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Combined value of white blood cell and total bilirubin on predicting MACE in STEMI patients treated with primary PCI

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Short title: White blood cell and total bilirubin predicting MACE in STEMI

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ABSTRACT:

Objectives: A combined equation based on white blood cell count (WBC) and total bilirubin (TB) was assessed for its ability to predict adverse clinical outcomes in acute ST-segment elevation myocardial infarction (STEMI) patients with primary percutaneous coronary intervention (PCI).

Design: A single-center, prospective cohort study.

Setting: The First Affiliated Hospital of Xinjiang Medical University.

Method: A total of 615 STEMI patients who received primary PCI were enrolled. WBC and TB were collected at admission. Logistic regression was used to determine the value of combined equation on the prognosis of in-hospital MACE. The primary endpoints were in-hospital mortality and major adverse cardiovascular events (MACE) during hospitalization and 36-months follow-up period.

Result: 77 patients occurred in MACE during hospitalization (17 in-hospital mortality). Patients in MACE group had significantly higher WBC (13.01±4.11 vs 10.31±2.85*10^9/L, P<0.001) and TB (17.21±6.49 vs 13.34±4.91umol/L, P<0.001) than the patients with non-MACE. When WBC and TB were taken as independent variables to make a category of logistic regression analysis of in-hospital MACE, the logistic regression model was: Logit (P) =-8.00+0.265 WBC+0.077 TB, the combination of WBC and TB were more valuable on evaluating the in-hospital MACE (AUC=0.804, 95% CI: 0.678-0.929, P<0.001), which showed significant differences (P<0.001). Multivarial logistic regression analysis showed that combined detection was an independent risk factor for in-hospital MACE (OR=5.85, 95% CI 3.425-9.990, P=0.032). Kaplan-Meier survival analysis showed that there was no correlation between the combined model and the long-term prognosis of STEMI patients (P=0.869).

Conclusion:The combination of WBC and TB may be an independent predictor for in-hospital outcomes in patients with STEMI than single detection.

Keywords: ST segment elevation myocardial infarction; White blood cell; Total bilirubin; in-hospital mortality; Major adverse cardiovascular events

Strengths and Limitations of the study

- This was the first study to indicate the combined effect of WBC and TB on predicting clinical prognosis in acute STEMI patients undergoing primary PCI.
- The combination of WBC and TB may be better than single biomarker, can be applied in the predicting in-hospital MACE of STEMI patients treated with primary PCI.
- A single-center prospective cohort study for a small number of patients was fail to adequately exclude the influence of unknown confounding factors on this study.
- The determination of bilirubin in patients with MI was only at a preoperative time point, and the dynamic changes of the bilirubin concentrations during hospitalization and follow-up were not clear.

INTRODUCTION

STEMI has been a hotspot in cardiology research with its urgent onset, rapid progress and high mortality. Although reperfusion therapy such as percutaneous coronary intervention (PCI) and fibrinolytic therapy had improved the survival rate of acute ST-segment elevation myocardial infarction (STEMI), the in-hospital mortality rate of STEMI is as high as 4%-12%.[1] It has always been a new challenge for clinician to identify high-risk patients in time, evaluate clinical prognosis accurately and prevent the occurrence of major adverse cardiovascular events (MACE). Screening a sensitive and specific biological index is of great significance for the treatment and prevention of STEMI complications.

In an attempt to identify STEMI patients at high risk of unfavorable outcomes, several predictors of adverse events in STEMI have been investigated. Research indicates that older age,[2] BNP,[3] D-dimer,[4] uric acid[5] and thrombolysis myocardial infarction (TIMI) risk score[6] are associated with a higher risk of adverse events in STEMI patients. However, there is no literature report on the combination of white blood cell count (WBC) and total bilirubin (TB) in the guiding significance for the prognosis of STEMI.

STEMI refers to the occurrence of plaque rupture, thrombosis or coronary artery spasm on the basis of coronary atherosclerosis, which results in a sharp decrease or interruption of coronary artery blood supply and a sustained and severe acute ischemia of the corresponding myocardium, leading to acute myocardial necrosis. Studies have shown that there are obvious inflammatory reactions and oxidative stress injury in STEMI.[7-8] Leukocyte elevation after STEMI is an important component of systemic inflammatory response and ischemic tissue repair mechanism. ischemia-induced chemokines activate white blood cells to chemotaxis to ischemic sites and remove necrotic tissues, white blood cells adhere to the injured vascular wall and form aggregates with blood cells, which eventually lead to thrombosis.[9] Activated white blood cells also produce oxygen free radicals, lysosomal enzymes and other substances, which cause local inflammatory response in ischemic sites.[10] White blood cell, as a marker of inflammation, has been proved to be closely related to the clinical prognosis of STEMI patients,[11] while bilirubin the end product of heme degradation, is also an endogenous oxidant in vivo, participates in the occurrence and development of myocardial infarction.[12] Heme oxygenase (HO) regulates the synthesis and catabolism of bilirubin, and keeps

the bilirubin content in a dynamic balance in human body.[13] HO-1 is an inducible isoform in response to diverse cellular stress such as oxidative stress, Inflammatory cytokines, heavy metals, cytokines, but is not expressed under normal conditions. Acute myocardial ischemia and hypoxia activates the stress process of the body and produces oxygen free radicals and oxides, infarction-related inflammatory factors, which significantly increase the activity of HO-1, and eventually leads to an increase in bilirubin. Studies has been suggested that bilirubin is elevated in patients with STEMI and has the effect of antioxidant stress, can be used as a biomarker for predicting clinical prognosis.[14]

Thus, there is a strong correlation between inflammatory response and oxidative stress, it's interact and influence each other to promote the development of STEMI. White blood cell and bilirubin, common and fast acquired biomarkers in routine blood tests, could reflect the level of inflammation and oxidative stress injury, respectively and that combined information from multiple inflammatory and oxidative stress injury might be more informative. Therefore, the present study firstly combined WBC (an inflammatory biomarker) and TB (oxidative stress biomarker) into a simplified equation and assessed whether this value was predictive of in-hospital MACE in patients with STEMI in order to find a simple and reliable auxiliary index to evaluate and predict the clinical prognosis of STEMI for early treatment.

METHODS

Study design

This was a single-center, prospective cohort study designed to assess whether admission WBC and TB could predict in-hospital and long-term outcomes in patients with STEMI treated with primary PCI. The study complied with the Declaration of Helsinki, and the study protocol was approved by the Human Ethical Committee of the First Affiliated Hospital of Xinjiang Medical University. All participants in this study were provided with written informed consent.

We enrolled consecutive patients with STEMI who treated with primary PCI at the Cardiac Center of the First Affiliated Hospital of Xinjiang Medical University between November 2011 and June 2017. STEMI diagnosis was in accordance with previously established guidelines [1]. All patients were treated with PCI within 12 hours after symptoms. Patients who did not have pre-PCI, bilirubin data, who had severe liver and kidney diseases, autoimmune system diseases, severe heart valve diseases, chronic inflammatory diseases, acute infectious diseases, malignant tumors, blood system diseases, active hemorrhage and other diseases, additionally, patients who had an infarct-related lesion unsuitable for stent implantation, and who were lost to follow-up were excluded from the current study.

Data collection

Investigators trained by professionals used uniformly designed questionnaires to collect patients' general information including age, gender, body mass index (BMI), history of smoking, dyslipidemia, hypertension, diabetes, laboratory examination results, angiographic examination results, in-hospital medication and the occurrence of MACE in our hospital by electronic medical records and paper cases.

Blood sampling and hematology measurements

Blood samples were collected from all participants immediately upon hospital admission for complete blood count analyses. In all patients, venous blood samples for laboratory analysis were collected at the time of presentation before the patients were transferred to the heart center. Peripheral blood samples were used for laboratory tests of several markers, including white blood cells, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein

cholesterol, total bilirubin, direct bilirubin, indirect bilirubin, creatine kinase isoenzyme, platelet, hemoglobin, creatinine and uric acid. The automatic blood analysis equipment (beckman LH750/DXC800 automatic blood analyzer) was used for the hematological analysis in our hospital testing center. The normal range for white blood cell is $(4\text{-}10)\times10^9$/L, total bilirubin is 5.5-27.5 umol/L.

All patients were assessed by transthoracic echocardiography within 48 h after primary PCI using Vivid 7 ultrasound system (GE Medical Systems, USA). Standard echocardiographic views were acquired and analyzed by two experienced cardiologists who were unware of grouping information. Left ventricular ejection fraction (LVEF) was recorded after the examination

Definition of cardiovascular risk factors

Hypertension was defined as self-reported use of antihypertensive medication within the past 2 week or having systolic blood pressure greater than 140 mmHg and/or diastolic blood pressure greater than 90mmHg. Diabetes was defined as fasting blood glucose of more than 7 mmol/L (or 2 hours after a meal), and/or, using of insulin or oral hypoglycemic agents, or a self-reported history of diabetes. Hyperlipidemia was defined as anyone of the four lipids abnormalities following or self-reported use of antihyperlipidemic medication, total cholesterol concentration>6.22mmol/L (240mg/dl), triglyceride concentration>2.26mmol/L (200mg/dl), low-density lipoprotein cholesterol concentration>4.14mmol/L (160mg/dl), high-density lipoprotein cholesterol concentration<1.04 mmol/L (40mg/dl).[15] GRACE score of patients was calculated using eight indicators of GRACE admission risk score according to the medical history, signs and laboratory examination results of admission,including Killip classification of cardiac function, age, heart rate, arterial systolic pressure, serum creatinine, ST segment changes of electrocardiogram, elevated myocardial markers, cardiac arrest at admission. Positive smoking history was defined as having smoked daily or occasionally in the past.

PCI and Medication

Patients were given loaded medication before operation: baiaspirin+clopidogrel (300 mg+300 mg) orally and secondary clinical prophylactic medication after PCI. Primary PCI was performed by two senior doctors in the Cardiac Center of the First Affiliated Hospital of Xinjiang Medical University.

At the same time, the results of angiography were recorded for each patient. The main vesssels were left main coronary artery, left anterior descending branch, left circumflex branch and right coronary descending branch. The degree of coronary artery stenosis was assessed by AXIOM Artis FC/BC cardiovascular computer quantitative analysis system in Germany. Cross-sectional projections of right and left coronary arteries were recorded. Most of the approaches were via radial artery, and a few were via femoral artery. There are at least 4 projection positions for the left coronary artery and at least 2 projection positions for the right coronary artery. If necessary, other postures are added to fully display each segment of the coronary artery. The imaging data of each patient undergoing coronal angiography are kept for reference. After angiography, more than two experienced interventional physicians including the surgeon discussed the results and filled in the detailed report of the results of coronary angiography after reaching consensus. The number of stenosis branches is defined as a single vessel>70% or two vessels more than 50%. PCI was performed by a professional cardiologist, and the stent implantation site, balloon type, pressure, stent size and type, TIMI blood flow classification were filled in detail after the operation.

Successful coronary angiography is defined as defined as residual stenosis<50% and thrombolysis in myocardial Infarction grade 3 flow after the procedure.

Clinical Outcomes and Definitions

The end point of this study was the occurrence of MACE. Follow-up was performed by telephone interview for all patients enrolled from November 2011 to July 2017. For those patients who reached at least one of the primary endpoints, recorded data and medical reports were evaluated to determine inclusion criteria. Follow-up via telephone was complete in 599 patients or family members. In-hospital MACE was a composite of cardiogenic death, cardiogenic shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction, etc.

Statistical Analysis

Patients were divided into two groups according to the occurrence of MACE in hospital or not.Based on EpiData 3.0 contact database and SPSS 22.0 software, statistical analysis was carried out. Counting data can be expressed by frequency or percentage (constituent ratio). If the data is normal distribution measurement data, it can be expressed in the form of mean±standard deviation. If

the analysis data do not conform to normal distribution measurement data, it can be analyzed by median method and quartile method. The comparison between two groups can be carried out by t (t') test of independent samples. If there are more than three groups and the data conform to normal distribution, F test is used. If the data do not conform to normal distribution, rank sum test is used. The comparison between groups can also be evaluated by X^2 test. Receiver operating characteristic (ROC) curve was used to evaluate WBC and TB of patients to predict the best clinical boundary of in-hospital mortality. Optional Logistic regression was used to analyze the influence of multiple factors. The ratio of risk degree of factors was expressed by odds ratio (OR) and confidence interval was expressed by confidence interval (CI). In the course of this study, all statistical tests were bilateral tests, and the significance level was 0.05.

Patients and public involvement

The study designed to assess whether admission WBC and TB could predict in-hospital and long-term outcomes in patients with STEMI treated with primary PCI. However, no patients or members of the public were included in the design, recruitment or conduct of the study. The results of measurements would be disseminated to participants after the study which was completed by the study team. The burden of the intervention will not be assessed by patients themselves.

RESULTS

Baseline clinical characteristics

A total of 615 STEMI patients (525 males and 90 female) with an average age of 58 years were included in this study. In-hospital MACE was found in 77 patients, including 17 patients with in-hospital mortality. The comparison of the MACE group and non-MACE group are summarized in ***Table 1,*** including 17 patients with in-hospital mortality (*Suppl Table S1)*

The patients of MACE group were older than that of non-MACE group (61.2±11.8 vs 57.8±12.2, *P*=0.019). The LVEF in MACE group was worse than that of non-MACE group (55.48±7.82 vs 58.52±6.58, *P*<0.001). The laboratory data of the patients showed that the WBC and TB was (13.01±4.11)×10^9/L and 17.21±6.49 mmol/L in the MACE group, and (10.31±2.85)×10^9/L and 13.34±4.91 mmol/L in the non-MACE group, respectively (*P*<0.001). Patients in MACE group also had significantly greater levels of uric acid (6.67±2.40 vs 5.27±1.70 umol/L, *P*<0.001), creatinine (83.95±24.38 vs 75.46±18.79 umol/L, *P*=0.004) and greater number of culprit vessels (3.21±1.25 vs 2.80±1.27, *P*=0.009). No significant differences were found between the MACE group and non-MACE group in terms of gender, BMI, smoking, hypertension, diabetes, hyperlipidemia, SBP, DBP, heart rate, GRACE score, useless of medication, direct bilirubin, indirect bilirubin, total cholesterol, triglycerides, HDL-C, LDL-C, CK-MB, platelet, hemoglobin and culprit vessels (*Table 2*).

Ability of WBC and TB to predict MACE

ROC curve was used to determine the predictive value of WBC and TB for the occurrence of in-hospital mortality in STEMI patients after primary PCI. The optimal cut-off point of WBC was 12.2×10^9/L with sensitivity 71%, and specificity 73.1% (AUC=0.765 95%CI:0.625-0.904), the best cut-off value of TB was 14.4 mmol/L with sensitivity 62.0% and specificity 75.1% (AUC=0.751 95%CI:0.660-0.842). Binomial logistic regression analysis was performed with in-hospital mortality as dependent variable and WBC and TB as independent variables, the logistic regression model was: Logit (P) =-8.00+0.265 WBC+0.077 TB ***(Table 3)***. The combination of WBC and TB were more valuable (AUC=0.804, 95%CI:0.678-0.929, *P*<0.001). The area under the curves of WBC, TB and logistic regression combined detection was 0.765, 0.751 and 0.804 separately, which showed

significant differences (P<0.001), the 95%CI of the combined detection was (0.678, 0.929), the sensitivity of logistic regression combined detection was 82.4%, the specificity was 64.7% (Table 2, ***Figure 1***). All patients were followed up for an average of 30.22 months, 172 patients occurred in MACE with the incidence of out-hospital MACE was 28.7%.

Logistic regression model was used to draw the ROC curve of in-hospital MACE, and Kaplan-Meier survival analysis of out-hospital MACE events was made by dividing the best truncation value into two groups. The results showed that there was no significant difference in the long-term incidence of MACE between the two groups (P=0.869, ***Figure 2***)

Multivariate Logistic Regression Analysis of in-hospital MACE

The independent risk factors affecting MACE in hospital were analyzed by logistic regression analysis. After adjusting for multiple clinical prognostic factors, including age, gender, smoking, hypertension, diabetes, heart rate, low-density lipoprotein cholesterol, creatine kinase isoenzyme, LVEF, culprit vessels, a significant association was noted between combined detection and the adjusted risk of in-hospital MACE (OR=5.85,95%CI 3.425-9.990, P=0.000). The results showed that the high value of equation is a independent predictor of worse in-hospital MACE of STEMI patients treated with primary PCI. Other independent predictors of in-hospital MACE were LVEF (OR=0.960, 95%CI:0.928-0.990, P=0.010) and number of culprit vessels (OR=1.25, 95%CI 1.019-1.529, P=0.032) ***(Table 4)***.

DISCUSSION

The current study marks is the first to indicate that the combined effect of WBC and TB could better predict prognosis (in terms of in-hospital mortality and in hospital MACE) in acute STEMI patients undergoing PCI compared to single predictor independently. Our studies suggesting that those with higher WBC and TB had a higher likelihood of in-hospital complications, and a higher hazard of in-hospital mortality, independent of potential confounding factors.

Inflammatory processes play an important role in the development of STEMI. It has been suggested that there is a positive correlation between leucocyte elevation and myocardial infarction area.[16-18] Kyne suggested that the increase of WBC count and neutrophilic granulocyte percentage in peripheral blood within12 hours was an independent predictor of heart failure in the first four days after acute myocardial infarction (AMI).[19] Kojima demonstrated an elevated WBC was significantly associated with higher risk of in-hospital mortality.[20] Nez reported that white blood cell count independently increased the risk of death for 30 days and 1 year in STEMI patients.[21] Çiçek G[22] examined that the incidence of MACE in STEMI patients with elevated white blood cell count increased significantly during hospitalization. This means that leucocyte elevation during myocardial infarction not only has a purely restorative physiological role, but also has a pathological role. Combined with myocardial infarction size, no coronary reflux and decreased cardiac function, increased white blood cells in STEMI patients may indicate poor clinical prognosis.

In addition to the inflammatory, oxidative stress injury plays an important role in the evolution of STEMI.[23] Okuhara showed that compared with non-AMI patients, the serum bilirubin concentration and Fe^2 in AMI patients increased temporarily 18-21 hours after the onset of the disease.[12] Elevated bilirubin has antioxidant capacity and the ability to remove peroxides, which can prevent the deterioration of disease, Sexual resistance to oxidative stress in the myocardium may be considered as a beneficial self-regulation in acute ischemia. Celik reported that total bilirubin levels in STEMI were independently associated with no-reflow coronary artery.[24] Halit reported that bilirubin levels in STEMI patients with impaired blood flow were higher than those in normal blood flow group. They suggested that the more severe the atherosclerosis, the higher the activity of HO-1 enzyme after myocardial infarction, the more obvious the increase of bilirubin levels.[25] The degree of increase was related to the severity of the lesion to a certain extent. Glu studied 1624 STEMI

patients and found that compared with the bilirubin group less than 9.0 mg/dl, the bilirubin group higher than 9.0 mg/dl had higher in-hospital mortality, but the bilirubin level was not related to long-term prognosis.[26] Chung followed up 1111 STEMI patients treated with PCI in hospital and 12 months after operation. The results showed that the incidence of MACE and cardiogenic mortality in hyperbilirubinemia group were higher (14.2% vs 4.2%, P<0.001) than those in low bilirubinemia group (13.9% vs 3.9%, P<0.001).[14] It can be concluded that the oxygen free radicals produced by oxidative stress injury and the damage of various oxides to the body under stress may exceed the protective and antioxidant effect of bilirubin on the body, which caused the protective effect of high bilirubin level is not significant. In contrast to stable coronary disease, the serum TB levels show different associations in stressful conditions.

Therefore, STEMI is a disease both with inflammatory and oxidative stress injury. As such, inflammatory biomarkers alone, or oxidative stress are not sufficient to capture the entire pathophysiologic process involved in STEMI. Instead, markers which combine inflammatory and oxidative stress information may be better at reflecting the process in STEMI. In present study, the area under the ROC curve for combined equation in predicting mortality was higher than those for WBC and TB, multivariate logistic regression analysis showed the high value of equation is a independent predicted of worse in-hospital MACE, which suggests that combining WBC and TB has a stronger predictive power for in-hospital MACE than individual markers, was of particular clinical importance for the subset of patients with STEMI at admission.

CONCLUSIONS

The combination of WBC and TB may be better than single biomarker, can be applied in the predicting in-hospital MACE of STEMI patients treated with primary PCI. the high value of equation is a independent predictor of worse in-hospital MACE, it can be used to identify high-risk STEMI patients and accurately predict their clinical prognosis for early treatment.

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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Table 1. Comparison between MACE group and non-MACE group.

