

The Predictive Values of White Blood Cell Indices (Lymphocyte and Eosinophilic Granulocyte) for Heart Failure in Acute Coronary Syndrome Patients Following Percutaneous Coronary Intervention: A Prospective Cohort Study

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Background: White blood cell (WBC) indices are strongly associated with cardiovascular disease, but data on the prognostic values of these parameters in patients with acute coronary syndrome (ACS) following percutaneous coronary intervention (PCI) are sparse. The current study aimed to investigate the relationship between baseline WBC indices levels and the incidence of heart failure (HF) in ACS patients after PCI and explore the predictive values over a 2-year follow-up period.

Methods: A total of 416 consecutive ACS patients treated with PCI were enrolled and received a median of 27.7 months follow-up. Univariate and multivariate Cox regression analyses and the receiver operating characteristic (ROC) curves were performed.

Results: Baseline lymphocyte (LYMPH) count, eosinophil (EO) count and eosinophil percentage (EO %) were higher in patients who experienced HF over a 2-year follow-up. In multivariate Cox proportional hazards analysis, LYMPH count, EO count and EO % were independently associated with the occurrence of HF (hazard ratio [HR] = 12.876, $P = 0.025$; HR = 16.625, $P = 0.004$; HR = 1.196, $P = 0.031$, respectively). The area under the ROC curve of baseline EO count predicting the occurrence of HF in ACS patients following PCI was 0.625 ($P = 0.037$). For patients aged 60 years and above, who had PCI or history of coronary artery bypass grafting, the higher EO count, the higher the risk of HF.

Conclusion: Elevated baseline LYMPH count, EO count and EO % were independently associated with the incidence of HF in ACS patients following PCI, suggesting that WBC indices might be available, simple, and cost-efficient biomarkers with predictive value, especially for patients aged more than 60 years.

Keywords: heart failure, acute coronary syndrome, lymphocytes, eosinophils, independent predictive factors

Background

Acute coronary syndrome (ACS), such as unstable angina (UA) and acute myocardial infarction (MI) caused by the rupture of the atherosclerotic plaque and subsequent thrombosis, is a serious form of cardiovascular disease with a high mortality risk worldwide.¹⁻³ Multiple studies have proved the influence of immune cells has moved into focus with respect to their involvement in ACS, especially the pathogenesis of atherosclerosis and thrombosis.^{4,5} Although revascularisation and antithrombotic strategies have achieved great success in decreasing mortality of patients with ACS, the outcomes are considered to be unsatisfactory.⁶ The occurrence of heart failure (HF) is closely related to long-

term survival of patients with ACS. There is a body of evidence suggesting that immune and inflammatory processes are involved in the pathophysiology of HF, and inflammation is currently discussed as a therapeutic target in HF.⁷

White blood cell (WBC) count is a marker of systemic inflammation. Inflammatory cytokines, which are closely related to the development of HF, are mainly secreted by WBC and, conversely, were also shown to impact WBC levels.⁸ Large amounts of clinical observations suggest that, besides classical players, WBC count is strongly associated with HF, additionally, associated with the risk factors of HF such as age and smoking.^{9,10} Several lines of evidence link lymphocytes (LYMPH) and eosinophils (EO) to cardiovascular events,^{11,12} but data on the association with the incident of HF are sparse, and the prognostic values of these WBC indices in ACS patients are still unclear. The aim of this study was to investigate the association of WBC indices, particularly its individual components such as LYMPH and EO counts, with the occurrence of HF in ACS patients following percutaneous coronary intervention (PCI) over a 2-year follow-up. We assume that WBC indices can be fast and convenient candidate markers to predict the risk of long-term adverse cardiovascular events in ACS patients after PCI.

Materials and Methods

Study Participants, Outcome and Follow-Up

The investigation presented a single-centre, prospective, cohort study as we previously described.¹³ Briefly, from January 2013 to February 2014, after screening of 2132 consecutive patients diagnosed with CAD at First Affiliated Hospital of Xi'an Jiaotong University, a total of 416 consecutive patients who were diagnosed with ACS and then underwent PCI were eventually enrolled in this study. The detailed inclusion and exclusion criteria are shown in Table 1. HF was defined as end point in the current study. Participants were eligible if they were in New York Heart Association (NYHA) functional class \geq II and were required to have a left ventricular ejection fraction (LVEF) \leq 40%, and were pharmacologically treated for HF. Patients with HF also have N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentration \geq 600 pg/mL (\geq 400 pg/mL if hospitalized for HF within the previous 12 months). All participants received follow-up through personal interviews by well-trained physicians until March 31, 2016. Follow-up ended on the date of end point (HF) occurrence. The median follow-up time was 27.7 months.

Ethics Statement

Approval of the Ethics Committee of Xi'an Jiaotong University (Ethical approval number: XJTU1AF2012LSK-312) was obtained, and the written informed consents were obtained from all the participants. The study conformed to the Declaration of Helsinki and collected the required data from the clinical records without clinical intervention to protect patients' privacy.

