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ORIGINAL RESEARCH COMMUNICATION

Interaction of MIF Family Proteins in Myocardial Ischemia/Reperfusion Damage and Their Influence on Clinical Outcome of Cardiac Surgery Patients

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Abstract

Aims: Cardiac surgery involves myocardial ischemia/reperfusion (I/R) with potentially deleterious consequences. Macrophage migration inhibitory factor (MIF) is a stress-regulating chemokine-like cytokine that protects against I/R damage, but functional links with its homolog, D-dopachrome tautomerase (MIF-2), and the circulating soluble receptor CD74 (sCD74) are unknown. In this study, we investigate the role of MIF, MIF-2, sCD74, and MIF genotypes in patients scheduled for elective single or complex surgical procedures such as coronary artery bypass grafting or valve replacement. ***Results:*** MIF and MIF-2 levels significantly increased intraoperatively, whereas measured sCD74 decreased correspondingly. Circulating sCD74/MIF complexes were detectable in 50% of patients and enhanced MIF antioxidant activity. Intraoperative MIF levels were independently associated with a reduced risk for the development of atrial fibrillation (AF) (odds ratio 0.99 [0.98–1.00]; $p = 0.007$). Circulating levels of MIF-2, but not MIF, were associated with an increased frequency of organ dysfunction and predicted the occurrence of AF (area under the curve [AUC] = 0.663; $p = 0.041$) and pneumonia (AUC = 0.708; $p = 0.040$). Patients with a high-expression *MIF* genotype exhibited a reduced incidence of organ dysfunction compared with patients with low-expression *MIF* genotypes (3 *vs.* 25; $p = 0.042$). ***Innovation:*** The current study comprehensively highlights the kinetics and clinical relevance of MIF family proteins and the *MIF* genotype in cardiac surgery patients. ***Conclusion:*** Our findings suggest that increased MIF levels during cardiac surgery feature organ-protective properties during myocardial I/R, while the soluble MIF receptor, sCD74, may enhance MIF antioxidant activity. In contrast, high MIF-2 levels are predictive of the development of organ dysfunction. Importantly, we provide first evidence for a gene–phenotype relationship between variant *MIF* alleles and clinical outcome in cardiac surgery patients. *Antioxid. Redox Signal.* 23, 865–879.

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Innovation

As patients undergoing cardiac surgery are exposed to myocardial ischemia/reperfusion (I/R) that frequently triggers a systemic inflammatory response with development of organ dysfunction, the present study highlights the first comprehensive description of the kinetics and clinical relevance of macrophage migration inhibitory factor (MIF) family proteins, MIF, D-dopachrome tautomerase (MIF-2), and sCD74, including the *MIF* genotype, in the clinical setting of cardiac surgery. Elevated MIF levels during myocardial reperfusion were independently associated with a significantly reduced risk for the occurrence of atrial fibrillation (AF) in the postoperative period. In contrast, we revealed a link between elevated MIF-2 levels during surgery and the development of AF. Patients with high-expression *MIF* genotype showed a significantly reduced incidence of postoperative complications, which could render the assessment of this genotype useful in the preoperative risk stratification of patients exposed to myocardial I/R.

Introduction

Coronary heart disease is the leading cause of death worldwide with coronary artery bypass grafting as the preferred intervention for revascularization in patients with high-risk coronary lesions (7). Unfortunately, open-heart surgery remains associated with severe complications such as organ failure and death, especially in older patients with comorbidities such as diabetes and chronic renal insufficiency. A perioperative inflammatory response to myocardial ischemia and reperfusion (I/R) is considered a major contributor to cardiac surgery-associated complications.

Macrophage migration inhibitory factor (MIF) is an upstream regulator of the innate and adaptive immune response that is widely expressed and that exhibits chemokine-like activities. Numerous studies have shown MIF to play a role in the pathogenesis of inflammatory diseases, such as atherosclerosis, rheumatoid arthritis, sepsis, asthma, and acute respiratory distress syndrome (9). However, emerging evidence indicates that MIF is expressed by the ischemic myocardium and that it may exhibit an overall cardioprotective role during I/R injury (13, 23, 29, 36). Several mechanisms may contribute to cardioprotection by MIF. Autocrine/paracrine MIF activity triggers the CD74/CD44/AMPK receptor signaling pathway, resulting in an increased rate of glucose uptake in cardiomyocytes (19, 23). MIF also fosters myocardial protection by inhibition of c-Jun N-terminal kinase (JNK)-mediated cardiomyocyte apoptosis. Moreover, cardioprotection is mediated by the MIF intrinsic redox activity that is further enhanced during ischemia/reperfusion injury by *S*-nitrosylation (13, 18, 38, 40).

While both experimental and clinical studies suggest that MIF may be a protective factor in the injured myocardium, little is known about the recently characterized MIF family protein member, D-dopachrome tautomerase (MIF-2), and the circulating soluble form of the MIF receptor CD74 ectodomain (sCD74), which was recently demonstrated to have MIF-neutralizing activity relevant to autoimmunity (2). Interestingly, we lately observed that MIF-2 has MIF-like functions in septic conditions (22) and provides protective effects during myocardial ischemia/reperfusion in an experimental study (28, 37). Nevertheless, the clinical significance of the interplay between MIF, its close homolog MIF-2, and the soluble form of their common receptor, CD74, is largely unknown.

Increased repeat numbers of the *MIF* promoter microsatellite (−794 $CATT_{5–8}$, *rs5844572*) and a nearby single-nucleotide polymorphism (SNP) (−173 G/C, *rs755622*) are associated with higher *MIF* expression, resulting in higher circulating MIF concentrations in humans. Emerging evidence also indicates significant associations between the *MIF* genotype and outcome from inflammatory diseases (2, 44).

Recently, we have reported that MIF may have cardio- and nephroprotective properties in cardiac surgical patients (35). Interestingly, only those patients who underwent conventional cardiac surgery with the use of cardiopulmonary bypass (CPB) and cardioplegic arrest-induced I/R exhibited a postoperative increase in MIF compared with a patient group that underwent off-pump cardiac surgery. Both groups showed an increase in interleukin-6 (IL-6), but MIF release appeared to be primarily mediated by CBP-associated I/R stress and, to a lesser extent, by perioperative inflammation (35).

In this study, we aimed to assess the potential importance of the entire MIF ligand/receptor family in patients undergoing cardiac surgery and to characterize the underlying molecular events. The functional role of MIF, MIF-2, and sCD74, as well as the importance of the *MIF* genotype, was studied in a cohort of 100 cardiac surgical patients and their impact on the occurrence of postoperative organ dysfunction was evaluated.

Results

Patients

From 120 patients initially screened for eligibility, 100 were included and followed until the final analysis (Fig. 1). Baseline characteristics of the patients are shown in Supplementary Table S1; Supplementary Data are available online at

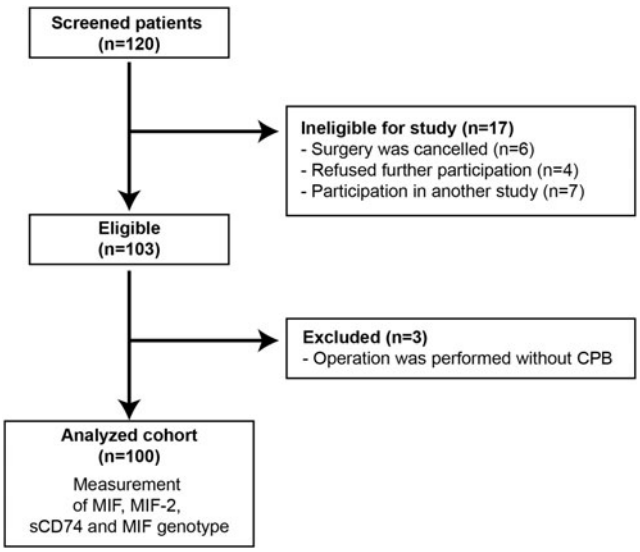


FIG. 1. Flowchart. Flowchart according to the Strobe recommendations for observational clinical trials.

www.liebertpub.com/ars. The included patients reflect a representative cohort of cardiac surgical patients from our center with typical comorbidities and comedication (7).

Serum levels of MIF, MIF-2, sCD74, and MIF/sCD74 complexes

To identify potential intraoperative triggers for MIF secretion, we measured circulating MIF levels in the serum samples of the patients at several predefined time intervals. The majority of the patients (68%) already showed significantly elevated baseline values of MIF before surgery in comparison with a group of sex- and age-matched healthy volunteers not undergoing any type of surgery (53 ± 57 *vs.* 5 ± 4 ng/ml; $p = 0.001$). This likely mirrors the underlying cardiac disease of the cardiac surgical patients in contrast to healthy volunteers.

