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ORIGINAL ARTICLE

Admission macrophage migration inhibitory factor predicts long-term prognosis in patients with ST-elevation myocardial infarction

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Aims We previously showed in patients with ST-segment elevated myocardial infarction (STEMI) that admission levels of macrophage migration inhibitory factor (MIF) predict infarct size. We studied whether admission MIF alone or in combination with other biomarkers is useful for risk assessment of acute and chronic clinical outcomes in STEMI patients.

Methods and results A total of 658 STEMI patients treated with primary percutaneous coronary intervention (PCI) were consecutively recruited. MIF level was determined at admission and echocardiography performed on day-3 and then 12 months post-MI. Patients were followed for a median period of 64 months. Major endpoints included ST-segment resolution, all-cause mortality, and major adverse cardiovascular events (MACE). High MIF level was associated with larger enzymatic infarct size, incomplete resolution of ST-segment elevation post-PCI, impaired left ventricular ejection fraction (LVEF), and poorer improvement of LVEF (all $P < 0.001$). After adjustment for classical risk factors standard biomarkers and day-3 LVEF, admission MIF remained independently prognostic for all-cause mortality [hazard ratio (HR) 2.27, 95% confidence interval (CI) 1.43–3.22], and MACE (HR 1.39, 95% CI 1.12–1.71, both $P < 0.05$). MIF was a significant additive predictor of all-cause mortality with a net reclassification improvement of 0.34 ($P = 0.02$). Furthermore, patients in high tertile of both admission MIF and day-3 Nt-proBNP had the highest mortality risk relative to other tertile groups (HR 11.28, 95% CI 4.82–26.94; $P < 0.001$).

Conclusion STEMI patients with high admission MIF level experienced a poorer recovery of cardiac function and worse long-term adverse outcomes. Combination of Nt-proBNP with MIF further improves prognostic capability.

Keywords Biomarker • Macrophage migration inhibitory factor • ST-segment elevation myocardial infarction • Prognosis • Major adverse cardiovascular events

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What's new?

- In ST-segment elevated myocardial infarction (STEMI) patients, admission macrophage migration inhibitory factor (MIF) level is in proportion with index of microvascular reperfusion injury.
- Admission MIF is an independent biomarker predicting the long-term adverse outcomes following STEMI.
- Admission MIF and day-3 Nt-proBNP in combination have improved prognostic value of adverse cardiovascular events in STEMI patients.

What's known?

- Macrophage migration inhibitory factor (MIF) is an upstream inflammatory cytokine and contributes to post-infarct inflammatory response.
- Myocardial ischaemia and infarct evokes release of MIF pre-stored in the myocardium into the circulation leading to rapid elevation of its blood levels. Admission MFI level is correlated with the mass of jeopardized myocardium.

Introduction

Current therapies including timely primary percutaneous coronary intervention (PCI) have significantly improved prognosis of patients with ST-segment elevated myocardial infarction (STEMI).[1–3] However, recurrent major adverse cardiovascular events (MACE) after STEMI are common.[3] Early identification of patients at high risk of long-term MACE is important for appropriate allocation and aggressiveness of therapy and care to improve their prognosis.[1] Recent studies in STEMI patients have provided evidence for the usefulness of biomarkers not only for early diagnosis, but also for risk prediction additional to that provided by traditional risk factors.[4–6] In addition, the prognostic value of a combination of different biomarkers outperformed a single measure.[7] Biomarkers of myocardial necrosis, such as high sensitive troponin (hs-Tn) and creatine kinase MB (CK-MB), are in routine use for infarct size evaluation based on serial measurements to identify the peak value or to construct area-under-curve. Studies have shown the prognostic value of biomarkers and imaging-based measurement of infarct size in STEMI patients.[8] Prognostic evaluation of patients with heart failure (HF) is feasible from measures such as N-terminal pro B-type natriuretic peptide (Nt-proBNP). Whilst some studies have indicated that measurement of Nt-proBNP during the index admission is prognostic of long-term outcome in STEMI patients, these have most often been 2–4 days after admission.[5,9,10] In addition, levels are influenced by age and renal function.[5]

A few groups, including ours, have studied plasma levels of macrophage migration inhibitory factor (MIF) as a biomarker in patients with STEMI or coronary artery disease, and reported increase of MIF levels lasting for approximately 2 weeks starting from admission.[11–15] In patients presenting with STEMI 3–6 h after symptoms, 71% of STEMI patients had admission MIF levels above the upper-limit of healthy subjects, a percentage significantly higher than that of admission levels of CK, myoglobin (20–30%) or TnI (50%), but comparable to hs-TnI (75%).[11] Furthermore, admission MIF level correlated with infarct size determined by cardiac magnetic resonance (CMR) imaging. In contrast, correlation was not seen between infarct size and admission levels of CK-MB and hs-TnI.[8,11] In STEMI patients, MIF levels measured prior to PCI were significantly higher in those with greater angiographic thrombus burden by TIMI reclassification.[13] In patients with coronary artery disease undergoing exercise stress test, those exhibiting positive signs (imaging) of myocardial ischaemia had elevated MIF level while levels of Tn and CRP were unchanged.[12] Thus, both preclinical and clinical studies are consistent with the notion that circulating MIF levels are elevated promptly in response to myocardial ischaemia and infarction in proportion to the mass of the affected myocardium. MIF is abundantly expressed by inflammatory cells.[16] We previously reported that cardiomyocytes express and store MIF at a high level,[11] and that at the time of admission in patients with STEMI, plasma MIF levels are significantly elevated whilst expression of MIF by circulating mononuclear cells remained unchanged,[17] indicating a cardiac source of circulating MIF in the acute phase of MI.

We hypothesized that a single measurement of admission MIF alone or in combination with other biomarkers, could predict long-term survival and nonfatal cardiovascular events in patients with STEMI. This possibility was considered likely as existing evidence for admission MIF in predicting infarct size and its pro-inflammatory nature.[18] We thus evaluated the prognostic performance and clinical correlates of MIF, assessed at the time of presentation, among patients with STEMI.

Methods

Patient population and study design

We consecutively recruited patients with STEMI receiving primary PCI at the Department of Cardiology, Third Hospital of Peking University from June 2010 to December 2013. Inclusion criteria were: (i) presentation with STEMI (typical symptoms for >30 min and <12 h plus persistent ST-segment elevation of ≥2 mV in at least two contiguous precordial electrocardiogram (ECG)-leads or ≥1 mV in at least two contiguous limb ECG-leads or a newly developed left bundle branch block; (ii) with invasive treatment by PCI; and (iii) availability of MIF measurements from blood samples on admission. Patients having one or more of the following criteria were excluded: (i) previous acute coronary syndrome within 1 month; (ii) rescue angioplasty; (iii) current infections, known malignant, inflammatory or autoimmune diseases; (iv) end-stage renal disease by estimated Glomerular Filtration Rate (eGFR) <30 mL/min/kg), and (v) unwillingness. The process of recruitment and study protocol are illustrated in *Figure 1*.

