Molecular Medicine

High Postoperative Blood Levels of Macrophage Migration Inhibitory Factor Are Associated with Less Organ Dysfunction in Patients after Cardiac Surgery

Christian Stoppe,[1,2]* Gerrit Grieb,[2,3]* Rolf Rossaint,[1] David Simons,[2,5] Mark Coburn,[1] Andreas Götzenich,[6] Tim Strüssmann,[2] Norbert Pallua,[3] Jürgen Bernhagen,[2] and Steffen Rex[1,4]

[1]Department of Anesthesiology, [2]Institute of Biochemistry and Molecular Cell Biology, and [3]Department of Plastic Surgery, Hand Surgery, Burn Unit, University Hospital, RWTH Aachen, Germany; [4]Department of Anesthesiology, University Hospitals Gasthuisberg, KU Leuven, Belgium; [5]Department of Radiology, German Cancer Research Center (DKFZ), Heidelberg, Germany; and [6]Department of Thoracic, Cardiac and Vascular Surgery, University Hospital, RWTH Aachen, Germany

Macrophage migration inhibitory factor (MIF) is an inflammatory cytokine that exerts protective effects during myocardial ischemia/reperfusion injury. We hypothesized that elevated MIF levels in the early postoperative time course might be inversely associated with postoperative organ dysfunction as assessed by the simplified acute physiology score (SAPS) II and sequential organ failure assessment (SOFA) score in patients after cardiac surgery. A total of 52 cardiac surgical patients (mean age (± SD) 67 ± 10 years; EuroScore: 7 (2–11)) were enrolled in this monocenter, prospective observational study. Serum levels of MIF and clinical data were obtained after induction of anesthesia, at admission to the intensive care unit (ICU), 4 h after admission and at the first and second postoperative day. To characterize the magnitude of MIF release, we compared blood levels of samples from cardiac surgical patients with those obtained from healthy volunteers. We assessed patient outcomes using the SAPS II at postoperative d 1 and SOFA score for the first 3 d of the eventual ICU stay. Compared to healthy volunteers, patients had already exhibited elevated MIF levels prior to surgery (64 ± 50 versus 13 ± 17 ng/mL; $p < 0.05$). At admission to the ICU, MIF levels reached peak values (107 ± 95 ng/mL; $p < 0.01$ versus baseline) that decreased throughout the observation period and had already reached preoperative values 4 h later. Postoperative MIF values were inversely correlated with SAPS II and SOFA scores during the early postoperative stay. Moreover, MIF values on postoperative d 1 were related to the calculated cardiac power index ($r = 0.420$, $p < 0.05$). Elevated postoperative MIF levels are inversely correlated with organ dysfunction in patients after cardiac surgery.
Online address: http://www.molmed.org
doi: 10.2119/molmed.2012.00071

INTRODUCTION

Macrophage migration inhibitory factor (MIF) is a pleiotropic inflammatory cytokine with chemokine-like functions that is rapidly released from preformed cytoplasmic pools of numerous cell types (including monocytes/macrophages and cardiomyocytes) in response to various noxious stimuli such as infection, inflammation and hypoxia (1–6). MIF is an upstream regulator of the initial immune response with various proinflammatory effects (1) that initiates and exacerbates various chronic and acute inflammatory disorders such as rheumatoid arthritis, atherosclerosis, acute respiratory distress syndrome and sepsis (3,5,6). Within the myocardium, MIF has been found to be induced by endotoxins, to initiate inflammatory responses with subsequent release of various proinflammatory cytokines, to induce apoptosis of cardiomyocytes and to promote cardiac dysfunction in sepsis and endotoxemia (7,8). Accordingly, inhibition of MIF proinflammatory activities resulted in an improvement of cardiomyocyte survival and myocardial function (7,8) and MIF was suggested to exacerbate ischemic tissue damage during myocardial ischemia and reperfusion (I/R) injury (9). In contrast, MIF has also been shown to protect the heart from I/R injury by attenuating oxidative stress, activating adenosine monophosphate–activated protein kinases (AMPK) and inhibiting c-Jun N-terminal kinase (JNK)-mediated apoptosis of cardiomyocytes (10,11). Furthermore, MIF has been found to promote neovascularization during hypoxic stress (12) and to confer protection against ox-
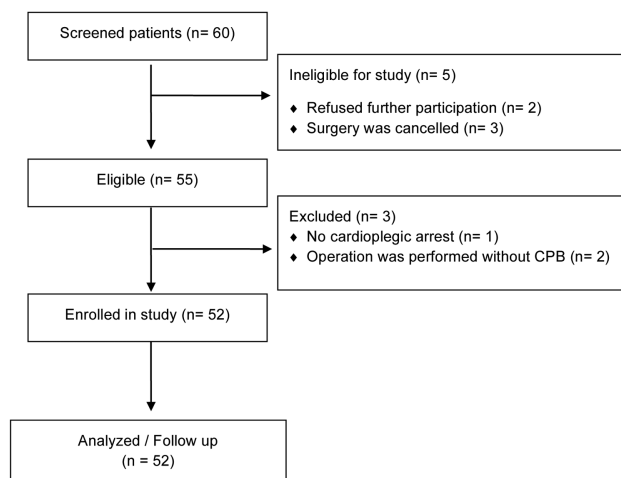
Figure 1. Flowchart according to the STROBE-recommendations (41). From the initially included 55 patients, 3 were excluded from further analysis because of an intraoperative switch of the surgical technique.

idative cell stress occurring during the early phase of reperfusion in myocardial infarction owing to its intrinsic thiol-protein oxidoreductase activity (13,14). These findings suggest an overall cardio-protective role for MIF in the setting of myocardial I/R injury.

