MEDICAL SCHOOL UNIVERSITY OF PÉCS

Macrophage migration inhibitory factor as a diagnostic and predictive biomarker in sepsis

DOCTORAL (PHD) THESIS

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List of abbreviations

ANOVA	analysis of variance
APACHE II	Acute Physiology and Chronic Health Evaluation II score
ARDS	adult respiratory distress syndrome
AUC	area under the ROC curve
CENTRAL	Cochrane Central Register of Controlled Trials
CI	confidence interval
COVID-19	Corona Virus Disease 2019
DAMP	damage-associated molecular pattern
DIC	disseminated intravascular coagulation
ELISA	enzyme-linked immunosorbent assay
ICU	intensive care unit
IQR	interquartile range
MIF	Macrophage Migration Inhibitory Factor
NA	not applicable
NR	not reported
PAMP	pathogen-associated molecular pattern
PICO	Patients, Indicator, Comparison, Outcome
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
RIFLE	acronym indicating Risk of renal dysfunction; Injury to the kidney; Failure of kidney function, Loss of kidney function, and End-stage kidney disease
ROC	receiver operating characteristic
SAPS II	Simplified Acute Physiology Score II
SD / SE	standard deviation / error
SIRS	systemic inflammatory response syndrome
SMD	standardized mean difference
SOFA	Sequential Organ Failure Assessment

Introduction

1.1 Sepsis and its global burden

Sepsis, a form of systemic inflammation, is defined as life-threatening organ dysfunction caused by dysregulation of the host's response to infectious noxa (Singer *et al.* 2016). Among the leading causes of sepsis are bacterial infections, but it can also be caused by viral infections, such as COVID-19 or influenza; fungal infections; or noninfectious insults, such as traumatic injury. Normally, the body releases chemical or protein immune mediators into the blood to combat the infection or insult (Garami *et al.* 2018). In an ideal scenario the systemic inflammatory response, which is often associated with fever, successfully eliminates the intruding pathogen from the host, thereby leading to survival. However, when the host organism is weakened by previous or simultaneous comorbidities or when the infection is too severe, then the outcome can be deadly, despite the adaptive (disease-tolerating) strategy of the host, which is characterized by hypothermia (Garami *et al.* 2018; Rumbus & Garami 2018). In the clinical setting, sepsis and septic shock are medical emergencies. Sepsis-induced tissue hypoperfusion is defined as acute organ dysfunction and involves also significant alterations in coagulation, as well as immunosuppression.

With regards to the clinical definitions of sepsis, in 1991, a consensus conference (Bone *et al.* 1992) determined initial definitions that focused on the view that sepsis resulted from the host's systemic inflammatory response syndrome (SIRS) to an infection. When sepsis was complicated by organ dysfunction, it was termed as *severe sepsis*, which could progress to *septic shock*, defined as "sepsis-induced hypotension persisting despite adequate fluid resuscitation." In 2001, a task force recognized limitations of these definitions, and expanded

the list of diagnostic criteria but did not offer alternatives because of the lack of supporting evidence (Levy *et al.* 2003). In effect, the definitions of sepsis, septic shock, and organ dysfunction have remained largely unchanged for more than 2 decades. The last revision, the Sepsis-3 definitions of sepsis and septic shock was a 2-year-long process that involved several components (Singer *et al.* 2016). Critical efforts in this process included a discussion of the concept of sepsis, identification of criteria alerting clinicians for the patient's risk to develop sepsis, and the development of the criteria to identify septic shock (Sartelli *et al.* 2018). The Sepsis-3 definitions suggest that patients with at least two of these three clinical variables may be prone for the poor outcome typical of sepsis (also called as the quick SOFA): (1) low blood pressure (systemic blood pressure of 100 mmHg or less), (2) high respiratory rate (≥ 22 breaths per min), or (3) altered mental state (Glasgow coma scale < 15) (Sartelli *et al.* 2018).

Even nowadays, sepsis and its related diseases constitute a major burden for the patients and healthcare providers, which is also indicated by the high incidence of hospital-treated sepsis cases across all regions (189/100000 person years) reported in 2020 (Rudd *et al*. 2020). Worldwide, sepsis is estimated to affect more than 100 million people annually and nowadays it is one of the major causes of death, posing a global health and financial burden for the society. According to a recent analysis of cause-of-death data from 109 million records in the Global Burden of Diseases, Injuries, and Risk Factors Study, almost 49 million incident cases of sepsis could be estimated around the world and 11 million sepsis-related deaths were reported (Rudd *et al*. 2020). In a cohort from 6 hospitals located in the US, sepsis was present in more than half of the hospitalizations and accounted for the highest

ratio (approx. one-third) among the causes of death (Rhee *et al.* 2019). While there was some evidence of a trend towards decreasing mortality rates in septic patients over the last decade, a continuous decline in mortality was not observed among patients with sepsis or septic shock in a recent systematic review (Bauer *et al.* 2020). These data warrant for the need of better sepsis management, which could be enhanced by improved diagnostic and prognostic options. In spite of the desperate need for reliable biomarker molecules in sepsis, the novel candidates require further validation before they can be incorporated into the clinical practice, as stated by the Sepsis-3 definition consensus (Singer *et al.* 2016).

The burden of sepsis is even further exaggerated in the intensive care unit (ICU). In one study, the estimated death rate in septic patients was as high as 26.7%, which was further increased to 41.9% when the patients were treated at the ICU (Rudd *et al*. 2020). Another study concluded that the estimated burden of sepsis worldwide is twice as much as what was thought previously (Rhee *et al.* 2019). Further increasing its burdens, sepsis was also associated with greater rehospitalization rates and higher healthcare costs compared to matched hospitalized controls (Bauer *et al.* 2020). The early diagnosis and assessment of severity could reduce the burdens of sepsis, which can be achieved through the discovery of reliable biomarker molecules, which are continuously being screened by many research groups. In 2010, an electronic search identified 178 sepsis-related biomarkers, but none of them was found eligible for routine use in clinical practice (Pierrakos & Vincent 2010). According to a more recent review by the same group (Pierrakos *et al.* 2020), the list of potential biomarkers in sepsis has expanded, and in 2020 it included more than 250

substances, but only a few of them were evaluated in a large patient population or in multiple studies, which still limits their clinical usability.