Baseline clinical data such as history of disease and medication were collected from medical records. Hypertension was defined as the current use of active treatment with antihypertensive agents or otherwise as a systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg on at least two separate occasions. Body mass index (BMI, kg/m^2) was obtained. Hypercholesterolaemia was defined as the

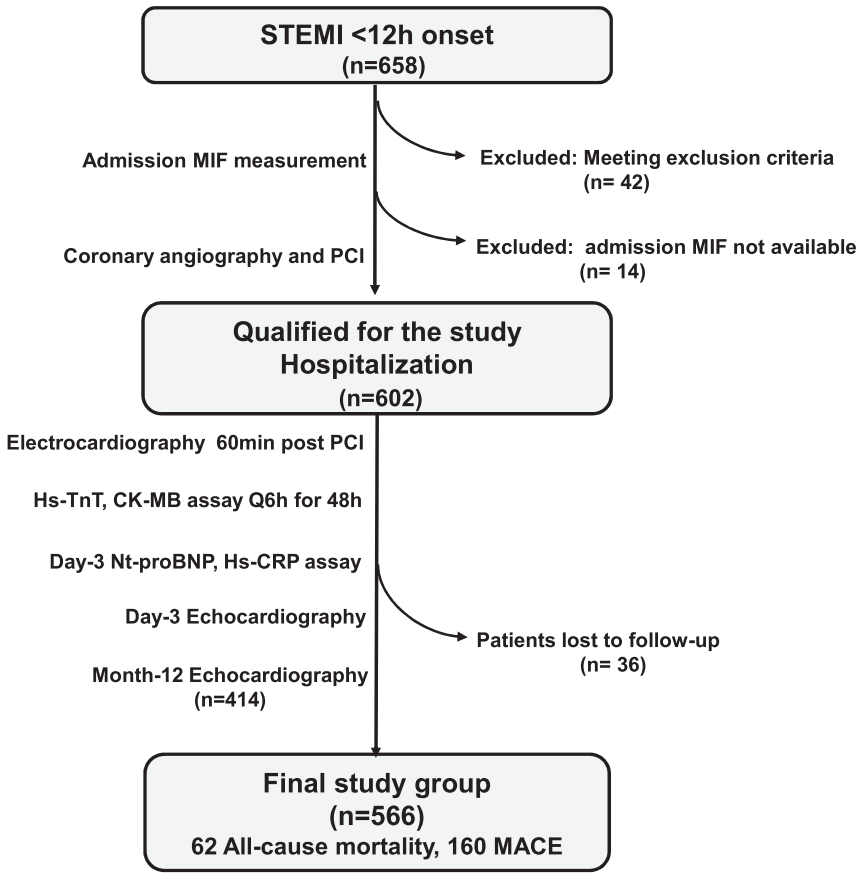

Figure 1 Study flow chart. A total of 658 patients with confirmed diagnosis of ST-elevation myocardial infarction were consecutively recruited into this prospective study. Of them, 42 patients were then excluded based on exclusion criteria and another 50 patients were omitted due to lack of admission migration inhibitory factor measure ($n = 14$) or lost during follow-up ($n = 36$), leading to a final study cohort of 566 patients. Echocardiography was performed at day-3 and then at 12 months during follow-up period. Biochemical assays included macrophage migration inhibitory factor (admission MIF), high sensitive troponin, and creatine kinase MB (within 48 hours), Nt-proBNP and Hs-CRP (both at day-3). CAG, coronary angiography; CK-MB, creatine kinase MB; CRP, C-reactive protein; hs-TnT, high sensitive troponin T; Nt-proBNP, N-terminal brain natriuretic peptide; PCI, primary percutaneous coronary intervention.

current use of active treatment with lipid-lowering drugs or value of total cholesterol ≥6.22 mmol/L or low density lipoprotein cholesterol ≥4.14 mmol/L. Current smokers were defined as those currently smoking any tobacco. Diagnosis of diabetes mellitus was confirmed by current treatment with antidiabetic medicine or with a fasting plasma glucose level ≥7 mmol/L or a non-fasting level of ≥11.1 mmol/L. Patients were prospectively classified according to maximum Killip class by three clinicians on admission and during hospitalization. This prospective cohort study was approved by the Human Ethical Committee, Peking University Health Science Centre and performed in accordance with the requirements of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Percutaneous coronary intervention and medication

After a loading dose of 300 mg aspirin and 600 mg clopidogrel coronary angiogram and PCI were performed. Quantitative coronary angiographic assessment was performed on images pre- and post-interventions. Details of culprit lesion, numbers of significantly stenosed vessels, TIMI classification pre- and post-PCI were recorded. Interventions were performed according to current guidelines.[19] Thrombus aspiration, use of glycoprotein IIb/IIIa inhibitor (Tirofiban) or intra-aortic balloon pump implantation were administered at the discretion of the operator. Two independent observers blinded to our study calculated ST-segment resolution by predefined criteria at 60 min after revascularization with a cut-off value <50% defined as incomplete ST-segment resolution.[20,21]

Following the PCI procedure, patients were prescribed enoxaparin sodium (100 U/kg/q12h for 3 days), and other secondary preventions including aspirin (100 mg/day), clopidogrel (75 mg/day for 12 months), cholesterol-lowering treatment (statins), β-blockers, and angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blocker (ARB). All patients received standard and individualized medical treatment and management at the discretion of an attending cardiologist.

Study endpoints and follow-up

Our study was designed to assess the ability of admission MIF levels in patients with STEMI to predict the occurrence of further cardiac events. Accordingly, the major end-points selected were those closely related to

post-infarct cardiac injury. The short-term endpoint was incomplete ST-segment resolution post-PCI as a surrogate of inefficient myocardial reperfusion. The long-term endpoints were all-cause and cardiac death, and the composite endpoint of MACE consisting of all-cause mortality, recurrent MI and rehospitalization due to HF as the primary reason. Long-term follow-up was accomplished by reviewing the hospital records and contacting patients or their relatives individually by telephone to collect information on occurrence of death due to cardiovascular causes and MACE. Recurrent MI was defined as accordance with the universal definition proposed in 2012.[22]

Echocardiography

Echocardiography was performed at day-3 and around 12 months of follow-up after MI using Vivid 7 (Vingmed, GE, Horten, Norway). Standard echocardiographic views were acquired under supervision of experienced cardiologists. Left ventricular (LV) end-diastolic dimensions (LVEDD) and ejection fraction (EF) were obtained using the modified biplane Simpson method.