Variables	Non-MACE (n=538)	MACE (n=77)	t/χ^2	*P*
Gender (men) (n,%)	465 (86.4)	63 (81.8)	1.180	0.277
Age (years)*	57.8±12.2	61.2±11.8	2.359	0.019
BMI (kg/m^2)	25.45±3.29	24.99±2.90	1.161	0.246
Smoking history (n,%)	302 (56.1)	47 (61.0）	0.660	0.416
Hypertension (n,%)	264 (49.1)	40 (51.9)	0.223	0.637
Diabetes (n,%)	115 (21.4)	13 (16.9)	0.825	0.364
Hyperlipemia (n,%)	396 (73.6)	61 (79.2)	1.112	0.292
SBP (mmHg)	121.88±19.08	118.75±18.45	1.348	0.178
DBP (mmHg)	76.58±13.13	75.18±13.33	0.870	0.385
Heart rate (/min)	81.76±13.64	84.69±17.93	1.686	0.092
LVEF (%)*	58.52±6.58	55.48±7.82	3.703	<0.001
TB (umol/L)*	13.34±4.91	17.21±6.49	5.040	<0.001
DBL (umol/L)	2.19±1.60	2.18±1.41	0.100	0.920
IBL (umol/L)	11.97±5.11	11.36±4.77	0.986	0.325
TC (mmol/L)	4.4±1.18	4.38±1.18	0.193	0.847
TG (mmol/L)	2.05±1.40	1.93±1.33	0.734	0.463
LDL (mmol/L)	2.86±0.85	2.8±0.84	0.544	0.587
HDL (mmol/L)	1.03±0.35	1.03±0.31	0.004	0.997
CK-MB (U/L)	277.73±221	306.14±204.18	1.065	0.287
WBC ($\times10^9$ /L)*	10.31±2.85	13.01±4.11	5.569	<0.001
Hb (g/L)	145.24±19.96	142.47±23.72	1.114	0.266
PLT ($\times10^9$ /L)	222.25±59.41	221.6±66.85	0.089	0.929
UC (mmol/L)*	5.27±1.70	6.67±2.40	4.921	<0.001
CR (umol/L)*	75.46±18.79	83.95±24.38	2.934	0.004
GRACE	132.89±27.74	137.7±32.29	1.394	0.164

LM (n,%)	51(9.5)	4(5.2)	1.519	0.218
LAD (n,%)	463(86.1)	71(92.2)	2.227	0.136
LCX (n,%)	334(62.1)	53(68.8)	1.315	0.251
RAD (n,%)	372(69.1)	61(79.2)	3.282	0.070
Culprit Vessels	2.80±1.27	3.21±1.25	2.631	0.009
Medication				
Aspirin	519(96.5)	73(94.8)	0.518	0.472
Clopidogrel*	498(92.6)	64(83.1)	7.635	0.006
β blockers	441(82.0)	62(80.5)	0.095	0.758
ACEI	382(71.0)	55(71.4)	0.006	0.939
Statins*	505(93.9)	66(85.7)	6.739	0.009

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, Left ventricular ejection fraction; TB, total bilirubin; DBI, direct bilirubin; IBL, indirect bilirubin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes; WBC, white blood cells; Hb, hemoglobin; PLT, Platelet; UC, uric acid; CR, creatinine; GRACE, globla registry of acute coronary events; LM, left main coronary artery; LAD, left anterior descending branch ; LCX, left circumflex branch; RAD, right coronary descending branch; ACEI, angiotensin-converting enzyme inhibitor.

Table 2. Admission of TB, WBC and combine as predictors of in-hospital mortality

Variables	Sensitivity (%)	Specificity (%)	AUC (0 to 1.0)	95% CI	X^2	P vale
TB (≥14.4 mmol/L)	76.5	62.0	0.751	0.660-0.842		
WBC (≥12.2 10^9/L)	71.0	73.1	0.765	0.625-0.904	8.410	0.014
Combine	82.4	64.7	0.804	0.678-0.9286		

Abbreviations: TB, total bilirubin; WBC, white blood cells; AUC, area under the curve; CI, confidence interval;

Table 3. Logistic regression analysis of in-hospital MACE

Variables	*b*	*OR*	*95% CI*	*P*
TB	0.077	1.080	-0.005-0.159	0.067
WBC	0.265	1.303	0.125-0.405	<0.001
combine	-8.004	0.000		<0.001

Abbreviations: TB, total bilirubin; WBC, white blood cells; OR, odds ratio; CI, confidence interval;

Table 4. Univariate and multivariate analysis of in-hospital MACE

Variables	Univariate		Multivariate	
	OR (95%CI)	*P* Value	OR (95%CI)	*P* Value
Gender (male)	1.69(0.763-3.767)	0.195		
Age (year)	1.02(1.003-1.055)	0.025	1.03(1.002-1.049)	0.028
BMI	0.98(0.896-1.072)	0.660		
Smoking	1.71(0.933-3.141)	0.083		
Hypoglycemic	0.87(0.506-1.494)	0.614		
Diabetes	0.77(0.3841.542)	0.460		
Heart rate	1.01(0.993-1.027)	0.249		
LVEF	0.96(0.926-0.990)	0.011	0.96(0.928-0.990)	0.010
LDL-C	0.88(0.640-1.205)	0.422		
CK-MB	1.00(0.999-1.001)	0.166		
Culprit Vessels	1.29(1.045-1.601)	0.018	1.25(1.019-1.529)	0.032
Combine	5.55(3.207-9.609)	0.000	5.85(3.425-9.990)	<0.001

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, Body mass index; LVEF, Left ventricular ejection fraction; LDL-C, low-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes.

Figure Legends

Figure 1. Admission of TB, WBC and Combine as predictors of in-hospital mortality.

Figure 2. Kaplan–Meier survival analysis of long-term MACE.

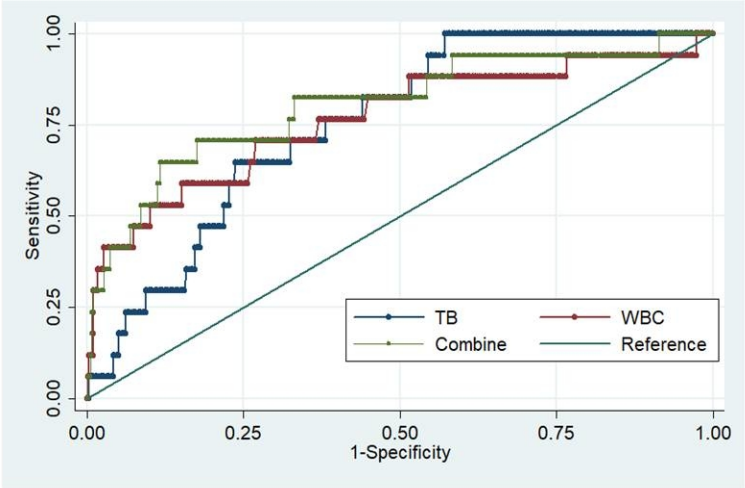

Figure 1. Admission of TB, WBC and Combine as predictors of in-hospital mortality.

275x190mm (96 x 96 DPI)

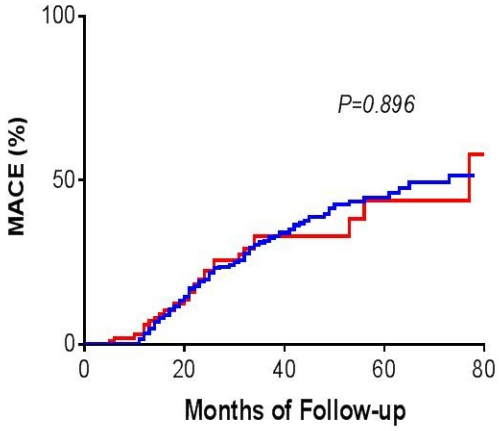

Figure 2. Kaplan–Meier survival analysis of long-term MACE.

275x190mm (96 x 96 DPI)

Supplementary materials

Combined value of white blood cell and total bilirubin on predicting MACE in STEMI patients treated with primary PCI

Munire-Tuxun, MD., Qian Zhao, PhD., Yang Xiang, MD., Fen Liu, PhD., Chun-Fang Shan, MD., Xin-Rong Zhou, MD., Ning Song, PhD., Ajiguli-Waisiding, MD., Xue-He, Zhang, MD., Gulandanmu-Aihemaiti, MD., Yi-Ning Yang, PhD., Xiao-Mei Li, PhD.

Supplementary Table S1 Comparison between in-hospital mortality group and survival group

Variables	Survival group(n=598)	Mortality group(n=17)	t/χ^2	*P*
Gender (men) (n,%)	516(86.3)	12(70.6)	3.550	0.067
Age (years)*	58.03±12.15	63.53±12.40	1.839	0.066
BMI (kg/m^2)	25.42±3.25	24.01±2.68	1.785	0.075
Smoking history (n,%)	338(56.5)	11(64.7)	0.451	0.502
Hypertension (n,%)	269(49.5)	8(47.1)	0.039	0.843
Diabetes (n,%)	125(20.9)	3(17.6)	0.106	0.744
Hyperlipemia (n,%)	443(74.1)	14(82.4)	0.593	0.441
SBP (mmHg)	121.59±18.89	119.76±23.65	0.378	0.706
DBP (mmHg)	76.46±13.08	74.18±15.91	0.707	0.480
Heart rate (/min)	81.90±13.88	90.06±23.31	2.333	0.020
LVEF(%)*	58.33±6.67	51.35±8.41	4.222	<0.001
TB (umol/L)*	13.69±5.22	18.47±5.59	3.718	<0.001
DBL (umol/L)	2.18±1.56	2.58±2.10	1.025	0.306
IBL (umol/L)	11.88±5.09	12.29±4.41	0.326	0.744
TC (mmol/L)	4.41±1.18	3.95±0.95	1.611	0.108
TG(mmol/L)	2.05±1.39	1.67±1.06	1.095	0.274
LDL (mmol/L)	2.86±0.85	2.51±0.90	1.686	0.092
HDL (mmol/L)	1.03±0.35	0.90±0.24	0.415	0.678
CKMB (U/L)	281.46±220.92	275.36±139.69	0.113	0.910
WBC (×10^9 /L)*	10.54±3.04	14.59±4.74	5.324	<0.001
Hb (g/L)	144.99±20.35	141.59±24.81	0.676	0.500
PLT (×10^9 /L)	222.58±60.33	207.59±60.52	1.010	0.313
UC (mmol/L)*	5.42±1.80	6.40±3.24	2.137	0.033
CR (umol/L)*	76.24±19.16	86.46±34.23	2.108	0.035
GRACE	133.06±28.01	148.71±36.61	2.250	0.025

LM (n,%)	54(9.0)	1(5.9)	0.201	0.54
LAD (n,%)	518(86.6)	16(94.1)	0.812	0.368
LCX (n,%)	375(62.7)	12(70.6)	0.440	0.507
RAD (n,%)	418(69.9)	15(88.2)	2.670	0.102
Culprit Vessels	2.84±1.27	3.41±1.42	1.840	0.066
Medication				
Aspirin	578(96.7)	14(82.4)	9.393	0.002
Clopidogrel*	553(92.5)	9(52.9)	32.806	0.000
β blockers	492(82.3)	11(64.7)	3.425	0.064
ACEI	429(71.7)	8(47.1)	4.896	0.027
Statins*	559(93.5)	12(70.6)	13.039	0.000

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, Left ventricular ejection fraction; TB, total bilirubin; DBI, direct bilirubin; IBL, indirect bilirubin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes; WBC, white blood cells; Hb, hemoglobin; PLT, Platelet; UC, uric acid; CR, creatinine; GRACE, globla registry of acute coronary events; LM, left main coronary artery; LAD, left anterior descending branch ; LCX, left circumflex branch; RAD, right coronary descending branch; ACEI, angiotensin-converting enzyme inhibitor.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Non
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6*
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	Non
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	8-9

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	11
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Non
		Cross-sectional study—Report numbers of outcome events or summary measures	Non
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11
		(*b*) Report category boundaries when continuous variables were categorized	Non
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The combined value of white blood cell and total bilirubin predict clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study

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The combined value of white blood cell and total bilirubin predict clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study

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Short title: White blood cell and total bilirubin predicting clinical outcomes in STEMI

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Words:4281 Tables: 4 Figures: 3

ABSTRACT:

Objectives: A combined equation based on white blood cell count (WBC) and total bilirubin (TB) was assessed for its ability to predict adverse clinical outcomes in acute ST-segment elevation myocardial infarction (STEMI) patients with primary percutaneous coronary intervention (PCI).

Design: A single-center, prospective cohort study.

Setting: The First Affiliated Hospital of Xinjiang Medical University.

Method: A total of 615 STEMI patients post primary PCI were enrolled. WBC and TB were collected at admission. Logistic regression was used to determine the combined equation. The primary endpoints were in-hospital mortality and major adverse cardiovascular events (MACE) during hospitalization and 36-months follow-up period.

Result: 77 patients occurred in MACE during hospitalization (17 in-hospital mortality). WBC and TB were taken as an independent variables to make a category of logistic regression analysis of in-hospital MACE, the logistic regression model was: Logit (P) =-8.00+0.265 WBC+0.077 TB, the combination of WBC and TB were more valuable on evaluating the in-hospital mortality (AUC=0.804, 95% CI: 0.678~0.929, P<0.001). Multivariate logistic regression analysis showed that combined detection was an independent risk factor for in-hospital MACE (OR=5.85, 95% CI 3.425~9.990, P=0.032). During the follow-up period, 172 patients (29.5%) developed MACE. But the combined detection didn't predict the long-term clinincal outcome.

Conclusion:The combination of WBC and TB may be an independent predictor for in-hospital outcomes in patients with STEMI than single detection.

Keywords: ST segment elevation myocardial infarction; white blood cell; total bilirubin; in-hospital mortality; major adverse cardiovascular events

1

2

Strengths and Limitations of the study

- This was the first study to indicate the combined effect of WBC and TB on predicting clinical prognosis in acute STEMI patients post primary PCI.
- The combination of WBC and TB may be better than single biomarker, can be applied in the predicting in-hospital MACE of STEMI patients treated with PCI.
- A single-center prospective cohort study for a small number of patients was fail to adequately exclude the influence of unknown confounding factors on this study.
- The determination of bilirubin in patients with STEMI was only at a preoperative time point, and the dynamic changes of the bilirubin concentrations during hospitalization and follow-up were not clear.

INTRODUCTION

STEMI has been a hot spot in cardiology research with its urgent onset, rapid progress and high mortality. Although reperfusion therapy such as percutaneous coronary intervention (PCI) and fibrinolytic therapy had improved the survival rate for acute ST-segment elevation myocardial infarction (STEMI), the in-hospital mortality rate of STEMI is as high as 4%-12%.[1] It has been a new challenge for clinician to identify high-risk patients timely, evaluate clinical prognosis accurately and prevent the occurrence of major adverse cardiovascular events (MACE). Screening a sensitive and specific biological index has great value for the treatment and prevention of STEMI complications.

In an attempt to identify STEMI patients at high risk of unfavorable outcomes, several predictors of adverse events in STEMI have been investigated. Research indicates that older age,[2] BNP, D-dimer, uric acid and thrombolysis myocardial infarction (TIMI) risk score are associated with a higher risk of adverse events in STEMI patients.[3-6] However, there is no literature report on the combination of white blood cell count (WBC) and total bilirubin (TB) in the guiding significance for the prognosis of STEMI.

STEMI refers to the occurrence of plaque rupture, thrombosis or coronary artery spasm on the basis of coronary atherosclerosis, which results in a sharp decrease or interruption of coronary artery blood supply and a sustained and severe acute ischemia of the corresponding myocardium, leading to acute myocardial necrosis. Studies have shown that there are obvious inflammatory reactions and oxidative stress injury in STEMI.[7-8] Leukocyte elevation after STEMI is an important component of systemic inflammatory response and ischemic tissue repair mechanism. Ischemia-induced chemokines activate white blood cells to chemotaxis to ischemic sites and remove necrotic tissues, white blood cells adhere to the injured vascular wall and form aggregates with blood cells, which eventually lead to thrombosis.[9] Activated white blood cells also produce oxygen free radicals, lysosomal enzymes and other substances, which cause local inflammatory response in ischemic sites.[10] White blood cell, as a marker of inflammation, has been proved to be closely related to the

clinical prognosis of STEMI patients,[11] while bilirubin, the end product of heme degradation, is also an endogenous oxidant in vivo, participates in the occurrence and development of myocardial infarction.[12] Heme oxygenase (HO) regulates the synthesis and catabolism of bilirubin, and keeps the bilirubin content in a dynamic balance in human body.[13] HO-1 is an inducible isoform in response to diverse cellular stress such as oxidative stress, Inflammatory cytokines, heavy metals, cytokines, but is not expressed under normal conditions. Acute myocardial ischemia and hypoxia activates the stress process of the body and produces oxygen free radicals, oxides and infarction-related inflammatory factors, which significantly increase the activity of HO-1, and eventually leads to an increase in bilirubin. Studies has been suggested that bilirubin is elevated in patients with STEMI and has the effect of antioxidant stress, can be used as a biomarker for predicting clinical prognosis.[14]

Thus, there is a strong correlation between inflammatory response and oxidative stress, it's interact and influence each other to promote the development of STEMI. White blood cell and bilirubin, common and fast acquired biomarkers in routine blood tests, could reflect the level of inflammation and oxidative stress injury respectively and that combined information from multiple inflammatory and oxidative stress injury might be more informative. Therefore, the present study firstly combined WBC (an inflammatory biomarker) and TB (oxidative stress biomarker) into a simplified equation and assessed whether this value was predictive of in-hospital MACE in patients with STEMI in order to find a simple and reliable auxiliary index to evaluate and predict the clinical prognosis of STEMI for early treatment.

METHODS

Study design

This was a single-center prospective cohort study designed to assess whether the combination of admission WBC and TB could predict in-hospital and long-term outcomes in patients with STEMI post primary PCI. The study complied with the Declaration of Helsinki, and the study protocol was approved by the Human Ethical Committee of the First Affiliated Hospital of Xinjiang Medical University. All participants in this study were provided with written informed consent.

We consecutively enrolled adult patients with STEMI who treated with primary PCI at the Cardiac Center of the First Affiliated Hospital of Xinjiang Medical University between June 2012 and June 2017. STEMI diagnosis was in accordance with previously established guidelines.[1] All patients were treated with PCI within 12 hours after symptoms. Patients who did not have pre-PCI, white blood cell and bilirubin data, who had severe liver and kidney diseases, autoimmune system diseases, severe heart valve diseases, chronic inflammatory diseases, acute infectious diseases, malignant tumors, blood system diseases, active hemorrhage and other diseases, additionally, patients who had an infarct-related lesion unsuitable for stent implantation, and who were lost to follow-up were excluded from the current study.

Data collection

Investigators trained by professionals used uniformly designed questionnaires to collect patients' general information including age, gender, body mass index (BMI), history of smoking, dyslipidemia, hypertension, diabetes, laboratory examination results, angiographic examination results, in-hospital medication and the occurrence of MACE in our hospital by electronic medical records and paper cases.

Blood sampling and hematology measurements

Blood samples were collected from all participants immediately upon hospital admission for complete blood count analyses. In all patients, venous blood samples for laboratory analysis were collected at the time of presentation before the patients were transferred to the heart center. Peripheral blood samples were used for laboratory tests of several markers, including WBC, total

cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), total bilirubin, direct bilirubin, indirect bilirubin, creatine kinase isoenzyme (CK-MB), platelet, hemoglobin, creatinine and uric acid. The automatic blood analysis equipment (Beckman LH750/DXC800 automatic blood analyzer) was used for the hematological analysis in our hospital testing center. The normal range for white blood cell is $(4\text{-}10)\times10^9$/L, total bilirubin is 5.5-27.5 umol/L.

All patients were assessed by transthoracic echocardiography within 48 h after primary PCI using Vivid 7 ultrasound system (GE Medical Systems, USA). Standard echocardiographic views were acquired and analyzed by two experienced cardiologists who were unware of grouping information. Left ventricular ejection fraction (LVEF) was recorded after the examination

Definition of cardiovascular risk factors

Hypertension was defined as self-reported use of antihypertensive medication within the past 2 week or having systolic blood pressure greater than 140 mmHg and/or diastolic blood pressure greater than 90mmHg. Diabetes was defined as fasting blood glucose of more than 7 mmol/L (or 2 hours after a meal), and/or, using of insulin or oral hypoglycemic agents, or a self-reported history of diabetes. Hyperlipidemia was defined as anyone of the four lipids abnormalities following or self-reported use of antihyperlipidemic medication, total cholesterol concentration>6.22 mmol/L (240 mg/dl), triglyceride concentration>2.26 mmol/L (200 mg/dl), low-density lipoprotein cholesterol concentration>4.14 mmol/L (160 mg/dl), high-density lipoprotein cholesterol concentration<1.04 mmol/L (40 mg/dl).[15] GRACE score of patients was calculated using eight indicators of GRACE admission risk score according to the medical history, signs and laboratory examination results of admission, including Killip classification of cardiac function, age, heart rate, arterial systolic pressure, serum creatinine, ST segment changes of electrocardiogram, elevated myocardial markers, cardiac arrest at admission. Positive smoking history was defined as having smoked daily or occasionally in the past.