1.2 Macrophage migration inhibitory factor (MIF)

MIF is a mediator molecule of the innate immune system (Garai *et al.* 2017), which is involved in a number of inflammatory processes and inflammation-associated disorders, such as autoimmune disorders (Grieb *et al.* 2010; Flaster *et al.* 2007), obesity (Grieb *et al.* 2010; Morrison & Kleemann 2015) and cancer (Grieb *et al.* 2010; (Bucala & Donnelly 2007). MIF, as a proinflammatory cytokine, is rapidly released into the bloodstream in various forms of acute systemic inflammation (Calandra & Roger 2003; Garai *et al.* 2017). It must be noted that the causes of acute systemic inflammation can be diverse, including infectious pathogens (e.g., sepsis, septic shock), as well as noninfectious disorders due to stress, autoimmune reaction, trauma, surgery, burns, etc. The elevated levels of MIF in the blood were reported in diseases with acute systemic inflammation caused by both infectious and noninfectious etiologies (Grieb *et al.* 2010), however, it has remained unclear whether the extent of the increase is similar or different in the two forms, therefore, if MIF can be used as a diagnostic tool in sepsis. The available literature data was controversial. In one study, a similar increase in MIF levels was observed in patients with systemic inflammation of septic and nonseptic (i.e., caused by major surgery) origin compared to the healthy controls (Lehmann *et al.* 2001), suggesting that MIF may serve as a biomarker for critical illness without the ability to differentiate between infectious and noninfectious causes. However, in other studies, MIF levels were markedly higher in sepsis than in patients with

other forms of systemic inflammation (Beishuizen *et al.* 2001; Brenner *et al.* 2010; Meawed *et al.* 2015), indicating that MIF can be used as a diagnostic biomarker for sepsis. It should be noted that according to the current clinical practice, MIF cannot be classified among the most common biomarkers for monitoring inflammatory processes. In intensive care, the monitoring of white blood cell count, fibrinogen, C-reactive protein, procalcitonin, and interleukin-6 levels is much more common, the trust invested in procalcitonin is particularly strong and proven (Papp *et al.* 2023). In addition to its diagnostic usability, the prognostic value of MIF has also remained controversial. High serum levels of MIF were found in septic patients and even higher MIF levels in patients with septic shock; however, the difference was not statistically significant ($p = 0.3$) (Calandra *et al.* 2000). Similarly, not significantly higher MIF levels were reported in septic patients with lung complications compared to those without it (Beishuizen *et al.* 2001). On the contrary, a significant correlation was not found between serum MIF levels and sepsis severity or mortality (Gao *et al.* 2007). Moreover, circulating MIF levels did not differ between sepsis survivors and nonsurvivors in one study (Lehmann *et al.* 2008), but nonsurvivors showed significantly higher MIF levels compared to survivors in another study (Beishuizen *et al.* 2001).

During my PhD studies, we were looking for a biomarker molecule in sepsis that has a past, but its future is not clear and the researchers' thoughts have not yet concluded with it. As explained above, MIF proved be an optimal candidate. To thoroughly investigate the potential diagnostic and prognostic biomarker value of MIF in sepsis, we used a dual approach. First, we performed a meta-analysis to summarize and amalgamate the current knowledge in the field. With the meta-analysis, we wanted to investigate the diagnostic and

prognostic biomarker value of the MIF blood level measured at the admission to the hospital based on literature data. Therefore, we analyzed its diagnostic value between septic and healthy, as well as septic and non-infectious systemic inflammation patients. We also looked at MIF's prognostic value by comparing its blood levels between less severe and more severe forms of sepsis as well as between survivors and nonsurvivors of the disease. As our second approach, we conducted a prospective, observational clinical trial in order to find answers to questions that could be not assessed by the meta-analysis of the literature data. In particular, we wanted to elucidate the kinetics of serum and urine MIF levels during the initial days of ICU admission, and to study whether the kinetics are similar or different between sepsis survivors and nonsurvivors.

In general, MIF is a proinflammatory cytokine produced in T-lymphocytes (but also an endocrine factor) and it is expressed in endothelial cells, eosinophils, and macrophages. Together with tumor necrosis factor, it promotes the inflammatory response. MIF not only inhibits the migration of macrophages (as its name suggests), but it can also increase macrophage surface adhesion and phagocytosis. In humans, MIF consists of 114 amino acids with a molecular weight of 12.5 kDa. Its expression was shown to increase in cancers, inflammation, and autoimmune disorders. It is also present in inflammatory processes of the lungs, for example, asthma, acute respiratory distress syndrome, tuberculosis, and Wegener's granulomatosis. In addition, higher MIF levels were also found in other, mostly inflammation-associated diseases, such as glomerulonephritis, ulcerative colitis and Chron's disease, dermatitis, psoriasis, systemic sclerosis, type 2 diabetes mellitus, pancreatitis, multiple sclerosis, atherosclerosis, lupus erythematosus, and endometriosis.

Aims

The ultimate goal of our present work was to evaluate the clinical importance of MIF in human patients in sepsis, and, thereby, to identify its biomarker value to help the diagnosis of sepsis, and to predict the outcome of the disease. Although MIF as a biomarker was investigated repeatedly in sepsis, previous clinical trials lead to contradictory results.

To achieve our ultimate goal, our specific aims were as follows:

2.1 Analysis of literature data about the biomarker role of MIF in septic humans (Toldi *et al.* 2021) to assess whether blood MIF levels are different between:

a) septic patients vs. healthy controls;

b) patients with sepsis vs. patients with noninfectious systemic inflammation;

c) septic patients with more severe vs. less severe forms of the disease; and

d) sepsis survivors vs. nonsurvivors.

As part of this aim, we also performed a receiver operating characteristic (ROC) curve analysis to evaluate the diagnostic performance of blood MIF levels in sepsis.

2.2 Prospective, observational clinical study (Toldi *et al.* 2023) in septic patients admitted to the ICU to investigate:

a) the kinetics of serum and urine MIF levels;

b) the characteristic kinetics in sepsis survivors vs. nonsurvivors;

c) intersex differences between serum and urine MIF kinetics; and

d) the influence of renal dysfunction on urine MIF kinetics.

Materials and Methods

3. Approach 1: meta-analysis of published human data

Our meta-analysis (Toldi *et al.* 2021) was conducted in accordance with the guidelines of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) statement (Moher *et al.* 2009). We formed our question for the analysis in the PICO [Patients, Indicator, Comparison, Outcome] format: in adult septic patients, we aimed at assessing the biomarker role of MIF in the diagnosis and prognosis of the disease. Our meta-analysis was registered with PROSPERO (CRD42020139137).

3.1 Search strategy

We searched the CENTRAL (Cochrane Central Register of Controlled Trials), Embase, and PubMed databases for original human studies from inception until December 2019 with the following search term: ("macrophage migration inhibitory factor" OR MIF) AND (sepsis OR septic). Similar to our previous meta-analysis on sepsis (Rumbus *et al.* 2017), publications on immunosuppressive conditions (e.g., organ transplantation, human immunodeficiency virus infection) were not included in the analysis. The search was carried out separately by two authors (János Toldi and András Garami), who also independently assessed study suitability and independently collected data from the selected studies. Disagreements were resolved by consensus with the help of a third party.

3.2 Study selection, data extraction, and risk of bias assessment

We screened the titles and abstracts of publications identified through the literature search, and then obtained the full text of potentially eligible articles. We included studies that reported blood MIF levels in two or more different groups of patients, at least one of which consisted of septic patients. In order to analyze the prognostic value, it was necessary to indicate the severity of the disease or the outcome (e.g., mortality rate) for the groups. From all included articles, we extracted the country of origin, the characteristics of the patient populations (sample size, sex ratio, age, severity score, mortality), as well as the reported MIF values in the blood of the patient groups. When necessary, the extracted values were converted to mean and standard deviation (SD) for the analysis. The different patient groups within the study (e.g., survivors and nonsurvivors, septic and nonseptic systemic inflammation) were extracted separately. The quality of each study included in the meta-analysis was evaluated using the Newcastle–Ottawa scale (Wells *et al.* 2000).

3.3 Statistical analysis

We calculated the difference between the blood MIF level of a septic patient group and that of another septic group or a control group for each included study. For the patient groups, the means were standardized (based on variances) to obtain standardized mean differences (SMD). For that reason, the means were divided by their corresponding SD values, which was necessary, because the different methods used to measure MIF could lead to different variances among the study groups and, therefore, influence the results. We used the random effect model by DerSimonian and Laird (DerSimonian & Laird 1986) to calculate the SMD

with 95% confidence intervals (CI), which were then compared by using standard meta-analysis tools (viz., forest plot).