MIF serum levels showed a significant intraoperative increase that was most pronounced during myocardial reperfusion. Serum levels of MIF showed a peak at 15 min after weaning from CPB, which was followed by a decrease to levels below baseline ($p < 0.001$, Fig. 2A). MIF shows unique characteristics regarding its rapid release profile in response to pathogenic stimuli (*e.g.*, hypoxia, infection, or inflammation) from several cell types, including T cells, macrophages, endothelial cells, thrombocytes, and cardiomyocytes, which are due to its storage in preformed intracellular pools. In particular, the latter characteristic of MIF significantly differs from other proinflammatory cytokines that first need to be transcriptionally activated and translated before secretion (7). In the past, various studies have characterized perioperative inflammation using classical markers of inflammation and myocardial damage (1, 8, 27). Accordingly, we here focused on the pre- and postoperative levels of markers such as procalcitonin (PCT), which did not rise until late after intensive care unit (ICU) admission. These findings may indicate that the release of MIF was mainly due to myocardial ischemia or to the earliest initiation phase of the inflammatory response. In contrast, the cardiomyocyte injury markers, CK-MB and troponin T, were found to be elevated 1 h after ICU admission and further increased over the following 24 h. Thus, MIF release during cardiac surgery does not simply reflect cardiomyocyte cell death (Supplementary Fig. S1).

We compared the serum level profile of MIF to that of its recently identified homolog, MIF-2 (22). Measured MIF-2 levels were fivefold lower than those of MIF. Interestingly, the time profile of circulating MIF-2 levels was similar to that of MIF, with both homologs showing maximal serum levels after myocardial reperfusion (Fig. 2A, B).

Given that many of the patients had received various commonly used cardiovascular medications, we next performed *in vitro* experiments to test the potential influence of some of these medications on the secretion of MIF in macrophages and cardiomyocytes. While macrophages did not show any significant MIF release upon stimulation (Fig. 2C), protamine, epinephrine, or norepinephrine triggered a significant release of MIF from cardiomyocytes (Fig. 2D). As inotropic medications (epinephrine and norepinephrine) are frequently used in patients after surgery with complex surgical procedures, this should be considered as a relevant and potentially confounding factor contributing to the postoperative release of MIF in patients with a complicated intra- and postoperative course.

A circulating soluble form of the MIF receptor CD74 (sCD74) has recently been characterized in patients with autoimmune liver disease and shown to modulate MIF signal transduction activity (2). In our current cardiac surgery study, soluble CD74 was detectable in the majority (90%) of the enrolled patients. The presence of sCD74 ($sCD74^+$) *versus* absence of sCD74 ($sCD74^-$) was not related to gender, age, or pre-existing diseases. In the $sCD74^+$ patients, sCD74 levels were slightly elevated *before* surgery when compared with a group of age- and sex-matched healthy volunteers; however, this difference did not reach statistical significance (52 ± 142 *vs.* 36 ± 41 ng/ml; $p = 0.342$).

In contrast to the profiles observed for circulating MIF and MIF-2, patient sCD74 serum levels showed a different behavior (Fig. 2E). In comparison with baseline values, circulating sCD74 levels decreased significantly upon myocardial reperfusion ($p = 0.013$), followed by an intermittent increase 15 min after weaning from CPB and another long-lasting decrease after termination of surgery ($p = 0.001$). These data indicated that sCD74 levels behaved—at least in part—inversely to those of MIF and MIF-2. Whereas the intraoperative serum levels of MIF correlated with the time of myocardial ischemia ($r = 0.355$; $p = 0.037$), no significant association was detected for the serum levels of MIF-2 and sCD74. Given the antidromic kinetics of MIF and sCD74 serum concentrations across the course of the cardiac surgical procedure, we considered the possibility that elevated MIF levels might act to obscure the detection of sCD74 in serum. However, ectopic addition of recombinant MIF to selected serum specimens in an *in vitro* experiment did not influence the detection of sCD74 (Supplementary Fig. S2A), verifying that MIF protein does not interfere with the sCD74 enzyme-linked immunosorbent assay (ELISA).

Various studies have demonstrated that myocardial I/R can lead to the release and alter the activity of different cathepsin isoforms, some of which are known to cleave the cytosolic tail of CD74 once MHC II/CD74 complexes have reached the endolysosomal compartment (33). Since cardiac surgery is frequently associated with cell injury and apoptosis, cathepsin S might as well contribute to perioperative increases in circulating sCD74 levels.

We measured the circulating levels of cathepsin S in our patient serum samples and detected a significant intraoperative increase that corresponded to the interim intraoperative increase of sCD74 after reperfusion (Supplementary Fig. S2B). Further studies are needed to mechanistically investigate the potential interaction between sCD74 and other mediators, including cathepsin, in the serum of cardiac surgery patients.

The CD74 ectodomain binds to MIF with nanomolar affinity (17). We thus hypothesized that stable circulating sCD74/MIF complexes may be detectable in patient serum. Utilizing a novel ELISA employing an anti-MIF capture combined with an anti-CD74 detection antibody, we calibrated serum immunoreactivity against a recombinant sCD74/MIF fusion protein. Circulating sCD74/MIF complexes were detected in $\sim 50\%$ of the studied patients. Interestingly, the concentration of the complexes did not change significantly over the observation period as indicated by an essentially unchanged ratio of sCD74/MIF from preoperative sampling to 12 h after admission to the ICU (Fig. 2F).

Significance of perioperative MIF and MIF-2 levels on postoperative outcome and underlying mechanisms

Next, we evaluated the correlation between MIF and MIF-2 levels and perioperative organ dysfunction. Peak MIF release in the reperfusion phase inversely correlated with the severity of organ dysfunction at postoperative day (POD)1 as assessed by the well-established Sequential Organ Failure Assessment (SOFA) scores ($r = -0.307$; $p = 0.006$). Moreover, we found an inverse correlation between the maximal intraoperative MIF release and postoperative serum creatinine levels ($r = -0.224$; $p = 0.048$). By contrast, intraoperative MIF-2 levels inversely varied with MIF and positively correlated with the MODS at POD1 ($r = 0.239$; $p = 0.017$). In addition, there was a positive correlation between intraoperative MIF-2 levels and postoperative serum levels of creatinine 24 h after surgery ($r = 0.416$; $p = 0.005$).

As pre-existing chronic kidney disease (CKD) may additionally influence the perioperative release of MIF, MIF-2, and sCD74, as well as the levels of sCD74/MIF complexes, we evaluated its relationship to the perioperative profiles of the MIF family proteins. Patients with CKD (representing 14% of our cohort) showed reduced perioperative MIF release, which was significantly lower at the time interval of myocardial reperfusion when compared with the MIF levels of all patients undergoing surgery (Fig. 2G).

