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Platelet Carcinoembryonic Antigen Cell Adhesion Molecule 5 (CEACAM5) as a Possible Novel Diagnostic Tool for Evaluation of Acute Coronary Syndrome

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF Wen Wan
AF Yujia Ye
BC Huawei Wang
DF Longjun Li
BC Yajuan Gu
BD Lai Yang
CD Lihong Yang
BD Han Liu
BC Chao Meng
BCD Deng Li
BC Zhe Wang
ABCDEFG Zhaohui Meng

Laboratory of Molecular Cardiology, Department of Cardiology, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, P.R. China

Corresponding Author: Zhaohui Meng, e-mail: zhhmeng@aliyun.com
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Background: Acute coronary syndrome (ACS) occurs approximately every 40 seconds, and was an underlying cause of death in 1 out of every 7 deaths. More accurate indicators are needed to distinguish patients with ACS from patients manifesting negative changes in electrocardiogram (ECG) and myocardial enzymes. This study aimed to investigate whether the expression of platelet carcinoembryonic antigen cell adhesion molecule-5 (CEACAM5/CEA/CD66e) could help predict ACS.

Material/Methods: We enrolled 82 participants (mean age 60 years, 33 females and 49 males). The expression of CEA on washed human platelets was assessed using two-color flow cytometry. The CEA levels on platelets and in serum of these 82 consecutive patients were detected using two-color whole-blood flow cytometry analysis and a custom-made Luminex multiplex assay, respectively.

Results: CEA was expressed on the surface of human platelets. The expression of platelet CEA ($P<0.01$), but not serum CEA ($P=0.30$), was significantly higher in patients with ACS compared to patients with normal coronary artery. Increased platelet CEA levels could serve as a new independent indicator for ACS ($P=0.0003$). Platelet CEA testing ($P=0.000002$), as well as cardiac troponin I (cTnI) ($P=0.0005$), can diagnose ACS with high sensitivity and specificity, and, combined with cTnI ($P<0.0001$), can improve the diagnostic value.

Conclusions: Platelet CEA expression was higher in individuals presenting with ACS. Hence, platelet CEA might be a novel and reliable biomarker for ACS. Large-scale studies are needed to confirm this hypothesis.

MeSH Keywords: Acute Coronary Syndrome • Biological Markers • Blood Platelets • Carcinoembryonic Antigen • Myocardial Infarction

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Background

Acute coronary syndrome (ACS) is an ischemic myocardial disorder defined by changes in electrocardiogram (ECG), cardiac necrosis biomarkers, and clinical manifestations of chest pain. ACS occurs approximately every 40 seconds, and is an underlying cause of death in 1 out of every 7 deaths. Its high morbidity and mortality have made it an international concern for decades [1].

Acute coronary syndrome is correlated with vulnerable coronary plaques. Rupture of luminal instable plaques results in platelet activation, thrombus formation, and even thrombotic occlusion. Platelets play a fundamental role in the progression, instability, and rupture of coronary plaques [2–4]. Moreover, platelet surface glycoproteins participate in platelet activation, adhesion, and aggregation, indirectly mediating the pathogenesis and clinical manifestations of ACS [5–7].

Historically, biomarkers from serum glutamic oxaloacetic transaminase (SGOT, now called AST) to cardiac troponins (cTn) were a fundamental tool used to evaluate and diagnose ACS. Troponin, as the classical biomarker for myocardial infarction, took several decades to achieve its current status [8–10]. Although cTn has high specificity and sensitivity to myocardial damage, it cannot recognize between myocardial ischemia, inflammation, or traumatic injury. There are several causes associated with elevation of cTn in the absence of ACS [10,11]. Therefore, researchers are continuing work on novel biomarkers that could help assess ACS much more specifically and sensitively. Moreover, research on biomarkers of ACS is not only limited to serum or plasma biochemical indicators, but also involves the expression of platelet membrane protein. Studies showed that platelet collagen receptor glycoprotein VI (GPVI), P-selectin, and carcinoembryonic antigen cell adhesion molecule-1 (CEACAM1/CD66a/Bgp) were expressed on the surface of platelets and exerted functions in platelet-mediated normal hemostasis and arterial thrombosis [3,12,13]. Additionally, the levels of P-selectin and GPVI on the platelet are increased in ACS and may serve as additional novel indicators of ACS [3,6,12–15].

CEACAM5, as well as CEACAM1, as a carcinoembryonic antigen cell adhesion molecule (CEACAM), plays an important role in cell adhesion, in intercellular and intracellular signaling, and in tumor progression [16,17]. CEA, a 180-kDa glycoprotein that is widely expressed on the surface of various cells, including epithelial cells, endotheliocytes, hemocytes, and immune cells, binds to the cell surface through a glycosylphosphatidylinositol linkage, and is composed of 6 constant C2-like domains as the extracellular structure, followed by the N-domain consisting of 1 variable (V)-like domain [18]. Moreover, CEA, which has been used for decades as a broad-spectrum tumor marker, can reflect the existence of various tumors. High expression of CEA in the serum of patients with cancers, such as colorectal cancer, breast cancer, and lung cancer, is closely related to tumor deterioration and subclinical metastases [17,19]. Platelets have crucial roles in tumor cell extravasation and metastasis formation [20,21]. However, little is known about the expression of CEA on platelets and its effect on platelet function. This study aimed to analyze whether CEA exerts a previously unknown function in ACS.

Material and Methods

Study population and enrolment criteria

This study enrolled 82 consecutive patients who complained of thoracic pain during the period December 2018 to March 2019 at the First Affiliated Hospital of Kunming Medical University. All patients underwent coronary angiography to make diagnosis according to the American Heart Association/American College of Cardiology (AHA/ACC) guidelines [22,23]. All processes were conducted according to the Declaration of Helsinki, and informed consent was obtained from all patients [24,25]. Of the 82 patients, 62 were diagnosed with ACS, including 21 patients with ST-segment elevation myocardial infarction (STEMI), 21 with non-ST-segment elevation myocardial infarction (NSTEMI), and 20 with unstable angina pectoris (UA). We enrolled a control group of 20 healthy participants with normal coronary arteries. We excluded patients aged less than 18 years, those presenting with false-positive myocardial necrosis markers, and those incapable of giving informed consent.

