Homocysteine enhances the predictive value of the GRACE risk score in patients with ST-elevation myocardial infarction

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Abstract

Objective: The present study aims to investigate whether the addition of homocysteine level to the Global Registry of Acute Coronary Events (GRACE) risk score enhances its predictive value for clinical outcomes in ST-elevation myocardial infarction (STEMI).

Methods: A total of 1143 consecutive patients with STEMI were included in this prospective cohort study. Homocysteine was detected, and the GRACE score was calculated. The predictive power of the GRACE score alone or combined with homocysteine was assessed by the receiver operating characteristic (ROC) analysis, methods of net reclassification improvement (NRI) and integrated discrimination improvement (IDI).

Results: During a median follow-up period of 36.7 months, 271 (23.7%) patients reached the clinical endpoints. It showed that the GRACE score and homocysteine could independently predict all-cause death [GRACE: HR=1.031 (1.024–1.039), p<0.001; homocysteine: HR=1.023 (1.018–1.028), p<0.001] and MACE [GRACE: HR=1.008 (1.005–1.011), p<0.001; homocysteine: HR=1.022 (1.018–1.025), p<0.001]. When they were used in combination to assess the clinical outcomes, the area under the ROC curve significantly increased from 0.786 to 0.884 (95% CI=0.067–0.128, Z=6.307, p<0.001) for all-cause death and from 0.678 to 0.759 (95% CI=0.055–0.108, Z=5.943, p<0.001) for MACE. The addition of homocysteine to the GRACE model improved NRI (all-cause death: 0.575, p<0.001; MACE: 0.621, p=0.008) and IDI (all-cause death: 0.083, p<0.001; MACE: 0.130, p=0.016), indicating effective discrimination and reclassification.

Conclusion: Both the GRACE score and homocysteine are significant and independent predictors for clinical outcomes in patients with STEMI. A combination of them can develop a more predominant prediction for clinical outcomes in these patients. *(Anatol J Cardiol 2017; 18: 182-93)*

Keywords: Global Registry of Acute Coronary Events risk score, homocysteine, ST-elevation myocardial infarction, all-cause death, major adverse cardiovascular events

Introduction

Patients with ST-elevation myocardial infarction (STEMI), a high-risk population, are heterogeneous in terms of clinical presentation as well as immediate- and long-term risks of adverse events. Identifying patients at higher risk for adverse outcomes after STEMI is a cornerstone of modern cardiovascular care (1). Consequently, accurate and comprehensive risk stratification is important for decision making when treating patients with STEMI. Currently, the Global Registry of Acute Coronary Events (GRACE) risk score (2, 3) is widely recommended as a means to evaluate the risks of death and death plus myocardial infarction (MI) in patients in hospital and within 6 months after discharge and to guide triage and management decisions in acute coronary syndrome (ACS) (4, 5). However, its predictive value in a longer period, for example >6 months after discharge, particularly in patients with STEMI, is not very clear. This scoring is a multivariable task that takes into account clinical characteristics together with electrocardiographic and cardiac enzymes/troponins as biomarkers. By doing so, the score reflects certain dimensions related to the clinical outcomes of ACS. Biomarkers may provide additional information of ACS pathophysiology, including STEMI. However, the biological variables considered in the GRACE system are limited to creatinine and cardiac enzymes/troponins.

Homocysteine, a toxic sulfhydryl-containing amino acid, is an intermediate metabolite product of methionine. It has been

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reported to play an important role in the pathogenesis of atherosclerosis and coagulation (6, 7). Epidemiological studies show that serum homocysteine concentration is associated with stroke, coronary heart disease, peripheral artery disease, and venous thrombosis (8). Prospective researches have demonstrated that homocysteine can predict mortality and other cardiovascular events in subjects with or without coronary artery disease (9, 10). Currently, homocysteine is identified as an intensive and independent risk factor and predictor for cardiovascular diseases (8). However, in spite of its important role in cardiovascular diseases, it is not considered in the GRACE risk score model.

Up to now, few studies have specifically evaluated the relationship between the homocysteine level and GRACE risk score. The present study aims to investigate the association between these two predictive factors and to determine whether a combination of homocysteine and the GRACE score model can better predict the longer clinical outcomes in patients with STEMI.

Methods

Study population

This prospective cohort study recruited consecutive patients with a confirmed diagnosis of STEMI admitted to the Department of Cardiology at two first-class hospitals in China between January 2010 and December 2012. STEMI was diagnosed according to the 2007 American College of Cardiology Foundation/American Heart Association Guidelines (1). The diagnostic criteria include the following: a) persistent symptoms of ischemia for at least 30 min; b) ST-segment elevation of at least 1 mm in at least two adjacent limb leads or at least 2 mm in at least two contiguous precordial leads or a new left bundle branch block in the electrocardiography; and c) elevated serum creatine kinase (CK) and creatine kinase-myocardial band (CK-MB) more than twice the upper limit of normal or elevated serum troponins (1). Patients with valvular heart disease, malignant tumors, or severe liver or kidney dysfunction needing instrumental replacement therapy were excluded. There were 1327 patients with STEMI, of which 1143 patients met the criteria. All of them agreed to participate. All management and treatment decisions were left to the discretion of the attending cardiologists according to the Guidelines. The research framework is shown in Figure 1.

This study was approved by the Ethics Committee and was performed in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from all patients. Before the implementation of this study, all the researchers were trained according to uniform standards, which guaranteed consistency in their observations. In addition, an independent committee was set up for quality control.

Laboratory detection and biomarker testing

Blood samples of the patients were centrifuged at 4°C and the obtained serum samples were stored in aliquots at –80°C. All laboratory parameters, including cardiac troponin, CK, CK-MB, plasma glucose, creatinine, uric acid, homocysteine, triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol concentrations were measured in the two hospitals using uniform equipment and reagents (Olympus AU640 Clinical Chemistry Analyzer, Olympus Diagnostica, Hamburg, Germany). Homocysteine was detected using high-performance liquid chromatography with fluorescence detection.