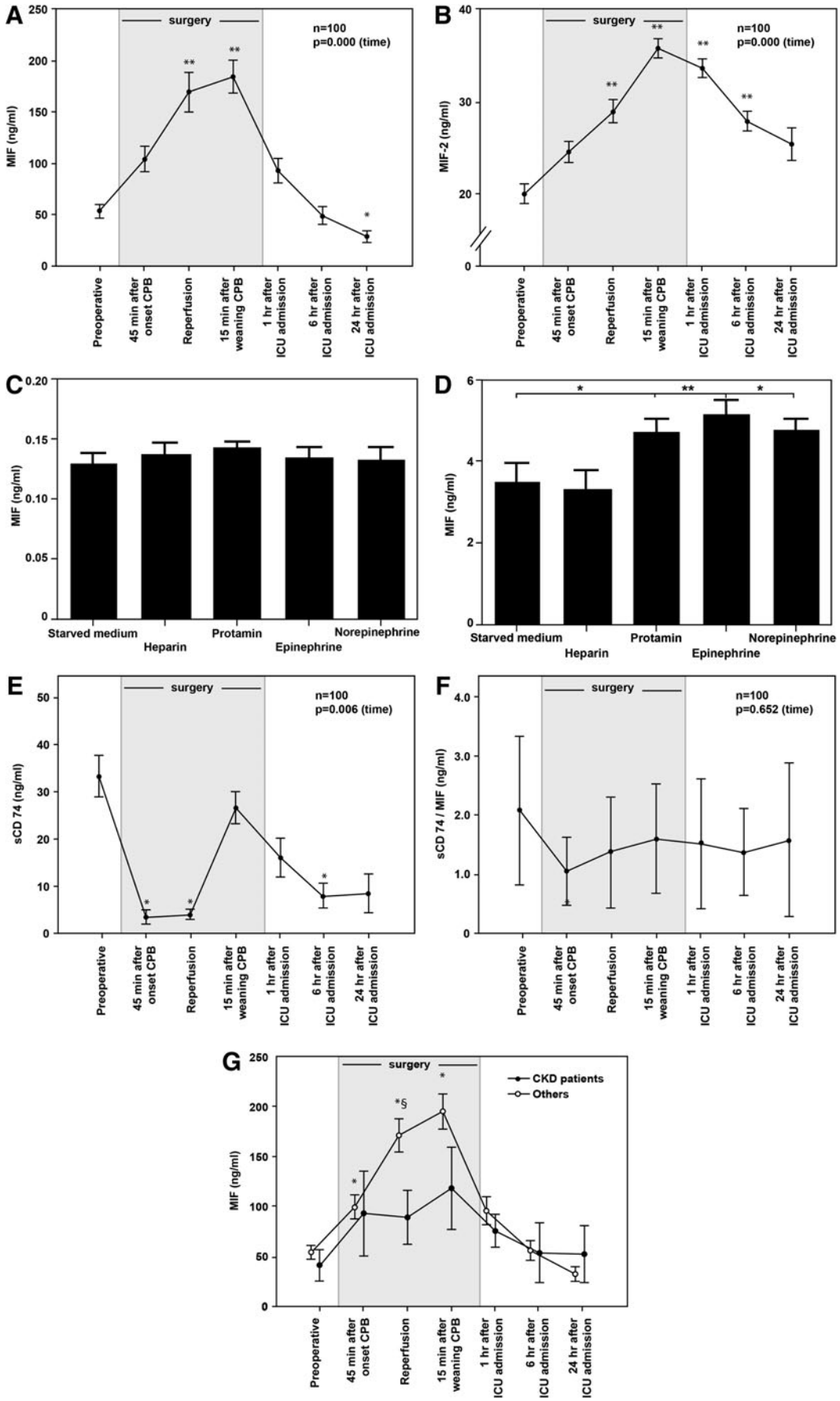
Importantly, intraoperative MIF values (at reperfusion) had a predictive value for freedom from postoperative atrial fibrillation (AF) (Fig. 3A). In contrast, intraoperative MIF-2 levels were predictive for development of AF (Fig. 3B) and were associated with an increased incidence of pneumonia (Fig. 3C). Moreover, MIF-2 levels showed a trend toward the prediction of acute kidney injury (AKI) area under the curve (AUC = 0.685; $p = 0.085$). These data were further supported by a multivariate analysis, which evaluated the effect of MIF and MIF-2 levels (measured at myocardial reperfusion) on the occurrence of AF. Patients' age, comedications (β-blocker, ACE inhibitors, and statins), and pre-existing diabetes mellitus were considered as potential confounding variables. Intraoperative MIF levels during myocardial reperfusion were independently associated with a reduced risk for the development of AF in the postoperative period (odds ratio 0.99 [0.98–1.00]; $p = 0.007$). In contrast, the data also indicated that high intraoperative MIF-2 levels (measured at reperfusion) were independently associated with the postoperative development of AF; however, the MIF-2 effect did not reach statistical significance (OR 1.03 [0.99–1.06]; $p = 0.131$). In summary, the MIF homologs, MIF and MIF-2, show a strikingly opposing behavior regarding the development of AF in the postoperative course.

To begin to investigate the underlying reasons for MIF-2-induced deleterious effects, we studied leukocyte migration behavior toward patient serum samples taken at the time point of maximal MIF and MIF-2 release after myocardial ischemia. According to the observed peaks of the MIF proteins during cardiac surgery and the known kinetics of leukocyte subset recruitment into the injured heart, peripheral blood monocytes (PBMCs), representing mid-acutely recruited inflammatory cells, were used to study the contribution of MIF proteins. Applying blocking antibodies against MIF or MIF-2, this analysis showed that MIF-2 contributed to the prochemotactic activity of perioperative patient serum on PBMC migration, an effect that appeared to be more pronounced compared with MIF (reduction in chemotactic index: from 11.3 [serum] to 3.6 [αMIF-2] *vs.* 8.7 for [αMIF]; αMIF *vs.* αMIF-2: $p = 0.057$; Fig. 3D). For comparison, further chemotaxis analyses were performed on acutely recruited inflammatory cells, that is, neutrophils, using blocking antibodies against prototypic neutrophil chemokines (Supplementary Fig. S3A). Expectedly, a significant inhibitory effect of anti-IL8/CXCL8-blocking antibodies on the migration of neutrophils was seen, while anti-MIP-1α had no effect. In addition, serum samples from cardiac surgical patients, perioperatively drawn across the entire time course, were probed for their prochemotactic potency (Supplementary Fig. S3B). While the significant reductions of serum-derived promigratory activity by the anti-MIF and anti-MIF-2 antibodies indicated that both MIF proteins contribute to monocyte recruitment into the myocardium and/or other organs during myocardial infarction, thereby enhancing regional inflammation, the analysis also revealed differences between MIF and MIF-2 that might point toward a differential role for the homologs in the recruitment of anti- *versus* proinflammatory monocytes, respectively, a notion that should require further attention in the future.

Significance of sCD74/MIF complexes on clinical outcome

Since the clinical significance of circulating sCD74/MIF complexes is unknown, we compared the clinical characteristics of patients with and without detectable sCD74 and sCD74/MIF complexes. We found a highly significant inverse correlation between the intraoperatively measured sCD74 levels and the duration of ICU stay ($r = -0.616$; $p = 0.001$; 45 min after CPB initiation). Furthermore, we

FIG. 2. Perioperative serum levels of MIF, MIF-2, sCD74, and sCD74/MIF complexes. (A, B): Comparison of the perioperative time courses of MIF **(A)** and MIF-2 **(B)** in the serum of patients that underwent cardiac surgery with the use of CPB. **(C, D)** Evaluation of MIF secretion profile from macrophages **(C)** and cardiomyocytes **(D)** after stimulation with commonly used medications. Isolated macrophages (80,000 cells/well) were incubated for 1 h on starved medium. Isolated cardiomyocytes from rats (200,000 cells/well) were incubated for 1 h on starved medium. Afterward, cells were stimulated with equimolar concentrations of protamine (300I. E./kgKG $\cong$ 200 μl), heparin (300I. E./kgKG $\cong$ 200 μl), epinephrine (100 μM), and norepinephrine (100 μM) for 90 min. **(E, F)** Time course of perioperative serum levels of sCD74 **(E)** and sCD74/MIF complex **(F)** in cardiac surgical patients. **(G)** Comparison of perioperative MIF levels between patients with CKD *versus* patients with no CKD. Differences between time points were calculated by use of one-way ANOVA. The differences between CKD patients were compared at single time points by using Student's *t*-test. Data represent mean ± SEM. The *gray* shaded area indicates duration of cardiac surgery. $^{*}p < 0.05$, $^{**}p < 0.01$ *versus* baseline. $^{\S}p < 0.05$ *versus* others at the corresponding time point (difference between groups). ANOVA, analysis of variance; CKD, chronic kidney disease; CPB, cardiopulmonary bypass; MIF, macrophage migration inhibitory factor; MIF-2, D-dopachrome tautomerase.


A
surgery
n=100
p=0.000 (time)
MIF (ng/ml)
B
surgery
n=100
p=0.000 (time)
MIF-2 (ng/ml)
Preoperative
45 min after onset CPB
Reperfusion
15 min after weaning CPB
1 hr after ICU admission
6 hr after ICU admission
24 hr after ICU admission
C
MIF (ng/ml)
Starved medium
Heparin
Protamin
Epinephrine
Norepinephrine
D
MIF (ng/ml)
E
surgery
n=100
p=0.006 (time)
sCD 74 (ng/ml)
F
surgery
n=100
p=0.652 (time)
sCD 74 / MIF (ng/ml)
G
surgery
CKD patients
Others
MIF (ng/ml)