Inter-study heterogeneity was tested with I-square (I^2) statistical test, where I^2 is the proportion of total variation attributable to inter-study variability (an I^2 value of more than 50% was considered as an indication of substantial heterogeneity), as suggested by the Cochrane Handbook for Systematic Reviews (Higgins & Green 2011). Publication bias was determined by visual inspection of funnel plots for the lack of asymmetry and evaluated quantitatively by Egger's test ($p < 0.1$ indicating publication bias). Sensitivity analysis (i.e., sequentially eliminating one study from the analysis, and then recalculating the SMD to investigate the impact of the given study on the summary estimate) was performed to test the impact of the individual studies. We used the Comprehensive Meta-Analysis (version 3.3; Biostat, Engelwood, MJ, USA) software to perform the meta-analyses.

As part of our meta-analysis, we constructed ROC curve to evaluate the diagnostic performance of blood MIF levels in sepsis. For that reason, individual blood MIF level data of septic patients and healthy controls were extracted with WebPlotDigitizer application from eligible papers (Leaver *et al.* 2010; Merk *et al.* 2011; Wiersinga *et al.* 2010), which presented the data in figures with linear scales. The area under the ROC curve (AUC) was calculated to assess the accuracy of blood MIF level measurement as a diagnostic test in sepsis. Within the range of 0.5 (no diagnostic ability) to 1.0 (perfect diagnostic ability), a higher AUC indicates better performance of a test. ROC curve analysis was performed using IBM SPSS Statistics for Windows, version 26 (IBM Corporation, Armonk, NY, USA).

4. Approach 2: prospective, observational clinical study

4.1 Patients

Between January 2012 and May 2015, we enrolled 51 septic patients into this prospective, observational study from our ICU (Department of Anesthesiology and Intensive Therapy, Medical School, University of Pecs, Pecs, Hungary). Our study protocol was approved by the Regional Research Ethical Committee of the University of Pecs (registration no.: 2406/2005), and the study was performed in accordance with the ethical standards in the 2008 Declaration of Helsinki. Following the detailed explanation of the study procedure, written informed consent was obtained from all study participants.

4.2 Inclusion and exclusion criteria

Sepsis was defined according to the most actual criteria at the time of patient enrollment by the International Sepsis Definitions Conference (Levy *et al.* 2003). Septic patients with elevated serum procalcitonin level at admission to the ICU were enrolled in the study. Patients were excluded if they were under 18 years or above 85 years of age or if they refused to participate in the study. Except for the measurements of MIF levels, the diagnostic and treatment procedures were conducted according to the sepsis guidelines in the patients.

4.3 Data collection

We collected demographic data (age and sex) from all enrolled patients. The mortality was followed up for 90 days from ICU admission. The following laboratory parameters were measured on days 0, 2, and 4 from ICU admission: blood cell counts, as well as levels of C-

reactive protein, creatinine, lactate, procalcitonin, and urea. On the same days, the urine concentrations of creatinine and total protein were also determined. The Acute Physiology and Chronic Health Evaluation (APACHE) II score (Knaus *et al.* 1985), the Sequential Organ Failure Assessment (SOFA) score (Jones *et al.* 2003), and the Simplified Acute Physiology Score (SAPS) II (Le Gall *et al.* 1993) was calculated on admission to the ICU. We determined the renal function disorder as more than 50% increase in serum creatinine levels above the baseline, which was in accordance with the RIFLE (acronym indicating Risk of renal dysfunction; Injury to the kidney; Failure of kidney function, Loss of kidney function, and End-stage kidney disease) criteria (Bellomo *et al.* 2004.)

4.4 Measurement of MIF concentration

Urine and venous blood samples were collected for the measurements of MIF levels on days 0, 2, and 4 from ICU admission. Blood was collected in Vacutainer serum tubes with silicon coating as clot accelerator (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and it was kept in the tubes at room temperature to clot for at least 60 min. Serum was collected after centrifugation at 1300 g for 10 min at room temperature, then it was aliquoted and stored at -70°C until the analysis. The levels of MIF were measured in urine and serum by using standard enzyme-linked immunosorbent assay (ELISA) kits (catalog number: DY289; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's recommendations as in a previous study (Marton *et al.* 2011). All measurements were performed in duplicates. The plates were read at 450 nm by using an iEMS MF microphotometer (Thermo Labsystem, Beverly, MA, USA). When studying renal dysfunction, the levels of urine MIF were also

calculated as ratios relative to the urine creatinine level based on earlier studies (Hong *et al.* 2012; Otukesh *et al.* 2009). The timing of the MIF level measurements and of the follow up period was based on the actual guidelines of our Department of Anesthesiology and Intensive Therapy and on the data obtained in our meta-analysis.

4.5 Statistical analysis

The R software was used to perform the statistical analysis of the collected data (version 3.6.1; R Development Core Team, Vienna, Austria). Significant differences in urine and serum MIF levels between survivors and nonsurvivors were studied by the Mann-Whitney test. In subgroup analysis, repeated measures ANOVA was performed with time and either sex or age as the independent variables, while either serum MIF or urine MIF as a dependent variable. Frequency tables for deaths were generated in groups with different patterns of MIF kinetics, and then the number of deaths were compared with the Fisher test between the groups. The data are reported in the mean ± standard error (SE) format, unless specified otherwise. Depending on the normal or nonnormal distribution of the data, we used repeated measures ANOVA or Mann-Whitney test, respectively. However, for better visual comparison, in most figures we present the results as box plots.

Results

Approach 1: meta-analysis of published human data

5.1 Study characteristics

Our literature search identified a total of 621 articles from the CENTRAL, Embase, and PubMed databases published until December 2019. When we enabled the online available filter for human studies and removed the duplicates, altogether 315 papers remained, which were screened for title and abstract. Thereafter, we obtained the full text of 45 articles, and, from those selected 21 papers that were eligible for our analyses (Ameen *et al.* 2016; Beishuizen *et al.* 2001; Bozza *et al.* 2004; Brenner *et al.* 2010; Calandra *et al.* 2000; Chuang *et al.* 2007; Chuang *et al.* 2014; de Mendonca-Filho *et al.* 2005; Emonts *et al.* 2007; Gando *et al.* 2007; Gao *et al.* 2007; Kofoed *et al.* 2006; Leaver *et al.* 2010; Lehmann *et al.* 2001; Lehmann *et al.* 2008; Meawed *et al.* 2015; Merk *et al.* 2011; Miyauchi *et al.* 2009; Payen *et al.* 2012; Pohl *et al.* 2017; Wiersinga *et al.* 2010). The flowchart of the study selection is presented in Figure 1. The analyzed papers included 1876 human subjects, among which there were 1206 septic patients, 134 patients with noninfectious systemic inflammation, and 536 healthy controls (i.e., subjects without known systemic inflammation). The characteristics of the studies and the patient population are summarized in Table 1.