Echocardiography

Comprehensive echocardiographic analysis of cardiac structure and function was performed by two experienced physicians in accordance with the recommendations of the American Society of Echocardiography (11). For a particular patient, the same operator analyzed the echocardiographic metrics. The physicians used the two-dimensional, M-mode, and biplane Simpson

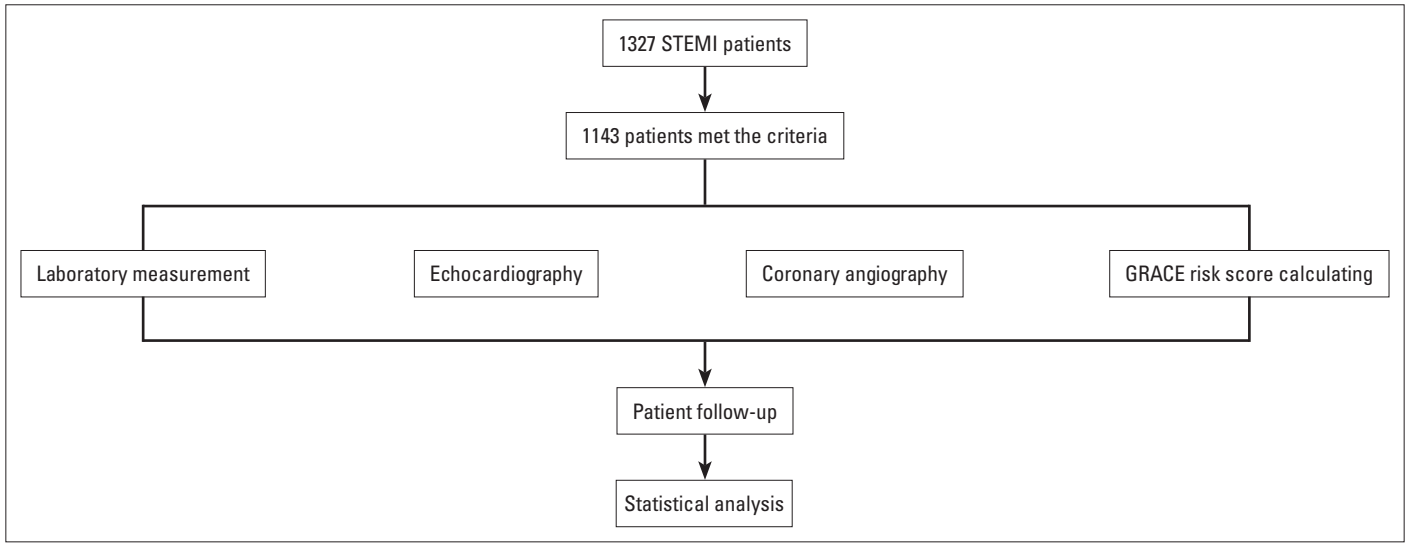


Figure 1. Study frame diagram

methods, as appropriate (11). All measurements were averaged over three cardiac cycles. The indexes of left ventricular end-diastolic diameter and left ventricular end-diastolic volume were normalized according to the body surface area. Left ventricular mass was calculated using the formula recommended by (11) and was expressed as the left ventricular mass index. Regional wall motion was assessed using a 16-segment model of the left ventricular and a 4-point grading scale: 1. normal contractility; 2. hypokinesia; 3. akinesia; and 4. dyskinesia (11). The wall motion score index was calculated as the sum of the score of each segment divided by the number of segments scored. Thus, a higher wall motion score index corresponds to a worse wall motion.

Coronary angiography

Coronary angiography was performed according to the standard method (12). There were two specialized physicians who read the images and decided the results. Coronary single vessel disease was defined as stenosis >50% in a major coronary artery (e.g., left anterior descending coronary artery, left circumflex coronary artery, or right coronary artery) and/or in its main branches. Multiple vessel disease was defined as stenosis >50% in more than one major coronary artery (12). The Gensini score was used to assess the severity of coronary artery stenosis because it has a close correlation with the lesion severity and is convenient to calculate (13). A higher score indicates a more severe lesion.

Data collection and the GRACE risk score calculation

Baseline data, including demographic data, clinical data, and medications, were collected using a standard case-report form. The GRACE risk prediction model was performed as described previously (2). The variables for estimation included age, heart rate, systolic blood pressure, creatinine level, history of congestive heart failure, in-hospital percutaneous coronary intervention, in-hospital coronary artery bypass graft surgery, previous MI, ST-segment depression, and elevated cardiac markers. Values of these variables were entered into the GRACE risk calculator to obtain estimates of the cumulative risks of all-cause death and major adverse cardiovascular events (MACE).

Clinical endpoint definition and patient follow-up

All the patients were followed up by telephone contacts or scheduled consultations to track the progress of the treatment and the occurrence of cardiovascular events. Follow-up information was completed for all the included patients. MACE included all-cause death, prehospitalization for heart failure or angina symptoms, recurrent nonfatal MI, repeated coronary revascularization, and stroke.

Statistical analysis

Continuous variables are presented as mean±standard deviation ($\overline{x}$±SD) or median (inter-quartile range). Categorical variables are presented as frequency (percentage). The normality of data distribution was tested using Kolmogorov–Smirnov analysis. Independent-samples t-test, Mann–Whitney U test, one-way analysis of variance, or Kruskal–Wallis H test was used to examine the differences between continuous variables, as appropriate. The Pearson χ^2 test or Fisher's exact test was used to determine the differences between categorical variables. Clinical outcomes were evaluated using the Kaplan–Meier method, and intergroup comparisons were conducted using the log-rank test. Univariate and multivariate Cox proportional hazard regression analysis was performed to identify predictors for adverse clinical outcomes. The potential correlation between the homocysteine level and the GRACE score were analyzed using the Spearman's rank correlation.

The predictive value of the combination of these two factors was estimated by the receiver operating characteristic (ROC) curve. Discrimination was assessed by the area under curve (AUC) and increase in AUC was tested for significance using the method previously proposed (14). Calibration was assessed with Hosmer–Lemeshow goodness-of-fit test (14). Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were performed to analyze the degree to which the addition of homocysteine improved the predictive ability of the GRACE model (15). NRI focuses on the reclassification constructed for with and without events, quantifying the correct movement in categories. IDI focuses on the difference between average sensitivity and "1-specificity" for models with or without homocysteine, which measures enhancement in average sensitivity without sacrificing average specificity from the addition of homocysteine to the GRACE system (15).

Statistical analyses were performed using SPSS (version 18.0), MedCalc (version 9.6.4.0), and R-programming language (version 3.1.2). All statistical tests were two-tailed, and a p-value of <0.05 was considered statistically significant.