ACS includes STEMI, NSTEMI, and UA. The diagnostic criteria were as described previously [26].

Sample collection

Blood samples were collected in Vacutainer tubes on admission. They were then prepared and assessed immediately by flow cytometry analysis, and the prepared serum was kept immediately at –80°C until assessed by Luminex, as described in previous studies [2,13,27].

Isolation and preparation of human platelets

Isolation of human platelet and preparation of washed platelets were conducted as described in a previous study; briefly, venous blood was collected into 2-mL Vacutainer tubes containing 300 µL 3.2% (w/v) trisodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation at 150 g for 10 min at 22°C. Washed platelets were generated by centrifuging PRP at 1000 g for 15 min at 22°C, and then we resuspended the platelets gently at 2–3×10^8 platelets/ml in Tyroades buffer (137 mmol/L NaCl, 2.8 mmol/L KCl, 10 mmol/L HEPES,

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1 mmol/L $MgCl_2$, 12 mmol/L $NaHCO_3$, 0.42 mmol/L NaH_2PO_4, 1 mg/ml glucose, and 2.5 mg/ml BSA, pH 7.4). The platelets were washed twice in this buffer [28]. The washed platelets were processed for two-color flow cytometry analysis [29].

Flow cytometry analysis

The assessment of expression of CEA on washed platelet surfaces was performed using two-color flow cytometry. Meanwhile, platelet CEA levels of different patients were assessed using two-color whole-blood flow cytometry [12,27]. All samples were labeled with PerCP-conjugated anti-human CD61 antibody (1: 10; BioLegend, USA; clone: VI-PL2) to recognize platelets, and then were labeled with either Fluorescein isothiocyanate (FITC)-conjugated anti-human CEA antibody (5 μg/mL; GeneTex, USA; clone: C365D3) or FITC-conjugated mouse immunoglobulin G1 (IgG1; 5 μg/mL; BD, USA; clone: mouse oligodendrocyte precursor cell-21) as homotypic controls. The samples were analyzed on a FACScan flow cytometer (BD FACSCanto II). Duplicate measurements were performed, and the expression levels of CEA are given as mean fluorescence intensity (MFI).

Measurement of serum CEA levels

The expression of serum CEA was analyzed with a custom-made Luminex multiplex assay (R&D Systems) following the manufacturer's protocols. Briefly, CEA in serum samples were measured by a multiplexed flow cytometric assay on a Luminex® system (MAGPIX® with xPONENT). Analysis was done according to the instructions of the manufacturer (MILLIPLEX® Analyst 5.1). Samples were measured in duplicate. The range of the standard curves for all CEA measured was 0–13 150 pg/ml. Based on the standard curves, the coefficient of variation (CV) was calculated and did not exceed 20%. One-to-two dilution was done for all samples.

Statistical analysis

We calculated the sample size by using PASS 15.0. All data were analyzed and processed using SPSS 21.0, GraphPad Prism 7.0, and MedCalc 15.10. A two-sided P value <0.05 was considered statistically significant. Normal distributions were checked using the Shapiro-Wilk normality test. Normal distribution data between groups were compared using the unpaired *t* test or one-way ANOVA, while abnormal distribution data were compared using the Mann-Whitney test or nonparametric test, respectively. The correlation between platelet CEA and serum CEA or clinical indexes was analyzed using the Spearman correlation test. Possible confounders were adjusted for by covariance analysis of Napierian logarithm of platelet CEA expression. The correlation between platelet CEA expression and ACS was found to be independent of sex, age, cardiovascular risk factors (CVF), and conventional laboratory indicators. The multivariable logistic regression analysis was performed to determine the association between the Napierian logarithm of platelet CEA expression and established biomarkers in all patients. The receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC) were calculated to evaluate the diagnostic value. Comparisons of different ROC curves were processed using MedCalc 15.10.

Results

We consecutively investigated platelet and serum CEA expression in a total of 82 patients. Of all the participants, 49 (59.8%) were males, 33 (40.2%) were females, and the mean age was 60 years. In all, 62 (75.6%) patients were diagnosed as having ACS, and 20 (24.4%) patients were treated as healthy controls. Of these patients, the median total cholesterol (TC) level was 4.41 (3.54-5.17) mmol/L, and the median low-density lipoprotein (LDL) level was 2.56 (3.54-5.17) mmol/L. The demographic particulars and medical treatment of the patients are shown in Table 1.

Expression of CEACAM5 on human platelets

The presence of CEACAM5 on the human platelet surface and whether the expression of CEA changed when activated by thrombin were detected using two-color flow cytometry analysis. As shown in Figure 1A and 1B, the MFI of resting human platelets was 2-fold higher than that of the isotype controls [MFI±standard error (SE): 146.7±12.25 *vs.* 98.33±4.33; P<0.01; n=3]. The expression levels of CEA increased when stimulated with different doses of thrombin (0.5–2 U/mL) in contrast to resting human platelets (MFI±SE: 214.7±13.3 *vs.* 290.3±10.41 *vs.* 195.7±29.76 *vs.* 146.7±12.25; P<0. 01; n=3; Figure 1C, 1D). The expression of CEA increased significantly at moderate thrombin concentrations (1 U/mL) but decreased evidently at higher thrombin concentrations (2 U/mL).