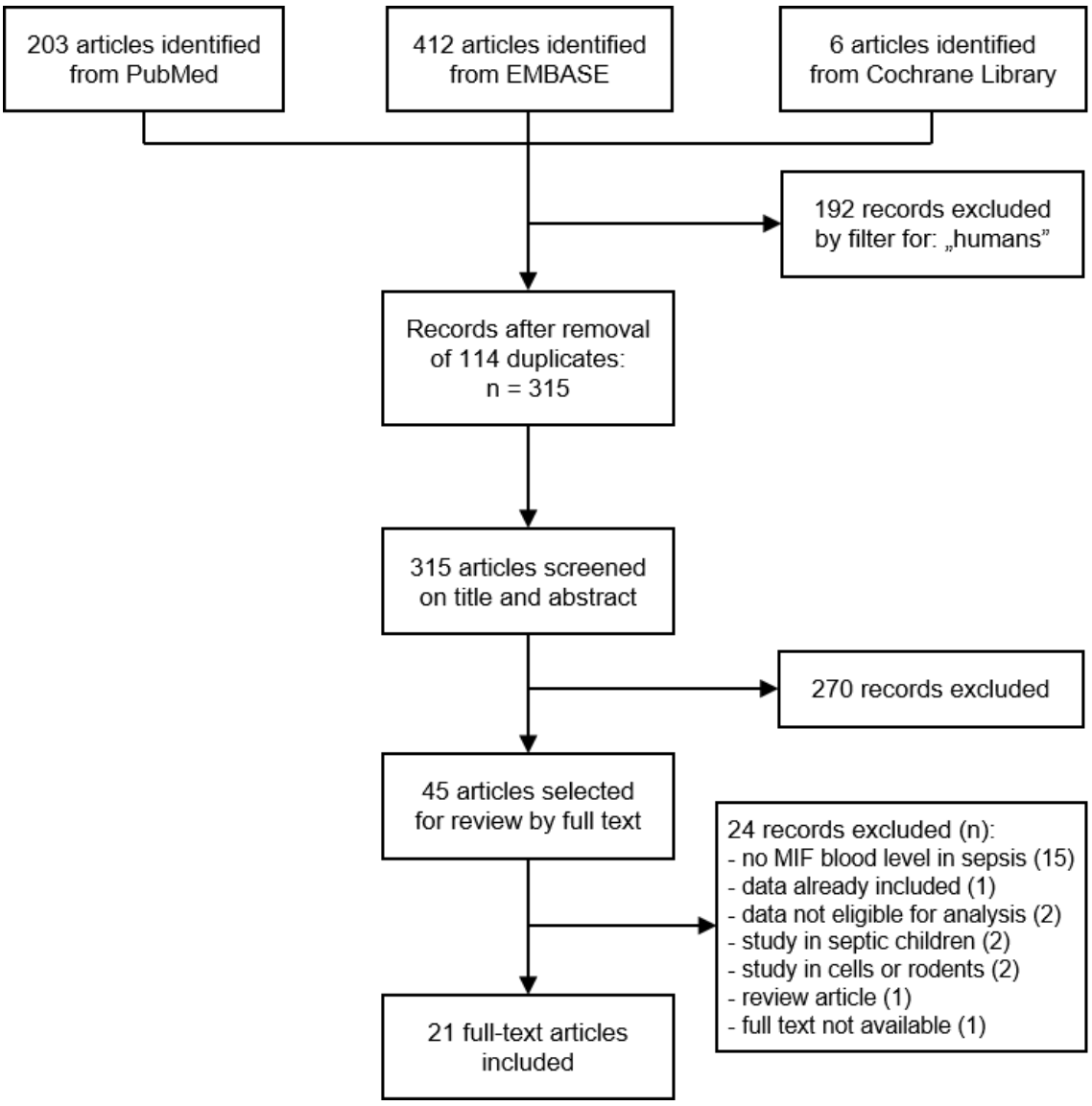

Figure 1. Flowchart of study selection and inclusion in our meta-analysis (Toldi *et al.* 2021).

***Table 1**. Characteristics of participants in the studies included in the meta-analysis (Toldi et al. 2021)*

Study report	Country	Study population	Population subgroups	N (males)	Mean years of age (SD)	Mean severity score (SD)	Deaths N (%)
Ameen et al. 2016	Kingdom of Saudi Arabia	Severe sepsis and septic shock	Survivor	22 (12)	59 (5)	APACHE = 25 (4)	0
			Nonsurvivor	17 (9)	64 (4)	APACHE = 21 (2)	17 (100)
Beishuizen et al. 2001	The Netherlands	Healthy control		41 (23)	62 (9)	NA	0
		Multiple trauma		8 (7)	52 (17)	APACHE II = 10 (2)	0
		Septic shock		32 (20)	64 (13)	APACHE II = 15 (6)	11 (34)
			Survivor	21 (NR)	61 (11)	APACHE II = 12 (5)	0
			Nonsurvivor	11 (NR)	67 (14)	APACHE II = 18 (5)	11 (100)
			Without ARDS	24 (NR)	59 (13)	APACHE II = 11 (6)	NR
			With ARDS	8 (NR)	64 (12)	APACHE II = 19 (4)	NR
Bozza et al. 2004	Brazil	Healthy control		11 (NR)	NR	NA	NR
		Sepsis		17 (10)	59 (23)	APACHE II = 17 (6)	3 (18)
		Septic shock		25 (15)	59 (27)	APACHE II = 21 (7)	13 (52)
Brenner et al. 2010	Germany	Healthy control		18 (10)	35 (9)	NA	0
		Major surgery		28 (12)	62 (14)	NR	0
		Severe sepsis and septic shock	Survivor and nonsurvivor	87 (51)	69 (12)	NR	44 (51)
Calandra et al. 2000	Switzerland	Healthy control		6 (NR)	median = 40	NA	NR
		Sepsis	Severe sepsis and septic shock	16 (13)	52 (18)	SAPS II = 45 (14)	6 (38)
Chuang et al. 2007	Taiwan	Severe sepsis	Survivor	81 (44)	67 (23)	APACHE II = 23 (8)	0
			Nonsurvivor	31 (24)			31 (100)
Chuang et al. 2014	Taiwan	Severe sepsis and septic shock	Survivor	109 (68)	71 (15)	APACHE II = 22 (8)	0
			Died in 48h	12 (6)	68 (18)	APACHE II = 27 (7)	12 (100)
			Died after 48h	32 (21)	74 (12)	APACHE II = 25 (8)	32 (100)