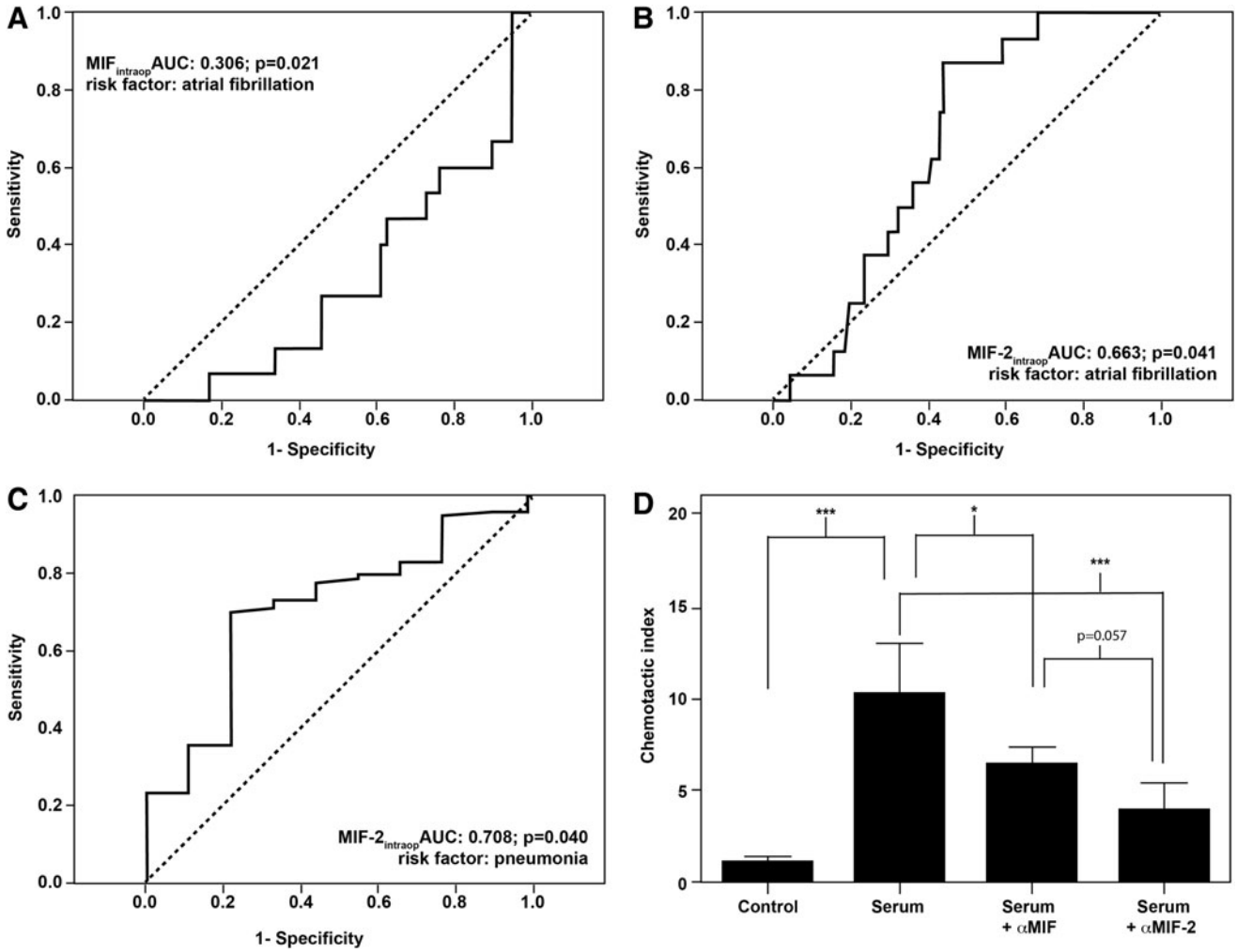


FIG. 3. Predictive value of intraoperative MIF and MIF-2 levels for the detection of postoperative AF and pneumonia. Receiver operating characteristic analysis: High intraoperative MIF levels show a predictive accuracy for freedom of AF **(A)**. In contrast, intraoperative MIF-2 levels show accuracy for the prediction of AF **(B)** and pneumonia **(C)** and thus demonstrate an oppositional behavior compared with MIF levels. **(D)** Migration experiments of PBMCs were performed in a Transwell chamber with diluted patient sera serving as chemoattractant. To assess the contribution of migration, sera at the time point of highest MIF or MIF-2 secretion were preincubated with blocking antibodies (100-fold molar excess) against MIF (120 μg/ml) and MIF-2 (3.5 μg/ml) for 30 min before migration. These concentrations were related to the measured concentrations of circulating MIF *versus* MIF-2, which differed. $*p < 0.05$, $**p < 0.01$ *versus* control and $***p < 0.0001$ *versus* control. Data represent mean ± SEM. AF, atrial fibrillation; AUC, area under the receiver operating curve; PBMC, peripheral blood monocyte.

observed a significantly reduced incidence of postoperative AKI in patients who had detectable sCD74/MIF complexes (Table 1). Last, we detected a reduced incidence of infectious complications in patients with detectable sCD74/MIF complexes (Table 1). These data may indicate a potential relevance for circulating sCD74 and sCD74/MIF complexes in recovery from elective cardiac surgery.

Circulating MIF may not only exert its functions by extracellular signal transduction (5) but also by an intrinsic antioxidant property (12, 13, 18, 40). These antioxidant effects of MIF are related to its thiol–protein oxidoreductase (TPOR) activity (11, 16, 35, 37). To this end, we recently showed that the MIF favorable property in the ischemic heart is further enhanced by *S*-nitrosylation of one of its cysteine residues during myocardial I/R (16). In the present study, we tested the effect of sCD74/MIF complex formation on MIF redox activity in the established 2-hydroxyethyl disulfide (HED) transhydrogenase activity assay. Soluble CD74/MIF complex formation markedly increased the redox activity of MIF in this assay (Fig. 4A). Moreover, while the survival of cardiomyocytes after exposure to peroxide-induced oxidative stress was already increased after the addition of recombinant MIF alone, this cellular antioxidant effect of MIF was further enhanced upon complexation of MIF with sCD74 (Fig. 4B). To confirm these findings, we evaluated the effect of sCD74/MIF complexes on cellular redox stress levels, that is, GSSG/GSH ratios, and confirmed that the extent of oxidative stress in peroxide-stressed cardiomyocytes is lowest in cells treated with MIF and sCD74 (Fig. 4C). Together, these findings insinuate that sCD74 may alter the conformational and functional properties of MIF to improve its intrinsic redox-protective effect.

Table 1. Perioperative Outcome in Patients Without and With Detection of sCD74/MIF Complexes During the Observation Period

	All patients (n = *100*)	*sCD74/MIF⁻* (n = *50*)	*sCD74/MIF⁺* (n = *50*)	p-*Value*
Type of postoperative complication and organ dysfunction				
Delirium, *n*	17 (17)	9 (18)	8 (16)	0.500
Acute kidney injury, *n*	8 (8)	7 (14)	1 (2)	**0.030**
Pneumonia, *n*	9 (9)	5 (10)	4 (8)	0.500
Stroke, *n*	2 (2)	1 (2)	1 (2)	0.753
Sepsis, *n*	4 (4)	2 (4)	2 (4)	0.691
Septic shock, *n*	3 (3)	2 (4)	1 (2)	0.500
Atrial fibrillation, *n*	20 (20)	9 (18)	11 (22)	0.402
Resternotomy (bleeding), *n*	3 (3)	2 (4)	1 (2)	0.558
Death, *n*	5 (5)	3 (6)	2 (4)	0.500
Composite outcome				
Infectious complications, *n*	7 (7)	4 (8)	3 (6)	0.500
Occurrence of organ dysfunction, *n*	29 (29)	19 (38)	10 (20)	**0.038**
Intraoperative data				
Duration of CPB, (min)	120 ± 50	119 ± 49	122 ± 52	0.723
Aortic cross-clamp (myocardial ischemia), (min)	80 ± 36	78 ± 32	82 ± 41	0.588

Data are presented as mean ± SD or as absolute numbers (with the percentage (%) of the whole). Significant values are bold.
CPB, cardiopulmonary bypass; MIF, macrophage migration inhibitory factor.

Significance of MIF gene polymorphisms

Previous studies have shown cellular MIF expression and protein production to correlate with the number of CATT repeats present within an MIF promoter microsatellite sequence (−794 $CATT_{5-8}$). High MIF expression also frequently correlates with the presence of a −173C SNP, which is in linkage disequilibrium with $CATT_{7X}$ (31). Given the previously shown clinical significance of *MIF* gene polymorphisms in various inflammatory diseases (10), we hypothesized that the *MIF* polymorphism may also influence MIF production during myocardial I/R in the setting of cardiac surgery. Accordingly, all patients were genotyped at the −794 $CATT_{5-8}$ and the −173 G/C loci. The distribution of alleles is summarized in Supplementary Table S2.

As expected, we observed a trend toward higher baseline serum levels of MIF before surgery in patients carrying the high-expression *MIF* genotype ($CATT_{7X}$) compared with other genotypes (77 ± 51 *vs.* 52 ± 55 ng/ml; $p = 0.080$) and with patients with the low-expression *MIF* genotype (Fig. 5A). To probe the impact of the *MIF* genotype on perioperative MIF release, we additionally computed the AUC of MIF serum levels from preoperative to ICU admission and other time intervals of interest (Fig. 5B). The AUC analysis revealed a significantly higher intraoperative MIF release (preoperative to ICU admission) in patients with the $CATT_{7X}$ genotype (Fig. 5B). Inversely, the low-expression *MIF* genotype ($CATT_{55}$) was associated with reduced MIF levels, although this difference was not statistically significant within the observation period. These data support the conclusion of an association between the *MIF* genotype and circulating serum levels of MIF in the setting of cardiac surgery.

