Macrophage Migration Inhibitory Factor for the Early Prediction of Infarct Size

William Chan, MBBS, PhD;* David A. White, BSc (Hons);* Xin-Yu Wang, MD;* Ru-Feng Bai, PhD; Yang Liu, PhD; Hai-Yi Yu, MBBS, PhD; You-Yi Zhang, MBBS, PhD; Fenling Fan, PhD; Hans G. Schneider, MBBS, MD; Stephen J. Duffy, MBBS, PhD; Andrew J. Taylor, MBBS, PhD; Xiao-Jun Du, MBBS, PhD; Wei Gao, MD;[†] Xiao-Ming Gao, MBBS, MD;[†] Anthony M. Dart, FRACP, DPhil[†]

Background—Early diagnosis and knowledge of infarct size is critical for the management of acute myocardial infarction (MI). We evaluated whether early elevated plasma level of macrophage migration inhibitory factor (MIF) is useful for these purposes in patients with ST-elevation MI (STEMI).

Methods and Results—We first studied MIF level in plasma and the myocardium in mice and determined infarct size. MI for 15 or 60 minutes resulted in 2.5-fold increase over control values in plasma MIF levels while MIF content in the ischemic myocardium reduced by 50% and plasma MIF levels correlated with myocardium-at-risk and infarct size at both time-points ($P<0.01$). In patients with STEMI, we obtained admission plasma samples and measured MIF, conventional troponins (TnI, TnT), high sensitive TnI (hsTnI), creatine kinase (CK), CK-MB, and myoglobin. Infarct size was assessed by cardiac magnetic resonance (CMR) imaging. Patients with chronic stable angina and healthy volunteers were studied as controls. Of 374 STEMI patients, 68% had elevated admission MIF levels above the highest value in healthy controls (>41.6 ng/mL), a proportion similar to hsTnI (75%) and TnI (50%), but greater than other biomarkers studied (20% to 31%, all $P<0.05$ versus MIF). Only admission MIF levels correlated with CMR-derived infarct size, ventricular volumes and ejection fraction (n=42, r=0.46 to 0.77, all $P<0.01$) at 3 day and 3 months post-MI.

Conclusion—Plasma MIF levels are elevated in a high proportion of STEMI patients at the first obtainable sample and these levels are predictive of final infarct size and the extent of cardiac remodeling. (*J Am Heart Assoc.* 2013;2:e000226 doi: 10.1161/JAHA.113.000226)

Key Words: infarct size • macrophage migration inhibitory factor • myocardial infarction

The use of plasma biomarkers has become central to the diagnosis and management of acute coronary syndromes and exclusion of myocardial necrosis.[1] Specifically,

From the Departments of Cardiovascular Medicine (W.C., S.J.D., A.J.T., A.M.D.) and Pathology (H.G.S.), Alfred Hospital, Melbourne, Australia; Baker IDI Heart and Diabetes Institute, Melbourne, Australia (X.-M.G., X.-J.D. D.A.W., R.-F.B., Y.L., F.F., A.M.D.); The Third Hospital, Peking University, Beijing, China (X.-Y.W., H.-Y.Y., Y.-Y.Z., W.G.); Departments of Medicine (S.J.D., A.J.T., X.-J.D., A.M.D.) and Surgery (X.-M.G.), Central Clinical School, Monash University, Melbourne, Australia.

*Drs Chan, White, and Wang contributed equally to this work.

[†]Drs W. Gao, X.-M. Gao, and Dart contributed equally to the work.

Correspondence to: Anthony M. Dart, FRACP, DPhil, Heart Centre, the Alfred Hospital, Heart Centre, the Alfred Hospital, 55 Commercial Road, Melbourne, Victoria 3004, Australia. E-mail: a.dart@alfred.org.au

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the diagnostic value of troponin I or T (TnI or TnT) elevation among patients with myocardial infarction (MI) is well established.[2,3] Long-term mortality and morbidity following MI are largely determined by infarct size, and the extent of left ventricular (LV) dysfunction.[4,5] Primary percutaneous coronary intervention (PCI) is now the established standard of treatment for patients with ST-elevation MI (STEMI)[6,7] to limit infarct size and mortality.[8] Improvement in the early diagnosis of MI has the potential to critically affect global health-service delivery.[9] Moreover, given its prognostic impact, early accurate prediction of infarct size, currently only possible after 36 to 48 hours of STEMI with either peak or cumulative release of troponins or creatine kinase (CK) or with imaging, in patients with MI would be advantageous in tailoring prompt treatment, particularly in complex patients, or where local healthcare resources are limited.

In addition to the electrocardiogram, measurement of circulating levels of cardiac biomarkers is routinely used for the early detection of myocardial necrosis. Several biomarkers are known to increase within the first 12 hours after

acute MI, including CK, troponins, and myoglobin.[10–12] Current routine laboratory troponin assay cannot definitively exclude acute MI on the basis of normal levels until at least 6 to 12 hours after symptom onset as the rise in plasma troponin can be delayed.[1,13] CK and myoglobin levels have been claimed to rise earlier after the onset of MI than troponins, albeit neither are cardiac-specific.[1] High-sensitive assays of troponins (hsTn) have significantly improved the sensitivity for early diagnosis of acute MI.[12,14,15] However, information on infarct size still requires serial measurements of biomarkers over the first 36 to 48 hours or with myocardial imaging techniques such as cardiac magnetic resonance during the first week post-STEMI.

Experimental studies have revealed a critical role for the inflammatory cytokine macrophage migration inhibitory factor (MIF) involved in the early response to acute ischemic stress and the ongoing inflammatory response following acute MI.[16–19] MIF is widely expressed and is present in cardiomyocytes in a preformed state.[20] Previous clinical studies have reported elevated MIF plasma levels detected as early as 4 to 6 hours after acute MI,[21,22] which remained elevated up to 2 weeks post-MI.[23] Although these clinical and experimental studies have indicated a close association between MIF and acute MI, to what extent very early circulating MIF levels correlate with the onset and extent of myocardial necrosis (ie, infarct size) has not been established.

In the current study, we performed experimental and clinical studies to examine our hypothesis that plasma MIF is elevated early after acute MI due to cardiac release and that MIF levels in the first obtainable plasma samples may predict infarct size.

Methods

Experiments on Mice With Acute Myocardial Infarction

A mouse model of MI was used to define changes in plasma MIF and its source during the acute phase of MI. Furthermore, we determined the relationship between plasma MIF levels and the size of myocardium-at-risk and infarct myocardium. TnI was similarly studied for comparison with MIF.