Routine laboratory measurements

Venous blood samples were collected at admission and then every 6 h for the first 2 days for assay of CK-MB and Hs-TnT. Peak concentrations were identified to estimate infarct size. Nt-proBNP and hs-CRP concentrations were determined on median day-3 post-MI, since their prognostic value at this time has outperformed earlier time-points during the acute phase.[5,9,10]

All routine biochemical assays were performed at the Clinical Biochemistry Department of Peking University Third Hospital within 30 min after collection of blood samples. hs-TnT and NT-pro-BNP were measured using E601 immunoassay analyser (Roche Diagnostics, Mannheim, Germany). CK-MB, hs-CRP, blood lipids and plasma creatinine concentration were analysed using an AU5400 automatic chemical analyser (Beckman Coulter, California, USA). eGFR was calculated according to Cockcroft-Gault formula. All the tests were conducted based on manufacturers' recommendation or literature.

Measurement of plasma concentration of macrophage migration inhibitory factor

Immediately after admission, venous blood samples were collected into vacutainer tubes containing heparin lithium prior to primary PCI. Within 30 min after collection, samples were centrifuged at 3000 rpm for 10 min at 4°C. Plasma was prepared and stored in aliquots at -80°C until analysis. Repeated freeze-thaw cycles were avoided. MIF level was measured, in duplicates, using Quantikine MIF ELISA kits (DMF00B, R&D Systems) according to manufacturer's specifications. The coefficient of variation for intra- and inter-assay variation was 2.8 ± 1.6% and 5.8 ± 1.3%, respectively. For comparison, we also measured MIF level of healthy people ($n = 65$) and of patients presenting to the emergency department of the Third Hospital with chest pain but excluded cardiac ischaemia as aetiology ($n = 600$). All assays were performed by personnel blinded to patient's identity and outcome.

Statistical analysis

Data was primarily analysed by identifying three tertiles of initial MIF measurement. Categorical variables were summarized as percentage and compared using χ^2 test between the MIF tertile groups. Continuous variables are presented as means ± SD or median with interquartile range (IQR) and the association between MIF tertile was tested by one-way ANOVA or Kruskal–Wallis rank-sum test. The association between MIF levels and other continuous variables (e.g. biomarkers, LVEF) was tested by Spearman's rank order correlation. Due to non-normal distribution, all biomarkers were logarithmically or log-2 transformed prior to entry into the statistical models. The primary endpoint (complete ST-segment resolution) was analysed with a logistic regression model. The Kaplan–Meier curves were generated to visualize the relationship of tertile MIF level with long-term prognosis using Log-rank test. Univariate and multivariate analyses were performed using the Cox proportional hazard models. Four models for the adjustment of covariates were utilized: *Model-1*, adjusted for age, sex and eGFR; *Model-2*, adjusted for all factors in Model-1 plus BMI, haemoglobin, previous MI, diabetes mellitus, hypertension, current smoking, hypercholesteremia, symptom-admission time <6 h, 3 vessel disease, Killip class >1, culprit lesion of left anterior descending (LAD), ST-segment resolution, thrombus aspiration, use of tilofiban during the PCI, and TIMI classification pre- and post-PCI; *Model-3*, adjusted for all factors in Model-2 plus conventional biomarkers including peak hs-TnT, Nt-proBNP and hs-CRP; *Model-4*, adjusted for all factors in Model-3 plus day-3 LVEF.

In further analyses, patients were classified into two groups based on the highest tertile of two or three biomarkers including MIF, day-3 NT-proBNP and peak hs-TnT. For example, a patient being in the highest tertile of both MIF and Nt-proBNP would be classified as positive and all other patients would be classified as negative (even though they might be in the highest tertile one or other of MIF or Nt-proBNP). With this approach, comparison was made between all possible combinations of two or three biomarkers. Discrimination was evaluated using C-statistics by Frank Harrell.[23] Continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were also calculated to quantify the degree of correct reclassification as a result of adding admission MIF to the clinical risk models.[24,25] All probability values were two-tailed and considered statistically significant if $P < 0.05$. Calculations of C-statistics, NRI and IDI were performed using package 'survIC1' and 'survIDINRI' in R programming 3.4.0 for Windows (R Development Core Team, 2016), other data analyses were performed using SPSS (version 22.0; SPSS, Inc. Chicago, IL, USA).

Results

Relationship between admission macrophage migration inhibitory factor and baseline characteristics and acute-phase markers

A total of 658 patients with confirmed diagnosis of STEMI were initially recruited. 56 patients were excluded on the basis of predefined exclusion criteria. Of them 14 patients did not have available measurement of admission MIF. Thirty-six patients were lost to follow-up. Thus, the final study cohort consisted of 566 patients (*Figure 1*). Their median age was 61 years and 79.9% were male. The median (IQR) of admission MIF was 55.1 (35.3–83.6) ng/mL, significantly higher than the two reference groups of healthy controls [16.9 (12.8–22.9) ng/mL] and chest pain patients presenting at the emergency department excluded an ischaemic aetiology [26.8 (21.7–34.6) ng/mL] (see Supplementary material online, *Figure S1*).

The clinical characteristics of this patient cohort according to MIF tertiles are summarized in *Tables 1* and *2*. There was no significant difference between those patients lost to follow-up and the study cohort (not shown). MIF levels were not associated with age, gender, eGFR, BMI, diastolic blood pressure or heart rate. Patients in the high MIF tertile group had higher prevalence of hypertension ($P = 0.029$) and culprit vessel lesion in LAD ($P = 0.001$), and were less likely to