Data Collection

The baseline information was composed of the demographic characteristics (age, gender, etc.) and partial known cardiovascular risk factors (smoking, family history, diabetes mellitus, hypertension, etc.). Clinical indexes (ejection fraction, systolic pressure, diastolic pressure, ACS type, NYHA classification, etc.) were recorded. After admission,

Table 1 Inclusion and Exclusion Criteria

Inclusion criteria	Consecutive patients who were diagnosed with ACS and underwent PCI in the First Affiliated Hospital of Medical College of Xi'an Jiaotong University between January 2013 and February 2014. The diagnosis of ACS included UA, NSTEMI and STEMI. (ACS was defined based on the criteria by the American Cardiology College)
Exclusion criteria	1. Patients with severe renal and liver diseases. 2. Patients with severe infections, immune system diseases. 3. Patients with malignant tumour, blood system diseases. 4. Patients with severe cerebrovascular diseases. 5. Patients who had prior history of surgical treatment within 2 weeks of this study. 6. Patients who were pregnancy.

Abbreviations: ACS, acute coronary syndromes; PCI, percutaneous coronary intervention; UA, unstable angina; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction.

peripheral blood samples of patients were collected from fasting state early in the morning before PCI. Baseline laboratory data containing partial cardiovascular risk factors (blood routine, blood lipids, creatine kinase isoenzymes MB, NT-proBNP, fibrinogen, blood glucose, and D-Dimer) were performed by experts from the biochemistry centre of the First Affiliated Hospital of Xi'an Jiaotong University, using standard biochemical techniques. More specifically, blood samples were harvested and blood routine examination including absolute and percentage counts (eg, blood neutrophil (NEUT) count, LYMPH count, EO count and EO percentage (EO %), etc.) was performed using the whole blood by an automatic hematology analyzer (Sysmex 2100, Japan). Echocardiographic parameters and other biochemical parameters were measured using standard methods.

Statistical Analysis

Statistical analyses were performed by SPSS 18.0 (SPSS Inc, Chicago, IL) and EmpowerStats (<http://www.empowerstats.com/>). Continuous variables are presented as the mean \pm standard deviation if normally distributed or median (lower quartile, upper quartile) otherwise. Categorical variables are presented as numbers (percentages). Variables of interest were compared using Student's *t*-test, one-way ANOVA or the Chi squared test as appropriate. For nonnormally distributed continuous variables, the Kruskal–Wallis analysis was used. Cox regression analysis was applied to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) to determine the independent predictors of HF. Multivariate and stratified analysis were used to identify predictors. Kaplan–Meier survival analysis and Log rank tests were performed to compare the adverse event-free survival rate. The area under the receiver operating characteristic (ROC) curves (AUCs) were used to indicate the predictive value of variables for HF. A value of $P < 0.05$ was considered statistically significant.

Results

Basic Characteristics of Participants

A total of 416 patients who were diagnosed with ACS and underwent the follow-up were enrolled in this investigation, with HF patients accounting for 9.9% (41/416) of the overall cohort. Table 2 displays the baseline characteristics of the whole patients, patients with HF and without HF. There were no significant differences observed in the baseline demographic, clinical parameters and laboratory data except for age, gender, the history of smoking, LYMPH count, EO count and EO % ($P < 0.05$). Of these, LYMPH count, EO count and EO % in patients with HF were significantly

Table 2 Basic Characteristics of Patients with HF, without HF and Overall

Variables	Overall (n = 416)	Non-HF (n = 375)	HF (n = 41)	P value
Demographic				
Age, years, mean (SD)	60.7 (10.5)	60.2 (10.6)	64.8 (8.6)	0.007
Male sex, n, %	324, 77.9%	300, 80.0%	24, 58.5%	0.002
BMI, kg/m ² , mean (SD)	25.0 (2.9)	25.0 (2.8)	25.0 (3.4)	0.929
Smoking, n, %	233, 56.1%	220, 58.8%	13, 31.7%	<0.001
Hypertension, n, %	214, 51.4%	192, 51.2%	22, 53.7%	0.765
DM, n, %	83, 20.0%	75, 20.0%	8, 19.5%	0.941
Family history, n, %	166, 39.9%	152, 40.5%	14, 34.1%	0.428
Past MI, n, %	68, 16.3%	59, 15.7%	9, 22.0%	0.307
Past PCI or CABG, %	78, 18.8%	68, 18.1%	10, 24.4%	0.330
Clinical				
SBP, mmHg, median (IQR)	120.0 (114.0–140.0)	120.0 (112.0–140.0)	130.0 (120.0–140.0)	0.374
DBP, mmHg, median (IQR)	80.0 (70.0–84.0)	80.0 (70.0–84.0)	80.0 (70.0–80.0)	0.732
LVEF, %, median (IQR)	61.0 (49.0–68.0)	61.0 (50.5–67.5)	62.0 (45.0–70.0)	0.766
ACS type				0.265
UA, n, %	233, 56.1%	206, 54.9%	27, 67.5%	
STEMI, n, %	125, 30.1%	115, 30.7%	10, 25.0%	
NSTEMI, n, %	57, 13.7%	54, 14.4%	3, 7.5%	

(Continued)

Table 2 (Continued).