Animals and surgery

All animal procedures were approved by a local Animal Ethics Committee and in accordance with guidelines set out in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Ed). Male C57Bl/6 mice at 12 weeks of age were subjected to open-chest surgery for coronary artery occlusion (CAO) for a period of 15 or 60 minutes, respectively (n=19 per group) or for sham procedure (n=6 per group) as we previously described.[24]

Quantification of the myocardium-at-risk and infarct myocardium

Myocardium-at-risk and infarct myocardium were identified as described previously with minor modifications.[16,25] Briefly, after cannulation of the ascending aorta, 5% Evans blue was injected into coronary arteries to stain nonischemic myocardium. The LV was then harvested and sectioned into 5 to 7 slices at a thickness of 0.7 mm from the apex to the base and then incubated in 1.5% triphenyltetrazolium chloride (TTC in PBS, 30 minutes at 37°C). The nonischemic zone (blue area), myocardium-at-risk (nonstained area by Evans blue), and infarct zone (nonstained area by TTC) were outlined and areas quantified digitally using Image J software. Since LV sections were at an equal thickness of 0.7 mm, volumes of myocardium-at-risk and infarct myocardium were also calculated by multiplying differently stained areas of each LV section with 0.7 mm, and expressed as mm^3 per heart. The size of infarct or myocardium-at-risk was expressed aspercentage of the LV.

Plasma and tissue preparation and assays of MIF and TnI

At the end of the ischemic period, blood was collected by cardiac puncture using heparin as an anticoagulant. Plasma was prepared and stored at −80°C until assay. In 2 subgroups of mice with CAO for 15 or 60 minutes, blood and hearts were collected. Under a dissecting microscope, the myocardium-at-risk and nonischemic LV tissues were carefully separated based on the pale color of the former and snap frozen. Tissue of 20 to 40 mg was homogenized and proteins extracted. Protein concentration was measured using Bradford assay.[16] MIF levels in mouse plasma (n=19 per group) and the myocardium (n=6 to 8 per group) were measured by Enzyme Linked Immunosorbent Assay (ELISA) in duplicates using a commercial mouse MIF kit (USCN Life Science & Technology Co) according to the manufacturer's instructions. The cardiac TnI assay (n=19 per group) was performed at the Alfred Hospital Pathology service using Chemiluminescent Microparticle Immunoassay (CMIA) on the Abbott Architect ci16200 (Abbott Laboratories).

Determination of cardiac MIF release by immunohistochemistry

Effect of myocardial ischemia on the cardiac MIF content was further examined by immunohistochemistry on fresh-frozen LV sections from mice with sham-operation, 15 or 60 minutes CAO (n=2 to 3 per group). We used rabbit antimouse primary anti-MIF antibody (1:2000, Invitrogen) and the secondary antibody was a biotinylated antirabbit IgG (1:200, Vector Laboratories). Antigen was visualized using 3,3′-Diaminobenzidine tetrahydrochloride (DAB).

Clinical Studies on Patients With Acute STEMI

Retrospective study (Beijing patient cohort)

This study was approved by the Human Ethics Committee, Peking University Health Science Centre and all participants provided written informed consent. We recruited consecutive patients with STEMI over 18 years of age who attended the Department of Cardiology, Third Hospital of Peking University during the period of 2008–2010. Patients with a diagnosis of STEMI (chest pain ≥30 minutes in duration, and electrocardiographic changes of new ischemia: ST elevation at the J point ≥0.1 mV in 2 contiguous leads) who underwent initial admission blood sampling within 6 hours after the onset of chest pain prior to any reperfusion interventions were eligible for inclusion. Patients having 1 or more of the following criteria were excluded: suspected or known previous MI in the same coronary artery territory as the current STEMI, rescue angioplasty, cardiogenic shock (systolic blood pressure <90 mm Hg), or renal failure (estimated glomerular filtration rate ≤30 mL/min). A total of 332 patients with STEMI meeting these inclusion and exclusion criteria were included in this report. We also recruited 58 healthy consecutive volunteers who had no evidence of cardiovascular and other systemic disease as determined on history, physical examination, and electrocardiogram from the Risk Assessment Clinic, the Third Hospital, and 26 patients with chronic stable angina who had evidence of coronary artery disease (CAD) verified by chart review (prior coronary angiograms or history of MI) and by their treating physicians and in whom there had not been any recent deterioration of anginal symptoms or CCS (Canadian Cardiovascular Society) class within the previous 6 months. Plasma samples were used for assay of MIF, TnT, CK-MB, and other routine laboratory analyses (see below). We included both a healthy control group and patients with chronic stable angina in order to investigate differences in not only MIF but also each of the other biomarkers of cardiomyocyte necrosis in each of the groups. This would serve to provide evidence for changes in MIF or other biomarker status according to different health states and clinical presentation. Compared to an apparently healthy phenotype, those patients with documented CAD might be expected to exhibit a different biomarker profile. Thus, it would be important to have baseline knowledge of different biomarker profiles when comparing against patients with STEMI.

Prospective study (Melbourne patient cohort)

This study was approved by the local hospital Human Research Ethics Committee in accordance with National Health and Medical Research Council's (NHMRC) National Statement on Ethical Conduct in Research Involving Humans and carried out at the Alfred Hospital between August 2008 and December 2010. All patients provided written informed consent. Admission plasma sampling was obtained prior to primary PCI for assay of MIF, TnI, hsTnI, CK, myoglobin, and high sensitive C-reactive protein (hsCRP). Plasma samples were then obtained every 6 hours for 48 hours for assay of CK and TnI to determine peak values and area under curve (AUC) determined by planimetry.[26] hsCRP at 48 hours post-MI was also analyzed. A total of 42 STEMI patients were recruited and all underwent primary PCI as per routine practice. Nine chronic stable angina patients and 50 healthy volunteers were recruited following the same standards as described in Beijing cohort during routine outpatient visits.

MIF ELISA

In both clinical studies, plasma MIF was measured in duplicates using Quantikine MIF ELISA kits (R&D Systems) according to manufacturer's specifications at the core laboratories in Baker IDI Heart and Diabetes Institute and in Cardiovascular Research Institute, Beijing University. The coefficient of variation for intra- and interassay variation was 2.2% and 3.6%, respectively. The highest value obtained from healthy control participants was used as the upper reference cut-off value. This was 32.9 ng/mL in the Beijing cohort and 41.6 ng/mL in the Melbourne cohort. The first obtainable blood/plasma sample post-admission was defined as the admission sample in both centers.

