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Plasma biomarkers and plaque strain predict long-term cardiovascular events in patients with acute coronary syndrome

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To test whether circulating and intracoronary biomarkers and coronary plaque strain have additive values to Global Registry of Acute Coronary Events (GRACE) score for predicting long-term cardiovascular events in ACS patients. One hundred ACS patients were enrolled and the GRACE score and plasma levels and intracoronary gradients of a number of biomarkers were measured. Coronary plaque burden and morphology in non-critical stenotic plaques were determined by intravascular ultrasound (IVUS) technique, and the maximal shear strain (SS_{max}) and maximal area strain (AS_{max}) were determined by intravascular ultrasound elastography (IVUSE) technique. Patients were followed for cardiovascular events and the predictive values of clinical characteristics, plasma biomarkers and plaque parameters were compared with GRACE score, and the incremental values of these measurements to the GRACE score were assessed. GRACE score, plasma biomarkers and plaque strain were independent predictors of cardiovascular events. Combination of GRACE score, plasma biomarkers and plaque strains significantly improved the predictive value of the GRACE score alone with the receiver-operating characteristic area increased from 0.457 to 0.667 (P=0.014). The combination of circulating and intracoronary biomarkers, plaque strain and GRACE score provides a better predictive tool than GRACE score alone in patients with ACS.

plasma biomarker, plaque strain, GRACE score, cardiovascular events, acute coronary syndrome

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INTRODUCTION

Pathological studies in the last decade have revealed that in the majority of patients with acute coronary syndrome (ACS), the catastrophic event is caused by atherosclerotic plaque rupture or erosion with subsequent intraluminal thrombosis, and vascular lesions prone to these events were deemed vulnerable plaque. Local inflammation plays a pivotal role in the pathogenesis of plaque formation, progression and disruption (Virmani et al., 2006), and a number of plasma biomarkers detected in the peripheral or coronary circulation have been reported to be associated with plaque inflammation and might be useful in predicating cardiovascular events (CVE) in patients with ACS (Wang et al., 2007;

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Antoniades et al., 2009; Ridker, 2007). However, as most of these studies on circulatory biomarkers were retrospective or cross sectional in nature without longitudinal follow-up results, the predictive values of these biomarkers remain inconclusive. Thus, exploration of novel circulatory biomarkers capable of predicting cardiovascular events induced by vulnerable plaque is still a major challenge. In addition, most vulnerable plaques are non-critical lesions evading detection by conventional coronary angiography (Virmani et al., 2000). Although intravascular ultrasonography (IVUS) has been used to evaluate the geometrical properties of plaque, lumen and vascular wall (Peters et al., 1994), assessment of plaque inflammation and deformation are beyond its capability. Recently, we have developed a novel algorithm for calculating two-dimensional strain of atherosclerotic plaques from IVUS images, constructed a new IVUS elastography (IVUSE) system and validated this technique with quantitative histological analysis in a rabbit model (Hu et al., 2012; Li et al., 2016; Li et al., 2017). However, it remains elusive whether this new technique can predict future cardiovascular events in patients with ACS.

Previous studies suggested that inflammation plays a key role in the initiation and development of atherosclerosis, and a number of inflammatory biomarkers such as secretory phospholipase A_2 ($sPLA_2$), phosphatidylcholine-specific phospholipase C (PC-PLC), soluble CD40 ligand (sCD40L), interleukin-6(IL-6), myeloperoxidase (MPO), monocyte chemoattractant protein-1 (MCP-1), tissue factor (TF), and YKL40 were investigated to reveal this procedure. Yet there is still room to further elucidate their additional value for different score system or models we adopted in daily clinical practice.

The GRACE risk score was initially developed to predict in-hospital mortality of patients with ACS in 2003 (Granger et al., 2003), and subsequently applied to predict 6-month mortality in a registry cohort of ACS in 2004 (Eagle et al., 2004). However, most patients with ACS today may receive percutaneous coronary intervention (PCI) and optimal medical therapy, with a consequence of very low in-hospital mortality, although a substantial proportion of patients continue to develop recurrent cardiovascular events on long-term follow-up. It is still an open question whether GRACE risk score can predict long-term multiple cardiovascular events such as cardiovascular death, non-fatal myocardial infarction, unstable angina and revascularization after ACS, and whether measurement of plasma inflammatory biomarkers and plaque morphology and strain may add incremental predictive value to GRACE score in patients with ACS.

In the present study, we aimed to evaluate the capability of GRACE score to predict long-term cardiovascular events, to compare the predictive value of GRACE score with plasma biomarkers in peripheral and coronary circulation, coronary plaque morphology, and coronary plaque strain, and to examine the additive predictive value of plasma biomarker and plaque measurements to GRACE score in ACS patients.

RESULTS

Baseline characteristics of study population

The demographic and clinical characteristics of enrolled patients were in Table 1. Among 100 ACS patients, 2 patients had ST segment elevation myocardial infarction (STEMI), 8 non-ST segment elevation myocardial infarction (NSTEMI) and 90 unstable angina pectoris (UAP) who aged 40 to 75 years (mean age 57.24±1.06 years) with the majority (64%) being males. Most of these patients had multiple risk factors for atherosclerosis such as hypertension, current smoking, diabetes and obesity, and received optimal medical therapies including aspirin, clopidogrel, beta-blockers and statins. More than half of these patients underwent PCI with implanted drug-eluting stents.

