model to determine the risk-adjusted predictors of clinical outcome with odds ratio (OR) and 95% confidence interval (CI). Cut-off points for Nogo-A and ROS were determined to predict clinical outcome using ROC curves. The area under the curve (AUC) was calculated based on the ROC curves. An AUC closer to 1 indicated a higher predictive power. We considered a p-value less than 0.05 as significant for all tests.

Results

Patients' characteristics

This study eventually included 244 CHD patients including 122 ACS patients, 101 UAP patients and 21 AMI patients. Table I summarizes the demographic, clinical and biochemical data

from these patients. The control group consisted of 56 age- and gender-matched healthy individuals. Among these parameters, TG, TC, HDL-c, LDL-c, SCr and hsCRP differed statistically significantly when compared with the healthy control group.

Comparison of Nogo-A and ROS levels among ACS, UAP and AMI groups

As shown in Table I and Figure 1, the serum concentrations of Nogo-A were significantly higher in patients with ACS, UAP and AMI (6.95 ±3.66; 6.93 ±3.72 and 7.05 ±3.43 ng/ml) than in control subjects (4.16 ±2.48 ng/ml) ($p < 0.001$; $p < 0.001$; $p = 0.001$, respectively).

Moreover, as shown in Figure 2, the plasma concentrations of ROS were higher in patients with ACS, UAP and AMI (991.82 ±773.38; 936.42 ±790.35 and 1258.25 ±636.66 IU/ml) than in the control group (580.64 ±369.87 IU/ml) ($p < 0.001$; $p = 0.002$; $p < 0.001$, respectively).

Diagnostic value of plasma Nogo-A for patients with CHD

The ROC curve identified the cutoff values of plasma Nogo-A concentrations that predict the CHD patients with high sensitivity and specificity values (Figure 3). The results indicate that Nogo-A has diagnostic value, with an optimal threshold of 5.466 ng/ml for maximized diagnostic performance (59% sensitivity and 78.6% specificity).

Predictive value of increased Nogo-A concentrations for CHD

We then calculated the OR and 95% CI using multiple logistic regression analysis to evaluate predictors of CHD for the entire study population (Table II).

Among these parameters, BMI (OR = 1.666, 95% CI: 1.294–2.145, $p = 0.001$), LDL-c (OR = 3.544, 95% CI: 1.420–8.848, $p = 0.007$), hsCRP (OR = 1.225, 95% CI: 1.046–1.436, $p = 0.012$) and Nogo-A (OR = 1.624, 95% CI: 1.125–2.293, $p = 0.009$) are predictive in the univariate analysis for CHD patients.

Discussion

The following are the major findings of the present study: 1) serum Nogo-A levels are higher in the CHD groups than in the control group; 2) serum ROS concentrations are higher in the CHD group than in the control subjects, indicating that oxidative stress may play a role in the pathogenesis of CHD; 3) plasma Nogo-A concentrations emerge as an independent predictor for CHD in a multivariate logistic regression model, with 59%

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Table I. Clinical and biochemical characteristics of patients with CHD