Table 1 Univariate associations with admission levels of MIF

	Total	Tertiles of admission MIF (ng/mL)			*P*-value
		<40.2 (low)	40.2~73.0 (median)	≥73.0 (high)	
number	566	189	189	188	
Age	60.1 ± 13.0	61.1 ± 12.0	60.2 ± 13.3	61.9 ± 13.5	0.448
Male gender, (%) *n*	80 (452)	75 (142)	84 (159)	80 (151)	0.091
Systolic BP (mmHg)	131 ± 21	131 ± 19	129 ± 19	133 ± 24	0.132
Diastolic BP (mmHg)	77 ± 15	79 ± 14	76 ± 14	77 ± 16	0.122
Heart rate (b.p.m.)	75 ± 15	73 ± 13	77 ± 16	75 ± 15	0.054
Body mass index (kg/m^2)	25.6 ± 3.3	25.6 ± 4.4	25.4 ± 2.6	25.8 ± 2.5	0.504
eGFR (mmol/L)	89 ± 26	87 ± 24	88 ± 24	92 ± 29	0.165
Admission time <3 h (%) *n*	43.8 (248)	42.3 (80)	42.3 (80)	46.8 (88)	0.599
History, (%) *n*					
Hypertension	57.6 (326)	54.0 (102)	53.4 (101)	65.4 (123)	0.029
Diabetes	24.6 (139)	27.0 (51)	25.9 (49)	20.7 (39)	0.322
Hypercholesteraemia	31.8 (180)	32.3 (61)	30.2 (57)	33.0 (62)	0.829
Smoking	67.3 (381)	65.1 (123)	68.8 (130)	68.1 (128)	0.717
Previous MI	6.9 (39)	10.1 (19)	6.9 (13)	3.7 (7)	0.053
Angiographic data, (%) *n*					
Culprit vessel LAD	46.1 (261)	46.0 (87)	36.5 (69)	55.9 (105)	0.001
3-Vessel lesion	37.5 (212)	34.4 (65)	41.3 (78)	36.7 (69)	0.372
Stents	97.3 (551)	96.3 (182)	97.8 (185)	97.8 (184)	0.543
Thrombus aspiration	16.2 (92)	16.4 (31)	18.0 (34)	14.4 (27)	0.633
Tirofiban	32.9 (186)	36.5 (69)	32.8 (62)	29.3 (55)	0.325
IABP *in situ*	3.4 (19)	2.1 (4)	3.7 (7)	4.3 (8)	0.488
TIMI = 0, before PCI	78.1 (442)	81.0 (153)	81.0 (153)	72.3 (136)	0.066
TIMI <3, After PCI	4.2 (24)	3.2 (6)	3.2 (6)	6.4 (12)	0.123
ST-segment resolution <50%	26.7 (151)	14.3 (27)	22.8 (43)	43.1 (81)	<0.001
LVEDD >55 mm (male), >50 mm (female)	16.4 (92)	14.4 (27)	10.6 (20)	24.3 (45)	0.001
LVEF <50%	36.4 (204)	23.0 (43)	29.8 (56)	56.8 (105)	<0.001
Killip Class II–IV	18.0 (102)	11.1 (21)	19.6 (37)	23.4 (44)	0.006
Medication, (%) *n*					
Clopidogrel	98.9 (560)	98.4 (186)	99.5 (188)	98.9 (186)	0.604
Aspirin	98.4 (557)	98.9 (187)	97.4 (184)	98.9 (186)	0.597
Statins	95.8 (542)	96.8 (183)	95.2 (180)	95.2 (179)	0.672
ACEI/ARBs	73.5 (416)	76.2 (144)	70.4 (133)	73.9 (139)	0.434
β-Blocker	72.8 (412)	76.7 (145)	70.9 (134)	70.7 (133)	0.331

Data are presented either as mean ± SD, percentage, or median (25th percentile; 75th percentile). Categorical variables are indicated as percentage (%) of patients.
P-values were derived from Mann–Whitney *U*-statistics, One-way ANOVA or χ^2 test for comparison among MIF tertile groups.
MI, myocardail infarction; eGFR, estimated glomerular filtration rate; LAD, left anterior descending; IABP, intra-aortic balloon pump; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; PCI, Percutaneous coronary intervention.

have previous MI ($P = 0.053$). Other atherosclerotic risk factors and angiographic findings were similar among the groups (*Table 1*). There was also no significant difference between the three groups in the proportion of patients treated with aspirin, clopidogrel, statins, ACEI or ARBs, and β-blockers on admission (not shown) or at discharge (*Table 1*).

Moderate but highly significant correlations were observed between concentrations of admission MIF and necrosis markers, peak hs-TnT ($r = 0.486$, $P < 0.001$) and peak CK-MB ($r = 0.343$, $P < 0.001$). MIF levels were also associated with inflammatory markers such as white blood cell (WBC) count ($r = 0.210$, $P < 0.001$) at admission and day-3 hs-CRP ($r = 0.154$, $P < 0.001$). Admission MIF correlated with non-fasting glucose levels ($r = 0.126$, $P = 0.006$) but not with haemoglobin, serum cholesterol or HbA1c%.

Admission macrophage migration inhibitory factor and acute or chronic left ventricular function

Patients in high tertile MIF group had a higher proportion of maximum Killip class >1 during hospitalization compared with those in the bottom tertile (23.4% vs. 11.1%, $P = 0.006$). High MIF levels were associated with elevated Nt-proBNP levels ($r = 0.190$, $P < 0.001$), impaired LVEF [$r = -0.298$, 95% confidence interval (CI) (-0.382,

Table 2 Laboratory associations with elevated levels of MIF

	Total	Tertiles of admission MIF (ng/mL)			*P*-value
		<40.2 (low)	40.2~73.0 (median)	≥73.0 (high)	
number	566	189	189	188	
White blood cells (10^9/L)	10.2 ± 3.3	9.4 ± 3.0	9.9 ± 3.0	11.2 ± 3.7	<0.001
Hemoglobin (g/L)	143 ± 21	141 ± 21	143 ± 18	144 ± 23	0.171
Platelets (10^9/L)	218 ± 48	214 ± 41	217 ± 51	224 ± 52	0.099
Non-fasting blood glucose (mmol/L)	6.3 (5.1–7.5)	6.0 (5.1–7.8)	6.5 (5.1–7.5)	6.7 (5.5–8.1)	0.024
HbA1c (%)	6.3 ± 1.3	6.2 ± 1.2	6.4 ± 1.4	6.3 ± 1.4	0.294
peak CK-MB (U/L)	195 (111–317)	161 (69–260)	213 (85–317)	275 (199–407)	<0.001
peak Hs-TnT (ng/ml)	4.6 (2.3–6.3)	2.8 (1.5–4.1)	4.5 (2.2–6.2)	6.3 (4.2–7.9)	<0.001
Nt-proBNP (pg/ml)	976 (433–2219)	718 (258–1897)	747 (395–1735)	1284 (625–3130)	0.002
Hs-CPR (pg/ml)	6.60 (3.60–12.16)	4.71 (2.02–12.81)	6.15 (2.79–17.85)	8.08 (3.20–16.32)	0.007
LDL-c (mmol/L)	2.89 ± 1.12	2.92 ± 0.89	2.86 ± 0.91	2.89 ± 1.46	0.893

Data are mean ± SD or median (25th percentile–75th percentile).
P-values were derived from Mann–Whitney *U*-test or One-way ANOVA for comparison among MIF tertile groups.
Nt-proBNP, N-terminal brain natriuretic peptide measured 2–3 days post-STEMI; LDL-c, low-density lipoprotein-cholesterol; CK-MB, creatine kinase MB fraction; CRP, C-reactive protein; hs-TnT, high sensitive-troponin T.