Results

Baseline characteristics of patients

The number of patients in the two study institutions was 578 and 565. As shown in Table 1, there were no differences in the baseline data of patients between the two hospitals. The included 1143 patients (85% male) had a mean age of 58 years (IQR, 50–67 years) and a median follow-up period of 36.7 months (IQR, 28.0–46.7 months). The patients were segregated into three groups according to the tertiles of homocysteine level at baseline (Tertile 1: ≤14.6 μmol/L; Tertile 2: 14.7–24.4 μmol/L; Tertile 3: ≥24.5 μmol/L). Demographic and clinical characteristics, biomarker concentrations, and medications during hospitalization are shown in Table 2. Intergroup comparisons showed that age (56.53±12.08 vs. 57.46±11.02 vs. 60.74±10.66, p<0.001), uric acid (294.10±91.90 vs. 307.10±84.61 vs. 314.04±96.77, p=0.009), multiple vessel disease (28.7% vs. 36.4% vs. 57.7%, p=0.001), the Gensini score (64.33±33.06 vs. 68.24±42.55 vs. 75.61±42.30, p<0.001) and the GRACE risk score (120.34±43.34 vs. 127.83±43.76 vs.

Table 1. Baseline data of patients in the two hospitals

	Hospital 1 (n=578)	Hospital 2 (n=565)	*P*
Clinical characteristics			
Age, years	58.20±11.15	58.29±11.67	0.888
Female sex	88 (15.2)	83 (14.7)	0.800
Body mass index, kg/m^2	24.07±2.89	23.84±2.65	0.165
Heart rate, min^{-1}	76.22±15.30	75.92±15.43	0.735
SBP, mm Hg	120.93±20.23	123.07±20.58	0.077
DBP, mm Hg	76.78±13.56	77.14±12.90	0.640
Smoking	421 (72.8)	383 (67.8)	0.062
Hypertension	247 (42.7)	246 (43.5)	0.783
Dyslipidemia	102 (17.6)	100 (17.7)	0.982
Diabetes	93 (16.1)	100 (17.7)	0.743
Anterior wall infarct	330 (57.1)	324 (57.3)	0.931
Killip classification			0.835
Class I	382 (66.1)	360 (63.7)	
Class II	150 (26.0)	154 (27.3)	
Class III	27 (4.7)	31 (5.5)	
Class IV	19 (3.3)	20 (3.5)	
Laboratory examinations			
Triglycerides, mmol/L	1.64±0.90	1.63±.95	0.868
Total cholesterol, mmol/L	4.14±1.01	4.09±1.52	0.529
LDL-C, mmol/L	2.43±0.78	2.34±0.85	0.078
Blood glucose, mmol/L	7.76±4.26	7.89±3.60	0.567
Uric acid, μmol/L	307.06±90.29	303.06±92.78	0.460
Creatinine, μmol/L	87.03±16.93	87.53±24.47	0.685
eGFR, mL/min/1.73m^2	112.41±38.20	113.28±52.37	0.747
Ultrasound cardiogram parameters			
LVEDDI, cm/m^2	3.03±0.41	3.01±0.42	0.392
LVEDVI, mL/m^2	58.49±13.92	58.54±14.18	0.947
LVMI, g/m^2	91.02±22.88	90.32±23.09	0.606
LVFS, %	29.54±7.80	29.36±7.84	0.682
LVEF, %	53.62±10.87	53.47±11.20	0.818
Wall motion score index	1.27±0.21	1.27±0.18	0.959
Coronary angiography characteristics			
Number of vessel disease			0.766
Single vessel disease	244 (42.2)	235 (41.6)	
Multiple vessel disease	334 (57.8)	330 (58.4)	
Gensini score	69.97±37.93	68.51±41.41	0.542
Homocysteine, μmol/L	21.68±13.92	20.91±15.80	0.382
GRACE risk score	97.55±28.53	97.55±28.93	0.996

DBP - diastolic blood pressure; eGFR - estimated glomerular filtration rate; GRACE - Global Registry of Acute Coronary Events; LDL-C - low-density lipoprotein cholesterol; LVEDDI - left ventricular end-diastolic dimension index; LVEDVI - left ventricular end-diastolic volume index; LVEF - left ventricular ejection fraction; LVFS - left ventricular fraction shortening; LVMI - left ventricular mass index; SBP - systolic blood pressure. eGFR is calculated according to the MDRD formula: eGFR (mL/min/1.73m^2 of body surface area)=186 x (SCr) – 1.154 x (age) – 0.203 (x0.742 for females). SCr is reported in mg/dL

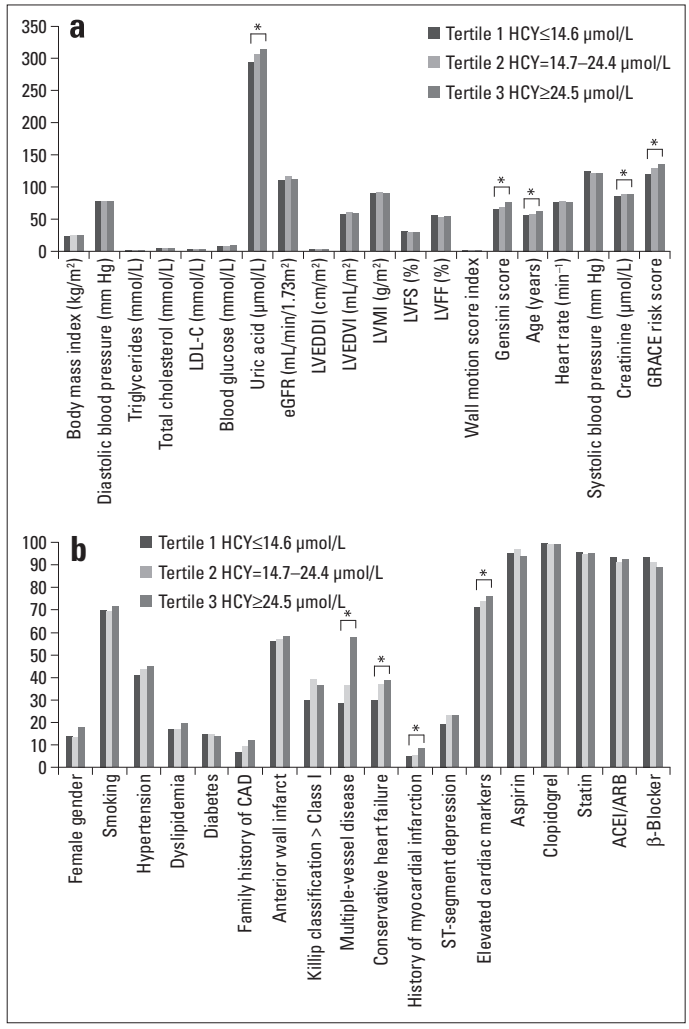


Figure 2. Baseline characteristics of patients grouped by tertiles of homocysteine level. (a) Continuous variables. (b) Categorical variables

134.08±43.98, p<0.001) increased with the increase in homocysteine level. There were no significant differences in other characteristics or variables among the three groups (Fig. 2).