| Variable	ACS (n = 122)	P-value	UAP (n = 101)	P-value	AMI (n = 21)	P-value	Control (n = 56)
Gender (male), n (%)	80 (65.57%)	/	64 (63.37%)	/	16 (76.19%)	/	24 (42.86%)
Age [years]	67.42 ±10.65	0.117	68.13 ±10.16	0.053	64.00 ±12.45	0.768	64.89 ±9.53
Weight [kg]	64.65 ±9.37	< 0.001	64.46 ±9.80	< 0.001	65.57 ±7.07	< 0.001	61.77 ±8.43
BMI [kg/m^2]	23.24 ±2.42	< 0.001	23.29 ±2.56	< 0.001	23.04 ±1.63	< 0.001	23.08 ±2.27
SBP [mm Hg]	142.56 ±101.18	0.927	147.41 ±110.35	0.701	119.24 ±18.73	< 0.001	141.68 ±19.31
DBP [mm Hg]	76.99 ±11.15	0.482	76.28 ±10.57	0.267	80.43 ±13.36	0.556	78.41 ±12.99
Pulse	76.29 ±13.22	0.848	76.11 ±12.91	0.910	77.14 ±14.95	0.735	75.86 ±14.19
Hypertension, n (%)	84 (68.85%)	/	70 (69.31%)	/	14 (66.67%)	/	36 (64.29%)
Diabetes mellitus, n (%)	25 (20.49%)	/	21 (20.79%)	/	4 (19.05%)	/	9 (16.07%)
Dyslipidemia, n (%)	35 (28.69%)	/	29 (28.71%)	/	6 (28.57%)	/	15 (26.79%)
Current smoking, n (%)	28 (22.95%)	/	21 (20.79%)	/	7 (33.33%)	/	10 (30.36%)
Family history, n (%)	21 (17.21%)	/	16 (15.84%)	/	5 (23.81%)	/	2 (3.57%)
ACE-I or ARB, n (%)	33 (27.05%)	/	27 (26.73%)	/	6 (28.57%)	/	7 (12.5%)
Statins, n (%)	10 (8.20%)	/	8 (7.92%)	/	2 (9.52%)	/	0 (0%)
β-blockers, n (%)	9 (7.38%)	/	8 (7.92%)	/	1 (4.76%)	/	2 (3.57%)
Aspirin, n (%)	11 (9.02%)	/	9 (8.91%)	/	2 (9.52%)	/	0 (0%)
LVSF (%)	38.06 ±8.16	0.672	38.86 ±7.85	0.780	34.19 ±8.75	0.047	38.54 ±6.40
LVEF (%)	66.10 ±10.76	0.199	67.62 ±9.98	0.699	58.80 ±11.57	0.002	68.21 ±8.59
LVIDd [cm]	4.93 ±0.53	0.017	4.92 ±0.52	0.027	4.98 ±0.57	0.096	4.74 ±0.49
LVIDs [cm]	3.12 ±0.60	0.021	3.07 ±0.59	0.089	3.35 ±0.58^	0.005	2.92 ±0.50
LA [cm]	3.91 ±0.58	0.046	3.92 ±0.60	0.045	3.86 ±0.46	0.266	3.72 ±0.59
TG [mmol/l]	1.59 ±0.77	0.005	1.54 ±0.69	0.015	1.87 ±1.03	0.001	1.28 ±0.49
TC [mmol/l]	4.33 ±1.06	0.001	4.29 ±1.03	0.001	4.53 ±1.17	0.002	3.77 ±0.84
HDL-c [mmol/l]	1.12 ±0.26	< 0.001	1.13 ±0.25	0.001	1.06 ±0.29	0.001	1.38 ±0.29
LDL-c [mmol/l]	2.43 ±0.73	< 0.001	2.42 ±0.73	< 0.001	2.50 ±0.70	0.001	1.94 ±0.61
SCr [μmol/l]	80.89 ±18.35	0.006	80.10 ±17.80	0.018	84.67 ±20.84	0.012	73.79 ±14.56
eGFR [ml/min/1.73 m^2]	78.20 ±15.67	0.239	78.19 ±15.37	0.249	78.26 ±17.43	0.506	81.16 ±15.37
FBG [mmol/l]	5.94 ±2.00	0.376	5.67 ±1.30	0.960	7.25 ±3.68	0.013	5.68 ±1.70
HbA$_{1c}$ (%)	5.99 ±1.07	0.166	5.89 ±0.92	0.415	6.46 ±1.53	0.062	5.76 ±1.00
Hemoglobin [g/l]	132.94 ±20.66	0.514	133.54 ±21.31	0.673	130.05 ±17.40	0.261	134.80 ±16.02
Fibrinogen [g/l]	2.77 ±0.81	0.383	2.68 ±0.72	0.972	3.23 ±1.07	0.035	2.68 ±0.56
hsCRP [mg/l]	9.67 ±14.16	0.004	6.71 ±6.28	0.003	23.90 ±27.56	< 0.001	4.11 ±2.21
Number of coronary arteries narrowed, n (%):							
1-vessel disease	50 (40.98%)	/	45 (44.55%)	/	5 (23.81%)	/	0
2-vessel disease	32 (26.23%)	/	30 (29.70%)	/	2 (9.52%)	/	0
3-vessel disease	40 (32.79%)	/	26 (25.74%)	/	14 (66.67%)	/	0
Nogo-A [ng/ml]	6.95 ±3.66	< 0.001	6.93 ±3.72	< 0.001	7.05 ±3.43	0.001	4.16 ±2.48
ROS [IU/ml]	991.82 ±773.38	< 0.001	936.42 ±790.35	0.003	1258.25 ±636.66	< 0.001	580.64 ±369.87