Expression levels of platelet CEA and serum CEA in patients with ACS

The expression levels of platelet CEA and serum CEA were continuously evaluated in a cohort of 82 patients with thoracic pain. The expression levels of platelet CEA [(MFI±SE): ACS 1459±149.8 *vs.* control 429.8±110; P<0.01], but not of serum CEA [(pg/ml; mean±SE): ACS 199.4±12.69 *vs.* control 173.5±16.77, P=0.30], increased significantly in patients with ACS compared to those with normal coronary arteries serving as controls (Figure 2A, 2B). Moreover, the expression of platelet CEA was markedly higher in patients with AMI and UA [(MFI±SE): AMI 1504±180.7 *vs.* UA 1364±273.3 *vs.* control 429.8±110; P<0.01] (Figure 2C). However, a slight difference was found in the expression of serum CEA [(pg/ml;

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Table 1. Baseline characteristics and medical treatment of patients on admission.

Characteristics	Total (N=82)	Control (n=20)	ACS (n=62)
Age (year)	60.0 ± 13.8*	53.7± 13.0*	62.0± 13.5*
Sex [n (%)]			
Female	33 (40.2%)	8 (40%)	25 (40.3%)
Male	49 (59.8%)	12 (60%)	37 (59.7%)
BMI (kg/m^2)	24.02±2.83*	25.56±3.22*	23.49±2.50*
CVRF [n (%)]			
Smoking	43.9	50	41.9
Arterial hypertension	51.2	25	59.7
Diabetes	26.8	20	29
Hyperlipidemia	19.5	25	17.7
CAD [n (%)]			
LM	6.1	0	8.1
3 Vessels	34.1	0	45.2
2 Vessels	13.4	0	17.7
1 Vessel	14.6	0	19.4
LVEF (%)			
Normal	36.6	60	29
Slightly reduced	11	0	14.5
Moderate	2.4	5	1.6
Low	1.2	5	0
Medication, n (%)			
Aspirin	52 (63.4%)	10 (50%)	42 (67.7%)
Clopidogrel	46 (56.1%)	8 (40%)	38 (61.3%)
Statins	44 (53.7%)	4 (20%)	40 (64.5%)
ACEI	32 (39.0%)	8 (40%)	24 (38.7%)
ARB	23 (28.0%)	7 (35%)	16 (25.8%)
β blockers	39 (47.6%)	9 (45%)	30 (48.4%)
Heparin	6 (7.3%)	1 (5%)	5 (8%)

BMI – body mass index; CAD – coronary artery disease; CVRF – cardiovascular risk factors; LVEF – left ventricular ejection fraction; ACEI – angiotensin converting enzyme inhibitors; ARB – angiotensin receptor blockers. * Mean±standard deviation.

mean±SE): AMI 217.9±16.49 *vs.* UA 161.4±16.25 *vs.* control 173.5±16.77, P=0.049] (Figure 2D). Finally, a significant increase was found in the expression levels of platelet CEA [(MFI±SE): STEMI 1445±309.6 *vs.* NSTEMI 1562±194.2 *vs.* UA 1364±273.3 *vs.* controls 429.8±110, P<0.01], but not serum CEA [(pg/ml; mean±SE): STEMI 224.3±23.42 *vs.* NSTEMI 211.1±23.73 *vs.* UA 161.4±16.25 *vs.* controls 173.5±16.77, P=0.11] in the 3 subgroups individuals of ACS (Figure 2E, 2F). Meanwhile, compared with healthy controls, the expression levels of platelet CEA of each subgroup were higher, but no significant statistical difference was observed between the 3 subgroups.

Correlation between the expression of platelet CEA and serum CEA, markers of myocardial necrosis, and markers of myocardial stretch

No statistically significant correlation was found between the expression levels of platelet CEA and serum CEA (Figure 3A). We found that the expression of platelet CEA was positively correlated with myocardial stretch marker such as brain-type natriuretic peptide (BNP) in all patients (r=0.27, P=0.021) (Figure 3B). Nevertheless, no statistically significant correlation was found between the expression levels of platelet CEA

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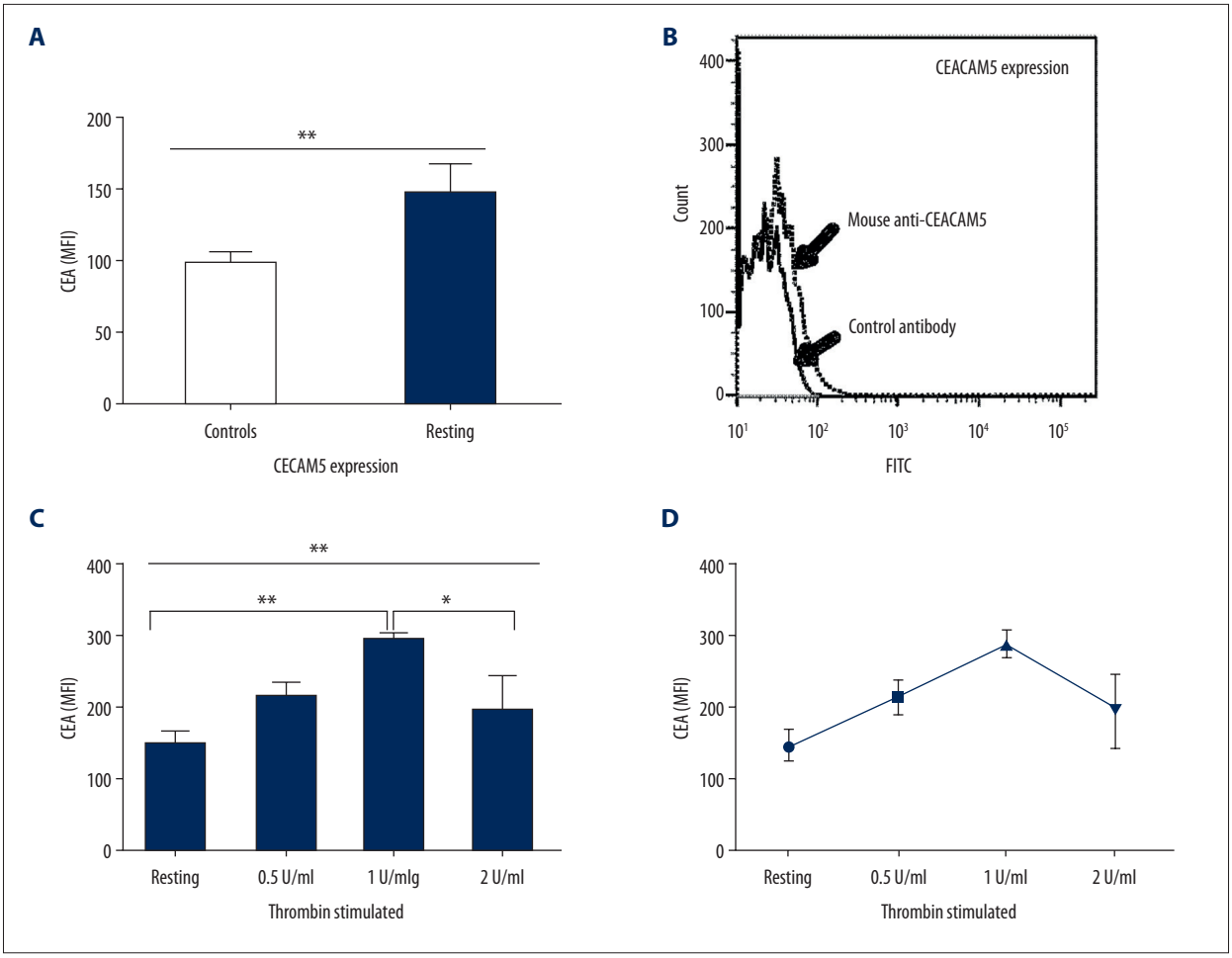