Variables	Overall (n = 416)	Non-HF (n = 375)	HF (n = 41)	P value
NYHA classification				0.073
I, n, %	153, 36.8%	142, 37.9%	11, 26.8%	
II, n, %	235, 56.5%	210, 56.0%	25, 61.0%	
III, n, %	19, 4.6%	17, 4.5%	2, 4.9%	
IV, n, %	9, 2.2%	6, 1.6%	3, 7.3%	
Medication at discharge				
Aspirin, n, %	416, 100%	375, 100%	41, 100%	
Clopidogrel, n, %	416, 100%	375, 100%	41, 100%	
Statin, n, %	408, 98.1%	368, 98.1%	40, 97.6%	0.800
ACEI/ARB, n, %	377, 90.6%	339, 90.4%	38, 92.7%	0.634
β -blocker, n, %	365, 87.1%	331, 88.3%	34, 82.9%	0.322
Blood				
RBC count, 10^{12} cells/L, median (IQR)	4.6 (3.7–6.6)	4.7 (3.7–6.6)	4.5 (3.6–6.7)	0.935
Hemoglobin, g/L, mean (SD)	138.9 (17.1)	138.8 (17.4)	140.1 (14.4)	0.653
MCV, fl, median (IQR)	93.0 (90.2–95.9)	93.1 (90.3–95.9)	92.1 (88.0–94.4)	0.054
MCH, pg, median (IQR)	31.1 (30.0–32.2)	31.1 (30.1–32.2)	30.6 (29.3–32.2)	0.066
MCHC, g/L, mean (SD)	334.0 (12.3)	334.0 (12.4)	334.0 (11.4)	0.810
PLT count, 10^3 cells/ μ L, median (IQR)	183.0 (152.0–218.0)	183.0 (151.0–217.5)	186.0 (160.0–227.0)	0.257
WBC count, 10^3 cells/ μ L, median (IQR)	6.5 (5.3–8.3)	6.5 (5.3–8.3)	7.2 (5.6–8.8)	0.064
NEUT count, cells/ μ L, median (IQR)	4245 (3315–5763)	4230 (3280–5705)	4440 (3620–6260)	0.194
LYMPH count, cells/ μ L, median (IQR)	1460 (1118–1930)	1440 (1100–1905)	1690 (1280–2390)	0.029
MONO count, cells/ μ L, median (IQR)	460 (338–610)	460 (335–610)	490 (340–590)	0.948
EO count, cells/ μ L, median (IQR)	90 (40–140)	80 (40–140)	150 (40–210)	0.008
EO percentage, %, median (IQR)	1.3 (0.6–2.3)	1.2 (0.6–2.2)	1.8 (0.8–3.4)	0.038
NEUT, %, mean (SD)	67.2 (10.3)	67.3 (10.2)	66.2 (10.5)	0.501
LYMPH, %, mean (SD)	24.1 (9.2)	24.0 (9.2)	25.2 (9.1)	0.410
MONO, %, median (IQR)	7.1 (5.5–8.7)	7.2 (5.6–8.7)	7.0 (4.4–8.2)	0.266
LDL-C, mmol/L, mean (SD)	2.2 (0.8)	2.2 (0.8)	2.2 (0.7)	0.556
NT-proBNP, pg/mL, median (IQR)	257.0 (104.5–797.0)	257.0 (104.4–838.2)	257.0 (109.7–528.5)	0.678
Blood glucose, mmol/L, median (IQR)	5.6 (4.9–6.8)	5.6 (4.9–6.9)	5.3 (5.0–6.1)	0.158
D-Dimer, mg/L, median (IQR)	0.5 (0.0–0.8)	0.5 (0.0–0.8)	0.6 (0.0–0.8)	0.397
Fibrinogen, g/L, median (IQR)	3.3 (2.8–3.8)	3.3 (2.8–3.8)	3.3 (3.0–3.7)	0.941
CKMB, U/L, median (IQR)	15.7 (11.5–33.8)	16.0 (11.6–34.3)	14.3 (11.2–21.4)	0.291

Note: Bold font denotes $P < 0.05$.

Abbreviations: HF, heart failure; SD, standard deviation; IQR, inter-quartile range; BMI, body mass index; MD, Diabetes Mellitus; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; ACS, acute coronary syndromes; UA, unstable angina; STEMI, ST-segment elevation myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; NYHA, New York Heart Association; ACEI, angiotensin-converting enzyme inhibition; ARB, angiotensin receptor blocker; RBC, red blood cell; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, Platelet; WBC, white blood cell; NEUT, Neutrophilic granulocyte; LYMPH, Lymphocyte; MONO, Monocyte; EO, Eosinophilic granulocyte; LDL-C, low-density lipoprotein cholesterol; NT-proBNP, N-terminal pro-B-type natriuretic peptide; CKMB, creatine kinase isoenzymes MB.

higher than those without HF (1690 (1280–2390) cells/ μ L vs 1440 (1100–1905) cells/ μ L, $P = 0.029$; 150 (40–210) cells/ μ L vs 80 (40–140) cells/ μ L, $P = 0.008$; 1.8 (0.8–3.4) vs 1.2 (0.6–2.2), $P = 0.038$, respectively). No significant differences were observed in other blood routine parameters (all had $P > 0.05$).

Univariate and Multivariate Cox Regression Analysis

To determine the clinical predictive values of baseline demographic, clinical and laboratory parameters we observed for over a median of 27.7 months of follow-up in the patients with ACS after PCI, univariate and multivariate Cox regression analysis were performed. Univariate Cox regression analysis reported that age and gender as well as baseline platelet (PLT) count, LYMPH count, EO count and EO % were positively correlated with the occurrence of HF in ACS patients

following PCI, whereas mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were negatively correlated with HF, over a 2-year follow-up (Table 3). Of note, EO count displayed significantly predictive value of HF in ACS patients after PCI (HR: 9.935, 95% CI: 2.136–46.215, $P = 0.003$).

