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Original Article

Epicardial adipose tissue thickness and plasma interleukin-35 predict acute myocardial infarction in patients with coronary artery disease

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Abstract: This study aimed to investigate the associations of echocardiographic epicardial adipose tissue (EAT) thickness and levels of plasma cytokines with acute coronary syndromes and extent of coronary atherosclerosis. The study enrolled 117 patients diagnosed with coronary artery disease (CAD) who underwent coronary angiography (May 2013 to April 2014) and 30 patients with chest pain and normal angiography (control group). Patients with CAD were divided into acute myocardial infarction (AMI, n = 40), unstable angina pectoris (UAP, n = 40) and stable angina pectoris (SAP, n = 37) groups. In a separate analysis, patients with CAD were divided into groups according to Gensini score (A: < 26; B: 26-54; C: > 54). EAT thickness and plasma concentrations of interleukin-35 (IL-35), YKL-40, adiponectin and high-sensitivity C-reactive protein (hs-CRP) were measured. Adiponectin, hs-CRP and YKL-40 levels correlated with coronary lesion severity. Mean EAT thicknesses in the control, SAP, UAP and AMI groups were 4.3 ± 0.8 mm, 5.1 ± 1.6 mm, 6.2 ± 1.8 mm and 7.9 ± 1.5 mm, respectively (P < 0.01). Multivariate logistic regression identified EAT thickness (OR: 2.196; 95% CI, 1.465-3.292), IL-35 (OR: 0.926; 95% CI, 0.891-0.963) and YKL-40 (OR: 1.114; 95% CI, 1.049-1.184) as independently associated with AMI. Using an optimal EAT thickness cut-off value of 6.75 mm, the sensitivity, specificity, positive and negative predictive values for predicting AMI were 82.5%, 81.3%, 62.3% and 92.6%, respectively. Measurements of EAT thickness and serum levels of interleukins, cytokines and adipokines may help identify patients needing a more aggressive approach for risk reduction. IL-35 gene regulation and EAT thickness reduction may be effective treatments for atherosclerosis and CAD.

Keywords: Epicardial adipose tissue, coronary artery disease, interleukin-35, Gensini score

Introduction

Epicardial adipose tissue (EAT) is located between the heart and pericardium and considered to be a metabolically active visceral fat tissue [1]. EAT in which coronary arteries are embedded covers more than three-quarters of the surface of the heart [2]. Side-branches of the coronary arteries provide nutrition for the EAT [3]. Since there is no anatomical barrier between the EAT and myocardium, they are both supplied by the same microcirculation. Recent studies have identified EAT as an active organ that secretes many proatherogenic and proinflammatory hormones and cytokines, which are thought to be involved in the initiation and progression of coronary artery disease (CAD) through endocrine and paracrine mechanisms [4]. EAT thickness has been found to be closely associated with the presence, severity and extent of atherosclerotic CAD [5]. EAT is presumed to play an important role in the development of subclinical atherosclerosis, which occurs years before the clinical manifestations of CAD [6]. We hypothesized that EAT could potentially be used to identify patients with stable angina pectoris (SAP) or unstable angina pectoris (UAP) who are at increased risk of acute myocardial infarction (AMI). Therefore, this study was designed to investigate the associations between EAT thickness and the levels of cytokines such as adipokines and interleukin-35 (IL-35) in patients with different clinical presentations of CAD (SAP, UAP and AMI)

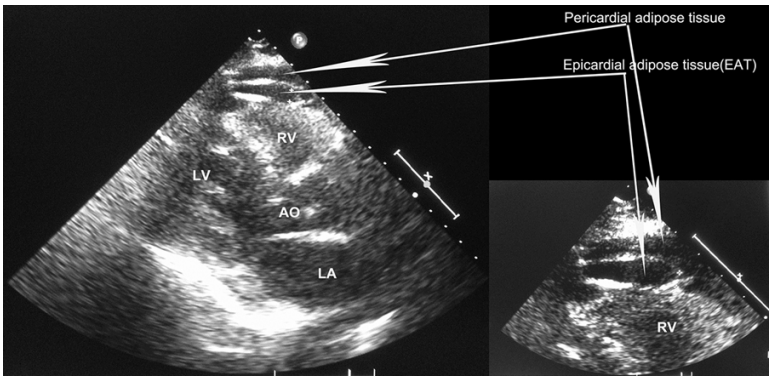

Figure 1. Transthoracic echocardiography image (parasternal long axis view) showing the measurement of epicardial adipose tissue thickness on the free wall of the right ventricle near the aortic annulus. AO: aorta; EAT: epicardial adipose tissue LA: left atrium; LV: left ventricle; RV: right ventricle.

and determine whether EAT thickness could be used to predict the risk of AMI in patients with CAD.

Materials and methods

Study population

This study enrolled a total of 117 consecutive hospitalized patients with a clinical diagnosis of CAD who underwent coronary angiography between May 2013 and April 2014. In addition, the study included 30 patients with chest pain who had normal angiography as a control group. The exclusion criteria were: history of either surgical or percutaneous prior revascularization; autoimmune disease; recent acute or chronic infection; serious impairment of liver or kidney function; malignant disease; myocarditis or cardiomyopathy; diseases of the blood system; serious heart failure; recent acute stroke; valvular heart disease; pericardial effusion; septicemia; on steroid therapy; and poor echocardiographic imaging. This study was approved by the institutional ethics committee of the Second Affiliated Hospital of Xuzhou Medical College.