Cardiovascular events

The median follow-up time was 28 months (range 6–51 months). We identified 32 cardiovascular events comprising 1 cardiac death, 29 recurrent unstable angina pectoris requiring hospitalization and 2 coronary revascularizations. The median duration of time from enrollment to cardiovascular events was 26 months (range 6–51 months).

All patients were divided into two groups based on the presence or absence of cardiovascular events during follow-up. There was no significant difference between patients with and without cardiac events in terms of age, gender, body weight index, risk factors including hypertension, diabetes and cigarette smoking, medications used, stents implanted, blood glucose, creatinine, uric acid, lipid profile, and cardiac troponin, as well as plasma levels of $sPLA_2$, PC-PLC, sCD40L, IL-6, MPO, MCP-1, TF and YKL40, except for that more patients received PCI in cases with cardiovascular events than without (Table 1). Likewise, there was no significant difference between the two groups of patients in terms of translesional concentration gradients of high-sensitive C-reactive protein (hsCRP), PC-PLC, sCD40L, IL-6, MPO, MCP-1, TF, YKL40 and dickkopf-1 (DKK1), as well as angiographic coronary stenosis, percentages of soft, fibrous, calcified, homogeneous and heterogeneous plaques, plaque area and area burden, plaque volume and volume burden, remodeling index and plaque eccentricity index. However, the plasma levels of hsCRP, SP-D and DKK1, intracoronary gradients of $sPLA_2$ and SP-D as well as plaque parameters of SS_{max}

Table 1 Clinical characteristics and peripheral plasma biomarkers in two groups of patients[a)]

	With events(n=32)	Without events(n=68)	Total (n=100)	P-value
age (year)	59.44±11.30	56.06±10.11	57.14±10.57	0.137
male (n, %)	23 (71.9%)	41 (60.3%)	64 (64)	0.372
BMI (kg m^{-2})	25.79±3.57	26.75±2.97	26.47±3.17	0.195
STEMI (n, %)	1 (3.13%)	1 (1.47%)	2 (2%)	
NSTEMI (n, %)	3 (9.38%)	5 (7.35%)	8 (8%)	0.802
UAP (n, %)	28 (87.5%)	62 (91.2%)	90 (90%)	
hypertension (n, %)	25 (80.6%)	41 (60.3%)	66 (66%)	0.065
diabetes (n, %)	8 (25%)	16 (23.5%)	24 (24%)	1.000
smoker (n, %)	16 (50.0%)	33 (48.5%)	49 (49%)	1.000
aspirin (n, %)	32 (100%)	68 (100%)	100 (100%)	1.000
clopidogrel (n, %)	16 (50%)	34 (50%)	50 (50%)	1.000
βblocker (n, %)	25 (78.1%)	45 (66.2%)	70 (70%)	0.252
ACEI/ ARB (n, %)	12 (37.5%)	23 (33.8%)	35 (35%)	0.823
statin (n, %)	26 (81.3%)	44 (67.4%)	70 (70%)	0.107
CCB (n, %)	8 (25%)	12 (17.6%)	20 (20%)	0.428
PCI (n, %)	27 (84.4%)	38 (55.9%)	65 (65%)	**0.007**
DES (n, %)	26 (96.3%)	37 (97.4%)	63 (63%)	1.000
TC (mmol L^{-1})	4.80±0.91	4.73±0.85	4.75±0.87	0.739
TG (mmol L^{-1})	1.74±0.48	1.76±0.54	1.75±0.52	0.901
LDL-C (mmol L^{-1})	2.70±0.72	2.70±0.59	2.70±0.63	0.995
HDL-C (mmol L^{-1})	1.11±0.19	1.16±0.24	1.15±0.23	0.316
BG (mmol L^{-1})	6.31±0.89	6.41±0.93	6.38±0.91	0.612
Cr (μmol L^{-1})	90.06±10.24	91.18±8.38	90.82±8.98	0.563
UA (μmol L^{-1})	320.96±43.21	323.44±43.12	322.65±42.94	0.789
cTNI (ng mL^{-1})	0.20±0.72	0.58±2.33	0.45±1.94	0.412
hsCRP (pg mL^{-1})	3.62±2.44	2.23±2.16	2.67±2.33	**0.006**
$sPLA_2$ (U mL^{-1})	696.19±310.81	661.84±279.22	673.06±288.76	0.583
PC-PLC (mU mL^{-1})	97.56±25.06	95.97±27.74	96.49±26.78	0.791
sCD40L (pg mL^{-1})	49.09±17.62	45.86±18.46	46.92±18.17	0.412
IL-6 (pg mL^{-1})	180.72±144.48	148.04±102.34	158.60±117.87	0.198
MPO (ng mL^{-1})	300.26±126.82	302.30±126.61	301.64±126.03	0.940
MCP-1 (pg mL^{-1})	117.13±22.49	110.98±32.21	112.99±29.41	0.277
SP-D (ng mL^{-1})	8.74±4.95	5.95±4.62	6.82 ±4.88	**0.008**
TF (pg mL^{-1})	45.10±16.79	39.66±12.76	41.42±14.33	0.077
YKL40 (ng mL^{-1})	29.88±22.67	26.82±21.17	27.76±21.57	0.521
DKK1 (pg mL^{-1})	923.97±416.70	693.95±341.05	765.98±379.70	**0.005**