Comparison of clinical characteristics between patients with and without MACE

During the period of follow-up, 271 (23.7%) patients reached the clinical endpoint, including 103 (9.0%) deaths, 75 (6.6%) heart failures, 51 (4.5%) unstable anginas, 32 (2.8%) MIs, 52 (4.5%) coronary revascularizations, and 16 (1.4%) strokes. The clinical characteristics of the patients with or without MACE are demonstrated in Table 3. Compared with the patients without adverse events, patients who experienced such events were older (62.55±11.47 vs. 56.91±11.06 years, p<0.001), more often females (19.9% vs. 13.4%, p=0.009), with a higher frequency of hypertension (49.4% vs. 41.2%, p=0.017), dyslipidemia (22.1% vs. 16.3%, p=0.029), and multiple vessel disease (73.1% vs. 40.7%, p<0.001). Moreover, these patients had higher heart rate (80.51±19.25 vs. 74.69±13.65 bpm, p<0.001), blood glucose (8.62±5.51 vs. 7.57±3.28 mmol/L, p<0.001), creatinine (91.83±31.22 vs. 85.86±16.34 μmol/L,

Table 2. Baseline characteristics of patients grouped by tertiles of homocysteine level

	Tertile 1 (≤14.6) (n=380)	Tertile 2 (14.7–24.4) (n=383)	Tertile 3 (≥24.5) (n=380)	*P*
Clinical characteristics				
Female sex	52 (13.7)	51 (13.3)	68 (17.9)	0.146
Body mass index, kg/m^2	23.83±2.68	24.05±2.92	23.99±2.72	0.537
Diastolic blood pressure, mm Hg	77.23±13.52	76.80±13.26	76.84±12.96	0.886
Smoking	266 (70.0)	267 (69.7)	271 (71.3)	0.871
Hypertension	155 (40.8)	168 (43.9)	170 (44.7)	0.519
Dyslipidemia	63 (16.6)	64 (16.7)	75 (19.7)	0.439
Diabetes	55 (14.5)	56 (14.6)	52 (13.7)	0.932
Family history of CAD	26 (6.8)	36 (9.4)	45 (11.8)	0.060
Anterior wall infarct	212 (55.8)	219 (57.2)	223 (58.7)	0.731
Killip classification				0.144
Class I	267 (70.3)	234 (61.1)	241 (63.4)	
Class II	89 (23.4)	111 (29.0)	104 (27.4)	
Class III	12 (3.2)	23 (6.0)	23 (6.1)	
Class IV	12 (3.2)	15 (3.9)	12 (3.2)	
Laboratory examinations				
Triglycerides, mmol/L	1.65±1.01	1.66±0.87	1.60±0.89	0.640
Total cholesterol, mmol/L	4.08±1.04	4.09±1.45	4.17±1.33	0.613
LDL-C, mmol/L	2.40±0.80	2.32±0.75	2.44±0.90	0.119
Blood glucose, mmol/L	7.70±3.37	7.67±3.69	8.09±4.67	0.256
Uric acid, μmol/L	294.10±91.90	307.10±84.61	314.04±96.77	0.009
eGFR, mL/min/1.73m^2	110.62±37.65	116.52±45.87	111.35±52.38	0.151
Ultrasound cardiogram parameters				
LVEDDI, cm/m^2	2.98±0.38	3.04±0.44	3.03±0.41	0.115
LVEDVI, mL/m^2	57.11±13.26	59.75±15.14	58.68±13.54	0.072
LVMI, g/m^2	89.02±22.48	92.01±23.62	90.99±22.76	0.188
LVFS, %	29.99±7.09	29.01±7.84	29.36±8.44	0.215
LVEF, %	54.64±10.40	52.79±11.43	53.22±11.17	0.053
Wall motion score index	1.27±0.18	1.28±0.20	1.27±0.20	0.703
Coronary angiography characteristics				
Number of vessel disease				0.001
Single vessel disease	271 (71.3)	244 (63.6)	161 (42.3)	
Multiple vessel disease	109 (28.7)	139 (36.4)	219 (57.7)	
Gensini score	64.33±33.06	68.24±42.55	75.61±42.30	<0.001
GRACE variables				
Age, years	56.53±12.08	57.46±11.02	60.74±10.66	<0.001
Heart rate, min^{-1}	75.23±13.06	76.60±16.20	76.38±16.57	0.421
Systolic blood pressure, mm Hg	123.00±21.21	121.60±20.55	121.36±19.48	0.491
Creatinine, μmol/L	85.03±16.63	88.18±20.67	88.31±24.75	0.038
Congestive heart failure	113 (29.7)	141 (36.8)	147 (38.7)	0.022
In-hospital PCI	380 (100)	372 (97.1)	352 (92.6)	<0.001
In-hospital CABG	–	–	–	–
History of myocardial infarction	19 (5)	20 (5.2)	32 (8.4)	0.091
ST-segment depression	74 (19.5)	89 (23.2)	87 (22.9)	0.381
Elevated cardiac markers	270 (71.1)	284 (74.2)	289 (76.1)	<0.001
GRACE risk score	120.34±43.34	127.83±43.76	134.08±43.98	<0.001
Medicine				
Aspirin	363 (95.5)	372 (97.1)	357 (93.9)	0.109
Clopidogrel	379 (99.7)	380 (99.2)	377 (99.2)	0.710
Statin	364 (95.8)	363 (94.8)	361 (95.0)	0.828
ACEI/ARB	355 (93.4)	350 (91.4)	352 (92.6)	0.572
β-Blocker	356 (93.7)	350 (91.4)	338 (88.9)	0.067

ACEI - angiotensin-converting enzyme inhibitor; ARB - angiotensin receptor blocker; CABG - coronary artery bypass grafting; CAD - coronary artery disease; eGFR - estimated glomerular filtration rate; GRACE - Global Registry of Acute Coronary Events; LDL-C - low-density lipoprotein cholesterol; LVEDDI - left ventricular end-diastolic dimension index; LVEDVI - left ventricular end-diastolic volume index; LVEF - left ventricular ejection fraction; LVFS - left ventricular fraction shortening; LVMI - left ventricular mass index; PCI - percutaneous coronary intervention. eGFR is calculated according to the MDRD formula: eGFR (mL/min/1.73m^2 of body surface area)=186 x (SCr) – 1.154 x (age) – 0.203 (x0.742 for females). SCr is reported in mg/dL

