

SERUM CYTOKINE NETWORKS INCLUDING IL-17A, VCAM-1, sIL-2R, IL-8, GATA1, IL-3, IL-5, AND GM-CSF PREDICT MAJOR ADVERSE CARDIAC EVENTS IN ST-ELEVATION MYOCARDIAL INFARCTION

SERUMSKE MREŽE CITOKINA KOJE UKLJUČUJU IL-17A, VCAM-1, sIL-2R, IL-8, GATA1, IL-3, IL-5 I GM-CSF U PREDVIĐANJU VELIKIH NEŽELJENIH KARDIOVASKULARNIH DOGAĐAJA KOD INFARKTA MIOKARDA SA ST ELEVACIJOM

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Summary

Background: Inflammation and immune dysregulation following ST-elevation myocardial infarction (STEMI) contribute to adverse remodelling and major adverse cardiac events (MACE). This study aimed to identify serum cytokine networks that predict 12-month MACE in STEMI patients, with a focus on IL-17A, VCAM-1, sIL-2R, IL-8, GATA1, IL-3, IL-5, and GM-CSF.

Methods: A prospective cohort of 320 STEMI patients undergoing primary percutaneous coronary intervention (pPCI) was enrolled. Serum levels of 18 cytokines, including the eight prespecified markers, were measured at admission, 24 hours, and 72 hours post-pPCI. The primary endpoint was MACE (cardiac death, recurrent MI, heart failure hospitalisation, or target vessel revascularisation) at 12 months. Cytokine networks were analysed using partial correlation matrices and machine learning (LASSO regression).

Results: MACE occurred in 71 patients (22.2%). A five-cytokine network comprising IL-17A, VCAM-1, sIL-2R, IL-8, and GATA1 at 24 hours post-pPCI most strongly predicted MACE (AUC 0.86, 95% CI 0.81–0.91). Elevated IL-17A (HR 2.45, $p < 0.001$), VCAM-1 (HR 1.98, $p = 0.003$), and sIL-2R (HR 2.12, $p = 0.001$) were independent predictors. GATA1 was detectable in 62% of patients at 24 hours and correlated with infarct size ($r = 0.54$, $p < 0.001$). IL-3, IL-5, and GM-CSF showed significant univariable associations with MACE, but these associations did not remain independent after multivariable adjustment.

Kratak sadržaj

Uvod: Inflamacija i imunološka disregulacija nakon infarkta miokarda sa ST elevacijom (STEMI) doprinose nepovoljnom remodelovanju srca i nastanku velikih neželjenih kardioloških događaja (MACE). Cilj ove studije je bio identifikovanje mreža serumskih citokina koje predviđaju pojavu MACE tokom 12 meseci kod pacijenata sa STEMI, sa posebnim fokusom na IL-17A, VCAM-1, sIL-2R, IL-8, GATA1, IL-3, IL-5 i GM-CSF.

Metode: U prospektivnu kohortnu studiju je uključeno 320 pacijenata sa STEMI koji su podvrgnuti primarnoj perkutanoj koronarnoj intervenciji (pPCI). Serumski nivoi 18 citokina, uključujući osam napred definisanih markera, mereni su pri prijemu, kao i 24 i 72 časa nakon pPCI. Primarni ishod je bio MACE (srčana smrt, reinfarkt miokarda, hospitalizacija zbog srčane insuficijencije ili revaskularizacija ciljnog krvnog suda) tokom 12 meseci. Mreže citokina su analizirane pomoću matrica parcijalne korelacije i metoda mašinskog učenja (LASSO regresija).

Rezultati: MACE se javio kod 71 pacijenta (22,2%). Mreža od pet citokina koju su činili IL-17A, VCAM-1, sIL-2R, IL-8 i GATA1, određena 24 časa nakon pPCI, najjače je predviđala MACE (AUC 0,86; 95% CI 0,81–0,91). Povišeni nivoi IL-17A (HR 2,45; $p < 0,001$), VCAM-1 (HR 1,98; $p = 0,003$) i sIL-2R (HR 2,12; $p = 0,001$) su bili nezavisni prediktori. GATA1 je bio detektabilan kod 62% pacijenata 24 časa nakon intervencije i korelirao je sa veličinom infarkta ($r = 0,54$; $p < 0,001$). IL-3, IL-5 i GM-CSF pokazali su značajnu povezanost sa MACE u univarijantnoj analizi,

Conclusion: A serum cytokine network comprising IL-17A, VCAM-1, sIL-2R, IL-8, and GATA1 at 24 hours post-pPCI robustly predicts MACE in STEMI. These findings support early immune profiling for risk stratification.