a) Values are mean±SD or n (%). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; BG, blood glucose; CCB, calcium channel blocker; Cr, creatinine; cTNI, cardiac troponin I; DES, drug eluting stent; DKK1, dickkopf-1; HDL-C, high-density lipoprotein cholesterols; hsCRP, high-sensitive C-reactive protein; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; MPO, myeloperoxidase; NSTEMI, non-ST segment elevation myocardial infarction; PCI, percutaneous coronary intervention; PC-PLC, phosphatidylcholine-specific phospholipase C; sCD40L, soluble CD40 ligand; SP-D, surfactant protein D; $sPLA_2$, secretory phospholipase A_2; STEMI, ST segment elevation myocardial infarction; TC, total cholesterol; TF, tissue factor; TG, triglycerides; UA, uric acid; UAP, unstable angina pectoris.

and AS_{max} were significantly higher in patients with than without cardiovascular events (Table 2).

Predictive values of GRACE score, plasma biomarkers and plaque strain

To assess the values of individual parameter for predicting cardiovascular events, measurements of GRACE score, circulating and intracoronary biomarkers and plaque strain were inputted into Cox proportion-hazards risk model. The results showed that GRACE score, plasma levels of SP-D and DKK1, intracoronary gradients of $sPLA_2$ and SP-D, as well as plaque parameters of SS_{max} and AS_{max}, may independently predict cardiovascular events (P<0.001–0.05).

Table 2 Intracoronary gradients of biomarkers and plaque parameters in two groups of patients[a)]

	With events (n=32)	Without events (n=68)	Total (n=100)	P-value
hsCRP gradient (pg mL^{-1})	0.15±0.24	0.15±0.25	0.15±0.25	0.948
sPLA$_2$ gradient (U mL^{-1})	31.25±17.03	20.24±22.19	23.83±21.20	**0.015**
PC-PLC gradient (mU mL^{-1})	0.49±0.82	0.89±0.97	0.77±0.94	0.062
sCD40L gradient (pg mL^{-1})	2.40±2.24	2.16±2.38	2.24±2.33	0.625
IL-6 gradient (pg mL^{-1})	8.36±7.21	8.90±7.35	8.73±7.27	0.732
MPO gradient (ng mL^{-1})	15.07±10.86	14.40±12.93	14.62±12.24	0.800
MCP-1 gradient (pg mL^{-1})	6.38±4.47	7.43±5.45	7.08±5.15	0.348
SP-D gradient (ng mL^{-1})	0.46±0.23	0.32±0.25	0.37±0.25	**0.012**
TF gradient (pg mL^{-1})	1.90±1.59	2.11±1.63	2.04±1.61	0.551
YKL40 gradient (ng mL^{-1})	1.46±1.18	1.61±1.27	1.56±1.24	0.584
DKK1 gradient (pg mL^{-1})	44.15±22.70	37.37±18.33	39.49±19.94	0.117
angiographicalstenosis (%)	47.81±9.06	47.94±8.34	47.90±8.53	0.944
soft plaque (%)	46.9	41.2	43	0.313
fibrous plaque (%)	39.6	28.0	30	0.136
calcified plaque (%)	13.5	30.8	27	0.065
homogeneous plaque (%)	40.6	30.9	34	0.371
heterogeneous plaque (%)	59.4	39.1	66	0.371
plaque volume (mm^3)	28.60±11.38	25.91±9.80	26.77±10.35	0.227
plaque volume burden (%)	56±10	54±10	55±10	0.314
plaque area (mm^2)	5.71±2.29	5.21±2.22	5.37±2.242	0.304
plaque area burden (%)	58±10	56±11	57±11	0.306
remodeling index	0.96±0.08	0.97±0.10	0.97±0.09	0.739
plaque eccentricity index	0.73±0.22	0.75±0.18	0.75±0.19	0.596
SS$_{max}$ (%)	5.09±2.83	3.93±1.64	4.30±2.15	**0.037**
AS$_{max}$ (%)	8.73±4.43	6.90±2.92	7.48±3.56	**0.039**

a) Values are mean±SD. AS$_{max}$, maximal area strain; DKK1, dickkopf-1; hsCRP, high-sensitive C-reactive protein; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MPO, myeloperoxidase; PC-PLC, phosphatidylcholine-specific phospholipase C; sCD40L, soluble CD40 ligand; SP-D, surfactant protein D; sPLA$_2$, secretory phospholipase A$_2$; SS$_{max}$, maximal shear strain; TF, tissue factor.

In addition, higher tertiles of SS$_{max}$, AS$_{max}$, intracoronary gradient of SP-D, and plasma levels of SP-D and TC were significantly associated with increased cumulative cardiovascular events (Figure 1). The GRACE score yielded a C-statistic of 0.457, and the C-statistic for plasma levels of SP-D and DKK1, intracoronary gradients of sPLA$_2$ and SP-D, and plaque parameters of SS$_{max}$ and AS$_{max}$ were 0.492, 0.505, 0.500, 0.504, 0.528 and 0.531, respectively, none of which was significantly higher than the C-statistic of GRACE score alone (Table S1 in Supporting Information).