Based on the results of the univariate analysis, we then used multivariate analysis to further explain whether PLT count, LYMPH count, EO count and EO % could independently be in predictive value for HF. We selected the confounders according to variables' associations with the outcomes of interest or a change in effect estimate of more than 10%. Multivariate analysis showed that PLT count had no value for prediction of HF in the patients with ACS after PCI. However, compared with crude regression analysis, the association between LYMPH count, EO count, EO % and the occurrence of HF were enhanced markedly even after adjusting for age, male sex, BMI, smoking, diabetes mellitus, past PCI or CABG, SBP, DBP and EF (Model 1), and further for red blood cell (RBC) count, MCV, WBC count, NEUT count, LYMPH %, MONO %, NT-proBNP, blood glucose and D-Dimer (Model 2), respectively, in the multivariable regression analysis (Table 4). Especially, the positive association of EO count was the most pronounced (Model 1: HR = 10.695, $P = 0.007$; Model 2: HR = 12.625, $P = 0.004$).

Table 3 Partial Univariate Cox Regression Analysis for Predictors of HF in ACS Patients Following PCI Over a 2-Year Follow-Up

Variables	HR	95% CI	P value
Age	1.048	1.017, 1.080	0.002
Male sex	2.376	1.269, 4.448	0.007
BMI	0.995	0.902, 1.097	0.915
Hypertension	1.331	0.717, 2.473	0.365
DM	0.964	0.444, 2.093	0.926
Family history	0.636	0.332, 1.221	0.174
Past MI	1.499	0.715, 3.143	0.284
Past PCI or CABG	1.434	0.701, 2.934	0.324
LVEF	0.994	0.970, 1.020	0.657
Hemoglobin	1.006	0.988, 1.025	0.538
RBC count	0.998	0.989, 1.007	0.636
MCV	0.929	0.886, 0.975	0.003
MCH	0.853	0.746, 0.976	0.020
MCHC	1.006	0.981, 1.031	0.642
PLT count	1.005	1.001, 1.010	0.023
WBC count	0.999	0.997, 1.002	0.806
NEUT count	1.086	0.962, 1.225	0.183
LYMPH count	1.801	1.270, 2.554	0.001
MONO count	1.108	0.305, 4.028	0.876
EO count	9.935	2.136, 46.215	0.003
EO %	1.164	1.008, 1.344	0.039
NEUT %	0.988	0.957, 1.020	0.453
LYMPH %	1.017	0.983, 1.053	0.329
MONO %	0.915	0.819, 1.022	0.114
CKMB	0.996	0.989, 1.002	0.199
NT-proBNP	1.000	0.999, 1.000	0.157
LDL-C	0.785	0.531, 1.162	0.226
Blood glucose	0.834	0.671, 1.036	0.101
D-Dimer	0.918	0.589, 1.431	0.706
Fibrinogen	0.901	0.653, 1.242	0.523

Note: Bold font denotes $P < 0.05$.

Abbreviations: HF, heart failure; ACS, acute coronary syndromes; PCI, percutaneous coronary intervention; HR, hazard ratio; CI, confidence interval; BMI, body mass index; DM, Diabetes Mellitus; MI, myocardial infarction; CABG, coronary artery bypass grafting; LVEF, left ventricular ejection fraction; RBC, red blood cell; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, Platelet; WBC, white blood cell; NEUT, Neutrophilic granulocyte; LYMPH, Lymphocyte; MONO, Monocyte; EO, Eosinophilic granulocyte; LDL-C, low-density lipoprotein cholesterol; NT-proBNP, N-terminal pro-B-type natriuretic peptide; CKMB, creatine kinase isoenzymes MB.

Table 4 Multivariate Cox Regression Analysis for PLT Count, LYMPH Count, EO Count and EO %

Variables	Model 1	Model 2
	HR (95% CI), P value	HR (95% CI), P value
PLT count	1.004 (1.000, 1.009), 0.050	1.001 (0.995, 1.006), 0.793
LYMPH count	1.906 (1.279, 2.839), 0.002	12.876 (1.373, 120.778), 0.025
EO count	10.695 (1.893, 60.417), 0.007	16.625 (2.461, 112.280) 0.004
EO %	1.171 (1.008, 1.360) 0.039	1.196 (1.016, 1.409) 0.031

Notes: Model 1: adjusted for age, male sex, BMI, smoking, DM, past PCI or CABG, SBP, DBP and LVEF. Model 2: further adjusted for RBC count, MCV, WBC count, NEUT count, LYMPH %, MONO %, NT-proBNP, blood glucose and D-Dimer. Bold font denotes $P < 0.05$.

Abbreviations: PLT, Platelet; LYMPH, Lymphocyte; EO, Eosinophilic granulocyte; HR, hazard ratio; CI, confidence interval; BMI, body mass index; DM, Diabetes Mellitus; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; RBC, red blood cell; MCV, mean corpuscular volume; WBC, white blood cell; NEUT, Neutrophilic granulocyte; MONO, Monocyte; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Kaplan–Meier Survival Analysis

Next, we explored the respective values for predicting HF in subgroups with different LYMPH count, EO count and EO % levels. Patients were equally divided into two groups (low-level and high-level) based on LYMPH count, EO count and EO % levels. Kaplan–Meier survival analysis was conducted to determine the long-term prognostic value of LYMPH count, EO count and EO % in ACS patients following PCI. With a median follow-up of 27.7 months, our results showed that high-level EO count was significantly associated with increased incidence of HF (Figure 1A, Log rank test: $P = 0.008$). As can be seen from [Supplementary Results](#), there were no statistically differences between high-level and low level of LYMPH count and EO % (Figure 1B and C), despite a similar trend with the result of Cox regression analysis based on the levels of LYMPH count, EO % ([Supplementary Table 1](#)).