The 117 patients with CAD were divided into three groups: AMI (n = 40), UAP (n = 40) and SAP (n = 37). Patients in the AMI group had myocardial infarction confirmed by significant elevations in troponin I and creatine kinase MB levels. Patients in the UAP group had chest pain at rest with definite ischemic electrocardiographic changes (ST-segment changes and/or T-wave inversions). Patients in the SAP group had typical exertion-induced chest discomfort associated with a downsloping or horizontal ST-segment depression of at least 1 mm in an exercise test. The 30 angiographically normal cases included as the control group had chest pain that was not accompanied by electrocardiographic changes, coronary stenosis, or coronary spasm when an intracoronary injection of acetylcholine was given during coronary angiography.

Blood sample collection and processing

Blood samples were taken from the antecubital vein. In the AMI group, blood samples were collected just after the patients had arrived at the emergency unit. For all groups, blood samples after fasting for a minimum of 12 hours were collected the morning after admission, with the subjects in a seated position and rested for 20 min. Plasma glucose, triglycerides, HDL cholesterol, total cholesterol, LDL cholesterol and high-sensitivity C-reactive protein were measured. In addition, samples were collected into sodium heparin Vacutainers and processed within 1 hour of collection by centrifugation for 15 min at 1000 × *g*. The supernatant was then aliquoted and stored at -80°C until analysis. All samples were thawed only once.

Detection of the levels of plasma cytokines by enzyme-linked immunosorbent assay (ELISA)

The levels of plasma IL-35 and adiponectin (APN) were measured using an ELISA, in accordance with the manufacturer's instructions (Westtang Bio-tech, Shanghai, China). Intra-assay and inter-assay coefficients of variation for ELISA were 5% and 10%, respectively. All samples were measured in triplicate.

Echocardiography

All echocardiographic assessments of the study patients were performed by the same cardiologist, who was blinded to the patient's clinical information. Echocardiography was performed before coronary angiography, using a GE ViVid E7 ultrasonography machine (GE Healthcare, Chicago, IL, USA) with a 2.5-3.5 MHz transducer. Each examination was record-

Table 1. Distribution of general characteristics in the study population

Parameter	SAP (n = 37)	UAP (n = 40)	AMI (n = 40)	Control (n = 30)	Homo-geneity *p*-value	Variance analysis *p*-value	Post-hoc test	SAP vs control *p*-value	UAP vs control *p*-value	AMI vs control *p*-value	SAP vs UAP *p*-value	SAP vs AMI *p*-value	UAP vs AMI *p*-value
Gender, male %	56.8	57.5	72.5	56.7	-	0.403	K-W	-	-	-	-	-	-
Age, years	62.84 ± 9.12	65.65 ± 9.77	62.60 ± 13.21	60.13 ± 9.34	0.057	0.195	LSD	0.301	0.033	0.337	0.246	0.922	0.200
BMI, kg/m^2	23.79 ± 1.66	23.39 ± 1.65	24.08 ± 1.89	23.05 ± 1.85	0.517	0.081	LSD	0.088	0.430	0.017	0.312	0.484	0.082
Smoking, %	51.4	52.5	57.5	50.0	-	0.924	K-W	-	-	-	-	-	-
Alcohol, %	48.6	50.0	52.5	46.7	-	0.969	K-W	-	-	-	-	-	-
DM, %	16.2	20.0	17.5	16.7	-	0.974	K-W	-	-	-	-	-	-
Hypertension, %	48.6	55.0	52.5	46.7	-	0.898	K-W	-	-	-	-	-	-
HDL, mg/dL	1.07 ± 0.20	0.99 ± 0.20	1.04 ± 0.18	1.16 ± 0.28	0.887	0.016	LSD	0.112	0.002	0.023	0.102	0.481	0.339
LDL, mg/dL	2.48 ± 0.79	3.00 ± 0.90	2.97 ± 0.77	2.51 ± 0.80	0.741	0.006	LSD	0.889	0.014	0.021	0.006	0.010	0.874
VLDL-C, mmol/L	0.80 ± 0.37	0.73 ± 0.42	0.65 ± 0.29	0.75 ± 0.31	0.151	0.336	LSD	0.556	0.835	0.266	0.393	0.071	0.328
TG, mmol/L	1.86 ± 0.80	1.48 ± 0.82	1.39 ± 0.63	1.49 ± 0.75	0.826	0.035	LSD	0.047	0.948	0.555	0.028	0.006	0.571
TC, mmol/L	3.81 ± 1.00	4.82 ± 1.53	4.59 ± 1.12	4.19 ± 0.99	0.765	0.002	LSD	0.195	0.032	0.171	< 0.001	0.005	0.397
Glucose, mmol/L	5.35 ± 0.78	6.01 ± 1.58	6.44 ± 1.89	4.94 ± 0.89	< 0.001	< 0.001	Tamhane	0.284	0.004	< 0.001	0.122	0.009	0.854

Data presented as mean ± standard deviation or %. AMI: acute myocardial infarction; BMI: body mass index; DM: diabetes mellitus; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; LSD: least significant difference; K-W: Kruskal-Wallis; SAP: stable angina pectoris; TC: total cholesterol; TG: triglycerides; UAP: unstable angina pectoris; VLDL-C: very low density lipoprotein cholesterol.

