Regulatory T Cell as Predictor of Intramyocardial Hemorrhage in STEMI Patients After Primary PCI

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Research Article

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Abstract

Background: Intramyocardial hemorrhage (IMH) is a result of ischemia-

reperfusion injury in ST-segment elevation myocardial infarction (STEMI) after primary percutaneous coronary intervention (PPCI). Despite patients with IMH show poorer prognoses, studies investigating predictors of IMH occurrence are scarce. This study firstly investigated the effectiveness of regulatory T cell (Treg), peak value of Creatine Kinase MB (pCKMB), high-sensitivity C-reactive protein (hsCRP), and left ventricular end-systolic diameter (LVESD) as predictors for IMH in STEMI patients received PPCI.

Methods: A prospective observational cohort study was performed in STEMI patients with cardiac magnetic resonance examination 6.3±2.3 days after PPCI. Logistic regression analysis was used to screen the risk factors for IMH. The predictive ability of risk factors for IMH were determined by Receiver operating characteristic curves, net reclassification improvement (NRI), integrated discrimination improvement (IDI) and C-index.

Results: Of the 182 patients, 80 patients (44.0%) developed IMH. On multivariable analysis, all 4 biomarkers were independent predictors of IMH [odds ratio (OR) and 95% confidence interval (CI): 0.350(0.202-0.606) for Treg, 1.004(1.001-1.006) for pCKMB, 1.060(1.022-1.100) for hsCRP, and 3.329(1.346-8.236) for LVESD]. After propensity score matching, the biomarkers individually and together significantly predicted IMH with areas under the curve of 0.750 for Treg, 0.721 for pCKMB, 0.656 for hsCRP, 0.633 for LVESD, and 0.821 for the integrated 4-marker panel. The addition of integrated 4-marker panel to a baseline risk model had an incremental effect on the predictive value for IMH [NRI: 0.197 (0.039 to 0.356); IDI: 0.200 (0.142 to 0.259); C-index: 0.806 (0.744 to 0.869), all $p < 0.05$].

Conclusions: Treg individually or in combination with pCKMB, hsCRP, and LVESD can effectively predict the existence of IMH in STEMI patients received PPCI.

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Introduction

Current guidelines recommend primary percutaneous coronary intervention (PPCI) as the gold standard of treatment for ST-segment elevation myocardial infarction (STEMI) patients^{1,2}, and PPCI restores thrombolysis in myocardial infarction flow 3 (TIMI 3) in over 90% of patients. Despite the recovery of the epicardial coronary circulation, however, additional injury caused by PPCI such as microvascular injury, also known as the no-reflow phenomenon, cannot be ignored. Studies have confirmed that up to 40–50% of STEMI patients underwent PPCI may experience no-reflow phenomenon, including microvascular obstruction (MVO) and Intramyocardial hemorrhage (IMH)^{3,4}. Among them, ischemic injury of capillaries leads to the occurrence of endothelial gaps and loss of integrity of capillary wall, and extensive erythrocyte extravasation leads to IMH^{5,6}. Studies demonstrated that IMH was closely related to infarct size, MVO and impaired left ventricular (LV) function, and major adverse cardiac events (MACEs)^{7–12}. Therefore, accurate diagnosis of IMH is of great importance in the clinical. According to the recommendations of the guidelines, cardiac magnetic resonance (CMR) imaging is considered the reference diagnostic method for the evaluation of IMH^{1,13}. However, we found some disadvantages of CMR that should not be ignored in the process of practice. First of all, CMR is time-consuming, and it is dangerous for STEMI patients to go for such time-consuming test without electrocardiogram monitoring in acute phase. Second, for those patients with claustrophobia or other contraindications to CMR, it is not feasible to assess IMH by CMR. Finally, CMR is expensive, which will undoubtedly increase the cost of hospitalization for patients. Therefore, considering the above reasons, we tried to find a safe, simple and effective method to predict the presence of IMH.

It is well known that myocardial ischaemia/reperfusion injury (MIRI) is a common cause of no-reflow¹⁴, in which inflammatory response plays an important role^{15,16}. Although innate immune response plays an important role in IRI, T lymphocytes including T-helper 1(Th1), Th2, Th17 and regulatory T (Treg) cells, are also involved in the pathogenesis of IRI. In this study, we focused on the role of Treg cells in MIRI. As far as I know, Treg can be capable of suppressing the innate immune response by inhibiting the macrophage inflammatory phenotype and neutrophil function¹⁷, thereby playing an anti-inflammatory effect in MIRI. Previous studies have reported that Treg can ameliorate IRI in kidney and brain^{18,19}. Recent studies have also reported the protective effect of Treg in mouse MIRI^{20,21}. In this study, we analyzed the circulating Treg level and other common laboratory indicators, and hypothesized that Treg individually or in combination with other indicators, such as left ventricular end-systolic diameter (LVESD), high-sensitivity C-reactive protein (hsCRP) and the peak value of Creatine Kinase MB (pCKMB), can be used to predict the presence of IMH in STEMI patients received PPCI.