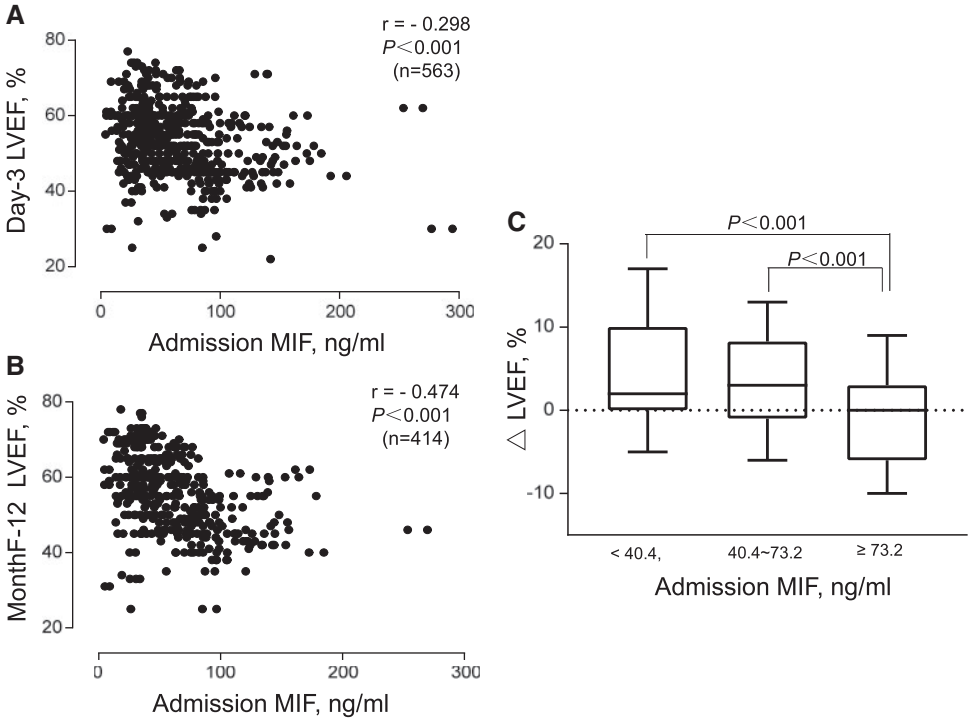

Figure 2 Admission macrophage migration inhibitory factor (MIF) levels correlate with left ventricular ejection fraction of acute or chronic phase post-ST-elevation myocardial infarction and improvement of left ventricular ejection fraction. Admission migration inhibitory factor levels were negatively correlated with left ventricular ejection fraction by echocardiography performed on day-3 and 12 months post-ST-elevation myocardial infarction (*A* and *B*). Patients were divided into three groups according to migration inhibitory factor tertiles. After calculating differences of left ventricular ejection fraction (ΔLVEF) of the two time-points, patients with high tertile migration inhibitory factor showed lack of spontaneous improvement of left ventricular ejection fraction relative to other two tertile groups ($P < 0.001$).

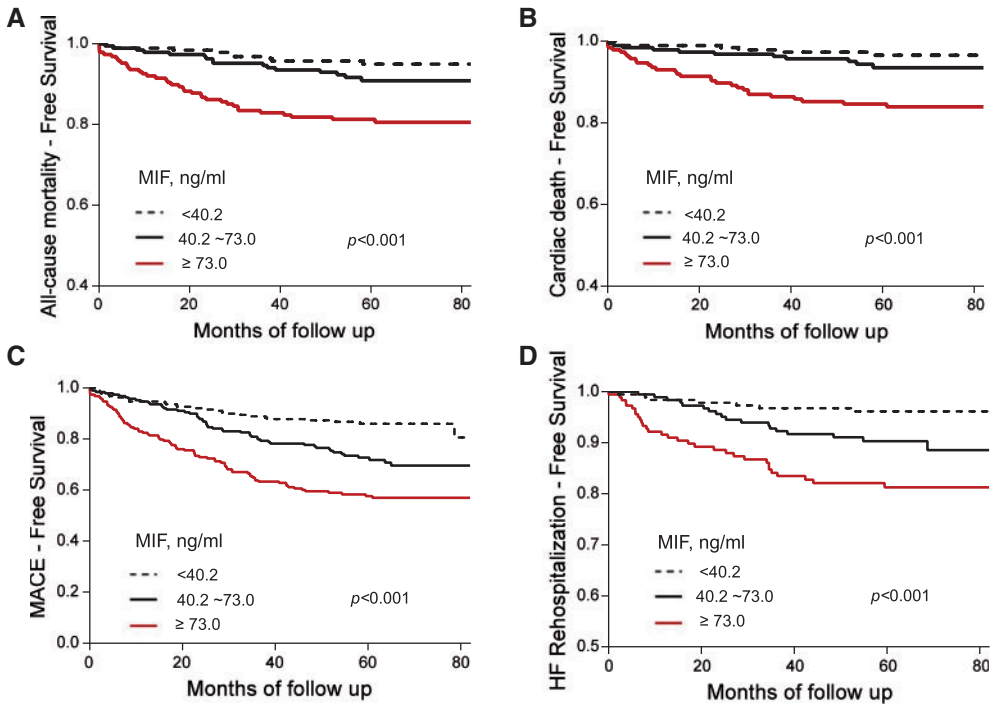

Figure 3 All-cause mortality, cardiovascular death, major adverse cardiovascular events and heart failure re-hospitalization according to tertiles of admission macrophage migration inhibitory factor (MIF) levels. The Kaplan–Meier event-free survival curves for all-cause death (*A*), cardiovascular death (*B*), major adverse cardiovascular events (*C*), and heart failure readmission (*D*) in ST-elevation myocardial infarction patients according to tertile migration inhibitory factor. Patients of high tertile migration inhibitory factor levels (red line) were compared with those of median tertile (black line) and low tertile (black dotted line). *P*-values indicate among group difference.

-0.215), $P < 0.001$, *Figure 2A*] and enlarged LVDD ($r = 0.115$, $P = 0.006$) on day-3 post-STEMI. There were stronger correlations between MIF and LVEF [$r = -0.474$, 95% CI (-0.550, -0.384), $P < 0.001$), *Figure 2B*] or LVDD ($r = 0.266$, $P < 0.001$) at 12-month post-STEMI. After calculating changes in LVEF (ΔLVEF) during day-3 and 12 months, our data revealed that admission MIF levels correlated negatively with ΔLVEF ($r = -0.261$, $P < 0.001$, $n = 414$) and that patients of high tertile MIF exhibit a lack of improvement of LVEF ($P < 0.001$) at 12 months relative to the day-3 value (*Figure 2C*).

Macrophage migration inhibitory factor and incomplete ST-segment resolution

In the subgroup of patients in high tertile MIF, the incidence of ST-segment resolution <50% at 60 min post-PCI was 3.3-fold and 1.9-fold higher than that of the low or median tertile groups ($P < 0.001$, *Table 1*). In contrast, admission hs-TnT or CK-MB were not associated with incomplete ST-segment resolution ($P = 0.263$ or $P = 0.486$). In multivariate logistics analyses, admission MIF as log-2 transformed continuous variable, was an independent predictor for incomplete resolution of ST-segment elevation with odds ratio (OR) 1.72 (95% CI 1.35–2.18; $P < 0.001$) per doubling in MIF concentration after adjustment for age, gender, eGFR, symptom-to-admission time <6 h, infarct location, previous history of diabetes, current smoking, levels of CK-MB, hs-TnT, and WBC at admission. The other remaining independent predictor was anterior infarct location (OR 2.02, 95% CI 1.35–3.01; $P = 0.001$) and WBC (OR 1.07, 95% CI 1.01–1.14; $P = 0.047$).