Figure 1. Expression of CEA on resting or stimulated human platelets was detected by flow cytometric analysis. (**A, B**) Expression of CEA on resting human platelets. The results are represented as MFI±standard error (SE) (n=3; ** P<0.01). The representative histogram profiles are shown in **B**. (**C, D**) Expression of CEA after stimulation with different doses of thrombin (n=3; * P<0.05, ** P<0.01).

and initial myocardial necrosis biomarkers such as cTnI, creatine phosphate kinase isoenzyme (CKMB), and myoglobin (MYO) values (cTnI: r=0.17, P=0.14; CKMB: r=0.11, P=0.34; MYO: r=0.13, P=0.26) (Figure 3C–3E). Then, the relationship between patients with AMI and healthy controls was explored. The expression of platelet CEA was positively correlated with myocardial injury and stretch biomarkers in these participants (BNP: r=0.26, P=0.014; cTnI: r=0.30, P=0.018; CKMB: r=0.27, P=0.035; MYO: r=0.26, P=0.041) (Figure 3F–3I).

Expression levels of platelet CEA in subgroups of patients with ACS

The expression levels of platelet CEA in patients with ACS were investigated under several conditions: sex, smoking, hypertension, diabetes mellitus, hyperlipemia, and the number of diseased vessels. No statistically significant difference was observed in each subgroup of patients with ACS (P>0.05; Supplementary Figure 1).

Expression of platelet CEA was an independent risk factor for ACS

Cardiovascular risk factors and myocardial necrosis biomarkers are independent risk factors for ACS, which are possible confounders influencing the expression of platelet CEA expression in ACS. Therefore, possible confounders were adjusted for by covariance analysis of platelet CEA expression. We found that the correlation between platelet CEA expression and ACS was independent of sex, age, cardiovascular risk factors (CVF), and conventional laboratory indicators (Table 2). We also found that the expression of CEA was an independent risk factor for ACS (P=0.0003; OR=5.98; 95% CI, 2.27–15.76). Then, a logistic regression analysis was conducted to determine if the expression of platelet CEA was independent of established biomarkers

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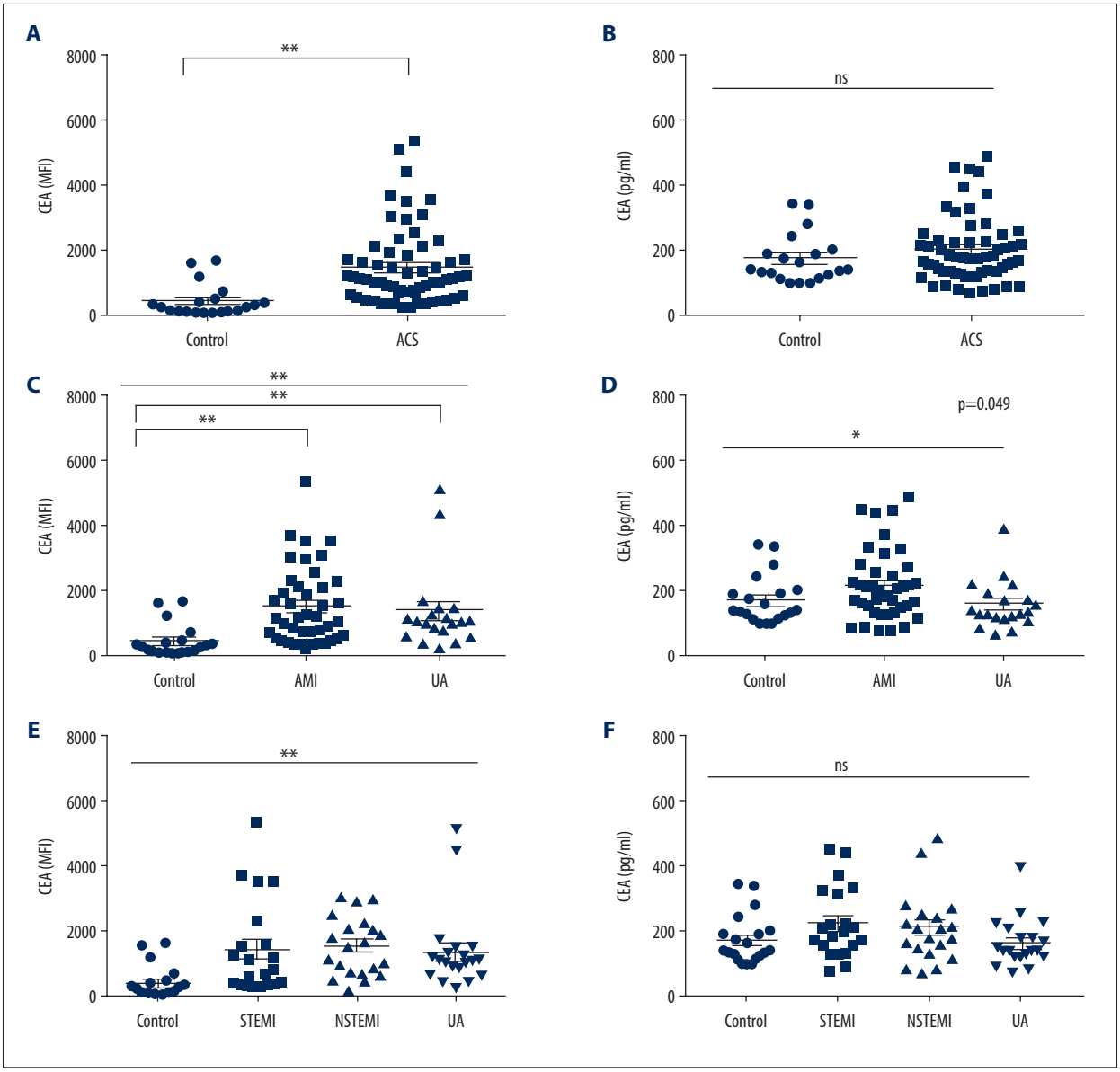