PCI and Medication

Patients were given loaded medication before operation: spirin+clopidogrel (300 mg+300 mg)

orally and secondary clinical prophylactic medication after PCI. Primary PCI was performed by two senior doctors in the Cardiac Center of the First Affiliated Hospital of Xinjiang Medical University. At the same time, the results of angiography were recorded for each patient. The main vessel was left main coronary artery, left anterior descending branch, left circumflex branch and right coronary descending branch. The degree of coronary artery stenosis was assessed by AXIOM Artis FC/BC cardiovascular computer quantitative analysis system in Germany. Cross-sectional projections of right and left coronary arteries were recorded. Most of the approaches were via radial artery, and a few were via femoral artery. There are at least 4 projection positions for the left coronary artery and at least 2 projection positions for the right coronary artery. If necessary, other postures are added to fully display each segment of the coronary artery. The imaging data of each patient undergoing coronal angiography are kept for reference. After angiography, more than two experienced interventional physicians including the surgeon discussed the results and filled in the detailed report of the results of coronary angiography after reaching consensus. The number of stenosis branches is defined as a single vessel>70% or two vessels more than 50%. PCI was performed by a professional cardiologist, and the stent implantation site, balloon type, pressure, stent size and type, TIMI blood flow classification were filled in detail after the operation.

Successful coronary angiography is defined as defined as residual stenosis<50% and thrombolysis in myocardial Infarction grade 3 flow after the procedure.

Clinical Outcomes and Definitions

In-hospital endpoints: (1) In-hospital mortality; (2) Major adverse cardiovascular events (MACE) during hospitalization including cardiac death, cardiogenic shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction.

Long-term follow-up endpoints: MACE including cardiac death, angina pectoris readmission, non-fatal myocardial infarction, malignant arrhythmia (ventricular tachycardia and ventricular fibrillation), severe cardiac insufficiency (cardiac III-IV level), stent restenosis, target vessels reascularization. Follow-up was performed by telephone interview for all patients enrolled. For those

patients who reached at least one of the primary endpoints, recorded data and medical reports were evaluated to determine inclusion criteria.

Statistical Analysis

Data were collected using Epidata3.1 (Odense, Denmark) and double checked. Analyses were carried out using Stata 15.0 software (Stata Corp LP, College Station, TX, USA). Continuous variables with a Gaussian distribution are presented as mean±standard deviation (SD), and those with a non-Gaussian distribution are presented as median values with corresponding 25th to 75th percentiles. The differences between groups were evaluated using Student's unpaired *t* test, one-way ANOVA or the Mann-Whitney rank test. Categorical variables were expressed as numbers and frequencies and the difference between groups was detected by Chi-square test. Logistic regression was used to determine the combined equation Receiver operating characteristic (ROC) curve was used to evaluate the combined value of WBC and TB to predict the best clinical boundary of in-hospital mortality. Univariate and multivariable logistic regression was used to analyze all potential influencing factors associated with in-hospital MACE. Odds ratio (OR) are shown with 95% confidence intervals (CIs). Kaplan-Meier plots were generated, and the log-rank test was used to compare the resulting curves. All statistical tests were bilateral tests, and the significance level was 0.05.

We included the subjects according to the sample size calculation formula. The sample size calculation formula in this research is $n = 2\overline{pq}(u_a + u_\beta)^2 / (p_1 - p_0)^2$

Based on the sample size of the current study, the power of the research results using *Power and sample size calculation* is 81.2%.

Patients and public involvement

The study designed to assess whether the combination of admission WBC and TB could predict in-hospital and long-term outcomes in patients with STEMI treated with primary PCI. However, no patients or members of the public were included in the design, recruitment or conduct of the study.

The results of measurements would be disseminated to participants after the study which was completed by the study team. The burden of the intervention will not be assessed by patients themselves.

RESULTS

Baseline clinical characteristics

During June 2012 and June 2017, we consecutively recruited 647 adult STEMI patients who underwent primary PCI. Of these patients 32 were excluded according to the exclusion criteria. A total of 615 STEMI patients (525 males and 90 female) with an average age of 58 years were included in this study. In-hospital MACE was found in 77 patients, including 17 patients with in-hospital mortality. Figure 1 depicts the flowchart of the study design. The comparison of the MACE group and non-MACE group are summarized in ***Table 1,*** including 17 patients with in-hospital mortality (*Suppl Table S1).*

The patients with MACE were older than without MACE group (61.2±11.8 vs 57.8±12.2, *P*=0.019). The LVEF in MACE group was worse than that of non-MACE group (55.48±7.82 vs 58.52±6.58, *P*<0.001). The laboratory data of the patients showed that the WBC and TB was (13.01±4.11)×10^9/L and 17.21±6.49 mmol/L in the MACE group, and (10.31±2.85)×10^9/L and 13.34±4.91 mmol/L in the non-MACE group, respectively (*P*<0.001). Patients in MACE group also had significantly greater levels of uric acid (6.67±2.40 vs 5.27±1.70 umol/L, *P*<0.001), creatinine (83.95±24.38 vs 75.46±18.79 umol/L, *P*=0.004) and greater number of culprit vessels (3.21±1.25 vs 2.80±1.27, *P*=0.009). The TIMI flow grade after PCI in non-MACE group was higher than MACE group (2.8±0.3 vs 2.7±0.6, *P*=0.019). The usage rates of clopidogrel (92.6% vs83.1%, *P*=0.006) and statins (93.9% vs 85.7%, *P*=0.009) in the MACE group were lower than those in the non-MACE group.

No significant differences were found between the MACE group and non-MACE group in terms of gender, BMI, smoking, hypertension, diabetes, hyperlipidemia, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, Global Registry of Acute Coronary Events (GRACE) score, direct bilirubin, indirect bilirubin, total cholesterol, triglycerides, HDL-C, LDL-C, CK-MB, platelet, hemoglobin and culprit vessels location and other drug utilization rates **(*Table 1*).**

Ability of WBC and TB to predict short-term clinical outcome

WBC and TB were combined using logistic regression. Binomial logistic regression analysis was performed with in-hospital mortality as dependent variable and WBC and TB as independent variables, the logistic regression model was: Logit (*P*) =-8.00+0.265 WBC+0.077 TB ***(Table 2)***, so the "combined" value refers to Logit (P) in our report. The recommended cut-off value for combined value based on the maximum of Youden's index on the ROC curve was 1.40 and it had 82.4% sensitivity and 64.7% specificity in predicting in-hospital mortality. ROC curve was used to determine the predictive value of WBC and TB for the occurrence of in-hospital mortality in STEMI patients after primary PCI. The optimal cut-off point of WBC was 12.2×10^9/L with sensitivity 71%, and specificity 73.1% (AUC=0.765 95%CI:0.625-0.904), the best cut-off value of TB was 14.4 mmol/L with sensitivity 62.0% and specificity 75.1% (AUC=0.751 95%CI:0.660-0.842).The combination of WBC and TB were more valuable (AUC=0.804, 95%CI:0.678-0.929, *P*<0.001). The area under the curves of WBC, TB and logistic regression combined detection was 0.765, 0.751 and 0.804 separately, which showed significant differences (*P*<0.001) (Table 3, ***Figure 2***). Further, the c-statistic was increased from 0.778 to 0.835 when a combination of WBC and TB.

WBC and TB levels predicted clinical outcomes

Based on the cut-off value of combined for WBC and TB, STEMI patients were further divided into the high- and low-risk groups. The independent risk factors affecting MACE in hospital were analyzed by logistic regression analysis. We first performed univariate logistics analysis, and in the multivariable logistic regression, we select the traditional risk factors for atherosclerosis, including age, body mass index(BMI), gender, smoking, hypertension, diabetes, LDL-c. we also chose the variables with positive study structure in univariate logistics analysis, such as combined detection, LVEF, culprit vessels and also includes some variables which can affect outcomes and has already identified by the researchers based on the literature and clinical work, such as heart rate, CK-MB. After adjusting, a significant association was noted between combined detection and the adjusted risk of in-hospital MACE (OR=5.85, 95%CI 3.425~9.990, *P*<0.001). The results showed that the higher value of equation is an independent predictor for in-hospital MACE of STEMI patients received primary PCI. Other independent predictors of in-hospital MACE were age (OR=1.03, 95% CI:

1.002~1.049, *P*=0.028), LVEF (OR=0.960, 95% CI: 0.928~0.990, *P*=0.010) and number of culprit vessels (OR=1.25, 95% CI: 1.019~1.529, *P*=0.032) ***(Table 4)***.

All patients were followed up for an average of 30.2 months, follow-up via telephone was complete in 583 patients or family members, 15 (2.5%) patients were lost to follow-up. After following 172 patients occurred in MACE with the incidence of out-hospital MACE was 29.5%. Kaplan-Meier survival analysis of long-term MACE was made by dividing the best truncation value into two groups. The results showed that there was no significant difference in the long-term incidence of MACE between the two groups (*P*=0.869, ***Figure 3***).

DISCUSSION

The current study is the first to indicate that the combined effect of WBC and TB could better predict prognosis (in terms of in-hospital mortality and in-hospital MACE) in acute STEMI patients undergoing PCI compared to single predictor independently. Our studies suggesting that those with higher WBC and TB had a higher likelihood of in-hospital complications, and a higher hazard of in-hospital mortality, independent of potential confounding factors.

Inflammatory processes play an important role in the development of STEMI. It has been suggested that there is a positive correlation between leucocyte elevation and myocardial infarction area.[16-18] White blood cells in the unstable plaque lesions adhesion, aggregation and release the tissue factor and promote blood clotting enzyme molecules, prompt production platelet activation and fibrin thrombi. When great quantities of white blood cell infiltrate in the ischemic area , because of its weak deformation, slow through the capillary, capillaries are more likely to block the ischemia area, increase myocardial microcirculation, led to a lack of oxygen to microvascular no reflow and aggravate ischemic infarction area,[16, 19]The increase of WBC count has an impact on the occurrence, development and prognosis of acute myocardial infarction, and the relevant mechanism is generally believed that the capillary is blocked by a large number of white blood cells, which causes no reflow of microvessels and increases the size of myocardial infarction,[20] Studies have proposed a positive correlation between leukocyte elevation and myocardial infarction area. [21]

There is indeed a lot of literature on the relationship between leukocytes and prognosis of ischemic heart disease. Kyne suggested that the increase of WBC count and neutrophilic granulocyte percentage in peripheral blood within12 hours was an independent predictor of heart failure in the first four days after acute myocardial infarction (AMI).[22] Kojima demonstrated an elevated WBC was significantly associated with higher risk of in-hospital mortality.[23] Nez reported that white blood cell count independently increased the risk of death for 30 days and 1 year in STEMI patients.[24] Çiçek G examined that the incidence of MACE in STEMI patients with elevated white blood cell count increased significantly during hospitalization.[25].

This means that leucocyte elevation during myocardial infarction not only has a purely

restorative physiological role, but also has a pathological role. Combined with myocardial infarction size, non coronary reflux and decreased cardiac function, increased white blood cells in STEMI patients may indicate poor clinical prognosis.

In addition to the inflammatory, oxidative stress injury plays an important role in the evolution of STEMI.[26] When STEMI occurs, the surface of coronary artery intima and lipid plaques are damaged. Plaque rupture and thrombosis leads to acute myocardial ischemia and hypoxia, activates the stress process cells and cytoplasm produce large amounts of oxygen free radicals through enzymatic and non-enzymatic systems, which leads to the oxidation of macromolecular protein, cell membrane lipid peroxidation and DNA damage, and finally cause the death of heart muscle cells. Myocardial cells after STEMI are stimulated by ischemia and hypoxia to produce large amounts of oxygen free radicals and oxidizing substances, a large amount of antioxidant substances are consumed so that the body loses its inhibiting effect on the formation of oxidative substances, and the balance between antioxidant substances and oxidative substances is broken, which will cause cell structure damage. [27]Oxidative stress injury can cause plaque instability, rupture and erosion, leading to thrombosis and complete infarction-related artery occlusion.[28] So when STEMI occurs, the body needs antioxidants to deal with the damage caused by oxidative stress.

Bilirubin is an endogenous oxidant in vivo. In recent years, more and more attention has been paid to the role of bilirubin in the pathophysiology of major cardiovascular diseases such as myocardial infarction,[29] atherosclerosis[30] and cardiovascular complications of diabetes.[31]As an antioxidant in the body, bilirubin is involved in the occurrence and development of myocardial infarction and plays an anti-oxidative role. Okuhara showed that compared with non-AMI patients, the serum bilirubin concentration and Fe^2 in AMI patients increased temporarily 18-21 hours after the onset of the disease.[12] Elevated bilirubin has antioxidant capacity and the ability to remove peroxides, which can prevent the deterioration of disease, Sexual resistance to oxidative stress in the myocardium may be considered as a beneficial self-regulation in acute ischemia. Celik reported that total bilirubin levels in STEMI were independently associated with no-reflow coronary artery.[32] Halit reported that bilirubin levels in STEMI patients with impaired blood flow were higher than those in normal blood flow group. They suggested that the more severe the atherosclerosis, the

higher the activity of HO-1 enzyme after myocardial infarction, the more obvious the increase of bilirubin levels.[33] The degree of increase was related to the severity of the lesion to a certain extent. Glu studied 1624 STEMI patients and found that compared with the bilirubin group less than 9.0 mg/dl, the bilirubin group higher than 9.0 mg/dl had higher in-hospital mortality, but the bilirubin level was not related to long-term prognosis.[34] Chung followed up 1111 STEMI patients treated with PCI in hospital and 12 months after operation. The results showed that the incidence of MACE and cardiogenic mortality in hyperbilirubinemia group were higher than those in low bilirubinemia group.[14] It can be concluded that the oxygen free radicals produced by oxidative stress injury and the damage of various oxides to the body under stress may exceed the protective and antioxidant effect of bilirubin on the body, which caused the protective effect of high bilirubin level is not significant. In contrast to stable coronary disease, the serum TB levels show different associations in stressful conditions.

Therefore, STEMI is a disease both with inflammatory and oxidative stress injury. As such, inflammatory biomarkers alone, or oxidative stress are not sufficient to capture the entire pathophysiologic process involved in STEMI. Instead, markers which combine inflammatory and oxidative stress information may be better at reflecting the process in STEMI.

In present study, the area under the ROC curve for combined equation in predicting mortality was higher than those for WBC and TB, multivariate logistic regression analysis showed the high value of equation is an independent predicted of worse in-hospital MACE. In addition to the elevation of white blood cells and bilirubin in the MACE group, TIMI blood flow was also worse than that in the non- MACE group. Integrate previous studies, we think the increase of WBC count causes no reflow of micro vessels and increases the size of myocardial infarction and the increase of TB levels reflect the severity of coronary damage, which suggests that combining WBC and TB has a stronger predictive power for in-hospital MACE than individual markers. Previous studies have shown that the in-hospital mortality of STEMI in China between 2001 and 2011 was 7.0%.[35] In our study, in-hospital MACE was found in 77 (12.5%) patients and there were 17 (2.8%) patients occurred in death in-hospital among the 615 STEMI patients, the in-hospital mortality was comparable to the China's national level, so we think our results are reliable. We did not use troponin

levels and other biomarkers that are recommended generally to predict clinical outcomes. As we know, troponin test fee will be more expensive and testing requirements for basic-level hospitals is too high, not all hospitals have this condition, while white blood cell and bilirubin, common and fast acquired biomarkers in routine blood tests, can be detected by most hospitals because of low test cost .So we still suggested that combining WBC and TB was of particular clinical importance for the subset of patients with STEMI at admission.

There are some limitations in our study. This study is a single center experiment for a few patients which have the limitation of prospective cohort design. In our study bilirubin was measured in patients at only one time point before PCI in this study. The dynamic changes of serum leucocyte count and bilirubin concentration during hospitalization and follow-up were not clear. In this study, oxidative stress related indicators and HO-1 enzyme activity were not directly tested, and various inflammatory markers such as C-reactive protein, neutrophils and lymphocytes were not included. In addition, to determine the exact role of leukocytes and bilirubin in patients for long-term prognosis more experimental basic studies are needed.

CONCLUSIONS

The combination of WBC and TB may be better than single biomarker, can be applied in the predicting in-hospital MACE of STEMI patients treated with primary PCI. The high value of equation is a independent predictor of worse in-hospital MACE, it can be used to identify high-risk STEMI patients and accurately predict their clinical prognosis for early treatment.

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CONTRIBUTORS

Conceived and designed the experiments: Xiaomei Li, Yining Yang, Analyzed the data: Qian Zhao, Fen Liu. Contributed reagents/materials/analysis tools: Chun-Fang Shan, Xin-Rong Zhou, Ning Song, Ajiguli-Waisiding, Xue-He, Zhang, Gulandanmu-Aihemaiti. Quality control the study and revision: Xiaomei Li, Yining Yang, Yang Xiang. Wrote the paper: Munire-Tuxun, Qian Zhao. All authors read and approved the final manuscript.

ETHICS APPROVAL

This study was carried out in accordance with the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China) (approval ID:20141201-03-1701A).

DATA AVAILABILITY STATMENT

All data relevant to the study are included in the article or uploaded as supplementary information. No additional data are available.

FOUNDING

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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Table 1. Comparison between MACE group and non-MACE group.

Variables	Non-MACE (n=538)	MACE (n=77)	t/χ^2	*P*
Gender (men) (n,%)	465 (86.4)	63 (81.8)	1.180	0.277
Age (years)*	57.8±12.2	61.2±11.8	2.359	0.019
BMI (kg/m^2)	25.45±3.29	24.99±2.90	1.161	0.246
Smoking history (n,%)	302 (56.1)	47 (61.0）	0.660	0.416
Hypertension (n,%)	264 (49.1)	40 (51.9)	0.223	0.637
Diabetes (n,%)	115 (21.4)	13 (16.9)	0.825	0.364
Hyperlipemia (n,%)	396 (73.6)	61 (79.2)	1.112	0.292
SBP (mmHg)	121.88±19.08	118.75±18.45	1.348	0.178
DBP (mmHg)	76.58±13.13	75.18±13.33	0.870	0.385
Heart rate (/min)	81.76±13.64	84.69±17.93	1.686	0.092
LVEF (%)*	58.52±6.58	55.48±7.82	3.703	<0.001
TB (umol/L)*	13.34±4.91	17.21±6.49	5.040	<0.001
DBL (umol/L)	2.19±1.60	2.18±1.41	0.100	0.920
IBL (umol/L)	11.97±5.11	11.36±4.77	0.986	0.325
TC (mmol/L)	4.4±1.18	4.38±1.18	0.193	0.847
TG (mmol/L)	2.05±1.40	1.93±1.33	0.734	0.463
LDL (mmol/L)	2.86±0.85	2.8±0.84	0.544	0.587

HDL (mmol/L)	1.03±0.35	1.03±0.31	0.004	0.997
CK-MB (U/L)	277.73±221	306.14±204.18	1.065	0.287
WBC ($\times 10^9$ /L)*	10.31±2.85	13.01±4.11	5.569	<0.001
Hb (g/L)	145.24±19.96	142.47±23.72	1.114	0.266
PLT ($\times 10^9$ /L)	222.25±59.41	221.6±66.85	0.089	0.929
UC (mmol/L)*	5.27±1.70	6.67±2.40	4.921	<0.001
CR (umol/L)*	75.46±18.79	83.95±24.38	2.934	0.004
GRACE	132.89±27.74	137.7±32.29	1.394	0.164
LM (n,%)	51(9.5)	4(5.2)	1.519	0.218
LAD (n,%)	463(86.1)	71(92.2)	2.227	0.136
LCX (n,%)	334(62.1)	53(68.8)	1.315	0.251
RAD (n,%)	372(69.1)	61(79.2)	3.282	0.070
Culprit Vessels*	2.80±1.27	3.21±1.25	2.631	0.009
TIMI grade post-PCI*	2.8±0.3	2.7±0.6	2.335	0.019
Medication				
Aspirin	519(96.5)	73(94.8)	0.518	0.472
Clopidogrel*	498(92.6)	64(83.1)	7.635	0.006
β blockers	441(82.0)	62(80.5)	0.095	0.758
ACEI	382(71.0)	55(71.4)	0.006	0.939
Statins*	505(93.9)	66(85.7)	6.739	0.009

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, Left ventricular ejection fraction; TB, total bilirubin; DBI, direct bilirubin; IBL, indirect bilirubin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes; WBC, white blood cells; Hb, hemoglobin; PLT, Platelet; UC, uric acid; CR, creatinine; GRACE, globla registry of acute coronary events; LM, left main coronary artery; LAD, left anterior descending branch ; LCX, left circumflex branch; RAD, right coronary descending branch; TIMI, thrombolysis in myocardial infarction; PCI, percutaneous coronary intervention; ACEI, angiotensin-converting enzyme inhibitor.