Materials And Methods

Study population

The study population for this observational study was identified from the Cardiovascular Center of Beijing Friendship Hospital. The data collection process was approved by the Institutional Review Board of Beijing Friendship Hospital affiliated to Capital Medical University and was in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients. The inclusion criteria for this study were STEMI patients within 12h of symptom onset who underwent PPCI at our hospital. The exclusion criteria included (1) previous myocardial infarction or revascularization [PCI or coronary artery bypass graft (CABG)]; (2) atrial fibrillation; (3) congestive heart failure (HF) with left ventricular ejection fraction (LVEF) < 40%; (4) estimated glomerular filtration rate (eGFR)<30mL/min/1.73m²; (5) rheumatic immune system disease or malignant tumor; (6) acute infectious disease within nearly 3 months; (7) claustrophobia or contraindications to CMR; and (8) disagree to be included in the study. A total of 182 patients were enrolled from October 30, 2019 to September 20, 2021.

Blood samples and data collections

Blood samples were obtained from all patients in a fasting state on the following morning of the admission day. A part of venous blood was prepared into peripheral blood mononuclear cells (PBMCs) by Ficoll density gradient method, and stored at -80°C for the following flow cytometric analysis. The remaining blood sample was sent to the Central Laboratory of Beijing Friendship Hospital to be tested by professional laboratory physicians for other indicators, including hsCRP. In order to obtain the peak value of myocardial enzymes such as pCKMB, fasting venous blood was taken every morning within 5 days after PPCI to detect the levels of myocardial injury markers.

An ultrasound cardiogram was performed within 24 hours after PPCI to obtain indicators of cardiac structure and function, such as LVESD and LVEF. Five to seven days after reperfusion, IMH was assessed by CMR using T2-weighted imaging. In addition, Patients' demographics, medical history, other laboratory indicators, and angiographic evaluation results were also collected.

Flow cytometric analysis of Treg

Cell preparation

For analysis of Treg, PBMCs were suspended in complete culture medium. The cell suspension was resuscitated in an incubator set at 37°C under a 5% CO₂ environment for 1 hour. The cells were then centrifuged at 2000 rpm for 5 min. For analysis of Treg, PBMCs were aliquoted into tubes for further staining.

Surface and intracellular staining

Treg commonly identified by their expression of CD4 and CD25 on the cell surface and the transcription factor Forkhead box P3 (Foxp3) in the nucleus [22]. For Treg analysis, the cells were incubated with Fluorescein isothiocyanate (FITC) anti-human CD4 and Allophycocyanin (APC) anti-human CD25. After the surface staining, the cells were stained with phycoerythrin(PE) anti-human Foxp3 for Treg detection after fixation and permeabilization according to the manufacturer's instructions. Isotype controls were

given to enable correct compensation and confirm antibody specificity. All of the antibodies were from Biolegend. Stained cells were detected by the Attune NxT cytometer and analyzed by the FlowJo 10 software.

CMR protocol and analysis

T2-weighted imaging was performed, and myocardium with a signal intensity >2 standard deviation (SD) above the mean signal intensity of remote noninfarcted myocardium was considered the area at risk (AAR). IMH was defined as the hypoenhanced region within the AAR. The CMR instrument used in this study is a 3.0-T scanner (MAGNETOM Prisma; Siemens Healthcare, Erlangen, Germany). CMR data were transferred to CV142 software (Release 5.12.2, Circle Cardiovascular Imaging, Calgary, Canada) and evaluated twice by an experienced CMR analyst. Another expert in CMR repeated the data evaluation.