Routine laboratory analyses

All routine laboratory assays including full blood examination and renal function were performed using commercially available automated platform. Fourth generation TnT and CK-MB were measured using a Roche Cobas E601 Immunology Analyzer (Diamond Diagnostics) at the Clinical Chemistry Service of the Third Hospital, Beijing University. hsTnI, TnI, CK, and hsCRP were measured using Abbott Architect ci16200 (Abbott Laboratories) at the Alfred Hospital Pathology by laboratory technicians blinded to patient details. In the Melbourne cohort, myoglobin was measured in whole blood using a fluorescence immunoassay (Triage Cardiac Panel, Biosite Diagnostics).[11] Based on manufacturers' recommendation or literature, the upper reference limits for biomarkers studied were set as follows: for the Beijing cohort, CK-MB 24 U/L and TnT 0.03 μg/L;[9] for the Melbourne cohort: CK 200 U/L, TnI 0.03 μg/L,[9] hsTnI 0.03 μg/L,[27] and myoglobin 200 μg/L.[11]

Cardiac resonance magnetic imaging for infarct size and LV volumes and function

Cardiac resonance magnetic (CMR) examination post-MI was completed in all 42 patients of the Melbourne cohort at day 3

and in 36 patients at 3 months using a clinical 1.5-T CMR scanner (Sigma HDx 1.5-T, GE Healthcare) following the method previously described.[26,28] Briefly, LV function was assessed by a standard steady state free precession technique called Fast Imaging Employing Steady State Acquisition (FIESTA). LV ejection fraction (LVEF) was calculated by volumetric analysis from a contiguous short axis FIESTA stack covering the LV and right ventricle using the summation of disc method[29] by 2 cardiologists. For determination of myocardium-at-risk, LV short-axis slices covering the whole LV using a T2-weighted triple-inversion recovery breath-hold fast spinecho pulse sequence were obtained using a body coil. Myocardium-at-risk was quantified by manual delineation of myocardium with bright signal intensity 2 SD above the mean signal obtained in the remote, noninfarct myocardium and multiplying the slice thickness and the myocardial density of 1.05 g/mL, expressed as a percentage of LV mass. Late enhancement images covering the whole LV were acquired ≈15 minutes after intravenous administration of a bolus of gadolinium-diethylene triamine penta-acetic acid (DTPA) to identify regional necrosis/fibrosis for infarct size quantification using an inversion recovery gradient echo technique. The area of hyperenhanced myocardium (bounded by endocardial and epicardial contours) on each of the short-axis slice was manually traced then multiplied by the slice thickness and the myocardial density of 1.05 g/mL to obtain the infarct mass, and expressed as a percentage of LV mass (infarct size).[30] All analyses were performed offline on dedicated workstations running AW SDC 4.4 and IDL version 6.3 with ReportCARD version 3.6 by fully blinded observers with excellent reproducibility (r=0.98) for infarct size assessment.[26,29]

Statistical Analysis

For the animal studies, results are presented as mean±SEM. For clinical studies, categorical data are presented as numbers and percentages. Continuous data are presented as mean±SD. All continuous variables were subjected to D'Agostino & Pearson omnibus or the 1-sample Kolmogorov–Smirnov normality test. If variables failed to pass the test, nonparametric Kruskal–Wallis test was used to detect significance among groups with Dunns post-hoc test, and Spearman correlation was performed to detect the correlation between parameters. If variables passed the test, Pearson correlation or standard linear regression was performed to detect the correlation and continuous variables were compared with either unpaired Student's t test or 1-way analysis of variance (ANOVA) with Newman-Keuls post-hoc test. When multiple independent terms were included in regression analyses, stepped entry and removal was used with p for inclusion (PIN) and p for removal (POUT)

set at 0.05 and 0.1, respectively. $P<0.05$ was considered statistically significant. All data analyses were performed with SPSS version 16 or Graphpad Prism 5.0.

Results

Experimental Findings From the Mouse Model of MI

Rapid myocardial MIF release following MI and correlation between plasma MIF levels and the extent of myocardial injury

First, we observed a 2.5-fold increase in plasma MIF level in mice subjected to CAO for 15 or 60 minutes (Figure 1A). We then studied whether the early rise of circulating MIF was cardiac in origin. Indeed, we found ≈50% reduction relative to the respective sham values in MIF content of the jeopardized myocardium at both time-points studied (Figure 1A, both $P<0.05$). MIF content in the noninfarct myocardium collected at 1 hour after MI showed no change (data not shown). Rapid loss of MIF from the ischemic myocardium was also indicated by immunohistochemistry for MIF in LV tissues of mice with CAO for 15 or 60 minutes (Figure 1B). Plasma TnI levels were modestly elevated at 15 minutes but robustly elevated after a 60-minute period of CAO (Figure 1A).

Myocardial necrosis, determined by TTC staining, was evident in LVs within a 60-minute period of CAO but not from those with CAO for 15 minutes (Figure 1C). Plasma MIF levels measured at either 15 or 60 minutes positively correlated with the size of infarct and myocardium-at-risk (Figure 1D), while plasma TnI at either 15 or 60 minutes post-MI showed no correlation with the size of infarct or myocardium-at-risk (Figure 1D).