Table 2. The combined equation of logistic regression of in-hospital mortality

Variables	*b*	*OR*	*95% CI*	*P*
TB	0.077	1.080	-0.005-0.159	0.067
WBC	0.265	1.303	0.125-0.405	<0.001
Constant term	-8.004	0.002		<0.001

Abbreviations: TB, total bilirubin; WBC, white blood cells; OR, odds ratio; CI, confidence interval;

Table 3. Admission of TB, WBC and combine as predictors of in-hospital mortality

Variables	Sensitivity (%)	Specificity (%)	AUC (0 to 1.0)	95% CI	X^2	P vale
TB (≥14.4 mmol/L)	76.5	62.0	0.751	0.660-0.842		
WBC (≥12.2 10^9/L)	71.0	73.1	0.765	0.625-0.904	8.410	0.014
Combine (≥1.4)	82.4	64.7	0.804	0.678-0.9286		

Abbreviations: TB, total bilirubin; WBC, white blood cells; AUC, area under the curve; CI, confidence interval;

Table 4. Univariate and multivariate logistic analysis of in-hospital MACE

Variables	Univariate		Multivariate	
	OR (95%CI)	*P* Value	OR (95%CI)	*P* Value
Gender (male)	1.69(0.763-3.767)	0.195		
Age (year)	1.02(1.003-1.055)	0.025	1.03(1.002-1.049)	0.028
BMI	0.98(0.896-1.072)	0.660		
Smoking	1.71(0.933-3.141)	0.083		
Hypoglycemic	0.87(0.506-1.494)	0.614		
Diabetes	0.77(0.3841.542)	0.460		
Heart rate	1.01(0.993-1.027)	0.249		
LVEF	0.96(0.926-0.990)	0.011	0.96(0.928-0.990)	0.010
LDL-C	0.88(0.640-1.205)	0.422		
CK-MB	1.00(0.999-1.001)	0.166		
Culprit Vessels	1.29(1.045-1.601)	0.018	1.25(1.019-1.529)	0.032
Combine (cutoff=1.4)	5.55(3.207-9.609)	0.000	5.85(3.425-9.990)	<0.001

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, Body mass index; LVEF, Left ventricular ejection fraction; LDL-C, low-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes.

1

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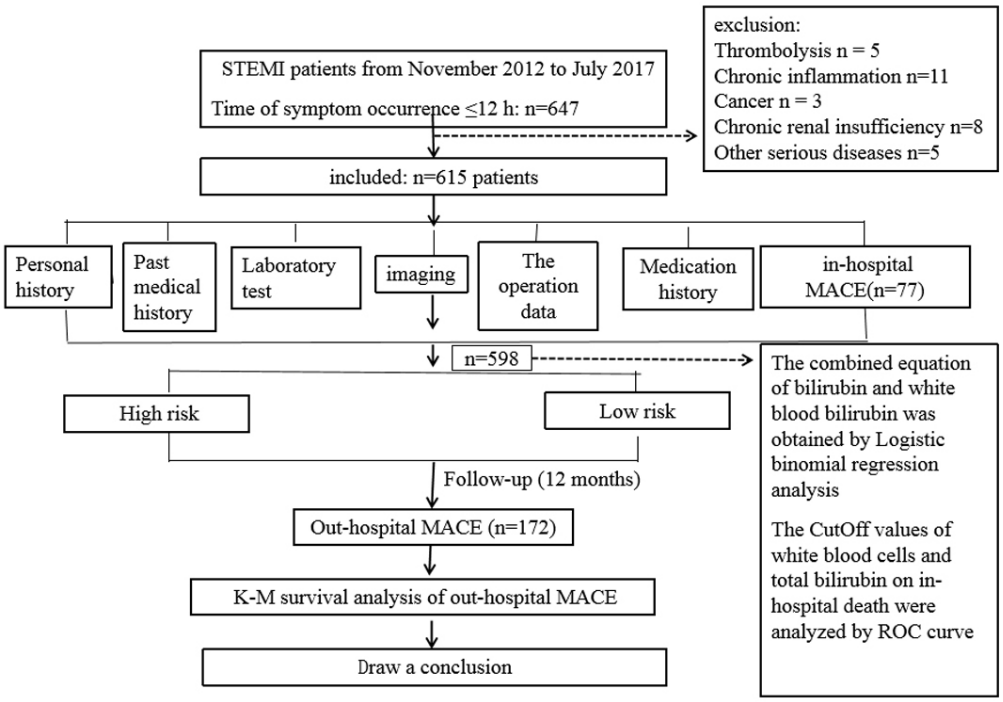
Figure Legends

Figure 1. The flowchart of study design with including and excluding procedures.

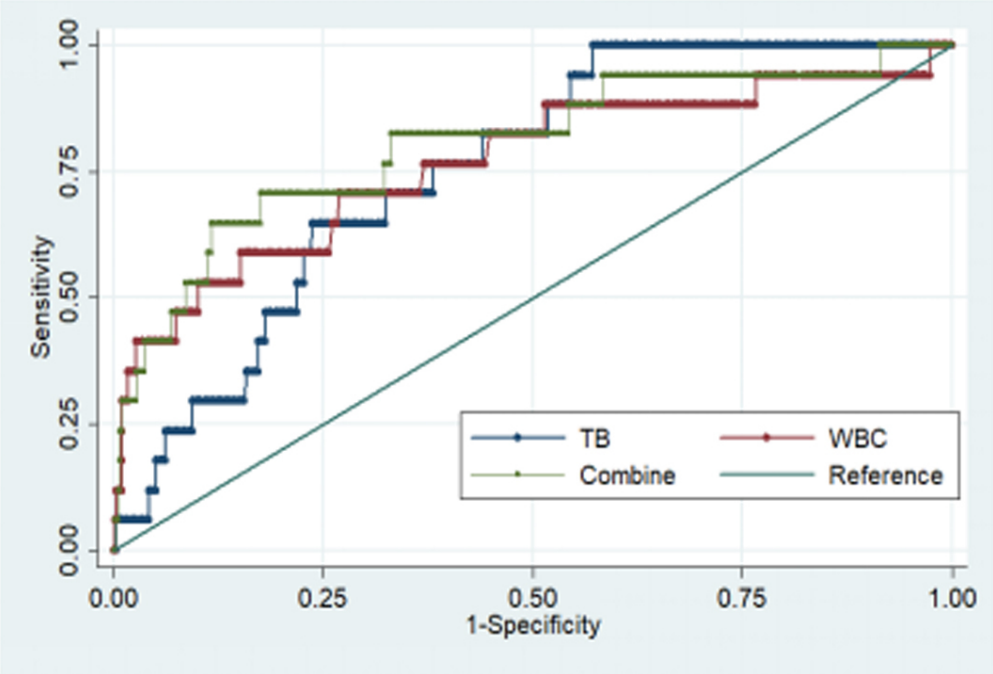
Figure 2. Admission of TB, WBC and Combine as predictors of in-hospital mortality.

Figure 3. Kaplan–Meier survival analysis of long-term MACE.



The flowchart of study design with including and excluding procedures.

90x90mm (300 x 300 DPI)



Admission of TB, WBC and Combine as predictors of in-hospital mortality.

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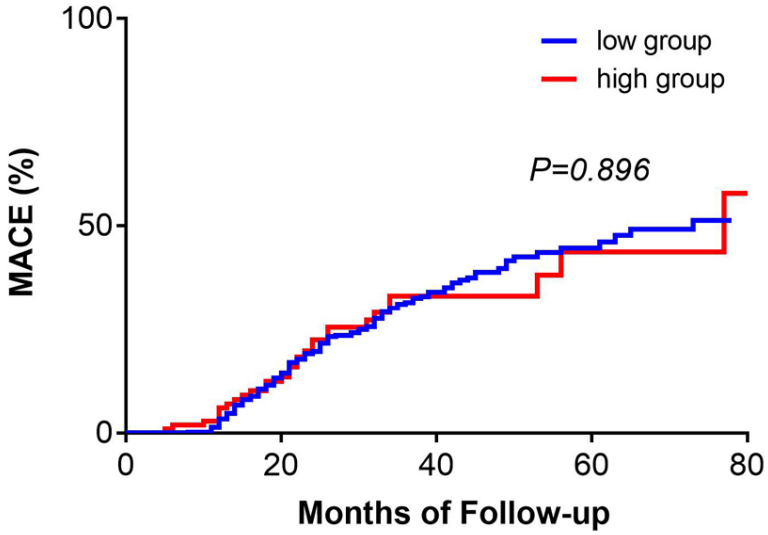

Number at risk					
Low group	505	353	127	42	0
High group	110	76	27	8	1

Kaplan–Meier survival analysis of long-term MACE.

90x90mm (300 x 300 DPI)

The combined value of white blood cell and total bilirubin predict clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study

Supplementary materials

Munire-Tuxun, MD., Qian Zhao, PhD., Yang Xiang, MD., Fen Liu, PhD., Chun-Fang Shan, MD., Xin-Rong Zhou, MD., Ning Song, PhD., Ajiguli-Waisiding, MD., Xue-He, Zhang, MD., Gulandanmu-Aihemaiti, MD., Yi-Ning Yang, PhD., Xiao-Mei Li, PhD.

Supplementary Table S1 Comparison between in-hospital mortality group and survival group

Variables	Survival group(n=598)	Mortality group(n=17)	t/χ^2	*P*
Gender (men) (n,%)	516(86.3)	12(70.6)	3.550	0.067
Age (years)*	58.03±12.15	63.53±12.40	1.839	0.066
BMI (kg/m^2)	25.42±3.25	24.01±2.68	1.785	0.075
Smoking history (n,%)	338(56.5)	11(64.7)	0.451	0.502
Hypertension (n,%)	269(49.5)	8(47.1)	0.039	0.843
Diabetes (n,%)	125(20.9)	3(17.6)	0.106	0.744
Hyperlipemia (n,%)	443(74.1)	14(82.4)	0.593	0.441
SBP (mmHg)	121.59±18.89	119.76±23.65	0.378	0.706
DBP (mmHg)	76.46±13.08	74.18±15.91	0.707	0.480
Heart rate (/min)	81.90±13.88	90.06±23.31	2.333	0.020
LVEF(%)*	58.33±6.67	51.35±8.41	4.222	<0.001
TB (umol/L)*	13.69±5.22	18.47±5.59	3.718	<0.001
DBL (umol/L)	2.18±1.56	2.58±2.10	1.025	0.306
IBL (umol/L)	11.88±5.09	12.29±4.41	0.326	0.744
TC (mmol/L)	4.41±1.18	3.95±0.95	1.611	0.108
TG(mmol/L)	2.05±1.39	1.67±1.06	1.095	0.274
LDL (mmol/L)	2.86±0.85	2.51±0.90	1.686	0.092
HDL (mmol/L)	1.03±0.35	0.90±0.24	0.415	0.678
CKMB (U/L)	281.46±220.92	275.36±139.69	0.113	0.910
WBC (×10^9 /L)*	10.54±3.04	14.59±4.74	5.324	<0.001
Hb (g/L)	144.99±20.35	141.59±24.81	0.676	0.500
PLT (×10^9 /L)	222.58±60.33	207.59±60.52	1.010	0.313
UC (mmol/L)*	5.42±1.80	6.40±3.24	2.137	0.033
CR (umol/L)*	76.24±19.16	86.46±34.23	2.108	0.035
GRACE	133.06±28.01	148.71±36.61	2.250	0.025

LM (n,%)	54(9.0)	1(5.9)	0.201	0.54
LAD (n,%)	518(86.6)	16(94.1)	0.812	0.368
LCX (n,%)	375(62.7)	12(70.6)	0.440	0.507
RAD (n,%)	418(69.9)	15(88.2)	2.670	0.102
Culprit Vessels	2.84±1.27	3.41±1.42	1.840	0.066
Medication				
Aspirin	578(96.7)	14(82.4)	9.393	0.002
Clopidogrel*	553(92.5)	9(52.9)	32.806	0.000
β blockers	492(82.3)	11(64.7)	3.425	0.064
ACEI	429(71.7)	8(47.1)	4.896	0.027
Statins*	559(93.5)	12(70.6)	13.039	0.000

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, Left ventricular ejection fraction; TB, total bilirubin; DBI, direct bilirubin; IBL, indirect bilirubin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes; WBC, white blood cells; Hb, hemoglobin; PLT, Platelet; UC, uric acid; CR, creatinine; GRACE, globla registry of acute coronary events; LM, left main coronary artery; LAD, left anterior descending branch ; LCX, left circumflex branch; RAD, right coronary descending branch; ACEI, angiotensin-converting enzyme inhibitor.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Non
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6-7*
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	Non
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	11

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	10-11
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Non
		Cross-sectional study—Report numbers of outcome events or summary measures	Non
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11
		(*b*) Report category boundaries when continuous variables were categorized	Non
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The combined value of white blood cell and total bilirubin predict clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study

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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	Coronary heart disease < CARDIOLOGY, Ischaemic heart disease < CARDIOLOGY, Myocardial infarction < CARDIOLOGY

The combined value of white blood cell and total bilirubin predict clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study

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Short title: White blood cell and total bilirubin predicting clinical outcomes in STEMI

# These authors contribute equally to the work.

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Words:4757 Tables: 4 Figures: 3

ABSTRACT:

Objectives: A combined equation based on white blood cell count (WBC) and total bilirubin (TB) was assessed for its ability to predict adverse clinical outcomes in acute ST-segment elevation myocardial infarction (STEMI) patients with primary percutaneous coronary intervention (PCI).

Design: A single-center, prospective cohort study.

Setting: The First Affiliated Hospital of Xinjiang Medical University.

Method: A total of 615 STEMI patients post primary PCI were enrolled. WBC and TB were collected at admission. Logistic regression was used to determine the combined equation. The primary endpoints were in-hospital mortality and major adverse cardiovascular events (MACE) which composed of cardiac death, cardiac shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction,angina pectoris readmission, severe cardiac insufficiency (cardiac III-IV level), stent restenosis, target vessels reascularization during hospitalization and 36-months follow-up period.

Result: 77 patients occurred in MACE during hospitalization (17 in-hospital mortality). WBC and TB were taken as an independent variables to make a category of logistic regression analysis of in-hospital MACE, the logistic regression model was: Logit (P) =-8.00+0.265 WBC+0.077 TB, the combination of WBC and TB were more valuable on evaluating the in-hospital mortality (AUC=0.804, 95% CI: 0.678~0.929, P<0.001). Multivariate logistic regression analysis showed that combined detection was an independent risk factor for in-hospital MACE (OR=5.85, 95% CI 3.425~9.990, P=0.032). During the follow-up period, 172 patients (29.5%) developed MACE. But the combined detection didn't predict the long-term clinincal outcome.

Conclusion:The combination of WBC and TB is an independent predictor for in-hospital outcomes in patients with STEMI than single detection.

Keywords: ST segment elevation myocardial infarction; white blood cell; total bilirubin; in-hospital

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Strengths and Limitations of the study

- This was the first study to indicate the combined effect of WBC and TB on predicting clinical prognosis in acute STEMI patients post primary PCI.
- The combination of WBC and TB may be better than single biomarker, can be applied in the predicting in-hospital MACE of STEMI patients treated with PCI.
- A single-center prospective cohort study for a small number of patients was fail to adequately exclude the influence of unknown confounding factors on this study.
- The determination of bilirubin in patients with STEMI was only at a preoperative time point, and the dynamic changes of the bilirubin concentrations during hospitalization and follow-up were not clear.

INTRODUCTION

STEMI has been a hot spot in cardiology research with its urgent onset, rapid progress and high mortality. Although reperfusion therapy such as percutaneous coronary intervention (PCI) and fibrinolytic therapy had improved the survival rate for acute ST-segment elevation myocardial infarction (STEMI), the in-hospital mortality rate of STEMI is as high as 4%-12%.[1] It has been a new challenge for clinician to identify high-risk patients timely, evaluate clinical prognosis accurately and prevent the occurrence of major adverse cardiovascular events (MACE). Screening a sensitive and specific biological index has great value for the treatment and prevention of STEMI complications.

In an attempt to identify STEMI patients at high risk of unfavorable outcomes, several predictors of adverse events in STEMI have been investigated. Research indicates that older age,[2] BNP, D-dimer, uric acid and thrombolysis myocardial infarction (TIMI) risk score are associated with a higher risk of adverse events in STEMI patients.[3-6] However, there is no literature report on the combination of white blood cell count (WBC) and total bilirubin (TB) in the guiding significance for the prognosis of STEMI.

STEMI refers to the occurrence of plaque rupture, thrombosis or coronary artery spasm on the basis of coronary atherosclerosis, which results in a sharp decrease or interruption of coronary artery blood supply and a sustained and severe acute ischemia of the corresponding myocardium, leading to acute myocardial necrosis. Studies have shown that there are obvious inflammatory reactions and oxidative stress injury in STEMI.[7-8] Leukocyte elevation after STEMI is an important component of systemic inflammatory response and ischemic tissue repair mechanism. Ischemia-induced chemokines activate white blood cells to chemotaxis to ischemic sites and remove necrotic tissues, white blood cells adhere to the injured vascular wall and form aggregates with blood cells, which eventually lead to thrombosis.[9] Activated white blood cells also produce oxygen free radicals, lysosomal enzymes and other substances, which cause local inflammatory response in ischemic sites.[10] White blood cell, as a marker of inflammation, has been proved to be closely related to the

clinical prognosis of STEMI patients,[11] while bilirubin, the end product of heme degradation, is also an endogenous oxidant in vivo, participates in the occurrence and development of myocardial infarction.[12] Heme oxygenase (HO) regulates the synthesis and catabolism of bilirubin, and keeps the bilirubin content in a dynamic balance in human body.[13] HO-1 is an inducible isoform in response to diverse cellular stress such as oxidative stress, Inflammatory cytokines, heavy metals, cytokines, but is not expressed under normal conditions. Acute myocardial ischemia and hypoxia activates the stress process of the body and produces oxygen free radicals, oxides and infarction-related inflammatory factors, which significantly increase the activity of HO-1, and eventually leads to an increase in bilirubin. Studies has been suggested that bilirubin is elevated in patients with STEMI and has the effect of antioxidant stress, can be used as a biomarker for predicting clinical prognosis.[14]

Thus, there is a strong correlation between inflammatory response and oxidative stress, it's interact and influence each other to promote the development of STEMI. White blood cell and bilirubin, common and fast acquired biomarkers in routine blood tests, could reflect the level of inflammation and oxidative stress injury respectively and that combined information from multiple inflammatory and oxidative stress injury might be more informative. Therefore, the present study firstly combined WBC (an inflammatory biomarker) and TB (oxidative stress biomarker) into a simplified equation and assessed whether this value was predictive of in-hospital MACE in patients with STEMI in order to find a simple and reliable auxiliary index to evaluate and predict the clinical prognosis of STEMI for early treatment.

METHODS

Study design

This was a single-center prospective cohort study designed to assess whether the combination of admission WBC and TB could predict in-hospital and long-term outcomes in patients with STEMI post primary PCI. The study complied with the Declaration of Helsinki, the study protocol was first approved in December 2014 by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China) (approval ID: 20141201-03) and ethics review was conducted in January 2017 (approval ID:20141201-03-1701A). All patients provided written informed consent.

We consecutively enrolled adult patients with STEMI who treated with primary PCI at the Cardiac Center of the First Affiliated Hospital of Xinjiang Medical University between June 2012 and June 2017. STEMI diagnosis was in accordance with previously established guidelines.[1] All patients were treated with PCI within 12 hours after symptoms. Patients who did not have pre-PCI, white blood cell and bilirubin data, who had severe liver and kidney diseases, autoimmune system diseases, severe heart valve diseases, chronic inflammatory diseases, acute infectious diseases, malignant tumors, blood system diseases, active hemorrhage and other diseases, additionally, patients who had an infarct-related lesion unsuitable for stent implantation, and who were lost to follow-up were excluded from the current study.

Data collection

Investigators trained by professionals used uniformly designed questionnaires to collect patients' general information including age, gender, body mass index (BMI), history of smoking, dyslipidemia, hypertension, diabetes, laboratory examination results, angiographic examination results, in-hospital medication and the occurrence of MACE in our hospital by electronic medical records and paper cases.

Blood sampling and hematology measurements

Blood samples were collected from all participants immediately upon hospital admission for complete blood count analyses. In all patients, venous blood samples for laboratory analysis were collected at the time of presentation before the patients were transferred to the heart center.