Table 2. Plasma concentrations of cytokines and EAT thickness in each group

Parameter	SAP (n = 37)	UAP (n = 40)	AMI (n = 40)	Control (n = 30)	Homo-geneity *p*-value	Variance analysis *p*-value	Post-hoc test	SAP vs control *p*-value	UAP vs control *p*-value	AMI vs control *p*-value	SAP vs UAP *p*-value	SAP vs AMI *p*-value	UAP vs AMI *p*-value
IL-35, pg/mL	74.06 ± 21.08	63.05 ± 17.65	52.76 ± 16.85	92.31 ± 24.42	0.622	< 0.001	LSD	< 0.001	< 0.001	< 0.001	0.016	< 0.001	0.022
YKL-40, ng/mL	48.92 ± 12.51	55.08 ± 11.36	65.18 ± 9.07	38.58 ± 8.08	0.055	< 0.001	LSD	< 0.001	< 0.001	< 0.001	0.011	< 0.001	< 0.001
APN, μg/mL	4.95 ± 1.08	4.06 ± 1.01	3.81 ± 1.01	6.90 ± 1.41	0.174	< 0.001	LSD	< 0.001	< 0.001	< 0.001	0.001	< 0.001	0.334
EAT, mm	5.12 ± 1.59	6.17 ± 1.76	7.94 ± 1.51	4.27 ± 0.81	0.002	< 0.001	Tamhane	0.037	< 0.001	< 0.001	0.045	< 0.001	< 0.001
hs-CRP, mg/L	3.86 ± 1.10	5.36 ± 1.17	5.93 ± 1.29	2.47 ± 0.77	0.055	< 0.001	LSD	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.024

Data presented as mean ± standard deviation. AMI: acute myocardial infarction; APN: adiponectin; EAT: epicardial adipose tissue thickness; hs-CRP: high-sensitivity C-reactive protein; IL-35: interleukin-35; LSD: least significant difference; SAP: stable angina pectoris; UAP: unstable angina pectoris; YKL-40: chitinase-3-like protein-1.

ed, and two other cardiologists who were blinded to the clinical presentation interpreted the results off-line. Parasternal and apical projections were obtained according to the recommendations of the American Society of Echocardiography [7]. EAT thickness on the free wall of the right ventricle was measured at end-systole from the parasternal long axis view, using the aortic annulus as an anatomic reference [8]. We chose to measure EAT thickness on the area above the right ventricle because this region is known to have the thickest EAT layer. This region was identified as the echo-free space between the outer wall of the myocardium and the visceral layer of the pericardium (**Figure 1**, Supplementary Data). The thickest point of the EAT was measured each time and the average value of 3 cardiac cycles was recorded.

Coronary angiography

Selective left and right coronary angiography was performed via the radial or femoral artery using the standard Judkins technique with 6 Fr catheters (MediCath, Barcelona, Spain) and an Axiom Artis dFA system (Siemens Corp., Berlin, Germany). The severity of coronary stenosis was estimated using the modified Gensini score. The Gensini score was computed by assigning a severity score to each coronary stenosis according to the degree of luminal narrowing and its geographic importance. This scoring system grades luminal narrowing of the coronary arteries as 1 for stenosis of 1-25%, 2 for stenosis of 26-50%, 4 for stenosis of 51-75%, 8 for stenosis of 76-90%, 16 for stenosis of 91-99%, and 32 for total occlusion. For the location scores, 5 points were assigned for the left main coronary artery; 2.5 for the proximal left anterior descending (LAD) and left circumflex (LCX) artery; 1.5 for the mid-segment LAD and LCX; 1 for the distal segment of the LAD and LCX, first diagonal branch, first obtuse marginal branch, right coronary artery, posterior descending artery and intermediate artery; and 0.5 for others [9]. The points were summated to give the total Gensini score for each patient. All patients with CAD were divided into three groups based on Gensini score: group A (Gensini score < 26), group B (Gensini score 26-54) and group C (Gensini score > 54).

Statistics

Data were analyzed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as means ± standard deviations, and categorical variables are presented as numbers and percentages. Data were tested for a normal distribution using the Kolmogorov-Smirnov test. Mean values were compared between different groups using analysis of variance (ANOVA). The X^2 test was used for comparisons of categorical variables between groups. Spearman correlation analysis was used to identify correlations between two variables. Univariate and multivariate logistic regression analyses were used to identify factors that were independently associated with AMI, with calculation of odds ratios (ORs) and 95% confidence intervals (95% CIs). Factors identified as significant in the univariate analysis ($P < 0.05$) were entered into the multivariate analysis, which was performed using the enter method (i.e. standard logistic regression analysis). An optimal cut-off value for EAT thickness for predicting AMI was determined by receiver operating characteristics (ROC) curve analysis, with calculations of the area under curve (AUC), sensitivity, specificity, positive predictive value and negative predictive value. All tests of significance were two-tailed. Statistical significance was defined as $P < 0.05$.