Restoration of coronary blood flow after termination of cardioplegic arrest in cardiac surgery with cardiopulmonary bypass (CPB) provokes myocardial I/R injury that contributes to perioperative inflammation and morbidity (15). An early increase of circulating MIF has already been previously demonstrated in patients after cardiac surgery with CPB (16–19). Given the recent evidence suggesting a prominent cardioprotective role for MIF in the setting of myocardial I/R injury, we hypothesized that elevated MIF levels during the early postoperative time course might be associated with less postoperative organ dysfunction in patients undergoing cardiac surgery with the use of cardioplegic arrest and CPB.

MATERIALS AND METHODS

Patients

The present study was designed and performed as a prospective clinical trial at the university hospital of Aachen. The local institutional review board approved this study. After public registration of the study (ClinicalTrials.gov identifier: NCT01412619) and obtainment of written informed consent, 52 patients undergoing elective cardiac surgery with the use of CPB were consecutively enrolled.

Patients with emergency operations, pregnancy, age less than 18 years or failure to give informed consent were excluded from this investigation.

Anesthesia

In all patients, anesthesia was performed according to our institutional routine (oral premedication 45 min prior to patient transfer to the operating room: midazolam 7.5 mg; intravenous induction of anesthesia: etomidate [0.1 mg/kg] and sufentanil [0.5–1 μg/kg]) (20). After administration of rocuronium (1 mg/kg), tracheal intubation was performed. General anesthesia was maintained with sufentanil infusion (0.5–1 μg/kg·per h) in combination with sevoflurane inhalation (0.7–1 volume%).

Cardiopulmonary Bypass

CPB was performed with the patient in moderate hypothermia (28°C–32°C) on a conventional CPB circuit with a pump flow of 2.2 L/min per m². Cardiac arrest was induced by the antegrade infusion of cold crystalloid cardioplegic solution. Prior to CPB, 300 U/kg heparin was administered to achieve an activated clotting time of >400 s. After the patient was weaned from CPB, heparin was antagonized with protamine at a ratio of 1:1.

Hemodynamic Management

Basic fluid substitution was accomplished with 1 mL/kg per h balanced crystalloid solutions. Packed red blood cells were transfused when the hemoglobin content was below 7.5 g/dL. The administration of additional fluids, vasopressors or inotropic drugs was left to the discretion of the attending physicians.

Intensive Care Unit

After completion of surgery, all patients were transferred to the intensive care unit (ICU). Tracheal extubation was performed when standard extubation criteria were fulfilled. After full recovery and completion of our standardized discharge criteria, patients were discharged from the ICU and transferred to standard care units.

Data Collection

Baseline characteristics were assessed and documented prior to surgery. The simplified acute physiology score (SAPS II) was evaluated on the first postoperative day (POD) (21). Subsequently the sequential organ failure assessment (SOFA) score was determined for the daily assessment of organ dysfunction throughout the ICU stay (22). Furthermore, the duration of ventilation and the length of ICU and hospital stays were documented separately.

In 14 patients, a pulmonary artery catheter had been placed intraoperatively as deemed necessary by the attending physician. In these patients, we calculated the cardiac power index (CPI) after induction of anesthesia, after admission to the ICU and at the morning of the first POD using the following formula: CPI = mean arterial pressure × cardiac index/451 (W/m²) (23).

Laboratory Tests

Serum samples for the determination of MIF were taken from the supernatant of blood collected for routine laboratory analyses after induction of anesthesia, 4 h after admission to the ICU and on POD 1 and 2 and subsequently stored at –80°C until further analysis.

We determined the serum levels of MIF according to our institutional standard using an enzyme-linked immunosorbent assay (ELISA) as previously described (24). The human MIF ELISA was performed with capture antibody MAB289 and detection antibody BAF289 from R&D Systems (Wiesbaden, Germany).

To describe the magnitude of MIF release in patients undergoing cardiac surgery, MIF values were compared with those obtained in healthy volunteers (age 29 ± 8 years; males = 18 [60%]), in which no evidence for any cardiovascular diseases was present (25).

Statistical Analysis

All data were statistically analyzed with a commercially available software package (SPSS 19.0; SPSS Inc., Chicago, IL, USA).

As the primary endpoint we evaluated serum MIF concentrations prior to surgery and during the early postoperative course until POD 2.