Peripheral blood samples were used for laboratory tests of several markers, including WBC, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), total bilirubin, direct bilirubin, indirect bilirubin, creatine kinase isoenzyme (CK-MB), platelet, hemoglobin, creatinine and uric acid. TB, direct bilirubin, indirect bilirubin were measured by Hitachi 7060 automatic biochemical analysis and the automatic blood analysis equipment (sysmex XE-5000 automatic blood analyzer) was used for WBC in our hospital testing center. The normal range for white blood cell is (4-10)×109/L, total bilirubin is 5.5-27.5 umol/L.

All patients were assessed by transthoracic echocardiography within 48 h after primary PCI Standard echocardiographic views were acquired and analyzed by two experienced cardiologists who were unware of grouping information. Left ventricular ejection fraction (LVEF) was measured by B mode echocardiography.

Definition of cardiovascular risk factors

Hypertension was defined as self-reported use of antihypertensive medication within the past 2 week or having systolic blood pressure greater than 140 mmHg and/or diastolic blood pressure greater than 90mmHg. Diabetes was defined as fasting blood glucose of more than 7 mmol/L (or 2 hours after a meal), and/or, using of insulin or oral hypoglycemic agents, or a self-reported history of diabetes. Hyperlipidemia was defined as anyone of the four lipids abnormalities following or self-reported use of antihyperlipidemic medication, total cholesterol concentration>6.22 mmol/L (240 mg/dl), triglyceride concentration>2.26 mmol/L (200 mg/dl), low-density lipoprotein cholesterol concentration>4.14 mmol/L (160 mg/dl), high-density lipoprotein cholesterol concentration<1.04 mmol/L (40 mg/dl).[15] GRACE score of patients was calculated using eight indicators of GRACE admission risk score according to the medical history, signs and laboratory examination results of admission, including Killip classification of cardiac function, age, heart rate, arterial systolic pressure, serum creatinine, ST segment changes of electrocardiogram, elevated myocardial markers, cardiac arrest at admission. Positive smoking history was defined as having smoked daily or occasionally in the past.

PCI and Medication

Patients were given loaded medication before operation: aspirin+clopidogrel (300 mg+300 mg) orally and secondary clinical prophylactic medication after PCI. In our study , angiograms were independently reviewed by two interventional cardiologists who were blinded of patients' information in the Cardiac Center of the First Affiliated Hospital of Xinjiang Medical University. The authors of this article were not involved in the PCI treatment of these patients. At the same time, the results of angiography were recorded for each patient. The main vessel was left main coronary artery, left anterior descending branch, left circumflex branch and right coronary descending branch. The degree of coronary artery stenosis was assessed by AXIOM Artis FC/BC cardiovascular computer quantitative analysis system in Germany. Cross-sectional projections of right and left coronary arteries were recorded. Most of the approaches were via radial artery, and a few were via femoral artery. There are at least 4 projection positions for the left coronary artery and at least 2 projection positions for the right coronary artery. If necessary, other postures are added to fully display each segment of the coronary artery. The imaging data of each patient undergoing coronal angiography are kept for reference. After angiography, more than two experienced interventional physicians including the surgeon discussed the results and filled in the detailed report of the results of coronary angiography after reaching consensus. The number of stenosis branches is defined as a single vessel>70% or two vessels more than 50%. PCI was performed by a professional cardiologist, and the stent implantation site, balloon type, pressure, stent size and type, TIMI blood flow classification were filled in detail after the operation.

Successful coronary angiography is defined as defined as residual stenosis<50% and thrombolysis in myocardial Infarction grade 3 flow after the procedure.

Clinical Outcomes and Definitions

In-hospital endpoints: (1) In-hospital mortality; (2) Major adverse cardiovascular events (MACE) during hospitalization including cardiac shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction.

Long-term follow-up endpoints: MACE including cardiac death, angina pectoris readmission, non-fatal myocardial infarction, malignant arrhythmia (ventricular tachycardia and ventricular fibrillation), severe cardiac insufficiency (cardiac III-IV level), stent restenosis, target vessels reascularization. Follow-up was performed by telephone interview for all patients enrolled. For those patients who reached at least one of the primary endpoints, recorded data and medical reports were evaluated to determine inclusion criteria.

Statistical Analysis

Data were collected using Epidata3.1 (Odense, Denmark) and double checked. Analyses were carried out using Stata 15.0 software (Stata Corp LP, College Station, TX, USA). Continuous variables with a Gaussian distribution are presented as average±standard deviation (SD), and those with a non-Gaussian distribution are presented as median values with corresponding 25th to 75th percentiles. The differences between groups were evaluated using Student's unpaired *t* test, one-way ANOVA or the Mann-Whitney rank test. Categorical variables were expressed as numbers and frequencies and the difference between groups was detected by Chi-square test. Logistic regression was used to determine the combined equation Receiver operating characteristic (ROC) curve was used to evaluate the combined value of WBC and TB to predict the best clinical boundary of in-hospital mortality. Univariate and multivariable logistic regression was used to analyze all potential influencing factors associated with in-hospital MACE. Odds ratio (OR) are shown with 95% confidence intervals (CIs). Kaplan-Meier plots were generated, and the log-rank test was used to compare the resulting curves. All statistical tests were bilateral tests, and the significance level was 0.05.

We included the subjects according to the sample size calculation formula. The sample size calculation formula in this research is $n = 2\bar{p}\bar{q}(u_a + u_\beta)^2 / (p_1 - p_0)^2$

Based on the sample size of the current study, the power of the research results using *Power and sample size calculation* is 81.2%.

Patients and public involvement

The study designed to assess whether the combination of admission WBC and TB could predict in-hospital and long-term outcomes in patients with STEMI treated with primary PCI. However, no patients or members of the public were included in the design, recruitment or conduct of the study.

The results of measurements would be disseminated to participants after the study which was completed by the study team. The burden of the intervention will not be assessed by patients themselves.

RESULTS

Baseline clinical characteristics

During June 2012 and June 2017, we consecutively recruited 647 adult STEMI patients who underwent primary PCI. Of these patients 32 were excluded according to the exclusion criteria. A total of 615 STEMI patients (525 males and 90 female) with an average age of 58 years were included in this study. In-hospital MACE was found in 77 patients, including 17 patients with in-hospital mortality. Figure 1 depicts the flowchart of the study design. The comparison of the MACE group and non-MACE group are summarized in ***Table 1,*** including 17 patients with in-hospital mortality (*Suppl Table S1).*

The patients with MACE were older than without MACE group (61.2±11.8 vs 57.8±12.2, *P*=0.019). The LVEF in MACE group was worse than that of non-MACE group (55.48±7.82 vs 58.52±6.58, *P*<0.001). The laboratory data of the patients showed that the WBC and TB was (13.01±4.11)×10^9/L and 17.21±6.49 mmol/L in the MACE group, and (10.31±2.85)×10^9/L and 13.34±4.91 mmol/L in the non-MACE group, respectively (*P*<0.001). Patients in MACE group also had significantly greater levels of uric acid (6.67±2.40 vs 5.27±1.70 umol/L, *P*<0.001), creatinine (83.95±24.38 vs 75.46±18.79 umol/L, *P*=0.004) and greater number of culprit vessels (3.21±1.25 vs 2.80±1.27, *P*=0.009). The TIMI flow grade after PCI in non-MACE group was higher than MACE group［3(1-3) vs 2(1-3), *P*=0.023］. The usage rates of clopidogrel (92.6% vs83.1%, *P*=0.006) and statins (93.9% vs 85.7%, *P*=0.009) in the MACE group were lower than those in the non-MACE group.

No significant differences were found between the MACE group and non-MACE group in terms of gender, BMI, smoking, hypertension, diabetes, hyperlipidemia, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, Global Registry of Acute Coronary Events (GRACE) score, direct bilirubin, indirect bilirubin, total cholesterol, triglycerides, HDL-C, LDL-C, CK-MB, platelet, hemoglobin and culprit vessels location and other drug utilization rates **(*Table 1*).**

Ability of WBC and TB to predict short-term clinical outcome

WBC and TB were combined using logistic regression. Binomial logistic regression analysis was performed with in-hospital mortality as dependent variable and WBC and TB as independent variables, the logistic regression model was: Logit (*P*) =-8.00+0.265 WBC+0.077 TB ***(Table 2)***, so the "combined" value refers to Logit (P) in our report. The recommended cut-off value for combined value based on the maximum of Youden's index on the ROC curve was 1.40 and it had 82.4% sensitivity and 64.7% specificity in predicting in-hospital mortality. ROC curve was used to determine the predictive value of WBC and TB for the occurrence of in-hospital mortality in STEMI patients after primary PCI. The optimal cut-off point of WBC was 12.2×10^9/L with sensitivity 71%, and specificity 73.1% (AUC=0.765 95%CI:0.625-0.904), the best cut-off value of TB was 14.4 mmol/L with sensitivity 76.5% and specificity 62% (AUC=0.751 95%CI:0.660-0.842).

We made a comparison between new predictive model and traditional predictive model based on hypersensitive troponinns (hs-TnT), The recommended cut-off value for peak troponins on the ROC curve was 0.87ug/ml and it had 85.2% sensitivity and 77.8% specificity in predicting in-hospital mortality(AUC=0.894 95%CI:0.831-0.961). The combination of WBC and TB were more valuable (AUC=0.804, 95%CI:0.678-0.928, *P*<0.001) than single WBC and TB but not as valuable as hs-TnT. The area under the curves of WBC, TB and logistic regression combined detection was 0.765, 0.751 and 0.804 separately, which showed significant differences (*P*<0.05) (Table 3, ***Figure 2***). Further, the c-statistic was increased from 0.778 to 0.835 when a combination of WBC and TB.

WBC and TB levels predicted clinical outcomes

Based on the cut-off value of combined for WBC and TB, STEMI patients were further divided into the high- and low-risk groups. The independent risk factors affecting MACE in hospital were analyzed by logistic regression analysis. We first performed univariate logistics analysis, and in the multivariable logistic regression, we included a total of 12 indicators. To avoid the influence of traditional factors on the results, we select the traditional risk factors for atherosclerosis, including age, body mass index (BMI), gender, smoking, hypertension, diabetes and low-density lipoprotein cholesterol. We also choose age, diabetes, hypertension, heart rate because they are included in the TIMI risk score, with left ventricular ejection fraction (LVEF), culprit vessels and combine detection,

has clinical significance in the univariate analysis. And we choose CK-MB, because CK-MB represents the area of myocardial necrosis and we considered there is a relationship between CK-MB and the poor prognosis of patients. After adjusting, a significant association was noted between combined detection and the adjusted risk of in-hospital MACE (OR=5.85, 95%CI 3.425~9.990, *P*<0.001). The results showed that the higher value of equation is an independent predictor for in-hospital MACE of STEMI patients received primary PCI. Other independent predictors of in-hospital MACE were age (OR=1.03, 95% CI: 1.002~1.049, *P*=0.028), LVEF (OR=0.960, 95% CI: 0.928~0.990, *P*=0.010) and number of culprit vessels (OR=1.25, 95% CI: 1.019~1.529, *P*=0.032) ***(Table 4)***.

All patients were followed up for an average of 30.2 months, follow-up via telephone was complete in 583 patients or family members, 15 (2.5%) patients were lost to follow-up. After following 172 patients occurred in MACE with the incidence of out-hospital MACE was 29.5%. Kaplan-Meier survival analysis of long-term MACE was made by dividing the best truncation value into two groups. The results showed that there was no significant difference in the long-term incidence of MACE between the two groups (*P*=0.869, ***Figure 3***).

DISCUSSION

The current study is the first to indicate that the combined effect of WBC and TB could better predict prognosis (in terms of in-hospital mortality and in-hospital MACE) in acute STEMI patients undergoing PCI compared to single predictor independently. Our studies suggesting that those with higher WBC and TB had a higher likelihood of in-hospital complications, and a higher hazard of in-hospital mortality, independent of potential confounding factors.

Inflammatory processes play an important role in the development of STEMI. It has been suggested that there is a positive correlation between leucocyte elevation and myocardial infarction area.[16-18] White blood cells in the unstable plaque lesions adhesion, aggregation and release the tissue factor and promote blood clotting enzyme molecules, prompt production platelet activation and fibrin thrombi. When great quantities of white blood cell infiltrate in the ischemic area , because of its weak deformation, slow through the capillary, capillaries are more likely to block the ischemia area, increase myocardial microcirculation, led to a lack of oxygen to microvascular no reflow and aggravate ischemic infarction area,[16, 19]The increase of WBC count has an impact on the occurrence, development and prognosis of acute myocardial infarction, and the relevant mechanism is generally believed that the capillary is blocked by a large number of white blood cells, which causes no reflow of microvessels and increases the size of myocardial infarction,[20] Studies have proposed a positive correlation between leukocyte elevation and myocardial infarction area. [21]

There is indeed a lot of literature on the relationship between leukocytes and prognosis of ischemic heart disease. Kyne suggested that the increase of WBC count and neutrophilic granulocyte percentage in peripheral blood within12 hours was an independent predictor of heart failure in the first four days after acute myocardial infarction (AMI).[22] Kojima demonstrated an elevated WBC was significantly associated with higher risk of in-hospital mortality.[23] Nez reported that white blood cell count independently increased the risk of death for 30 days and 1 year in STEMI patients.[24] Çiçek G examined that the incidence of MACE in STEMI patients with elevated white blood cell count increased significantly during hospitalization.[25]

This means that leucocyte elevation during myocardial infarction not only has a purely

restorative physiological role, but also has a pathological role. Combined with myocardial infarction size, non coronary reflux and decreased cardiac function, increased white blood cells in STEMI patients may indicate poor clinical prognosis.

In addition to the inflammatory, oxidative stress injury plays an important role in the evolution of STEMI.[26] Under physiological conditions, antioxidant substances in the body are in equilibrium with oxygen free radicals. When STEMI occurs, the surface of coronary artery intima and lipid plaques are damaged. Plaque rupture and thrombosis leads to acute myocardial ischemia and hypoxia, activates the stress process cells and cytoplasm produce large amounts of oxygen free radicals through enzymatic and non-enzymatic systems, which leads to the oxidation of macromolecular protein, cell membrane lipid peroxidation and DNA damage, and finally cause the death of heart muscle cells. Myocardial cells after STEMI are stimulated by ischemia and hypoxia to produce large amounts of oxygen free radicals and oxidizing substances, a large amount of antioxidant substances are consumed so that the body loses its inhibiting effect on the formation of oxidative substances, and the balance between antioxidant substances and oxidative substances is broken, which will cause cell structure damage. [27]Oxidative stress injury can cause plaque instability, rupture and erosion, leading to thrombosis and complete infarction-related artery occlusion.[28] So when STEMI occurs, the body needs antioxidants to deal with the damage caused by oxidative stress.

Antioxidants and markers which can represent oxidative reactions in vivo have been reported in the previous literature. Malondialdehyde (MDA) is the metabolite of unsaturated fatty acids in biofilms after oxidative damage, while advanced oxidation protein products (AOPP) is the metabolite of proteins after free radical attack and oxidative reaction, which can reflect the degree of oxidative reaction.[29] Superoxide dismutase (SOD) and glutathione (GSH), as important antioxidant substances in the body, can remove excess oxygen free radicals in the body and maintain the reduced state of cells[30]. When acute coronary syndrome occurs, GSH and SOD in serum are reduced.[31-32] Previous studies have reported impaired oxidation and antioxidant balance and increased oxidative stress in STEMI patients.[33] Gur showed that total antioxidant capacity (TAC) levels were significantly lower and total oxidation state (TOS), oxidative stress index (OSI),lipid hydroperoxide

(LOOH) were significantly higher in patients with no-reflow compared to normal flow group in patients with anterior STEMI undergoing primary PCI .[8] SARDAR showed that in the development process from unstable angina (UA) to STEMI, total sialic acid, oxidative modified protein and other oxidation substances were gradually increased, and the antioxidant status was gradually decreased.[34] These oxidation substances were more significant in STEMI patients. However, these substances require a certain amount of technology and time to test, and not all hospitals have the capability.

Bilirubin is an endogenous oxidant in vivo. In recent years, more and more attention has been paid to the role of bilirubin in the pathophysiology of major cardiovascular diseases such as myocardial infarction,[35] atherosclerosis[36] and cardiovascular complications of diabetes.[37]As an antioxidant in the body, bilirubin is involved in the occurrence and development of myocardial infarction and plays an anti-oxidative role. Okuhara showed that compared with non-AMI patients, the serum bilirubin concentration and Fe^2 in AMI patients increased temporarily 18-21 hours after the onset of the disease.[12] Elevated bilirubin has antioxidant capacity and the ability to remove peroxides, which can prevent the deterioration of disease, Sexual resistance to oxidative stress in the myocardium may be considered as a beneficial self-regulation in acute ischemia. Celik reported that total bilirubin levels in STEMI were independently associated with no-reflow coronary artery.[38] Halit reported that bilirubin levels in STEMI patients with impaired blood flow were higher than those in normal blood flow group. They suggested that the more severe the atherosclerosis, the higher the activity of HO-1 enzyme after myocardial infarction, the more obvious the increase of bilirubin levels.[39] The degree of increase was related to the severity of the lesion to a certain extent. Glu studied 1624 STEMI patients and found that compared with the bilirubin group less than 9.0 mg/dl, the bilirubin group higher than 9.0 mg/dl had higher in-hospital mortality, but the bilirubin level was not related to long-term prognosis.[40] Chung followed up 1111 STEMI patients treated with PCI in hospital and 12 months after operation. The results showed that the incidence of MACE and cardiogenic mortality in hyperbilirubinemia group were higher than those in low bilirubinemia group.[14] It can be concluded that the oxygen free radicals produced by oxidative stress injury and the damage of various oxides to the body under stress may exceed the protective and antioxidant effect of bilirubin on the body, which caused the protective effect of high bilirubin level is not

significant. In contrast to stable coronary disease, the serum TB levels show different associations in stressful conditions

Therefore, STEMI is a disease both with inflammatory and oxidative stress injury. As such, inflammatory biomarkers alone, or oxidative stress are not sufficient to capture the entire pathophysiologic process involved in STEMI. Instead, markers which combine inflammatory and oxidative stress information is a better at reflecting the process in STEMI.

In present study, the area under the ROC curve for combined equation in predicting mortality was higher than those for WBC and TB, multivariate logistic regression analysis showed the high value of equation is an independent predicted of worse in-hospital MACE. In addition to the elevation of white blood cells and bilirubin in the MACE group, TIMI blood flow was also worse than that in the non-MACE group. Integrate previous studies, we think the increase of WBC count causes no reflow of micro vessels and increases the size of myocardial infarction and the increase of TB levels reflect the severity of coronary damage, which suggests that combining WBC and TB has a stronger predictive power for in-hospital MACE than individual markers. Previous studies have shown that the in-hospital mortality of STEMI in China between 2001 and 2011 was 7.0%.[41] In our study, in-hospital MACE was found in 77 (12.5%) patients and there were 17 (2.8%) patients occurred in death in-hospital among the 615 STEMI patients, the in-hospital mortality was comparable to the China's national level, so we think our results are reliable. We did not use troponin levels and other biomarkers that are recommended generally to predict clinical outcomes. As we know, troponin test fee will be more expensive and testing requirements for basic-level hospitals is too high, not all hospitals have this condition, while white blood cell and bilirubin, common and fast acquired biomarkers in routine blood tests, can be detected by most hospitals because of low test cost .So we still suggested that combining WBC and TB was of particular clinical importance for the subset of patients with STEMI at admission.

There are some limitations in our study. This study is a single center experiment for a few patients which have the limitation of prospective cohort design. In our study bilirubin was measured in patients at only one time point before PCI in this study. The dynamic changes of serum leucocyte

count and bilirubin concentration during hospitalization and follow-up were not clear. In this study, oxidative stress related indicators and HO-1 enzyme activity were not directly tested, and various inflammatory markers such as C-reactive protein, neutrophils and lymphocytes were not included. In addition to determine the exact role of leukocytes and bilirubin in patients for long-term prognosis more experimental basic studies are needed.

CONCLUSIONS

The combination of WBC and TB is better than single biomarker, can be applied in the predicting in-hospital MACE of STEMI patients treated with primary PCI. The high value of equation is a independent predictor of worse in-hospital MACE, it can be used to identify high-risk STEMI patients and accurately predict their clinical prognosis for early treatment.

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CONTRIBUTORS

Conceived and designed the experiments: Xiaomei Li, Yining Yang, Analyzed the data: Qian Zhao, Fen Liu. Contributed reagents/materials/analysis tools: Chun-Fang Shan, Xin-Rong Zhou, Ning Song, Ajiguli-Waisiding, Xue-He, Zhang, Gulandanmu-Aihemaiti. Quality control the study and revision: Xiaomei Li, Yining Yang, Yang Xiang. Wrote the paper: Munire-Tuxun, Qian Zhao. All authors read and approved the final manuscript.

ETHICS APPROVAL

This study was carried out in accordance with the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China) (approval ID:20141201-03-1701A).