Additive predictive values of plasma biomarkers and plaque strain to GRACE score

The C-statistics were calculated using 7 different models: model 1 with GRACE score and plasma level of DKK-1, model 2 with GRACE score and plasma level of SP-D, model 3 with GRACE score and intracoronary gradient of SP-D, model 4 with GRACE score and intracoronary gradient of sPLA2, model 5 with GRACE score and SS$_{max}$, model 6 with GRACE score and AS$_{max}$, and model 7 with GRACE score, plasma levels of SP-D and DKK1, intracoronary gradients of sPLA$_2$ and SP-D, and plaque parameters of SS$_{max}$ and AS$_{max}$ in combination. The result showed that the C-statistic for model 1, model 2, model 3, model 4, model 5 and model 6 was 0.506, 0.531, 0.539, 0.528, 0.548 and 0.557, respectively, none of which was statistically different from that for GRACE score. However, the C-statistic for model 7 reached 0.667, which was significantly higher than that for GRACE score alone (Figure 2, Table 3). The sensitivity and specificity for model 7 were 68% and 64%, respectively, as revealed by ROC analysis.

DISCUSSION

In this study, we calculated GRACE score and measured the plasma levels and intracoronary gradients of novel biomarkers as well as plaque strain parameters using IVUSE technique in 100 patients with ACS. We compared the pre-

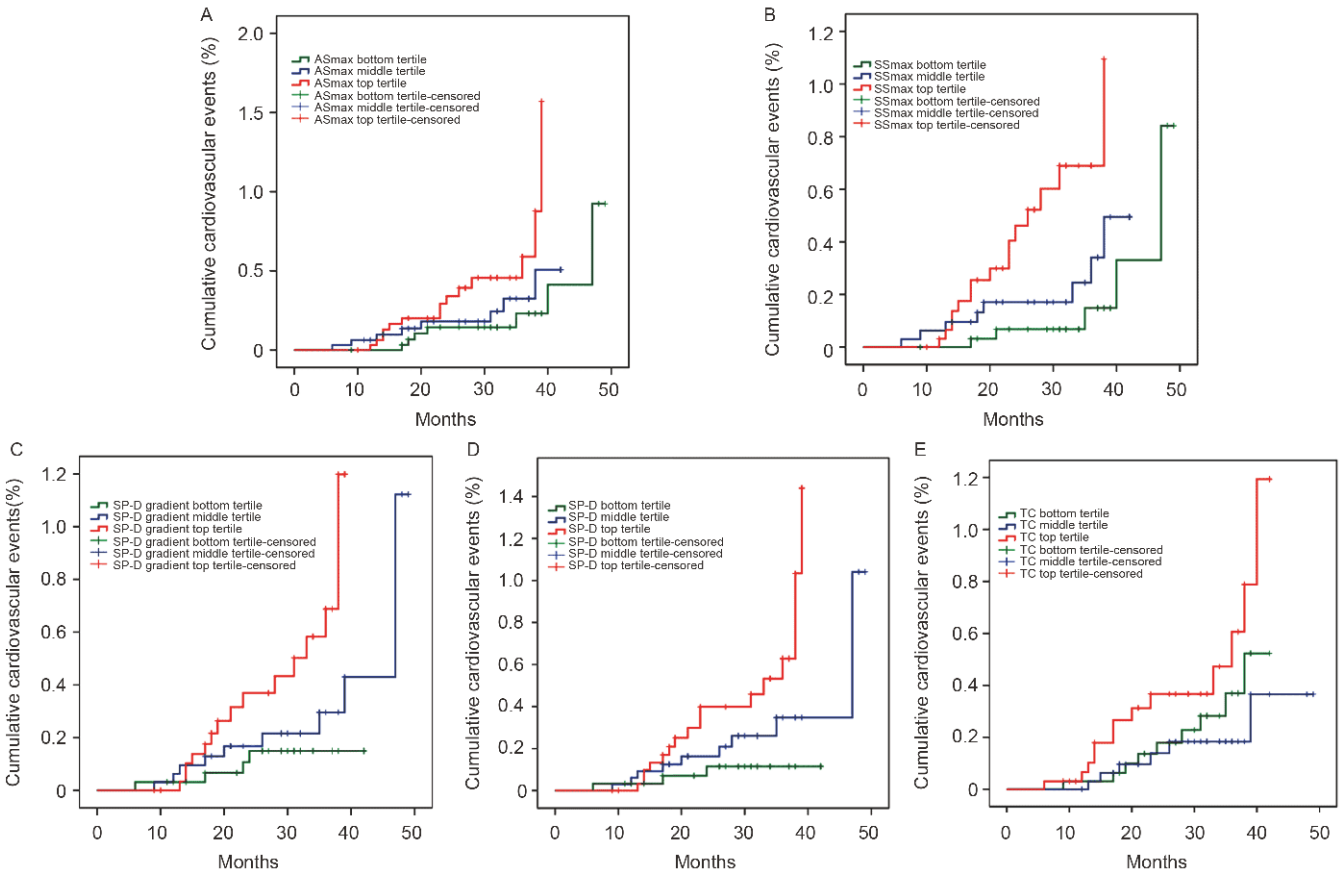

Figure 1 Relationship between tertiles of plaque strain and biochemical parameters, and cumulative cardiovascular events during follow-up. A and B, Kaplan-Meier curves showing the relation between ASmax and SSmax tertiles, and cumulative cardiovascular events during follow-up (P=0.037 and P<0.001, respectively, log rank test for trend). C, D and E, Kaplan-Meier curves showing the relation between SP-D gradient, SP-D and TC tertiles and cumulative cardiovascular events during follow-up (P=0.012, P=0.008 and P=0.042, respectively, log rank test for trend).