Study report	Country	Study population	Population subgroups	N (males)	Mean years of age (SD)	Mean severity score (SD)	Deaths N (%)
de Mendonca-Filho et al. 2005	Brazil	Sepsis	Negative microbiology	24 (16)	70 (2)	APACHE II = 15 (1)	5 (21)
			Positive microbiology	25 (17)	71 (2)	APACHE II = 16 (1)	12 (48)
Emonts et al. 2007	Switzerland and The Netherlands	Healthy control		196 (NR)	NR	NA	NR
		Sepsis, severe sepsis, and septic shock	Survivor	36 (18)	47 (17)	NR	0
			Early death	20 (17)	53 (14)	NR	20 (100)
			Late death	12 (9)	61 (13)	NR	12 (100)
Gando et al. 2007	Japan	Healthy control		10 (NR)	NR	NA	NR
		SIRS and sepsis	Without DIC	28 (17)	56 (3)	APACHE II = 17 (1)	1 (4)
			With DIC	20 (8)	51 (5)	APACHE II = 27 (2)	12 (60)
Gao et al. 2007	USA	Healthy control		53 (NR)	NR	NA	NR
		Sepsis		36 (NR)	NR	NR	NR
		Sepsis-induced acute lung injury		53 (NR)	NR	NR	19 (36)
Kofoed et al. 2006	Denmark	Healthy control		10 (NR)	NR	NA	NR
		Sepsis		10 (NR)	NR	NR	NR
Leaver et al. 2010	UK	Healthy control		20 (10)	NR	NA	NR
		Severe sepsis and septic shock		35 (22)	62 (22)	19 (6)	10 (29)
Lehmann et al. 2001	Germany	Healthy control		10 (NR)	NR	NA	NR
		Nonseptic critically ill		18 (17)	60 (18)	SOFA = 2 (1)	NR
		Severe sepsis		19 (14)	44 (16)	SOFA = 10 (2)	NR
Lehmann et al. 2008	Germany	Healthy control		34 (NR)	NR	NA	NR
		Nonseptic critically ill		10 (7)	61 (17)	SOFA = 3 (1)	0
		Severe sepsis	Survivor	23 (NR)	55 (11)	SOFA = 9 (3)	0
			Nonsurvivor	14 (NR)		SOFA = 16 (3)	14 (100)
Meawed et al. 2015	Egypt	Nonseptic systemic inflammation		28 (19)	50 (5)	NR	NR
		Sepsis	Survivor and nonsurvivor	25 (15)	53 (6)	APACHE II = 17 (3)	4 (16)
		Severe sepsis		27 (16)	63 (7)	APACHE II = 20 (3)	15 (56)

Study report	Country	Study population	Population subgroups	N (males)	Mean years of age (SD)	Mean severity score (SD)	Deaths N (%)
Merk et al. 2011	Canada	Healthy control		85 (NR)	NR	NA	NR
		Severe sepsis and septic shock		37 (22)	60 (17)	APACHE II = 22 (7)	10 (27)
Miyauchi et al. 2009	Japan	Sepsis	Normal adrenal response	22 (14)	63 (17)	APACHE II = 26 (6)	6 (27)
			Adrenal insufficiency	19 (16)	66 (15)	APACHE II = 26 (10)	6 (32)
Payen et al. 2012	France	Severe sepsis and septic shock	Without acute kidney injury	47 (30)	median = 60	median SOFA = 5	6 (12)
			Mild acute kidney injury	75 (47)	median = 61	median SOFA = 7	20 (26)
			Severe acute kidney injury	54 (34)	median = 63	median SOFA = 10	22 (41)
Pohl et al. 2017	Germany	Healthy control		10 (NR)	NR	NA	NR
		Nonseptic critically ill		42 (28)	69 (13)	APACHE II = 24 (9)	35 (83)
		Severe sepsis and septic shock		30 (19)	69 (11)	APACHE II = 26 (9)	13 (43)
Wiersinga et al. 2010	Thailand	Healthy controls		32 (23)	41 (9)	NA	NR
		Sepsis	Survivor and nonsurvivor	34* (17)	52 (16)	NR	15* (44)

*MIF levels were reported for 29 septic and 10 survivor patients. ARDS, adult respiratory distress syndrome; APACHE, acute physiology and chronic health evaluation score; DIC, disseminated intravascular coagulation; NA, not applicable; NR, not reported; SAPS, simplified acute physiology score; SIRS, systemic inflammatory response syndrome; SOFA, sequential (sepsis-related) organ failure assessment score.

5.2 The diagnostic performance of blood MIF levels in sepsis

When we studied the difference in blood MIF levels between septic patients and healthy control subjects, we included 14 studies, which contained data from 579 septic patients and 536 healthy participants. The relative weight of the studies used in the forest plot was similar, ranging between 5 and 8% (Figure 2).

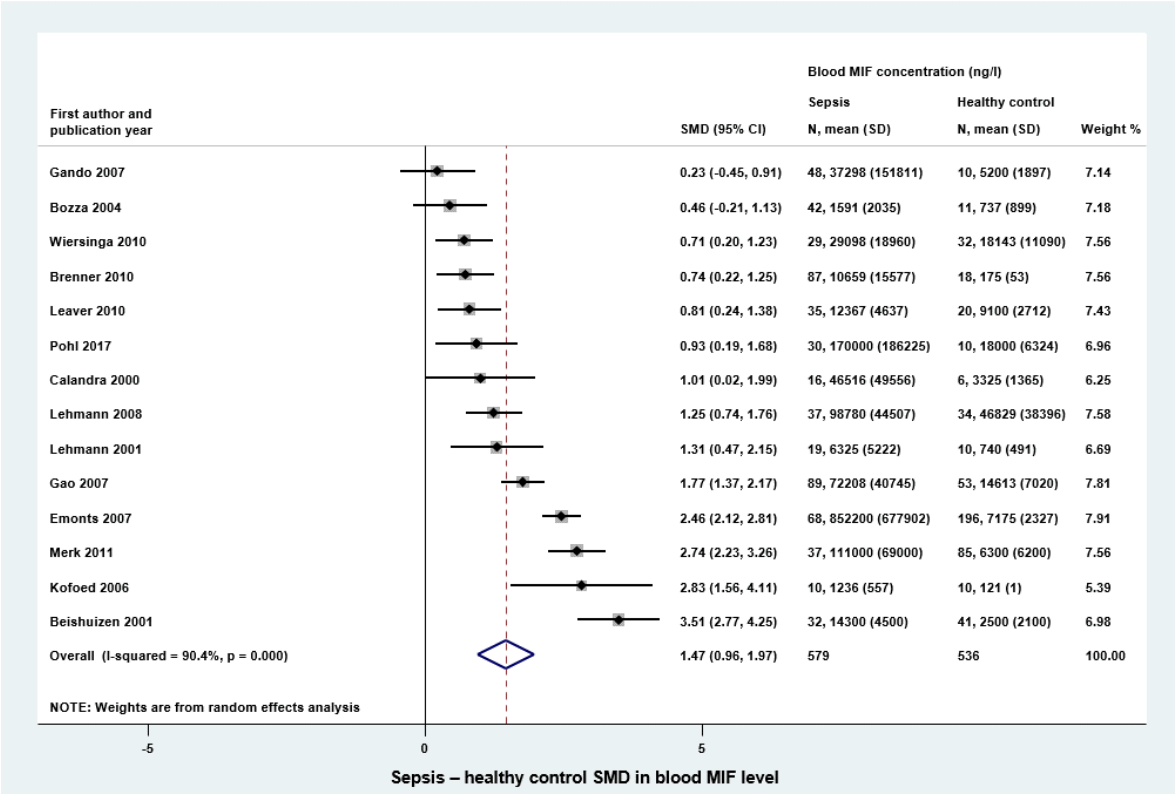

Figure 2. Forest plot of SMDs in blood levels of MIF between septic patients and healthy controls (Toldi *et al.* 2021). Here, and in Figures 4-6 black diamonds represent the SMD for each study, while the left and right horizontal arms of the diamonds indicate the corresponding 95% CIs. The size of the gray box surrounding the diamond is proportional to the relative weight of the study. The open rhombus on the bottom represents the average SMD calculated from the SMDs of all individual studies. The left and right vertices of the rhombus represent the CIs of the average SMD, while the vertical diagonal and the dashed line indicate the average SMD of all studies in the forest plot. A negative SMD indicates higher MIF levels in healthy controls, whereas an SMD greater than zero indicates increased MIF levels in sepsis.