Keywords: STEMI, cytokine network, IL-17A, GATA1, MACE, risk prediction, inflammation

Introduction

ST-elevation myocardial infarction (STEMI) is still a major cause of morbidity and mortality worldwide. However, progress has been made in reperfusion therapy (1, 2). Major adverse cardiac events (MACE), including cardiac death, recurrent infarction, heart failure, and the need for target vessel revascularisation, take place in 15–25% of patients in the first year after a STEMI (3, 4). Besides conventional risk factors, the inflammatory response following ischemia is a major determinant of infarct healing, left ventricular remodelling, and long-term patient outcomes (5, 6).

Cytokines are necessary for the immune response after myocardial infarction. Proinflammatory cytokines such as IL-1 β , IL-6, and TNF- α are involved in neutrophil recruitment and matrix degradation, whereas the anti-inflammatory cytokine IL-10 reduces tissue damage (7, 8). Nevertheless, measuring single cytokines does not always provide a complete picture of the complex interactions underlying post-STEMI immune dysregulation (9). Therefore, modern studies increasingly advocate analysing cytokine networks and propose that, rather than isolated biomarkers, interlinked cytokine modules may offer better prognostic value for patient outcomes (10).

Some of these lesser-studied cytokines and immune markers may also have significant physiological effects. Signature cytokines produced by T helper 17 (Th17) cells, such as IL-17A, are known to induce neutrophil infiltration and have been linked to maladaptive cardiac remodelling in preclinical models (11, 12). Soluble VCAM-1 is a marker of endothelial activation and has been linked to recurrent ischemia (13). sIL-2R, the soluble IL-2 receptor, is a well-known marker of T-cell activation (14). IL-8 enhances neutrophil chemotaxis and is correlated with infarct size (15). GATA binding protein 1 (GATA1), an erythroid/megakaryocytic transcription factor that normally does not circulate in serum, has been hypothesised to be released during processes of myocardial necrosis (16). Furthermore, cytokines associated with eosinophils, such as IL-3, IL-5 and GM-CSF, have been examined to a lesser extent in the setting of STEMI (17).

ali ove povezanosti nisu ostale nezavisne nakon multivarijantnog prilagođavanja.

Zaključak: Mreža serumskih citokina koju čine IL-17A, VCAM-1, sIL-2R, IL-8 i GATA1, određena 24 časa nakon pPCI, pouzdano predviđa MACE kod pacijenata sa STEMI. Ovi nalazi ukazuju na značaj ranog imunološkog profilisanja za stratifikaciju rizika.

Ključne reči: STEMI, mreža citokina, IL-17A, GATA1, MACE, predikcija rizika, inflamacija

In this prospective cohort study, we aimed to identify serum cytokine networks predicting 12-month MACE in patients with STEMI, pre-specified to include IL-17A, VCAM-1, sIL-2R, IL-8, GATA1, IL-3, IL-5 and GM-CSF.

Materials and Methods

Study design and population

We conducted a prospective, single-centre, observational cohort study of 344 consecutive patients with ST-elevation myocardial infarction (STEMI) who underwent successful primary percutaneous coronary intervention (pPCI) from January 2022 to June 2024. Among this initial group, 320 patients met the angiographic criteria for successful pPCI, defined as final Thrombolysis in Myocardial Infarction (TIMI) flow grade 3 in the infarct-related artery with residual stenosis <30%, and comprised the analytic cohort (Figure 1). STEMI diagnosis was based on the Fourth Universal Definition of Myocardial Infarction, requiring chest pain of <12 hours' duration, persistent ST-segment elevation of ≥ 1 mm in ≥ 2 contiguous leads, and a subsequent rise in cardiac troponin I. Successful pPCI was also defined post-procedural TIMI flow grade 3 and residual stenosis <30%.

Angiographic definition of pPCI success was restoration of normal epicardial blood flow in the culprit coronary artery corresponding to post-procedure TIMI flow grade 3 and residual stenosis <30% after stent implantation or balloon angioplasty. TIMI flow grade 3 refers specifically to normal antegrade flow into the distal coronary bed. Grades 0 to 2 refer to absent, low or partial flow. Therefore, patients with a final TIMI flow grade <3 were systematically excluded from the study.