dictive values for cardiovascular events between GRACE score alone and the combination of GRACE score with plasma levels and intracoronary gradients of novel biomarkers and plaque strain parameters, and found that the predictive value of GRACE score, plasma levels and intracoronary gradients of novel biomarkers and plaque strain parameters in combination was superior to GRACE score alone. To our knowledge, our study is the first to demonstrate the additive value of novel biomarkers and plaque strain to classical GRACE score for predicting long-term cardiovascular events in patients with ACS.

During the last two decades, GRACE score has been most favorable and highly recommended by the latest international practice guidelines because it allows more accurate stratification and discrimination of risk during both admission and discharge of patients with ACS (Roffi et al., 2016). GRACE score was initially developed to predict in-hospital mortality of patients with ACS and soon transformed into a predictor of 6 month mortality in these cohorts. However, the performance of GRACE score to predict long-term cardiovascular events remains elusive. In the present study, the capability of GRACE score for predicting long-term cardiovascular events in our cohort of patients was not satisfactory probably due to the following reasons. First, the GRACE score was derived from a cohort of high-risk patients with a mean age of 66.3 years, an in-hospital mortality of 4.5%, a positive rate of 31.6% of initial cardiac markers and an incidence of 35.3% of ST segment elevation. In contrast, the mean age was 57.1 years, mortality only 1% and the incidence of myocardial infarction only 10% in our cohort; Second, patients in the GRACE score study received less intensive treatment with PCI in only 14% of subjects, aspirin in 43% and statins in 20.4%, whereas in our study, 65% received PCI, 100% aspirin, 70% β-blockers and 70% statins; Third, the GRACE score investigators followed their patients for in-hospital mortality only during hospitalization, while we followed our patients for cardiovascular events for a median duration of 28 months. By comparison, the baseline characteristics in our cohort of patients may better reflect current practice in the detection and treatment of ACS than those in the GRACE score population.

One major limitation inherent in GRACE score is that this model heavily relies on clinical status of patients at presentation and lacks parameters reflecting the pathological

