Association between new circulating proinflammatory and anti-inflammatory adipocytokines with coronary artery disease

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Background The aim of this study was to evaluate the diagnostic and risk predictive value of emerging proinflammatory and anti-inflammatory adipocytokines on coronary artery disease (CAD).

Patients and methods The study involved 259 inpatients suspected acute coronary syndrome who underwent coronary angiography. Demographic, clinical characteristics, and coronary artery stenosis rated by Gensini score were collected by cardiovascular doctors. The levels of serum inflammatory adipocytokines were evaluated by ELISA. The correlations of the cytokines with clinical parameters were assessed. Receiver operating characteristic curves were constructed for the diagnosis of CAD.

Results The 259 inpatients were assigned to the CAD ($n=180$) and control groups ($n=79$). Compared with the control group, the CAD group displayed significantly higher serum levels of retinol-binding protein-4 (RBP4), pentraxin 3 (PTX3), galectin-3 (GAL-3), and plasminogen activator inhibitor (PAI-1), and significantly lower levels of netrin-1 (NTN1), interleukin-37 (IL-37), and adiponectin (ADP) (all $P<0.05$). PAI-1 was significantly upregulated, and IL-37 and ADP were significantly downregulated in the three-vessels CAD subgroup compared to the one- and two-vessels CAD subgroups ($P<0.05$). The RBP4, PTX3, GAL-3, PAI-1, and IL-37 inflammatory cytokines were significantly positively correlated with Gensini score, and ADP was negatively correlated (all $P<0.001$). IL-37 was a more accurate anti-inflammatory biomarker than NTN1 and ADP. Combining cytokines significantly increased the sensitivity and specificity.

Conclusion The inflammatory adipocytokines GAL-3, RBP4, PTX3, NTN1, and IL-37 were more effective than the classical biomarkers PAI-1 and ADP in the diagnosis and risk assessment of CAD patients. *Coron Artery Dis* 30:528–535 Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

Introduction

Accumulating evidence has suggested that inflammation acts as a novel risk against myocardial ischemia, cardiovascular remodeling, and atherosclerosis [1]. Cytokines play a pivotal role in the initiation and progression of atherosclerosis [2]. The serum concentrations of cytokines might reflect the intensity of coronary artery plaques and their vulnerability to rupture [3]. Many previous studies have suggested that adipocytokines were associated with coronary artery disease (CAD), similar to classical risk factors. Although several biomarkers have been emerging as novel risk factors of CAD, only a few studies have focused on their diagnostic and prognostic value.

Pentraxin 3 (PTX3) is a multimeric acute phase protein of the pentraxin superfamily that serves as a proinflammatory biomarker. It is usually secreted in several cells, including macrophages, fibroblasts, vascular smooth muscle cells, and endothelial cells [4]. In CAD patients, PTX3 is released from activated platelets and neutrophils [5] and is associated with the progress of coronary atherosclerotic lesions [6]. Galectins and β-galactoside-binding animal lectins are differentially excreted by various immune cells and by a wide range of other cells. The galectins are divided into three types: prototype, chimera, and tandem. Galectin-3 (GAL-3) is one of the chimera members. It is secreted by activated macrophages [7] and is crucial in physiological and

pathological processes, including cell growth, angiogenesis, carcinogenesis, and inflammation. GAL-3 might be closely correlated with heart failure, cardiovascular death, and all-cause mortality [8]. As a well-known potent inhibitor of plasminogen activation and fibrinolysis, the concentration of plasma plasminogen activator inhibitor (PAI-1) increases in the patients with atherosclerosis [9], making it a putative CAD risk factor. Recently, retinol-binding protein-4 (RBP4) has been linked to obesity, insulin resistance, and type 2 diabetes, and it can transport the protein for vitamin A [10]. Moreover, RBP4 is crucial in the pathogenesis of arteriosclerosis in CAD patients [11]. Adiponectin (ADP) is a classical anti-inflammatory cytokine, a peptide hormone, and a member of the adipokines family that was first discovered in 1995 [12]. ADP can protect the cardiovascular systems from atherosclerosis by inhibiting the production of tumor necrosis factor-alpha (TNF-α), reducing monocyte adhesion, and repairing endothelial cells [13,14]. As a new member of the interleukin (IL) family, IL-37 often decreases in peripheral blood in healthy individuals. The overexpression of IL-37 in murine macrophage-like RAW264.7 cells leads to the reduction in lipopolysaccharide-stimulated proinflammatory cytokines [15]. Netrins play a vital role in cell migration and axon guidance. Netrin-1 (NTN1) is one of the five types of netrins, which acts as a bifunctional regulator in neuron migration [16]. In addition, NTN1 is involved in angiogenesis, atherosclerosis, and anti-ischemia reperfusion injury, exerting a cardioprotective effect in CAD [17].

In this study, we aimed to investigate the association of some new proinflammatory and anti-inflammatory adipocytokines, including serum levels of PTX-3, PAI-1, GAL-3, RBP-4, ADP, IL-37, and NTN1, with the development and exacerbation of CAD and deduce the array of inflammatory biomarkers for the diagnosis and risk assessment in clinical practice.

