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Original Article

Peripheral blood memory T cells and follicular helper T cells as potential biomarkers for assessing disease severity and in-hospital outcomes in acute myocardial infarction patients

Xuming Bi[1], Zhenhong Zhang[1], Zhenping Zhang[1], Hongwei Lian[2], Wei Gao[3]

[1]*Department of Dermatology and Cardiology, Shidao People's Hospital, Rongcheng 264300, Shandong, China;* [2]*Rongcheng City Center for Disease Control and Prevention, Rongcheng 264300, Shandong, China;* [3]*Department of Dermatology and Emergency, Shidao People's Hospital, Rongcheng 264300, Shandong, China*

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Abstract: Background: Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality worldwide. Understanding its pathogenesis is essential for improving treatment and outcomes. Memory T cells and follicular helper T cells (Tfh) may play a role in the immune response to AMI. This study aimed to investigate the association of these cells with AMI severity and hospital outcomes, exploring their potential as biomarkers. Methods: We studied 403 AMI patients admitted to Shidao People's Hospital, Rongcheng City, between January 2019 and July 2023. Based on Gensini scores assessing coronary artery damage, patients were split into low-risk (LR) and high-risk (HR) groups. Additionally, we categorized patients by hospital outcomes: adverse outcomes (AO, including death or complications) or non-adverse outcomes (NAO). Memory T cell subsets and Tfh cells were quantified using flow cytometry. Heart function and inflammation markers were also measured. Statistical analyses were performed to correlate immune cell levels with AMI severity and outcomes. Results: Patients in the HR group exhibited higher percentage and absolute numbers of CD3+ CD8+ effector memory (Tem) cells and Tfh cells. Elevated levels of these cells were associated with more severe AMI and poorer hospital outcomes. Higher levels of inflammation markers (ischemia-modified albumin [IMA], hypersensitive C-reactive protein [hs-CRP], pentraxin 3 [PTX3]) correlated with worse disease severity. Conversely, lower levels of CD4+ resting memory T cells were linked to worse outcomes, suggesting a potential protective role. Increases in central memory and effector memory T cells were also associated with poor outcomes. Conclusion: Our findings show clear links between specific types of T cell subsets and AMI severity and outcomes, highlighting the immune system's critical role in AMI pathogenesis.

Keywords: Acute myocardial infarction, memory T cells, follicular helper T cells, disease severity, biomarkers, in-hospital outcomes

Introduction

Acute myocardial infarction (AMI) remains a leading cause of morbidity and mortality worldwide, despite advances in clinical management and therapeutic interventions [1]. Characterized by the sudden occlusion of coronary blood supply, which leads to myocardial tissue necrosis, AMI involves complex mechanisms encompassing both acute inflammatory responses and chronic immune system dysregulation [2, 3]. Identifying biomarkers that accurately reflect disease severity and predict clinical outcomes is critical for improving management strategies and patient prognoses [4]. Recent research has highlighted that immune cell subsets, specifically memory T cells and Tfh, play significant roles in modulating the inflammatory processes in AMI and influencing patient outcomes [5].

Memory T cells, including both central and effector memory T cell subsets, are key players in adaptive immunity, known for their longevity and rapid response upon antigen re-exposure [6, 7]. In cardiovascular diseases, their role extends beyond pathogen clearance to modulation of inflammatory responses at sites of vas-

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cular injury, influencing both disease progression and repair mechanisms [8]. Investigations reveal that different memory T cell subpopulations contribute distinctly to the inflammatory milieu in the myocardium following ischemic events [9]. While central memory T cells are primarily involved in systemic immune surveillance, effector memory T cells exert localized effects at sites of tissue damage [10].

Tfh cells represent another important cellular subset due to their pivotal role in humoral immunity [11]. By assisting B cells in antibody production, Tfh cells have been implicated in the development of immune-mediated conditions and chronic inflammatory disorders, where an exaggerated humoral response contributes to tissue damage [12]. In the context of AMI, it has been hypothesized that Tfh cells may exacerbate inflammatory responses through autoantibody production or the perpetuation of chronic inflammation. Previous investigations have indicated that inflammatory activity associated with Tfh cells correlates with adverse cardiovascular outcomes, suggesting a potential predictive role for these cells in AMI [13].

While the relationship between immune cell dynamics and cardiovascular diseases has been progressively elucidated, data specifically examining the interplay between peripheral blood memory T cells, Tfh cells, and clinical outcomes in AMI remain limited. A deeper understanding of these interactions could reveal novel biomarkers for disease risk stratification and therapeutic intervention, facilitating personalized treatment approaches [14]. Moreover, investigating the impact of these immune cell subsets on both myocardial damage severity and recovery trajectory could offer valuable insights into the development of immunomodulatory treatments aimed at reducing inflammation-driven myocardial injury [15].

This study aims to investigate the correlation between peripheral blood memory T cells, Tfh cells, and both disease severity and in-hospital outcomes in AMI patients. By elucidating these associations, we aim to identify novel biomarkers for early risk stratification and inform targeted immunomodulatory therapies to improve clinical outcomes in AMI.

Methods

Research protocol

This retrospective study included 403 patients diagnosed with AMI who were admitted to Shidao People's Hospital, Rongcheng City between January 2019 and July 2023. Data were collected from existing medical records post-discharge, ensuring comprehensive data collection without participant loss. AMI severity was evaluated using the Gensini score, which quantifies coronary artery stenosis and lesion location. The sample size was calculated using G*Power software, assuming a medium effect size ($d = 0.5$) and a two-tailed significance level ($\alpha = 0.05$). A minimum of 105 patients per group was required to reject the null hypothesis of equal means with 95% statistical power, based on a two-sided, two-sample t-test assuming equal variances.

According to the Gensini scoring system, a score < 30 was indicative of mild lesions, 30-59 indicated moderate lesions, and ≥ 60 indicated severe lesions [16]. Patients with mild or moderate lesions (n = 276) were categorized as the low-risk (LR) group, whereas those with severe lesions (n = 127) were classified as the high-risk (HR) group. Additionally, patients were further classified into two groups according to their in-hospital outcomes. Those who experienced adverse events - such as death, major bleeding, recurrent AMI, cardiogenic shock, malignant arrhythmia, heart failure, or stroke - were assigned to the adverse outcome (AO) group (n = 94), while those without these events were assigned to the non-adverse outcome (NAO) group (n = 309). Adverse events were confirmed based on clinicians' documented diagnoses in medical records.

Ethical approval was granted by the Institutional Review Board and the Ethics Committee at Shidao People's Hospital, Rongcheng City. Given the retrospective nature of the study and the use of anonymized patient records, the requirement for informed consent was exempted according to the applicable regulatory and ethical guidelines.

Inclusion and exclusion criteria

Inclusion criteria: (1) patients meeting the diagnostic criteria for AMI [17, 18] age ≥ 18 years;

(3) newly diagnosed AMI; (4) normal liver and kidney function; (5) complete clinical case data available.