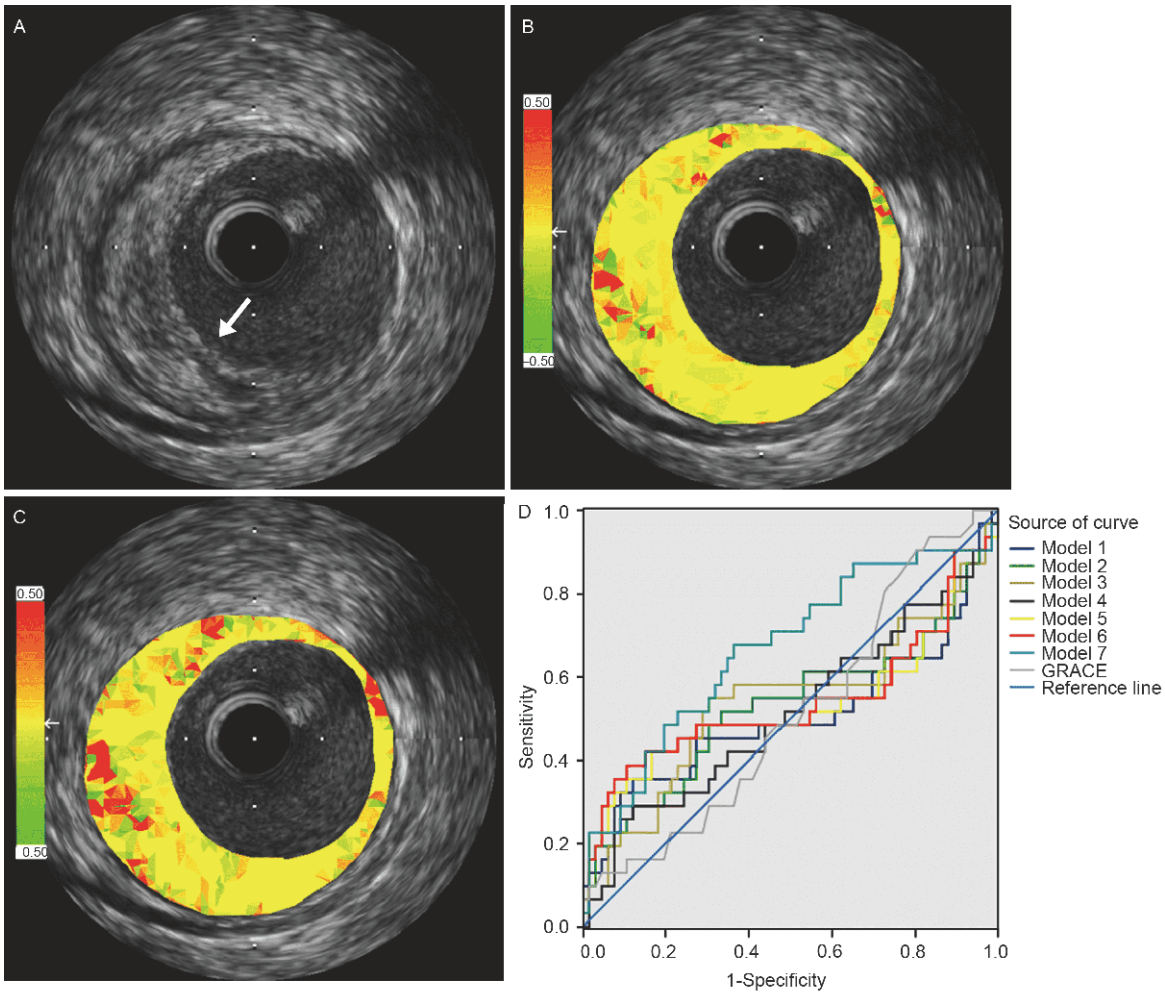

Figure 2 IVUSE construction, strain calculation for plaque and ROC analysis of different cox regression models. A, IVUS imaging of a coronary plaque (white arrow). B and C, IVUSE construction and illustration of shear strain and area strain respectively in the same plaque as in panel A. Different colors represent different levels of strain and correspond with the values illustrated in the color bar on the left. D, Model 1 was with GRACE score and plasma levels of DKK-1, model 2 with GRACE score and plasma levels of SP-D, model 3 with GRACE score and intracoronary gradient of SP-D, model 4 with GRACE score and intracoronary gradient of sPLA2, model 5 with GRACE score and SS_{max}, model 6 with GRACE score and AS_{max}, and model 7 with GRACE score, plasma levels of SP-D and DKK1, intracoronary gradients of $sPLA_2$ and SP-D, and plaque parameters of SS_{max} and AS_{max} in combination. The area under the ROC curve (AUC) was significantly larger for model 7 than that for GRACE (0.667 vs. 0.457, P=0.014). No significant difference was observed in AUC between other models and GRACE.

Table 3 The predictive power of different Cox regression models[a)]

	c statistics for GRACE (95% CI)	c statistics for combination (95% CI)	*P*-value
model 1	0.457 (0.320–0.594)	0.506 (0.364–0.649)	0.628
model 2	0.457 (0.320–0.594)	0.531 (0.392–0.670)	0.458
model 3	0.457 (0.320–0.594)	0.539 (0.402–0.676)	0.407
model 4	0.457 (0.320–0.594)	0.528 (0.397–0.660)	0.464
model 5	0.457 (0.320–0.594)	0.548 (0.407–0.690)	0.365
model 6	0.457 (0.320–0.594)	0.557 (0.415–0.698)	0.319
model 7	0.457 (0.320–0.594)	0.667 (0.544–0.789)	**0.014**

a) CI, confidence interval; GRACE, Global Registry of Acute Coronary Events. Model 1 was with GRACE score and plasma levels of DKK-1, model 2 with GRACE score and plasma levels of SP-D, model 3 with GRACE score and intracoronary gradient of SP-D, model 4 with GRACE score and intracoronary gradient of sPLA2, model 5 with GRACE score and SS_{max}, model 6 with GRACE score and AS_{max}, and model 7 with GRACE score, plasma levels of SP-D and DKK1, intracoronary gradients of sPLA2 and SP-D, and plaque parameters of SS_{max} and AS_{max} in combination.

nature of coronary plaques, which may explain why GRACE score predicts short-term outcome better than long-term one. Previous studies in our laboratory suggested that inflammation plays a key role in initiating and accelerating the pathological process leading to plaque instability and rupture (Zhang et al., 2007). In the present study, we measured lipid profile, cardiac troponin, and plasma levels and intracoronary gradients of a number of inflammatory biomarkers. However, we did not find any significant difference in lipid profile, cardiac troponin, plasma levels of $sPLA_2$, PC-PLC, sCD40L, IL-6, MPO, MCP-1, TF and YKL40, and intracoronary gradients of hsCRP, PC-PLC, sCD40L, IL-6, MPO, MCP-1, TF, YKL40, and DKK1 in patients with and without cardiovascular events, although in previous basic and clinical studies, these biomarkers were reported to be linked with plaque instability or cardiovascular events (Zhang et al., 2010a; Li et al., 2013). In contrast, the plasma levels of hsCRP, SP-D and DKK1, and intracoronary gradients of $sPLA_2$ and SP-D increased significantly in patients with than without cardiovascular events. It was reported that sPLA2 modified low density lipoprotein cholesterol (LDL-C) and accumulated in the arterial intima both extracellularly and intracellularly. In addition, the lipolytic products sPLA2 may promote atherosclerosis via monocyte/macrophage recruitment and inducing cell death (Oörni and Kovanen, 2009). Clinically, sPLA2 activity predicted all-cause mortality in elderly subjects, and death or myocardial infarction in post-infarction patients (Lind et al., 2012). SP-D, a multimeric collectin involved in innate immune defense, is synthesized by type 2 pneumocytes in the lungs and endothelium throughout the vasculature. SP-D was found proatherogenic through affecting lipid metabolism in mice (Sorensen et al., 2006), and associated with coronary artery calcification and carotid intima-media thickness (Hu et al., 2016). Our recent studies found that DKK-1 promoted plaque formation and vulnerability by inducing apoptosis in endothelial cells through activating JNK-endoplasmic reticulum stress pathway and inhibiting canonical Wnt signaling (Di et al., 2017). We also found that the plasma levels of DKK-1 had predictive values in patients with ACS (Wang et al., 2013). Although plasma biomarkers are associated with plaque inflammation, the plasma levels of these cytokines are subject to interference of insidious infection or systemic inflammation. In view of our previous findings that the intracoronary gradient of inflammatory biomarkers was higher in patients with unstable angina than with stable angina, we measured intracoronary gradient of a series of inflammatory biomarkers and found that plasma levels of SP-D and DKK1 and intracoronary gradients of $sPLA_2$ and SP-D may independently predict cardiovascular events with a C-statistic similar to that of GRACE score, which lent support to previous research findings from our and others' laboratories.