Findings From Clinical Studies

Baseline clinical data and clinical characteristics of STEMI patients

The baseline clinical characteristics of both patient cohorts are presented in Table 1. In both cohorts, a higher proportion of STEMI patients were male and smokers compared to healthy controls. The prevalence of smoking, hypertension, diabetes, dyslipidemia, and family history of CAD was significantly higher in STEMI and chronic stable angina patients than that in healthy controls. Patients with chronic stable angina had the largest body mass index in the Beijing cohort, and were significantly older than the other 2 groups in the Melbourne cohort. The clinical and procedural characteristics of the STEMI patients are summarized in Table 2. The mean values of MIF, TnT, and CK-MB at admission, were higher than their respective upper reference limits in the
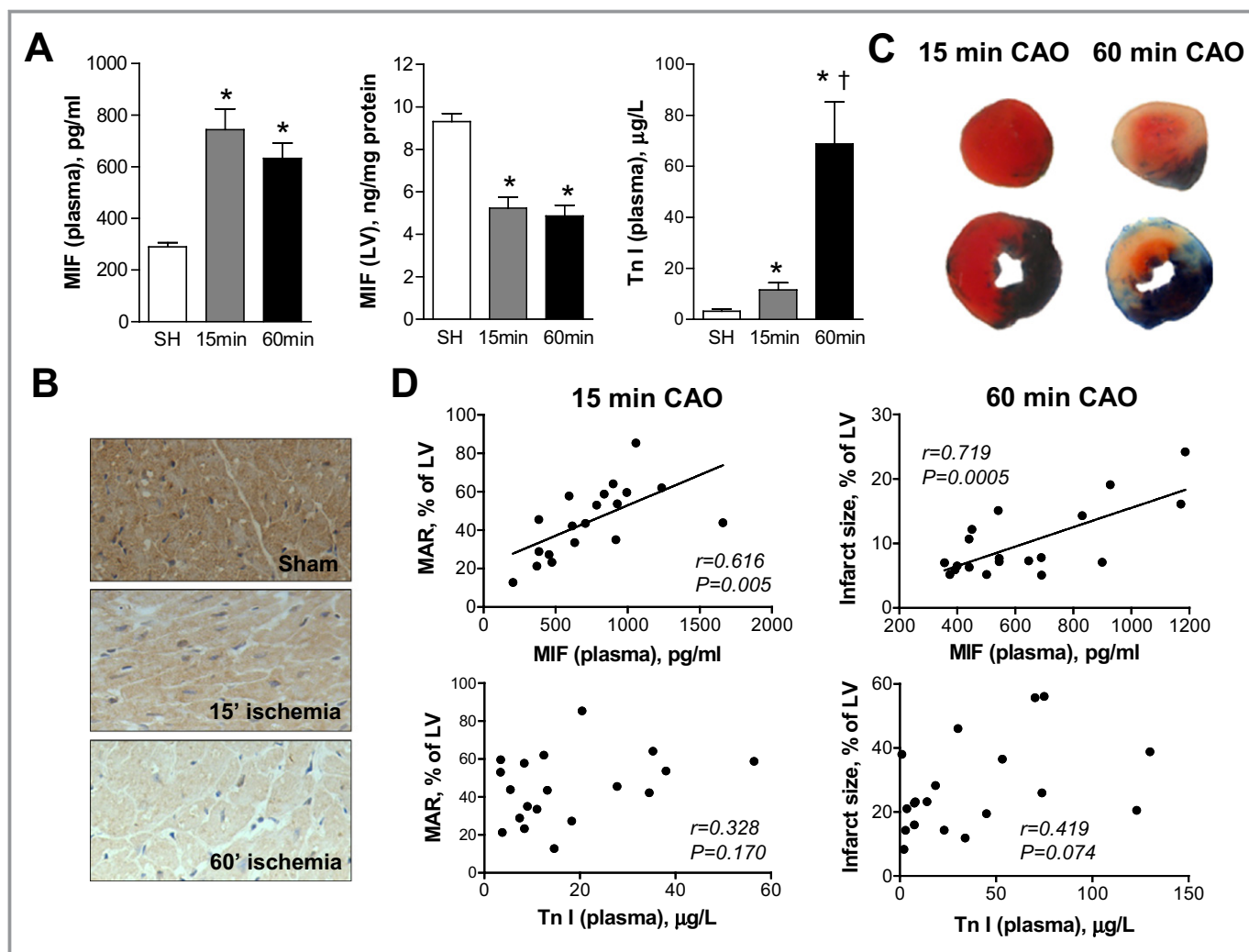
Figure 1. Changes in mouse plasma and cardiac macrophage migration inhibitory factor (MIF) following coronary artery occlusion (CAO) and correlation of plasma MIF levels with size of ischemic or infarct myocardium at 15 and 60 minutes of CAO. A, Rapid elevation of plasma MIF together with reduction in myocardial MIF at 15- and 60-minute periods of CAO. In contrast, plasma levels of troponin I (TnI) increased modestly at 15 minutes but markedly at 60 minutes, when myocardial necrosis developed. n=19 per CAO group, n=6 for sham-operated group, n=6 to 8 per group for myocardial MIF measurement. One-way ANOVA with Newman-Keuls post hoc test was used to detect significance between groups. *P<0.01 vs sham-operated control (SH). [†]P<0.01 vs 15 minutes CAO. B, Representative immunohistochemical staining for MIF (brown color) in the myocardium from mice subjected to sham-operation or with CAO for 15 and 60 minutes, respectively, showing a reduction of MIF density. n=2 to 3 per group. C, Representative left ventricular (LV) sections from mice subjected to CAO for 15 or 60 minutes. LV sections were dual stained to identify ischemic but viable (pink) myocardium and necrotic (pale) myocardium, dark blue stained areas denote normal perfused areas. D, Pearson correlation was performed to detect correlation between plasma levels of MIF or TnI and the size of myocardium-at-risk (MAR) or infarct size determined by serial LV sections with dual staining, as shown in (C). n=19 per group.

Beijing cohort. For the Melbourne cohort, admission mean levels of MIF, TnI, hsTnI, and myoglobin, except CK, were above their respective upper reference limits. In the Beijing STEMI cohort, patients with admission MIF value above 32.9 ng/mL were older, more likely to be male, and had a higher BMI (all P<0.05) than those whose MIF values were below 32.9 ng/mL. The Melbourne cohort showed the same trend with respect to age and male gender, but differences did not reach statistical significance. The median symptom-to-blood sampling time was 216±83 minutes and 211±75 minutes in the Beijing and the Melbourne cohorts, respectively.

Early elevation of plasma MIF levels in patients with STEMI

The distribution of plasma MIF and other biomarkers from the admission blood samples in patients with STEMI are shown in Figure 2. In the Beijing cohort, 67.5% of patients exhibited plasma MIF levels above the upper reference limit (32.9 ng/mL) of healthy controls (Figure 2A), a proportion significantly higher

Table 1. Baseline Characteristics of Study Participants

	Beijing Cohort			Melbourne Cohort		
	Healthy Control	CSA	STEMI	Healthy Control	CSA	STEMI
Number	58	26	332	50	9	42
Age, y	54.0±10.0	64.8±9.4*	60.7±12.9*	57.8±10.9	73.7±5.1*	59.6±10.9[†]
Male gender, %	62	58	82*[†]	52	80*	76*
BMI, kg/m^2	22.5±2.5	32.0±11.5*	25.2±3.0[†]	25.3±4.1	28.6±3.8	27.7±5.4
Current smoking, %	8	42*	72*[†]	10	22	45*
Hypertension, %	—	73*	54*	—	44*	38*
Diabetes, %	—	35*	25*	—	33*	14*
Dyslipidemia, %	—	46*	30*	—	44*	33*
Family history of CAD, %	—	42*	21*[†]	22	55*	45*

Values are expressed as mean±SD or percentage. BMI indicates body mass index; CAD, coronary artery disease; CSA, chronic stable angina; STEMI, ST-segment elevation myocardial infarction.

*P<0.05 vs healthy control.

[†]P<0.05 vs CSA.

than that of plasma CK-MB (30.6%, Figure 2C), or TnT (24%, individual data not shown, all P<0.05 versus MIF). In the Melbourne cohort, 71.4% of patients with STEMI had elevated admission MIF level above the upper reference limit (41.6 ng/mL, Figure 2B), a figure similar to hsTnI (75%) or TnI (50%, P=0.073, individual data not shown), but significantly higher than that of CK (19.5%) and myoglobin (26.8%, both P<0.05 versus MIF, Figure 2D through 2F).