Several criteria led to the exclusion of patients from the study. This included a previous history of MI, presentation with cardiogenic shock (Killip class IV), or an active infection or systemic inflammatory disease such as rheumatoid arthritis or lupus. Patients with known malignancy within the last 5 years or those receiving chronic immunosuppressive or anti-inflammatory treatment (including steroid dos-

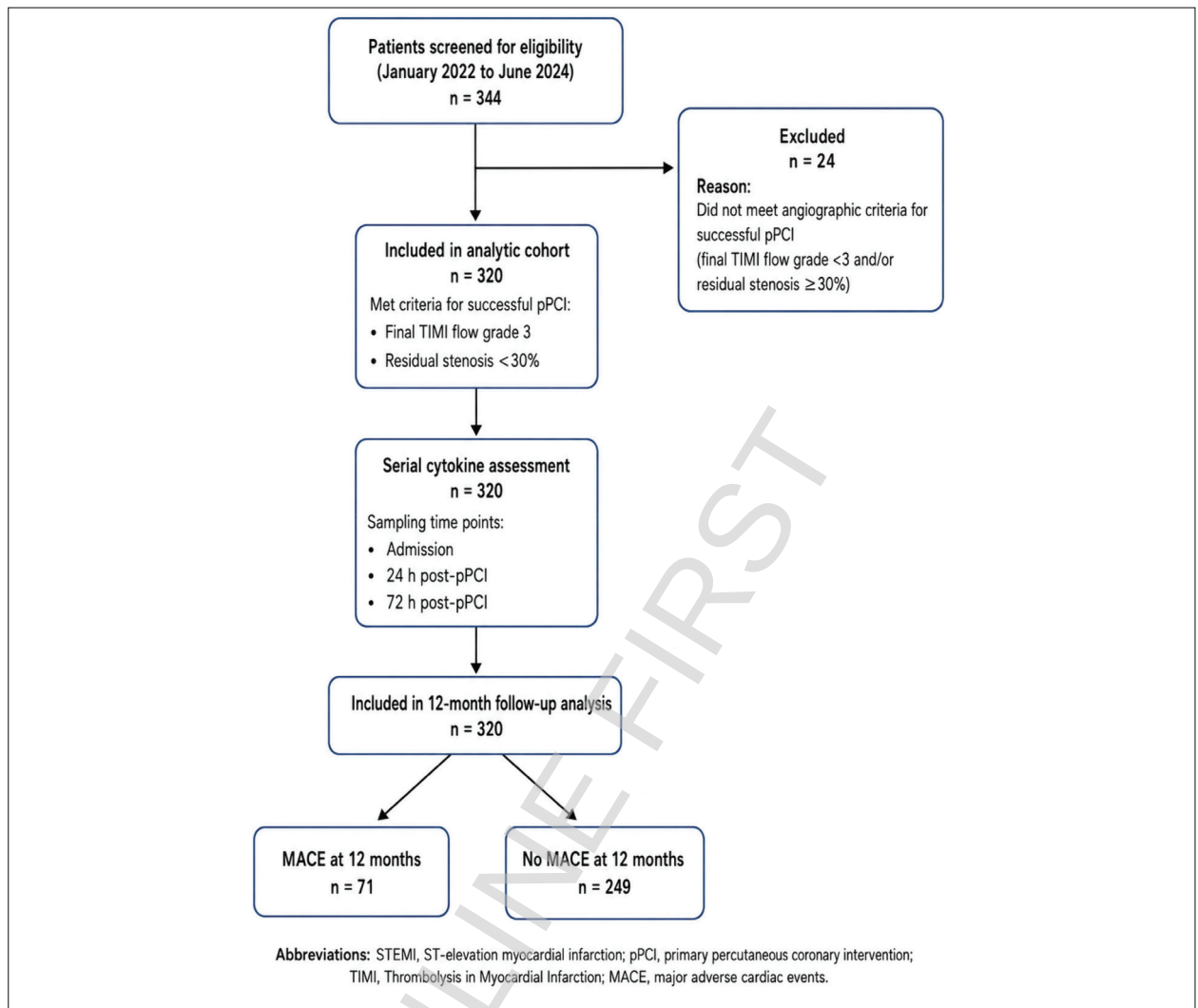


Figure 1 Study flow diagram of participants.

es >10 mg/day) were also excluded. Severe liver or renal failure, defined as an estimated glomerular filtration rate <30 mL/min/1.73 m², was another exclusion criterion, as was the inability to give informed consent.

Blood sampling and multiplex cytokine assays

Peripheral venous blood specimens were collected in serum separator tubes at three standardised time points: on admission before primary percutaneous coronary intervention (pPCI); 24 hours (plus or minus 2 hours) after pPCI; and 72 hours (plus or minus 4 hours) after pPCI. Samples were centrifuged at 1500 g for 15 min within 30 min of collection. The serum was then divided into aliquots, stored at -80 °C, and processed in batch runs to minimise inter-assay variability.