Secondary endpoints were clinically relevant outcome parameters, that is, SAPS II score on the POD 1; daily SOFA scores; CPI after induction of anesthesia, at ICU admission and at POD 1; duration of mechanical ventilation; and hospital and ICU length of stay. We assessed the degree of respiratory dysfunction using the ratio of partial arterial oxygen tension (PaO_2) over the fraction of inspired oxygen (FiO_2) (Horowitz index).

The Shapiro–Wilk *W* test was used to test all data for normal distribution. We used the Student *t* test to compare normally distributed results of single measurements and the Mann–Whitney *U* test to compare non–normally distributed data.

To describe the time course of perioperative MIF levels, we used analysis of variance (ANOVA) to compare repeated measurements. To compare the time course of perioperative MIF levels between different groups, we statistically analyzed the repeated measurements using a repeated measurement ANOVA to take into account the correlated observations within the groups with the within-factor time and the grouping factor age and sex, respectively. In case of significant results, we used the Bonferroni test to perform *post hoc* testing (26).

The impact of MIF on postoperative organ dysfunction was additionally investigated by computing the area under the curve (AUC) of MIF serum levels from admission until POD 1 (MIF_{AUC}). This type of analysis was performed to approach the dynamic and interindividu- ally different conditions of cytokine release expected to occur in the postoperative period. The true extent of MIF release is probably better quantified by integrating the MIF levels over the observation period, rather than by reporting the blood levels at some arbitrary points in time.

Proportions were compared by use of the χ^2 test. For correlation studies, linear regression analysis with calculation of the Spearman-rho coefficient (for non–normally distributed data) or Pearson coefficient (for normally distributed data) was performed. In all cases, a level of $p < 0.05$ was considered statistically significant.

All supplementary materials are available online at www.molmed.org.

Table 1. Baseline characteristics and data on surgery.[a]

	All patients (n = 52)
Biometric/demographic data	
Age, years	67.6 ± 9.7
Male sex, n (%)	35 (67)
Height, cm	174.5 (159–189)
Weight, kg	75.5 (52–112)
EuroScore	7 (2–11)
Prior or preexisting disease, n (%)	
CAD	38 (73)
Hypertension	41 (79)
Pulmonary hypertension	8 (15)
Chronic pulmonary disease	11 (21)
Chronic kidney disease	3 (6)
Diabetes	7 (14)
Type of surgery, n (%)	
Isolated coronary artery bypass grafting	23 (44)
Isolated valvular surgery	12 (23)
Aortic surgery, n (%)	8 (15)
Combined procedure, n (%)	9 (17)
Duration of surgery, min	
Total time	217 ± 83
Ischemia time	76 ± 33
CPB time	119 ± 58.6
Cross-clamp time	70.5 ± 40.5
Recirculation time	34.2 ± 22.3
Outcome values	
Duration of ventilation, h	11.4 ± 4.3
ICU length of stay, d	1 (1–8)
Hospital length of stay, d	12 (7–52)
Mortality, n (%)	0 (0)

[a]Data are presented as median (range) (non–normally distributed data), as mean ± SD (normally distributed data) or as absolute numbers (% of the whole).
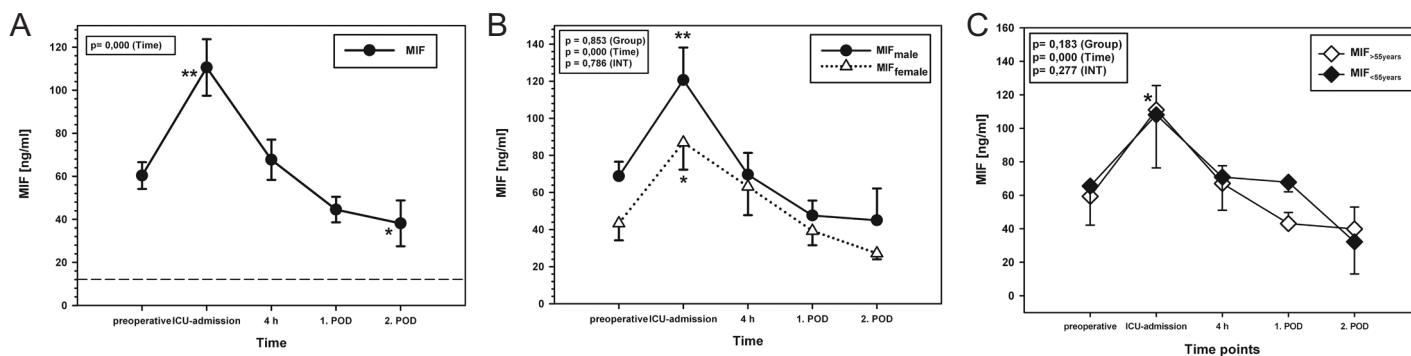
Figure 2. (A) Perioperative time course of serum MIF levels in cardiac surgical patients (n = 52 until first POD). The dashed line indicates the mean value of MIF as obtained in a healthy control group. (B) Comparison of perioperative time course of serum MIF values between male (n = 35) and female (n = 17) patients. (C) Comparison of perioperative time course of serum MIF values between young (age 46 ± 8 years; n = 12) and old patients (age 71 ± 6 years; n = 40). Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$ versus baseline.