Statistical analysis

Continuous variables were presented as mean $\pm$ SD or median (IQR). Comparisons between the two study groups were analyzed by Student's *t* test or Mann-Whitney U-test. Categorical variables are expressed as number and percentage and compared using the Pearson Chi square test. To find the factors that could independently predict IMH, variables that were significant in the univariate logistic regression analysis were incorporated into the multivariate logistic regression analysis. In addition, intercorrelations among variables were taken into consideration in the multivariate analysis. The receiver operating characteristic (ROC) curve analysis was used to evaluate the discriminatory capability of the biomarkers for IMH. To control the confounding factors, 1:1 propensity score matching analysis was performed. The cutoff value was defined for the maximum Youden index. We also calculated net reclassification improvement (NRI), integrated discrimination improvement (IDI) and C-index to determine the extent to which the addition of new prediction model improves the predictive power of existing baseline risk model. The nomogram was made to calculate the predicted value of an individual suffering from IMH. All analyses were two-tailed and *p* value <0.05 was considered statistically significant. Data were analyzed using IBM SPSS statistics 24, MedCalc and R Programming Language.

Results

Baseline characteristics of patients

According to the CMR results, patients were divided into IMH group (*n*=80) and no-IMH group (*n*=102). The study patients had an average age of 58.0 $\pm$ 11.6 years and 150 (82.4%) patients were male. As shown in **Table 1**, the frequencies of Treg in the IMH group [0.8(0.3, 1.1)] was significantly lower than that in the no-IMH group [1.3(0.7, 1.8)] (*p*<0.001). There were significant differences (*p* < 0.05) among the 2 groups in terms of medical history [angiotensin-converting enzyme inhibitor/

angiotensin receptor blocker (ACEI/ARB)], diagnosis at admission(anterior MI) , white blood cell (WBC), hsCRP, fasting plasma glucose (FPG), myocardial enzyme [pCKMB, peak value of myoglobin (pMYO) and peak value of troponin I (pTNI)], peak value of N-terminal pro-brain natriuretic peptide (pNT-pro BNP), LVESD and LVEF. No significant difference was found in the other indicators.

Figure 1 showed the CMR images and flow scatter diagrams of representative cases of STEMI in patients with IMH group and no-IMH group, respectively. The hypoenhanced region in “panel E” (red arrows) was the area of IMH.

Predictors of MVO

Table 2 showed the results of the univariate and multivariate logistic regression analyses. In univariate analysis, decreased Treg frequency, increased hsCRP, pCKMB and LVESD were significantly associated with IMH, as were decreased LVEF, no previous use of ACEI/ARB, anterior MI diagnosed at admission, increased WBC, FPG and pTNI. Correlation analysis showed that pCKMB was significantly correlated with pTNI(*r*=0.623, *p*<0.001). In addition, hsCRP was correlated with WBC(*r*=0.684, *p*<0.001). Therefore, pTNI and WBC were not included in the multivariate model. After multivariable adjustment, decreased Treg frequency [OR(95% CI): 0.350(0.202-0.606), *p*<0.001], increased hsCRP [OR(95% CI): 1.060(1.022-1.100), *p*=0.002], pCKMB [OR(95% CI): 1.004(1.001-1.006), *p*=0.002] and LVESD [OR(95% CI): 3.329(1.346-8.236), *p*=0.009]were determined to be independent predictors of IMH in STEMI patients received PPCI.

ROC curve analysis of 4 predictors

Before propensity score matching, the results of the ROC analysis detailed in **Table 3** and **Figure 2** revealed that all 4 biomarkers significantly predicted the presence of IMH (area under the ROC curve [AUC]: Treg 0.701, pCKMB 0.684, hsCRP 0.658, and LVESD 0.646; all $p < 0.01$). According to the maximum Youden indexes, the cutoff values for Treg, pCKMB, hsCRP, and LVESD were 1.07%, 137.5ng/ml, 5.74mg/L, and 3.52cm, respectively. The AUC for the combination of Treg, pCKMB, hsCRP, and LVESD was 0.786 ($p < 0.001$), indicating very good discriminative ability for the prediction of IMH. Notably, the discriminatory capability for IMH of the 4-biomarker panel was stronger than those of the individual biomarkers ($*p < 0.05$).

After propensity score matching, the age, gender, BMI, medical history(ACEI/ARB), diagnosis at admission(anterior MI), and FPG were not statistically different between the IMH and no-IMH groups. The results of ROC analysis (**Table 3, Figure 2**) still showed that the discriminatory capability of the 4-biomarker panel was good (AUC 0.821, 95% CI 0.747-0.894) and stronger than that of the pCKMB, hsCRP and LVESD [with AUC values of 0.721 for pCKMB, 0.656 for hsCRP, and 0.633 for LVESD] ($*p < 0.05$). The AUC value of Treg was 0.750, and there is no significant difference in the discriminatory capability for MVO between Treg and 4-biomarker panel. According to the maximum Youden indexes, the cutoff values for Treg, pCKMB, hsCRP and LVESD were 1.07%, 73.2ng/ml, 5.20mg/L, and 3.52cm, respectively.