Multiplex bead-based immunoassay (Bio-Plex Pro™ Human Cytokine 18-plex Panel, Bio-Rad, Hercules, CA, USA) was used to quantify IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17A, GM-CSF, IFN-γ, TNF-α, soluble IL-2 receptor (sIL-2R) and soluble vascular cell adhesion molecule-1 (VCAM-1). Because GATA1 is not typically included in commercial multiplex panels, its serum concentrations were measured separately using a high-sensitivity custom sandwich ELISA kit (ab227938, Abcam, Cambridge, UK). The assay had a detection range of 1.5–300 pg/mL. Intra- and inter-assay coefficients of variation (CVs) were <8% and <10%, respectively. All assays were performed in duplicate, and the average of duplicates was used for analysis. Each plate also included quality control samples for assay reliability.

Clinical data collection and follow-up

Baseline demographics, established cardiovascular risk factors (including hypertension, diabetes mellitus, dyslipidaemia, smoking status), Killip class on admission, symptom-to-balloon time, and specific procedural details (including stent length, number of stents deployed, and whether glycoprotein IIb/IIIa inhibitors were administered) were recorded. Relevant laboratory data recorded at the same time included peak troponin I, high-sensitivity C-reactive protein (hs-CRP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP). The left ventricular ejection fraction (LVEF) was measured by echocardiography 72 h after pPCI, in particular using the biplane Simpson method.

The primary outcome of interest was the occurrence of major adverse cardiac events (MACE) over 12 months. The composite endpoint was: cardiac death, defined as death due to myocardial infarction, heart failure, or sudden cardiac death; non-fatal recurrent myocardial infarction, defined as typical chest pain or electrocardiographic changes with elevation of troponin above the 99th percentile; hospitalisation for heart failure, defined as symptoms and evidence of pulmonary congestion by chest radiograph or echocardiogram; or target vessel revascularisation, defined as ischemia-driven percutaneous coronary intervention or coronary artery bypass grafting of the treated vessel. Clinical follow-up assessments were performed at 1, 6 and 12 months by outpatient clinic visits or telephone interviews by cardiologists blinded to the cytokine data. An independent clinical events committee adjudicated all observed events.

Cytokine network and statistical analysis

Cytokine networks were constructed from partial correlation matrices using graphical LASSO (glasso) regularisation. This approach enables the estimation of sparse inverse covariance matrices that can reveal direct or conditional interactions among cytokines, after controlling for the effects of all other measured analytes. Network plots were generated using the qgraph package in R. Network density (global connectivity) was compared between the MACE and non-MACE cohorts using permutation tests.

To select predictors, the least absolute shrinkage and selection operator (LASSO) logistic regression was applied to the 24-hour cytokine data. We accomplished this with a 10-fold cross-validation procedure, aimed to minimise binomial deviance. The optimal lambda (λ) was chosen as that within 1 standard error of the minimum. Adjusted odds ratios (ORs) and hazard ratios (HRs), along with their corresponding 95% confidence intervals (CIs), were obtained using multivariable logistic regression and Cox proportional hazards models, respectively. Potential confounders were selected a priori and adjusted for: age, sex, diabetes, hypertension, Killip class ≥ 2 , logarithmically transformed peak troponin I, and 72-hour LVEF. The proportional hazards assumption was empirically checked using Schoenfeld residuals. Receiver operating characteristic curves and area under the curve (AUC) were compared by using DeLong's test. Statistical significance was defined as a two-sided p-value < 0.05 . All statistical analyses were carried out in R version 4.3.1.

Table 1 Baseline and clinical characteristics of STEMI patients with vs without MACE.

Characteristic	No MACE (n=249)	MACE (n=71)	p-value
Age (years)	58.4 $\pm$ 11.2	65.1 $\pm$ 9.8	<0.001
Male sex, n (%)	178 (71.5)	48 (67.6)	0.53
Hypertension, n (%)	122 (49.0)	41 (57.7)	0.20
Diabetes mellitus, n (%)	65 (26.1)	32 (45.1)	0.002
Current smoker, n (%)	98 (39.4)	25 (35.2)	0.53
Killip class ≥ 2 , n (%)	42 (16.9)	27 (38.0)	<0.001
Peak troponin I (ng/mL), median (IQR)	32.5 (15.2–68.4)	89.2 (41.3–154.0)	<0.001
LVEF at 72h (%), mean $\pm$ SD	52.1 $\pm$ 8.5	44.3 $\pm$ 10.2	<0.001
Symptom-to-balloon (min), median (IQR)	185 (120–295)	210 (135–330)	0.08

*Data are mean $\pm$ SD, median (IQR), or n (%). MACE: major adverse cardiac events; LVEF: left ventricular ejection fraction. Bold p-values indicate statistical significance ($p < 0.05$).