We additionally examined whether commonly used medications such as metoprolol, a β-adrenergic receptor blocker, may affect circulating MIF levels in cardiac surgery patients. This was done by evaluation of the MIF release profile with respect to comedication of β-blockers. We observed that β-blockers had no influence on the MIF levels in general. However, the MIF release curve in the patient group taking β-blockers showed a delayed increase and MIF levels were significantly lower 45 min after onset of CPB compared with the group that was not treated with β-blockers (Fig. 5C). Notably, a majority of patients (85%) with long-term β-blocker usage had a low-expression *MIF* genotype group. To further investigate this observation, we performed mechanistic studies in a cardiomyocyte cell line treated by hypoxia in the presence *versus* absence of metoprolol. We noticed that a transient stimulation of β_1-receptors by epinephrine affects MIF secretion in cardiomyocytes (Supplementary Fig. S4). Interestingly, metoprolol significantly reduced MIF secretion (Fig. 5D) induced by hypoxia, which represents one major stimulus for MIF secretion during cardiac surgery (9, 38). These data provide a potential explanation for the observed delayed MIF secretion in patients treated with β-blocker.

To characterize the impact of the *MIF* genotype on the development of postoperative organ dysfunction, we evaluated its significance on clinical outcome data of the enrolled patients (Table 2). Of note, we observed a significantly higher incidence of AKI in patients carrying the low-expression *MIF* genotype, $CATT_{55}$, when compared with all other patients ($CATT_{XX}$; chi-square: 33 *vs.* 6%; $p = 0.018$) (Table 2 and Supplementary Table S3). In contrast, patients with a high-expression *MIF* genotype ($CATT_{7X}$) showed a trend toward a reduced incidence of postoperative complications ($p = 0.068$) as assessed by a composite outcome, encompassing the occurrence of any organ dysfunction and/or death (Table 2 and Supplementary Table S4). These data were further strengthened by comparison of patients with the GC SNP *versus* all others that indicated a trend toward reduced postoperative complications (Table 2 and Supplementary Table S5). Importantly, patients with the MIF haplotype, −794 $CATT_{7X}$

plus −173 C, showed a significantly reduced incidence of postoperative complications (Table 2 and Supplementary Table S6). The odds ratio for freedom from postoperative complications was 9.00 for the high *MIF* expression genotype, $CATT_{7X}$, but this observation should be considered cautiously given the small patient cohort and the wide 95% confidence interval (1.14–71.04). No additional information was obtained when comparing the allele sum.

Discussion

The present study provides the first description of the kinetics and potential clinical relevance of the MIF ligand/receptor family *in toto*, as well as the *MIF* genotype in myocardial I/R in humans. Our findings of increased MIF expression in the serum of cardiac surgical patients are consistent with prior experimental data that MIF may offer cardioprotection during myocardial I/R (36). The interaction of MIF with its soluble receptor, sCD74, which circulates at increased levels in a subgroup of patients, may enhance MIF protective redox properties. We additionally obtained first evidence that high-expression *MIF* genotypes are associated with a reduced incidence of postoperative organ dysfunction, an observation that is consistent with an overall tissue-protective effect of MIF. In contrast, circulating MIF-2 levels appear to correlate with postoperative complications and end-organ injury, suggesting a different expression pattern functional profile for this MIF family member.

Open-heart surgery is performed annually in ~1 million patients worldwide and remains associated with appreciable morbidity and mortality rates (7, 11). While reperfusion of the myocardium by surgical revascularization, in particular, after terminating cardioplegic arrest, causes direct injury to the ischemic heart, I/R also elicits a systemic inflammatory response that can propagate the initial noxious stimulus to organs primarily not affected by ischemia, including lungs, brain, kidneys, and intestine (39). It is therefore important to characterize the molecular events and mediators that drive this inflammatory response, and vice versa, to identify protective factors that could provide improved therapeutic options.

We and others have recently gathered evidence for a protective role of the pleiotropic chemokine-like innate cytokine MIF in myocardial I/R injury (13, 18, 23, 29, 36, 38). In ischemic cardiomyocytes, MIF activates the CD74/AMP kinase pathway, resulting in an increase of glucose uptake *via* the translocation of GLUT4 (19, 23). Furthermore, MIF protects the myocardium by inhibition of JNK-mediated cardiomyocyte apoptosis and through its antioxidant activity that is enhanced by *S*-nitrosylation (13, 18, 23, 30, 38). While the cardioprotective role of MIF is thus well established in

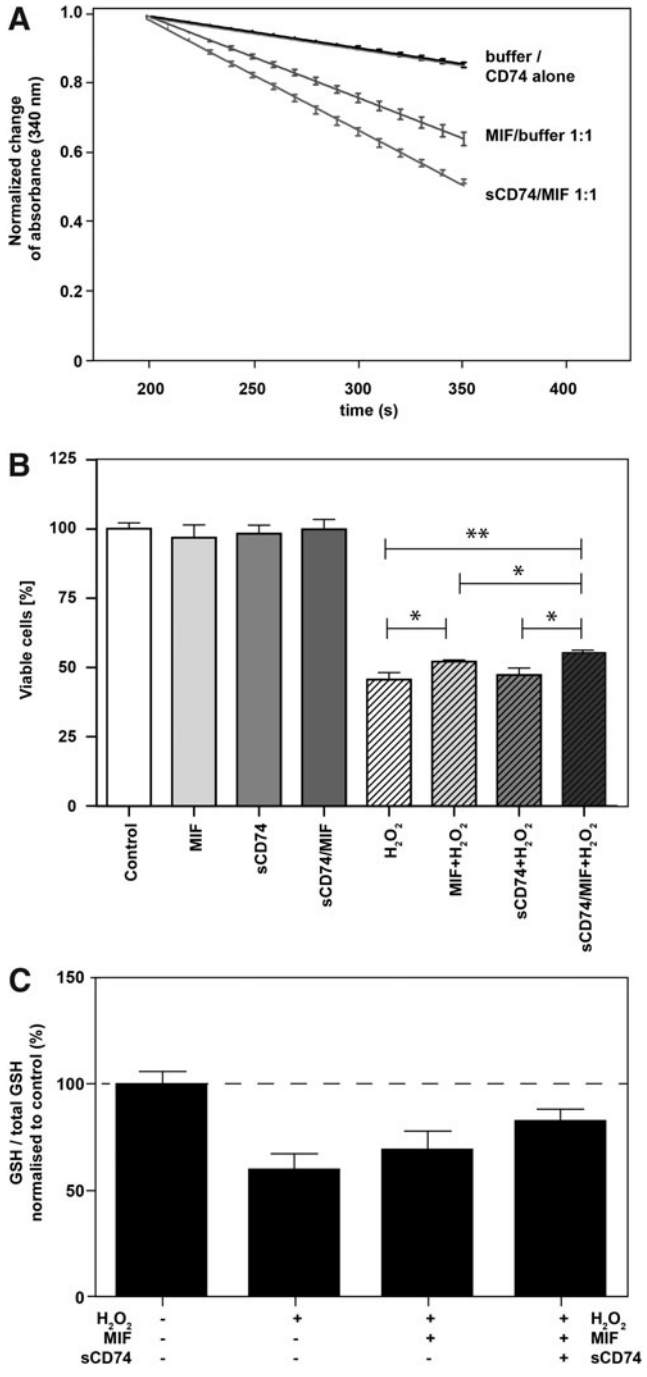


FIG. 4. sCD74 improves the redox activity of MIF. (A) sCD74/MIF complex formation increases MIF catalytic TPOR activity in an MIF-catalyzed HED transhydrogenase assay. Activity is expressed as a decrease of NADPH absorbance at 340 nm over time normalized to 1 at the time of addition of MIF or MIF/CD74 (1:1) complexes. **(B)** sCD74/MIF complexes increase rat cardiomyocyte survival after exposure to H_2O_2. Cardiomyocytes were pretreated with MIF, sCD74, or sCD74/MIF complexes (200-fold molar excess) for 30 min at 37°C before oxidative stress was induced by addition of 1.5 mMH_2O_2 for 90 min at 37°C. Cell viability was assessed by trypan blue staining and is normalized to untreated cells. **(C)** Complexation of MIF with sCD74 further enhances MIF capacity to reduce oxidative stress as measured by the GSH/GSSG ratio. Cardiomyocytes were pretreated with PBS, MIF, or sCD74/MIF complexes (fivefold molar excess) for 30 min at 37°C. After incubation with 1 m*M* H_2O_2 for 2 h, reduced GSH as well as total GSH was measured in the cell lysates. Measured GSH/total GSH ratios are normalized to untreated cells. $*p < 0.05$, $**p < 0.01$ *versus* H_2O_2 treatment. Data represent mean ± SEM of six independent experiments. HED, 2-hydroxyethyl disulfide; PBS, phosphate-buffered saline; TPOR, thiol–protein oxidoreductase.