It has been well established that risk of plaque rupture is related to both intrinsic plaque vulnerability and extrinsic hemodynamic triggers, and plaque rupture occurs when external mechanical forces outmatch the tensile strength of the fibrous cap. As local strain measures relative deformation in a given vascular lesion, these parameters actually depicts the net result of the local stress imposed on and the elastic property of the plaque. Our previous studies demonstrated that plaque strain predicted plaque rupture in animals (Zhang et al., 2010b) and ischemic cerebrovascular events in patients (Zhang et al., 2009). Recently, we constructed a new IVUSE system and validated this technique with quantitative histological analysis in rabbit (Hu et al., 2012). In the present study, we measured coronary stenosis severity, percentages of soft, fibrous, calcified, homogeneous and heterogeneous plaques, plaque area and area burden, plaque volume and volume burden, remodeling index and plaque eccentricity index using IVUS technique and found that there was no significant difference in these IVUS parameters between the two groups of patients, nor did these parameters enable to predict cardiovascular events during follow-up. These results were different from those in Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) trial where a plaque burden of 70% or greater or a minimal luminal area of 4.0 mm^2 predicted cardiovascular events in patients with ACS, and this discrepancy was probably due to the relative small plaque burden in our patients (Stone et al., 2011). Recently, Brugaletta S performed palpography to measure the surface strain of coronary plaques in a subgroup of patients in the PROSPECT trial but failed to identity high risk plaques with this technique (Brugaletta et al., 2012). In the current trial, we measured the maximal area strain (AS_{max}) and maximal shear strain (SS_{max}) of an entire plaque in a given cross section based on a novel algorithm, which had been validated against histopathology (Hu et al., 2012), and found that all these strain parameters were able to predict independently cardiovascular events with a C-statistic similar to that of GRACE score.

Among the 32 clinical endpoints recorded in this trial, 29 were caused by recurrent unstable angina pectoris requiring hospitalization which we believe represented *de novo* coronary stenosis rather than instent thrombosis or restenosis for the following reasons. First, patients with instent thrombosis are usually manifestd with sudden death or ST-segment elevation myocardial infarction, which did not occur in our patients. Second, most of our patients undergoing PCI were treated with a drug eluting stent (63 out of 65 cases), who were unlikely to develop significant instent restenosis in 2 or 3 years after the procedure. Third, a small proportion of our patients with unstable angina pectoris underwent coronary computed tomography demonstrating that the clinical events were induced by *de novo* lesions rather than instent rest-

enosis.

The most important finding in this study was the additive value of novel biomarkers and plaque strain to classical GRACE score for predicting long-term cardiovascular events in patients with ACS. To compare the additive values of different plasma biomarker and plaque strain parameters for predicting cardiovascular events, we used 7 different models and found that although the C-statistics for model 1 to model 6 were all higher than that of GRACE score, the difference did not reach statistical significance. On the other hand, the C-statistic for model 7 was significantly higher than that of GRACE score. These results indicated that the combination model is superior to GRACE score for predicating long-term cardiovascular events in patients with ACS.

There were several limitations in our study. First, the patient sample was relatively small mainly due to demanding techniques used in the present study, although the cardiovascular event rate was high during follow-up (32%). Thus, our conclusions warrant further verification in a multicenter, large-scale clinical trial. Second, the combination model (model 7) requires measurement of plasma level and intracoronary gradient of biomarkers and plaque strain parameters, which necessitates invasive investigation and makes risk prediction more complex and expensive than GRACE score alone. However, in patients undergoing coronary angiography, our combination model provides a useful approach to accurate risk stratification and precise treatment strategy. Furthermore, the results derived from our study can be generalized to a larger population with ACS because the predictive power of model 7 was superior to that of widely used GRACE score. Third, the majority of cardiovascular events during follow-up were unstable angina requiring hospitalization despite the fact that most patients (65%) received coronary drug-eluting stents during their initial hospitalization for ACS, which reflects current clinical situation that patients with ACS often have multivessel non-critical lesions resulting in future cardiovascular events. Further studies are needed to test whether our model can predict cardiovascular death better than GRACE score.