Macrophage migration inhibitory factor and long-term adverse outcomes

During a median follow-up period of 64 months (ranging from 0.03 to 83 months), 160 patients had a MACE. Of them, 62 patients died with 46 due to cardiovascular causes. There were 56 patients re-admitted due to HF, and 42 experienced recurrent MI. The admission MIF level was found to be closely associated with long-term adverse outcomes. As shown in *Figure 3*, Kaplan–Meier survival curves and log-rank analyses demonstrated different incidence distributions, according to MIF tertiles, of all-cause mortality, cardiovascular death, MACE and HF re-hospitalization (all $P < 0.001$). To explore the independency of MIF in prognostic prediction, we applied univariate and multivariate Cox-regression analyses using different models (*Table 3*). In all four clinical risk models tested including clinical characteristics and conventional biomarkers such as Nt-proBNP, peak hs-TnT, hs-CRP, and day-3 LVEF, MIF remained as an independent predictor of all-cause mortality, cardiovascular death and MACE. By C-statistics, inclusion of peak hs-TnT did not significantly alter the prognostic value of admission MIF ($P > 0.05$).

Estimated by C-statistics, our data showed that inclusion of MIF in a clinical risk model (including: age, sex, eGFR, haemoglobin, previous MI, diabetes mellitus, hypertension, current smoking, symptom-

Table 3 Multivariable Cox regression analyses for predictive value of admission MIF for Risk of MACE, all-cause mortality, cardiovascular death, or HF rehospitalization

		MACE	All-cause mortality	Cardiovascular death	HF rehospitalization
Unadjusted	HR (95% CI)	1.91 (1.57–2.31)	2.49 (1.80–3.44)	2.86 (1.95–4.20)	2.00 (1.43–2.79)
	P-value	<0.001	<0.001	<0.001	<0.001
Model 1	HR (95% CI)	1.71 (1.40–2.08)	2.54 (1.81–3.55)	2.88 (1.92–4.32)	1.97 (1.36–2.77)
	P-value	<0.001	<0.001	<0.001	<0.001
Model 2	HR (95% CI)	1.68 (1.38–2.05)	2.47 (1.78–3.42)	2.60 (1.74–3.87)	1.93 (1.38–2.69)
	P-value	<0.001	<0.001	<0.001	<0.001
Model 3	HR (95% CI)	1.46 (1.10–1.95)	2.37 (1.70–3.31)	2.55 (1.53–4.26)	1.84 (1.30–2.60)
	P-value	0.009	0.001	0.001	0.001
Model 4	HR (95% CI)	1.39 (1.12–1.71)	2.27 (1.42–3.22)	2.49 (1.52–4.24)	1.58 (1.13–2.24)
	P-value	0.002	0.010	0.004	0.008

Model 1: adjusted for age, sex and eGFR.
Model 2: Model-1 plus BMI, hemoglobin, previous MI, diabetes mellitus, hypertension, current smoking, hypercholesteremia, symptom-admission time <6 h, 3 vessel disease, Killip class >1, culprit lesion of LAD, ST-segment resolution, use of tirofiban during the PCI, TIMI classification pre- and post-PCI.
Model 3: Model-2 plus Nt-proBNP, peak TnT and hs-CRP.
Model 4: Model-3 plus LVEF.

admission time <6 h, culprit lesion of LAD, 3 vessel disease, Killip class >1, ST-segment resolution, TIMI class pre- and post-PCI, peak hs-TnT, and day-3 LVEF), significantly improved predictive ability for all-cause mortality [0.84 (0.77–0.91) vs. 0.89 (0.83–0.94), $P = 0.020$] and MACE [0.72 (0.67–0.77) vs. 0.74 (0.70–0.79), $P = 0.047$]. NRI and IDI were calculated to evaluate whether the addition of MIF to the clinical risk model led to any significant risk reclassification of the end points. Continuous NRI was significantly increased with 0.34 (95% CI 0.04–0.47) for all-cause mortality and 0.24 (95% CI 0.11–0.34) for MACE. Meanwhile, IDI (reflecting the changes in discrimination slope) yielded similar improvement with 0.06 (95% CI 0.00–0.144) for all-cause mortality and 0.05 (95% CI 0.01–0.09) for MACE (see Supplementary material online, *Table S1*).

Combined prognostic value of macrophage migration inhibitory factor and Nt-proBNP

The prognostic merit of MIF relative to peak hs-TnT, CRP, and Nt-proBNP was compared by C-statistics. We found that MIF (C-statistics 0.71, 95% CI 0.64–0.78) provided better prognostic information than peak hs-TnT (C-statistics 0.63, 95% CI 0.56–0.71; $P = 0.03$), or hs-CRP (C-statistics 0.53, 95% CI 0.46–0.60; $P < 0.001$), but was comparable to day-3 Nt-proBNP (C-statistics 0.70, 95% CI 0.62–0.75; $P = 0.33$) in all-cause mortality. Cox regression analysis revealed that, after adjustment for risk factors including standard biomarkers Nt-proBNP, peak hs-TnT and hs-CRP (*Figure 3*), only admission MIF and day-3 Nt-proBNP were independent predictors for adverse outcomes of STEMI patients. After adjustment for Model-4 with addition of day-3 LVEF, Nt-proBNP remains significant only for cardiovascular death and HF rehospitalization (see Supplementary material online, *Table S2*).

To investigate additive prognostic value of combination of MIF and Nt-proBNP, risk stratification for the endpoints in STEMI patients was made according to tertiles of MIF and NT-proBNP levels. The risk of the all-cause mortality (26.0% vs. 0.0%, $P < 0.001$) and MACE

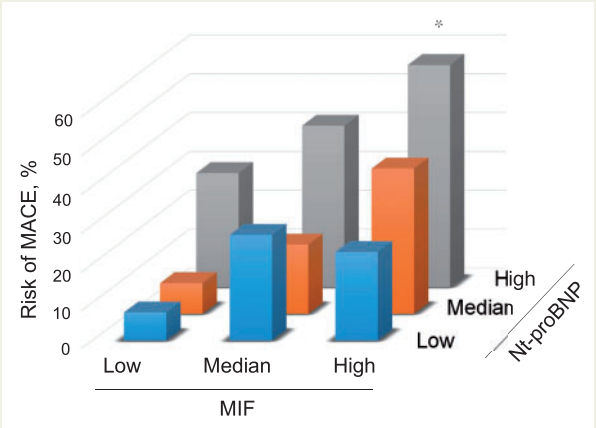

Figure 4 Risk stratification of major adverse cardiovascular events in ST-elevation myocardial infarction patients according to tertiles of macrophage migration inhibitory factor (MIF) and Nt-proBNP levels. Combination of admission macrophage migration inhibitory factor (MIF) and day-3 Nt-proBNP identified sub-groups of patients with increased risk of major adverse cardiovascular events during the follow-up period. Patients were re-grouped according to tertile of MIF or Nt-proBNP. The risk of major adverse cardiovascular events significantly increased in patients with both biomarkers in high tertile compared with patients with both biomarkers in the low tertile (*$P < 0.001$).