DATA AVAILABILITY STATMENT

All data relevant to the study are included in the article or uploaded as supplementary information. No additional data are available.

FOUNDING

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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Table 1. Comparison between MACE group and non-MACE group.

Variables	Non-MACE (n=538)	MACE (n=77)	t/χ^2	*P*
Gender (men) (n,%)	465 (86.4)	63 (81.8)	1.180	0.277
Age (years)*	57.8±12.2	61.2±11.8	2.359	0.019
BMI (kg/m^2)	25.45±3.29	24.99±2.90	1.161	0.246
Smoking history (n,%)	302 (56.1)	47 (61.0）	0.660	0.416
Hypertension (n,%)	264 (49.1)	40 (51.9)	0.223	0.637
Diabetes (n,%)	115 (21.4)	13 (16.9)	0.825	0.364
Hyperlipemia (n,%)	396 (73.6)	61 (79.2)	1.112	0.292
SBP (mmHg)	121.88±19.08	118.75±18.45	1.348	0.178
DBP (mmHg)	76.58±13.13	75.18±13.33	0.870	0.385
Heart rate (/min)	81.76±13.64	84.69±17.93	1.686	0.092
LVEF (%)*	58.52±6.58	55.48±7.82	3.703	<0.001
TB (umol/L)*	13.34±4.91	17.21±6.49	5.040	<0.001
DBL (umol/L)	2.19±1.60	2.18±1.41	0.100	0.920
IBL (umol/L)	11.97±5.11	11.36±4.77	0.986	0.325
TC (mmol/L)	4.4±1.18	4.38±1.18	0.193	0.847
TG (mmol/L)	2.05±1.40	1.93±1.33	0.734	0.463
LDL (mmol/L)	2.86±0.85	2.8±0.84	0.544	0.587

HDL (mmol/L)	1.03±0.35	1.03±0.31	0.004	0.997
CK-MB (U/L)	277.73±221	306.14±204.18	1.065	0.287
WBC ($\times 10^9$ /L)*	10.31±2.85	13.01±4.11	5.569	<0.001
Hb (g/L)	145.24±19.96	142.47±23.72	1.114	0.266
PLT ($\times 10^9$ /L)	222.25±59.41	221.6±66.85	0.089	0.929
UC (mmol/L)*	5.27±1.70	6.67±2.40	4.921	<0.001
CR (umol/L)*	75.46±18.79	83.95±24.38	2.934	0.004
GRACE	133(115~148)	136(116~158)	1.250	0.211
LM (n,%)	51(9.5)	4(5.2)	1.519	0.218
LAD (n,%)	463(86.1)	71(92.2)	2.227	0.136
LCX (n,%)	334(62.1)	53(68.8)	1.315	0.251
RAD (n,%)	372(69.1)	61(79.2)	3.282	0.070
Culprit Vessels*	2.80±1.27	3.21±1.25	2.631	0.009
TIMI grade post-PCI*	3 (1~3)	2 (1~3)	2.567	0.023
Medication				
Aspirin	519(96.5)	73(94.8)	0.518	0.472
Clopidogrel*	498(92.6)	64(83.1)	7.635	0.006
β blockers	441(82.0)	62(80.5)	0.095	0.758
ACEI	382(71.0)	55(71.4)	0.006	0.939
Statins*	505(93.9)	66(85.7)	6.739	0.009

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, Left ventricular ejection fraction; TB, total bilirubin; DBI, direct bilirubin; IBL, indirect bilirubin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes; WBC, white blood cells; Hb, hemoglobin; PLT, Platelet; UC, uric acid; CR, creatinine; GRACE, globla registry of acute coronary events; LM, left main coronary artery; LAD, left anterior descending branch ; LCX, left circumflex branch; RAD, right coronary descending branch; TIMI, thrombolysis in myocardial infarction; PCI, percutaneous coronary intervention; ACEI, angiotensin-converting enzyme inhibitor.

Table 2. The combined equation of logistic regression of in-hospital mortality

Variables	*b*	*OR*	*95% CI*	*P*
TB	0.077	1.080	-0.005-0.159	0.067
WBC	0.265	1.303	0.125-0.405	<0.001
Constant term	-8.004	0.002		<0.001

Abbreviations: TB, total bilirubin; WBC, white blood cells; OR, odds ratio; CI, confidence interval;

Table 3. Admission of TB, WBC and combine as predictors of in-hospital mortality

Variables	Sensitivity (%)	Specificity (%)	AUC (0 to 1.0)	95% CI	C-statistic	P vale
TB (≥14.4 mmol/L)	76.5	62.0	0.751	0.660-0.842		
WBC (≥12.2 10^9/L)	71.0	73.1	0.765	0.625-0.904	7.989	0.032
Combine (≥1.4)	82.4	64.7	0.804	0.678-0.928		
hs-TnT (≥0.87 μg/ml)	85.2	77.8	0.894	0.831-0.961		

Abbreviations: TB, total bilirubin; WBC, white blood cells; hs-TnT: hypersensitive troponin; AUC, area under the curve; CI, confidence interval;

Table 4. Univariate and multivariate logistic analysis of in-hospital MACE

Variables	Univariate		Multivariate	
	OR (95%CI)	*P* Value	OR (95%CI)	*P* Value
Gender (male)	1.69(0.763-3.767)	0.195		
Age (year)	1.02(1.003-1.055)	0.025	1.03(1.002-1.049)	0.028
BMI	0.98(0.896-1.072)	0.660		
Smoking	1.71(0.933-3.141)	0.083		
Hypoglycemic	0.87(0.506-1.494)	0.614		
Diabetes	0.77(0.3841.542)	0.460		
Heart rate	1.01(0.993-1.027)	0.249		
LVEF	0.96(0.926-0.990)	0.011	0.96(0.928-0.990)	0.010
LDL-C	0.88(0.640-1.205)	0.422		
CK-MB	1.00(0.999-1.001)	0.166		
Culprit Vessels	1.29(1.045-1.601)	0.018	1.25(1.019-1.529)	0.032
Combine (cutoff=1.4)	5.55(3.207-9.609)	0.000	5.85(3.425-9.990)	<0.001

Abbreviations: OR, odds ratio; CI, confidence interval; BMI, Body mass index; LVEF, Left ventricular ejection fraction; LDL-C, low-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes.

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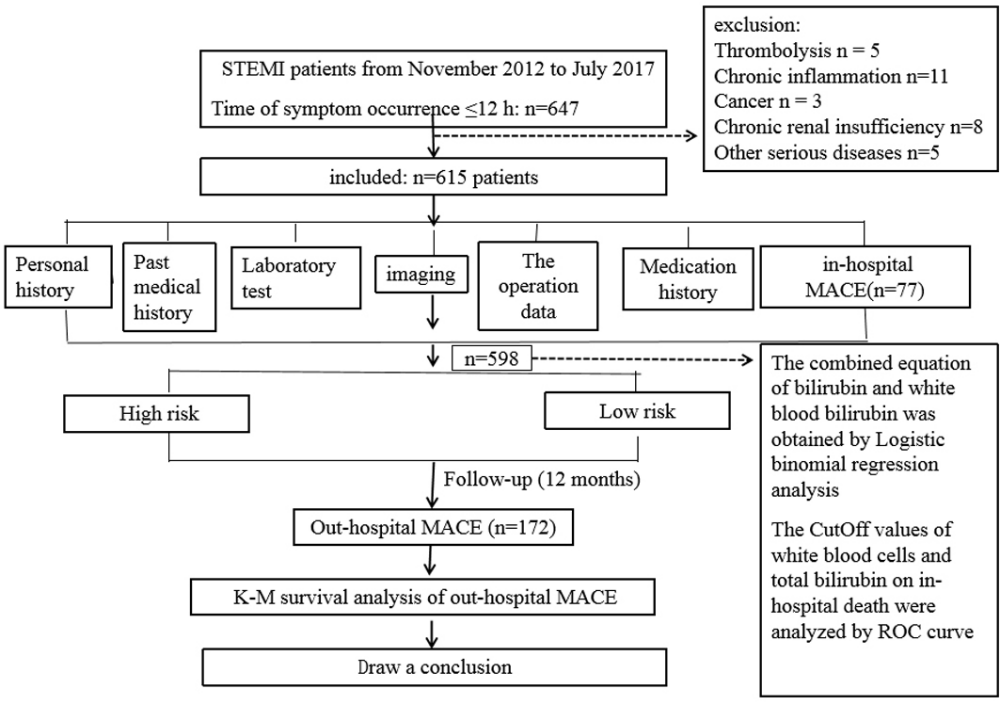
Figure Legends

Figure 1. The flowchart of study design with including and excluding procedures.

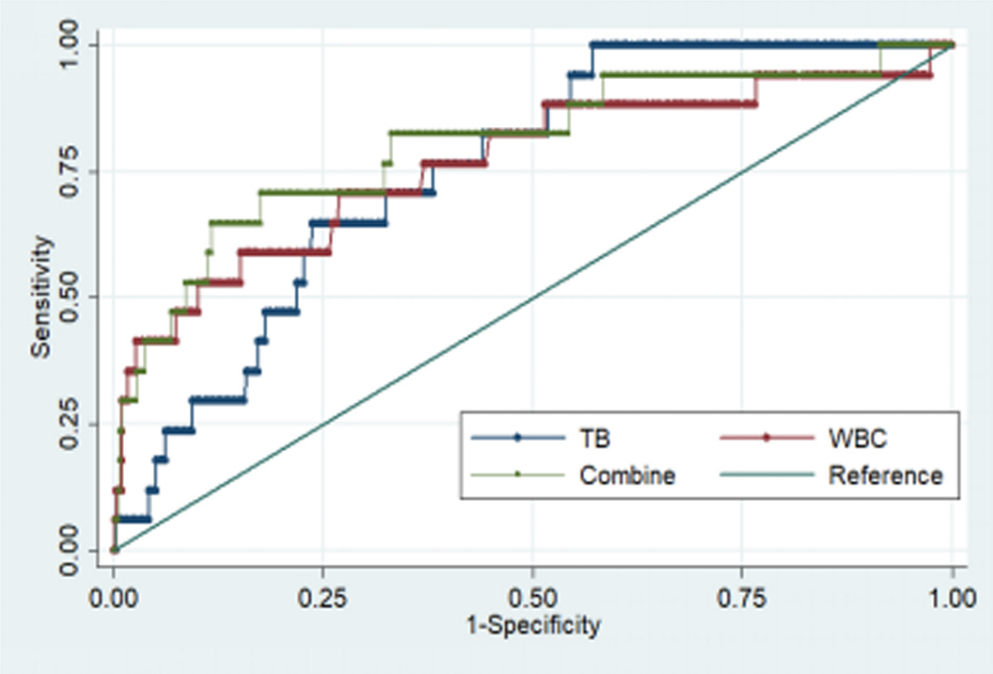
Figure 2. Admission of TB, WBC and Combine as predictors of in-hospital mortality.

Figure 3. Kaplan–Meier survival analysis of long-term MACE.



The flowchart of study design with including and excluding procedures.

90x90mm (300 x 300 DPI)



Admission of TB, WBC and Combine as predictors of in-hospital mortality.

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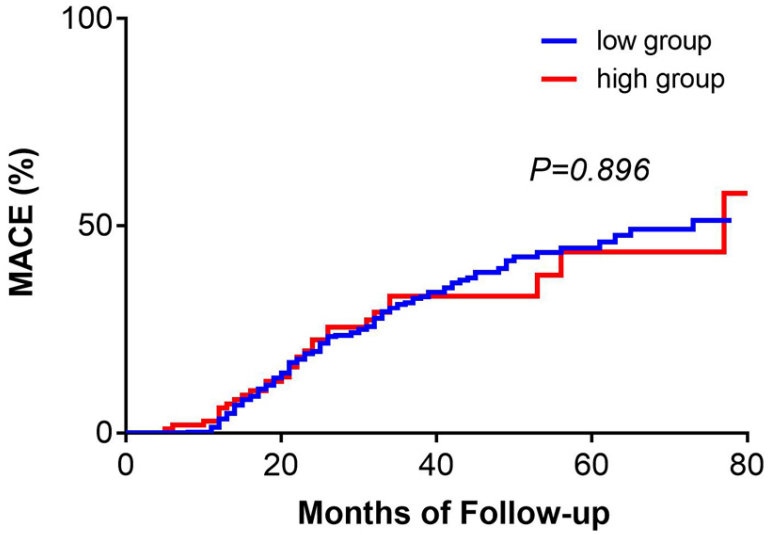

Number at risk					
Low group	505	353	127	42	0
High group	110	76	27	8	1

Kaplan–Meier survival analysis of long-term MACE.

90x90mm (300 x 300 DPI)

The combined value of white blood cell and total bilirubin predict clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study

Supplementary materials

Munire-Tuxun, MD., Qian Zhao, PhD., Yang Xiang, MD., Fen Liu, PhD., Chun-Fang Shan, MD., Xin-Rong Zhou, MD., Ning Song, PhD., Ajiguli-Waisiding, MD., Xue-He, Zhang, MD., Gulandanmu-Aihemaiti, MD., Yi-Ning Yang, PhD., Xiao-Mei Li, PhD.

Supplementary Table S1 Comparison between in-hospital mortality group and survival group

Variables	Survival group(n=598)	Mortality group(n=17)	t/χ^2	*P*
Gender (men) (n,%)	516(86.3)	12(70.6)	3.550	0.067
Age (years)*	58.03±12.15	63.53±12.40	1.839	0.066
BMI (kg/m^2)	25.42±3.25	24.01±2.68	1.785	0.075
Smoking history (n,%)	338(56.5)	11(64.7)	0.451	0.502
Hypertension (n,%)	269(49.5)	8(47.1)	0.039	0.843
Diabetes (n,%)	125(20.9)	3(17.6)	0.106	0.744
Hyperlipemia (n,%)	443(74.1)	14(82.4)	0.593	0.441
SBP (mmHg)	121.59±18.89	119.76±23.65	0.378	0.706
DBP (mmHg)	76.46±13.08	74.18±15.91	0.707	0.480
Heart rate (/min)	81.90±13.88	90.06±23.31	2.333	0.020
LVEF(%)*	58.33±6.67	51.35±8.41	4.222	<0.001
TB (umol/L)*	13.69±5.22	18.47±5.59	3.718	<0.001
DBL (umol/L)	2.18±1.56	2.58±2.10	1.025	0.306
IBL (umol/L)	11.88±5.09	12.29±4.41	0.326	0.744
TC (mmol/L)	4.41±1.18	3.95±0.95	1.611	0.108
TG(mmol/L)	2.05±1.39	1.67±1.06	1.095	0.274
LDL (mmol/L)	2.86±0.85	2.51±0.90	1.686	0.092
HDL (mmol/L)	1.03±0.35	0.90±0.24	0.415	0.678
CKMB (U/L)	281.46±220.92	275.36±139.69	0.113	0.910
WBC (×10^9 /L)*	10.54±3.04	14.59±4.74	5.324	<0.001
Hb (g/L)	144.99±20.35	141.59±24.81	0.676	0.500
PLT (×10^9 /L)	222.58±60.33	207.59±60.52	1.010	0.313
UC (mmol/L)*	5.42±1.80	6.40±3.24	2.137	0.033
CR (umol/L)*	76.24±19.16	86.46±34.23	2.108	0.035
GRACE	133.06±28.01	148.71±36.61	2.250	0.025

LM (n,%)	54(9.0)	1(5.9)	0.201	0.54
LAD (n,%)	518(86.6)	16(94.1)	0.812	0.368
LCX (n,%)	375(62.7)	12(70.6)	0.440	0.507
RAD (n,%)	418(69.9)	15(88.2)	2.670	0.102
Culprit Vessels	2.84±1.27	3.41±1.42	1.840	0.066
Medication				
Aspirin	578(96.7)	14(82.4)	9.393	0.002
Clopidogrel*	553(92.5)	9(52.9)	32.806	0.000
β blockers	492(82.3)	11(64.7)	3.425	0.064
ACEI	429(71.7)	8(47.1)	4.896	0.027
Statins*	559(93.5)	12(70.6)	13.039	0.000

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, Left ventricular ejection fraction; TB, total bilirubin; DBI, direct bilirubin; IBL, indirect bilirubin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes; WBC, white blood cells; Hb, hemoglobin; PLT, Platelet; UC, uric acid; CR, creatinine; GRACE, globla registry of acute coronary events; LM, left main coronary artery; LAD, left anterior descending branch ; LCX, left circumflex branch; RAD, right coronary descending branch; ACEI, angiotensin-converting enzyme inhibitor.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Non
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6-7*
Bias	9	Describe any efforts to address potential sources of bias	7-8
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	Non
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	13

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	13
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	11
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Non
		Cross-sectional study—Report numbers of outcome events or summary measures	Non
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12
		(*b*) Report category boundaries when continuous variables were categorized	Non
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Predicting value of white blood cell and total bilirubin on clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study

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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	Coronary heart disease < CARDIOLOGY, Ischaemic heart disease < CARDIOLOGY, Myocardial infarction < CARDIOLOGY

Predicting value of white blood cell and total bilirubin on clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study

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Short title: White blood cell and total bilirubin predicting clinical outcomes in STEMI

# These authors contribute equally to the work.

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Words:4777 Tables: 4 Figures: 3

ABSTRACT:

Objectives: A combined equation based on white blood cell count (WBC) and total bilirubin (TB) was assessed for its ability to predict adverse clinical outcomes in acute ST-segment elevation myocardial infarction (STEMI) patients with primary percutaneous coronary intervention (PCI).

Design: A single-center, prospective cohort study.

Setting: The First Affiliated Hospital of Xinjiang Medical University.

Method: A total of 615 STEMI patients post primary PCI were enrolled. WBC and TB were collected at admission. Logistic regression was used to determine the combined equation. The primary endpoints were in-hospital mortality and major adverse cardiovascular events (MACE) which composed of cardiac death, cardiac shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction,angina pectoris readmission, severe cardiac insufficiency (cardiac III-IV level), stent restenosis, target vessels reascularization during hospitalization and 36-months follow-up period.

Result: 77 patients occurred in MACE during hospitalization (17 in-hospital mortality). WBC and TB were taken as an independent variables to make a category of logistic regression analysis of in-hospital MACE, the logistic regression model was: Logit (P) =-8.00+0.265 WBC+0.077 TB, the combination of WBC and TB were more valuable on evaluating the in-hospital mortality (AUC=0.804, 95% CI: 0.678~0.929, P<0.001). Multivariate logistic regression analysis showed that combined detection was an independent risk factor for in-hospital MACE (OR=5.85, 95% CI 3.425~9.990, P=0.032). During the follow-up period, 172 patients (29.5%) developed MACE. But the combined detection didn't predict the long-term clinincal outcome.

Conclusion:The combination of WBC and TB is an independent predictor for in-hospital outcomes in patients with STEMI than single detection.

Keywords: ST segment elevation myocardial infarction; white blood cell; total bilirubin; in-hospital mortality; major adverse cardiovascular events

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Strengths and Limitations of the study

- This was the first study to indicate the combined effect of WBC and TB on predicting clinical prognosis in acute STEMI patients post primary PCI.
- The combination of WBC and TB may be better than single biomarker, can be applied in the predicting in-hospital MACE of STEMI patients treated with PCI.
- A single-center prospective cohort study for a small number of patients was fail to adequately exclude the influence of unknown confounding factors on this study.
- The determination of bilirubin in patients with STEMI was only at a preoperative time point, and the dynamic changes of the bilirubin concentrations during hospitalization and follow-up were not clear.

INTRODUCTION

STEMI has been a hot spot in cardiology research with its urgent onset, rapid progress and high mortality. Although reperfusion therapy such as percutaneous coronary intervention (PCI) and fibrinolytic therapy had improved the survival rate for acute ST-segment elevation myocardial infarction (STEMI), the in-hospital mortality rate of STEMI is as high as 4%-12%.[1] It has been a new challenge for clinician to identify high-risk patients timely, evaluate clinical prognosis accurately and prevent the occurrence of major adverse cardiovascular events (MACE). Screening a sensitive and specific biological index has great value for the treatment and prevention of STEMI complications.

In an attempt to identify STEMI patients at high risk of unfavorable outcomes, several predictors of adverse events in STEMI have been investigated. Research indicates that older age,[2] BNP, D-dimer, uric acid and thrombolysis myocardial infarction (TIMI) risk score are associated with a higher risk of adverse events in STEMI patients.[3-6] However, there is no literature report on the combination of white blood cell count (WBC) and total bilirubin (TB) in the guiding significance for the prognosis of STEMI.