In accordance with the function of MIF as a proinflammatory cytokine (Calandra & Roger 2003), in sepsis the levels of MIF in the blood were higher than in healthy conditions with SMDs ranging from 0.23 to 3.51 between the septic and healthy groups. Overall, in septic patient groups blood MIF levels were significantly higher than in healthy controls with an SMD of 1.47 (95% CI: 0.96–1.97) (Figure 2). In the included studies, the authors used different methods to determine blood MIF levels, which may explain why the values varied greatly even in healthy controls. The detailed description and comparison of the used methods would be beyond the scope of the current work, and it must be also noted that such list would be most probably incomplete, because the authors did not always provide detailed description about the applied methods. Nevertheless, our results confirm that MIF is elevated in sepsis compared to controls. Next, we also wanted to see its diagnostic performance based on ROC curve analysis. We found three studies which presented blood MIF level values of individual participants (Leaver *et al.* 2010; Merk *et al.* 2011; Wiersinga *et al.* 2010). From these, we could extract the data of 101 septic patients and 141 healthy controls. Our ROC curve analysis of these data resulted in an AUC of 0.850 (Figure 3), which demonstrates that blood MIF level measurement shows good sensitivity and specificity for the diagnosis of sepsis.

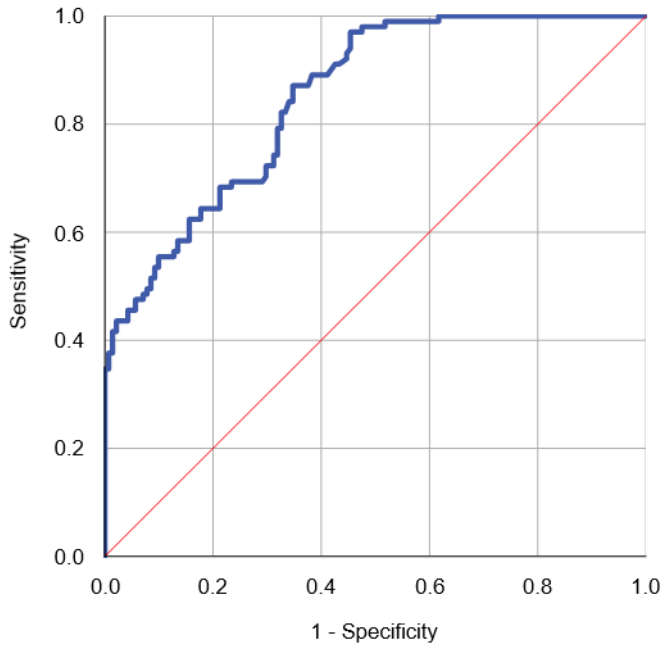

Figure 3. ROC curve analysis of the diagnostic performance of MIF levels in sepsis (Toldi *et al.* 2021). The individual data of septic patients (N = 101) and healthy controls (N = 141) were extracted from previously published studies (Leaver *et al.* 2010; Merk *et al.* 2011; Wiersinga *et al.* 2010). The area under the blue ROC curve was 0.850. The diagonal red line serves as a reference line corresponding to the ROC curve of a diagnostic test that randomly classifies the condition (i.e., a test that has no diagnostic ability).

Then, perhaps as the most interesting approach in assessment of the diagnostic value of MIF, we studied whether the magnitude of the elevation of blood MIF levels are different between sepsis and systemic inflammation due to noninfectious etiologies. We included six studies in our meta-analysis, which reported data from 257 septic patients and 134 patients with nonseptic systemic inflammation (Figure 4).

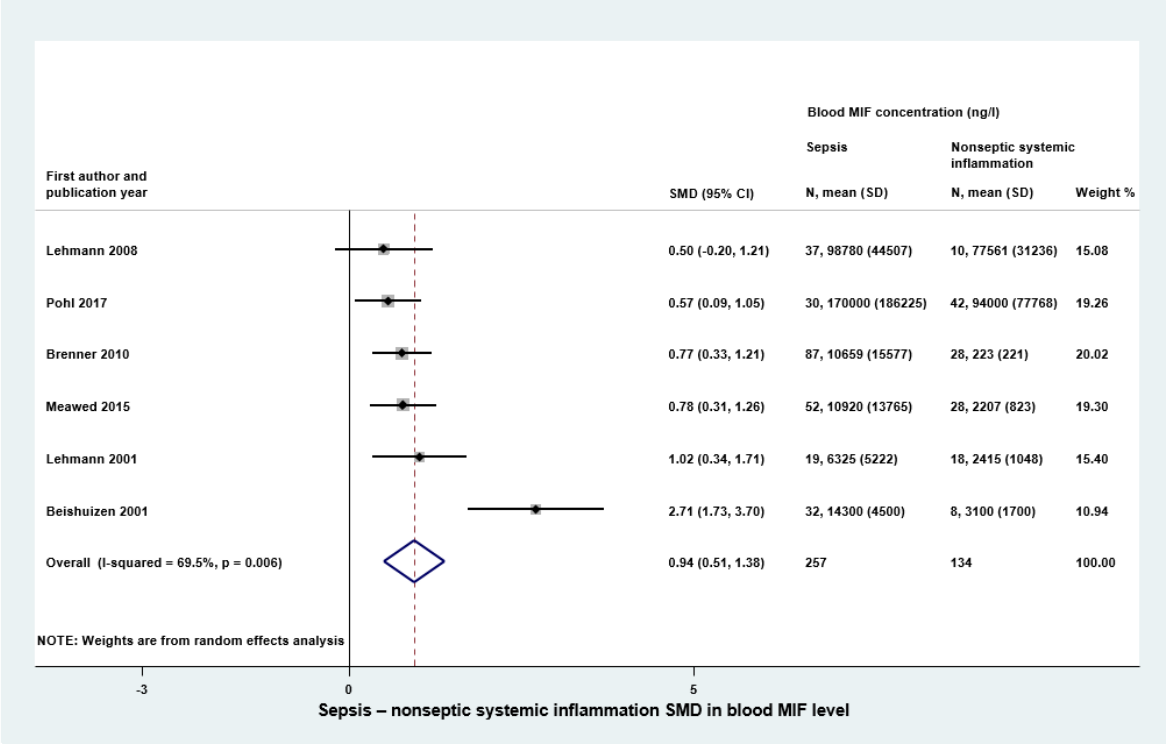

Figure 4. Forest plot of SMDs in blood levels of MIF between septic patients and patients with systemic inflammation due to noninfectious causes (Toldi *et al.* 2021).

In the latter group, the cause of systemic inflammation was either surgery (Lehmann *et al.* 2001; Brenner *et al.* 2010; Lehmann *et al.* 2008) or multiplex traumatic injury (Beishuizen *et al.* 2001), or fever not related to sepsis (Meawed *et al*. 2015), or critical illness (Pohl *et al.* 2017) (see also Table 1). The relative weight of the studies ranged from 11 to 20%. The MIF levels in the blood were higher in septic patients than in patients with nonseptic systemic inflammation in all of the analyzed individual studies. Importantly, the overall SMD was 0.94 (95% CI: 0.51–1.38), which was significantly different between the two groups (Figure 4). Unfortunately, we could not collect enough individual patient data from the literature or from the authors that would have allowed us to perform a ROC curve

analysis for diagnostic performance (i.e., sensitivity and specificity) of MIF between the septic and nonseptic patient groups.