(57.1% vs. 7.4%, $P < 0.001$; *Figure 4*) increased significantly in patients with both biomarkers in the highest tertile compared with those with both biomarkers in the bottom tertile. Furthermore, to study prognostic value of different combinations, STEMI patients were divided into positive group (+) if individual biomarkers in top tertile and as negative group (-) if in median or low tertile. Compared with those in both negative group, the hazard ratio of patients in Nt-proBNP (+) MIF (+) group was over 11-fold in total mortality [hazard ratio (HR)

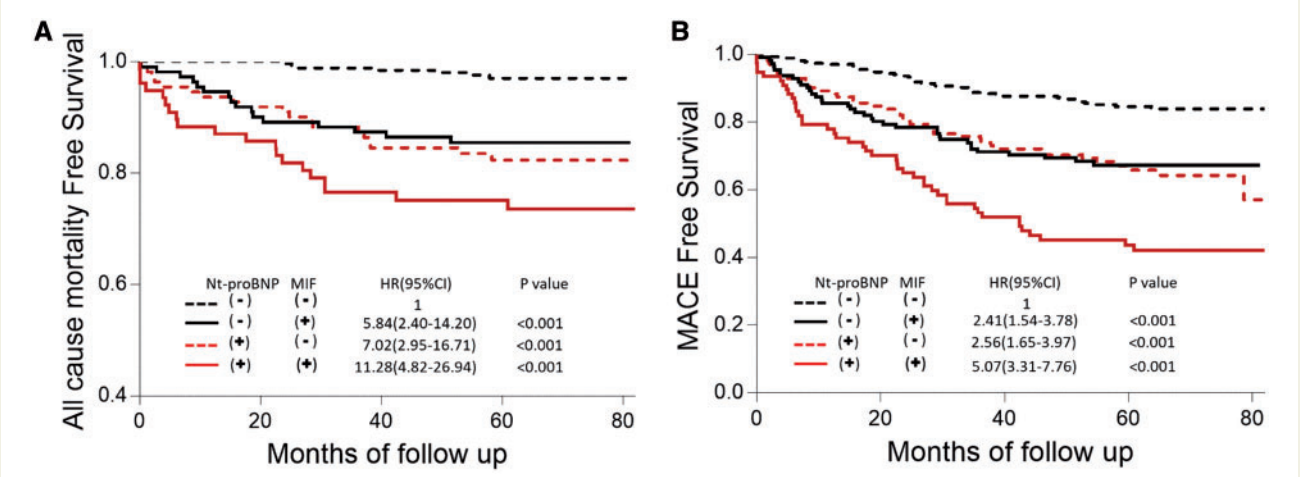

Figure 5 Comparison of all-cause mortality and major adverse cardiovascular events in ST-elevation myocardial infarction patients based on macrophage migration inhibitory factor (MIF) and Nt-proBNP tertiles. The Kaplan–Meier event-free survival curves for (*A*) all-cause mortality and (*B*) major adverse cardiovascular events in patients based on migration inhibitory factor and Nt-proBNP levels. Patients were divided into tertile groups separately and defined as positive (+) group with high tertile, negative group with median or low tertile level. Four groups came into being as Nt-proBNP(+) MIF(+) (red line, $n = 77$), Nt-proBNP(-) MIF(+) (black line, $n = 111$), Nt-proBNP(+) MIF(-) (dotted red line, $n = 111$) and Nt-proBNP(-) MIF(-) (dotted black line, $n = 267$). *P*-values in inserts indicate difference vs. the Nt-proBNP(-) MIF(-) group.

11.28; 95% CI 4.82–26.94; $P < 0.001$, *Figure 5*], similar to that of Triple (+) groups (HR 11.39; 95% CI 4.29–29.68; $P < 0.001$). However, the hazard ratio of patients in peak hs-TnT(+) MIF(+) or Nt-proBNP(+) peak hs-TnT(+) group was 4.12 (95% CI 2.16–7.85) or 6.60 (95% CI 3.32–13.10). Similar results were shown in Nt-proBNP(+) MIF(+) group with regard to the risk of MACE (*Figure 5*, and Supplementary material online, *Table S3*).

Discussion

This is the first prospective study to demonstrate that MIF level on admission has prognostic value across the spectrum of STEMI patients. *First*, we showed that admission MIF levels were predictive of later changes in necrotic markers (peak CK-MB and peak hs-TnT) and inflammatory parameters (hs-CRP, white blood cell count). Further, admission MIF is an independent risk factor of impaired restoration of myocardial reperfusion (ST-segment resolution <50% by 60 min post-PCI). *Second*, recovery in LVEF by 12 months post-STEMI was impaired in subgroup with a high MIF level. *Third*, by multivariate analysis, admission MIF as a continuous variable remained an independent predictor of long-term all-cause mortality, cardiovascular death and MACE after adjustments for established risk factors and biomarkers. *Finally*, we demonstrated in our STEMI patients that risk stratification of all-cause mortality and MACE was improved by combination of MIF and day-3 Nt-proBNP. These findings suggest that admission MIF level provides useful information beyond what currently is available from clinical and angiographic characteristics in patients with STEMI.

We confirmed our previous report that elevated admission MIF, but not admission levels of Tn or CK-MB, correlated significantly with estimated infarct size, impaired LVEF and LV dilatation in patients with STEMI.[11] We showed that admission MIF level closely correlated not only with cardiac biomarkers routinely used for estimation of infarct size, but was also related with inadequacy of ST-segment resolution during the acute phase. Patients with higher MIF levels had a greater degree of inflammation, indicated by greater white blood cell counts and CRP levels. We have furthered our previous findings[11] by showing that admission MIF levels were not only correlated with LVEF measured at acute and chronic time-points following STEMI, but also associated with dynamic change of LVEF. Specifically, patients in the high MIF tertile showed a lack of improvement in LVEF by 12 months post-MI. This finding may account for the prognostic value of initial MIF in predicting long term outcomes such as re-admission with HF. Although renal dysfunction has been associated with elevated circulatory MIF level, we found predictive ability of MIF unchanged when adjusted for eGFR, a finding in keeping with several clinical trials.[26,27]

Myocardial ischaemia/reperfusion injury remains a common event in patients with STEMI.[28,29] Despite optimal angiographic revascularization, microvascular damage, manifested in the form of no-reflow phenomenon, is detected in 30–60% of STEMI patients.[29,30] According to current guideline and clinical practice, ST-segment resolution post-PCI is used as a non-invasive but powerful indicator for the assessment of reperfusion efficacy and microvascular obstruction following STEMI.[1,20] We have now shown that patients presenting with an initial MIF in the high tertile have a 2.5-fold greater prevalence of incomplete ST-segment resolution compared with those in the low tertile, and that MIF was an independent predictive risk factor of incomplete ST-segment resolution. This is the first demonstration that level of plasma MIF prior to primary PCI is predictive of reperfusion efficacy and microvascular obstruction. The ability of admission MIF level to predict reperfusion damage may contribute to its infarct size predictive nature. This finding calls for future studies on microvascular damage involving use of CMR imaging.