Patients and methods
Study cohort
A total of 259 inpatients with chest pain and suspected acute coronary syndrome who had undergone coronary angiography were consecutively enrolled in this study from January to June 2017 at The Affiliated Hospital of Chengde Medical University. The patients were assigned to the CAD group ($n = 180$) or the control group ($n = 79$). CAD was defined as stenosis of at least 50% of the luminal diameter in at least one major coronary artery branch, while the control group did not have luminal stenosis after coronary angiography. The clinical types of CAD were diagnosed according to the universal American College of Cardiology guidelines. The study was approved by the Institutional Review Boards of The Affiliated Hospital of Chengde Medical University. All subjects provided written informed consent. The exclusion criteria were infectious diseases, malignant tumors, hematopoietic or immune system diseases, and connective tissue diseases with coronary artery vasculitis.

Baseline demographic and clinical characteristics
Data regarding the gender, age, past medical history, smoking status, BMI, blood pressure, heart rate (HR), complete blood count (CBC), serum cholesterol, homocysteine (Hcy), liver and kidney function tests, thyroid gland function, echocardiographic examination, and medication were collected for all subjects. The diagnostic criteria of the classical risk factors including dyslipidemia [18], hypertension [19], and type 2 diabetes mellitus [20] were followed according to the authoritative international guidelines

Coronary angiography
All the subjects underwent coronary angiography by an experienced team of cardiologists. The cardiologists were blinded to the groups when collecting the data. The severity of coronary artery stenosis was quantitated by the Gensini score, with a score of 0 for no stenosis, 1 for <25% stenosis, 2 for 25–50% stenosis, 4 for 50–75% stenosis, 8 for 75–90% stenosis, 16 for 90–99% stenosis, and 32 for 100% stenosis. The scores were multiplied by a factor based on the position of the lesion. For example, five for the left main coronary artery, 2.5 for proximal left circumflex coronary artery (LCX) or proximal left anterior descending (LAD) coronary artery, 1.5 for the mid-region of LAD, 1.0 for distal right coronary artery posterolateral branch of LAD, first diagonal branch, and mid-distal region of the LCX or obtuse branch, and 0.5 for the other segments [21].

Estimation of serum inflammatory adipocytokines by ELISA
Blood samples were withdrawn in EDTA-containing tubes from a clean venipuncture during coronary angiographysurgery. The sera were centrifuged at 5000*g* for 10 minutes and preserved at –80°C. The levels of RBP4, PTX3, PAI-1, NTN1, IL-37, GAL-3, and ADP were measured using ELISA kits.

Statistical analyses
Continuous variables are reported as mean±SD for normally distributed data or median and quartiles (Q1;Q3) for non-normally distributed data. Discrete variables were expressed as frequency and percent, and compared using the Chi-square test. The correlations between inflammatory cytokines and coronary stenosis as well as clinical parameters were assessed using Spearman's rank order test. Receiver operating characteristic (ROC) curves were constructed, and the area under the curve (AUC) was calculated to obtain the cutoff values for the diagnosis of CAD. A two-sided *P* value less than 0.05 was considered statistically significant. The statistical computations were performed using SPSS software, version 19.0 (SPSS, Chicago, Illinois, USA).

Results
Baseline demographic and clinical characteristics
The cohort of 259 inpatients comprised 180 in the CAD group and 79 in the control group. The CAD group displayed angiographic coronary stenosis with one-vessel involvement in 63 (35.0%), two-vessels in 54 (30.0%), and

three-vessels in 63 (35.0%). In addition, the CAD group was significantly older (60.5±9.8 vs 57.5±9.7 years; $P < 0.05$) and had more male patients ($n = 135$, 75.0% vs $n = 49$, 62.0%; $P < 0.05$) than the control group. Similarly, the incidence of chest pain, hyperlipidemia, hypertension, and type 2 diabetes mellitus was significantly higher in the CAD group than that in the control group ($P < 0.05$). The CAD group had significantly higher incidences of smoking, decreased ejection fraction, and abnormal ventricular wall motion in echocardiography than the control group (all $P < 0.05$). However, no significant differences were detected in the CBC, thyroid gland function, and liver and kidney function tests between the two groups (all $P > 0.05$; Table 1).

Proinflammatory and anti-inflammatory cytokines between coronary artery disease and control groups

Significant increases were evident in the CAD group compared with the control group in the serum levels of the proinflammatory cytokines RBP4 (5.79 vs 3.60 ng/mL), PTX3 (3.93 vs 1.56 ng/mL), GAL3 (3.35 vs 1.14 ng/mL), and PAI-1 (770.89 vs 709.79 pg/mL) ($P < 0.05$). Conversely, the concentrations of the anti-inflammatory cytokines NTN1 (50.45 vs 89.28 pg/mL), IL-37 (87.74 vs 185.38 pg/mL), and ADP (8.13 vs 11.24 ng/mL) were significantly lower in the CAD group than the control group (all $P < 0.05$; Table 2 and Supplementary Fig. S1, Supplemental digital content 1, *http://links.lww.com/MCA/A268*).