5.3 The prognostic value of blood MIF levels in sepsis

So far, we have studied the usability of blood MIF levels as a biomarker for the diagnosis of sepsis. Nevertheless, we also wanted to know whether the increased blood MIF levels can predict the clinical progression of the disease. We found eligible data to address this question from two approaches: (1) by comparing patient groups with less severe and more severe forms of sepsis based on different parameters (e.g., the absence or presence of organ dysfunction) within the same study; and (2) by comparing survivor and nonsurvivor septic patient groups within the same study. In eleven included studies the blood MIF levels were reported in different severity groups of sepsis. The classification of the severity of the disease into more severe and less severe groups was based on the presence of one of the following conditions: severe sepsis (Meawed *et al.* 2015), septic shock (Calandra *et al.* 2000; Bozza *et al.* 2004), DIC (Gando *et al.* 2007), organ damage (pulmonary, renal or adrenal gland dysfunction) (Beishuizen *et al.* 2001; Gao *et al* 2007; Miyauch *et al.* 2009; Payen *et al.* 2012), early fatality (Emonts *et al.* 2007; Chuang *et al.* 2014), or positive hemoculture (de Mendonca-Filho *et al.* 2005) (also see Table 1). As it could be expected, in most cases, the clinical severity scores were higher in the patient groups with more severe disease. Altogether, 347 patients were categorized as the more severe and 274 patients as the less severe septic groups. The relative weight of the studies was similar, ranging between 7 and 11%. Our forest plot showed that blood MIF level was significantly higher in the more severe

forms of sepsis than in the less severe forms with an overall SMD of 0.84 (95% CI: 0.45–1.24) (Figure 5).

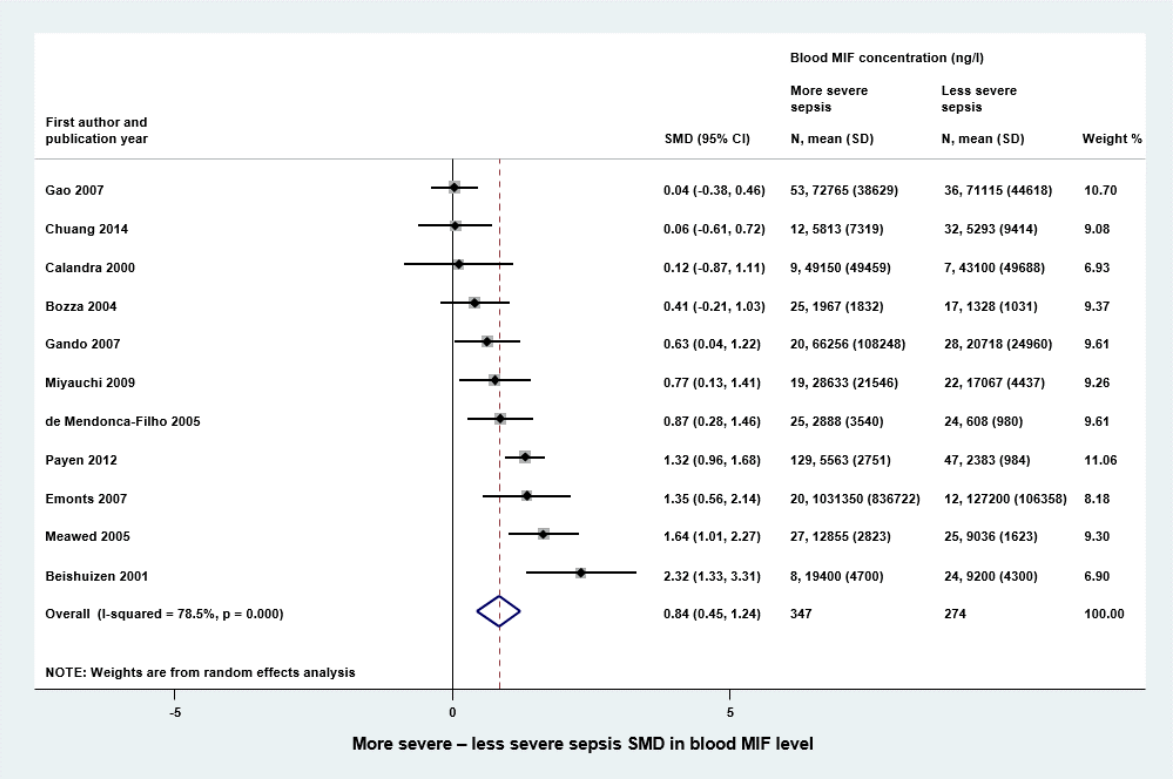

Figure 5. Forest plot of SMDs in blood levels of MIF between patients with more severe and less severe forms of sepsis (Toldi *et al.* 2021).

In our second approach to investigate the prognostic usability of MIF in sepsis, the blood MIF levels were compared between survivors and nonsurvivors of sepsis. For that, we found 11 studies, which included 447 survivors and 257 nonsurvivors of sepsis. As in our former forest plot, these studies had similar relative weights, ranging from 7 to 11%. We calculated the SMD by subtracting the mean blood MIF level of sepsis survivors from that of sepsis nonsurvivors. Thus, a positive result indicated higher MIF levels in patients who died,

whereas negative values would have indicated higher levels in the survivors. It should be noted, however, that the SMD was not negative in any of the analyzed studies. With regards to the summed difference, we found that the overall SMD was significantly higher than zero (0.75, 95% CI: 0.40–1.11) (Figure 6), which demonstrated that blood MIF levels were markedly higher in nonsurvivors than in survivors of sepsis.