RESULTS

Enrolled Patients and Baseline Characteristics

From 60 initially screened patients, a total of 55 consecutive patients were enrolled in this prospective observational monocenter study (Figure 1). From these 55 patients, three patients were excluded from further analysis because the surgical procedure was intraoperatively switched to a beating-heart procedure. Patients were followed during the ICU stay until discharge from the ICU. Baseline characteristics and surgical data of the included patients are presented in Table 1.

Perioperative Time Course of Serum MIF Levels

Prior to surgery, the majority of patients (n = 47) already showed significantly elevated MIF levels in comparison to reference values obtained from the control group of healthy individuals (age 29 ± 8 years; 60% male) (64 ± 49 ng/mL versus 13 ± 17 ng/mL; $p < 0.01$).

MIF values peaked at the end of surgery and then already decreased within 4 h after admission to the ICU. At the beginning of POD 1, MIF levels further decreased and reached values that were significantly lower than at baseline (Figure 2A). To assess sex-specific differences with respect to perioperative MIF release, MIF values were analyzed separately for male and female patients (Figure 2B). Whereas overall ANOVA showed no significant interaction associated with patient sex, graphical depiction of the data indicates a trend to lower MIF levels in the female patient group, at least at baseline and admission to the ICU. In the group of healthy volunteers, we could not detect any sex-specific differences for MIF (female: 14 ± 23 ng/mL; male: 11 ± 9 ng/mL; $p = 0.59$).

Accordingly, we analyzed potential age-specific differences within the enrolled patients (young [< 55years], 46 ± 8 years, n = 12, versus old [>55years], 71 ± 6 years, n = 40). However, we could not detect any significant differences between these cohorts (Figure 2C).

MIF levels at admission to the ICU were significantly correlated with the duration of CPB ($r = 0.305$, $p = 0.044$) and the time interval between the end of cardioplegic arrest and termination of CPB ($r = 0.366$, $p = 0.015$).

To further characterize the time course of MIF release within the perioperative inflammatory response, circulating levels of serum procalcitonin (PCT) and whole blood leukocytes (WBC) were analyzed for comparison and are illustrated in Supplementary Figure S1. Of note, MIF was the first inflammatory parameter to peak. In contrast, PCT and WBC increased only after admission to the ICU, when MIF levels were already decreasing again.

Postoperative Outcome and Organ Dysfunction

Thirty-seven patients were discharged from the ICU at POD 1, whereas 14 patients stayed for a longer time (2 [3–8] d). Different types of organ failure are depicted in Table 2. No patient died throughout the observation period.

Analysis of serum levels of MIF$_{preoperative}$ showed a strong positive correlation with SOFA score on POD 1, whereas the correlation to SAPS II at POD1 failed to reach statistical significance ($r = 0.224$; $p = 0.110$).

MIF peak values (that is, at admission) revealed a significant inverse correlation to the SOFA score on the third POD (Table 3) and the SAPS II on the second and third POD (also see Supplementary Figures S2A, B). In addition, we have included the MIF levels over the early postoperative observation period (MIF$_{AUC}$). We observed that MIF$_{AUC}$ was inversely correlated with the SAPS II and SOFA scores on POD 1 (Figure 3 and Table 3). MIF$_{admission}$ ($r = 0.296$; $p = 0.041$) and MIF$_{4h}$ ($r = 0.367$; $p = 0.012$) correlated inversely with the paO$_2$/FiO$_2$ ratios at POD 1. Moreover, MIF$_{4h}$ was inversely correlated to the SOFA score at POD 2 and 3 (Table 3).

To assess the predictive value of postoperative MIF values for the ICU length of stay, duration of mechanical ventilation and SAPS II or SOFA score through-

out the postoperative period, a receiver-operating characteristic analysis was performed, but failed to show any significant results.

In addition, MIF values on POD 1 were closely related to CPI at this time point (Figure 4), whereas no other correlation was found between any MIF levels and CPI at any further predefined time point.

The time course of cardiomyocyte-specific troponin T was assessed (Figure 5) to show the extent of myocardial damage. The measured troponin T values demonstrated an immediate increase after admission to the ICU that reached peak values at the first POD and again decreased throughout the further observation period. MIF serum values 4 h after admission to the ICU showed a significant inverse correlation with troponin T levels at admission to the ICU and at POD 1 (admission: $r = -0.257$, $p = 0.082$; POD 1: $r = -0.290$, $p = 0.044$). Likewise, MIF and troponin T values at POD 1 were inversely correlated ($r = -0.311$, $p = 0.033$).

DISCUSSION

In the present study we found that early elevation in circulating serum protein levels of MIF are associated with less organ dysfunction as reflected by SAPS II, SOFA score and PaO_2/FiO_2 ratio during the early postoperative course in patients after cardiac surgery. Moreover, CPI on POD 1 was closely related to the circulating MIF values on the same day.