Results

Study population

The general characteristics of the study population are shown in **Table 1**, Supplementary Data. There were no significant differences between groups with respect to gender, prevalence of diabetes or hypertension, or proportion of patients who smoked or drank alcohol. Age was significantly higher in the UAP group than in the control group ($P < 0.05$), and body mass index was significantly higher in the AMI group than in the control group ($P < 0.05$), while there were no significant differences for any other pairwise comparisons for these variables between groups. The prevalences of dyslipidemia and fasting glucose were higher in patients in the UAP and AMI groups than in the SAP and control groups ($P < 0.05$).

Plasma concentrations of cytokines and EAT thickness in the control, SAP, UAP and AMI groups

The plasma concentrations of cytokines and EAT thickness in each group are shown in **Table 2**, Supplementary Data. The concentrations

Table 3. Plasma concentrations of cytokines and EAT thickness in patients with coronary artery disease stratified according to the Gensini coronary score

Parameter	Group A (Gensini score < 26) (n = 44)	Group B (Gensini score 26-54) (n = 40)	Group C (Gensini score > 54) (n = 33)	Homogeneity *p*-value	Variance analysis *p*-value	Post-hoc test	A vs B *p*-value	A vs C *p*-value	B vs C *p*-value
hs-CRP, mg/L	3.81 ± 1.06	5.17 ± 0.76	6.68 ± 0.83	0.230	< 0.001	LSD	< 0.001	< 0.001	< 0.001
IL-35, pg/mL	62.77 ± 20.68	65.58 ± 22.03	60.22 ± 17.77	0.410	0.534	LSD	0.530	0.587	0.266
YKL-40, ng/mL	45.49 ± 9.81	58.63 ± 6.91	68.90 ± 8.85	0.104	< 0.001	LSD	< 0.001	< 0.001	< 0.001
APN, μg/mL	5.27 ± 0.78	4.10 ± 0.54	3.10 ± 0.82	0.057	< 0.001	LSD	< 0.001	< 0.001	< 0.001
EAT, mm	5.04 ± 1.61	6.53 ± 1.37	8.20 ± 1.62	0.632	< 0.001	LSD	< 0.001	< 0.001	< 0.001

Data presented as mean ± standard deviation. APN: adiponectin; EAT: epicardial adipose tissue thickness; hs-CRP: high-sensitivity C-reactive protein; IL-35: interleukin-35; LSD: least significant difference; YKL-40: chitinase-3-like protein-1.

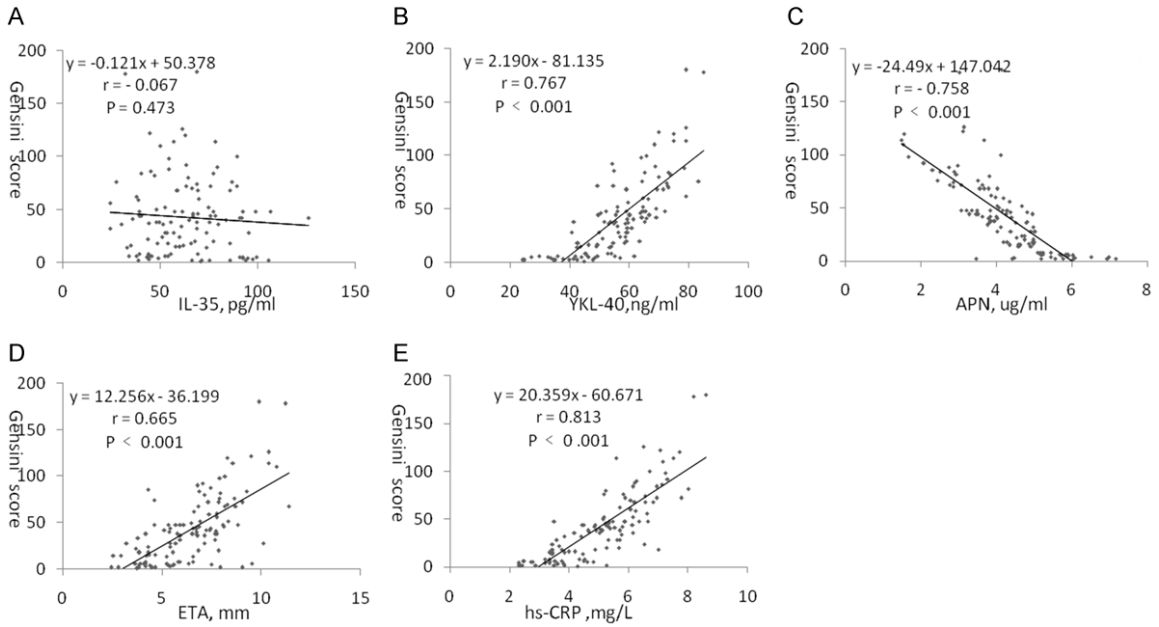

Figure 2. The correlations of plasma cytokine concentrations and EAT thickness with Gensini coronary score in patients with CAD. A: There was no significant correlation between the plasma level of interleukin-35 (IL-35) and Gensini coronary score (r = -0.067, P = 0.473). B: Plasma level of chitinase-3-like protein-1 (YKL-40) was positively correlated with Gensini coronary score (r = 0.767, P < 0.001). C: Plasma level of adiponectin (APN) was negatively correlated with Gensini coronary score (r = -0.758, P < 0.001). D: EAT thickness was positively correlated with Gensini coronary score (r = 0.665, P < 0.001). E: Plasma level of high-sensitivity C-reactive protein (hs-CRP) was positively correlated with Gensini coronary score (r = 0.813, P < 0.001).