Figure 2. Expression levels of platelet CEA and serum CEA in patients with ACS. Expression of platelet CEA or serum CEA was evaluated using two-color whole-blood flow cytometry or a custom-made Luminex multiplex assay, respectively. The results are presented as MFI±SE, or mean (pg/ml)±SE. (**A**) Expression of platelet CEA and (**B**) serum CEA in patients with ACS and healthy controls. (**C**) Expression of platelet CEA and (**D**) serum CEA in patients with AMI, UA, and healthy control. (**E**) Expression of platelet CEA and (**F**) serum CEA in patients with STEMI, NSTEMI, UA, and healthy controls. (** P<0.01, ns – no significance, n=82).

for ACS (Table 3). We found that the expression of CEA on human platelet surfaces was independently correlated with ACS (P=0.002; OR=8.56; 95% CI, 2.19–33.436).

Diagnostic value of CEA for ACS

The ROC analyses (Figure 4A) demonstrated that the expression of platelet CEA could help diagnose ACS, with high sensitivity and specificity, as indicated by the AUC value of 0.86 (P=0.000002). Although the AUC of platelet CEA expression was not better than that of cTnI levels for ACS diagnosis (AUC: 0.86 *vs.* 0.76; P>0.05), combining the results of platelet CEA and cTnI levels provided a significantly better measurement for ACS identification compared with the cTnI test alone (AUC: 0.91; P=0.0025, Figure 4B). Nevertheless, for AMI, the expression of platelet CEA was also of significantly high diagnostic value (AUC: 0.87; P=0.000002, Figure 4C). The AUC from the platelet CEA plus cTnI testing was superior to platelet CEA

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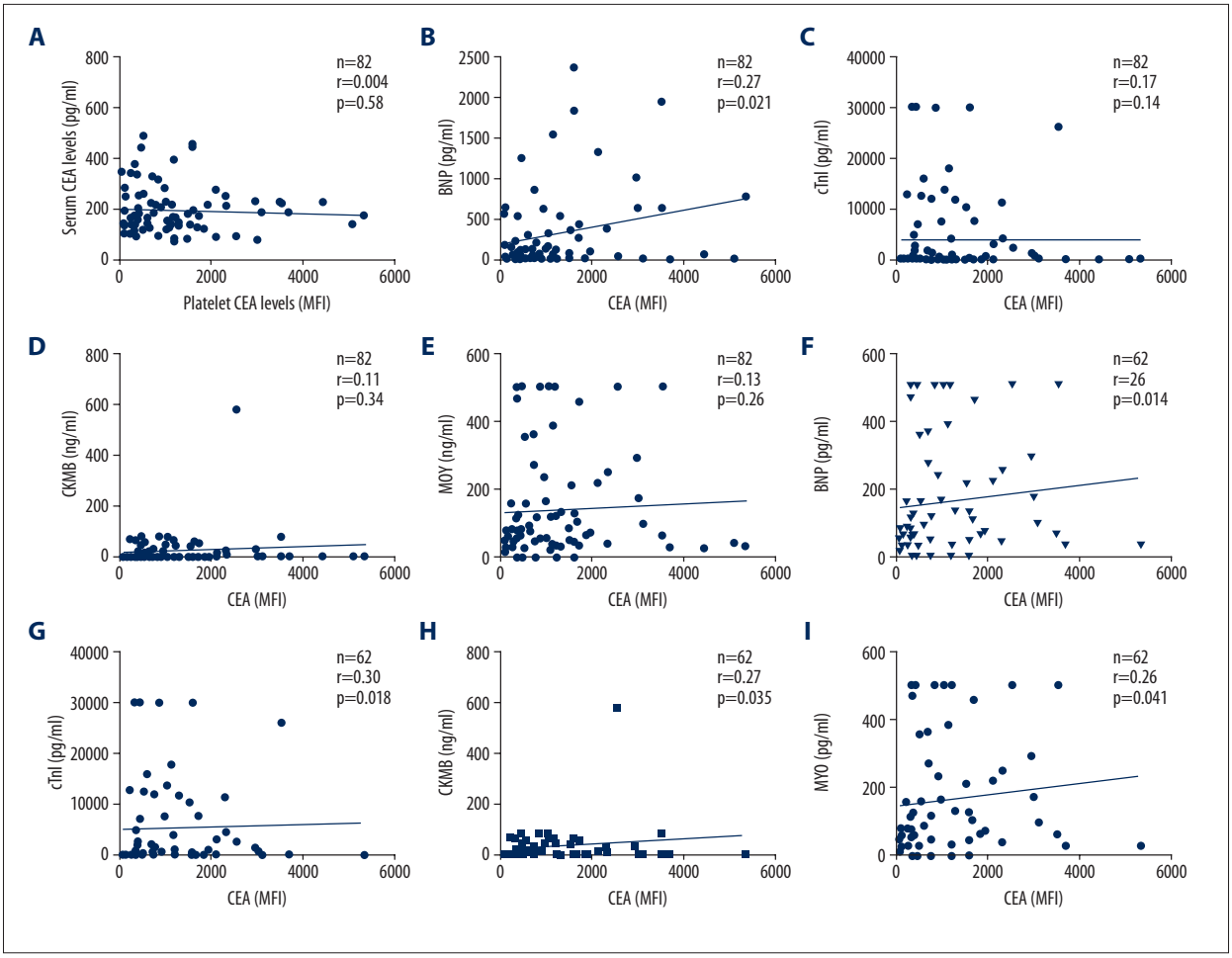