Exclusion criteria: (1) patients with other types of heart disease such as congenital heart disease or rheumatic heart disease [18]; (2) patients with cancer, infectious diseases, or autoimmune deficiencies; (3) patients with coagulation disorders; (4) patients with acute metabolic disorders; (5) patients with cerebrovascular or peripheral vascular diseases; (6) patients with psychiatric disorders involving sensory disturbances; (7) patients discharged alive within 24 hours of admission; (8) patients who died or discontinued treatment within 24 hours of admission.

Data collection

Medical data were retrieved from the clinical information system, encompassing basic demographic information, routine blood tests, markers of cardiac injury, cardiovascular inflammatory indicators, and levels of peripheral blood memory T cell subsets and Tfh cells. Cardiac function parameters, including left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDD), and left ventricular end-systolic diameter (LVESD), were assessed using a Philips iu22 color Doppler ultrasound diagnostic system (Philips Medical Systems, Ltd., Eindhoven, the Netherlands) with a probe frequency of 7-12 MHz.

Measurement of routine blood tests, cardiac injury markers and cardiovascular inflammatory indicators

Peripheral venous blood samples (3 mL) were obtained from AMI patients after an overnight fast, approximately 24 hours following admission. These samples were processed using a low-temperature high-speed centrifuge (model TLD 12A, Hunan Xiangxi Scientific Instrument Factory) at 3,000 rpm for 10 minutes with a centrifugal radius of 10 cm. The supernatant was then collected and stored at -80°C for subsequent analysis.

Routine blood parameters, including red blood cells (RBC), white blood cells (WBC), platelets (PLT), and hemoglobin (Hb) levels, were assessed using a fully automated hematology analyzer (model BC6800, Mindray Bio-Medical Electronics Co., Ltd., Shenzhen) and a fully automated coagulation analyzer (model ACLT-OP750, Werfen Medical Products Trading Beijing Co., Ltd.). High-sensitivity C-reactive protein (hs-CRP) levels were measured using immunoturbidimetry with a kit from Shenzhen Jinrui Biotechnology Co., Ltd. Ischemic modification albumin (IMA) levels were evaluated using the free cobalt colorimetric method with a kit from Nanjing Norman Biotechnology Co., Ltd. Pentraxin 3 (PTX3) levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit provided by Shanghai Saiposen Biotechnology Co., Ltd. (SPS-15170, China). All procedures adhered strictly to the manufacturers' instructions.

Detection of peripheral blood memory T cell and follicular T cell levels

Peripheral venous blood (5 mL) was collected in ethylenediaminetetraacetic acid (EDTA) tubes. Peripheral blood mononuclear cells (PBMCs) were isolated from 3 mL of whole blood using Lymphoprep. Fresh EDTA-anticoagulated blood specimens were mixed with physiological saline and lymphocyte separation medium in a 1:1:1 ratio. The mixture was centrifuged at 1500 rpm for 20 minutes at room temperature utilizing a low-temperature high-speed centrifuge (model TLD 12A, Hunan Xiangxi Scientific Instrument Factory). Following centrifugation, PBMCs were collected with a pipette, resuspended in physiological saline in a 1:1 ratio, then centrifuged again at 1200 rpm for 8 minutes at room temperature. The supernatant was discarded, and the cells were resuspended in RPMI 1640 medium, adjusting the cell concentration to 1 × 10^6 cells/mL.

A 100 μL aliquot of PBMCs was transferred to a flow cytometry tube, to which a dead cell exclusion dye and human Fc receptor blocking solution were added. After thorough mixing, the sample was incubated in the dark for 15 minutes. Fluorescently labeled antibodies specific for T cell subsets were then added. Flow cytometric analysis was conducted using a FACSVerse flow cytometer (BD Biosciences) to identify CD3+ CD8+ Tem, CD4+ memory T cells, CD4+ CD45RO+ memory T cells, and Tfh cells. All peripheral blood samples were processed on the same day they were collected.

Statistical analysis

Statistical analysis was conducted using SPSS 29.0 software (SPSS Inc., Chicago, IL, USA). Categorical variables were reported as counts and percentages [n (%)]. The chi-square test was used for the sample with size ≥ 40 and expected frequencies (T) ≥ 5, with the test statistic denoted as χ^2. For samples with sizes ≥ 40 but expected frequencies between 1 and < 5, the chi-square test was corrected using the appropriate formula. If the sample sizes was < 40 or T < 1, Fisher's exact test was applied.

Continuous variables were initially evaluated for normality using the Shapiro-Wilk test. Data with a normal distribution were presented as means and standard deviations (X ± s). For data not following a normal distribution, the Wilcoxon rank-sum test was applied, with results presented as medians and interquartile ranges [median (25% quartile, 75% quartile)]. Statistical significance was set at a *P*-value below 0.05. Correlation analysis was conducted using Pearson's correlation for continuous variables and Spearman's correlation for categorical variables. This analysis examined the relationship between peripheral blood memory T cells, follicular T cells, disease severity, and in-hospital outcomes.

Results

Basic data

This study investigated the association between peripheral blood memory T cells, Tfh cells, and AMI severity by comparing the demographic and clinical characteristics between the LR group (n = 276) and the HR group (n = 127) (**Table 1**). No significant differences were observed in age (58.5 ± 8.26 vs. 59.25 ± 8.18 years; P = 0.395) or gender distribution (female/male: 43.84%/56.16% vs. 45.67%/54.33%, P = 0.731) between the two groups. Body mass index (BMI) was comparable between groups, as were lifestyle factors such as smoking and alcohol consumption history (P > 0.05). Medical histories, including hypertension, diabetes, and hyperlipidemia, did not differ significantly between groups (P > 0.05). Educational level and marital status were also comparable between groups. The distribution of myocardial infarction types (STEMI vs. NSTEMI) did not differ significantly between groups (44.2%/55.8% vs. 40.94%/59.06%, P = 0.540). No significant differences were observed in medication usage, including aspirin, statins, nitrate lipid, β-receptor antagonists, clopidogrel, ACEI/ARB, and CCB (all P > 0.05). These analyses demonstrate that the demographic and clinical backgrounds of the two groups were well-matched, supporting the subsequent analyses of immunological parameters and clinical outcomes related to AMI severity.

The LVEF was higher in the LR group compared to the HR group (50.08% ± 6.37% vs. 48.74% ± 4.65%, P = 0.018), indicating better cardiac function in the LR group (**Table 2**). Additionally, the LVEDD was larger in the HR group compared to the LR group (52.28 ± 2.34 mm vs. 51.66 ± 2.65 mm, P = 0.024). The LVESD was also significantly greater in the HR group (35.65 ± 2.67 mm vs. 34.99 ± 2.34 mm, P = 0.013). These findings suggest that impaired cardiac function, as indicated by LVEF, LVEDD, and LVESD, is correlated with increased disease severity in AMI patients.