of IL-35 and APN were significantly lower in patients with CAD than in the control group and significantly lower in the UAP and AMI groups than in the SAP group (all P < 0.05). The mean concentrations of YKL-40 in the SAP, UAP, AMI and control groups were 48.92 ± 12.51 ng/mL, 55.08 ± 11.36 ng/mL, 65.18 ± 9.07 ng/mL and 38.58 ± 8.08 ng/mL, respectively, and the differences were significant for all pairwise comparisons between groups (P < 0.05). EAT thickness was significantly higher in CAD patients than in control groups. The mean EAT thicknesses in the SAP, UAP, AMI and control groups were 5.12 ± 1.59 mm, 6.17 ± 1.76 mm, 7.94 ± 1.51 mm and 4.27 ± 0.81 mm, respectively, and the differences were significant for all pairwise comparisons between groups (P < 0.05).

Plasma concentrations of cytokines and EAT thickness in patients with CAD stratified according to the Gensini coronary score

When data were compared between three groups based on Gensini coronary score, the mean plasma concentrations of hs-CRP and YKL-40 and the mean EAT thickness increased as the Gensini score increased, whereas the

mean plasma concentration of APN decreased as the Gensini score increased, with significant differences ($P < 0.001$) observed for all pairwise comparisons between groups (**Table 3**, Supplementary Data). By contrast, plasma levels of IL-35 did not differ significantly between groups (**Table 3**, Supplementary Data). Spearman correlation analysis (**Figure 2**, Supplementary Data) revealed that plasma levels of hs-CRP and YKL-40 were positively correlated with Gensini coronary score ($r = 0.833$, $P < 0.01$ and $r = 0.814$, $P < 0.01$, respectively), while plasma levels of APN were negatively correlated with Gensini coronary score ($r = -0.844$, $P < 0.01$). EAT thickness was positively correlated with Gensini coronary score ($r = 0.664$, $P < 0.01$). There was no significant correlation between plasma levels of IL-35 and Gensini coronary score.

Variation of study parameters with increasing EAT thickness in patients with CAD

The study parameters were further compared between three subgroups of patients with CAD based on EAT thickness (< 5 mm, 5-7 mm and > 7 mm); the results are presented in **Table 4**, Supplementary Data. These EAT cut-off values were used to define the groups because they have been utilized for data comparisons in previous studies [10, 11]. Pairwise comparisons revealed significant differences between all the EAT thickness groups with regard to plasma levels of hs-CRP, YKL-40 and APN ($P < 0.05$), with plasma concentrations of hs-CRP and YKL-40 increasing with greater EAT thickness and plasma level of APN decreasing with greater EAT thickness.

Logistic regression analysis of factors associated with AMI in patients with CAD

Univariate logistic regression analysis showed that EAT thickness, plasma level of IL-35 and plasma level of YKL-40 were associated with AMI (**Table 5**, Supplementary Data). Multivariate logistic regression analysis found that EAT thickness, plasma level of IL-35 and plasma level of YKL-40 were all independently associated with AMI (**Table 6**, Supplementary Data).

ROC curve analysis of the utility of EAT thickness for predicting AMI in patients with CAD

ROC curve analysis (**Figure 3**, Supplementary Data) identified the optimal cut-off EAT thickness value for predicting AMI as 6.75 mm (AUC: 0.838; 95% CI: 0.765-0.911; $P < 0.001$). Using a cut-off value of 6.75 mm for EAT thickness, the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for predicting AMI were 82.5%, 81.3%, 62.3%, 92.6% and 82.4%, respectively.

Discussion

The present study yielded several important findings regarding the associations of EAT thickness and plasma level of IL-35 with atherosclerotic disease in patients with CAD. First, this study revealed associations between EAT thickness and the presence and severity of CAD. Second, EAT thickness emerged as an independent predictor of CAD among other well-known risk factors. Third, ROC curve analysis identified 6.75 mm as the optimal cut-off value for EAT thickness for predicting the presence of AMI in patients admitted with a wide range of chest pain syndromes. Indeed, the most striking finding of this study was the identification of EAT as a predictor of AMI with satisfactory sensitivity, specificity and negative predictive values. Fourth, although there was no association between plasma level of IL-35 and Gensini score, the concentrations of both IL-35 and APN were lower in the UAP and AMI groups than in the SAP group. We suggest that EAT thickness and plasma level of IL-35 may help to identify patients with a high risk of AMI and predict coronary findings prior to coronary angiography. Furthermore, EAT thickness and plasma level of IL-35 potentially could be used as follow-up parameters in clinical practice.