Results

Baseline characteristics and outcomes

Among 320 enrolled patients, 71 (22.2%) experienced ≥ 1 major adverse cardiovascular event (MACE) within 12 months. Rates of specific events were: cardiac death 5.6% (18 patients), recurrent myocardial infarction 6.9% (22 patients), hospital-

isation for heart failure 5.9% (19 patients) and target vessel revascularisation 8.1% (26 patients). The patients with MACE were significantly older (65.1 ± 9.8 years vs 58.4 ± 11.2 years, $p < 0.001$), had a higher prevalence of diabetes mellitus (45.1% vs 26.1%, $p = 0.002$), and a higher prevalence of Killip class ≥ 2 (38.0% vs 16.9%, $p < 0.001$). The peak troponin I levels were more than 2.5-fold higher in the MACE cohort (89.2 [interquartile range, IQR: 41.3–154.0]

Table II Serum levels of prespecified cytokines/markers at three time points.

Marker	Time	No MACE (n=249)	MACE (n=71)	p-value
IL-17A (pg/mL)	Admission	12.4 (8.1–18.2)	13.1 (8.5–19.0)	0.45
	24h	18.2 (12.5–25.3)	34.7 (22.1–48.3)	<0.001
	72h	15.1 (10.0–22.4)	28.5 (18.2–40.1)	<0.001
VCAM-1 (ng/mL)	Admission	215 (178–265)	222 (185–275)	0.38
	24h	289 (235–345)	456 (378–542)	<0.001
	72h	265 (215–320)	398 (334–478)	<0.001
sIL-2R (pg/mL)	Admission	145 (110–195)	152 (115–210)	0.42
	24h	198 (155–250)	345 (268–432)	<0.001
	72h	182 (142–235)	298 (242–375)	<0.001
IL-8 (pg/mL)	Admission	8.2 (5.1–12.5)	8.9 (5.5–13.2)	0.39
	24h	14.3 (9.5–20.1)	28.6 (18.8–42.3)	<0.001
	72h	11.2 (7.2–16.8)	22.4 (14.2–34.0)	<0.001
GATA1 (pg/mL)	Admission	0 (0–2.1)	0 (0–2.5)	0.88
	24h	5.2 (2.1–10.5)	18.3 (9.8–32.1)	<0.001
	72h	3.2 (1.0–8.2)	12.5 (6.1–24.0)	<0.001
IL-3 (pg/mL)	Admission	2.1 (1.0–3.8)	2.3 (1.1–4.0)	0.56
	24h	3.0 (1.5–5.2)	4.8 (2.5–7.8)	0.02
	72h	2.5 (1.2–4.5)	3.9 (2.0–6.5)	0.03
IL-5 (pg/mL)	Admission	3.5 (1.8–6.1)	3.7 (1.9–6.5)	0.62
	24h	4.2 (2.2–7.0)	6.9 (3.5–10.2)	0.01
	72h	3.8 (1.9–6.5)	5.8 (3.0–9.0)	0.02
GM-CSF (pg/mL)	Admission	1.8 (0.9–3.2)	1.9 (0.9–3.5)	0.71
	24h	2.5 (1.2–4.5)	5.2 (2.8–8.5)	0.008
	72h	2.1 (1.0–4.0)	4.0 (2.0–7.0)	0.01

*Data are median (IQR). Bold p-values indicate statistically significant differences ($p < 0.05$). Note that IL-17A, VCAM-1, sIL-2R, IL-8, and GATA1 show the biggest differences at 24h ($p < 0.001$).

Table III Multivariable logistic regression for 12-month MACE.

Variable (measured at 24h post-pPCI)	Adjusted OR	95% CI	p-value
IL-17A (per 10 pg/mL)	1.89	1.42–2.51	<0.001
VCAM-1 (per 50 ng/mL)	1.34	1.12–1.62	0.002
sIL-2R (per 50 pg/mL)	1.45	1.18–1.78	<0.001
IL-8 (per 5 pg/mL)	1.28	1.05–1.56	0.01
GATA1 (per 10 pg/mL)	1.52	1.18–1.96	0.001
Age (per 10 years)	1.40	1.10–1.78	0.006
Diabetes (yes vs no)	2.10	1.20–3.68	0.009
LVEF (per 5% decrease)	1.25	1.08–1.45	0.003

*OR: odds ratio; CI: confidence interval; LVEF: left ventricular ejection fraction. Model adjusted for age, sex, diabetes, hypertension, Killip class ≥ 2 , peak troponin I (log-transformed), and LVEF at 72h. Bold indicates $p < 0.05$.

vs 32.5 [IQR: 15.2–68.4] ng/mL, $p < 0.001$). The left ventricular ejection fraction (LVEF) at 72 hours was significantly reduced ($44.3 \pm 10.2\%$ vs. $52.1 \pm 8.5\%$, $p < 0.001$). On the other hand, no significant differences were found between groups in sex, hypertension, smoking status and symptom-to-balloon time.