Incremental effect of 4 predictors on predictive value for MVO

Table 4 showed that compared with the LVESD, hsCRP, pCKMB and Treg, the addition of the 4-biomarker panel (combination of Treg, pCKMB, hsCRP and LVESD) significantly improved the reclassification and discrimination ability beyond the baseline risk model with NRI of 0.197, and IDI of 0.200 (both $p < 0.05$). In addition, the C-index of the baseline risk model changed after addition of the 4-biomarker panel [0.806(0.744 to 0.869), $p < 0.001$]. The nomogram in **Figure 3** was used to calculate the predicted value of an individual suffering from IMH.

Association between number of abnormal biomarker levels and IMH

The data presented in **Figure 4** illustrate the association between the number of abnormal biomarker levels and the risk of IMH. Using the cutoff values derived from the ROC analysis to define biomarker levels as normal or abnormal, the risk of IMH increased significantly with an increasing number of abnormal biomarker levels. In a logistic regression model, the odds of IMH were increased by 11-fold or 39-fold respectively, if patients presented with abnormal levels of 2 or ≥ 3 biomarkers compared with ≤ 1 biomarkers (**Table 5**). This finding implies that abnormal levels of the biomarkers at presentation may facilitate better IMH risk stratification.

Discussion

As far as I know, this is the first study to explore the predictive ability of Treg individually or in combination with other 3 biomarkers, including pCKMB, hsCRP and LVESD, for IMH in STEMI patients underwent PPCI. The major findings were these: (1) in STEMI patients received PPCI, IMH group had lower level of Treg

frequency than no-IMH group; (2) Treg frequency, pCKMB, hs-CRP and LVESD were independent risk factors for IMH; (3) the predictive ability of the 4-biomarker panel for IMH was stronger than those of the biomarker individually; and (4) the risk of IMH increased significantly with an increasing number of abnormal biomarker levels (Treg<1.07%, pCKMB $\geq$ 73.2ng/mL, hsCRP $\geq$ 5.20mg/L, and LVESD $\geq$ 3.52cm). Based on the results of this study, we confirmed the effectiveness of Treg individually or in combination with pCKMB, hsCRP and LVESD in predicting IMH. For those patients who cannot undergo CMR examination, Treg undoubtedly is a simple, safe and effective method to predict IMH in clinical practice.

Acute myocardial infarction, including non-ST elevated myocardial infarction and STEMI, is the leading cause of cardiovascular disease and mortality worldwide²³.

In STEMI, the coronary artery is often acutely completely blocked, and it is pivotal to open the infarct-related artery (IRA) urgently. Therefore, the preferred treatment strategy for STEMI patients within 12h of symptom onset is coronary revascularization by PPCI

in combination with antithrombotic therapy ^{1,2}.

Nevertheless, despite re-established epicardial blood flow in the IRA, a sizable proportion of patients continue to experience hypoperfusion of myocardial tissue after PPCI, called no-reflow phenomenon. IMH is a form of no-reflow phenomenon resulting from the extensive erythrocyte aggregation and extravasation because of the damaged endothelial walls ^{5,6}. IMH can be visualized by T2-weighted CMR because breakdown products of hemoglobin are paramagnetic and influence regional magnetic tissue properties ²⁴. However, not all patients are suitable for CMR to clarify the presence of IMH, such as those with serious condition or contraindications.

Therefore, we urgently need to find a simple and effective method to predict the presence of IMH. Carrick et al. showed that IMH was more closely associated with adverse outcomes than MVO ¹¹. Husser et al. found that in patients with IMH, the crude MACEs rate was significantly higher compared to patients without IMH (22% vs. 12%, $p=0.04$) and the Kaplan Meier analysis revealed a significant longer MACE-free survival for patients without IMH, and IMH after STEMI predicts adverse remodeling ²⁵. In addition, Amier et al. also demonstrated that presence and extent of IMH was associated with larger infarct size, greater extent of MVO, larger left ventricular dimensions, and lower LVEF (all $p<0.001$) ³. Therefore, in this study, we analyzed various indexes of myocardial enzymes and left ventricular structure, and found that pCKMB and LVESD were independent predictors of IMH. Although the molecular mechanisms involved in the initiation and progression of MIRI are still not completely understood, accumulating evidences have suggested that excessive inflammation plays a predominant role in it ^{15,26}. And Carrick et al. also found that IMH was associated with markers of inflammation, including peak monocyte count and peak neutrophil count ¹¹. In this study, we also found that inflammatory markers were significantly higher in patients with IMH than in patients without IMH, and hsCRP was an independent predictor of IMH. Considering that Treg cells have been verified to playing an anti-inflammatory effect in MIRI by inhibiting the macrophage inflammatory phenotype and neutrophil function in animal experiments ^{17,20,21}, we hypothesized that the circulating Treg levels are related to the presence of IMH in STEMI patients. The final results were consistent with our expectation that Treg is a protective factor of IMH and can independently predict IMH occurrence in STEMI patients received PPCI. Moreover, we proposed for the first time that Treg can be combined with pCKMB, hsCRP and LVESD to predict the presence of IMH. These findings not only indirectly indicated that Treg may have a certain protective effect on human MIRI, but also provided us with a simpler method to predict MVO than CMR, especially for those who can't finish the CMR.