STEMI refers to the occurrence of plaque rupture, thrombosis or coronary artery spasm on the basis of coronary atherosclerosis, which results in a sharp decrease or interruption of coronary artery blood supply and a sustained and severe acute ischemia of the corresponding myocardium, leading to acute myocardial necrosis. Studies have shown that there are obvious inflammatory reactions and oxidative stress injury in STEMI.[7-8] Leukocyte elevation after STEMI is an important component of systemic inflammatory response and ischemic tissue repair mechanism. Ischemia-induced chemokines activate white blood cells to chemotaxis to ischemic sites and remove necrotic tissues, white blood cells adhere to the injured vascular wall and form aggregates with blood cells, which eventually lead to thrombosis.[9] Activated white blood cells also produce oxygen free radicals, lysosomal enzymes and other substances, which cause local inflammatory response in ischemic sites.[10] White blood cell, as a marker of inflammation, has been proved to be closely related to the clinical prognosis of STEMI patients,[11] while bilirubin, the end product of heme degradation, is also an endogenous oxidant in vivo, participates in the occurrence and development of myocardial infarction.[12] Heme oxygenase

(HO) regulates the synthesis and catabolism of bilirubin, and keeps the bilirubin content in a dynamic balance in human body.[13] HO-1 is an inducible isoform in response to diverse cellular stress such as oxidative stress, Inflammatory cytokines, heavy metals, cytokines, but is not expressed under normal conditions. Acute myocardial ischemia and hypoxia activates the stress process of the body and produces oxygen free radicals, oxides and infarction-related inflammatory factors, which significantly increase the activity of HO-1, and eventually leads to an increase in bilirubin. Studies has been suggested that bilirubin is elevated in patients with STEMI and has the effect of antioxidant stress, can be used as a biomarker for predicting clinical prognosis.[14]

Thus, there is a strong correlation between inflammatory response and oxidative stress, it's interact and influence each other to promote the development of STEMI. White blood cell and bilirubin, common and fast acquired biomarkers in routine blood tests, could reflect the level of inflammation and oxidative stress injury respectively and that combined information from multiple inflammatory and oxidative stress injury might be more informative. Therefore, the present study firstly combined WBC (an inflammatory biomarker) and TB (oxidative stress biomarker) into a simplified equation and assessed whether this value was predictive of in-hospital MACE in patients with STEMI in order to find a simple and reliable auxiliary index to evaluate and predict the clinical prognosis of STEMI for early treatment.

METHODS

Study design

This was a single-center prospective cohort study designed to assess whether the combination of admission WBC and TB could predict in-hospital and long-term outcomes in patients with STEMI post primary PCI. The study complied with the Declaration of Helsinki, the study protocol was first approved in December 2014 by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China) (approval ID: 20141201-03) and ethics review was conducted in January 2017 (approval ID:20141201-03-1701A). All patients provided written informed consent.

We consecutively enrolled adult patients with STEMI who treated with primary PCI at the Cardiac Center of the First Affiliated Hospital of Xinjiang Medical University between June 2012 and June 2017. STEMI diagnosis was in accordance with previously established guidelines.[1] All patients were treated with PCI within 12 hours after symptoms. Patients who did not have pre-PCI, white blood cell and bilirubin data, who had severe liver and kidney diseases, autoimmune system diseases, severe heart valve diseases, chronic inflammatory diseases, acute infectious diseases, malignant tumors, blood system diseases, active hemorrhage and other diseases, additionally, patients who had an infarct-related lesion unsuitable for stent implantation, and who were lost to follow-up were excluded from the current study.

Data collection

Investigators trained by professionals used uniformly designed questionnaires to collect patients' general information including age, gender, body mass index (BMI), history of smoking, dyslipidemia, hypertension, diabetes, laboratory examination results, angiographic examination results, in-hospital medication and the occurrence of MACE in our hospital by electronic medical records and paper cases.

Blood sampling and hematology measurements

Blood samples were collected from all participants immediately upon hospital admission for complete blood count analyses. In all patients, venous blood samples for laboratory analysis were collected at the time of presentation before the patients were transferred to the heart center. Peripheral blood samples were used for laboratory tests of several markers, including WBC, total cholesterol,

triglycerides, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), total bilirubin, direct bilirubin, indirect bilirubin, creatine kinase isoenzyme (CK-MB), platelet, hemoglobin, creatinine and uric acid. TB, direct bilirubin, indirect bilirubin were measured by Hitachi 7060 automatic biochemical analysis and the automatic blood analysis equipment (sysmex XE-5000 automatic blood analyzer) was used for WBC in our hospital testing center. The normal range for white blood cell is (4-10)×109/L, total bilirubin is 5.5-27.5 umol/L.

All patients were assessed by transthoracic echocardiography within 48 h after primary PCI Standard echocardiographic views were acquired and analyzed by two experienced cardiologists who were unware of grouping information. Left ventricular ejection fraction (LVEF) was measured by B mode echocardiography.

Definition of cardiovascular risk factors

Hypertension was defined as self-reported use of antihypertensive medication within the past 2 week or having systolic blood pressure greater than 140 mmHg and/or diastolic blood pressure greater than 90mmHg. Diabetes was defined as fasting blood glucose of more than 7 mmol/L (or 2 hours after a meal), and/or, using of insulin or oral hypoglycemic agents, or a self-reported history of diabetes. Hyperlipidemia was defined as anyone of the four lipids abnormalities following or self-reported use of antihyperlipidemic medication, total cholesterol concentration>6.22 mmol/L (240 mg/dl), triglyceride concentration>2.26 mmol/L (200 mg/dl), low-density lipoprotein cholesterol concentration>4.14 mmol/L (160 mg/dl), high-density lipoprotein cholesterol concentration<1.04 mmol/L (40 mg/dl).[15] GRACE score of patients was calculated using eight indicators of GRACE admission risk score according to the medical history, signs and laboratory examination results of admission, including Killip classification of cardiac function, age, heart rate, arterial systolic pressure, serum creatinine, ST segment changes of electrocardiogram, elevated myocardial markers, cardiac arrest at admission. Positive smoking history was defined as having smoked daily or occasionally in the past.

PCI and Medication

Patients were given loaded medication before operation: aspirin+clopidogrel (300 mg+300 mg) orally and secondary clinical prophylactic medication after PCI. In our study , angiograms were independently reviewed by two interventional cardiologists who were blinded of patients' information in the Cardiac Center of the First Affiliated Hospital of Xinjiang Medical University. The authors of this article were not involved in the PCI treatment of these patients. At the same time, the results of angiography were recorded for each patient. The main vessel was left main coronary artery, left anterior descending branch, left circumflex branch and right coronary descending branch. The degree of coronary artery stenosis was assessed by AXIOM Artis FC/BC cardiovascular computer quantitative analysis system in Germany. Cross-sectional projections of right and left coronary arteries were recorded. Most of the approaches were via radial artery, and a few were via femoral artery. There are at least 4 projection positions for the left coronary artery and at least 2 projection positions for the right coronary artery. If necessary, other postures are added to fully display each segment of the coronary artery. The imaging data of each patient undergoing coronal angiography are kept for reference. After angiography, more than two experienced interventional physicians including the surgeon discussed the results and filled in the detailed report of the results of coronary angiography after reaching consensus. The number of stenosis branches is defined as a single vessel>70% or two vessels more than 50%. PCI was performed by a professional cardiologist, and the stent implantation site, balloon type, pressure, stent size and type, TIMI blood flow classification were filled in detail after the operation.

Successful coronary angiography is defined as defined as residual stenosis<50% and thrombolysis in myocardial Infarction grade 3 flow after the procedure.

Clinical Outcomes and Definitions

In-hospital endpoints: (1) In-hospital mortality; (2) Major adverse cardiovascular events (MACE) during hospitalization including cardiac shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction.

Long-term follow-up endpoints: MACE including cardiac death, angina pectoris readmission, non-fatal myocardial infarction, malignant arrhythmia (ventricular tachycardia and ventricular

fibrillation), severe cardiac insufficiency (cardiac III-IV level), stent restenosis, target vessels reascularization. Follow-up was performed by telephone interview for all patients enrolled. For those patients who reached at least one of the primary endpoints, recorded data and medical reports were evaluated to determine inclusion criteria.

Statistical Analysis

Data were collected using Epidata3.1 (Odense, Denmark) and double checked. Analyses were carried out using Stata 15.0 software (Stata Corp LP, College Station, TX, USA). Continuous variables with a Gaussian distribution are presented as average±standard deviation (SD), and those with a non-Gaussian distribution are presented as median values with corresponding 25th to 75th percentiles. The differences between groups were evaluated using Student's unpaired *t* test, one-way ANOVA or the Mann-Whitney rank test. Categorical variables were expressed as numbers and frequencies and the difference between groups was detected by Chi-square test. Logistic regression was used to determine the combined equation Receiver operating characteristic (ROC) curve was used to evaluate the combined value of WBC and TB to predict the best clinical boundary of in-hospital mortality. Univariate and multivariable logistic regression was used to analyze all potential influencing factors associated with in-hospital MACE. Odds ratio (OR) are shown with 95% confidence intervals (CIs).The multivariate analysis controlled for all factors with significant associations emerging from the univariate analysis and the traditional risk factors for atherosclerosis.Kaplan-Meier plots were generated, and the log-rank test was used to compare the resulting curves. All statistical tests were bilateral tests, and the significance level was 0.05.

We included the subjects according to the sample size calculation formula. The sample size calculation formula in this research is $n = 2\overline{pq}(u_\alpha + u_\beta)^2 / (p_1 - p_0)^2$

Based on the sample size of the current study, the power of the research results using *Power and sample size calculation* is 81.2%.

Patients and public involvement

The study designed to assess whether the combination of admission WBC and TB could predict in-hospital and long-term outcomes in patients with STEMI treated with primary PCI. However, no patients or members of the public were included in the design, recruitment or conduct of the study.

The results of measurements would be disseminated to participants after the study which was completed by the study team. The burden of the intervention will not be assessed by patients themselves.

RESULTS

Baseline clinical characteristics

During June 2012 and June 2017, we consecutively recruited 647 adult STEMI patients who underwent primary PCI. Of these patients 32 were excluded according to the exclusion criteria. A total of 615 STEMI patients (525 males and 90 female) with an average age of 58 years were included in this study. In-hospital MACE was found in 77 patients, including 17 patients with in-hospital mortality. Figure 1 depicts the flowchart of the study design. The comparison of the MACE group and non-MACE group are summarized in ***Table 1,*** including 17 patients with in-hospital mortality (*Suppl Table S1).*

The patients with MACE were older than without MACE group (61.2±11.8 vs 57.8±12.2, *P*=0.019). The LVEF in MACE group was worse than that of non-MACE group (55.48±7.82 vs 58.52±6.58, *P*<0.001). The laboratory data of the patients showed that the WBC and TB was (13.01±4.11)×10^9/L and 17.21±6.49 mmol/L in the MACE group, and (10.31±2.85)×10^9/L and 13.34±4.91 mmol/L in the non-MACE group, respectively (*P*<0.001). Patients in MACE group also had significantly greater levels of uric acid (6.67±2.40 vs 5.27±1.70 umol/L, *P*<0.001), creatinine (83.95±24.38 vs 75.46±18.79 umol/L, *P*=0.004) and greater number of culprit vessels (3.21±1.25 vs 2.80±1.27, *P*=0.009). The TIMI flow grade after PCI in non-MACE group was higher than MACE group［3(1-3) vs 2(1-3), *P*=0.023］. The usage rates of clopidogrel (92.6% vs83.1%, *P*=0.006) and statins (93.9% vs 85.7%, *P*=0.009) in the MACE group were lower than those in the non-MACE group.

No significant differences were found between the MACE group and non-MACE group in terms of gender, BMI, smoking, hypertension, diabetes, hyperlipidemia, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, Global Registry of Acute Coronary Events (GRACE) score, direct bilirubin, indirect bilirubin, total cholesterol, triglycerides, HDL-C, LDL-C, CK-MB, platelet, hemoglobin and culprit vessels location and other drug utilization rates **(*Table 1*).**

Ability of WBC and TB to predict short-term clinical outcome

WBC and TB were combined using logistic regression. Binomial logistic regression analysis was performed with in-hospital mortality as dependent variable and WBC and TB as independent variables,

the logistic regression model was: Logit (*P*) =-8.00+0.265 WBC+0.077 TB ***(Table 2)***, so the "combined" value refers to Logit (P) in our report. The recommended cut-off value for combined value based on the maximum of Youden's index on the ROC curve was 1.40 and it had 82.4% sensitivity and 64.7% specificity in predicting in-hospital mortality. ROC curve was used to determine the predictive value of WBC and TB for the occurrence of in-hospital mortality in STEMI patients after primary PCI. The optimal cut-off point of WBC was 12.2×10^9/L with sensitivity 71%, and specificity 73.1% (AUC=0.765 95%CI:0.625-0.904), the best cut-off value of TB was 14.4 mmol/L with sensitivity 76.5% and specificity 62% (AUC=0.751 95%CI:0.660-0.842).

We made a comparison between new predictive model and traditional predictive model based on hypersensitive troponinns (hs-TnT), The recommended cut-off value for peak troponins on the ROC curve was 0.87ug/ml and it had 85.2% sensitivity and 77.8% specificity in predicting in-hospital mortality(AUC=0.894 95%CI:0.831-0.961). The combination of WBC and TB were more valuable (AUC=0.804, 95%CI:0.678-0.928, *P*<0.001) than single WBC and TB but not as valuable as hs-TnT. The area under the curves of WBC, TB and logistic regression combined detection was 0.765, 0.751 and 0.804 separately, which showed significant differences (*P*<0.05) (Table 3, ***Figure 2***). Further, the c-statistic was increased from 0.778 to 0.835 when a combination of WBC and TB.

WBC and TB levels predicted clinical outcomes

Based on the cut-off value of combined for WBC and TB, STEMI patients were further divided into the high- and low-risk groups. The independent risk factors affecting MACE in hospital were analyzed by logistic regression analysis. We first performed univariate logistics analysis, and in the multivariable logistic regression, we included a total of 12 indicators. To avoid the influence of traditional factors on the results, we select the traditional risk factors for atherosclerosis, including age, body mass index (BMI), gender, smoking, hypertension, diabetes and low-density lipoprotein cholesterol. We also choose age, diabetes, hypertension, heart rate because they are included in the TIMI risk score, with left ventricular ejection fraction (LVEF), culprit vessels and combine detection, has clinical significance in the univariate analysis. And we choose CK-MB, because CK-MB represents the area of myocardial necrosis and we considered there is a relationship between CK-MB

and the poor prognosis of patients. After adjusting, a significant association was noted between combined detection and the adjusted risk of in-hospital MACE (OR=5.85, 95%CI 3.425~9.990, P<0.001). The results showed that the higher value of equation is an independent predictor for in-hospital MACE of STEMI patients received primary PCI. Other independent predictors of in-hospital MACE were age (OR=1.03, 95% CI: 1.002~1.049, P=0.028), LVEF (OR=0.960, 95% CI: 0.928~0.990, P=0.010) and number of culprit vessels (OR=1.25, 95% CI: 1.019~1.529, P=0.032) ***(Table 4)***.

All patients were followed up for an average of 30.2 months, follow-up via telephone was complete in 583 patients or family members, 15 (2.5%) patients were lost to follow-up. After following 172 patients occurred in MACE with the incidence of out-hospital MACE was 29.5%. Kaplan-Meier survival analysis of long-term MACE was made by dividing the best truncation value into two groups. The results showed that there was no significant difference in the long-term incidence of MACE between the two groups (P=0.869, ***Figure 3***).

DISCUSSION

The current study is the first to indicate that the combined effect of WBC and TB could better predict prognosis (in terms of in-hospital mortality and in-hospital MACE) in acute STEMI patients undergoing PCI compared to single predictor independently. Our studies suggesting that those with higher WBC and TB had a higher likelihood of in-hospital complications, and a higher hazard of in-hospital mortality, independent of potential confounding factors.

Inflammatory processes play an important role in the development of STEMI. It has been suggested that there is a positive correlation between leucocyte elevation and myocardial infarction area.[16-18] White blood cells in the unstable plaque lesions adhesion, aggregation and release the tissue factor and promote blood clotting enzyme molecules, prompt production platelet activation and fibrin thrombi. When great quantities of white blood cell infiltrate in the ischemic area , because of its weak deformation, slow through the capillary, capillaries are more likely to block the ischemia area, increase myocardial microcirculation, led to a lack of oxygen to microvascular no reflow and aggravate ischemic infarction area,[16, 19]The increase of WBC count has an impact on the occurrence, development and prognosis of acute myocardial infarction, and the relevant mechanism is generally believed that the capillary is blocked by a large number of white blood cells, which causes no reflow of microvessels and increases the size of myocardial infarction,[20] Studies have proposed a positive correlation between leukocyte elevation and myocardial infarction area. [21]

There is indeed a lot of literature on the relationship between leukocytes and prognosis of ischemic heart disease. Kyne suggested that the increase of WBC count and neutrophilic granulocyte percentage in peripheral blood within12 hours was an independent predictor of heart failure in the first four days after acute myocardial infarction (AMI).[22] Kojima demonstrated an elevated WBC was significantly associated with higher risk of in-hospital mortality.[23] Nez reported that white blood cell count independently increased the risk of death for 30 days and 1 year in STEMI patients.[24] Çiçek G examined that the incidence of MACE in STEMI patients with elevated white blood cell count increased significantly during hospitalization.[25]

This means that leucocyte elevation during myocardial infarction not only has a purely restorative physiological role, but also has a pathological role. Combined with myocardial infarction size, non

coronary reflux and decreased cardiac function, increased white blood cells in STEMI patients may indicate poor clinical prognosis.

In addition to the inflammatory, oxidative stress injury plays an important role in the evolution of STEMI.[26] Under physiological conditions, antioxidant substances in the body are in equilibrium with oxygen free radicals. When STEMI occurs, the surface of coronary artery intima and lipid plaques are damaged. Plaque rupture and thrombosis leads to acute myocardial ischemia and hypoxia, activates the stress process cells and cytoplasm produce large amounts of oxygen free radicals through enzymatic and non-enzymatic systems, which leads to the oxidation of macromolecular protein, cell membrane lipid peroxidation and DNA damage, and finally cause the death of heart muscle cells. Myocardial cells after STEMI are stimulated by ischemia and hypoxia to produce large amounts of oxygen free radicals and oxidizing substances, a large amount of antioxidant substances are consumed so that the body loses its inhibiting effect on the formation of oxidative substances, and the balance between antioxidant substances and oxidative substances is broken, which will cause cell structure damage. [27]Oxidative stress injury can cause plaque instability, rupture and erosion, leading to thrombosis and complete infarction-related artery occlusion.[28] So when STEMI occurs, the body needs antioxidants to deal with the damage caused by oxidative stress.

Antioxidants and markers which can represent oxidative reactions in vivo have been reported in the previous literature. Malondialdehyde (MDA) is the metabolite of unsaturated fatty acids in biofilms after oxidative damage, while advanced oxidation protein products (AOPP) is the metabolite of proteins after free radical attack and oxidative reaction, which can reflect the degree of oxidative reaction.[29] Superoxide dismutase (SOD) and glutathione (GSH), as important antioxidant substances in the body, can remove excess oxygen free radicals in the body and maintain the reduced state of cells[30]. When acute coronary syndrome occurs, GSH and SOD in serum are reduced.[31-32] Previous studies have reported impaired oxidation and antioxidant balance and increased oxidative stress in STEMI patients.[33] Gur showed that total antioxidant capacity (TAC) levels were significantly lower and total oxidation state (TOS), oxidative stress index (OSI),lipid hydroperoxide (LOOH) were significantly higher in patients with no-reflow compared to normal flow group in patients with anterior STEMI undergoing primary PCI .[8] SARDAR showed that in the development process from unstable

angina (UA) to STEMI, total sialic acid, oxidative modified protein and other oxidation substances were gradually increased, and the antioxidant status was gradually decreased.[34] These oxidation substances were more significant in STEMI patients. However, these substances require a certain amount of technology and time to test, and not all hospitals have the capability.