Admission MIF levels correlated with infarct size, myocardium at risk, LV remodeling and dysfunction

The relationship between admission MIF levels and infarct size and LV remodeling and function was further evaluated in the prospective study conducted in Melbourne. Admission plasma MIF levels correlated significantly with CMR-derived infarct size at day-3 post-MI whereas other biomarkers measured did not (Figure 3). Moreover, admission MIF levels also correlated with peak level and AUC for TnI (r=0.669 and 0.678, both P<0.001) and CK (r=0.513 and 0.695, both P<0.001). Day-3 infarct size closely correlated with peak level and AUC for CK (r=0.589 and 0.775, both P<0.001) and TnI (r=0.660 and 0.859, both P<0.001) as well, suggesting that MIF is a reliable marker for infarct size prediction. Importantly, admission MIF levels also correlated with myocardium-at-risk at day 3 (Figure 4A) and chronic infarct size 3 months post-MI (Figure 4B). Furthermore, standard multiple regression analysis revealed that among the 5 admission biomarkers tested (eg, MIF, hsTnI, CK, myoglobin, and hsCRP) only MIF emerged as a significant independent predictor of both day 3 and 3 months infarct size (data not shown). Notably, admission MIF levels also exhibited a significant relationship with CMR-derived LV end-diastolic and end-systolic volume indexed

(LVEDVI, LVESVI) and LVEF determined at 3 days and 3 months post-MI (all P<0.01, Figure 4). All significant associations reported for admission MIF were still present and essentially unchanged when examined in regression analyses, which also included age, gender, and body mass index in addition to admission MIF as independent variables. In these analyses, age was significantly related to LVEF (r=0.048) and body mass index to LVEDVI (r=0.037) at day-3 post-MI, but there were no other significant associations with age, gender, or body mass index.

hsCRP assay was also performed in patients with STEMI (the Melbourne cohort). Plasma hsCRP levels were unchanged at admission but increased by 10-fold at 48 hours post-MI. Furthermore, admission hsCRP levels neither correlated with admission MIF levels nor with infarct size measured by CMR at 3 days (Figure 5), while hsCRP levels at 48 hours post-MI significantly correlated with admission MIF levels and day-3 infarct size (all P<0.05, Figure 5). However, there were no significant associations involving hsCRP at 48 hours post-MI when these were examined using log$_e$ transformed hsCRP values in regression analyses which also included age, gender, and body mass index.

Discussion

Three important findings have been made in the present study. First, we demonstrated in the mouse MI model that there was a rapid increase in circulating MIF, which occurred earlier than that of TnI and was accompanied by a reciprocal reduction in myocardial MIF content suggesting release of MIF from the ischemic myocardium into circulation. Elevated plasma MIF levels correlated with the mass of ischemic and

Table 2. Baseline Clinical and Procedural Characteristics in STEMI Patients

	Beijing Cohort	Melbourne Cohort	P Value
Number	332	42	—
Heart rate, beats/min	76±16	73±16	0.25
Systolic blood pressure, mm Hg	137±27	122±23	0.0006
Diastolic blood pressure, mm Hg	79±18	77±14	0.49
Serum creatine, μmol/L	88±28	85±25	0.51
Symptom-to-sampling, min	216±83	211±75	0.983
Admission MIF, ng/mL	55±40	66±36	0.091
Admission troponins, μg/L	0.08±0.26 (TnT)	0.10±0.21 (TnI)	0.63
Admission hsTnI, μg/L	—	0.38±0.19	—
Admission CK, U/L	—	133±111	—
Admission CK-MB, U/L	31±51	—	—
Admission myoglobin, μg/L	—	367±913	—
Pre-PCI medication, n (%)			
Aspirin	100%	100%	—
Clopidogrel	5%	54%	<0.001
β-blocker	0	2%	0.219
ACEi/ARB	0	27%	<0.001
Statin	28.6%	10%	0.014
Infarct-related artery			
Left anterior descending artery, n (%)	49%	37%	0.24
Right coronary artery, n (%)	35%	39%	0.82
Left circumflex artery, n (%)	15%	24%	0.22
Number of stenosed vessels	2.1±0.88	1.7±0.8	0.005
Stent type BMS/DES, %/	3/97	67/33	—

Values are expressed as mean±SD, percentage or exact number. ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMS, bare-metal stent; CK, creatine kinase; DES, drug-eluting stent; hsTnI, high sensitive troponin I; MIF, macrophage migration inhibitory factor; PCI, percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction.

infarcted myocardium. Second, elevation of plasma MIF was observed in 68% of patients with STEMI at the earliest available samples after admission, and this proportion was similar to hsTnI or TnI, but significantly higher than other conventional cardiac biomarkers measured (19% to 30%) at the same time. Third, in patients with STEMI, admission levels of MIF, but not other biomarkers, correlated positively with infarct size and LV chamber size, and negatively with LVEF measured both acutely (day 3) and at 3 months. Our experimental and clinical findings indicate that a single MIF assay at admission could be a useful biomarker for early prediction of final infarct size and might have utility in the early detection of myocardial necrosis.

Previous clinical studies reported an increase in plasma MIF levels in patients with acute MI 4 to 6 hours after admission,[22] which remained elevated over the next 2 weeks.[21] However, it remains unclear if MIF circulating levels might be detectable earlier than 4 to 6 hours after