Our study had several limitations. First, this was a single-center study with a small sample size; thus, generalization of the findings should be cautious. Second, laboratory parameters were only measured once after hospital admission, which could cause potential bias due to measurement error. Third, this study did not include the follow-up data, and it is not clear whether Treg levels can predict prognosis of STEMI patients. In future work, we will not only continue to expand the sample size to confirm the stability of the conclusions, but also collect the follow-up data to further explore the impact of Treg level on the prognosis.

Conclusions

In conclusion, the current study firstly showed that Treg individually or in combination with pCKMB, hsCRP, and LVESD can effectively predict the presence of IMH in STEMI patients after PPCI.

Abbreviations

IMH, intramyocardial hemorrhage; STEMI, ST-segment elevation myocardial infarction; PPCI, primary percutaneous coronary intervention; Treg, regulatory T cell; pCKMB, the peak value of Creatine Kinase MB; hsCRP, high-sensitivity C-reactive protein; LVESD; left ventricular end-systolic diameter; OR, odds ratio; CI, confidence interval; NRI, net reclassification improvement; IDI, integrated discrimination improvement; TIMI, thrombolysis in myocardial infarction flow; MVO, microvascular obstruction; LV, left ventricular; MACEs, major adverse cardiac events; CMR, cardiac magnetic resonance; MIRI, myocardial ischaemia/reperfusion injury; Th1, T-helper 1; Th17, T-helper 17; CABG, coronary artery bypass graft; HF, heart failure; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; PBMCs, peripheral blood mononuclear cells; Foxp3, transcription factor Forkhead box P3; FITC, fluorescein isothiocyanate; APC, allophycocyanin; PE, phycoerythrin; SD, standard deviation; AAR, area at risk; ROC, receiver operating characteristic; ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; WBC,

white blood cell; FPG, fasting plasma glucose; pMYO, the peak value of myoglobin; pTNI, the peak value of troponin I; pNT-pro BNP, the peak value of N-terminal pro-brain natriuretic peptide; AUC, area under the ROC curve; IRA, infarct-related artery.

Declarations

Ethics approval and consent to participate

The study data collections were approved by the Institutional Review Board of Beijing Friendship Hospital affiliated to Capital Medical University, and written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YZ participated in the design of the project, the implementation of basic experiments, the statistics of data and the writing of manuscripts. HG, LL and SYL participated in the collection of blood samples. YH, JSL, BH and DHL participated in the analysis of CMR. WPL contributed discussion and edited manuscript. HWL provided funding support, designed study and reviewed manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Clinical characteristics of the 2 groups.