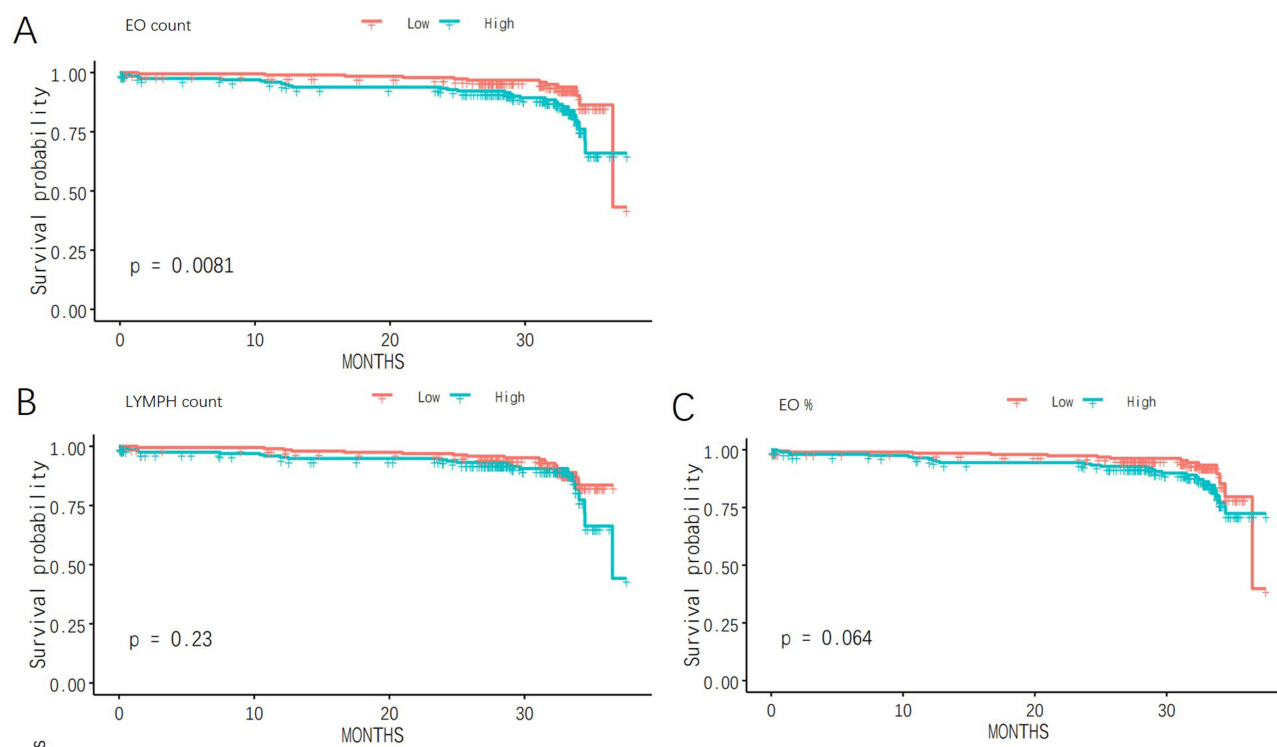


Figure 1 Kaplan-Meier survival analysis of the occurrence of HF based on EO count (A), LYMPH count (B), and EO% (C) levels (low-level and high-level) in ACS patients during the 2-year follow-up period.

Stratified Cox Regression Analysis

Considering that LYMPH count, EO count and EO % levels are closely related to age, gender, history of smoking and hypertension, we conducted stratification analysis further (Table 5). Our results demonstrated that, compared to patients younger than 60 years, both LYMPH and EO counts had greater predictive power for HF in those older than 60 years (HR: 1.588 vs 2.099; 9.242 vs 31.328, respectively). Compared to non-smoker, LYMPH count showed a stronger capacity to predict HF in patients who had a smoking history (HR: 1.650 vs 2.091). Moreover, for patients who had PCI or CABG history, EO count was typically a stronger predictor for HF.

Receiver-Operating Characteristic (ROC) Curve Analysis

The AUCs of LYMPH count, EO count and EO % for predicting the occurrence of HF in ACS patients after PCI over a 2-year follow-up are shown in Table 6. The AUC of EO count was 0.625 ($P = 0.037$), with a sensitivity of 0.705 and a specificity of 0.575. The ROC curves of EO count as a marker to predict HF in ACS patients after PCI are illustrated in Figure 2A.

Table 5 Stratified Cox Regression Analysis for LYMPH Count, EO Count and EO % in Predicting of HF in ACS Patients Following PCI Over a 2-Year Follow-Up