Table 3. Clinical characteristics of patients with or without MACE

	Without MACE (n=872)	With MACE (n=271)	*P*
Clinical characteristics			
Age, years	56.91±11.06	62.55±11.47	<0.001
Female sex	117 (13.4)	54 (19.9)	0.009
Body mass index, kg/m^2	23.96±2.80	23.93±2.68	0.873
Heart rate, min^{-1}	74.69±13.65	80.51±19.25	<0.001
SBP, mm Hg	122.21±19.79	121.26±22.35	0.502
DBP, mm Hg	77.24±12.89	76.04±14.28	0.194
Smoking	623 (71.4)	180 (66.4)	0.128
Hypertension	359 (41.2)	134 (49.4)	0.017
Dyslipidemia	142 (16.3)	60 (22.1)	0.029
Diabetes	126 (14.4)	37 (13.7)	0.767
Family history of CAD	90 (10.3)	17 (6.7)	0.055
Anterior wall infarct	496 (56.9)	158 (58.3)	0.725
Killip classification			<0.001
Class I	602 (69.0)	140 (51.7)	
Class II	221 (25.3)	83 (30.6)	
Class III	31 (3.6)	27 (10.0)	
Class IV	18 (2.1)	21 (7.7)	
Laboratory examinations			
Triglycerides, mmol/L	1.65±0.91	1.57±0.95	0.210
Total cholesterol, mmol/L	4.11±1.22	4.13±1.49	0.768
LDL-C, mmol/L	2.37±0.81	2.43±0.83	0.273
Blood glucose, mmol/L	7.57±3.28	8.62±5.51	<0.001
Uric acid, µmol/L	299.26±84.40	323.83±109.45	<0.001
Creatinine, µmol/L	85.86±16.34	91.83±31.22	<0.001
eGFR, mL/min/1.73m^2	111.89±37.11	115.91±66.29	0.207
Echocardiogram parameters			
LVEDDI, cm/m^2	3.00±0.40	3.07±0.44	0.014
LVEDVI, mL/m^2	58.11±13.97	59.84±14.22	0.076
LVMI, g/m^2	89.27±22.23	95.18±24.73	<0.001
LVFS, %	29.90±7.75	27.99±7.84	<0.001
LVEF, %	54.14±10.95	51.64±11.07	0.001
Wall motion score index	1.27±0.19	1.29±0.20	0.119
Coronary angiography characteristics			
Number of vessel disease			<0.001
Single vessel disease	517 (59.3)	73 (26.9)	
Multiple vessel disease	355 (40.7)	198 (73.1)	
Gensini score	65.64±38.19	82.70±42.31	<0.001
Homocysteine, mmol/L	18.55±10.02	30.14±22.57	<0.001
GRACE risk score	121.61±41.67	146.09±46.19	<0.001

CAD - coronary artery disease; DBP - diastolic blood pressure; eGFR - estimated glomerular filtration rate; GRACE - Global Registry of Acute Coronary Events; LDL-C - low-density lipoprotein cholesterol; LVEDDI - left ventricular end-diastolic dimension index; LVEDVI - left ventricular end-diastolic volume index; LVEF - left ventricular ejection fraction; LVFS - left ventricular fraction shortening; LVMI - left ventricular mass index; MACE - major adverse cardiovascular events; SBP - systolic blood pressure. eGFR is calculated according to the MDRD formula: eGFR (mL/min/1.73m^2 of body surface area)=186 x (SCr) – 1.154 x (age) – 0.203 (x0.742 for females). SCr is reported in mg/dL

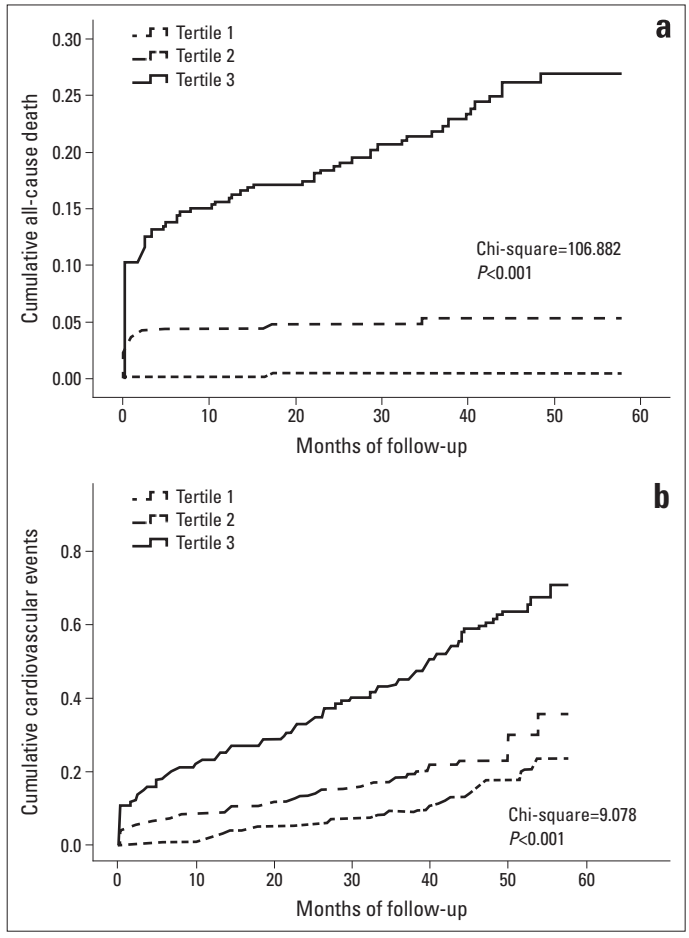


Figure 3. Kaplan–Meier survival curve analysis. The probability of all-cause death (a) and major adverse cardiovascular events (b) increased with the increase in homocysteine level

p<0.001), uric acid (323.83±109.45 vs. 299.26±84.40 µmol/L, p<0.001), Killip classification (Class I: 51.7% vs. 69.0%, p<0.001), left ventricular mass (95.18±24.73 vs. 89.27±22.23 g/m^2, p<0.001), and Gensini score (82.70±42.31 vs. 65.64±38.19, p<0.001) and a lower left ventricular fraction shortening (27.99%±7.84% vs. 29.90%±7.75%, p<0.001) and ejection fraction (51.64%±11.07% vs. 54.14%±10.95%, p=0.001). It was noteworthy that the homocysteine level (30.14±22.57 vs. 18.55±10.02 µmol/L, p<0.001) and the GRACE risk score (146.09±46.19 vs. 121.61±41.67, p<0.001) were significantly higher in patients with MACE than in those without MACE.