Total	No-IMH	IMH
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	(n=182)	(n=102)	(n=80)	<i>p</i> value
Lym/PBMCs(%)	69.5(59.9,77.3)	69.7(59.1,78.6)	69.2(59.4,76.3)	0.522
CD4+ T cells/Lym(%)	34.5(27.3,45.1)	34.1(26.5,45.6)	33.9(27.6,45.0)	0.868
Treg/CD4+ T cells(%)	1.0(0.5,1.5)	1.3(0.7,1.8)	0.8(0.3,1.1)	<0.001
Age, years	58.0±11.6	59.1±10.2	56.7±13.1	0.235
Male gender	150(82.4)	84(82.4)	66(82.5)	0.979
BMI, kg/m ²	25.9±3.6	26.1±3.6	25.7±3.7	0.346
SBP, mmHg	124.9±20.0	125.8±20.8	123.9±19.1	0.628
DBP, mmHg	77.1±13.3	77.3±13.2	76.8±13.5	0.805
Heart rate,bpm	74.8±14.3	73.1±13.9	76.8±14.7	0.191
Medical history				
Current/ex-Smoker	132(72.5)	73(71.6)	59(73.8)	0.744
Diabetes mellitus	63(34.6)	34(33.3)	29(36.3)	0.681
Hypertension	119(65.4)	67(65.7)	52(65.0)	0.923
Stroke	20(11.0)	11(10.8)	9(11.3)	0.921
Dyslipidemia	139(76.4)	79(77.5)	60(75.0)	0.699
Antiplatelet agent	16(8.8)	7(6.9)	9(11.3)	0.300
ACEI/ARB	44(24.2)	31(30.4)	13(16.3)	0.027
Beta-blocker	16(8.8)	11(10.8)	5(6.3)	0.284
Statins	12(6.6)	6(5.9)	6(7.5)	0.662
Diagnosis at admission				
Anterior MI	87(47.8)	41(40.2)	46(57.5)	0.020
In-hospital treatment				
PCI/CABG	182(100.0)	102(100.0)	80(100.0)	1.000
Antiplatelet agent	182(100.0)	102(100.0)	80(100.0)	1.000
ACEI/ARB	79(43.4)	48(47.1)	31(38.8)	0.262
Beta-blocker	146(80.2)	79(77.5)	67(83.8)	0.290
Statins	159(87.4)	93(91.2)	66(82.5)	0.080
In-hospital time(d)	8.9±2.6	8.7±2.8	9.3±2.3	0.144
Laboratory values WBC, 10 ⁹ /L				
	9.2±2.6	8.8±2.6	9.7±2.5	0.034
Hemoglobin, g/L	147.8±15.2	145.7±15.6	150.3±14.3	0.154
HsCRP, mg/L	4.8(2.3,15.4)	3.5(2.1,8.7)	8.0(3.3,23.2)	<0.001
FPG, mmol/L	6.3(5.5,8.9)	6.0(5.3,8.3)	6.7(5.8,9.4)	0.009
RBG,mmol/L	8.4(7.2,12.1)	8.2(7.0,12.2)	8.6(7.3,12.1)	0.465
HbA1c, %	6.6±1.7	6.4±1.5	6.7±1.9	0.102
Albumin, g/L	41.7±4.2	41.1±4.8	42.4±3.2	0.053
ALT, U/L	26.5(18.0,38.3)	24.5(17.0,34.8)	28.0(19.3,39.8)	0.152
Creatinine, umol/L	70.8±18.1	70.0±18.3	70.5±18.0	0.663

Table 1 (continued)

	Total (n=182)	No-IMH (n=102)	IMH (n=80)	<i>p</i> value
eGFR, ml/min/1.73m ²	99.5±19.6	98.8±19.2	100.4±19.3	0.441
TC, mmol/L	5.0±1.1	5.0±1.1	5.0±1.1	0.910
TGs, mmol/L	1.6(1.2,2.4)	1.6(1.2,2.5)	1.6(1.1,2.2)	0.579
LDL-C, mmol/L	3.0±0.7	3.0±0.7	3.1±0.7	0.799
HDL-C, mmol/L	1.00±0.21	1.00±0.21	0.99±0.21	0.832
Apo-A1, g/L	1.18±0.20	1.19±0.20	1.16±0.20	0.403
Apo-B, g/L	0.92±0.24	0.91±0.23	0.93±0.24	0.690
Apo-E, mg/dL	4.1±1.7	4.1±2.0	4.1±1.3	0.956
Lp(a), mg/L	107.0(48.8,224.5)	97.0(49.8,214.5)	125.5(47.3,213.5)	0.687
Myocardial enzyme				
pCKMB, ng/ml	189(101,348)	137(47,294)	244(139,486)	<0.001
pMyo, ng/ml	89(49,204)	76(40,186)	112(59,300)	0.039
pTNI, ng/ml	30(26,50)	25(13,50)	42(33,50)	<0.001
pNT-pro BNP, pg/ml	1433(789,2610)	1131(576,2132)	1952(946,3162)	0.002
Echocardiography				
LA, cm	3.81±0.41	3.80±0.43	3.82±0.38	0.813
LVEDD, cm	5.0±0.4	4.9±0.4	5.1±0.4	0.054
LVESD, cm	3.5±0.4	3.4±0.4	3.7±0.4	0.002
LVEF, %	51.1±7.6	52.3±8.1	49.6±6.6	0.034
Angiography findings				
Multi-vessel/LM	113(62.1)	65(63.7)	48(60.0)	0.607
Proximal LAD	74(40.7)	36(35.3)	38(47.5)	0.096
CTO	14(7.7)	7(6.9)	7(8.8)	0.635

Dates are presented as mean±SD, median (IQR) or number (%).