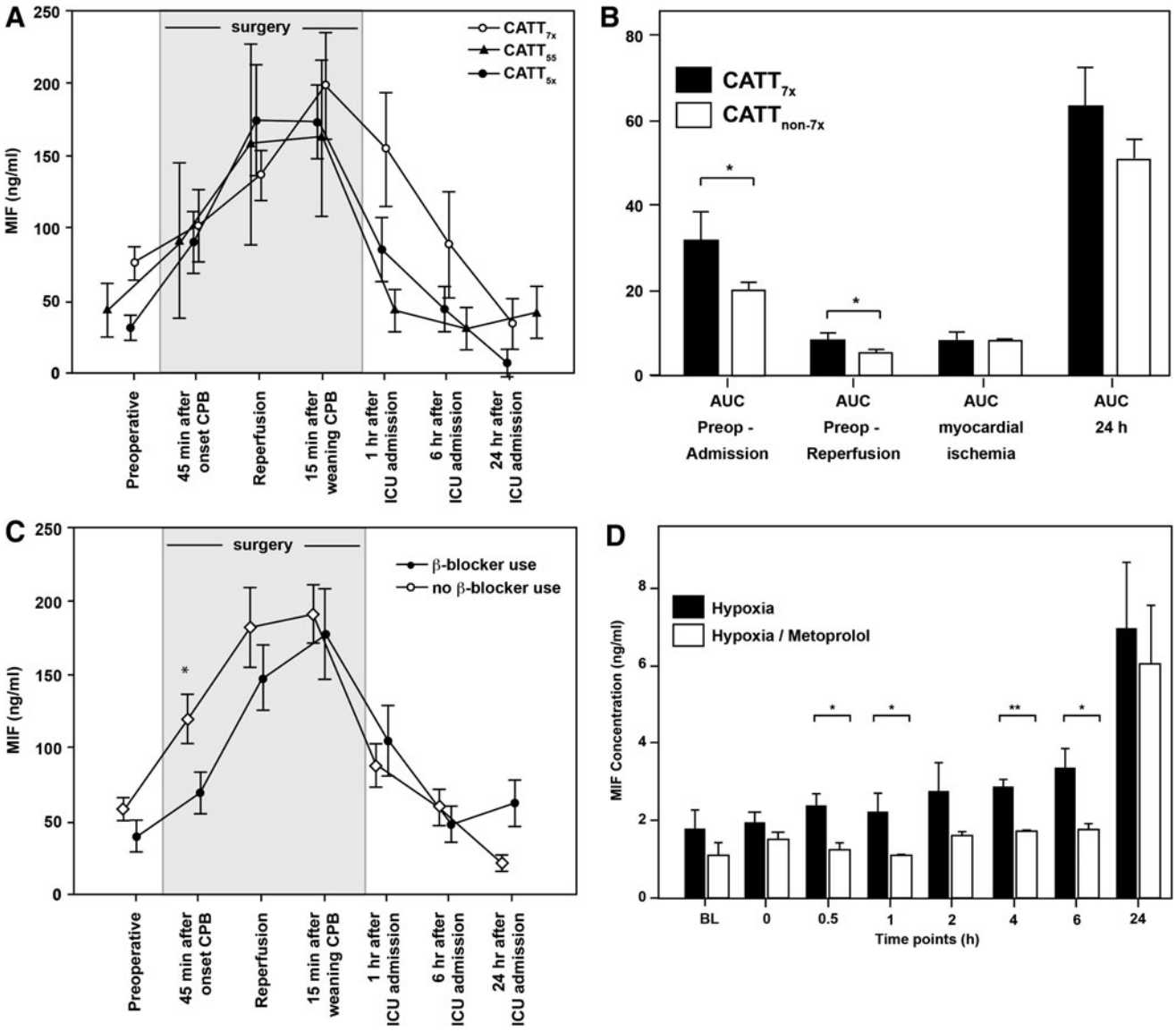


FIG. 5. Significance of *MIF* gene polymorphism and *β*-blockers on MIF secretion. All patients were genotyped at the −794 CATT locus and at the −173 G/C SNP. MIF secretion was compared between patients with the high MIF expression genotype ($CATT_{7x}$) and low MIF expression genotypes ($CATT_{55}$ and $CATT_{5X}$) **(A)**. **(B)** AUC analysis at different peri- and postoperative time points. The impact of *MIF* genotype on perioperative MIF release was additionally investigated by computing the AUC of MIF serum levels from preoperative to ICU admission ($AUC_{preop\text{-}admission}$), preoperative to myocardial reperfusion ($AUC_{preop\text{-}reperfusion}$), connection to CPB to termination of CPB ($AUC_{myocardial\ ischemia}$), and preoperative to 24 h after admission to ICU (AUC_{24h}). This type of analysis was performed to approach the dynamic and interindividually different conditions of cytokine release expected to occur in the perioperative period. **(C)** Comparison of MIF secretion kinetics from patients taking *β*-blockers with patients not treated with *β*-blockers. **(D)** MIF secretion from the cardiomyocyte cell line, HL-1, after a brief hypoxic stimulus (1 h) was measured by ELISA. Cells were treated with the *β*-blocker metoprolol (0.5 μg/ml) or buffer control for 30 min at 37°C before being subjected to hypoxia. $*p < 0.05$, $**p < 0.01$ *versus* preoperative; Data represent mean ± SEM of six independent experiments. ELISA, enzyme-linked immunosorbent assay; ICU, intensive care unit; SNP, single-nucleotide polymorphism.

experimental models of myocardial I/R, data in human subjects are sparse. Additionally, there is no information regarding the newly characterized MIF homolog, MIF-2, in human myocardial I/R injury, although this cytokine was shown recently to similarly protect murine hearts in a model of acute ischemia (28). While data about kinetics of MIF release in noncardiac surgery are lacking, an increasing number of clinical trials have investigated the kinetics and clinical significance of MIF levels during cardiac surgery. In this context, we have recently demonstrated that myocardial I/R represents the major trigger for perioperative MIF release and that only patients undergoing conventional CPB in combination with cardioplegia-induced cardiac arrest exhibited a significant release of MIF (38, 41).

Table 2. Significance of *MIF* Genotype for Postoperative Outcome (Summary of Results)

	Group	*Clinical findings*	p-*Value*
Type of postoperative complication and organ dysfunction			
Acute kidney injury	$CATT_{55\text{-}7}$	Significantly increased incidence	**0.018**
Atrial fibrillation	C-SNP	No effect	0.292
Delirium	$CATT_{7X}$ and C-SNP	Trend to reduced incidence	0.130
Pneumonia	$CATT_{7X}$	Trend to reduced incidence	0.116
Septic shock	$CATT_{55}$	Significantly increased incidence	**0.043**
Death	C-SNP	No effect	0.197
Composite outcome			
Occurrence of any organ dysfunction + death	$CATT_{7X}$	Trend to reduced incidence	0.068
	C-SNP	Trend to reduced incidence	0.065
	$CATT_{7X}$ and C-SNP	Significantly reduced incidence	**0.032**
Infectious complications	C-SNP	Trend to reduced incidence	0.123

Significant values are bold.

Kinetics and trigger of perioperative MIF release

The present study demonstrates similar increases in the circulating levels of MIF and MIF-2 in response to cardiac surgery that occurred remarkably earlier than those of PCT, affirming that MIF is an upstream mediator, potentially contributing to the initiation of reactive host responses (9). In accordance with previous findings, we found circulating MIF levels to correlate with the duration of cardioplegic arrest, which may suggest myocardial ischemia to be the primary trigger of perioperative MIF release (35, 36). After termination of surgery, both MIF and MIF-2 levels decreased rapidly, an effect that may be attributed to a reduced production rate from the ischemic tissue or to an increased clearance rate by various host mechanisms that in some subjects may involve interaction with a circulating form of their common receptor, sCD74 (2, 22).

Several downstream effects of MIF are mediated by high-affinity binding to and signal transduction through CD74 (16, 17, 34), and some studies indicate that an upregulation of CD74 expression may occur concomitantly with an increase in MIF expression (14, 21). The underlying shedding mechanisms of CD74 have recently been shown to be mediated by the intramembrane protease, SPPL2a (3, 4, 32). Moreover, a truncated circulating form of CD74 has been demonstrated in patients with autoimmune hepatitis and was shown to neutralize MIF-dependent signal transduction (2). Yet, data on the interaction between sCD74 and MIF in clinical settings remain largely elusive. Assis *et al.* suggested that circulating serum CD74 is a membrane-truncated protein, which is released following intramembrane cleavage (2). Since patients undergoing cardiac surgery are frequently exposed to myocardial I/R with an ensuing inflammatory response and systemic release of MIF, we considered this clinical setting to be an opportunity to characterize the relevance of sCD74 and sCD74/MIF complexes. Notably, we detected circulating sCD74/MIF complexes in about 50% of patients. In this study, the detection of sCD74/MIF showed a significantly reduced incidence of postoperative kidney injury and overall complications, which are known to result from increased oxidative stress and its sequels (38).