Temporal cytokine profiles

As shown in Table II, there were no significant differences between the groups for any of the eight prespecified markers at admission. However, at 24 hours after primary percutaneous coronary intervention (pPCI), all five core network cytokines, IL-17A, VCAM-1, sIL-2R, IL-8 and GATA1, were significantly increased in MACE patients (all $p < 0.001$). For example, the median level of IL-17A increased from 18.2 pg/mL in the non-MACE group to 34.7 pg/mL in the MACE group, and GATA1 was detectable in 86% of future MACE patients and 48% of non-MACE patients. At 72 hours, although still significantly higher in the MACE group, there was a trend towards lower levels, suggesting a peak at 24 hours. Differences in the magnitudes of IL-3, IL-5, and GM-CSF were smaller (p -values 0.008–0.03), suggesting weaker associations.

Cytokine network architecture

The partial correlation network constructed at 24 h showed a highly interconnected module with a mean absolute partial correlation (r) of 0.52, including IL-17A, VCAM-1, sIL-2R, IL-8, and GATA1. In particular, GATA1 showed strong and specific associations with IL-17A ($r = 0.61$) and IL-8 ($r = 0.55$), suggesting biological crosstalk. Global network

connectivity was also significantly higher in the MACE group than in the non-MACE group (global strength 12.4 vs. 7.8, $p < 0.001$). We found a separate, weaker module with IL-3, IL-5 and GM-CSF (mean $r = 0.31$) that correlated with peripheral blood eosinophil counts ($r = 0.42$, $p = 0.008$). This module did not independently predict MACE after adjustment for confounders.

Lasso and multivariable predictors

IL-17A, VCAM-1, sIL-2R, IL-8 and GATA1 were identified as the best combination of predictors among five cytokines at 24 h by LASSO regression. In the subsequent multivariable logistic regression analysis (Table III), each of these markers remained independently associated with MACE after adjustment for relevant clinical confounders. The strongest effect was seen for IL-17A (per 10 pg/mL: adjusted odds ratio [OR] 1.89; 95% confidence interval [CI] 1.42–2.51; $p < 0.001$), followed by GATA1 (per 10 pg/mL: OR 1.52; 95% CI 1.18–1.96; $p = 0.001$) and sIL-2R (per 50 pg/mL: OR 1.45; 95% CI 1.18–1.78; $p < 0.001$). Most prominent clinical predictors were diabetes (OR 2.10) and lower LVEF (per 5% decrease: OR 1.25).

In the Cox proportional hazards model (Table IV), patients in the top tertile of IL-17A had >2 -fold increased hazard of MACE (hazard ratio [HR] 2.45; 95% CI 1.52–3.95; $p < 0.001$) with similar results for VCAM-1 (HR 1.98), sIL-2R (HR 2.12) and GATA1 (HR 2.01). Kaplan-Meier curves diverged early within the first 3 months in the time-to-event analysis for the high-risk cytokine network score (log-rank $p < 0.001$).

Table IV Cox proportional hazards model for time-to-mace.

Predictor (top tertile at 24h vs lower two tertiles)	HR	95% CI	p-value
IL-17A (top tertile)	2.45	1.52–3.95	<0.001
VCAM-1 (top tertile)	1.98	1.26–3.11	0.003
sIL-2R (top tertile)	2.12	1.35–3.33	0.001
IL-8 (top tertile)	1.78	1.14–2.78	0.01
GATA1 (top tertile)	2.01	1.28–3.16	0.002
IL-3 (top tertile)	1.42	0.92–2.20	0.11
IL-5 (top tertile)	1.35	0.88–2.07	0.17
GM-CSF (top tertile)	1.48	0.96–2.28	0.08

*HR: hazard ratio; CI: confidence interval. The multivariate model includes all eight cytokines, plus age, sex, diabetes, Killip class, and LVEF. The combined five-cytokine network score (IL-17A, VCAM-1, sIL-2R, IL-8, GATA1) remained significant in an additional model (global $p < 0.001$). Bold indicates $p < 0.05$.

Predictive performance and validation

The area under the receiver operating characteristic curve (AUC) for the five-cytokine panel at 24 hours for prediction of 12-month MACE was 0.86 (95% CI 0.81–0.91). This performance was much better than that of a clinical model including age, diabetes, Killip class and LVEF (AUC 0.71; $p < 0.001$), peak troponin I alone (AUC 0.73; $p = 0.002$), and hs-CRP alone (AUC 0.68; $p < 0.001$). At the optimal Youden index cut-off, the panel had a sensitivity of 82%, a specificity of 78%, and a negative predictive value of 93%. The addition of this cytokine panel to the clinical model improved net reclassification improvement (NRI) by 0.38 ($p < 0.001$) and integrated discrimination improvement (IDI) by 0.12 ($p < 0.001$).