Table 1   Baseline characteristics of demographic and clinical findings

Factors	CAD group (N=180)	Control group (N=79)	χ^2	P value
Male, n (%)	135 (75.0)	49 (62.0)	4.490	0.034
BMI (kg/m^2)	25.5±3.1	24.5±3.3	2.219	0.028
Age, years	60.5±9.8	57.5±9.7	2.260	0.024
STEMI, n (%)	39 (21.7)			
NSTEMI, n (%)	27 (15.0)			
UA, n (%)	110 (61.1)			
SAP, n (%)	4 (2.2)			
Chest pain, n (%)	149 (82.8)	20 (25.3)	79.950	<0.001
Hyperlipidemia, n (%)	77 (42.8)	15 (19.0)	13.570	<0.001
Hypertension, n (%)	123 (68.3)	41 (51.9)	6.390	0.012
Type 2 diabetes mellitus, n (%)	70 (38.9)	16 (20.2)	8.600	0.003
Stroke, n (%)	37 (20.6)	14 (17.7)	0.279	0.597
Smoking, n (%)	110 (61.1)	28 (35.4)	14.530	<0.001
SBP, mmHg	138.0 (125.0–153.75)	140.0 (124.0–145.0)	−1.593	0.111
DBP, mmHg	81.0 (75.0–90.0)	80.0 (75.0–90.0)	−0.261	0.794
HR, bpm	70.0 (62.0–79.5)	70.0 (61.0–78.0)	−0.228	0.819
Left atrium, mm	32.0 (34.0–37.0)	34.0 (32.0–37.0)	−0.495	0.621
LVEDD, mm	50.5 (47.0–55.0)	50.0 (47.0–53.75)	−0.904	0.366
LVESD, mm	34.0 (31.0–37.0)	33.0 (32.0–36.0)	−0.949	0.343
Ejection fraction, %	57.0 (51.0–62.0)	61.0 (57.0–64.0)	−3.399	0.001
Abnormal ventricular wall motion, n (%)	100 (55.6)	19 (24.1)	20.480	<0.001
WBC, 10^{12}/L	6.75 (5.81–8.36)	6.89 (5.62–8.10)	−0.480	0.634
HGB, g/L	144.0 (133.0–153.0)	144.0 (132.0–152.0)	−0.220	0.824
HCT, %	41.05 (38.93–43.8)	41.4 (38.4–44.1)	−0.470	0.746
Platelets, 10^9/L	208.0 (179.5–258.8)	221.5 (175.0–254.3)	−0.324	0.746
Lymphocytes, %	28.0 (20.2–33.5)	29.9 (23.8–35.8)	−1.485	0.138
Neutrophils, %	64.0 (56.6–71.4)	63.1 (55.5–69.4)	−1.019	0.308
TC, mmol/L	4.16±0.97	3.92±0.87	1.998	0.047
Triglyceride, mmol/L	1.7 (1.2–2.5)	1.4 (1.1–2.1)	−2.127	0.033
HDL-C, mmol/L	1.08±0.26	1.15±0.32	−1.669	0.096
LDL-C, mmol/L	2.14±0.87	1.90±0.76	2.138	0.034
Hcy, mmol/L	13.4 (12.0–21.0)	19.4 (10.6–34.5)	−0.285	0.775
Creatinine, µmol/L	72.27±18.05	67.08±12.25	2.315	0.021
Uric acid, mmol/L	320.9 (264.9–365.0)	324.4 (262.2–385.6)	−0.024	0.981
BUN, mmol/L	5.47 (4.54–6.90)	5.51 (4.70–6.58)	−0.042	0.966
AST, U/L	28.00 (21.50–39.50)	25.00 (21.00–36.00)	−0.942	0.346
ALT, U/L	37.97±26.42	38.55±36.12	−1.684	0.094
FT3, nmol/L	4.55±0.73	4.37±0.75	0.808	0.422
FT4, nmol/L	16.18±3.07	16.34±2.08	−0.248	0.804
TSH, mIU/L	2.21 (1.38–4.25)	2.08 (1.47–3.83)	−0.221	0.825
Coronary angiography				
One vessel	63 (35.0)			
Two vessels	54 (30.0)			
Three vessels	63 (35.0)			
Medicine, all %				
Aspirin	178 (98.9)	40 (50.6)	95.945	<0.001
Clopidogrel	167 (92.8)	2 (2.5)	197.209	<0.001
Beta blocker	106 (58.9)	27 (34.2)	13.421	<0.001
ACEI/ARB	91 (50.6)	16 (24.1)	20.793	<0.001
Statins	179 (99.4)	45 (57.0)	84.782	<0.001
CCB	51 (28.3)	10 (12.7)	7.492	0.006

t-values: ACEI/ARB, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CAD, coronary artery disease; CCB, calcium-channel blockers; DBP, diastolic blood pressure; FT3, free triiodothyronine; FT4, free thyroxine; Hcy, homocysteine; HDL-C, high-density lipoprotein cholesterol; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; SBP, systolic blood pressure; TC, total cholesterol; TSH, thyroid-stimulating hormone; WBC, white blood cell.