Routine blood results among different levels of disease severity

The HR group exhibited significantly higher WBC counts than the LR group (10.54 ± 3.04 × 10^9/L vs. 9.78 ± 2.77 × 10^9/L, P = 0.013), suggesting a potential association with increased inflammatory response and disease severity (**Table 3**). However, other routine blood metrics, including red blood cell (RBC) counts (4.89 ± 1.38 × 10^{12}/L vs. 5.04 ± 1.86 × 10^{12}/L, P = 0.401), platelet count (PLT) (242.68 ± 23.3 × 10^9/L vs. 244.49 ± 25.87 × 10^9/L, P = 0.485), and hemoglobin (Hb) levels (157.38 ± 11.73 g/L vs. 159.71 ± 17.52 g/L, P = 0.175), did not show marked distinctions among the groups. These results emphasize WBC count as a marker of inflammation, correlating with AMI severity.

Cardiac injury markers and cardiovascular inflammatory indicators among different levels of disease severity

Ischemia modified albumin (IMA) levels were notably higher in the HR group compared to the LR group (102.16 ± 11.56 U/mL vs. 99.66 ± 8.68 U/mL, P = 0.031), indicating greater ischemic stress (**Table 4**). Similarly, hypersensitive C-reactive protein (hs-CRP) levels were elevat-

Table 1. Comparison of demographic characteristics between the two groups

Parameters	LR group (n = 276)	HR group (n = 127)	t/x^2	P
Age (years)	58.5 ± 8.26	59.25 ± 8.18	0.851	0.395
Female/Male	121 (43.84%)/155 (56.16%)	58 (45.67%)/69 (54.33%)	0.118	0.731
Body Mass Index (kg/m^2)	23.34 ± 2.52	23.28 ± 2.24	0.220	0.826
Smoking history	82 (29.71%)	42 (33.07%)	0.461	0.497
Alcohol consumption history	79 (28.62%)	44 (34.65%)	1.488	0.223
Hypertension	81 (29.35%)	42 (33.07%)	0.569	0.451
Diabetes	75 (27.17%)	31 (24.41%)	0.343	0.558
Hyperlipidemia	83 (30.07%)	42 (33.07%)	0.365	0.545
Educational level (high school or below/college or above)	63 (22.83%)/213 (77.17%)	32 (25.2%)/95 (74.8%)	0.271	0.602
Marital Status (Single/Married/Divorced)	73 (26.45%)/167 (60.51%)/36 (13.04%)	31 (24.41%)/76 (59.84%)/20 (15.75%)	0.604	0.739
Types of myocardial infarction (STEMI/NSTEMI)	122 (44.2%)/154 (55.8%)	52 (40.94%)/75 (59.06%)	0.376	0.540
Medication situation				
Aspirin	229 (82.97%)	100 (78.74%)	1.039	0.308
Statins	249 (90.22%)	112 (88.19%)	0.383	0.536
Nitrate lipid	191 (69.2%)	82 (64.57%)	0.855	0.355
β-receptor antagonist	151 (54.71%)	64 (50.39%)	0.651	0.420
Clopidogrel	144 (52.17%)	74 (58.27%)	1.301	0.254
ACEI/ARB	98 (35.51%)	55 (43.31%)	2.247	0.134
CCB	39 (14.13%)	19 (14.96%)	0.049	0.825

STEMI: ST segment elevation myocardial infarction; NSTEMI: Non-ST segment elevation myocardial infarction; ACEI/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker; CCB: calcium channel blockers.

Table 2. Comparison of cardiac function indicators between the two groups

Parameters	LR group (n = 276)	HR group (n = 127)	t	P
LVEF (%)	50.08 ± 6.37	48.74 ± 4.65	2.371	0.018
LVEDD (mm)	51.66 ± 2.65	52.28 ± 2.34	2.261	0.024
LVESD (mm)	34.99 ± 2.34	35.65 ± 2.67	2.498	0.013

LVEF: left ventricular ejection fraction; LVEDD: left ventricular end diastolic diameter; LVESD: left ventricular end systolic diameter.

Table 3. Comparison of routine blood characteristics between the two groups

Parameters	LR group (n = 276)	HR group (n = 127)	t	P
RBC/(10^{12}/L)	4.89 ± 1.38	5.04 ± 1.86	0.841	0.401
WBC/($\times10^9$/L)	9.78 ± 2.77	10.54 ± 3.04	2.493	0.013
PLT ($\times10^9$/L)	242.68 ± 23.3	244.49 ± 25.87	0.698	0.485
Hb (g/L)	157.38 ± 11.73	159.71 ± 17.52	1.363	0.175

RBC: red blood cell; WBC: white blood cell; PLT: platelets; Hb: hemoglobin.

Table 4. Comparison of cardiac injury markers and cardiovascular inflammatory indicators between the two groups

Parameters	LR group (n = 276)	HR group (n = 127)	t	P
IMA (U/mL)	99.66 ± 8.68	102.16 ± 11.56	2.170	0.031
Hs-CRP (mg/L)	22.65 ± 2.53	23.7 ± 5.21	2.168	0.032
PTX3 (ng/mL)	2.87 ± 0.84	3.06 ± 0.93	2.019	0.044

IMA: ischemia modified albumin; hs-CRP: hypersensitive C-reactive protein; PTX3: pentraxin 3.

ed in the HR group (23.7 ± 5.21 mg/L vs. 22.65 ± 2.53 mg/L, P = 0.032), reflecting a heightened inflammatory response associated with increased disease severity. Pentraxin 3 (PTX3) concentrations were also marked higher in the HR group (3.06 ± 0.93 ng/mL vs. 2.87 ± 0.84 ng/mL, P = 0.044), further supporting the presence of increased cardiovascular inflammation linked to worse clinical outcomes. These data underscore the potential utility of these biomarkers as indicators of disease AMI severity.

Peripheral blood memory T cell and follicular T cell levels among different levels of disease severity

The HR group exhibited a higher percentage of CD3+ CD8+ Tem cells compared to the LR group (40.88% ± 13.85% vs. 37.21% ± 10.35%, P = 0.008), as well as a greater absolute number (11.73 ± 5.27 × 10^7/L vs. 10.34 ± 3.04 × 10^7/L, P = 0.006) (**Figures 1**, **2**). Similarly, the percentage of Tfh cells was marked increased in the HR group (23.48% ± 6.24% vs. 21.57% ± 5.43%, P = 0.002), along with a higher absolute number (19.63 ± 7.45 × 10^7/L vs. 17.86 ± 4.26 × 10^7/L, P = 0.013). These analyses demonstrate that elevated levels of these immune cells were associated with greater disease severity and could potentially serve as markers for risk stratification in AMI patients.

In the evaluation of CD4+ memory T cell subpopulations, the HR groups showed a notable reduction in the percentage of CD4+ resting memory T cells compared to the LR group (30.67% ± 10.42% vs. 33.67% ± 10.63%, P = 0.008) (**Figures 3**, **4**). This reduction suggests a potential association between lower levels of CD4+ resting memory T cells and increased disease severity. Conversely, the percentage of CD4+ activated memory T cells did not differ significantly between the groups (2.02% ± 0.67% vs. 1.94% ± 0.62%, P = 0.233), indicating that activation status may not be a distinguishing factor in this context. These findings highlight the potential of CD4+ resting memory T cells as potential biomarkers for assessing risk and severity in AMI.