IMH, intramycardial hemorrhage; Lym, Lymphocyte; PBMCs, peripheral blood mononuclear cells; Treg, regulatory T cell; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; MI, myocardial infarction; PCI/CABG, percutaneous coronary intervention/coronary artery bypass graft; WBC, white blood cell; HsCRP, hypersensitive C-reactive protein; FPG, fasting plasma glucose; RBG, random blood glucose; HbA1c, glycated hemoglobin; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TGs, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; Apo-E, apolipoprotein E; Lp(a), lipoprotein(a); pCKMB, the peak value of creatine kinase MB; pMYO, the peak value of myoglobin; pTNI, the peak value of troponin I; NT-pro BNP, N-terminal pro-brain natriuretic peptide; LA, left atrium; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LM, left main coronary artery; LAD, left anterior descending; CTO, chronic total occlusions.

Table 2. Univariate and multivariate logistic regression analysis of IMH occurrence in patients with STEMI-PPCI.

	Univariate OR (95% CI)	<i>p</i> Value	Multivariate Adjusted OR (95% CI)	<i>p</i> Value
Treg/CD4+ T cells(%)	0.387(0.245-0.613)	<0.001	0.350(0.202,0.606)	<0.001
Age, years	0.985(0.960,1.010)	0.234		
Male gender	1.010(0.468,2.179)	0.979		
Medical history				
ACEI/ARB	0.444(0.214,0.921)	0.029	0.504(0.213,1.192)	0.119
Diagnosis at admission				
Anterior MI	2.012(1.111,3.650)	0.021	2.066(0.962,4.425)	0.063
WBC, 10 ⁹ /L	1.128(1.008,1.263)	0.036		
HsCRP, mg/L	1.048(1.019,1.078)	0.001	1.060(1.022,1.100)	0.002
FPG, mmol/L	1.141(1.027,1.267)	0.014	1.042(0.915,1.187)	0.537
Myocardial enzyme				
pCKMB, ng/ml	1.004(1.002,1.006)	<0.001	1.004(1.001,1.006)	0.002
pMyo,ng/ml	1.001(1.000,1.003)	0.123		
pTNI, ng/ml	1.105(1.063,1.149)	<0.001		
pNT-pro BNP, pg/ml	1.001(0.998,1.002)	0.261		
Echocardiography				
LVESD, cm	2.978(1.438,6.165)	0.003	3.329(1.346,8.236)	0.009
LVEF, %	0.960(0.923,0.997)	0.037	1.054(0.996,1.115)	0.069

IMH, intramyocardial hemorrhage; STEMI, ST-segment elevation myocardial infarction; PPCI, primary percutaneous coronary intervention; Treg, regulatory T cell; ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; MI, myocardial infarction; WBC, white blood cell; HsCRP, hypersensitive C-reactive protein; FPG, fasting plasma glucose; pCKMB, the peak value of creatine kinase MB; pMYO, the peak value of myoglobin; pTNI, the peak value of troponin I; NT-pro BNP, N-terminal pro-brain natriuretic peptide; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction.

Table 3. Pre- and postmatching receiver operating characteristic curve analysis of Treg, hsCRP, pCKMB and LVESD for the prediction of IMH.

	Cutoff value	AUC	95% CI	<i>p</i> value	Sensitivity	Specificity	Youden Index
Pre-matching							
LVESD, cm	3.52	0.646*	0.566-0.726	0.001	0.663	0.608	0.270
HsCRP, mg/L	5.74	0.658*	0.579-0.738	<0.001	0.613	0.657	0.270
pCKMB, ng/ml	137.5	0.684*	0.608-0.761	<0.001	0.763	0.549	0.312
Treg, %	1.07	0.701*	0.625-0.777	<0.001	0.800	0.618	0.418
Treg+pCKMB+HsCRP+LVESD	-/-	0.786	0.721-0.851	<0.001	0.825	0.618	0.443
Post-matching							
LVESD, cm	3.52	0.633*	0.537-0.729	0.009	0.625	0.625	0.250
HsCRP, mg/L	5.20	0.656*	0.561-0.751	0.002	0.609	0.687	0.297
pCKMB, ng/ml	73.2	0.721*	0.633-0.809	<0.001	0.922	0.453	0.375
Treg, %	1.07	0.750	0.663-0.836	<0.001	0.797	0.719	0.516
Treg+pCKMB+HsCRP+LVESD	-/-	0.821	0.747-0.894	<0.001	0.813	0.734	0.547

Treg, regulatory T cell; hsCRP, hypersensitive C-reactive protein; pCKMB, the peak value of creatine kinase MB; LVESD, left ventricular end-systolic diameter; IMH, intramyocardial hemorrhage. Note: Compared with Treg+pCKMB+HsCRP+LVESD **p* <0.05.