CONCLUSIONS

In conclusion, plasma levels of SP-D and DKK1, intracoronary gradients of $sPLA_2$ and SP-D, and plaque strain parameters SS_{max} and AS_{max} can independently predict long-term cardiovascular events in patients with ACS similar to GRACE score. The combination of circulating and intracoronary biomarkers, plaque strain and GRACE score provides a better prediction than GRACE score alone. This combinational approach may pave a new avenue to precise risk stratification in ACS patients although multicenter, large-scale clinical trials are warranted to further verify our preliminary findings.

MATERIALS AND METHODS

Study design and participants

This study was a multicenter, prospective and long-term follow-up trial. From April 2009 to December 2012, 100 ACS patients with complete clinical, biochemical, coronary angiographic and IVUS information were enrolled from Shandong University Qilu Hospital and Shenyang Military Region General Hospital, who satisfied the following inclusion criteria: (1) All patients had a diagnosis of ACS on admission based on ACC/AHA 2007 guidelines for ACS (Anderson et al., 2007); (2) Patients underwent selective coronary angiography and completed IVUS studies of three major coronary arteries; (3) Patients had a TIMI grade 3 flow after percutaneous coronary stenting for a culprit coronary stenosis; (4) Patients had at least one non-critical stenosis defined by coronary angiography as lumen diameter stenosis of 30%–70% without intervention; (5) Patients received optimal medical treatment including antiplatelets, β-blockers or statins according to ACC/AHA 2007 guidelines for ACS; (6) Patients had a complete GRACE score registration. Patients with the following conditions were excluded: (1) moderate to severe valvular heart disease; (2) arrhythmias including ventricular tachycardia, atrial fibrillation, atrial flutter, sick sinus syndrome, and type 2 second-degree or third-degree atrioventricular block; (3) heart failure with NYHA ≥Ⅲ or LVEF≤40%; (4) uncontrolled severe hypertension (SBP≥180 mmHg and/or DBP≥110 mmHg); (5) other non-cardiovascular diseases including active liver disease, severe anemia, acute or chronic infection, expected lifetime due to non-cardiovascular diseases < 3 years, and serious mental disease (Figure S1 in Supporting Information). The study protocol was approved by the Ethics Committee of Shandong University Qilu Hospital and informed consent was obtained from all patients.

Biochemical assay

The plasma levels of lipids, blood glucose, creatinine, uric acid, cardiac troponin, and circulating and intracoronary levels of biomarkers including $sPLA_2$, PC-PLC, sCD40L, IL-6, MPO, MCP-1, TF and YKL40 were measured (Materials in Supporting Information).

IVUS imaging and analysis

During the process of selective coronary angiography, all patients underwent IVUS imaging of coronary arteries and IVUS image analysis was conducted to derive a number of

plaque parameters (see Materials in Supporting Information for details).

IVUSE construction and analysis

A validated two-dimensional IVUSE technique was used to measure maximal shear strain (SS_{max}) and maximal area strain (AS_{max}) of a plaque from IVUS images (Figure 2, Materials in Supporting Information).

GRACE score calculation

The GRACE score included eight clinical variables during hospitalization (age, heart rate, systolic blood pressure, plasma creatinine concentration, Killip class at presentation, cardiac arrest on admission, ST-segment deviation, and elevated cardiac enzymes/markers). Values for these variables on admission were entered into the GRACE risk calculator (available at http://www.outcomes-umassmed.org/grace) to obtain estimates of the cumulative risk of all-cause mortality and pre-specified composite endpoint of cardiovascular events.

Follow-up and endpoint

All patients were followed up clinically or by telephone every 6 months after enrollment for 3 years. Routine physical checkup, bioassay of blood glucose and plasma lipid profile and electrocardiography (ECG) were performed at each follow-up and cardiovascular event and event times were recorded. The clinical endpoints included sudden cardiac death, non-fatal myocardial infarction, unstable angina pectoris requiring hospitalization, and coronary revascularization, which were verified by telephone call and in-hospital case records.

Statistical analysis

Statistical analysis involved use of SPSS 16.0 (SPSS, Chicago, USA). Continuous data were expressed as mean±SD and were analyzed by independent *t* test, and categorical data were expressed as percentages and were analyzed by Chi-square test. For numerical variables of skew distribution, Mann-Whitney U test was applied to compare the between-group difference. The Cox proportion risk model, which takes into account the time to first cardiovascular event, was adopted to assess the predictive power of variables for cardiovascular events, and the hazard ratio (HR) and 95% confidence interval (95%CI) were calculated. Each parameter was first introduced into the Cox regression model to test its predictive value (Table S1 in Supporting Information). Parameters with significant predictive power were then introduced into combination models to assess the additive predictive values of plasma biomarkers and plaque strain to GRACE score by applying C-statistics. The Kaplan-Meier curves were plotted for the relationship between tertiles of each independent parameter and cumulative cardiovascular events during follow-up. The log-rank test for trends was used to examine the statistical significance. $P<0.05$ was considered statistically significant.

Compliance and ethics *The author(s) declare that they have no conflict of interest. This study was conformed with the Helsinki Declaration of 1975 (as revised in 2008) concerning human and animal rights.*

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SUPPORTING INFORMATION

Figure S1 Schematic diagram of the research.

Table S1 The predictive power of GRACE score, clinical characteristics, biomarkers and plaque parameters in two groups of patients

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