Additionally, the HR group demonstrated a statistically higher percentage of central memory T cells (52.81% ± 13.67%) compared to the LR group (49.23% ± 8.37%) (P = 0.007) (**Table 5**; **Figure 5**). Additionally, Tem cells were also elevated in the HR group (16.96% ± 4.66%) compared to the LR group (15.44% ± 3.98%) (P = 0.002). These analyses suggest that increased proportions of central and effector memory T cells are connected to greater disease severity and could serve as immune markers of risk in AMI patients.

Peripheral blood memory T cell and follicular T cell levels among different in-hospital outcomes

In patients with AMI, a comparison between the NAO group and the AO group revealed significant differences in the levels of peripheral

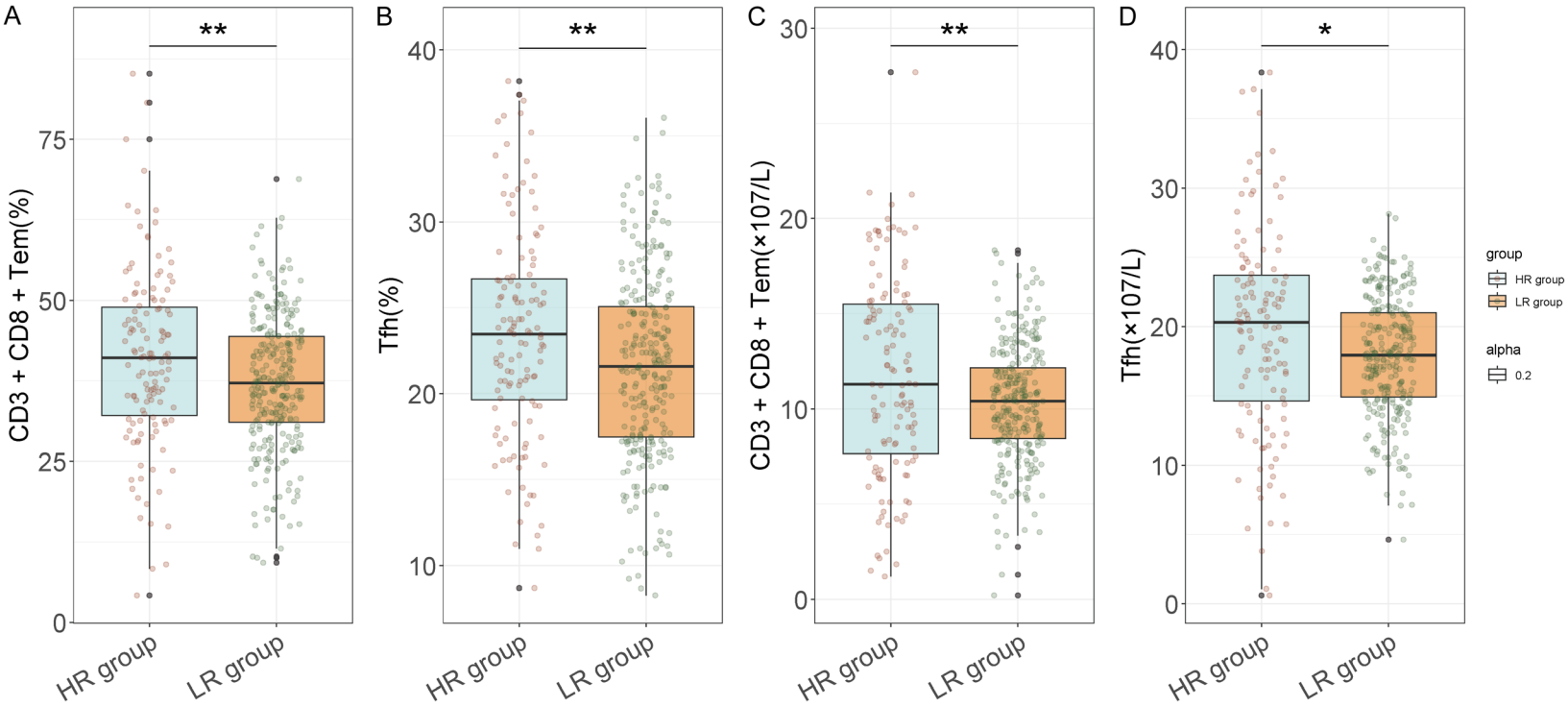


Figure 1. Comparison of CD3+ CD8+ Tem, Tfh cell percentage and absolute number between the LR and HR groups. Tem: effector memory T cells; Tfh: follicular helper T; + indicates positive. *: P < 0.05; **: P < 0.01.