Figure 3. Correlation between the expression of platelet CEA and serum CEA levels, myocardial injury markers, and myocardial stretched markers. (**A**) Correlation between the expression of platelet CEA and serum CEA in patients with ACS and healthy controls (n=82). (**B–E**) Correlation between the expression of platelet CEA and BNP, cTnI, CKMB, and MYO in patients with ACS and healthy controls (n=82). (**F–I**) Correlation between the expression of platelet CEA and BNP, cTnI, CKMB, and MYO in patients with AMI and healthy controls (n=62).

Table 2. Multifactorial analysis of covariance for CEA.

Category	Factor	P value
CVRF	Smoking	0.625
	Hypertension	0.230
	Diabetes	0.508
	LDL	0.055
Conventional laboratory Markers	cTnI	0.154
	BMI	0.455
Sex	Male *vs.* Female	0.853
Age	Years	0.305
Groups	ACS *vs.* controls	0.0003*

CVRF – cardiovascular risk factors; CAD – coronary artery disease.

testing alone (AUC: 0.96 *vs.* 0.87; P=0.039), but about equal to the cTnI testing (AUC: 0.96 *vs.* 0.90; P>0.05, Figure 4D).

Discussion

The major findings we demonstrated are as follows: (1) CEA was expressed on the human platelet surface, and increased when stimulated with different doses of thrombin; (2) The expression of platelet CEA was increased in individuals presenting with ACS, which might be a powerful and reliable biochemical marker for ACS diagnosis; (3) Platelet CEA expression was equal to cTnI testing in identifying patients with ACS, and the combination of both was superior to cTnI testing alone. These findings suggest that the expression of platelet CEA would significantly improve diagnosis of ACS.

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Table 3. Predictive value of CEA for ACS.

Parameters	P value	Odds ratio	95% CI for the odds ratio	
			Lower	Upper
CEA (MFI)	0.002*	8.56	2.19	33.436
CTnI (pg/mL)	0.987	1.00	0.999	1.001
CKMB (ng/mL)	0.075	1.516	0.959	2.396
CRP (mg/L)	0.877	1.006	0.934	1.083
Smoking	0.284	0.338	0.046	2.461
Hypertension	0.246	3.03	0.466	19.72
Diabetes	0.705	1.42	0.231	8.743
Hyperlipidaemia	0.203	3.878	0.481	31.267