symptom onset in patients with acute MI (STEMI). Our findings from mice and patients with acute MI demonstrated a much earlier elevation of plasma MIF levels than previously reported. In mice, a brief period of ischemia (15 minutes) induced a significant increase in circulating MIF level. Specifically, admission plasma MIF levels determined at a median of 216 minutes (Beijing cohort) or 211 minutes (Melbourne cohort) of symptom-to-sampling time were already elevated in 68% to 71% of patients with STEMI compared to 19% to 50% for other biomarkers studied with the exception of hsTnI (75%). Although release of MIF by cardiac tissue or cardiomyocytes has been reported in in vitro settings of hypoxia, ischemia or oxidative stress,[18,21] our in vivo animal model further documented the cardiac source of circulating MIF manifested by reciprocal changes in plasma MIF levels and in the ischemic myocardium. A reduction of MIF density in the ischemic myocardium was observed by immunohistochemistry.
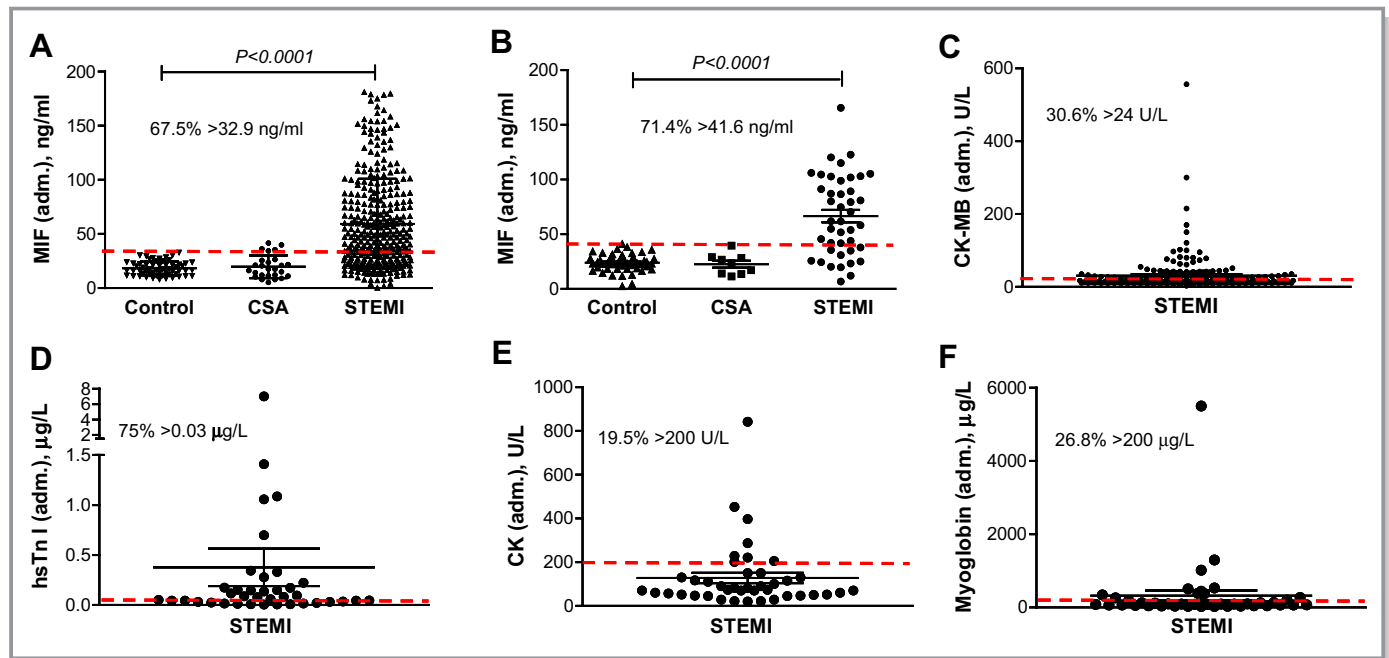
Figure 2. Admission plasma levels of macrophage migration inhibitory factor (MIF) and other biomarkers in patients with ST-elevation myocardial infarction (STEMI) and chronic stable angina (CSA), and healthy controls. Broken lines denote upper reference normal limit for respective biomarker. A and C, Data from the Beijing cohort. n=58, 26, 332 for control, CSA, and STEMI, respectively. B, D, E, F, Data from the Melbourne cohort. n=50, 9, 42 for control, CSA, and STEMI, respectively. One-way ANOVA with Newman-Keuls post hoc test was used to detect significance between groups in both cohorts. ANOVA indicates analysis of variance; CK, creatine kinase; hsTnI, high sensitive troponin I.

There are several reasons why a biomarker that could predict final infarct size might be desirable in the clinical management of patients presenting with STEMI. Early changes in circulating levels of this biomarker would facilitate decision-making about the timeliness of reperfusion, particularly in regions of the world where healthcare resources are limited. It would also facilitate appropriate resource allocation for specific patients who are likely to need more intensive medical attention. Currently available biomarkers require serial assays to determine peak values and/or AUC for infarct size quantification. In contrast, MIF levels obtained

from our experimental mouse MI model, correlated with ischemic and infarct mass at 15 and 60 minutes post–CAO, whereas TnI assay showed no such correlation, Similarly, in our clinical studies, while there was no predictive value for infarct size by CK, hsTnI, TnI, and myoglobin, at the time of hospital presentation, an impressive correlation between a single admission MIF level and infarct size determined by CMR either acutely or at 3 months was observed. These findings raise the prospect that MIF may prove to be useful in the clinical setting, especially in the emergency department or coronary care unit. Release of conventional biomarkers from
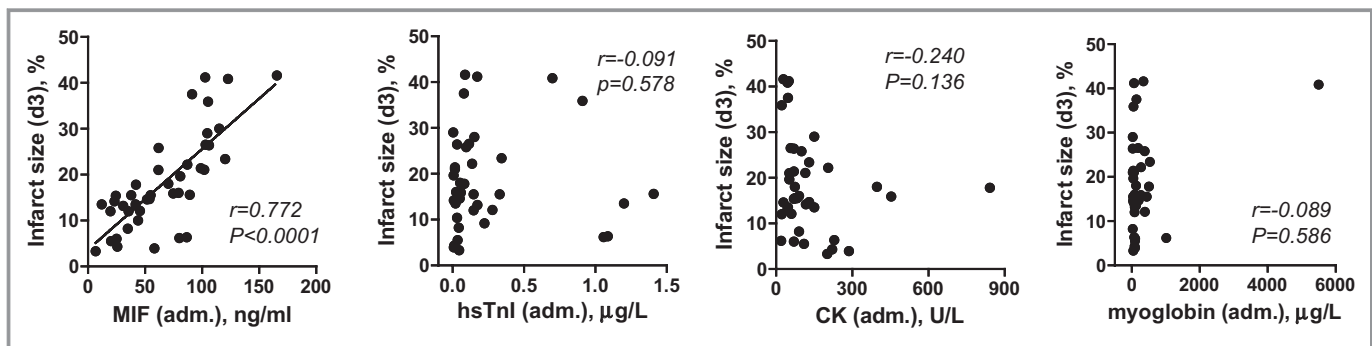