Outcome

An inverse correlation was observed between circulating intraoperative MIF levels and the extent of perioperative organ injury as assessed by established organ dysfunction scores, including the SOFA score (15, 20, 42). Moreover, intraoperative MIF levels predicted freedom from AF and showed an inverse correlation with postoperative serum creatinine levels, hence suggesting both cardio- and renoprotective effects for MIF. While MIF has been demonstrated to exhibit cardioprotective properties in experimental models, data on MIF-mediated renal protection are sparse (25). Since oxidative stress can significantly contribute to the development of postoperative AKI (41), the observed renoprotective effects of MIF may be attributed to its well-known antioxidant properties (38). By contrast, we found intraoperatively elevated MIF-2 levels to correlate with the development of AKI and the occurrence of AF. Although we did not notice a causal relationship in our small cohort, the presence of CKD or prolonged duration of CPB may be considered as a causative factor for the development of AKI in the postoperative course.

The mechanistic reasons for the diverging effects of MIF and MIF-2 remain to be elucidated and could be manifold: (i) divergent effects of MIF and MIF-2 may relate to differences in their respective antioxidant capacities: MIF-2 lacks two of three conserved cysteines (Cys 60 and Cys 81) that mediate MIF redox activity and contribute to several MIF functional and beneficial properties (12, 22, 26); (ii) both cytokines are known to have chemokine-like functions (22) on leukocyte motility and a contribution of both MIF and MIF-2 to cardiac surgery patient serum-mediated leukocyte chemotaxis was demonstrated in our current study, but the mechanisms and subtype-specific profiles may differ; in fact, MIF-2 lacks the *pseudo*-(E)LR motif known to be required for MIF/CXCR2-mediated leukocyte recruitment responses; (iii) while molecular evidence is still elusive, MIF-2 effects on leukocyte recruitment may thus be mediated through CXCR4, CXCR4/CD74 complexes, or indirectly through an induction of secondary chemotactic mediators such as downstream chemokines; (iv) alternatively, the MIF effect on subacute inflammatory cell recruitment into the injured cardiac tissue may be overcompensated by its redox-mediated cardioprotective effects,

whereas for MIF-2, which intrinsically lacks the protective antioxidant properties, disease-aggravating effects on leukocyte recruitment would predominate; and (v) last, MIF and MIF-2, for which effects on cardiomyocyte apoptosis have not yet been studied in detail, may differ in their effects on cardiomyocyte injury and survival in the setting of myocardial I/R, and this may in turn lead to different patterns in the release of proinflammatory cytokines and chemokines from cardiac tissue.

In fact, the chemotactic potential of cardiac surgery patient-derived serum was demonstrated in our current study. Although circulating chemokines do not strictly reflect local chemokine concentration gradients in a given tissue, chemokine serum levels are an adequate surrogate marker of the local process, that is, in the cardiac tissue, which cannot be further investigated in humans. Additional experimental studies are needed to mechanistically address the discussed underlying functional differences in the future.

Considering the clinical significance of circulating sCD74, patients with detectable sCD74/MIF complexes exhibited a reduced incidence of AKI and overall organ dysfunction. These observations may be due to an enhanced antioxidant capacity of MIF. In line with this notion, sCD74 interaction with MIF increased the TPOR activity of MIF, leading to an increased reduction of HED and improving redox stress-challenged cardiomyocyte survival and a decrease in oxidative stress as measured by cellular GSH/GSSG ratios *in vitro*. However, these first findings should be considered with caution due to the limitations of an exploratory analysis, while encouraging further studies to mechanistically investigate the MIF/sCD74 interaction.

Our human genetic data also are the first to suggest a genotype–phenotype relationship between commonly occurring variant *MIF* alleles and clinical outcomes in cardiac surgery patients. Notably, we found patients with a high-expression *MIF* genotype ($CATT_{7X}$) to show significantly higher intraoperative MIF release and to experience a significantly lower incidence of organ dysfunction. No postoperative infections were documented in the high-expression *MIF* genotype. These preliminary results, if extended by additional large-scale prospective studies, could assist in the risk stratification of patients and potentially enable a pharmacogenomic approach to augment MIF action intraoperatively, as has been suggested by the preclinical application of small-molecule MIF agonists (43).

In conclusion, the present study highlights the first description of the kinetics, interaction, and clinical relevance of the MIF family proteins, MIF, MIF-2, and sCD74, as well as the *MIF* promoter genotype, in patients undergoing cardiac surgery that may be used in future for preoperative risk stratification of patients exposed to myocardial I/R.

Materials and Methods

Study design and patients

The study was approved by the institutional review board (Ethics committee, RWTH Aachen University, Germany) and registered at ClinicalTrials.gov (ClinicalTrials.gov identifier: NCT0126772). One hundred patients were consecutively enrolled in this prospective observational study after written informed consent had been obtained. All included patients were scheduled for elective conventional cardiac surgery with the use of aortic cross-clamp with cardioplegic arrest following initiation of CPB. Exclusion criteria were emergency operations, known or suspected pregnancy, age less than 18 years, and failure to obtain informed consent.

Anesthesia

All patients received balanced anesthesia according to our institutional routine. With exception of metformin, ACE inhibitors, and AT_2 receptor antagonists, preoperative medication was continued until the day of surgery. Induction of anesthesia was performed with propofol ($1.5\,mg\cdot kg^{-1}$) and sufentanil ($0.5–1\,\mu g\cdot kg^{-1}$). Muscle relaxation was obtained with rocuronium ($1\,mg\cdot kg^{-1}$). Anesthesia was maintained by continuous infusion of sufentanil ($1\,\mu g\cdot kg^{-1}\cdot h^{-1}$) and sevoflurane (0.5–1 MAC) throughout the whole procedure. Basic fluid substitution was performed with $1\,ml\cdot kg^{-1}\cdot h^{-1}$ balanced crystalloid solutions. Packed red blood cells were transfused when the hemoglobin content was below $7.5\,g\cdot dl^{-1}$. Additional fluids, vasopressors, or inotropic drugs were administered according to the discretion of the attending physicians.

Surgical procedure

All patients underwent conventional cardiac surgery with the use of CPB. After midline sternotomy, dissection of the internal mammary artery, and harvesting of the venous conduits, heparin was administered ($300\,IE\cdot kg^{-1}$) to obtain an activated clotting time of >400 s. The extracorporeal circulation was performed with a nonpulsatile pump flow of $2.2\,L\cdot min^{-1}\cdot m^{-2}$, and blood pressure was maintained between 50 and 70 mm Hg. A single antegrade infusion of cold crystalloid cardioplegic solution was used for induction of cardiac arrest (Custodiol™; Köhler Chemie) in the aortic root immediately after cross-clamping. Patient temperatures were either kept warm or allowed to drift to 32°C during CPB. After weaning from CPB, the heparin was antagonized with protamine in a ratio of 1:1, and aspirin was administered p.o. starting 8 h postoperatively.

Intensive care unit

After the end of surgery, all patients were transferred to the ICU and received standardized treatment according to our institutional guidelines. The patients were weaned from ventilation when standard extubation criteria were fulfilled. After completion of our standardized discharge criteria, patients were transferred to standard care units for further recovery.

Data collection and blood sample acquisition

In addition to clinical routine measurements, serum samples were obtained after induction of anesthesia (preoperative), 45 min after institution of CPB, 2 min after opening of cross-clamp (myocardial reperfusion), 15 min after weaning from CPB, at 1 h after admission to the ICU, 6 h after admission, and 24 h after admission to the ICU for the laboratory determination of MIF, MIF-2, and sCD74. Serum samples were used instead of plasma to provide comparable data with previous studies, which determined the time course of MIF during cardiac surgery (38). All blood samples were immediately centrifuged (3000 rpm, 10 min) and

the supernatants were transferred to cryotubes. Subsequently, the serum samples were stored at −80°C until final analysis. In addition, whole blood samples were collected before surgery (EDTA tubes) for later genotype analysis.

The incidence of organ dysfunction, systemic inflammatory response syndrome, sepsis, severe sepsis, and septic shock was recorded according to the ACCP/SCCM consensus conference criteria (6): Arterial hypoxemia ($PaO_2/FiO_2 < 300$ mmHg), coagulation abnormalities in the absence of pharmacologic anticoagulation (INR > 1.5 or a PTT > 60 s), hypotension (MAP < 70 mmHg) despite adequate fluid replacement, thrombocytopenia (platelet count $<100{,}000 \cdot L^{-1}$), hyperbilirubinemia (plasma total bilirubin $> 2\,mg \cdot dl^{-1}$ or 34.2 μM), and neurological dysfunction (Glasgow Coma Score below 15 in the absence of sedation or metabolic impairment). AKI was defined according to the RIFLE criteria (twofold increase of creatinine within the first 48 h after surgery or glomerular filtration rate <50% or urine output $<0.5\,ml \cdot kg^{-1} \cdot h^{-1}$ for at least 12 h).