Table 2 Levels of serum inflammatory adipocytokines between the two groups

Factors	CAD group (*N*=180)	Control group (*N*=79)	Z	P value
RBP4, ng/mL	5.79 (4.49–6.96)	3.60 (2.07–5.25)	−6.6	<0.001
PTX3, ng/mL	3.93 (3.18–5.27)	1.56 (0.55–2.34)	−10.5	<0.001
PAI-1, pg/mL	770.89 (719.27–851.58)	709.79 (592.0–775.90)	−5.07	<0.001
NTN1, pg/mL	50.45 (30.83–69.78)	89.28 (60.08–126.26)	−7.02	<0.001
IL-37, pg/mL	87.74 (72.14–108.28)	185.38 (153.32–218.21)	−10.49	<0.001
GAL-3, ng/mL	3.35 (2.10–4.47)	1.14 (0.56–1.99)	−8.71	<0.001
ADP, ng/mL	8.13 (6.88–9.21)	11.24 (10.18–12.14)	−9.446	<0.001

ADP, adiponectin; CAD, coronary artery disease; GAL-3, galectin-3; IL-37, interleukin-37; NTN1, Netrin-1; PAI-1, plasminogen activator inhibitor-1; PTX3, Pentraxin3; RBP4, retinol-binding protein-4.

Table 3 Levels of serum inflammatory adipocytokines in the one-, two-, three-vessels coronary artery disease subgroups

Group	N	RBP4	PTX3	PAI-1	NTN1	IL-37	GAL-3	ADP
Control group	82	3.72 (2.08–5.31)	1.67 (0.55–2.39)	711.00 (597.41–783.56)	88.31 (61.54–126.26)	184.58 (149.45–217.69)	1.15 (0.58–2.00)	11.23 (10.10–12.12)
CAD group								
One vessel	60	5.18 (4.35–6.35)*	3.55 (2.99–4.41)*	740.10 (677.13–797.14)*	50.80 (37.02–77.88)*	101.68 (89.08–124.38)*	2.91 (1.56–3.61)*	8.91 (7.97–9.68)*
Two vessels	54	6.08 (4.30–6.96)***	4.24 (3.21–5.64)*	760.56 (723.80–819.33)***	43.44 (27.05–69.01)*	87.00 (71.16–100.54)***	3.17 (1.96–4.64)*	8.22 (6.97–8.77)***
Three vessels	63	6.35 (5.03–7.89)***	4.04 (3.32–5.29)***	822.18 (748.45–929.55)******	44.87 (20.56–64.57)*	79.10 (43.26–92.47)******	4.22 (3.34–5.96)******	7.03 (5.50–8.17)******

Compared to the control group, **P*<0.05; Compared to the one-vessel lesion group, ***P*<0.05; Compared to the two-vessel lesions group, ****P*<0.05.
ADP, adiponectin; CAD, coronary artery disease; GAL-3, galectin-3; IL-37, interleukin-37; NTN1, Netrin-1; PAI-1, plasminogen activator inhibitor-1; PTX3, Pentraxin3; RBP4, retinol-binding protein-4.

Proinflammatory and anti-inflammatory adipocytokines in one-, two-, and three-vessels coronary artery disease subgroups

PAI-1 was significantly elevated in the three-vessels CAD subgroup than in the control, one-vessel, and two-vessels subgroups. On the other hand, IL-37 and ADP levels were significantly downregulated in the three-vessels CAD subgroup than in the control, one-vessel, and two-vessels subgroups (*P*<0.05). The other adipocytokines did not differ significantly among the groups (*P*>0.05).

Association between inflammatory adipocytokines and coronary stenosis

To further explore the association among the novel cytokines, Gensini score, and clinical parameters, we analyzed an array of correlations (Tables 4–6, and Supplementary Fig. S2, Supplemental digital content 2, *http://links.lww.com/MCA/A269*). The inflammatory cytokines, RBP4 (*r*=0.313, *P*<0.001), PTX3 (*r*=0.278, *P*<0.001), GAL-3 (*r*=0.67, *P*<0.001), PAI-1 (*r*=0.524, *P*<0.001), and IL-37 (*r*=−0.65, *P*<0.001), showed a significantly positive correlation with the Gensini score. However, ADP exhibited a negative correlation with the Gensini score (*r*=−0.493, *P*<0.001).