EAT is present on the surface of the heart between the myocardium and visceral pericardium. EAT is concentrated in the atrioventricular and interventricular grooves, along the major branches of the coronary arteries, around the atria, over the free wall of the right ventricle and over the apex of the left ventricle [12] in the normal adult population. EAT is supplied by side-branches of the coronary arteries, similar to the microcirculation of the myocardium. Therefore, EAT can locally modulate both the myocardium and coronary arteries [13]. Both adipocytes and tissue macrophages in EAT have secretory properties and are capable of producing adipokines and cytokines, as in omental and mesenteric visceral adipose tissue. These adipokines and cytokines influence

Table 4. Variation of study parameters with increasing EAT thickness in patients with coronary artery disease

Parameter	Epicardial adipose tissue thickness			Homogeneity	Variance analysis	Post-hoc	< 5 mm vs 5-7 mm	< 5 mm vs > 7 mm	5-7 mm vs > 7 mm
	< 5 mm (n = 30)	5-7 mm (n = 41)	> 7 mm (n = 46)	*p*-value	*p*-value	test	*p*-value	*p*-value	*p*-value
Gender, male %	56.70	56.10	71.70	-	0.247	KW	-	-	-
Age, years	63.30 ± 10.09	63.30 ± 9.42	62.37 ± 12.49	0.202	0.391	LSD	0.395	0.717	0.178
BMI, kg/m^2	23.11 ± 1.47	23.66 ± 1.56	24.25 ± 1.95	0.111	0.018	LSD	0.181	0.005	0.110
Alcohol, %	43.30	53.70	52.20	-	0.662	KW	-	-	-
Smoking, %	46.70	48.80	63.00	-	0.274	KW	-	-	-
Diabetes mellitus, %	13.30	19.50	19.60	-	0.749	KW	-	-	-
Hypertension, %	50.00	65.90	41.30	-	0.072	KW	-	-	-
HDL, mg/dL	1.03 ± 0.21	1.04 ± 0.18	1.03 ± 0.19	0.487	0.931	LSD	0.777	0.973	0.724
LDL, mg/dL	2.52 ± 0.92	2.88 ± 0.83	2.98 ± 0.78	0.553	0.064	LSD	0.078	0.022	0.591
VLDL-C, mmol/L	0.82 ± 0.41	0.71 ± 0.35	0.68 ± 0.34	0.576	0.216	LSD	0.191	0.088	0.683
TG, mmol/L	1.69 ± 0.89	1.67 ± 0.76	1.41 ± 0.70	0.462	0.180	LSD	0.901	0.118	0.116
TC, mmol/L	4.18 ± 1.23	4.46 ± 1.16	4.54 ± 1.45	0.946	0.484	LSD	0.367	0.240	0.783
Glucose, mmol/L	5.73 ± 1.35	5.67 ± 1.39	6.33 ± 1.84	0.043	0.100	LSD	0.872	0.102	0.050
hs-CRP, mg/L	4.02 ± 1.31	4.77 ± 1.06	6.05 ± 1.26	0.870	< 0.001	LSD	0.011	< 0.001	< 0.001
IL-35, pg/mL	62.81 ± 18.31	65.63 ± 20.23	60.81 ± 21.78	0.606	0.547	LSD	0.565	0.678	0.274
YKL-40, ng/mL	48.04 ± 10.68	56.44 ± 10.78	62.29 ± 12.86	0.484	< 0.001	LSD	0.003	<0.001	0.021
APN, µg/mL	4.89 ± 1.15	4.37 ± 0.88	3.74 ± 1.11	0.416	< 0.001	LSD	0.042	< 0.001	0.005

Data presented as mean ± standard deviation or %. APN: adiponectin; BMI: body mass index; HDL: high-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; IL-35: interleukin-35; LDL: low-density lipoprotein cholesterol; LSD: least significant difference; K-W: Kruskal-Wallis; TC: total cholesterol; TG: triglycerides; VLDL-C: very low density lipoprotein cholesterol; YKL-40: chitinase-3-like protein-1.

Table 5. Univariate logistic regression analysis of factors associated with acute myocardial infarction

Independent variable	Odds ratio	95% confidence interval	*p*-value
Age, years (per unit increase)	0.967	0.902-1.037	0.350
Gender (female vs male)	0.631	0.103-3.883	0.620
BMI, kg/m^2 (per unit increase)	0.761	0.470-1.233	0.268
Alcohol consumption (yes vs no)	0.937	0.156-5.613	0.943
Smoking (yes vs no)	1.200	0.265-5.443	0.813
Hypertension (yes vs no)	0.608	0.126-2.929	0.535
Diabetes mellitus (yes vs no)	1.173	0.149-9.211	0.879
EAT thickness, mm (per unit increase)	2.814	1.457-5.433	0.002
IL-35, pg/mL (per unit increase)	0.920	0.877-0.966	0.001
YKL-40, ng/mL (per unit increase)	1.158	1.059-1.267	0.001
APN, μg/mL (per unit increase)	2.401	0.860-6.703	0.094
hs-CRP, mg/L (per unit increase)	1.360	0.554-3.337	0.503
Glucose, mmol/L (per unit increase)	1.134	0.724-1.779	0.583
TG, mmol/L (per unit increase)	0.967	0.144-6.477	0.972
TC, mmol/L (per unit increase)	1.138	0.604-2.143	0.688
HDL, mg/dL (per unit increase)	1.597	0.041-62.521	0.802
LDL, mg/dL (per unit increase)	0.718	0.256-2.019	0.530
VLDL-C, mmol/L (per unit increase)	1.770	0.038-82.790	0.771

APN: adiponectin; BMI: body mass index; EAT: epicardial adipose tissue; HDL: high-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; IL-35: interleukin-35; LDL: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides; VLDL-C: very low density lipoprotein cholesterol; YKL-40: chitinase-3-like protein-1.