MIF and MIF-2 and sCD74 ELISA measurement

Serum levels of MIF were assessed with an ELISA technique as previously described (38), using capture antibody MAB289 and detection antibody BAF289 (R&D Systems). Serum levels of MIF-2 were measured using an ELISA technique as previously described (22).

For detection of circulating CD74, a competitive sandwich ELISA was developed as part of this study. As capture antibody, anti-CD74 (250 ng/ml, clone C-16, SC-5438; Santa Cruz) was used, and for detection clone, LN-2 (400 ng/ml, sc-6262; Santa Cruz) was used. The rhCD74 protein standard used for the ELISA was purchased from R&D Systems (3590-CD). The interassay variability was 25%, and the assay sensitivity was 1.56 ng/ml. Patients with serum levels below the detection limit were excluded from further analysis with respect to its clinical significance.

Sandwich ELISA to measure sCD74/MIF complexes

A competitive sandwich ELISA to detect circulating MIF-sCD74 complexes was developed using a rabbit polyclonal anti-MIF antibody (R102; 1:300 dilution) for coating and anti-CD74 (400 ng/ml, clone LN-2, sc-6262; Santa Cruz) for detection. Immunoreactivity was calibrated against a recombinant sCD74/MIF fusion protein purified from a pET28 *Escherichia coli* plasmid engineered with a coding sequence corresponding to $sCD74^{73–232}$-GSGSGS-$MIF^{2–114}$.

Hydroxyethyl disulfide transhydrogenase assay

Catalytic redox activity of MIF in a complex with sCD74 was measured by the HED transhydrogenase assay, which was performed as described previously (24). Briefly, reduction of HED by reduced glutathione was measured in an MIF-catalyzed reaction. Oxidation of NADPH by oxidized glutathione was then recorded photometrically in a coupled step ($\lambda = 340$ nm). For each measurement, 2 μg rhMIF in 20 mM sodium phosphate buffer (pH 7.2) was used and was preincubated with sCD74 (R&D Systems) at a 1:1 molar ratio for 30 min on ice. The transhydrogenase reaction was started by adding MIF or MIF/CD74 complexes to the prewarmed reaction mixture (30°C, final volume: 100 μl; final MIF concentration: 1.6 μM) and the reaction was recorded in a Jasco V650 spectrophotometer. Spectra were recorded for 10 min at 30°C, and MIF activity was calculated from the linearized slope of the curve ($\lambda = 340$ nm).

GSH/GSSG assay

The GSH/GSSG assay was performed in accordance with the manufacturer's guidelines (Abcam). First, isolated cardiomyocytes were preincubated for 30 min with MIF (150 μg/ml), MIF/sCD74 complexes (sCD74 in fivefold molar excess), or phosphate-buffered saline (PBS), respectively. Afterward, 1 mM H_2O_2 was added for 2 h and subsequently the redox activity of MIF and MIF in complex with sCD74 was assessed by the GSH/total GSH ratio in accordance with the manufacturer's description.

Assessment of the effect of sCD74/MIF on survival of cardiomyocytes

To measure the effect of murine MIF and sCD74 on the survival of rat cardiomyocytes challenged by H_2O_2 stress, rat cardiomyocytes were isolated enzymatically from neonatal rats and plated on fibronectin/gelatin-coated 48-well plates.

In these experiments, eight conditions were used in parallel. The control group received only PBS, whereas the other groups were stimulated with either 100 ng/ml MIF, a concentration that reflects the intraoperatively measured MIF concentration and a fivefold molar excess of sCD74, or preincubated sCD74/MIF complexes (30 min on ice) to ensure an efficient interaction and complex formation. To induce oxidative stress, all four groups received either 1.5 mM H_2O_2 or PBS (control) for 90 min. After the treatment with H_2O_2, the supernatant as well as the cells detached by accutase treatment was incubated with 0.4% trypan blue dye. Cell survival was assessed by a cell counter (BioRad TC20™ Automated Cell Counter).

Migration assay with neutrophilic granulocytes or PBMCs

Migration assays were performed using a modified Transwell chamber (Corning, Inc.) in a 24-well plate format and cell culture inserts containing filters with a pore size of either 3 μm (for neutrophilic granulocytes) or 5 μm (for PBMCs). The PBMCs were detached using a cell scraper. The neutrophilic granulocytes were used right after isolation. For the migration assay, either 50,000 cells (PBMCs) or 400,000 cells (neutrophilic granulocytes) per well were used in a volume of 200 μl. Lower chambers contained starved medium alone (RPMI 1640 containing 0.5% bovine serum albumin and 1% penicillin/streptomycin) or medium mixed with patient serum and one of the following antibodies: anti-MIF (120 μg/ml), anti-D-DT (3.5 μg/ml), anti-IL-8/CXCL8 (570 ng/ml), or anti-MIP-1α (42 ng/ml).

After either 3 h (PBMC) or 1 h (neutrophilic granulocytes) of migration at 37°C and 5% CO_2, all cell culture inserts were removed. To optimize counting conditions, cells were fixed and stained with Hoechst dye diluted in 3.6% PFA (1:1000). Finally, the fixed cells were incubated overnight. Pictures were taken under a microscope (100× magnification) the next morning for a final cell counting using the semiautomized software ImageJ (National Institutes of Health).

MIF polymorphism analysis

DNA was extracted from serum samples using the Easy-DNA Kit (Invitrogen). The MIF −794 CATT5-8 (*rs5844572*) polymorphism analysis was performed as previously described (44), with TET-labeled amplicons resolved using an ABI 310 Genetic Analyzer (Applied Biosystems). The MIF −173 G/C SNP (*rs755622*) analyses were performed by a predeveloped TaqMan assay for allelic discrimination.

Statistical analysis

All data were statistically analyzed using a commercially available software package (SPSS 19.0; SPSS, Inc.). Apart from the separated analysis of any organ dysfunction, the assessment of overall postoperative complications was assessed as composite outcome that comprises the occurrence of any postoperative organ dysfunction and/or death until the 30th POD. The 30-day time frame is commonly used in the intensive care literature because there is virtually no loss to follow-up during this period and there are only about 5% of outlying patients who remain alive and dependent on life-sustaining therapy after (sometimes months after) 30 days.

All metric data were tested for normal distribution using the Shapiro–Wilk W test. Normally distributed results of single measurements were compared between the groups using Student's *t*-test. Results of repeated measurements were compared using a repeated measurement analysis of variance (One-Way ANOVA). Nonparametric data were compared using the Wilcoxon signed-rank test. In case of significant results, *post hoc* testing was performed using the Bonferroni test and (for not normally distributed data) the Wilcoxon signed-rank test with the Bonferroni adjustment for multiple measurements, respectively. Proportions were compared using Fisher's exact test. For correlation studies, linear regression analysis was used. The predictive value of MIF and MIF-2 for the development of AF and pneumonia at any time during ICU stay was calculated from the AUC using receiver operating characteristics. Logistic regression was used to analyze the influence of intraoperative serum levels of MIF (respectively, DDT) on postoperative events (AF, organ dysfunction) controlling for possible confounders (age, medication, diabetes mellitus). In all cases, a level of $p < 0.05$ was considered statistically significant.

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Author Disclosure Statement

Drs. Lin Leng, Richard Bucala, and Jürgen Bernhagen are coinventors on issued patents or patent applications describing the therapeutic utility of MIF modulation. All other authors state that no competing financial interests exist.

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Abbreviations Used

AF = atrial fibrillation
AKI = acute kidney injury
AMPK = activating adenosine monophosphate-activated protein kinase
AUC = area under the curve
CABG = coronary artery bypass grafting
CK = creatine kinase
CPB = cardiopulmonary bypass
DDT, MIF-2 = D-dopachrome tautomerase
ELISA = enzyme-linked immunosorbent assay
EuroSCORE = European system for cardiac operative risk evaluation
HED = hydroxyethyl disulfide
ICU = intensive care unit
I/R = ischemia and reperfusion
IL = interleukin
JNK = c-Jun N-terminal kinase
LDH = lactate dehydrogenase
LVEF = left ventricular ejection fraction
MIF = macrophage migration inhibitory factor
MODS = multiorgan dysfunction score
PBMC = peripheral blood monocyte
PBS = phosphate-buffered saline
PCT = procalcitonin
POD = postoperative day
sCD74 = circulating receptor CD74 (sCD74)
SNP = single-nucleotide polymorphism
SPPL2a = signal peptide peptidase-like 2a
SOFA = sequential organ failure assessment
TPOR = thiol–protein oxidoreductase