MIF plays a pivotal role in the control of innate and acquired immunity (1–4). Owing to its inflammatory activities, MIF has been reported to promote the progression of atherosclerosis by activating proinflammatory atherogenic pathways (27). In this context, recent evidence suggests a major role of MIF in the development of coronary artery disease (CAD) (28), and in fact, circulating MIF levels were recently found to be elevated in patients with chronic CAD (29). Accordingly, the majority of cardiac surgical patients in the present study already exhibited remarkably increased MIF lev-

Table 2. Different types of postoperative organ failure.[a]

	All patients, POD 1 (n = 52)	Patients in ICU, POD 2 (n = 14)	Patients in ICU, POD 3 (n = 10)
Type of organ dysfunction, n (%)			
Myocardial	4 (8)	0 (0)	1 (1)
Cerebral	16 (31)	6 (42)	6 (60)
Thrombocytopenia	10 (19)	7 (50)	5 (50)
Pulmonary	16 (31)	9 (64)	9 (90)
Shock	3 (6)	2 (14)	1 (10)
Acute kidney injury	0 (0)	0 (0)	0 (0)
Hyperbilirubinemia	5 (10)	2 (14)	0 (0)
Other	8 (15)	1 (7)	0 (0)
Incidence, n (%)			
Systemic inflammatory response syndrome (SIRS)	24 (46)	2 (14)	3 (30)
Sepsis	0 (0)	0 (0)	0 (0)
Septic shock	0 (0)	0 (0)	0 (0)

[a]Data are presented as absolute numbers (% of the whole).

Table 3. Correlations between perioperative MIF serum levels and SOFA score.[a]

MIF serum levels	SOFA POD 1	SOFA POD 2	SOFA POD 3
Preoperative			
Correlation r	**0.327**	0.310	0.078
p	**0.018**	0.281	0.831
n	**52**	14	10
Admission to the ICU			
Correlation r	–0.190	–0.444	**–0.634**
p	0.178	0.111	**0.049**
n	52	14	**10**
4 h after admission to the ICU			
Correlation r	–0.392	**–0.537**	**–0.755**
p	0.401	**0.048**	**0.012**
n	52	**14**	**10**
MIF$_{AUC}$			
Correlation r	**–0.360**	–0.175	–0.446
p	**0.009**	0.551	0.196
n	**52**	14	10
POD 1			
Correlation r	–0.248	**–0.583**	**–0.734**
p	0.077	**0.029**	**0.016**
n	52	**14**	**10**

[a]Bold items were significant ($P < 0.05$).

els prior to surgery compared with healthy volunteers. Given the central role of MIF in proatherogenic signaling and stress-related stimuli, preoperatively elevated MIF levels in patients scheduled for cardiovascular surgery might reflect severity of disease and/or systemic stress response (30). In fact, preoperative MIF serum levels in our patients were

significantly higher than those observed by De Mendonça-Filho *et al.* after induction of anesthesia (16,17). Of note, patients included in the study populations of the latter investigators had a significantly lower preoperative risk profile (as assessed by the EuroScore) and hence severity of disease than the patients analyzed in the present study. Moreover,
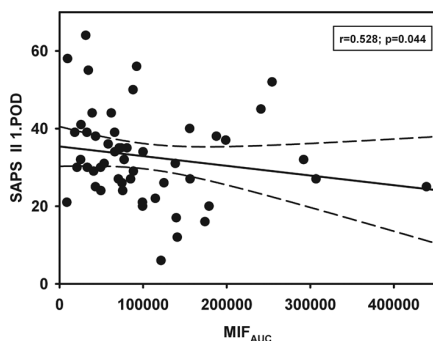
Figure 3. Correlations of MIF$_{AUC}$ with SAPS II score on the first POD. Data are depicted as linear regression (black line) with 95% confidence intervals (long dashed line).

Figure 4. Correlation between MIF values and cardiac power index on the first POD. Insertion of the pulmonary artery catheter was performed intraoperatively as deemed necessary by the attending physician. In these patients (n = 14), the CPI was calculated after induction of anesthesia, after admission to the ICU and at the morning of the first POD with the following formula: CPI = mean arterial pressure × cardiac index/451 (W/m^2) (23). Data are depicted as linear regression (black line) with 95% confidence interval (long dashed line).

Figure 5. Perioperative release of myocardial specific troponin T. **$p < 0.05$ versus baseline.

preoperative MIF levels in our study showed a positive correlation to SAPS II and SOFA score at POD 1, probably predicting a more complicated postoperative course in sicker patients and in those patients requiring more extensive surgery. We acknowledge that preoperative MIF levels in our study population might also have been affected by confounding factors unrelated to the severity of cardiovascular disease. Of note, the control group consisting of healthy volunteers was not age matched. Although we could not detect a significant difference in perioperative MIF release between patients aged <55 years and >55 years, several studies have demonstrated that MIF serum levels increase with age (31). In addition, recent studies revealed higher MIF levels associated with female sex (32). However, the proportion of females was nearly identical in the study group compared with the control group. Moreover, statistical analysis revealed no significant sex-related effect on perioperative MIF release. In any case, the origin and significance of preoperatively elevated MIF values warrant further investigation.