Relative to a single biomarker, the use of two or more biomarkers has been shown to improve predictive power in STEMI patients.[7] However, previous investigations had not demonstrated whether admission MIF could further improve the predictive capability of conventional biomarkers for STEMI. This was tested in our study by combination of admission MIF and peak hs-TnT, hs-CRP (day-3) and Nt-proBNP (day-3). Using *C*-statistics and Cox regression, single prognostic value was reached for combination in pairs. Our data demonstrated that combination of admission MIF and day-3 Nt-proBNP significantly optimized risk stratification compared with single biomarker models. Interestingly, no significant improvement was found after inclusion into this combination of peak hs-TnT as a biomarker of infarct size. In this regard, peak hs-TnT derived from repeated measures does not have additional contribution. Indeed, peak hs-TnT is not an independent prognostic indicator by multivariate analyses. Thus, for STEMI patients, admission MIF and Nt-proBNP levels could effectively predict their long-term outcomes.

There are studies indicating cardioprotection by MIF in the setting of ischaemia.[31–36] However, such action conferred by MIF appears only to be evident in the specific experimental setting of a brief period of ischaemia (10–20 min) followed by reperfusion.[31–36] When the period of ischaemia is extended to over 30 min followed by reperfusion, the infarct size-limiting effect of MIF is lost.[31,37–39] One study in patients undergoing cardiac surgery indicates that elevation of intra-operative MIF level is inversely related to post-surgery day-1 organ dysfunction,[35] while another study on patients with cardiac arrest resuscitation indicates that increased MIF level reflect organ injury and higher mortality.[40] There has been no clinical report on such inverse association between post-STEMI admission MIF levels and acute or chronic prognosis. Our findings of a strong positive association between admission MIF levels and subsequent adverse cardiac events does not support a protective action of MIF in patients with STEMI.

Several other mechanisms need to be considered in explaining the mechanism(s) by which initial MIF levels are predictive of acute and chronic clinical outcomes following STEMI. These include: (i) the nature of cardiac MIF release upon MI and likely upon reperfusion[11,12]; (ii) its pro-inflammatory property,[11,37,41] and (iii) its regulation of fibrotic healing and interstitial fibrosis.[11,41,42] The myocardium has high content of MIF[12] forming the major source of circulating MIF upon ischaemia and infarction. Cardiac MIF release is a rapid process without requirement of *de novo* synthesis and is accompanied by a reciprocal reduction in cardiac MIF content.[11,18,41] This explains the rise of circulating MIF occurring much earlier than other cardiac biomarkers with its admission level correlated with the ischaemic mass and infarct size, as reported in our previous studies in both mice and human patients with STEMI.[11,41] However, MIF remains as an independent predictor of adverse cardiac events after inclusion of peak hs-TnT as a biomarker of infarct size, which suggests that other reasons also contribute to MIF's predictive power.

As an upstream pro-inflammatory cytokine, MIF plays a key role in various settings of pathological processes such as atherosclerosis, plaque instability, inflammation, and stroke.[37,41,43–46] MI-evoked inflammatory and fibrotic processes are critical for the clinical course by determining the extent of chronic LV remodelling and cardiac function following MI.[47] Whilst release of cardiac MIF mediates cardioprotection following a brief period of ischaemia,[18,48] following a prolonged ischaemia and formation of necrosis, MIF exacerbates cardiac damage by activating macrophage and other immune cells to migrate to the infarcted myocardium, upregulating inflammatory response and leading to increased synthesis of other pro-inflammation cytokines.[18,37,41,49,50] Similarly, in the ischaemia–reperfusion model, treatment with anti-MIF antibody or use of MIF knockout mice reduced collagen content of the infarct region one week post MI.[38,41] There is also evidence that the interstitial fibrosis of non-ischaemic myocardium following MI is regulated by MIF.[42] In STEMI patients, MIF levels measured at 2.5 day after MI positively correlated with the degree of CMR-sign of ECM expansion, a measure of interstitial fibrosis of the remote non-ischaemic myocardium.[42] It is also shown in patients with non-ischaemic HF undergoing endomyocardial biopsy that elevated myocardial MIF content associated with severe myocardial fibrosis forming an independent predictor of adverse cardiac outcomes.[51] All these findings indicate that MIF is an endogenous regulator of cardiac remodelling and fibrosis, providing additional basis for the association of admission MIF and chronic cardiac events post-STEMI.

A few limitations of this study need to be discussed. First, this prospective cohort study had a small sample size and was conducted only in Chinese population. However, sample size calculation using PASS for multivariant Cox regression analysis indicates that 566 observations is adequate for an anticipated event rate of 0.2500 with a power of 0.97 (1-β) and α of 0.05000. The predictive value of MIF for adverse outcomes needs to be further confirmed in larger patient cohorts with different ethnic backgrounds. Second, CMR was not performed in patients to evaluate infarct size or microvascular obstruction, although we estimated both by peak Tn value, angiographic TIMI and ST-segment resolution post-PCI. Third, with the exception of CRP, we did not assay additional inflammatory biomarkers that might have allowed for relating admission MIF to subsequent inflammatory responses. In addition, previous studies have revealed polymorphisms of MIF gene or its promoter region that might be associated with risk for inflammatory or coronary artery disease.[18,35,52] The potential association of such polymorphisms and post-infarct cardiac risk remains to be investigated. Finally, we only studied STEMI patients and it remains to be investigated whether the predictive power of MIF is similarly applicable to non-STEMI patients.

Collectively, the present study documents the prognostic value of admission MIF in patients with STEMI. We have demonstrated that admission MIF predicts microvascular damage, adverse progression of LV systolic dysfunction, long-term mortality and MACE, independent of clinical established risk factors, acute LVEF and routinely measured biomarkers. Furthermore, combination of admission MIF and day-3 Nt-proBNP provides a better prognostic prediction than either alone. The current study adds to our previous studies,[11,12] establishing admission MIF as a useful biomarker for short- and long-term prognosis in STEMI patients. Admission MIF levels allow for risk stratification of high-risk STEMI patients, who would potentially benefit from more comprehensive diagnostic evaluation and intensified therapy for secondary prevention. Furthermore, the ability to predict severity of outcome at the time of admission would permit allocation of actual resources where they are limited and to add decision on whether urgent patient transfer is required, although this would require development of a rapid MIF assay. Our findings call for large scale studies on STEMI as well as non-STEMI patients for further

confirmation to achieve biomarker-guided management strategies to subgroup of patients who would have poor long-term prognosis.

Supplementary material

Supplementary material is available at *European Heart Journal – Quality of Care and Clinical Outcomes* online.

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