Table 6. Multivariate stepwise logistic regression analysis of factors independently associated with acute myocardial infarction

Independent variable	Odds ratio	95% confidence interval	*p*-value
EAT thickness, mm (per unit increase)	2.196	1.465-3.292	< 0.001
IL-35, pg/mL (per unit increase)	0.926	0.891-0.963	< 0.001
YKL-40, ng/mL (per unit increase)	1.114	1.049-1.184	< 0.001

EAT: epicardial adipose tissue; IL-35: interleukin-35; YKL-40: chitinase-3-like protein-1.

the myocardium and coronary arteries via paracrine and vasocrine mechanisms. Cytokines released from EAT can traverse the coronary wall by diffusion (from outside to inside) and interact with cells in each layer of the vessel wall in a paracrine fashion [14]. On the other hand, adipokines and free fatty acids might be released from EAT directly into vasa vasorum and be transported downstream into the coronary wall to participate in vasocrine signaling [15]. EAT has cardioprotective properties through local secretion of anti-inflammatory and anti-atherogenic adipokines such as APN, which can improve endothelial function but is under-expressed in the EAT in patients with CAD [16]. Recently, down-regulation of these adipokines was demonstrated in chronic CAD [17]. In the present study, we found that the concentration of APN was lower in patients with CAD than in those without CAD. Furthermore, the concentration of APN was lower in the UAP and AMI groups than in the SAP group. Additionally, the plasma concentration of APN gradually decreased as the severity of CAD increased. Therefore, the plasma concentration of APN can reflect the presence and severity of CAD to a certain extent.

An increase in the quantity of EAT is associated with incident CAD and major adverse cardiac events [18], and the associations are independent of body mass index and other traditional risk factors. EAT is one of the factors contributing to CAD, as compared with other visceral adipose tissue [19]. A recent meta-analysis of 2872 patients concluded that EAT thickness and volume were significantly increased in patients with CAD compared to those without CAD [4]. Furthermore, patients in the higher EAT volume tertile (≥ 100 mL) were more likely to have CAD compared to those in the lower EAT tertile (< 100 mL) (risk ratio: 0.69; 95% CI: 0.52-0.92; P = 0.01). The authors pointed out that patients with coronary plaques had increased EAT volume compared with patients without coronary plaques. Another study demonstrated that EAT had significant correlation with both non-stenotic lesions and non-calcified plaques, and a greater EAT volume was found in patients with non-calcified plaques than in patients with calcified plaques [20]. This may be relevant for the development of acute coronary syndromes as

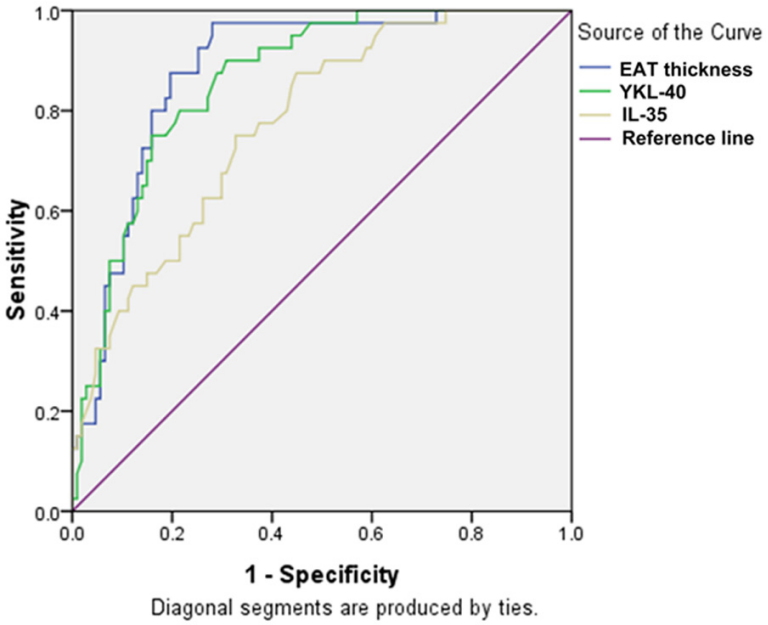

Figure 3. Receiver operating characteristic (ROC) curves for epicardial adipose tissue (EAT) thickness, plasma level of chitinase-3-like protein-1 (YKL-40) and plasma level of interleukin-35 (IL-35) in the prediction of acute myocardial infarction. The area under the curve was 0.838 (95% confidence interval: 0.765-0.911, P < 0.001) for EAT thickness, 0.812 (95% confidence interval: 0.734-0.890; P < 0.001) for YKL-40 and 0.290 (95% confidence interval: 0.194-0.386; P < 0.001) for IL-35.