All values are mean ± SD; a,b same letters indicate no statistical significance based on Tukey's HSD post-hoc test or Wilcoxon's rank-sum test. ACS – acute coronary syndrome, UAP – unstable angina pectoris, AMI – acute myocardial infarction, BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, ACE-I – angiotensin-converting enzyme inhibitor, ARB – angiotensin receptor blocker, LVSF – left ventricular shortening fraction, LVEF – left ventricular ejection fraction, LVIDd – left ventricular internal diastolic diameter, LVIDs – left ventricular internal dimension systole at end-diastole, LA – left atrium, TG – triglyceride, TC – total cholesterol, HDL-c – HDL cholesterol, LDL-c – LDL cholesterol, SCr – serum creatinine, eGFR – estimated glomerular filtration rate, FBG – fasting blood glucose.

sensitivity and 78.6% specificity; 4) ROS could not improve the predictive values of CHD.

Reactive oxygen species and antioxidants are in equilibrium in the human body. The disturbance of this equilibrium by the increase of ROS or decrease of antioxidant capacity causes oxidative stress. It is widely accepted that the occurrence of oxidative stress promotes CHD and increases plaque vulnerability [20]. Reactive oxygen species are continuously generated in the body as a consequence of aerobic metabolism or as part of the body's defense against microorganisms. Overproduction of ROS will consequently increase mitochondrial permeability transition, decrease mitochondrial membrane potential, release pro-apoptotic factors, and lead to activation of caspase-3 and apoptosis [21, 22]. Protection of mitochondrial function is therefore a promising therapeutic strategy for the treatment of CHD.

To the best of our knowledge, this is the first report to demonstrate the relationship between Nogo-A expression and CHD. Nogo-A is generally considered to be the most important inhibitor of axonal growth in models of CNS [23]. In addition to its significant role in neuronal regeneration in the CNS, Nogo-A is reported to be involved in glucose homeostasis. Nogo-A knockout mice present increased insulin secretion, resulting in higher glucose clearance [24]. Moreover, knockdown of Nogo-A specifically inhibits the growth of hepatocellular carcinoma SMMC-7721 cell line [25]. Nevertheless, little is known regarding the role of Nogo-A expression in CHD. Molecular and biochemical analysis has demonstrated that Nogo-A is expressed in adult human heart [26], and increasing evidence has shown that Nogo may serve as a novel indicator of heart failure [14]. Moreover, amino-Nogo-A, a long specific region (amino acids 186-1004) of Nogo-A, showed a strong cytoprotective effect against H_2O_2-induced oxidative stress in vitro [7]. Taken together with these results, we hypothesize that Nogo-A may affect ROS production, and subsequently induce the oxidative stress that was responsible for CHD. Thus, it drove us to analyze the association between Nogo-A, ROS and CHD.

The present study was designed to evaluate the association of Nogo-A and ROS with CHD and also investigate the relationships between plasma Nogo-A and the risk of CHD. Since this is the first study to assess the role of Nogo-A expression in CHD, we believe that Nogo-A may be used as a potential biomarker for clinical and molecular diagnosis of CHD, with a threshold of 5.466 ng/ml for diagnostic performance (59% sensitivity and 78.6% specificity), whereas Nogo-B has a threshold of 821.96 pg/ml for maximized diagnostic
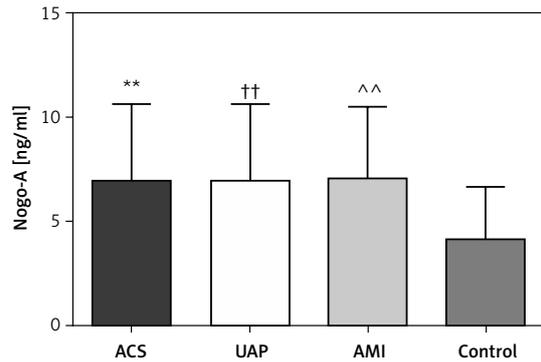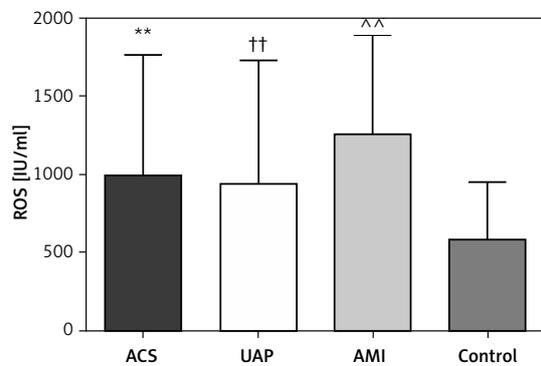