Correlations of inflammatory adipocytokines with clinical and biochemical parameters

A significant correlation was observed among different inflammatory adipocytokines (*P*<0.05; Table 5). In addition, different inflammatory adipocytokines were significantly correlated with traditional cardiovascular risk factors, such as hyperlipidemia, hypertension, type 2

Table 4 Association between inflammatory adipocytokines and coronary stenosis

	Gensini score	
Adipocytokines	R	P value
RBP4	0.313	<0.001
PTX3	0.278	<0.001
PAI-1	0.524	<0.001
NTN1	−0.138	0.068
IL-37	−0.65	<0.001
GAL-3	0.67	<0.001
ADP	−0.493	<0.001

ADP, adiponectin; GAL-3, galectin-3; IL-37, interleukin-37; NTN1, Netrin-1; PAI-1, plasminogen activator inhibitor-1; PTX3, Pentraxin3; RBP4, retinol-binding protein-4.

diabetes mellitus, and smoking. Furthermore, all inflammatory adipocytokines showed a significant correlation with brain natriuretic peptide (BNP), GAL-3 (*r*=0.204, *P*<0.05), PTX3 (*r*=0.193, *P*<0.05), RBP4 (*r*=0.187, *P*<0.05), and ADP (*r*=−0.181, *P*<0.05). In addition, creatinine was significantly correlated with PTX3 (*r*=0.165, *P*<0.05) and inversely with IL-37 (*r*=−0.154, *P*<0.05) and ADP (*r*=−0.151, *P*<0.05) (Table 6).

Receiver operating characteristic curve analysis of the inflammatory adipocytokines with coronary artery disease

Tables 7 and 8 and Supplementary Figs. S3 and S4; Supplemental digital content 3 and 4, *http://links.lww.com/MCA/A270*; *http://links.lww.com/MCA/A271*, illustrated the ROC of NTN1, IL-37, ADP, RBP4, PTX3, PAI-1, and GAL-3 for the diagnosis of CAD. The data in Supplementary Fig. S3, Supplemental digital content

Table 5 Correlation between different inflammatory adipocytokines

	PAI-1 r	PAI-1 P value	NTN1 r	NTN1 P value	IL-37 r	IL-37 P value	GAL-3 r	GAL-3 P value	ADP r	ADP P value	PTX3 r	PTX3 P value	RAP4 r	RAP4 P value
PAI-1	1.000		−0.160	0.011	−0.409	<0.001	0.321	<0.001	−0.321	<0.001	0.265	<0.001	0.191	0.002
NTN1			1.000		0.322	<0.001	−0.378	<0.001	0.263	<0.001	−0.245	0.034	−0.253	<0.001
IL-37					1.000		−0.587	<0.001	0.533	<0.001	−0.565	<0.001	−0.393	<0.001
GAL-3							1.000		−0.452	<0.001	0.535	<0.001	0.325	<0.001
ADP									1.000		−0.399	<0.001	−0.202	<0.001
PTX3											1.000		0.400	<0.001
RBP4													1.000	

ADP, adiponectin; GAL-3, galectin-3; IL-37, interleukin-37; NTN1, Netrin-1; PAI-1, plasminogen activator inhibitor-1; PTX3, Pentraxin3; RBP4, retinol-binding protein-4.