Bilirubin is an endogenous oxidant in vivo. In recent years, more and more attention has been paid to the role of bilirubin in the pathophysiology of major cardiovascular diseases such as myocardial infarction,[35] atherosclerosis[36] and cardiovascular complications of diabetes.[37]As an antioxidant in the body, bilirubin is involved in the occurrence and development of myocardial infarction and plays an anti-oxidative role. Okuhara showed that compared with non-AMI patients, the serum bilirubin concentration and Fe^2 in AMI patients increased temporarily 18-21 hours after the onset of the disease.[12] Elevated bilirubin has antioxidant capacity and the ability to remove peroxides, which can prevent the deterioration of disease, Sexual resistance to oxidative stress in the myocardium may be considered as a beneficial self-regulation in acute ischemia. Celik reported that total bilirubin levels in STEMI were independently associated with no-reflow coronary artery.[38] Halit reported that bilirubin levels in STEMI patients with impaired blood flow were higher than those in normal blood flow group. They suggested that the more severe the atherosclerosis, the higher the activity of HO-1 enzyme after myocardial infarction, the more obvious the increase of bilirubin levels.[39] The degree of increase was related to the severity of the lesion to a certain extent. Glu studied 1624 STEMI patients and found that compared with the bilirubin group less than 9.0 mg/dl, the bilirubin group higher than 9.0 mg/dl had higher in-hospital mortality, but the bilirubin level was not related to long-term prognosis.[40] Chung followed up 1111 STEMI patients treated with PCI in hospital and 12 months after operation. The results showed that the incidence of MACE and cardiogenic mortality in hyperbilirubinemia group were higher than those in low bilirubinemia group.[14] It can be concluded that the oxygen free radicals produced by oxidative stress injury and the damage of various oxides to the body under stress may exceed the protective and antioxidant effect of bilirubin on the body, which caused the protective effect of high bilirubin level is not significant. In contrast to stable coronary disease, the serum TB levels show different associations in stressful conditions

Therefore, STEMI is a disease both with inflammatory and oxidative stress injury. As such,

inflammatory biomarkers alone, or oxidative stress are not sufficient to capture the entire pathophysiologic process involved in STEMI. Instead, markers which combine inflammatory and oxidative stress information is a better at reflecting the process in STEMI.

In present study, the area under the ROC curve for combined equation in predicting mortality was higher than those for WBC and TB, multivariate logistic regression analysis showed the high value of equation is an independent predicted of worse in-hospital MACE. In addition to the elevation of white blood cells and bilirubin in the MACE group, TIMI blood flow was also worse than that in the non-MACE group. Integrate previous studies, we think the increase of WBC count causes no reflow of micro vessels and increases the size of myocardial infarction and the increase of TB levels reflect the severity of coronary damage, which suggests that combining WBC and TB has a stronger predictive power for in-hospital MACE than individual markers. Previous studies have shown that the in-hospital mortality of STEMI in China between 2001 and 2011 was 7.0%.[41] In our study, in-hospital MACE was found in 77 (12.5%) patients and there were 17 (2.8%) patients occurred in death in-hospital among the 615 STEMI patients, the in-hospital mortality was comparable to the China's national level, so we think our results are reliable. We did not use troponin levels and other biomarkers that are recommended generally to predict clinical outcomes. As we know, troponin test fee will be more expensive and testing requirements for basic-level hospitals is too high, not all hospitals have this condition, while white blood cell and bilirubin, common and fast acquired biomarkers in routine blood tests, can be detected by most hospitals because of low test cost .So we still suggested that combining WBC and TB was of particular clinical importance for the subset of patients with STEMI at admission.

There are some limitations in our study. This study is a single center experiment for a few patients which have the limitation of prospective cohort design. In our study bilirubin was measured in patients at only one time point before PCI in this study. The dynamic changes of serum leucocyte count and bilirubin concentration during hospitalization and follow-up were not clear. In this study, oxidative stress related indicators and HO-1 enzyme activity were not directly tested, and various inflammatory markers such as C-reactive protein, neutrophils and lymphocytes were not included. In addition to determine the exact role of leukocytes and bilirubin in patients for long-term prognosis more experimental basic studies are needed.

CONCLUSIONS

The combination of WBC and TB is better than single biomarker, can be applied in the predicting in-hospital MACE of STEMI patients treated with primary PCI. The high value of equation is a independent predictor of worse in-hospital MACE, it can be used to identify high-risk STEMI patients and accurately predict their clinical prognosis for early treatment.

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CONTRIBUTORS

Conceived and designed the experiments: Xiaomei Li, Yining Yang, Analyzed the data: Qian Zhao, Fen Liu. Contributed reagents/materials/analysis tools: Chun-Fang Shan, Xin-Rong Zhou, Ning Song, Ajiguli-Waisiding, Xue-He, Zhang, Gulandanmu-Aihemaiti. Quality control the study and revision: Xiaomei Li, Yining Yang, Yang Xiang. Wrote the paper: Munire-Tuxun, Qian Zhao. All authors read and approved the final manuscript.

ETHICS APPROVAL

This study was carried out in accordance with the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China) (approval ID:20141201-03-1701A).

DATA AVAILABILITY STATMENT

All data relevant to the study are included in the article and are not publicly available.

FOUNDING

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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Table 1. Comparison between MACE group and non-MACE group.

Variables	Non-MACE (n=538)	MACE (n=77)	t/χ^2	*P*
Gender (men) (n,%)	465 (86.4)	63 (81.8)	1.180	0.277
Age (years)*	57.8±12.2	61.2±11.8	2.359	0.019
BMI (kg/m^2)	25.45±3.29	24.99±2.90	1.161	0.246
Smoking history (n,%)	302 (56.1)	47 (61.0)	0.660	0.416
Hypertension (n,%)	264 (49.1)	40 (51.9)	0.223	0.637
Diabetes (n,%)	115 (21.4)	13 (16.9)	0.825	0.364
Hyperlipemia (n,%)	396 (73.6)	61 (79.2)	1.112	0.292
SBP (mmHg)	121.88±19.08	118.75±18.45	1.348	0.178
DBP (mmHg)	76.58±13.13	75.18±13.33	0.870	0.385
Heart rate (/min)	81.76±13.64	84.69±17.93	1.686	0.092
LVEF (%)*	58.52±6.58	55.48±7.82	3.703	<0.001
TB (umol/L)*	13.34±4.91	17.21±6.49	5.040	<0.001
DBL (umol/L)	2.19±1.60	2.18±1.41	0.100	0.920
IBL (umol/L)	11.97±5.11	11.36±4.77	0.986	0.325
TC (mmol/L)	4.4±1.18	4.38±1.18	0.193	0.847
TG (mmol/L)	2.05±1.40	1.93±1.33	0.734	0.463
LDL (mmol/L)	2.86±0.85	2.8±0.84	0.544	0.587
HDL (mmol/L)	1.03±0.35	1.03±0.31	0.004	0.997
CK-MB (U/L)	277.73±221	306.14±204.18	1.065	0.287
WBC (×10^9 /L)*	10.31±2.85	13.01±4.11	5.569	<0.001

Hb (g/L)	145.24±19.96	142.47±23.72	1.114	0.266
PLT ($\times 10^9$ /L)	222.25±59.41	221.6±66.85	0.089	0.929
UC (mmol/L)*	5.27±1.70	6.67±2.40	4.921	<0.001
CR (umol/L)*	75.46±18.79	83.95±24.38	2.934	0.004
GRACE	133(115~148)	136(116~158)	1.250	0.211
LM (n,%)	51(9.5)	4(5.2)	1.519	0.218
LAD (n,%)	463(86.1)	71(92.2)	2.227	0.136
LCX (n,%)	334(62.1)	53(68.8)	1.315	0.251
RAD (n,%)	372(69.1)	61(79.2)	3.282	0.070
Culprit Vessels*	2.80±1.27	3.21±1.25	2.631	0.009
TIMI grade post-PCI*	3 (1~3)	2 (1~3)	2.567	0.023
Medication				
Aspirin	519(96.5)	73(94.8)	0.518	0.472
Clopidogrel*	498(92.6)	64(83.1)	7.635	0.006
β blockers	441(82.0)	62(80.5)	0.095	0.758
ACEI	382(71.0)	55(71.4)	0.006	0.939
Statins*	505(93.9)	66(85.7)	6.739	0.009

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, Left ventricular ejection fraction; TB, total bilirubin; DBI, direct bilirubin; IBL, indirect bilirubin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes; WBC, white blood cells; Hb, hemoglobin; PLT, Platelet; UC, uric acid; CR, creatinine; GRACE, globla registry of acute coronary events; LM, left main coronary artery; LAD, left anterior descending branch ; LCX, left circumflex branch; RAD, right coronary descending branch; TIMI, thrombolysis in myocardial infarction; PCI, percutaneous coronary intervention; ACEI, angiotensin-converting enzyme inhibitor.

1

Table 2. The combined equation of logistic regression of in-hospital mortality

Variables	*b*	*OR*	*95% CI*	*P*
TB	0.077	1.080	-0.005-0.159	0.067
WBC	0.265	1.303	0.125-0.405	<0.001
Constant term	-8.004	0.002		<0.001

Abbreviations: TB, total bilirubin; WBC, white blood cells; OR, odds ratio; CI, confidence interval;

Table 3. Admission of TB, WBC and combine as predictors of in-hospital mortality

Variables	Sensitivity (%)	Specificity (%)	AUC (0 to 1.0)	95% CI	C-statistic	P vale
TB (≥14.4 mmol/L)	76.5	62.0	0.751	0.660-0.842		
WBC (≥12.2 10^9/L)	71.0	73.1	0.765	0.625-0.904	7.989	0.032
Combine (≥1.4)	82.4	64.7	0.804	0.678-0.928		
hs-TnT (≥0.87 μg/ml)	85.2	77.8	0.894	0.831-0.961		

Abbreviations: TB, total bilirubin; WBC, white blood cells; hs-TnT: hypersensitive troponin; AUC, area under the curve; CI, confidence interval;

Table 4. Univariate and multivariate logistic analysis of in-hospital MACE

Variables	Univariate		Multivariate	
	OR (95%CI)	*P* Value	OR (95%CI)	*P* Value
Gender (male)	1.69(0.763-3.767)	0.195		
Age (year)	1.02(1.003-1.055)	0.025	1.03(1.002-1.049)	0.028
BMI	0.98(0.896-1.072)	0.660		
Smoking	1.71(0.933-3.141)	0.083		
Hypoglycemic	0.87(0.506-1.494)	0.614		
Diabetes	0.77(0.3841.542)	0.460		
Heart rate	1.01(0.993-1.027)	0.249		
LVEF	0.96(0.926-0.990)	0.011	0.96(0.928-0.990)	0.010
LDL-C	0.88(0.640-1.205)	0.422		
CK-MB	1.00(0.999-1.001)	0.166		
Culprit Vessels	1.29(1.045-1.601)	0.018	1.25(1.019-1.529)	0.032
Combine (cutoff=1.4)	5.55(3.207-9.609)	0.000	5.85(3.425-9.990)	<0.001

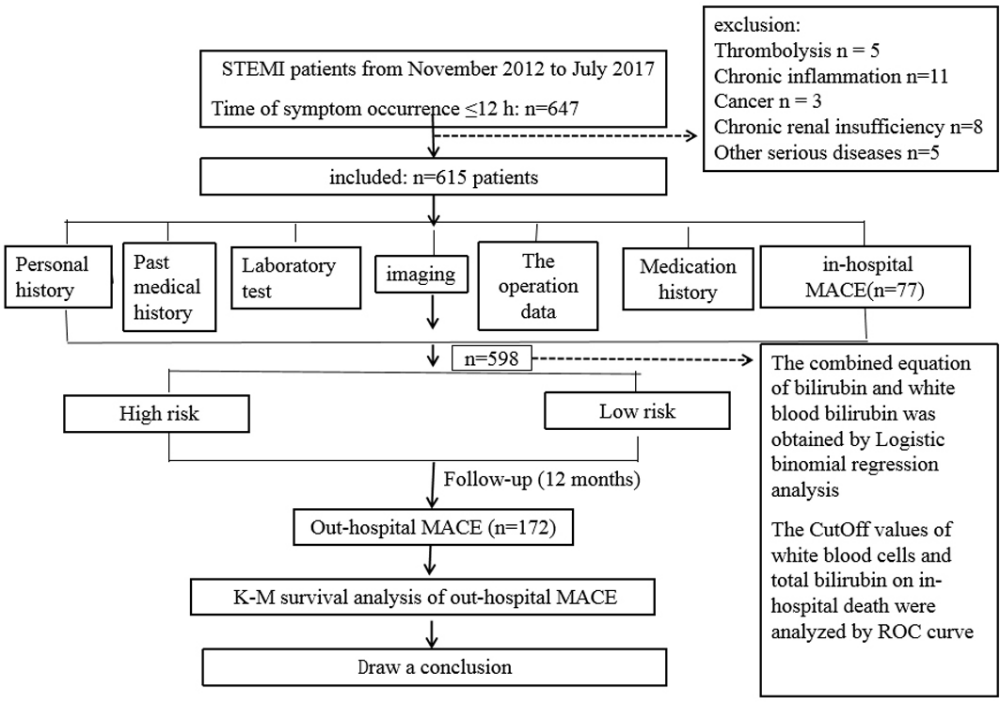
Abbreviations: OR, odds ratio; CI, confidence interval; BMI, Body mass index; LVEF, Left ventricular ejection fraction; LDL-C, low-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes.

Figure Legends

Figure 1. The flowchart of study design with including and excluding procedures.

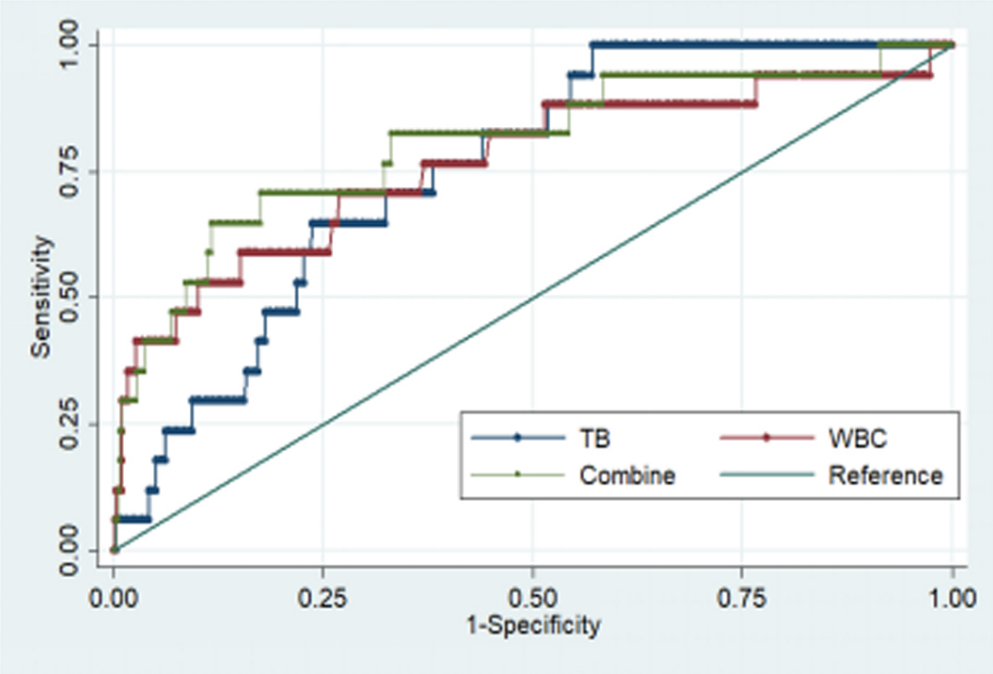
Figure 2. Admission of TB, WBC and Combine as predictors of in-hospital mortality.

Figure 3. Kaplan–Meier survival analysis of long-term MACE.



The flowchart of study design with including and excluding procedures.

90x90mm (300 x 300 DPI)



Admission of TB, WBC and Combine as predictors of in-hospital mortality.

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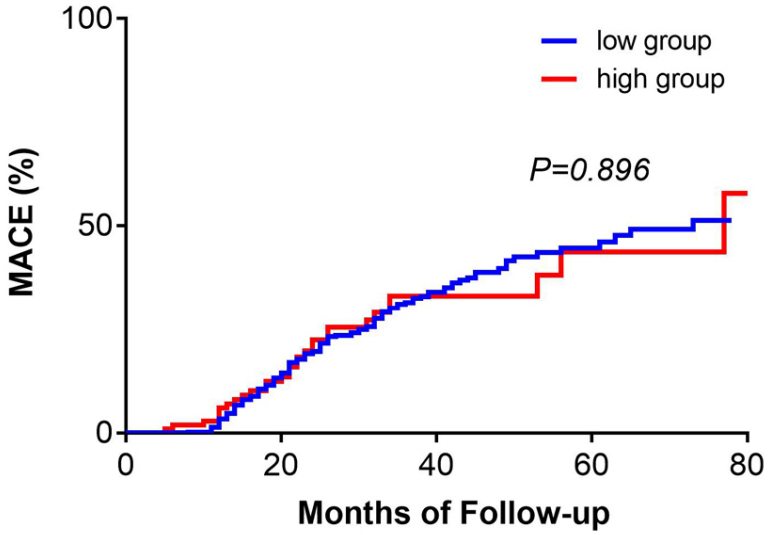

Number at risk					
Low group	505	353	127	42	0
High group	110	76	27	8	1

Kaplan–Meier survival analysis of long-term MACE.

90x90mm (300 x 300 DPI)

The combined value of white blood cell and total bilirubin predict clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study

Supplementary materials

Munire-Tuxun, MD., Qian Zhao, PhD., Yang Xiang, MD., Fen Liu, PhD., Chun-Fang Shan, MD., Xin-Rong Zhou, MD., Ning Song, PhD., Ajiguli-Waisiding, MD., Xue-He, Zhang, MD., Gulandanmu-Aihemaiti, MD., Yi-Ning Yang, PhD., Xiao-Mei Li, PhD.

Supplementary Table S1 Comparison between in-hospital mortality group and survival group

Variables	Survival group(n=598)	Mortality group(n=17)	t/χ^2	*P*
Gender (men) (n,%)	516(86.3)	12(70.6)	3.550	0.067
Age (years)*	58.03±12.15	63.53±12.40	1.839	0.066
BMI (kg/m^2)	25.42±3.25	24.01±2.68	1.785	0.075
Smoking history (n,%)	338(56.5)	11(64.7)	0.451	0.502
Hypertension (n,%)	269(49.5)	8(47.1)	0.039	0.843
Diabetes (n,%)	125(20.9)	3(17.6)	0.106	0.744
Hyperlipemia (n,%)	443(74.1)	14(82.4)	0.593	0.441
SBP (mmHg)	121.59±18.89	119.76±23.65	0.378	0.706
DBP (mmHg)	76.46±13.08	74.18±15.91	0.707	0.480
Heart rate (/min)	81.90±13.88	90.06±23.31	2.333	0.020
LVEF(%)*	58.33±6.67	51.35±8.41	4.222	<0.001
TB (umol/L)*	13.69±5.22	18.47±5.59	3.718	<0.001
DBL (umol/L)	2.18±1.56	2.58±2.10	1.025	0.306
IBL (umol/L)	11.88±5.09	12.29±4.41	0.326	0.744
TC (mmol/L)	4.41±1.18	3.95±0.95	1.611	0.108
TG(mmol/L)	2.05±1.39	1.67±1.06	1.095	0.274
LDL (mmol/L)	2.86±0.85	2.51±0.90	1.686	0.092
HDL (mmol/L)	1.03±0.35	0.90±0.24	0.415	0.678
CKMB (U/L)	281.46±220.92	275.36±139.69	0.113	0.910
WBC (×10^9 /L)*	10.54±3.04	14.59±4.74	5.324	<0.001
Hb (g/L)	144.99±20.35	141.59±24.81	0.676	0.500
PLT (×10^9 /L)	222.58±60.33	207.59±60.52	1.010	0.313
UC (mmol/L)*	5.42±1.80	6.40±3.24	2.137	0.033
CR (umol/L)*	76.24±19.16	86.46±34.23	2.108	0.035
GRACE	133.06±28.01	148.71±36.61	2.250	0.025

LM (n,%)	54(9.0)	1(5.9)	0.201	0.54
LAD (n,%)	518(86.6)	16(94.1)	0.812	0.368
LCX (n,%)	375(62.7)	12(70.6)	0.440	0.507
RAD (n,%)	418(69.9)	15(88.2)	2.670	0.102
Culprit Vessels	2.84±1.27	3.41±1.42	1.840	0.066
Medication				
Aspirin	578(96.7)	14(82.4)	9.393	0.002
Clopidogrel*	553(92.5)	9(52.9)	32.806	0.000
β blockers	492(82.3)	11(64.7)	3.425	0.064
ACEI	429(71.7)	8(47.1)	4.896	0.027
Statins*	559(93.5)	12(70.6)	13.039	0.000

Abbreviations: BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, Left ventricular ejection fraction; TB, total bilirubin; DBI, direct bilirubin; IBL, indirect bilirubin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CKMB, creatine kinase isoenzymes; WBC, white blood cells; Hb, hemoglobin; PLT, Platelet; UC, uric acid; CR, creatinine; GRACE, globla registry of acute coronary events; LM, left main coronary artery; LAD, left anterior descending branch ; LCX, left circumflex branch; RAD, right coronary descending branch; ACEI, angiotensin-converting enzyme inhibitor.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	Non
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6-7*
Bias	9	Describe any efforts to address potential sources of bias	7-8
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	Non
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	13

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	13
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	11
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	Non
		Cross-sectional study—Report numbers of outcome events or summary measures	Non
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12
		(*b*) Report category boundaries when continuous variables were categorized	Non
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Non
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.