Figure 3. Admission (adm.) macrophage migration inhibitory factor (MIF) levels, but not other cardiac biomarkers, for example, high sensitive troponin I (hsTnI), creatine kinase (CK), and myoglobin, correlated with cardiac magnetic resonance–derived infarct size determined at day 3 (d3) after acute ST-elevation myocardial infarction. Data were from the Melbourne cohort. Pearson correlation for MIF and Spearman correlation for other biomarkers were performed to detect a correlation with infarct size.
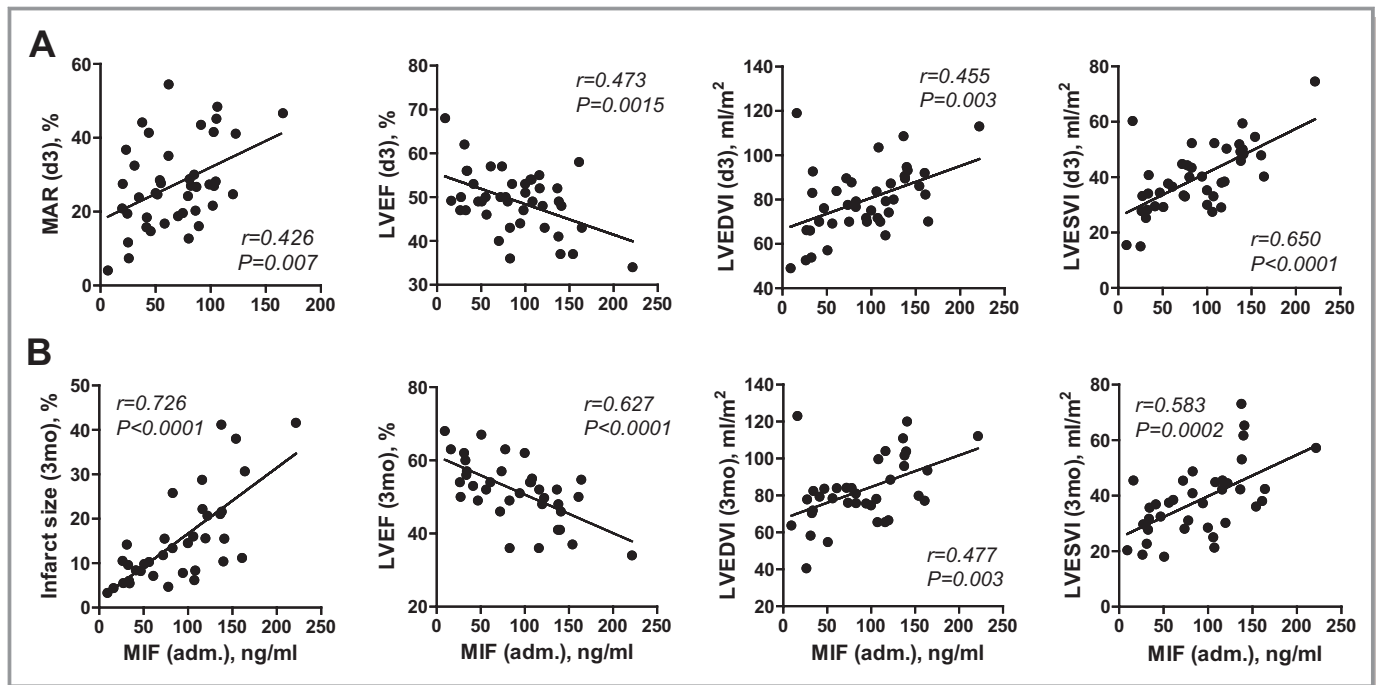
Figure 4. Admission (adm.) macrophage migration inhibitory factor (MIF) levels correlated with cardiac magnetic resonance (CMR)-derived size of myocardium-at-risk (MAR), infarct size, left ventricular (LV) indexed end-diastolic or end-systolic volumes (LVEDVI, LVESVI) and LV ejection fraction (LVEF) measured at day 3 (d3) and 3-month (3 mo) after acute ST-elevation myocardial infarction (MI). Data were from the Melbourne cohort, n=42 for day 3 and n=36 for 3 months post-MI. Standard linear logistic regression between admission MIF levels and CMR-derived parameters was performed.

cardiomyocytes is known to require degradation of sarcomere contractile proteins (such as troponins) and membrane disintegration with leakage of cytosolic enzymes (such as CK),[12] and therefore a significant delay relative to that of MIF is required for adequate detection. Our observation that both peak and cumulative release of CK and TnI following PCI appear to be strong predictors of infarct size is consistent with the literature.[31–33] However, this information would not be available at least for another 24 to 36 hours for peak levels and 48 hours for AUC.[1] Currently, no admission biomarkers appear to be able to predict final infarct size when used within the first hours of onset of symptoms. The current study indicates that plasma MIF levels may offer such potential.

As MIF is a proinflammatory cytokine,[34] and acute MI is associated with a systemic and regional inflammatory responses, the question raised as to whether the rise in MIF also reflect a state of inflammation. A recent study reported that the plasma level of CRP at 2 days after acute MI correlated with an inflammatory marker, interleukin-6, and infarct size.[35] We therefore elected to study the relationship between MIF and a surrogate marker of inflammation, hsCRP, in the Melbourne Cohort. Unlike MIF, plasma hsCRP level was not elevated at the time of admission but increased at 48 hours after STEMI and only hsCRP levels at 48 hours correlated with admission MIF levels and with day-3 infarct size, similar to the previous report.[35] The lack of a correlation

between admission MIF and hsCRP levels suggests that a rapid release of cardiac MIF is a supra-acute reaction to the ischemic insult. However, it is possible that the subsequent sustained rise of circulating MIF reported previously[22,23] may be related to a systemic inflammation and this requires a further investigation.