Despite substantial technological advances in the past decades, cardiac surgery with the use of CPB regularly elicits an extensive sepsis-like immune response, which can lead to severe systemic inflammation and postoperative organ dysfunction and multiorgan fail-
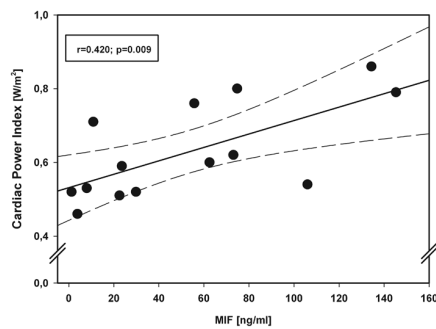
ure (15). Part of the inflammatory response to cardiac surgery is triggered by the reestablishment of coronary blood flow after cardioplegic arrest and hence by global myocardial I/R injury.

The involvement of MIF in the inflammatory response to cardiac surgery has already been investigated in previous studies. Confirming our findings, several investigators reported a significant increase of circulating MIF after cardiac surgery, with MIF release being associated with time of cardioplegic arrest, duration of CPB time and/or time of recirculation on CPB (17,19). Accordingly, we detected a comparable initial elevation of MIF values upon ICU admission, followed by a significant reduction at the first and second POD. The exact mechanism of MIF increase in the perioperative inflammatory reaction still remains speculative. MIF may be released from macrophages, monocytes and T lymphocytes (1) as a response to inflammatory stimuli such as CPB and surgical trauma. Alternatively/additionally, MIF may be liberated from preformed pools in cardiomy-
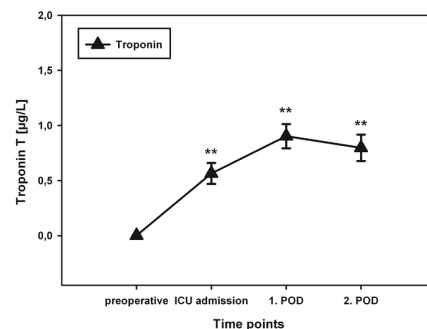
ocytes as a consequence of ischemia and reperfusion injury (9–11). In the present study, the correlation between the duration of CPB and MIF concentrations at admission to the ICU might indicate a potential association between MIF release and the inflammatory stimulus elicited by CPB. At the end of surgery, the cessation of inflammatory stimulation most probably results in a remarkable decrease of MIF release. Moreover, the subsequent reuptake (33) of MIF might also contribute to the significant decrease of circulating MIF concentrations throughout the further observation period. Further studies are warranted to elucidate the corresponding underlying mechanisms and to determine the half-life of circulating MIF, which still is unknown.

Previous studies demonstrated a close relationship between postoperatively elevated MIF levels and the occurrence of postoperative organ dysfunction after cardiac surgery (16,17). These findings were attributed to the plethora of proinflammatory actions exerted by MIF; for an overview, see (1). Binding of MIF to CD74 results in the phosphorylation of the extracellular signal-regulated kinase 1/2 proteins, promoting cell growth. MIF also upregulates the expression of the Toll-like receptor 4 (TLR4) by macrophages. Activation of TLR4 by endotoxin initiates the release of various inflammatory mediators such as cytokines, nitric oxide and arachidonic acid derivatives. Moreover, MIF counteracts immunosuppression ex-

erted by glucocorticoids at transcriptional and posttranscriptional levels.

Interestingly, we could not confirm the association between postoperatively elevated MIF values and the occurrence of postoperative organ dysfunction in patients undergoing cardiac surgery. In contrast, our data indicate a rather beneficial role for MIF, because we observed an inverse correlation between early postoperative elevations of MIF serum levels and various indicators of organ dysfunction at POD 1–3. In particular, MIF was directly correlated with CPI, which in humans was found to be the strongest predictor of survival in patients with cardiogenic shock secondary to left ventricular dysfunction after acute myocardial ischemia (23). Our observations are in line with recent findings from experimental and clinical investigations revealing various protective mechanisms of MIF in the setting of I/R injury and inflammation. First, MIF release has been found to activate AMPK in an autocrine/paracrine manner (35). AMPK exerts cardioprotection by activating anaerobic glycolysis in the ischemic heart, initiating glucose transporter-4 translocation, increasing glucose uptake and limiting myocardial damage and apoptosis (34,36). Second, the release of endogenous MIF from cardiomyocytes has been demonstrated to inhibit the activation of the JNK pathway (37). JNK modulates multiple cellular functions, including proliferation, differentiation and apoptosis and thus promotes myocardial damage after ischemia and reperfusion (37). Accordingly, animal studies with JNK-knockout mouse hearts showed reduced myocardial necrosis and apoptosis in comparison to JNK wild-type mice. Third, via its intrinsic thiol-protein oxidoreductase activity, MIF can modulate cellular redox homeostasis through c-Jun N-terminal activation domain binding protein-1 (JAB-1). MIF/JAB-1 interaction blocks JNK activity, which provides protection against oxidative cell stress, particularly that occurring during the early phase of reperfusion in myocardial ischemia