Variables	LYMPH Count		EO Count		EO %	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age <60 years	1.588 (1.037, 2.433)	0.034*	9.242 (1.588, 89.198)	0.003*	1.113 (0.856, 1.446)	0.425
≥60 years	2.099 (1.166, 3.779)	0.013*	31.328 (3.424, 286.653)	0.002*	1.217 (1.019, 1.453)	0.030
Male	1.804 (1.176, 2.767)	0.007	14.867 (2.163, 102.175)	0.006	1.165 (0.955, 1.422)	0.131
Female	2.133 (0.921, 4.939)	0.078	5.764 (0.358, 92.787)	0.217	1.174 (0.925, 1.492)	0.188
Smoker	2.091 (1.185, 3.691)	0.011*	373.67 (1.432, 97,529.946)	0.037	1.065 (0.708, 1.601)	0.762
Non-smoker	1.650 (1.013, 2.688)	0.044*	4.100 (0.758, 22.186)	0.102	1.133 (0.975, 1.316)	0.120
Hypertensives	1.582 (0.854, 2.931)	0.145	6.149 (1.104, 34.259)	0.038	1.267 (0.996, 1.367)	0.055
Non-hypertensives	1.921 (0.890, 3.995)	0.093	44.353 (0.352, 5587.592)	0.124	1.070 (0.779, 1.471)	0.676
Family history: Yes	2.792 (1.514, 5.151)	0.001	8.758 (0.923, 83.127)	0.059	1.206 (0.977, 1.489)	0.082
Family history: No	1.391 (0.858, 2.257)	0.181	19.473 (1.791, 211.747)	0.015	1.152 (0.949, 1.398)	0.153
Past MI: Yes	2.170 (0.971, 4.851)	0.059	84.280 (0.037, 189,939)	0.260	1.168 (0.644, 2.118)	0.610
Past MI: No	1.731 (1.149, 2.607)	0.009	10.540 (2.059, 53.950)	0.015	1.177 (1.011, 1.370)	0.036
Past PCI or CABG: Yes	1.582 (0.669, 3.740)	0.296	801.325 (1.074, 598,072)	0.048*	1.477 (0.950, 2.296)	0.083
Past PCI or CABG: No	1.842 (1.257, 2.699)	0.002	8.713 (1.566, 48.491)	0.013*	1.135 (0.962, 1.340)	0.134

Notes: All significant results (HR and 95% CI) are in bold. Asterisks indicate meaningful differences ($P < 0.05$).

Abbreviations: LYMPH, Lymphocyte; EO, Eosinophilic granulocyte; HF, heart failure; ACS, acute coronary syndromes; PCI, percutaneous coronary intervention; HR, hazard ratio; CI, confidence interval; MI, myocardial infarction; CABG, coronary artery bypass grafting.

Table 6 ROC Analysis for Predicting the Occurrence of HF in ACS Patients Following PCI Over a 2-Year Follow-Up

Variables	AUC	Sensitivity	Specificity	C Index	P value
LYMPH count	0.492	0.869	0.187	0.625	0.020
EO count	0.625	0.705	0.575	0.828	0.037
EO %	0.539	0.620	0.546	0.600	0.067
Model 1	0.627	0.639	0.566	0.664	<0.001
Model 2	0.642	0.631	0.591	0.710	<0.001

Notes: Model 1 consisted of EO count and age. Model 2 consisted of EO count, LYMPH count, EO %, age and male sex. Bold characters indicate the best performances.

Abbreviations: ROC, receiver operating characteristic; HF, heart failure; ACS, acute coronary syndromes; PCI, percutaneous coronary intervention; AUC, area under the receiver curve; LYMPH, Lymphocyte; EO, Eosinophilic granulocyte.