Homocysteine and GRACE score as significant predictors for clinical outcomes

The cumulative incidences of all-cause death and MACE in the three groups of patients are illustrated using the Kaplan–Meier survival curves in Figure 3. The curves revealed significantly worse clinical outcomes in patients with homocysteine above the third percentile compared with those below the third percentile. Log-rank test on the curves identified significant differences among the three groups (all-cause death: χ^2=106.882, p<0.001; MACE: χ^2=96.078, p<0.001).

Table 4. Cox proportional hazard regression analyses for all-cause death

	Univariate analysis		Multivariable analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.083 (1.062–1.104)	<0.001	1.070 (1.043–1.097)	<0.001
Female sex	2.399 (1.561–3.686)	<0.001	1.669 (1.061–2.625)	0.027
Body mass index	0.945 (0.882–1.013)	0.108		
Heart rate	1.030 (1.021–1.039)	<0.001	1.016 (1.003–1.029)	0.018
Systolic blood pressure	0.991 (0.982–1.001)	0.093		
Smoking	1.154 (0.408–1.899)	0.113		
Hypertension	1.129 (0.766–1.663)	0.541		
Dyslipidemia	1.220 (0.725–2.053)	0.453		
Diabetes	1.507 (1.093–1.921)	0.036		
Family history of CAD	1.007 (0.093–1.921)	0.136		
Anterior wall infarct	1.409 (0.940–2.112)	0.097		
Killip classification	2.047 (1.699–2.466)	<0.001		
Triglycerides	1.226 (0.520–1.932)	0.115		
LDL-C	0.978 (0.767–1.247)	0.859		
Blood glucose	1.068 (1.039–1.099)	<0.001		
Uric acid	1.004 (1.002–1.006)	<0.001		
eGFR	1.004 (1.001–1.007)	0.010		
LVEDVI	1.012 (0.999–1.024)	0.064		
LVEF	0.956 (0.939–0.973)	<0.001	0.968 (0.946–0.992)	0.008
Gensini score	1.011 (1.007–1.016)	<0.001	1.007 (1.002–1.013)	0.009
Homocysteine	1.026 (1.022–1.030)	<0.001	1.023 (1.018–1.028)	<0.001
GRACE risk score	1.040 (1.033–1.047)	<0.001	1.031 (1.024–1.039)	<0.001

CAD - coronary artery disease; CI - confidence interval; eGFR - estimated glomerular filtration rate; GRACE - Global Registry of Acute Coronary Events; HR - hazard ratio; LDL-C - low-density lipoprotein cholesterol; LVEDVI - left ventricular end-diastolic volume index; LVEF - left ventricular ejection fraction

Cox proportional hazard regression analyses were performed to identify the predictive factors for adverse clinical outcomes. Table 4 summarizes the results of univariate and multivariate Cox proportional hazard regression analyses for all-cause death and Table 5 for MACE in this cohort of patients. Univariate analysis showed that both the GRACE risk score [all-cause death: HR=1.040 (1.033–1.047), p<0.001; MACE: HR=1.012 (1.009–1.015), p<0.001] and homocysteine level [all-cause death: HR=1.026 (1.022–1.030), p<0.001; MACE: HR=1.023 (1.019–1.026), p<0.001] were associated with higher risks of all-cause death and MACE. After adjusting for potential confounding factors, such as age, sex, body mass index, heart rate, blood pressure, smoking, hypertension, dyslipidemia, diabetes, anterior wall infarct location, and so on, the GRACE risk score [all-cause death: HR=1.031 (1.024–1.039), p<0.001; MACE: HR=1.008 (1.005–1.011), p<0.001] and homocysteine level [all-cause death: HR=1.023 (1.018–1.028), p<0.001; MACE: HR=1.022 (1.018–1.025), p<0.001] remained significant predictors.

Correlation between homocysteine and GRACE risk score

The correlation between homocysteine and clinical variables was analyzed by Spearman's rank correlation test, and the results showed that the homocysteine level was significantly positively correlated with the GRACE risk score (r=0.134, p<0.001) as well as age (r=0.148, p<0.001), Gensini score (r=0.089, p=0.003). Figure 4 illustrates these correlations.

Combination of GRACE score with homocysteine in predicting clinical outcomes

ROC analysis was performed to assess whether a combination of the GRACE risk score and homocysteine level could better predict the adverse clinical outcomes. As shown in Figure 5, AUC significantly increased when the GRACE risk score was coupled with the homocysteine level (all-cause death: AUC=0.786 vs. 0.884, 95% CI=0.067–0.128, Z=6.307, p<0.001; MACE: AUC=0.678 vs. 0.759, 95% CI=0.055–0.108, Z=5.943, p<0.001). More importantly, the inclusion of homocysteine into the GRACE model was associated with an NRI of 57.5%

Table 5. Cox proportional hazard regression analyses for major adverse cardiovascular events

	Univariate analysis		Multivariable analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.043 (1.032–1.055)	<0.001	1.023 (1.009–1.037)	0.001
Female sex	1.616 (1.199–2.177)	0.002		
Body mass index	0.992 (0.950–1.034)	0.695		
Heart rate	1.020 (1.013–1.026)	<0.001	1.015 (1.008–1.021)	<0.001
Systolic blood pressure	0.998 (0.992–1.004)	0.587		
Smoking	1.284 (0.590–1.977)	0.132		
Hypertension	1.376 (1.084–1.746)		0.009	
Dyslipidemia	1.267 (0.951–1.688)	0.106	1.418 (1.062–1.893)	0.018
Diabetes	0.951 (0.672–1.345)	0.775		
Family history of CAD	0.670 (0.410–1.096)	0.111		
Anterior wall infarct	1.092 (0.858–1.391)	0.475		
Killip classification	1.673 (1.470–1.905)	<0.001		
Triglycerides	0.889 (0.768–1.029)	0.115		
LDL-C	1.031 (0.892–1.190)	0.683		
Blood glucose	1.052 (1.028–1.076)	<0.001		
Uric acid	1.003 (1.002–1.004)	<0.001		
eGFR	1.002 (1.000–1.005)	0.073		
LVEDVI	1.009 (1.001–1.017)	0.027		
LVEF	0.979 (0.969–0.990)	<0.001		
Gensini score	1.009 (1.007–1.012)	<0.001		
Homocysteine	1.023 (1.019–1.026)	<0.001	1.022 (1.018–1.025)	<0.001
GRACE risk score	1.012 (1.009–1.015)	<0.001	1.008 (1.005–1.011)	<0.001