(4,13,14,38). The potential of MIF to convey cardioprotection in the setting of I/R injury is highlighted by our observations of an inverse relationship between the magnitude of troponin T release and postoperatively assessed MIF levels. In addition, in infectious disease, recently reported findings add to evidence indicating a potentially beneficial role of MIF in certain conditions and time windows. Pollak *et al*. demonstrated an increased susceptibility to bacterial superinfection after MIF neutralization in a murine model of peritonitis (36). Intriguingly, Yende *et al*. observed that MIF gene polymorphism with high MIF expression was associated with a significant reduction in death following community-acquired pneumonia (39).

It is not obvious why, in our cardiac surgical patients, MIF release was associated with a more favorable clinical evolution on POD 1-3, whereas several other studies found MIF elevations to parallel postoperative organ injury (16–19). The reasons for this apparent contrast are speculative and may relate to the geographic origin of included patients (Western Europe, Japan [18], South America [16,17] and North America [19]). In fact, the MIF genotype is probably influenced by geographic origin and is known to significantly affect perioperative release of MIF and the extent of perioperative inflammation (40). Other factors that may have contributed to the contrasting findings include the application of aprotinin in one study (19) and the use of different cardioplegia protocols in all studies.

The present results must be viewed in light of the limitations of a small-size pilot study. From the 60 initially screened patients we could include only 52 patients to consider the surgical procedure as standardized. Moreover, the predefined time points for blood sampling may reflect the intraoperative kinetics of MIF release only inadequately. The possibility cannot be excluded that major changes and peak values of MIF release were obscured by the application of arbitrarily chosen blood sampling intervals that, from a retrospective point of view,

may have been too broad. However, the chosen time intervals correspond with those applied in the available literature (16,17). Last, in our study we were not able to identify/analyze the mechanism underlying the observed protection. Further studies are warranted to elucidate the pleiotropic effects of MIF in clinical settings of myocardial I/R injury.

CONCLUSION

In summary, we conclude that MIF has an ambivalent mode of action, which is apparently dependent on the time pattern of its release. The acute release of MIF in response to myocardial I/R injury may convey protective effects, because in the present study we found the early postoperative elevation of serum MIF levels to be associated with less postoperative organ dysfunction in patients after cardiac surgery.

DISCLOSURE

The authors declare they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

REFERENCES

1. Calandra T, Roger T. (2003) Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat. Rev. Immunol.* 3:791–800.
2. Roger T, David J, Glauser MP, Calandra T. (2001) Mif regulates innate immune responses through modulation of toll-like receptor 4. *Nature*. 414:920–4.

3. Zernecke A, Bernhagen J, Weber C. (2008) Macrophage migration inhibitory factor in cardiovascular disease. *Circulation*. 117:1594–602.

4. Nguyen MT, *et al*. (2003) The cytokine macrophage migration inhibitory factor reduces prooxidative stress-induced apoptosis. *J. Immunol*. 170:3337–47.

5. Morand EF, Leech M, Bernhagen J. (2006) MIF: a new cytokine link between rheumatoid arthritis and atherosclerosis. *Nat. Rev. Drug. Discov*. 5:399–410.

6. Donnelly SC, *et al*. (1997) Regulatory role for macrophage migration inhibitory factor in acute respiratory distress syndrome. *Nat. Med*. 3:320–3.

7. Garner LB, *et al*. (2003) Macrophage migration inhibitory factor is a cardiac-derived myocardial depressant factor. *Am. J. Physiol. Heart Circ. Physiol*. 285:2500–9.

8. Chagnon F, Metz CN, Bucala R, Lesur O. (2005) Endotoxin-induced myocardial dysfunction: effects of macrophage migration inhibitory factor neutralization. *Circ. Res*. 96:1095–102.

9. Gao XM, *et al*. (2011) Deletion of macrophage migration inhibitory factor protects the heart from severe ischemia-reperfusion injury: a predominant role of anti-inflammation. *J. Mol. Cell. Cardiol*. 50:991–9.

10. Miller EJ, *et al*. (2008) Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic heart. *Nature*. 451:578–82.

11. Qi D, *et al*. (2009) Cardiac macrophage migration inhibitory factor inhibits JNK pathway activation and injury during ischemia / reperfusion. *J. Clin. Invest*. 119:3807–16.

12. Amin MA, *et al*. (2003) Migration inhibitory factor mediates angiogenesis via mitogen-activated protein kinase and phosphatidylinositol kinase. *Circ. Res*. 93:321–9.

13. Thiele M, Bernhagen J. (2005) Link between macrophage migration inhibitory factor and cellular redox regulation. *Antioxid. Redox Signal*. 7:1234–48.