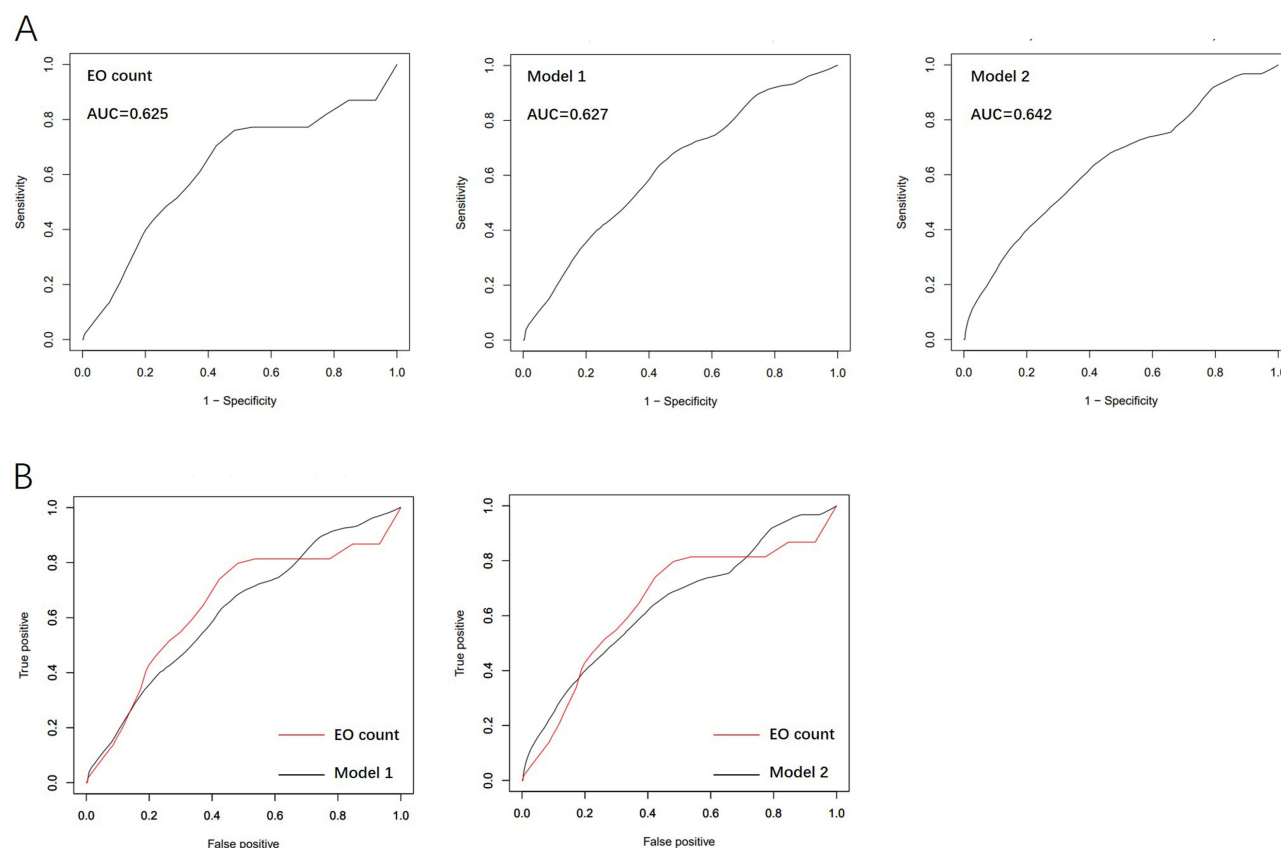


Figure 2 Receiver-operating characteristic (ROC) curve analysis. **(A)** ROC curve analysis of EO count, Model 1 and Model 2 (Model 1: EO count combined with age; Model 2: EO count combined with LYMPH count, EO %, age and gender) in ACS patients following PCI over a 2-year follow-up. **(B)** Comparisons of the ROC curve of EO count and ROC curves of model 1 or model 2, respectively.

To find the best predictive index for HF, we next evaluated various combinations of these four blood cell indices with age and gender. The results revealed that only two of these combinations (Model 1 and Model 2) were found to have statistical significance. The AUC of EO count combined with age (Model 1) was 0.627 ($P < 0.001$), and the AUC of EO count combined with LYMPH count, EO %, age and gender (Model 2) was 0.642 ($P < 0.001$), which were higher than that of each blood cell index. Regrettably, no difference between ROC curve of EO count and ROC curve of model 1 was observed neither ROC curve of EO count and ROC curve of model 2 (Figure 2B). In a word, the combinations were not more valuable than EO count alone. EO count had a high long-term prognostic value in the parameters observed.

Discussion

We conducted this study, enrolling 416 ACS patients after PCI and making follow-up (the median follow-up time was 27.7 months), to explore the clinical predictive values of WBC indices (LYMPH and EO) for the occurrence of HF. The results indicated that LYMPH count, EO count and EO % were higher in ACS patients who suffered HF. Secondly, LYMPH count, EO count and EO % were independent predictors for HF in patients with ACS undergoing PCI over a 2-year of prospective observation, even after adjustment for potential confounding factors. Of these, for smokers aged 60 years and above, who had PCI or CABG history, the higher EO count, the higher the incidence of HF. Furthermore, EO count had highest long-term prognostic value among all the parameters we observed. To the best of our knowledge, this study is the first time to uncover the relationship between EO count and EO % and 2-year adverse cardiovascular events in ACS patients after PCI in China.

Our data are important as they uncover the causal relationships to provide the supplementation that earlier observational studies have reported, indicating associations between peripheral WBC and HF. In our previous studies,¹⁴ we have reported that MCV and MCH, two of the RBC indices included in routine blood examination which is widely used in

clinic, related to increased long-term major adverse cardiovascular events (MACEs) in ACS patients. The results combined with our present data, which displayed high value of RBC and WBC indices, help to highlight the potential importance of routine blood examination, the most basic but most easily ignored laboratory examination.

Although an orchestrated and strongly controlled immune response is a novel step for an adequate repair of the infarcted myocardium, a deregulated and excessive activation of the immune response leading to inflammatory cascade has been demonstrated to mediate myocardial damage and in turn associated with worse clinical outcomes in ACS patients.^{12,15} Traditionally, WBC count determined by blood routine examination has been used as a biomarker to explore the association. Of these, neutrophil, monocyte, lymphocyte and, more recently, eosinophils cell counts have been gradually gained considerable attention.¹⁶ As an important effector of immune system, T lymphocyte-mediated immune response has a vital role in the progression of cardiovascular disease.^{17,18} In congestive HF, autoreactive T helper (Th) cells with specificity for an antigen expressed in cardiomyocytes can promote the progression from hypertrophy to HF in response to pressure overload, and the activation of the immune system leads to increased production and release of proinflammatory cytokines.¹⁶ Patients with congestive HF show increased pro-inflammatory CD4⁺ Th1 and Th17 cells, as well as lower anti-inflammatory T regulatory cells (Treg), and these features are related to disease severity.¹⁹ In chronic ischemic cardiomyopathy, systemic expansion of CD4⁺ and CD8⁺ T lymphocyte and CD4⁺ Th1, Th2, Th17, and Treg subsets can be found in the heart, circulation, and lymphoid organs. Moreover, various studies have shown that T lymphocytes play an important role in the progression of cardiac remodeling after MI.¹⁶ In addition, CD4/CD28-null T cells are observed to accumulate preferentially in unstable ruptured coronary plaques and have been suggested to promote plaque instability, which predisposes ACS patients to recurrent acute coronary events and indicates a poor prognosis.²⁰ At the same time, we have observed a correlation between lymphocyte count and HF in ACS patients. The results of the current study indicate that baseline lymphocyte count is an independent predictor of HF in patients with ACS undergoing percutaneous coronary intervention (PCI) over a 2-year period of prospective observation, even after adjustment for potential confounding factors. Thus, our findings provide pathophysiological insights into the development of HF, and reducing T lymphocyte infiltration in ACS patients may be a necessary translational target in the treatment of HF, albeit after immediate revascularization and antithrombotic strategies. At the same time, we have observed relations between lymphocyte and HF in ACS patients. The result in the current study indicated that baseline LYMPH count was independent predictors for HF in patients with ACS undergoing PCI over a 2-year of prospective observation, even after adjustment for potential confounding factors. Thus, our findings provide pathophysiological insight into the development of HF, and the reduction of T lymphocyte infiltration in ACS patients may be a necessary translational target in HF although after immediate revascularisation and antithrombotic strategies.