CAD - coronary artery disease; CI - confidence interval; eGFR - estimated glomerular filtration rate; GRACE - Global Registry of Acute Coronary Events; HR - hazard ratio; LDL-C - low-density lipoprotein cholesterol; LVEDVI - left ventricular end-diastolic volume index; LVEF - left ventricular ejection fraction

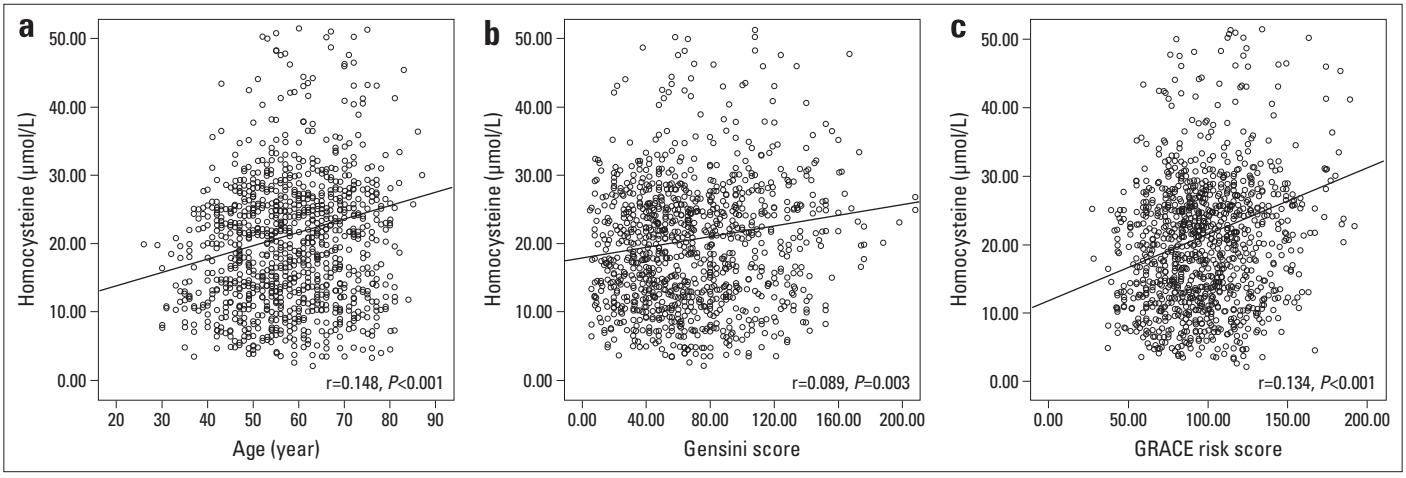
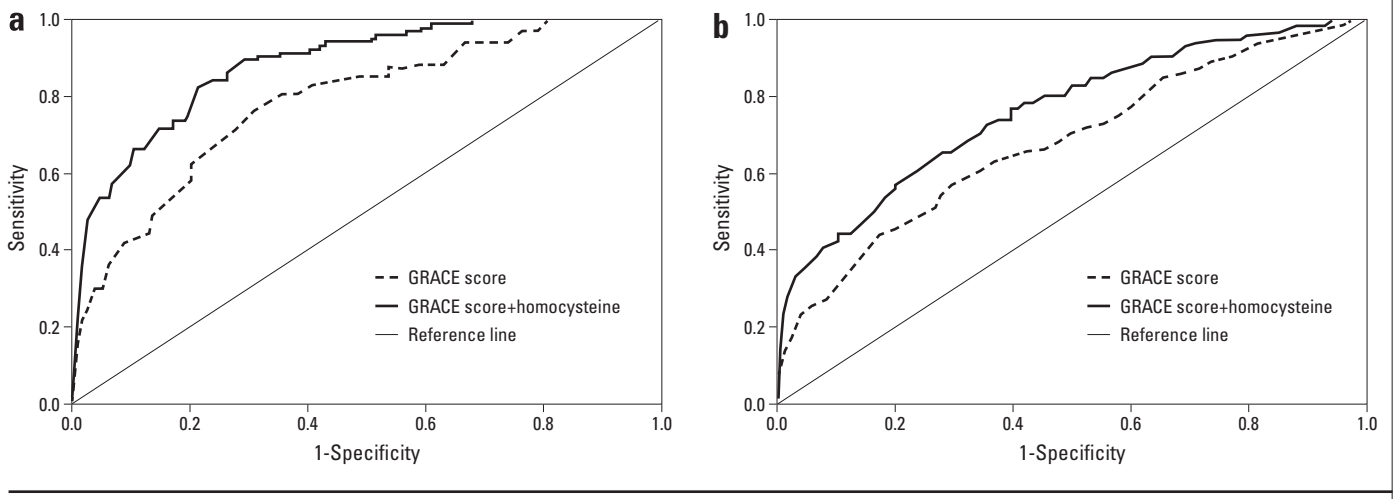


Figure 4. Spearman's rank correlation analysis. Homocysteine level was significantly positively correlated with age (a), the Gensini score (b), and the GRACE risk score (c)

(p<0.001) for all-cause death and 62.1% (p=0.008) for MACE, indicating effective reclassification. IDI again showed that the model diagnostic performance was significantly improved by the addition of homocysteine to the GRACE system (all-cause death: IDI=0.083, p<0.001; MACE: IDI=0.130, p=0.016). Thus, it indicated that the combination of the GRACE risk score and homocysteine level developed a more predominant prediction for clinical outcomes in patients with STEMI.