Figure 1. Nogo-A concentrations in ACS, UAP, AMI control subjects

**p < 0.001, for ACS group compared with controls,
††p < 0.001, for UAP group compared with controls,
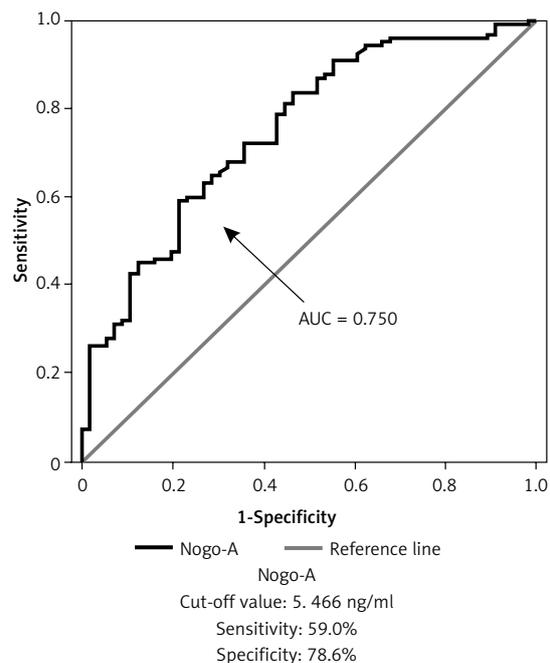*^^p = 0.001, for AMI group compared with controls.*

Figure 2. ROS concentrations in ACS, UAP, AMI and control subjects

**P < 0.001, for ACS group compared with controls,
††p = 0.002, for UAP group compared with controls,
^^p < 0.001, for AMI group compared with controls.

Nogo-A
Cut-off value: 5. 466 ng/ml
Sensitivity: 59.0%
Specificity: 78.6%

Figure 3. ROC curve analysis of the diagnostic value of plasma Nogo-A for CHD

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Table II. Univariate and multivariate logistic regression model for prediction of CHD

Parameter	Univariate analysis OR (95% CI)	*P*-value	Multivariate analysis OR (95% CI)	*P*-value
Age [years]	1.024 (0.993–1.056)	0.132	Not selected	
BMI [kg/m²]	1.735 (1.419–2.121)	< 0.001	1.666 (1.294–2.145)	0.001
SBP [mm Hg]	1.000 (0.996–1.004)	0.948	Not selected	
HbA$_{1c}$ (%)	1.275 (0.894–1.819)	0.179	Not selected	
eGFR [ml/min/1.73 m²]	0.988 (0.967–1.008)	0.240	0.983 (0.955–1.012)	0.241
FBG [mmol/l]	1.088 (0.890–1.330)	0.409	1.163 (0.886–1.527)	0.276
TG [mmol/l]	2.207 (1.238–3.932)	0.007	Not selected	
TC [mmol/l]	1.837 (1.284–2.629)	0.001	1.496 (0.851–2.627)	0.162
HDL-c [mmol/l]	0.033 (0.009–0.121)	< 0.001	Not selected	
LDL-c [mmol/l]	3.022 (1.759–5.189)	< 0.001	3.544 (1.420–8.848)	0.007
hsCRP [mg/l]	1.178 (1.059–1.311)	0.003	1.225 (1.046–1.436)	0.012
Fibrinogen [g/l]	1.190 (0.762–1.860)	0.444	Not selected	
Nogo-A [ng/ml]	1.414 (1.217–1.643)	< 0.001	1.624 (1.125–2.293)	0.009
ROS [IU/ml]	1.001 (1.001–1.002)	0.001	0.999 (0.997–1.001)	0.320

BMI – body mass index, SBP – systolic blood pressure, eGFR – estimated glomerular filtration rate, FBG – fasting glucose, TG – triglyceride, TC – total cholesterol, HDL-c – HDL cholesterol, LDL-c – LDL cholesterol.

performance of ACS (42.2% sensitivity and 96.4% specificity). It seems that the combination examination of Nogo-A and Nogo-B may improve the diagnostic efficiency and accuracy for CHD.

The main limitation of this study is the relatively small population because of our exclusion criteria, and the lack of clinical follow-up with these subjects. Further studies with a larger sample size need to be performed.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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