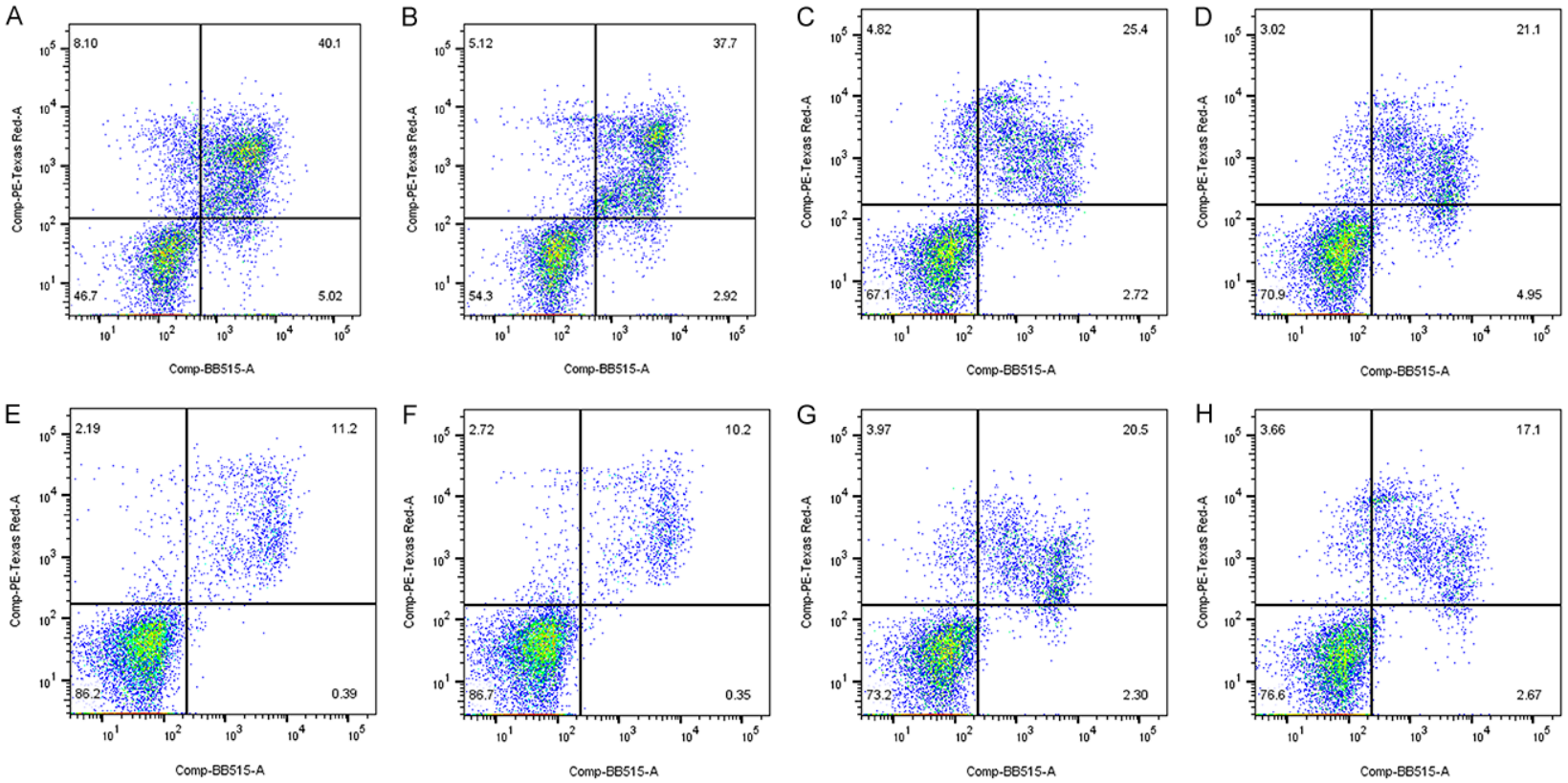


Figure 2. Flow cytometric analysis of CD3+CD8+ Tem cells and Tfh cells in high- and low-risk AMI patients. A. Percentage of CD3+CD8+ Tem cells in the HR group; B. Percentage of CD3+CD8+ Tem cells in the LR group; C. Percentage of Tfh cells in the HR group; D. Percentage of Tfh cells in the LR group; E. Absolute count of CD3+CD8+ Tem cells ($\times 10^7$/L) in the HR group; F. Absolute count of CD3+CD8+ Tem cells ($\times 10^7$/L) in the LR group; G. Absolute count of Tfh cells ($\times 10^7$/L) in the HR group; H. Absolute count of Tfh cells ($\times 10^7$/L) in the LR group; Tem: effector memory T cells; Tfh: follicular helper T cells.

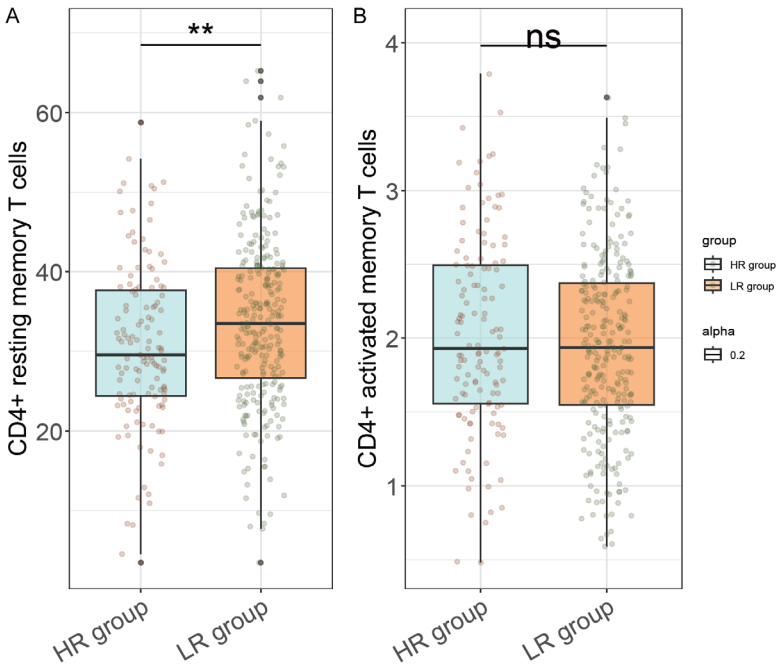


Figure 3. Comparison of CD4+ memory T cell subpopulations between the HR and LR groups (%). Ns: no significant; **: P < 0.01.

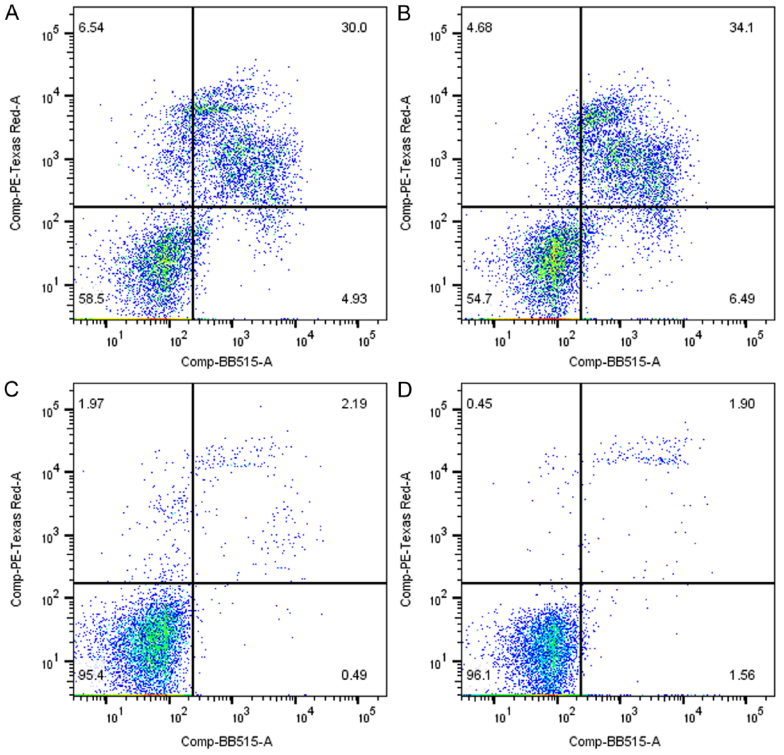


Figure 4. Flow cytometric analysis of CD4+ resting and activated memory T cells in high- and low-risk AMI patients. A. Percentage of CD4+ resting memory T cells in the HR group; B. Percentage of CD4+ resting memory T cells in the LR group; C. Percentage of CD4+ activated memory T cells in the HR group; D. Percentage of CD4+ activated memory T cells in the LR group.

blood memory T cells and Tfh cells (**Table 6**; **Figure 6**). The AO group showed significantly higher percentages and absolute numbers of CD3+ CD8+ Tem cells, with percentages of 39.72% ± 13.92% compared to 35.47% ± 10.72% in the NAO group (P = 0.007), and absolute numbers of 12.54 ± 5.72 × 10^7/L vs. 10.68 ± 3.77 × 10^7/L (P = 0.004). Additionally, Tfh cell levels were higher in the AO group, both in percentage (22.77% ± 6.53% vs. 20.39% ± 5.21%, P = 0.002) and absolute numbers (20.53 ± 8.84 × 10^7/L vs. 17.47 ± 4.43 × 10^7/L, P = 0.002). The AO group also demonstrated a marked reduction in CD4+ resting memory T cell percentages (31.05% ± 10.38% vs. 34.52% ± 10.47%, P = 0.005). No significant differences were observed in the percentages of CD4+ activated memory T cells. Furthermore, the AO group had elevated levels of CD4+ CD45RO+ central memory T cells (53.87% ± 13.45% vs. 50.23% ± 8.36%, P = 0.014) and Tem cells (17.73% ± 4.99% vs. 16.36% ± 4.23%, P = 0.017). These data indicate that higher levels of memory T cells and Tfh cells are associated with adverse in-hospital outcomes in AMI patients, highlighting their potential role as biomarkers for risk assessment.