Interestingly, plasma levels of MIF were already significantly increased in mice undergoing CAO within 15 minutes when myocardial necrosis was not yet evident. Plasma MIF in mice correlated with the extent of the ischemic myocardium. This observation implies that MIF is also released from ischemic-but-viable myocardium following severe ischemic stress. Although plasma TnI was also elevated at this time-point, there was no correlation between TnI level and size of myocardium-at-risk. Similarly, both admission MIF and hsTnI levels were elevated in STEMI patients, but only MIF levels closely correlated with CMR-derived size of myocardium-at-risk. These results suggest a predictive value of early circulating MIF levels with the extent of ischemic myocardium. Therefore, we propose that MIF may represent a new class of biomarker that is released by the myocardium upon acute ischemic insult but without the necessity for tissue necrosis. Furthermore, the predictive value of admission MIF levels was not only limited to acute and final infarct size, but was also associated with parameters of cardiac remodeling and dysfunction. CMR imaging performed in STEMI patients in
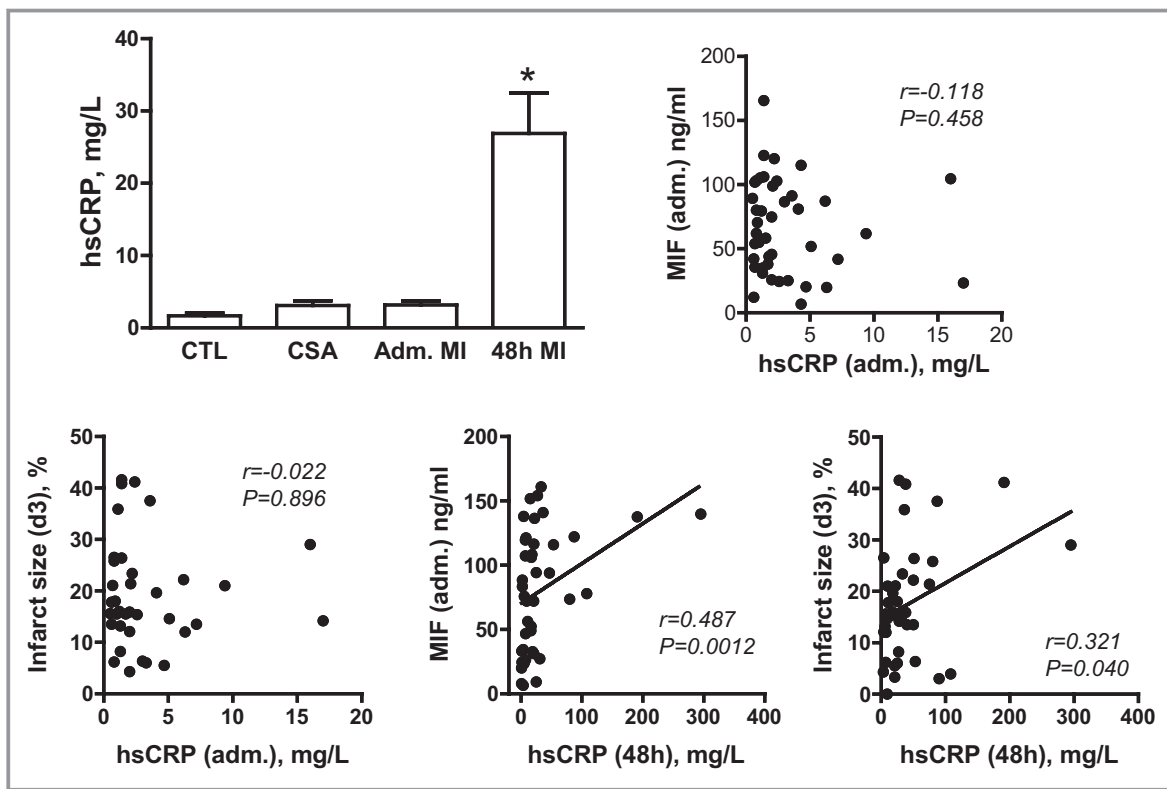
Figure 5. Plasma levels of high sensitive C-reactive protein (hsCRP) in healthy controls (CTL) and in patients with chronic stable angina (CSA) or with ST-elevation myocardial infarction (STEMI), and correlation between hsCRP and migration inhibitory factor (MIF) or cardiac magnetic resonance imaging-derived infarct size at 3 days post-MI. Plasma hsCRP was not elevated at admission (adm.) but was increased after 48 hours post-MI. Admission hsCRP correlated neither with admission MIF levels nor day 3 infarct size. However, plasma hsCRP at 48 hours post-MI correlated with admission MIF levels and infarct size. n=10, 9, 42 for healthy controls, CSA, and MI (both admission and 48 hours post-MI) groups, respectively. *P<0.01 vs other groups. Nonparametric Kruskal–Wallis test was used to detect significance in hsCRP between groups with Dunns post-hoc test. Spearman correlation was performed for correlation between MIF, hsCRP, or infarct size.

the Melbourne cohort demonstrated a strong correlation between admission MIF levels and LV volume index or LVEF measured by CMR acutely and chronically.

Limitations and Future Direction

First, in our current study only STEMI patients were investigated. Our hypothesis was that elevation in plasma MIF early after acute MI might be predictive of infarct size. Thus, inclusion of only STEMI patients was by study design. However, we demonstrated that the very early rise in plasma MIF levels is most likely due to release of preformed cardiomyocyte stores providing a plausible biologic explanation that closely couples the early release of MIF, which might be of use in early detection of myocardial necrosis, with infarct size insofar as reflecting the magnitude of overall myocardial injury or necrosis that is subsequently quantified by cardiac magnetic resonance imaging. The possibility that MIF might be of utility in the early detection of myocardial necrosis needs to be investigated in future studies in patients who present with non-ST elevation acute coronary syndrome,

including unstable angina, to properly assess the diagnostic performance of MIF against other biomarkers. Second, our study was not designed to specifically compare the diagnostic performance of MIF versus established biomarkers. We acknowledge that hsTns, which have a lower limit of detection with higher sensitivity for early diagnosis than conventional troponin assays, need to be compared against MIF in future studies. While the diagnostic performance of hsTn in detecting myocardial necrosis is excellent with superior sensitivity and precision over conventional troponin assays, there are still well recognized challenges and limitations in adopting and implementing the use of hsTn in early diagnosis of acute MI.[36] For example, the appropriate reference (99th percentile) value to use clinically is different for men versus women (a situation that might be similar to observed differences in MIF levels between men and women and also with increasing age); solitary elevations of hsTn values (>99th percentile) is thought to be inadequate for clinical decision making and serial changes in hsTn values will still be required to determine acute myocardial injury. Thus, each biomarker has advantages and limitations depending on the pretest

probability of myocardial injury and the specific assays used. In our study we did not use the MIF 99th percentile to define our upper reference limit but rather the highest value of healthy control participants. Although our study, to our knowledge, is the first study to publish the distribution of MIF levels in the largest sample of normal controls, the sample size of 50 (Melbourne cohort) and 58 (Beijing cohort) remains relatively small. We plan to undertake future studies involving larger numbers of normal controls in order to define the 99th MIF percentile. Third, MIF is not cardiac-specific, however, rapid rise of circulating MIF levels within a short time-window during the early phase post-MI period strongly supports a cardiac origin. Finally, we excluded patients with a prior infarct in order to minimize any confounding related to the interpretation of biomarker levels with myocardium-at-risk and infarct size. Patients with prior infarction are known to have a smaller myocardium-at-risk. Therefore, by only including patients without prior infarct, we believe this to be a more appropriate study design in our evaluation of the relationship between biomarkers (MIF) with myocardium-at-risk and infarct size. In addition, it is possible that patients with prior MI who present with their current MI might have a smaller rise in MIF levels as would be predicted by the size of MI imaged on CMR, which is a composite of old and new MI. Thus, by including only patients without prior MI this confounding effect could be minimized.

In conclusion, we demonstrate that plasma MIF levels were elevated in the majority of STEMI patients at the first obtainable sample post-admission, which predicted acute and final infarct size and also post-MI cardiac remodeling and dysfunction while other biomarkers, such as hsTnI, conventional TnI and TnT, CK, myoglobin and hsCRP, did not. This early rise in MIF levels may have significant implications for prognostication, patient management, and healthcare utilization.

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Disclosures

None.

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