GATA1 as a novel biomarker

Median GATA1 levels in both groups were 0 pg/mL at admission and were largely undetectable. However, by 24 hours, levels were detectable in 62% of all patients and 86% of the MACE group. Levels of GATA1 at 24 hours correlated significantly with peak troponin I (Spearman's $r = 0.54$, $p < 0.001$) and in a subgroup of 120 patients who underwent cardiac magnetic resonance imaging at 5 days, with infarct size expressed as a percentage of left ventricular mass ($r = 0.61$, $p < 0.001$). However, GATA1 did not correlate significantly with hs-CRP ($r = 0.12$, $p = 0.11$), suggesting that it serves as a marker of myocardial necrosis rather than systemic inflammation.

Discussion

This prospective study of 320 STEMI patients offers several novel insights. First, a specific network of five cytokines (IL-17A, VCAM-1, sIL-2R, IL-8, and GATA1) assessed at 24h after pPCI exhibits high predictive ability for 12-month MACE, with an AUC of 0.86, significantly better than clinical factors and conventional biomarkers. Second, the interconnectivity observed in this network, especially among patients presenting with MACE, supports a transition from single biomarker to multi-cytokine network methods for post-MI risk stratification. Third, based on previous observations that serum GATA1 may reflect myocardial necrosis, we demonstrate that GATA1 exhibits dynamic detectability following STEMI and is part of a broader cytokine network signature associated with both infarct burden and 12-month MACE.

IL-17A, mainly produced by Th17 and $\gamma\delta$ T cells, is linked to adverse cardiac remodelling. In murine models, IL-17A drives neutrophil infiltration, activation of matrix metalloproteinases, and fibroblast proliferation, all of which result in greater infarct expansion and impaired systolic function (18, 19). We found that IL-17A was the strongest independent predictor (HR 2.45), consistent with a recent study showing that increased IL-17A levels at 24 hours post-STEMI were associated with left ventricular thrombus formation (13). However, our network analysis shows that IL-17A is not a lone ranger; its strong partial correlations with sIL-2R (a marker of T-cell activation) and IL-8 (a neutrophil chemotactic mediator) suggest a concerted Th17-neutrophil axis that contributes to adverse outcomes.

Although IL-17A is commonly considered a Th17-associated cytokine, circulating IL-17A in STEMI can originate from a variety of cell populations, including conventional CD4⁺ Th17 cells, $\gamma\delta$ T cells, innate lymphoid cells, neutrophils, and other innate immune populations. This difference is biologically meaningful, as Th17-derived IL-17A is usually linked to IL-23-dependent adaptive immune activation, while $\gamma\delta$ T cells can rapidly produce IL-17A, showing an innate-like character that may be less dependent on IL-23 signalling. Hence, the association between serum IL-17A and MACE cannot be regarded as conclusive evidence for a mechanism exclusively specific for Th17. Instead, it suggests activation of an inflammatory axis associated with IL-17A. Future studies, including cellular immunophenotyping, intracellular cytokine staining, and upstream cytokines such as IL-23, are warranted to elucidate whether the prognostic IL-17A signal corresponds to Th17-cell activation, $\gamma\delta$ T-cell activation, or a mixed cellular response following STEMI.

Soluble IL-2 receptor is shed from activated T lymphocytes and indicates the magnitude of adaptive immune responses. In STEMI, necrotic antigens such as cardiac myosin and troponin induce rapid T-cell activation (14). Our data showed that sIL-2R at 24 h was an independent predictor of MACE with an HR of 2.12. Importantly, sIL-2R was not correlated with infarct size ($r=0.09$, $p=0.31$), suggesting that T-cell activation might not simply reflect more extensive necrosis but could represent an independent immunopathological process. This is consistent with the concept of post-MI autoimmunity in which regulatory T-cell dysfunction leads to an unrestrained effector T-cell response that contributes to exacerbated remodelling (20).

VCAM-1, present on activated endothelium, facilitates the adhesion of monocytes and lymphocytes. However, its soluble form, released by proteolytic cleavage, is a marker of endothelial activation and has been associated with recurrent atherothrombotic events (13, 21). In our study, MACE patients showed a 60% increase in VCAM-1 levels at 24 hours. The VCAM-1–IL-17A link ($r=0.48$) is biologically plausible, as IL-17A can induce VCAM-1 expression in endothelial cells via NF- κ B signalling (11). Prolonged increase of VCAM-1 at 72 hours in MACE patients indicates continuous endothelial dysfunction in the subacute phase. The presence of GATA1 in serum after STEMI is of note, as GATA1 is a nuclear transcription factor in principle, which is essential for erythroid and megakaryocytic differentiation (22). Its strong correlation with troponin I ($r=0.54$) and infarct size measured by MRI ($r=0.61$) suggests passive release from necrotic cardiomyocytes. However, alternative origins cannot be excluded: GATA1 is also expressed in megakaryocytes, and platelet activation in MI could result in the re-