non-calcified parts of a plaque contribute to plaque vulnerability [21]. Furthermore, EAT volume is a strong and independent determinant of the presence of total coronary occlusions [22]. EAT might affect coronary micro-vascular function, which is relevant to the mechanisms underlying coronary slow flow [23]. EAT was an independent predictor of diminished coronary flow reserve in women with angiographically normal coronary arteries [24], while increased EAT thickness was detected in patients with coronary slow flow phenomenon [25]. A community-based study determined that pericardial fat was associated with an increase in incident coronary events such as myocardial infarction, resuscitated cardiac events, angina and fatal coronary heart disease [19]. It was also reported that EAT thickness was higher in patients with unstable presentation than in those with atypical chest pain or stable angina [26]. A recent study reported that EAT thickness measured by echocardiography was associated with plaque vulnerability determined by virtual histology intravascular ultrasound in patients with significant CAD [27]. It was reported recently that EAT volume was associated with atherosclerotic plaque characteristics, classified as fibrous, calcific, lipid or necrotic, and there was an association between increased EAT volume and higher percentage of necrotic plaque tissue, which is potentially the most vulnerable type [28]. Plaque vulnerability may be the explanation for the association between increased EAT and AMI.

Inflammatory cells and pro-inflammatory cytokines are found both in early lesions and advanced lesions, as well as following plaque rupture and during thrombus formation. The imbalance of anti-inflammatory and pro-inflammatory cytokines results in the progression of atherosclerosis, plaque instability and the subsequent onset of acute coronary syndrome [29]. In the present study, we measured the levels of not only anti-inflammatory cytokines but also pro-inflammatory cytokines, namely hs-CRP, IL-35, YKL-40 and APN. Many studies have shown that IL-35 is an anti-inflammatory cytokine that can efficiently suppress effector T cell activity [30] and alter the progression of inflammatory [31] and autoimmune diseases [32]. A recent study found that EBI3 and p35 were expressed in almost all advanced plaque lesions and co-expressed in atheroma vascular smooth muscle cells, indicating that IL-35 may be secreted by vascular smooth muscle cells [33]. IL-35 secretion can be stimulated by the pro-inflammatory cytokines, TNF-a or IFN-c, and this effect was attenuated by pretreatment with the peroxisome proliferator-activated receptor-c (PPARc) agonist, rosiglitazone, suggesting a potential role of IL-35 in the progression of atherosclerosis. In the present study, the relationship between the levels of each cytokine and the severity of coronary artery stenosis was measured by Spearman correlation analysis. We found that the level of IL-35 was not related to the Gensini score, suggesting that changes in IL-35 level are associated with inflammatory status and

plaque destabilization in CAD but not the severity of coronary artery stenosis. Previous studies have confirmed that coronary lesions in patients with SAP were often characterized by severe luminal narrowing, a mild inflammatory response and a stable plaque, while coronary lesions in acute coronary syndromes were often characterized by moderate luminal narrowing, a strong inflammatory response and a vulnerable plaque. The present study found that the levels of anti-inflammatory cytokines (IL-35 and APN) were lower in the UAP and AMI groups than in the SAP group, while the levels of pro-inflammatory cytokines (hs-CRP and YKL-40) were significantly higher in the UAP and AMI groups. These variations in cytokine levels support the proposal that a strong inflammatory response participates in acute coronary syndromes. Therefore, measuring the plasma levels of cytokines may be a useful method for forecasting acute coronary syndromes. However, information regarding the plasma level of IL-35 and its potential role in CAD has been limited. Thus, our study has enriched this field of research and indicates that the level of serum IL-35 could be used to predict the incidence of cardiovascular events and the prognosis of patients with acute coronary syndrome.

Study limitations

The most important limitations of this study are the relatively small sample size and the cross-sectional design, which hampers the inferral of causality. Although we measured the serum levels of IL-35 and adipokines, our study was not designed to explore the underlying mechanisms. In addition, although physiopathologic properties, atherosclerotic plaque components and thrombotic milieu may differ between patients with ST-elevation AMI (STEMI) and those with non-STEMI, the AMI group in the present study was not analyzed separately as STEMI and NSTEMI because the number of patients in the subgroups would have been inadequate for a proper statistical analysis. Therefore, we preferred to allocate patients with proven myocardial damage into a single group. Patients without angiographic evidence of coronary artery stenosis were recruited as a control group, while patients with coronary artery stenosis ≥ 50% were enrolled as patients with CAD; hence, this study provides no information about patients with coronary stenosis ≤ 50%. Lastly, quantification of EAT was performed by echocardiography. Although assessment of EAT volume by computed tomography is a more precise method, echocardiography is cheap and easily available.

In conclusion, quantification of EAT thickness using echocardiography and measurement of serum levels of interleukins, cytokines and adipokines are relatively inexpensive and readily available methods that may prove beneficial for identifying patients needing a more aggressive approach to risk reduction. Furthermore, these variables potentially could be used as follow-up parameters. IL-35 gene regulation and EAT thickness reduction could be effective therapeutic approaches in the treatment of atherosclerosis and CAD.

Disclosure of conflict of interest

None.

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