Table 4. Evaluate the incremental predictive value and predictive power of various models with NRI, IDI and C-index.

	Categor Index	y-free NRI		- Index	IDI		C- index Index	95% CI	p value
		95% CI	p value		95% CI	p value			
Baseline risk model			Ref.			Ref.	0.661	0.580 to 0.741	<0.001
+LVESD	0.046	-0.068 to 0.161	0.428	0.037	0.010 to 0.064	0.008	0.695	0.617 to 0.772	<0.001
+HsCRP	0.058	-0.101 to 0.217	0.476	0.027	0.003 to 0.052	0.030	0.685	0.607 to 0.763	<0.001
+pCKMB	0.195	0.023 to 0.368	0.027	0.067	0.030 to 0.103	<0.001	0.725	0.651 to 0.799	<0.001
+Treg	0.178	0.005 to 0.352	0.044	0.093	0.053 to 0.132	<0.001	0.747	0.677 to 0.817	<0.001
+4-biomarker panel	0.197	0.039 to 0.356	0.015	0.200	0.142 to 0.259	<0.001	0.806	0.744 to 0.869	<0.001

Baseline risk model including diagnosis at admission (anterior myocardial infarction), FPG, ACEI/ARB used before admission and LVEF.

NRI, net reclassification improvement; IDI, integrated discrimination improvement; LVESD, left ventricular end-systolic diameter; HsCRP, hypersensitive

C-reactive protein; pCKMB, the peak value of creatine kinase MB; Treg, regulatory T cell; FPG, fasting plasma glucose; ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; LVEF, left ventricular ejection fraction. Ref., reference. The 4-biomarker panel represents the combination of Treg, pCKMB, hsCRP and LVESD.

Table 5. Logistic regression analysis of number of abnormal biomarker levels and the odds of developing IMH.

No.of Abnormal Biomarkers	No-IMH	IMH	OR(95% CI)	p value
≤1	35(92.1)	3(7.9)	1	-/-
2	16(50.0)	16(50.0)	11.7(3.0-45.8)	<0.001
≥3	13(22.4)	45(77.6)	40.4(10.7-152.8)	<0.001

Cutoff values for abnormal biomarker levels were LVESD≥3.52cm, HsCRP≥5.20 mg/L, pCKMB≥73.2ng/mL, and Treg≥1.07%.

IMH, intramyocardial hemorrhage; LVESD, left ventricular end-systolic diameter; HsCRP, hypersensitive C-reactive protein; pCKMB, the peak value of creatine kinase MB; Treg, regulatory T cell.

Figures

Figure 1

CMR examples with or without IMH burden and their circulating Treg frequencies.

Panels “A–D” show a case without IMH burden who is diagnosed as acute anterior myocardial infarction. Panels “E–H” show a case with IMH burden who is also diagnosed as acute anterior myocardial infarction. The red arrows in panel “E” represent the region of IMH. According to panels D and H, the Treg frequency in patients with IMH(0.75%) is significantly lower than that in patients without IMH(1.37%). CMR, cardiac magnetic resonance imaging; IMH, intramyocardial hemorrhage; Treg, regulatory T cell.

Figure 2

Pre-(A) and postmatching(B) receiver operating characteristic curve analysis of Treg, pCKMB, hsCRP and LVESD for the prediction of IMH.

IMH, intramyocardial hemorrhage; HsCRP, hypersensitive C-reactive protein; pCKMB, the peak value of creatine kinase MB; Treg, regulatory T cell; LVESD, left ventricular end-systolic diameter.

Figure 3

The proposed nomogram for predicting the risk probability of IMH.

Treg, regulatory T cell; pCKMB, the peak value of creatine kinase MB; HsCRP, hypersensitive C-reactive protein; LVESD, left ventricular end-systolic diameter; IMH, intramyocardial hemorrhage.

Figure 4

Association of the number of abnormal biomarker levels based on the identified cutoff values and the risk of IMH.

IMH, intramyocardial hemorrhage; LVEDD, left ventricular end-systolic diameter; HsCRP, hypersensitive C-reactive protein; pCKMB, the peak value of creatine kinase MB; Treg, regulatory T cell.