lease of megakaryocyte-derived microparticles containing GATA1 (23). Regardless of the mechanism, GATA1 showed better predictive performance for MACE than troponin I (AUC 0.80 vs 0.73, $p=0.04$). This could be due to the longer half-life of GATA1 in circulation, enabling it to integrate the total necrosis burden over time. Its tissue origin should be confirmed by further studies using mass spectrometry and immunohistochemistry.

IL-3, IL-5 and GM-CSF all share a common β chain receptor that promotes eosinophil differentiation, supports survival and enhances activation (24, 25). That these cytokines clustered as a separate network module and had limited predictive value (p -values 0.08–0.11 in adjusted models) suggests that eosinophil-driven pathways may not be major determinants of MACE among unselected STEMI patients. This finding contrasts with a study by Toor et al. (17), which reported impaired cardiac repair in eosinophil-deficient murine models. However, the human physiological context may differ. Eosinophil counts in our cohort were within the normal physiological range (median 150 cells/ μ L), with only 4% of patients having mild eosinophilia (>500 cells/ μ L). Thus, while eosinophils may modulate healing in experimental models, their clinical impact in typical STEMI appears to be modest.

Previous network analyses in acute coronary syndromes have mainly focused on conventional cytokines, including IL-6, TNF- α , and IL-10 (26). Our study expands on these observations by including less-studied markers (IL-17A, sIL-2R, GATA1) and by demonstrating that the time point 24 hours post-PCI, rather than admission or 72 hours, best reflects the peak of the predictive signal. This window in time is consistent with the ‘inflammatory burst’ phase following reperfusion, characterised by maximal neutrophil infiltration, complement activation, and the generation of reactive oxygen species (8, 27). From a clinical standpoint, a 24-hour blood draw is clinically feasible and lends itself to integration with routine post-PCI protocols.

Our five-cytokine panel might improve risk stratification in STEMI with external validation. Patients with high network scores (e.g., top tertile for ≥ 3 cytokines) may be candidates for more intensive surveillance (e.g., early echocardiography at 1 month) or for targeted anti-inflammatory interventions. For example, in atherosclerosis, IL-17A blockade (e.g., secukinumab) or inhibition of the IL-17A pathway is being investigated (28, 29). In parallel, inhibitors targeting VCAM-1 are currently in preclinical development (21). Further randomised trials are needed to determine whether therapeutic strategies targeting this cytokine network can effectively reduce the incidence of MACE.

Limitations

Limitations were associated with this research study. Firstly, it was a single-centre study with a moderate sample size and no external validation group. This indicates the need for additional independent studies with multicentre cohorts to validate the five-cytokine network. Secondly, cytokine levels were measured across three sampling periods. Collecting cytokine samples over a shorter time period will allow better elucidation of post-pPCI inflammatory dynamics.

Furthermore, there was a lack of mechanistic cellular analysis, including flow cytometric estimation of T helper 17 (Th17) cells, regulatory T cells, neutrophil subpopulations, eosinophils, and cytokine-producing immune cell populations. This means that the cellular origin and causal pathways of the identified cytokine network cannot be determined. Thirdly, the serum level of GATA1 was determined by ELISA, which does not exclude the possibility of cross-reactivity with other GATA family members or circulating protein fragments; validation by independent assays, such as mass spectrometry and immunoblotting, would be needed. Finally, patients with cardiogenic shock were excluded, although they are an important subgroup of patients with high-risk STEMI. The results obtained in this particular case are therefore not applicable to patients with Killip class IV or with profound haemo-

dynamic instability. Sixth, adjustment for major clinical confounders was performed. However, residual confounding from unmeasured variables, such as genetic polymorphisms, medication adherence, changes in therapy after discharge, dietary factors, and environmental exposures, cannot be ruled out. These limitations underscore the need for prospective external validation and mechanistic studies before clinical implementation of this cytokine-network approach.

Conclusions

A serum cytokine network comprising IL-17A, VCAM-1, sIL-2R, IL-8, and GATA1 at 24 hours post-pPCI independently and predicts in this single-centre cohort 12-month MACE in STEMI patients, outperforming clinical factors and traditional biomarkers. GATA1 emerges as a novel biomarker associated with myocardial necrosis. These findings support early immune profiling for risk stratification and suggest potential therapeutic targets. Prospective multicentre validation and mechanistic studies are required before clinical application.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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