14. Koga K, *et al*. (2010) Macrophage migration inhibitory factor provides cardioprotection during ischemia / reperfusion by reducing oxidative stress. *Antioxid. Redox Signal*. 14:1191–202.

15. Wan S, LeClerc JL, Vincent JL. (1997) Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. *Chest*. 112:676–692.

16. De Mendonça-Filho HT, *et al*. (2004) Circulating levels of macrophage migration inhibitory factor are associated with mild pulmonary dysfunction after cardiopulmonary bypass. *Shock*. 22:533–7.

17. De Mendonça-Filho HT, *et al*. (2006) Circulating inflammatory mediators and organ dysfunction after cardiovascular surgery with cardiopulmonary bypass: a prospective observational study. *Crit. Care*. 10:R46.

18. Gando S, *et al*. (2000) An increase in macrophage migration inhibitory factor release in patients with cardiopulmonary bypass surgery. *Surg. Today* 30:689–94.

19. Merchant S, *et al*. (2008) Macrophage migration inhibitory factor in pediatric patients undergoing surgery for congenital heart repair. *Mol. Med*. 14:124–30.

20. Stoppe C, *et al*. (2011) The intraoperative decrease of selenium is associated with the postoperative development of multiorgan dysfunction in cardiac surgical patients. *Crit. Care Med*. 39:1879–85.

21. Le Gall JR, Lemeshow S, Saulnier F. (1993) A new Simplified Acute Physiology Score (SAPS II) based on a European / North American multicenter study. *JAMA*. 270:2957–63.

22. Vincent JL, *et al*. (1996) The SOFA (Sequential Organ Failure Assessment) score to describe organ dysfunction / failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med*. 22:707–10.

23. Fincke R, *et al*. (2004) Cardiac power is the strongest hemodynamic correlate of mortality in cardiogenic shock: a report from the SHOCK trial registry. *J. Am. Coll. Cardiol*. 44:340–8.

24. Flieger O, *et al*. (2003) Regulated secretion of macrophage migration inhibitory factor is mediated by a non-classical pathway involving an ABC transporter. *FEBS Lett*. 551:78–86.

25. Grieb G, *et al*. (2010) Macrophage migration inhibitory factor-A potential diagnostic tool in severe burn injuries? *Burns*. 36:335–42.

26. Ludbrook J. (1998) Multiple comparison procedures updated. *Clin. Exp. Pharmacol. Physiol*. 25:1032–7.

27. Burger-Kentischer A, *et al*. (2002) Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis. *Circulation* 105:1561–6.

28. Boekholdt SM, *et al*. (2004) Macrophage migration inhibitory factor and the risk of myocardial infarction or death due to coronary artery disease in adults without prior myocardial infarction or stroke: the EPIC-Norfolk Prospective Population study. *Am. J. Med*. 117:390–7.

29. Herder C, *et al*. (2008) Macrophage migration inhibitory factor (MIF) and risk for coronary heart disease: results from the MONICA / KORA Augsburg case-cohort study, 1984–2002. *Atherosclerosis*. 200:380–8.

30. Flaster H, Bernhagen J, Calandra T, Bucala R (2007) The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity. *Mol. Endocrinol*. 21:1267–80.

31. Qian F, *et al*. (2012) Age-associated elevation in TLR5 leads to increased inflammatory responses in the elderly. *Aging Cell*. 11:104–10.

32. Hardman MJ, *al*. (2005): Macrophage migration inhibitory factor: a central regulator of wound healing. *Am. J. Pathol*. 167:1561–74.

33. Schwartz V, *et al*. (2011) Role for CD74 and CXCR4 in clathrin-dependent endocytosis of the cytokine MIF. *Eur J. Cell Biol*. 91:435–49.

34. Russell RR 3rd, *et al*. (2004) AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. *J. Clin. Invest*. 114:495–503.

35. Marsin AS, *et al*. (2000) Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. *Curr. Biol*. 10:1247–55.

36. Pollak N, Sterns T, Echtenacher B, Mannel DN. (2005) Improved resistance to bacterial superinfection in mice by treatment with macrophage migration inhibitory factor. *Infect. Immun*. 73:64884–92.

37. Qi D, *et al*. (2009) Cardiac macrophage migration inhibitory factor inhibits JNK pathway activation and injury during ischemia / reperfusion. *J. Clin. Invest*. 119:3807–16.

38. Kleemann R, *et al*. (2000) Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. *Nature*. 408:211–6.

39. Yende S, *et al*. (2009) The influence of macrophage migration inhibitory factor gene polymorphisms on outcome from community-acquired pneumonia. *FASEB J*. 8:2403–11.

40. Zhong XB, *et al*. (2005) Simultaneous detection of microsatellite repeats and SNPs in the macrophage migration inhibitory factor (MIF) gene by thin-film biosensor chips and application to rural field studies. *Nucleic Acids Res*. 33:121.

41. von Elm E, *et al*. (2007) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*. 370:1453–7.