Correlation analysis

Correlation analysis of peripheral blood memory T cells and Tfh cells along with routine biomarkers, with disease severity in AMI patients demonstrated several signifi-

Table 5. Comparison of CD4+CD45RO+ cells subpopulations between the two groups (%)

Parameters	LR group (n = 276)	HR group (n = 127)	t	P
Central memory T cells	49.23 ± 8.37	52.81 ± 13.67	2.729	0.007
Effector memory T cells	15.44 ± 3.98	16.96 ± 4.66	3.178	0.002

+ indicates positive.

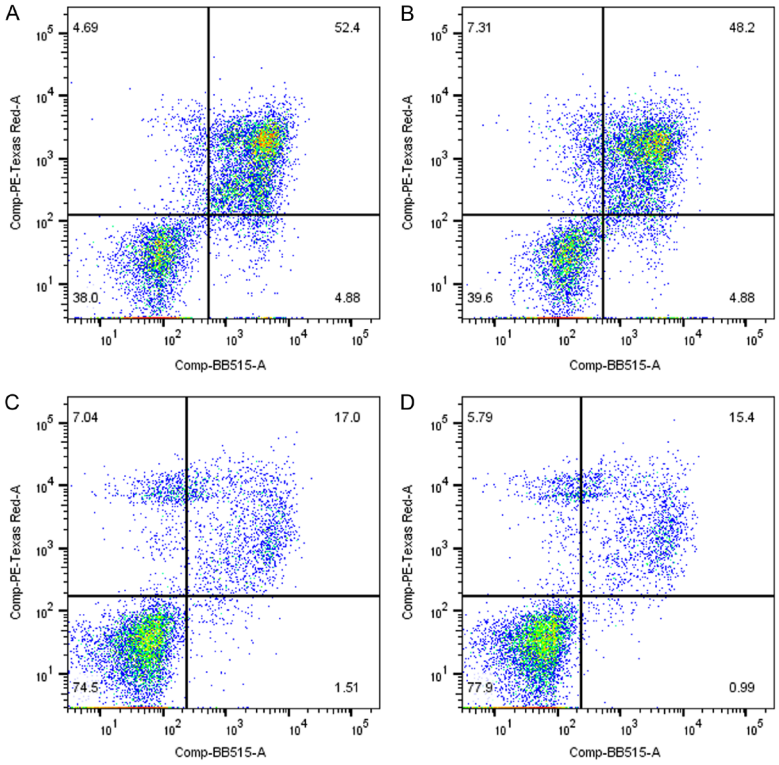


Figure 5. Flow cytometric evaluation of CD4+CD45RO+ central and effector memory T cells in high- and low-risk AMI patients. A. Percentage of CD4+CD45RO+ central memory T cells in the HR group; B. Percentage of CD4+CD45RO+ central memory T cells in the LR group; C. Percentage of CD4+CD45RO+ effector memory T cells in the HR group; D. Percentage of CD4+CD45RO+ effector memory T cells in the LR group.

cant associations (**Table 7**). WBC counts showed a modest positive correlation (rho = 0.106, P = 0.034) with disease severity. IMA levels (rho = 0.106, P = 0.033), hs-CRP (rho = 0.127, P = 0.011), and PTX3 (rho = 0.101, P = 0.042) also significantly correlated with increased severity. Notably, CD3+ CD8+ Tem cells, both as a percentage (rho = 0.134, P = 0.007) and in absolute numbers (rho = 0.127, P = 0.011), as well as Tfh cells (rho = 0.141 for percentage and rho = 0.142 for absolute count, P = 0.005 and P = 0.004, respectively), were positively correlated with greater disease severity. On the contrary, CD4+ resting memory T cells demonstrated a negative correlation (rho = -0.137, P = 0.006), indicating a possible protective role against disease progression. Additionally, both CD4+CD45RO+ central memory T cells (rho = 0.156, P = 0.002) and Tem cells (rho = 0.156, P = 0.002) were positively associated with disease severity. These findings underscore the potential of these immune cell subsets as important biomarkers for assessing AMI severity.

The correlation analysis examining the relationship between peripheral blood memory T cells, Tfh cells, and in-hospital outcomes in AMI patients revealed several significant associations (**Table 8**). The percentage of CD3+ CD8+ Tem cells was positively correlated with worse in-hospital outcomes (rho = 0.156, P = 0.002), as was the percentage of Tfh cells (rho = 0.155, P = 0.002). Similarly, the absolute number of CD3+ CD8+ Tem cells showed a positive correlation with AO (rho = 0.136, P = 0.006), and the absolute number of Tfh cells demonstrated an even stronger association (rho = 0.165, P < 0.001). In contrast, CD4+ resting memory T cells were negatively associated with adverse in-hospital outcomes (rho = -0.143, P = 0.004), indicating a potential protective role. Additionally, both CD4+CD45RO+ central memory T cells (rho = 0.132, P = 0.008) and Tem cells (rho = 0.112, P = 0.025) were linked with poorer outcomes. These findings suggest that specific memory T cell subsets can serve as critical biomarkers for predicting in-hospital outcomes in AMI, potentially guiding treatment strategies.

Discussion

The findings from our study provide significant insights into the intricate relationship between

Table 6. Comparison of peripheral blood memory T cell and follicular T cell levels between the two groups

Parameters	NAO group (n = 309)	AO group (n = 94)	t	P
CD3+ CD8+ Tem (%)	35.47 ± 10.72	39.72 ± 13.92	2.726	0.007
Tfh (%)	20.39 ± 5.21	22.77 ± 6.53	3.237	0.002
CD3+ CD8+ Tem ($\times 10^7$/L)	10.68 ± 3.77	12.54 ± 5.72	2.965	0.004
Tfh ($\times 10^7$/L)	17.47 ± 4.43	20.53 ± 8.84	3.233	0.002
CD4+ resting memory T cells (%)	34.52 ± 10.47	31.05 ± 10.38	2.821	0.005
CD4+ activated memory T cells (%)	1.79 ± 0.42	1.86 ± 0.52	1.248	0.214
CD4+CD45RO+ central memory T cells (%)	50.23 ± 8.36	53.87 ± 13.45	2.482	0.014
CD4+CD45RO+ effector memory T cells (%)	16.36 ± 4.23	17.73 ± 4.99	2.406	0.017

Tem: effector memory T cells; Tfh: follicular helper T; + indicates positive.

peripheral blood memory T cells, Tfh cells, and their potential role as biomarkers for disease severity and in-hospital outcomes in patients with AMI. Specifically, elevated levels of CD3+ CD8+ Tem and Tfh cells were observed in patients with higher disease severity and adverse in-hospital outcomes. These immune cell subsets may play crucial roles in the inflammatory and immune processes associated with AMI. The increased presence of CD8+ Tem cells in more severe cases likely reflects a robust immune response triggered by myocardial injury. Cytotoxic T cells, including Tem, are essential in driving inflammatory responses, which, if dysregulated, may contribute to cardiovascular damage.