Table 6 Correlations of inflammatory adipocytokines with clinical parameters

	PAI-1	NTN1	IL-37	GAL-3	ADP	PTX3	RAP4
Chest pain	0.146*	−0.216*	−0.360*	0.155*	−0.273*	0.380*	0.268*
Hyperlipidemia	0.084	−0.146*	−0.140*	0.152*	−0.218*	0.152*	0.055
Hypertension	0.120	−0.061	−0.143*	0.203*	−0.142*	0.180*	0.122*
Type 2 diabetes mellitus	0.104	−0.073	−0.186*	0.144*	−0.171*	0.142*	0.141*
Stroke	−0.031	0.079	−0.089	0.156*	−0.014	0.091	0.133*
Peripheral artery disease	−0.023	−0.070	−0.028	0.013	−0.083	0.100	0.106
Heart failure	−0.118	0.035	0.115	0.045	0.079	−0.063	0.010
Smoking	0.053	−0.139*	−0.177*	0.066	−0.139*	0.176*	0.121
Heavy alcohol intake	−0.061	0.086	0.059	−0.066	−0.064	0.015	−0.127*
Family history of CAD	−0.057	−0.045	−0.014	0.018	0.062	−0.014	−0.024
HR	−0.047	−0.035	−0.043	0.126*	−0.050	−0.007	−0.04
SBP, mmHg	0.049	−0.047	−0.089	0.133*	−0.108	0.033	0.060
DBP, mmHg	0.026	0.015	0.016	0.004	0.071	0.010	0.021
BNP, pg/mL	0.069	0.047	−0.133	0.204*	−0.181*	0.193*	0.187*
Left atrium, mm	0.080	0.076	−0.075	0.066	−0.037	0.097	0.040
LVEDD, mm	0.001	0.115	0.033	0.052	−0.129*	0.013	0.042
LVESD, mm	0.020	0.046	0.018	0.016	−0.136*	0.010	0.079
Ejection fraction, %	−0.084	0.123	0.110	−0.047	0.175*	−0.102	−0.154*
Abnormal ventricular wall motion, n (%)	0.118	−0.162*	−0.194*	0.226*	−0.234*	0.161*	0.135*
Aortic valve calcification	0.129*	−0.025	−0.048	−0.005	0.016	0.092	0.055
WBC, 10^{12}/L	0.045	0.016	−0.084	0.033	−0.121	−0.011	−0.013
HGB, g/L	0.015	0.073	−0.003	−0.092	−0.080	−0.064	−0.032
Platelet, 10^9/L	−0.001	−0.009	−0.054	−0.075	0.067	−0.087	−0.021
Neutrophils (%)	−0.156*	0.047	−0.086	0.015	−0.092	0.058	0.092
TC, mmol/L	0.077	−0.061	−0.124*	0.085	−0.132*	0.053	0.068
Triglyceride, mmol/L	0.105	−0.032	−0.099	0.026	−0.105	0.109	0.149*
HDL-C, mmol/L	−0.055	0.071	0.050	−0.075	0.041	−0.072	−0.154*
LDL-C, mmol/L	0.078	−0.106	−0.126*	0.114	−0.162*	0.056	0.054
Hcy, mmol/L	−0.237	−0.274	−0.041	0.117	0.132	−0.093	0.135
Creatinine, mmol/L	0.051	−0.026	−0.154*	0.108	−0.151*	0.165*	0.063
Uric acid, mmol/L	−0.021	−0.065	0.009	−0.026	−0.037	0.004	0.034
BUN, mmol/L	−0.018	−0.030	−0.077	0.031	−0.088	0.112	0.075
AST, U/L	−0.026	0.031	−0.100	−0.051	−0.202*	−0.008	0.004
ALT, U/L	−0.136*	−0.032	−0.016	−0.105	−0.043	−0.078	0.037
HbA1c, %	−0.360*	0.202	0.138	−0.011	0.025	−0.139	0.123
BMI, kg/m²	0.217*	−0.201*	0.129	0.115	−0.113	0.145	0.180*
FT3, nmol/L	0.166	−0.052	−0.047	−0.087	0.011	0.027	−0.088
FT4, nmol/L	0.161	−0.076	0.033	−0.087	0.058	−0.039	0.034
TSH, mIU/L	0.053	0.008	−0.064	0.184	−0.002	0.002	0.04

*$P<0.05$.
ACEI/ARB, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CAD, coronary artery disease; CCB, calcium-channel blockers; DBP, diastolic blood pressure; FT3, free triiodothyronine; FT4, free thyroxine; Hcy, homocysteine; HDL-C, high-density lipoprotein cholesterol; HGB, hemoglobin; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; SBP, systolic blood pressure; TC, total cholesterol; TSH, thyroid-stimulating hormone; WBC, white blood cell.

3, *http://links.lww.com/MCA/A270* and Table 7 suggested that the anti-inflammatory biomarkers, IL-37 [AUC 0.908, 95% confidence interval (CI): 0.863–0.952], was more accurate than NTN1 (AUC 0.771, 95% CI: 0.703–0.890) and ADP (AUC 0.870, 95% CI: 0.820–0.920), and the optimal cutoff value of IL-37 was 140.86 ng/mL. In the case of proinflammatory biomarkers (Supplementary Fig. S4, Supplemental digital content 4, *http://links.lww.com/MCA/A271* and Table 8), PTX3 (AUC 0.914, 95% CI:

0.875–0.943) was better than other biomarkers, such as RBP4 (AUC 0.769, 95% CI: 0.700–0.837), GAL-3 (AUC 0.853, 95% CI: 0.803–0.903), and PAI-1 (AUC 0.699, 95% CI: 0.629–0.769). The serum PTX-3 threshold at 4.03 ng/mL maximized the true-positive and false-negative results. Table 9 and Supplementary Fig. S5, Supplemental digital content 5, *http://links.lww.com/MCA/A272* present the AUC of combined proinflammatory and anti-inflammatory adipocytokines for the diagnosis of

Table 7 Receiver operating characteristic curve analysis of the anti-inflammatory biomarkers with coronary artery disease

Factors	AUC	P value	95% CI	Se (%)	Sp (%)	Cutoff
NTN1	0.771	<0.001	0.703–0.890	70.89	75.88	71.60
IL-37	0.908	<0.001	0.863–0.952	83.54	90.00	140.86
ADP	0.870	<0.001	0.820–0.920	82.28	85.37	9.80

ADP, adiponectin; AUC, area under the curve; CI, confidence interval; IL-37, interleukin-37; NTN1, Netrin-1.

Table 8 Receiver operating characteristic curve analysis of the inflammatory biomarkers with coronary artery disease

Factors	AUC	P value	95% CI	Se (%)	Sp (%)	Cutoff
RBP4	0.769	<0.001	0.700–0.837	91.76	61.27	4.03
PTX3	0.914	<0.001	0.875–0.943	96.47	82.19	2.41
PAI-1	0.699	<0.001	0.629–0.769	74.57	54.44	724.5
GAL-3	0.853	<0.001	0.803–0.903	68.64	86.08	2.66

AUC, area under the curve; CI, confidence interval; GAL-3, galectin3; PAI-1, plasminogen activator inhibitor-1; PTX3, Pentraxin3; RBP4, retinol-binding protein-4.