All-cause death	GRACE score vs. GRACE score+homocysteine
The area under ROC (AUC)	0.786 vs. 0.884 (Z=6.307, *P*<0.001)
Hosmere Lemeshow	
GRACE risk score	Chi-square=7.417, *P*=0.492
GRACE risk score+homocysteine	Chi-square=4.539, *P*=0.806
Net reclassification improvement	0.575 (*P*<0.001)
Integrated discrimination improvement	0.083 (*P*<0.001)

MACE	GRACE score vs. GRACE score+homocysteine
The area under ROC (AUC)	0.678 vs. 0.759 (Z=5.943, *P*<0.001)
Hosmere Lemeshow Chi-square	
GRACE risk score	Chi-square=12.643, *P*=0.125
GRACE risk score+homocysteine	Chi-square=4.974, *P*=0.760
Net reclassification improvement	0.621 (*P*=0.008)
Integrated discrimination improvement	0.130 (*P*=0.016)

Figure 5. ROC curve analysis. The addition of homocysteine improved the predictive power of the GRACE risk scoring system for all-cause death (a) and major adverse cardiovascular events (b)

Discussion

In the present study, we evaluated the predictive power of homocysteine level and the GRACE risk score, alone and in combination, in a cohort of patients with STEMI. The GRACE risk score is a widely recommended means to identify patients at higher risk for adverse outcomes in ACS. Homocysteine is a biomarker, which has been identified as a risk factor and predictor for cardiovascular diseases. Our hypothesis was that the predictive power of the GRACE scoring in STEMI could be enhanced by the addition of homocysteine level. In our study, we found that increased homocysteine levels are significantly associated with increased risks of all-cause death and MACE, verifying that homocysteine can serve as an independent predictor for adverse events in STEMI. The GRACE risk score and homocysteine level are positively correlated, indicating that the increase of one is always accompanied by an increase of the other. When the two predictors are jointly used to assess the clinical outcomes, the area under the ROC curve is significantly increased. The calibration, discriminatory capacity, and reclassification of the GRACE scoring are improved significantly when the homocysteine level is considered. Our data suggest that measurement of homocysteine level on admission may greatly enhance the predictive power of the GRACE risk score for cardiovascular events in patients with STEMI.

Risk stratification is an important part of the comprehensive management and treatment of patients following STEMI. Several models have been developed to execute risk stratification, such as the Thrombolysis in Myocardial Infarction trial, Platelet Glycoprotein IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy (PURSUIT) trial, and GRACE risk scoring system (16). The GRACE model is currently the most robust clinical risk stratification tool (4, 5). However, there is still room for improvement in its ability to discriminate clinical outcomes (17). Some biomarkers may provide additional information of pathophysiology in STEMI, but could not be considered in the GRACE model (16, 17).

Homocysteine is an intermediate metabolite of methionine and is a toxic amino acid containing a mercapto group (18, 19). Homocysteine has three metabolic pathways. In the first pathway, homocysteine can be catalyzed by the vitamin B6-dependent cystathionine beta synthetase, which is converted into cysteine by the transulfate pathway. In the second pathway, homocysteine can be methylated by betaine homocysteine methyltransferase to methionine. Lastly, homocysteine can be catalyzed by methionine synthase into methionine. Thus, the leading cause of elevated serum homocysteine levels may be the folate deficiency and/or the deficiency or gene mutations of key enzymes in homocysteine or folic acid metabolic pathways (18, 19). The homocysteine level may vary depending on age, diet, and genetic background (8). Homocysteine has been under a lot of speculation since its discovery in 1932 and has received increasing attention in recent years. An extraordinary number of epidemiological studies have found that an elevation of serum homocysteine is prevalent in patients with stroke, MI, peripheral vascular disease, and venous thrombosis (8). Many clinical studies have identified homocysteine as a significant and independent risk factor for cardiovascular diseases (20, 21). Moreover,

a significant association between hyperhomocysteinemia and cardiovascular events has been indicated in several large-scale prospective studies (9, 10, 22, 23). In addition, it has been reported that the Chinese population has a higher serum homocysteine level compared with the western populations (24). In our study, the average level of serum homocysteine in the Chinese patients with STEMI was 21.30±14.87 μmol/L. The baseline homocysteine concentrations were higher in patients with MACE than in those without MACE. The cumulative risk of adverse cardiovascular events increased with an increase in homocysteine level.

Atherosclerosis is the most common pathological process that leads to stroke, MI, heart failure, and claudication (25). Since McCully et al. (26) proposed that homocysteine could induce atherosclerosis in 1969, homocysteine has been widely studied. Now hyperhomocysteinemia is considered as an independent risk factor for atherosclerotic vascular diseases (6, 27). Some animal experiments showed that apoE-null mice fed with hyperhomocysteinemic diets developed atherosclerotic lesions in the aorta that were of significantly greater size and complexity compared with that of those developed in mice fed with control diets (28, 29). Serum homocysteine level is found to be correlated with arterial stiffness (30), carotid intima-media thickness (31), and the severity of coronary artery disease (32). Elevated serum homocysteine level is associated with a higher risk of coronary artery disease in patients with chronic renal dysfunction (33).

However, the exact biological mechanisms of atherogenic effects of homocysteine remain unclear. Some of the presumed mechanisms include endothelial dysfunction, promoting proliferation of vascular smooth muscle cells (8), dysregulating cholesterol and triglyceride metabolism, increasing the oxidative modification of LDL (34), activating inflammatory responses (28), oxidative damage, inhibiting endothelial nitric oxide synthase (eNOS) (35), enhancing synthesis of collagen and deterioration of arterial wall elastic material (36, 37), and augmenting thrombus formation (38). Our study demonstrates a positive correlation between homocysteine level and the Gensini score, meaning the higher homocysteine, the more severe coronary artery disease. This may be one of the reasons that homocysteine is correlated with adverse clinical outcomes.

Biomarkers, such as N-terminal pro–B-type natriuretic peptide (NT-proBNP) (39), C-reactive protein (CRP) (40), growth differentiation factor 15 (GDF-15) (41), cystatin C (CysC) (42), mean platelet volume (MPV) (43), neutrophil count (44), and red blood cell fatty acid (45), may enhance risk assessment beyond the GRACE risk scoring system as they reflect additional mechanisms. Our study demonstrates that the GRACE risk score and homocysteine concentration at baseline are significantly positively correlated. Either of them can independently predict the clinical outcomes, but their combination generates a stronger predictive power for cardiovascular events in patients with STEMI. This will help physicians to identify high-risk patients more accurately.

Study limitations

This study has several limitations. Firstly, homocysteine level may be influenced by age, diet, and genetic background. Secondly, the subjects were limited exclusively to Chinese patients. Due to the differences in diet and genetic background, the results of this study should be drawn cautiously to other ethnic populations. Lastly, the patients included in this study were from only two hospitals in the same area. Our findings need to be further proved by large multicenter research.

Conclusion

In conclusion, our study confirms that either the GRACE risk score or the homocysteine level can independently predict adverse cardiovascular events. The two predictors are positively correlated. Using them in combination derives a more robust predictive power for clinical outcomes in patients with STEMI.

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