Compared to other inflammatory parameters, eosinophil was seldom discussed, although the routine blood examination is a frequently used measure of immune responses in ACS patients. Cardiovascular manifestations of hyper-eosinophilic syndrome are the main cause of morbidity and mortality in the uncommon disorder, eosinophilic cardiomyopathy.²¹ The toxic effects of eosinophil cells in the pathophysiology of eosinophilic cardiomyopathy have been demonstrated. Pathological observations of the patients' hearts with sustained eosinophilia illustrate that both ventricles may be affected by the infiltration of eosinophil cells in the interstitial compartment, which is deleterious to cardiac tissue and associated with thickening of LV wall, fibrosis of the endocardium, thrombus formation, vasculitis, and myocardial necrosis.^{22,23} In recent years, emerging findings of eosinophils in cardiovascular diseases are focus on its effect on regulating the inflammation progress. Eosinophils act an important role in the initiation, progression, and rupture of thrombus.²⁴ For instance, the infiltration of eosinophils into the infarcted myocardial area is involved in atherosclerotic plaque rupture, a basic inflammatory pathogenesis of ACS. Eosinophils help platelets adhering to the injured vessel wall and secondary releasing cytokines such as interleukin (IL)-4, IL-10, and IL-13 which are suggested to modulate the inflammatory response.²⁵ Similar to our findings, some studies have reported that a higher EO count is associated with a higher risk of cardiac events after MI. For instance, Jiang concluded that circulatory eosinophils reflected the extent of MI.²⁶ Besides, study from Toor et al showed that augmented eosinophil count was associated with a higher death risk at long-term follow-up, and eosinophils were found to be a useful biomarker for risk stratification of CAD patients and predicting mortality.²⁷ However, Shiyovic et al found that increased circulating EO count after admission in ST-elevation myocardial infarction (STEMI) patients had association with a lower risk of death over

1-year follow-up.²⁸ Furthermore, since the single inflammatory cell is unable to not representative of the overall systematic inflammation, new indexes are proposed by combining different subtypes of WBC indices. For instance, eosinophil to leukocyte ratio was reported to be independently associated with MACEs in STEMI patients following PCI.²⁹ The authors urged that study methodology, study period or different cohorts may account for these discrepancies between studies. Our results, obtained in a homogeneous group of ACS patients undergoing PCI, suggesting an association between increased peripheral EO count after admission with high risk of the incidence of HF. The last but not least, stratified Cox regression analysis for EO count indicated that, when compared to ACS patients younger than 60 years, both LYMPH and EO counts had greater predictive power for HF in those older than 60 years, what is more, ROC curve analysis demonstrated that the combinations of EO count with patient' age had a higher long-term prognostic value for HF. These findings could be incorporated into our daily armamentarium to improve risk stratification of ACS patients.

Overall, WBC count is a new risk factor and biomarker for the development of HF; importantly, in contrast to IL-6, tumor necrosis factor- α , and high-sensitive C-reactive protein, WBC count is established as a routine laboratory parameter in clinical practice. First, our results further support WBC indices as strong independent risk factors for HF in ACS patients, even after PCI, which warrants evaluation as a target for intervention not only in manifest HF but also in prevention. Second, we add evidence suggesting the differences in age and gender in the development of HF, which might have implications for future trials on preventive interventions targeting inflammatory process. Additionally, we suggest high eosinophils count as a novel risk factor of HF which warrants further study in human population and animal model.

Limitation

(1) The current study was a single-center study with a small sample size limited to ACS patients following PCI from China. A larger cohort from more-centre and different populations would be needed to further refine our findings. (2) The duration of follow-up was likely too short that a longer follow-up visit is needed to further clarify the influence of the WBC indices on long-term prognoses. (3) There may be other possible factors which might have impacts on the outcomes but have not been adjusted. (4) Due to missing data of basophil count and percentage, statistical analyses were not performed. Further investigation in terms of basophil count is required to extend our findings.

Conclusion

Elevated baseline LYMPH count, EO count and EO % measured by routine blood examination were independently associated with the risk of heart failure in ACS patients following PCI, suggesting that WBC indices might be available, simple, and cost-efficient biomarkers with predictive values, and be worthy of further exploration of the underlying mechanism. As standard and easily obtained, parameters from routine blood examination could provide the possibility to help clinicians to identify high-risk patients, predict the long-term clinical outcomes, and optimize clinical decisions, especially for patients aged more than 60 years.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author Juan Zhou (Email: zj.7332@mail.xjtu.edu.cn) on reasonable request.

Ethics Approval and Consent to Participate

Approval of the Ethics Committee of Xi'an Jiaotong University (Ethical approval number: XJTU1AF2012LSK-312) was obtained, and the written informed consents were obtained from all the participants. The study conformed to the Declaration of Helsinki and collected the required data from the clinical records without clinical intervention to protect patients' privacy.

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Disclosure

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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