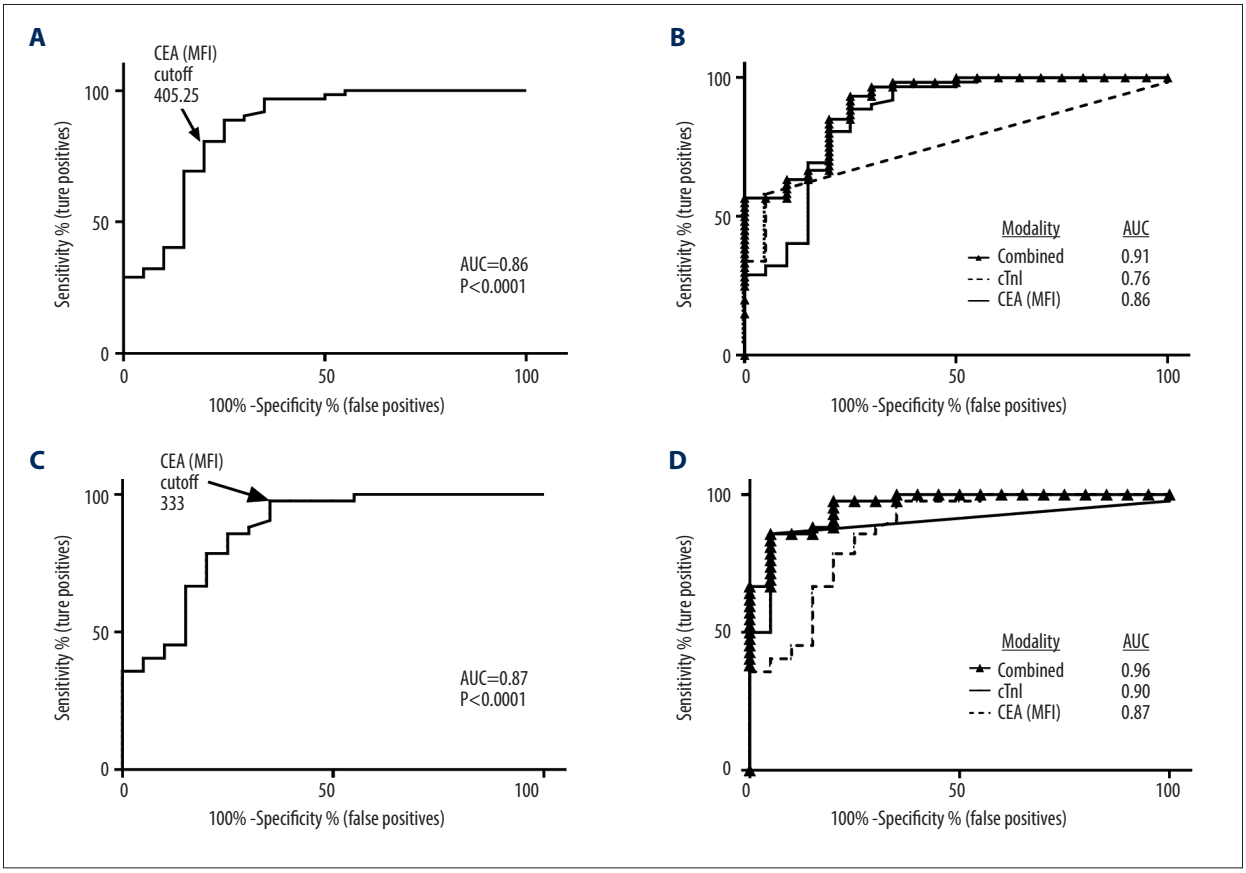

Figure 4. Diagnostic value of CEA for acute coronary syndrome. (**A**) Diagnostic value of the expression of platelet CEA for ACS, with a highly significant AUC of 0.86 (P<0.01); the cutoff value was 405.25. (**B**) Comparison of ROC of CEA versus cTnI for the diagnosis of ACS. The platelet CEA testing was similar to the cTnI analysis (AUC 0.86 *vs.* 0.76; P>0.05). The AUC obtained from CEA testing plus cTnI analysis was superior to cTnI measurement alone (AUC 0.91 *vs.* 0.76; P<0.01). (**C**) Expression of platelet CEA with a highly significant AUC of 0.87 for the diagnosis of AMI (P<0.01); the cutoff value was 333. (**D**) ROC comparison of the expression of platelet CEA versus cTnI for AMI diagnosis. The platelet CEA testing was similar to the cTnI analysis for AMI diagnosis (AUC 0.86 *vs.* 0.76; P>0.05). The AUC from CEA testing plus cTnI analysis was superior to the CEA testing alone (AUC: 0.96 *vs.* 0.87; P=0.039).

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Platelets exert a pivotal function in physiological hemostasis and pathological thrombogenesis [5]. Moreover, the amount of glycoproteins on the platelet surface appears to represent a critical mediator in these processes [30]. Denise et al. [12] found that CEACAM1 was expressed on the surface of platelets, and its expression was increased when stimulated with thrombin. CEA and CEACAM1 are both CEACAMs, and they share some similarities in structure and hemophilic or heterophilic ligands interaction. CEA is well known as a classic tumor marker and is widely expressed on the surfaces of various cells [16–18], but little research has focused on its expression on platelets. In the present study, we found that CEACAM5 was expressed on human platelet surfaces. Moreover, the expression levels of CEACAM5 on the platelet were elevated by agonist stimulation with thrombin. These findings reveal that CEA is a novel protein expressed on the surface of platelets, and the expression level of CEA appears to be correlated with platelet activation.

Biomarkers from not only serum, but also platelet surface, could help assess and discriminate ACS [11,31,32]. GPVI, as well as P-selectin, was expressed on the surface of platelets, and plays a vital role in the complex course of platelet activation, aggregation, adhesion, and even plaque formation [33]. The expression of platelet GPVI and P-selectin are associated with imminent acute coronary events [13,15]. In this study, we found that the expression of platelet CEA was significantly increased in individuals presenting with ACS when compared with healthy controls. Moreover, the expression level of platelet CEA was an independent risk factor for ACS, suggesting that platelet CEA expression could forecast the occurrence of ACS, and might be an additional potential biomarker for the diagnosis of ACS.

ACS is often accompanied by different degrees of myocardial ischemia, injury, necrosis, and inflammation. The progression of ACS has long been assessed by detecting changes in myocardial necrosis markers (e.g., myoglobin, CKMB, and cTnI), inflammatory markers (e.g., C-reactive protein and CRP), and myocardial stretch markers (e.g., BNP). Studies have speculated that as biomarkers of myocardial necrosis, inflammation or myocardial stretch might provide an earlier evaluation of risk stratification and adverse events for ACS [10,11]. Platelet P-selectin expression was positively correlated with cTnI and CKMB in ACS [3]. Meanwhile, platelet GPVI expression was increased when myocardial necrosis markers are still within the normal range and positively correlated with the peak levels of myocardial necrosis markers [13,15]. Here, we found that the expression of platelet CEA was positively correlated with the myocardial stretch biomarker BNP in ACS patients, but was correlated with myocardial stretch and injury biomarkers in patients with AMI. This demonstrates that the high expression of platelet CEA could help predict ventricular remodeling after myocardial ischemia or infarction in ACS. We considered that platelet CEA expression was not synchronous with the change in myocardial necrosis biomarkers at the onset of ACS, which means that it might be independent of initial myocardial injury biomarkers and may be another early marker to reflect platelet activation in ACS. However, platelet CEA might participate in the emergence of AMI and in the course of remodeling after myocardial infarction. Additionally, the increased levels of platelet CEA at admission were independent of cardiovascular risk factors and myocardial necrosis markers, indicating that CEA was an independent risk factor for ACS, and the increased levels of platelet CEA might serve as an early and reliable marker to predict imminent myocardial ischemia and could be a promising candidate for risk stratification and prediction of adverse events for patients with ACS.