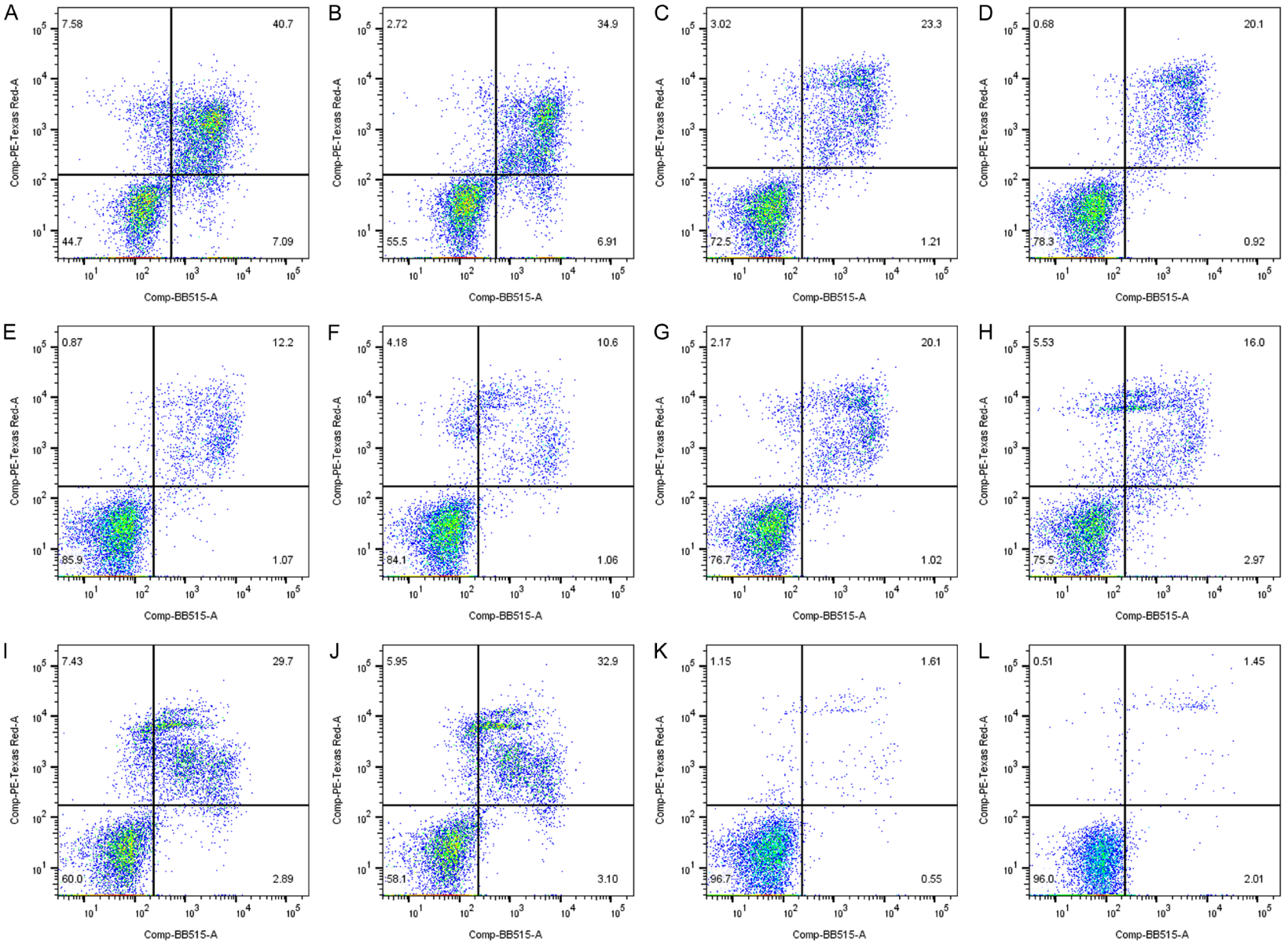
Furthermore, the positive correlation between Tfh cells with disease severity and adverse outcomes underscores their potential involvement in AMI pathogenesis. Tfh cells play a pivotal role in humoral immunity by aiding B cells and facilitating antibody production. In the context of AMI, elevated Tfh levels may indicate an upregulated humoral response, potentially exacerbating inflammation through autoantibody production or disruption of immune homeostasis.

Conversely, the inverse relationship between CD4+ resting memory T cells and both disease severity and AO suggests these cells may confer a protective effect against myocardial damage. CD4+ resting memory T cells are typically associated with immune regulation and the maintenance of immune tolerance. Their reduced levels in higher-risk patients may imply an impaired ability to suppress hyperactive immune responses, possibly resulting in unchecked inflammation and greater myocardial damage. This observation aligns with previous studies revealing that regulatory T cells and other subsets of CD4+ cells play protective roles in cardiovascular disease by modulating inflammation and preventing tissue injury.

Notably, the elevation of CD4+CD45RO+ central and effector memory T cells, which were associated with worsened outcomes and severity, might reflect a shift in immune cell populations towards an effector phenotype, promoting a more aggressive inflammatory response. Central memory T cells are known for their rapid response upon antigen re-exposure, leading to sustained inflammation and increased tissue damage in chronic inflammatory settings like AMI [19-21]. Effector memory T cells, in contrast, were typically involved in immediate defensive actions following injury, which can also perpetuate inflammation if not adequately regulated [22, 23].

In addition to immune cell dynamics, our study identified notable associations between WBC counts, IMA, hs-CRP, and PTX3 with disease severity. This reinforces the concept that systemic inflammation is intricately linked to the pathophysiology of AMI. Elevated WBC counts are well-recognized markers of systemic inflammation and have been consistently linked to adverse outcomes in cardiovascular diseases [24, 25]. The significance of IMA, hs-CRP, and PTX3 as inflammatory markers further emphasizes their crucial role in myocardial injury development and subsequent clinical outcomes.