Table 9 Receiver operating characteristic curve analysis of the combined inflammatory biomarkers with coronary artery disease

Factors	AUC	P value	95% CI
RBP4+PTX3+PAI-1+GAL-3	0.950	<0.001	0.921–0.980
NTN1+IL-37+ADP	0.957	<0.001	0.932–0.983

ADP, adiponectin; AUC, area under the curve; CI, confidence interval; GAL-3, galectin-3; IL-37, interleukin-37; NTN1, netrin-1; PAI-1, plasminogen activator inhibitor-1; PTX3, Pentraxin3; RBP4, retinol-binding protein-4.

Table 10 Sensitivity and specificity of combined inflammatory biomarkers with coronary artery disease

Factors		Se (%)	Sp (%)		Se (%)	Sp (%)
RBP4+PTX3+PAI-1+GAL-3	Parallel	99.9	27.8	Series	42.6	99.1
NTN1+IL-37+ADP		99.5	57.4		47.6	99.4

ADP, adiponectin; GAL-3, galectin-3; IL-37, interleukin-37; NTN1, netrin-1; PAI-1, plasminogen activator inhibitor-1; PTX3, Pentraxin3; RBP4, retinol-binding protein-4.

CAD, NTN1+IL-37+ADP (AUC 0.957, 95% CI: 0.932–0.983) and PAI-1 (AUC 0.950, 95% CI: 0.921–0.980). Interestingly, the sensitivity of the proinflammatory and anti-inflammatory adipocytokines profiles mushroomed extremely with connection in parallel, while the specificity of cytokines increased dramatically by compounding in series.

Discussion

In this cohort study, we explored the association of proinflammatory and anti-inflammatory adipocytokines, including the serum levels of RBP-4, PTX-3, GAL-3, PAI-1, ADP, IL-37, and NTN1, with the development of CAD and the severity of coronary stenosis and deduced the putative inflammatory biomarkers for the diagnosis and risk assessment in the clinical setting. Moreover, we demonstrated significantly increased levels of the proinflammatory cytokines RBP4, PTX3, PAI-1, and GAL-3 in the CAD group than in the control group in univariable and multivariable analyses. Conversely, the anti-inflammatory cytokines NTN1, IL-37, and ADP were downregulated in the CAD group as compared with the control group. In addition, except for NTN1, all the biomarkers were significantly correlated with the Gensini score in the CAD patients. Also, all the inflammatory adipocytokines were significantly correlated with BNP. Furthermore, some inflammatory adipocytokines were significantly correlated with traditional cardiovascular clinical risk factors, such as hyperlipidemia, hypertension, type 2 diabetes mellitus, and smoking. Notably, inflammation is crucial in the pathogenesis and progression of coronary artery atherosclerosis. Thus, the inflammatory biomarkers might provide critical clues for the diagnosis, risk, and prognosis assessment of CAD. To the best of our knowledge, the current study is the first comparison of the association among the array of new serum inflammatory biomarkers and the diagnosis and risk assessment of CAD. In this study, GAL-3 showed the strongest correlation with the Gensini score ($r = 0.67$) among all the proinflammatory markers in CAD patients. GAL-3 regulated the survival of cardiomyocytes through the antiapoptotic process in the endothelial cells [22]. The association between GAL-3 with the incidence and severity of CAD might demonstrate its vital role in the development of atherosclerosis process. The current finding of a significant association of GAL-3 with the attack and coronary stenosis in CAD agrees with the previous study in other populations, indicating a significant role of GAL-3 in cardiac dysfunction [23]. Moreover, the positive correlation between GAL-3 and BNP found in the present study also contributed to this view. Furthermore, data from other studies indicate the association of GAL-3 with other cardiovascular diseases, HR, and abnormal ventricular wall motions. The latter two contribute to persistent atrial fibrillation [24]. Interestingly, GAL-3 is a robust independent predictor of high-risk non-ST-elevation myocardial infarction and cardiovascular death, and so has been implicated as a promising biomarker in clinical diagnosis and treatment of the disease [25].

Similarly, we also found that RBP4 was correlated with CAD and coronary stenosis. Recently, Farjo *et al.* [26] illustrated the mechanisms underlying the association of RBP4 with other inflammatory biomarkers. However, the correlations between RBP4 and metabolic disorders remain controversial. Previous studies demonstrated that RBP4 was associated with hypertriglyceridemia and hyperinsulinemia. On the other hand, some reports suggested that RBP4 was not associated with insulin resistance. The findings of the current study are consistent with an association between RBP4 with metabolic factors [27].