The diagnosis of ACS in patients with complaints of thoracic pain or other symptoms is indefinite and time-consuming. Biomarkers of myocardial necrosis have high specificity to myocardial damage, but several causes are found to be associated with elevation of cTn in the absence of ACS [11]. Therefore, we need search new biomarkers to help distinguish ACS. Previous studies have focused on microRNAs and biochemical indicators of serum or platelet surface [10,11,31,32]. We showed that the expression levels of platelet CEA might be a new biomarker for the diagnosis of ACS, with the MFI cutoff value of 405.25 *vs.* 333 for AMI. In addition, combining it with cTnI testing could significantly improve the diagnostic value of ACS. Our results suggest that the combination of platelet CEA testing and cTnI examination could help make the clinical decision of ACS much more quickly and efficiently. Due to the laborious process and difficult clinical interpretation, the diagnostic value of platelet CEA is limited. Thus, improvements in the techniques for isolating and analyzing platelet surface protein expression are needed.

Conclusions

CEA was expressed on the surface of platelets. Increased levels of platelet CEA could reliably help diagnose ACS, and indicate a poorer prognosis of ACS. This was an exploratory study with small sample size and further studies are needed to enlarge the sample size, and to explore the mechanism underlying the increased platelet CEA expression in patients with ACS. Investigations are needed to determine whether specific inhibition of platelet CEA could weaken myocardial damage and alter prognosis.

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Conflicts of interest

None.

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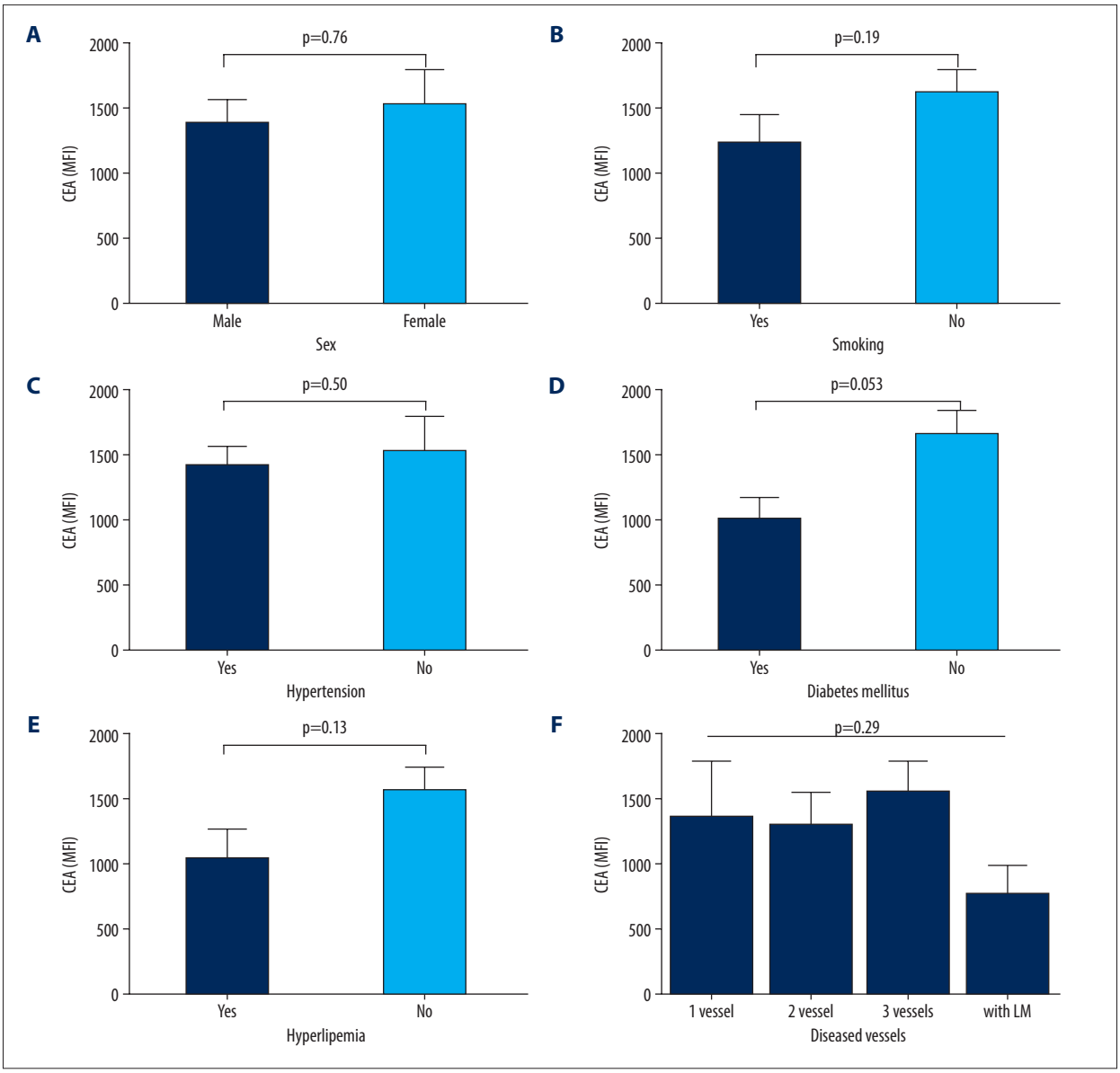
Supplementary Data



Supplementary Figure 1. Expression levels of platelet CEA in subgroups of patients with ACS. (**A–F**) Platelet CEA expression in subgroups of patients with ACS (sex, smoking, hypertension, diabetes mellitus, hyperlipemia, and the number of diseased vessels) demonstrating that cardiovascular risk factors had no influence on the expression of platelet CEA in patients with ACS.

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