The mechanisms underlying these immune responses are likely multifactorial, involving both innate and adaptive immune system


A
7.58
40.7
44.7
7.09
B
2.72
34.9
55.5
6.91
C
3.02
23.3
72.5
1.21
D
0.68
20.1
78.3
0.92
E
0.87
12.2
85.9
1.07
F
4.18
10.6
84.1
1.06
G
2.17
20.1
76.7
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H
5.53
16.0
75.5
2.97
I
7.43
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60.0
2.89
J
5.95
32.9
58.1
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K
1.15
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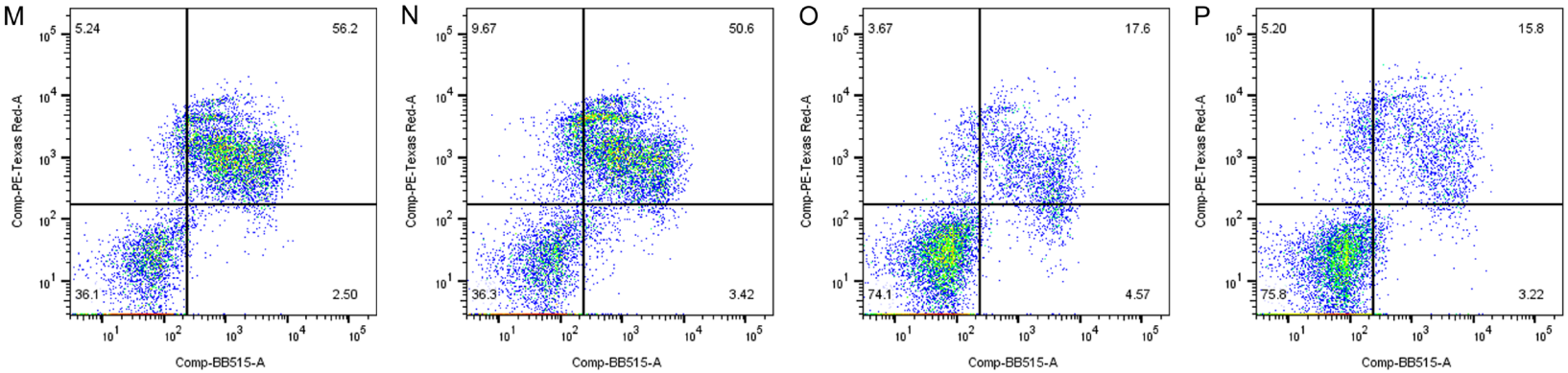

Figure 6. Flow cytometric analysis of peripheral blood memory T cell and Tfh cell subsets in AMI patients with AO and NAO. A. Percentage of CD3+CD8+ Tem cells in the AO group. B. Percentage of CD3+CD8+ Tem cells in the NAO group. C. Percentage of Tfh cells in the AO group. D. Percentage of Tfh cells in the NAO group. E. Absolute count of CD3+CD8+ Tem cells ($\times 10^7$/L) in the AO group. F. Absolute count of CD3+CD8+ Tem cells ($\times 10^7$/L) in the NAO group. G. Absolute count of Tfh cells ($\times 10^7$/L) in the AO group. H. Absolute count of Tfh cells ($\times 10^7$/L) in the NAO group. I. Percentage of CD4+ resting memory T cells in the AO group. J. Percentage of CD4+ resting memory T cells in the NAO group. K. Percentage of CD4+ activated memory T cells in the AO group. L. Percentage of CD4+ activated memory T cells in the NAO group. M. Percentage of CD4+CD45RO+ central memory T cells in the AO group. N. Percentage of CD4+CD45RO+ central memory T cells in the NAO group. O. Percentage of CD4+CD45RO+ effector memory T cells in the AO group. P. Percentage of CD4+CD45RO+ effector memory T cells in the NAO group. AO: adverse outcome; NAO: Non-adverse outcome; Tem: effector memory T cells; Tfh: follicular helper T.

Table 7. Correlation between peripheral blood memory T cells and follicular T cells and routine biomarkers with disease severity in AMI patients

Parameters	Rho	P
WBC/(×10^9/L)	0.106	0.034
IMA (U/mL)	0.106	0.033
Hs-CRP (mg/L)	0.127	0.011
PTX3 (ng/mL)	0.101	0.042
CD3+ CD8 + Tem (%)	0.134	0.007
Tfh (%)	0.141	0.005
CD3+ CD8+ Tem (×10^7/L)	0.127	0.011
Tfh (×10^7/L)	0.142	0.004
CD4+ resting memory T cells	-0.137	0.006
CD4+CD45RO+ central memory T cells (%)	0.156	0.002
CD4+CD45RO+ effector memory T cells (%)	0.156	0.002

WBC: white blood cell; IMA: ischemia modified albumin; hs-CRP: hypersensitive C-reactive protein; PTX3: pentraxin 3; Tem: effector memory T cells; Tfh: follicular helper T; + indicates positive.

Table 8. Correlation analysis of peripheral blood memory T cells and follicular T cells with in-hospital outcomes in AMI patients

Variables	Rho	P
CD3+ CD8+ Tem (%)	0.156	0.002
Tfh (%)	0.155	0.002
CD3+ CD8 + Tem (×10^7/L)	0.136	0.006
Tfh (×10^7/L)	0.165	$p < 0.001$
CD4+ resting memory T cells (%)	-0.143	0.004
CD4+CD45RO+ central memory T cells (%)	0.132	0.008
CD4+CD45RO+ effector memory T cells (%)	0.112	0.025

Tem: effector memory T cells; Tfh: follicular helper T; + indicates positive.

dynamics [26]. The initiation and progression of atherosclerosis, often preceding AMI, are heavily influenced by chronic inflammation and immune responses to oxidized lipids and other antigens within atherosclerotic plaques [27]. Upon plaque rupture and thrombosis, the subsequent ischemic injury results in the release of damage-associated molecular patterns (DAMPs), further activating immune pathways [28]. Additionally, myocardial damage from ischemia-reperfusion injury involves reactive oxygen species and inflammatory cytokine release, contributing to T-cell accumulation and activation at the injury site [29, 30]. This immune infiltration can exacerbate local inflammation and extend myocardial damage [31].

Understanding these dynamics is crucial for informing new therapeutic strategies aimed at modulating immune responses to improve outcomes after AMI. For instance, selectively targeting harmful immune responses while preserving or enhancing regulatory mechanisms could potentially mitigate excessive inflammation and its detrimental effects on cardiac tissue. Identifying individuals at higher risk for adverse outcomes could enable more personalized treatment approaches, optimizing both immediate and long-term patient care.

Despite these insights, several limitations should be considered. The observational design of the study precludes causal inferences, necessitating cautious interpretation of the observed associations. The sample size, although appropriate for preliminary findings, could fail to adequately reflect the diversity and complexity of patient immune responses; larger studies could provide more robust validation of our results. Additionally, the study population was limited to a specific geographic and demographic cohort, possibly impacting the generalizability of the findings. Furthermore, potential confounding factors such as concurrent medications, comorbid conditions, and genetic predispositions were not extensively controlled, which could impact immune cell profiles and outcomes. Addressing these limitations in future research would strengthen the evidence base and deepen our understanding of the complex immunological interactions in AMI.

Conclusion

In conclusion, this study illustrates the significant associations between specific T cell subsets and AMI severity and outcomes, accentuating the vital role of immune responses in the pathophysiology of the disease. Future research should focus on revealing the mechanisms by which these cells influence cardiovascular out-

comes and exploring targeted therapeutic strategies to leverage these pathways for clinical benefit. As our understanding of these immune interactions deepens, it may open new avenues for the management and treatment of AMI.

Disclosure of conflict of interest

None.

Address correspondence to: Wei Gao, Department of Dermatology and Emergency, Shidao People's Hospital, No. 199 Shuangbang East Road, Chishan Street, Rongcheng 264300, Shandong, China. E-mail: gw78256662024@126.com

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