The classical proinflammatory adipocytokine, PAI-1, has been proved associated with aortic valve calcification, which increases the risk of major adverse cardiovascular outcomes in acute coronary syndrome [28]. In addition, PAI-1 contributes to the formation of atherosclerotic

plaques [29]. PTX3 secreted by macrophages and neutrophils in advanced atherosclerotic lesions has been strongly associated with CAD, compared to GAL-3, RBP-4, and PAI-1 [30]. Herein, a positive correlation was discovered among the level of PTX3, chest pain, and several traditional clinical risk factors of CAD. Also, a positive association was noted between PTX3 and aortic valve calcification [31]. Intriguingly, markedly elevated PTX3 was an independent, long-term predictor of all-cause mortality in CAD, which was associated with vulnerable plaque burdens and resulting impairment of post-percutaneous coronary intervention myocardial perfusion [32,33].

Strikingly, the anti-inflammatory adipocytokine, IL-37, was negatively linked with the coronary stenosis ($r = -0.65$) with low expression in the three-vessels CAD subgroup, compared with the one-vessel and two-vessels CAD subgroups and control group. Another study also demonstrated a protective effect of IL-37 that was due to the modulation of the response of macrophages to liposomes and facilitation of the differentiation to anti-inflammatory M2 cells. Thus, assessing the stenosis of coronary artery and risk of CAD patients in clinical practice is a valuable step. These findings support the potential role for IL-37 as a protective diagnostic biomarker of CAD.

We also demonstrated that the level of NTN1 was negatively correlated with Gensini score ($r = -0.318$). A previous study reported the association of low serum NTN1 levels with obesity and type 2 diabetes mellitus [34]. Supplementary Fig. S3, Supplemental digital content 3, *http://links.lww.com/MCA/A270* and Table 7 show that IL-37 (AUC 0.908, 95% CI: 0.863–0.952) was more accurate than NTN1 (AUC 0.771, 95% CI: 0.703–0.890) and ADP (AUC 0.870, 95% CI: 0.820–0.920), with an optimal cutoff value of IL-37 of 140.86 ng/mL. IL-37 had the largest AUC among the three anti-inflammatory adipocytokines in CAD with 83.54% sensitivity and 90% specificity. The protective mechanism became effective through the suppression of ROCK activation [35]. The IL-37 level has been associated with severe coronary artery calcification [36]. Thus, IL-37 could be suggested as a novel biomarker to diagnose and assess CAD.

To the best of our knowledge, ADP and PAI-1 are classical adipocytokines associated with CAD. According to the present study, the emerging biomarkers have a robust effect in CAD patients as compared with ADP and PAI-1.

Compared with the control group, patients in the CAD group more often received aspirin, clopidogrel, and statins. Treatment with antiplatelet medications may influence inflammation by modulating leukocyte responses and reducing myocardial necrosis [37]. In patients with acute coronary syndrome, clopidogrelin addition to aspirin significantly decreases the levels of TNF-α and C-reactive protein compared to aspirin alone [38]. Additionally, statins have positive effects on the reduction

of inflammation through immunomodulation, oxidative stress, antithrombotic, and antiplatelet mechanisms in CAD. As a protective factor, statins reduce the serum levels of TNF-α and C-reactive protein [39]. Presently, the levels of proinflammatory adipocytokines were very high in the CAD group. Thus, it is obvious that inflammatory responses remain after treatment. Some previous studies have proved the substantial values of circulating inflammatory biomarkers on the therapeutic and prognostic assessment in patients with CAD. The IL-1β inhibitor canakinumab was recently shown to have anti-inflammatory activity and the capability to reduce plasma levels of IL-6 and C-reactive protein, which trigger a series of reactions in atherogenesis and vessel response to injury [40]. Canakinumab can abate cardiovascular events, which should be proved valuable in clinical practice [41]. Multiple strategies to reduce inflammation will continue play a vital role after the diagnostic value of the various biomarkers have been determined. IL-1 had an established role in the atherogenesis and vessel-response to injury. A phase II, double-blinded, randomized, placebo-controlled study demonstrated that the inflammatory markers were decreased in non-ST-elevation acute coronary syndromes with the treatment of IL-1 receptor antagonistm, which indicated the importance of IL-1 as a therapeutic target in ACS [42]. Canakinumab Anti-Inflammatory Thrombosis Outcomes Study provided a strong proof of concept evidence in humans that modulation of the IL-6 signaling pathway, at least with canakinumab, associated with reduced major adverse cardiovascular events rates, independent of lipid lowering [43]. Besides, 18F-fluorodeoxyglucose PET identified the precise point of action of canakinumab, which needed to be further studied [44].

The present findings indicate that the combination of these classical and novel biomarkers may have diagnostic benefits in CAD. Furthermore, the biomarker combinations may be beneficial in the evaluation of the severity of coronary stenosis and heart dysfunction, and in the clinical outcome. Multibiomarker research are expected to provide new insights concerning diagnosis and risk assessment in CAD patients.

Conclusion
The newly identified proinflammatory and anti-inflammatory adipocytokines RBP4, PTX3, GAL-3, NTN1, and IL-37 were more effective than the classical biomarkers, PAI-1 and ADP, for the diagnosis and risk assessment in CAD patients.

Acknowledgments

Conflicts of interest

There are no conflicts of interest.

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