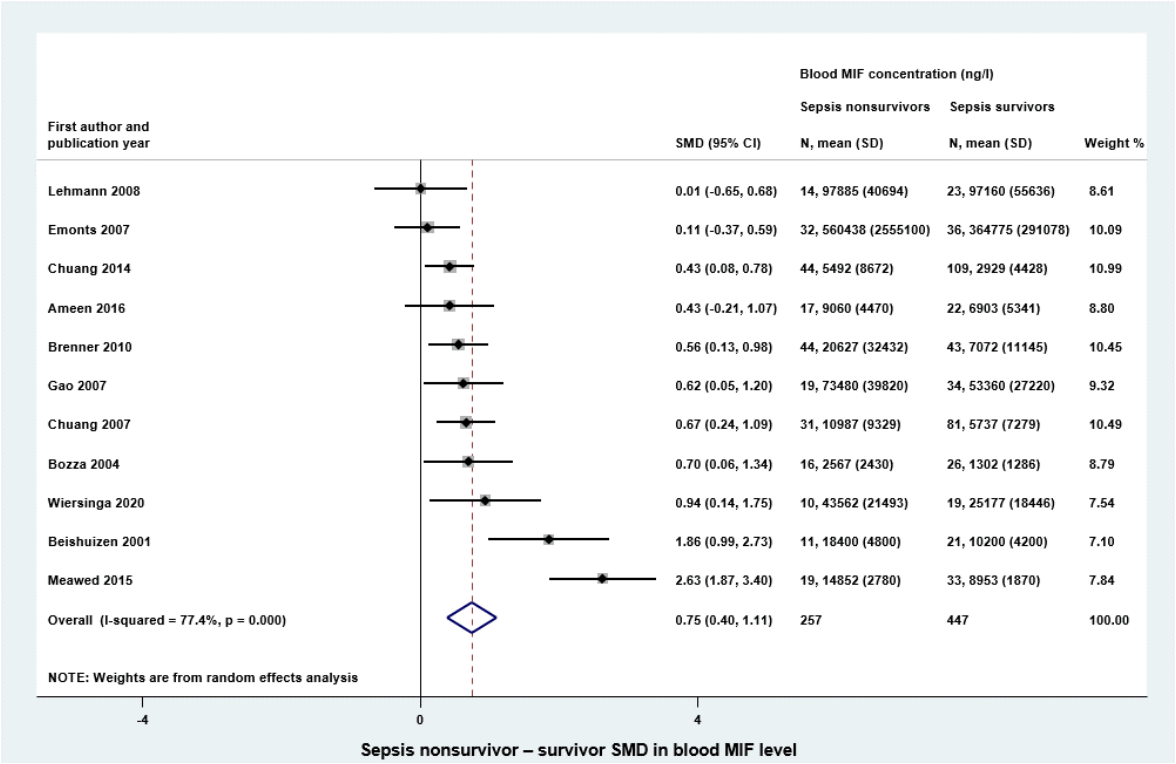

Figure 6. Forest plot of SMDs in blood levels of MIF between sepsis nonsurvivors and survivors (Toldi *et al.* 2021).

6 Approach 2: prospective, observational clinical study

The results of our meta-analysis presented as Approach 1 above, clearly indicated that the blood level of MIF on the day of hospital admission can be used as a valuable biomarker for the diagnosis of sepsis and for prediction of the severity of the disease. However, we did not find enough eligible data to answer further important questions related to the prognostic

biomarker value in sepsis, such as 1) how are the kinetics of blood MIF after ICU admission? 2) are the kinetics of urine MIF similar to those in the blood? 3) are the kinetics different between sepsis survivors and nonsurvivors? 4) are there any intersex differences in the kinetics?. To find answers to these questions, we conducted a single-center prospective, observational study with repeated measurements of MIF in serum and urine on days 0, 2, and 4 from admission to the ICU at the University of Pecs, Hungary.

6.1 Patient enrollment and characteristics

Fifty-nine patients were found eligible for the study according to the inclusion criteria during the study period, but only 51 patients could be enrolled, because 8 of them refused to participate in the study. In addition, one patient had to be excluded, because the outcome could not be assessed at the end of the 90-day follow up. In sum, we included data from 50 patients in the final analysis (for the flow diagram, see Figure 7); their baseline characteristics can be found in Table 2 together with the statistical comparison of the parameters between survivors (N = 21) and nonsurvivors (N = 29). The death rate was 58% in this study population, which is comparable with recent data reported in the literature (Bauer *et al.* 2020). The sex and age distribution of the patients were similar in the two groups, so was the number of cases with renal dysfunction as assessed by the RIFLE criteria (Bellomo *et al.* 2004). Except for the SAPS II and SOFA scores, which tended to be higher in nonsurvivors than in survivors ($p = 0.15$ and 0.16, respectively), as it could be expected, we did not detect any meaningful difference between the two outcome groups at admission to the ICU. As mentioned before, the timing of the MIF level measurements and of the follow

up period was based on the actual guidelines of our Department of Anesthesiology and Intensive Therapy and on the data obtained in our meta-analysis**.**

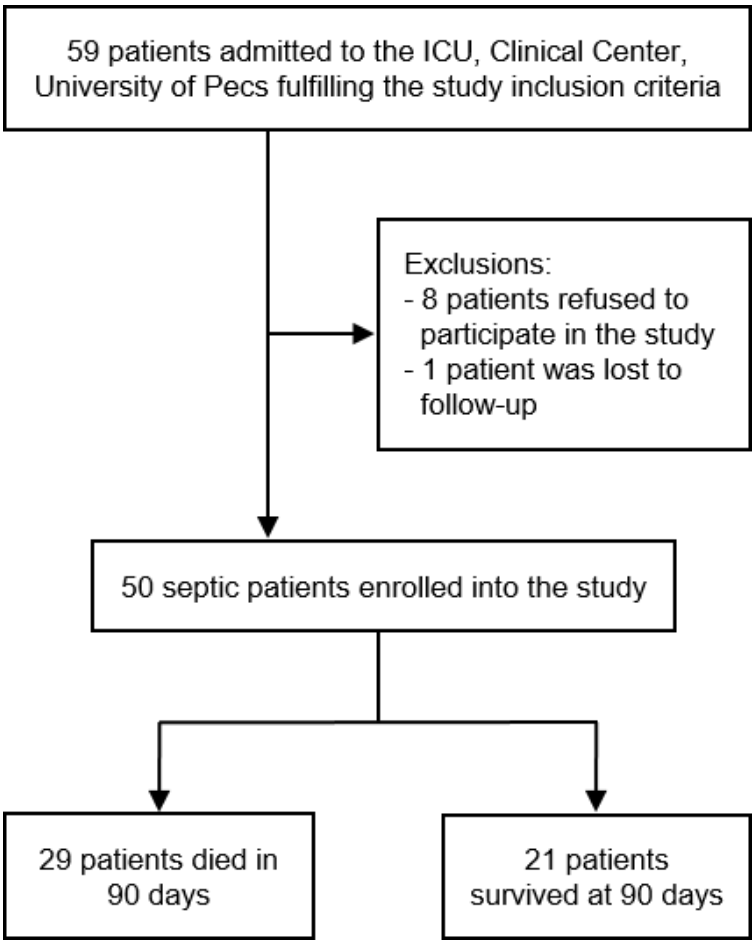

Figure 7. Flow diagram of patient enrollment in our clinical study (Toldi *et al.* 2023).

***Table 2**. Baseline characteristics of the survivor and nonsurvivor septic patients enrolled into our clinical study (Toldi et al. 2023)*

Parameters (unit)	**Survivors (N = 21)**	**Nonsurvivors (N = 29)**	**All (N = 50)**	**p value**
Demographic characteristics				
Age (years)	67 ± 3	66 ± 3	66 ± 2	0.78
65 years old or older, n (%)	12 (57)	17 (59)	29 (58)	1.00
Female, n (%)	12 (57)	11 (38)	23 (46)	0.57
Blood test results				
Red blood cell count (10^{12}/l)	3.7 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	0.17
White blood cell count (10^9/l)	13.6 ± 0.2	15.1 ± 2.1	14.5 ± 1.5	0.63
Neutrophil percentage (%)	12 ± 2	14 ± 2	13 ±1	0.68
C-reactive protein (mg/l)	232.4 ± 28.1	253.5 ± 21.8	244.7 ± 17.2	0.56
Procalcitonin (ng/ml)	23.62 ± 10.99	40.22 ± 10.19	33.86 ± 7.60	0.27
Lactate (mmol/l)	4.4 ± 1.3	4.0 ± 1.3	4.2 ± 0.9	0.81
Creatinine (µmol/l)	180.2 ± 31.0	173.8 ± 23.1	176.5 ± 18.5	0.87
Urea (mmol/l)	13.7 ± 1.8	15.6 ± 1.7	14.8 ± 1.2	0.47
Estimated glomerular filtration rate (ml/min/1.73 m^2)	37.6 ± 5.1	39.6 ± 4.4	38.8 ± 3.3	0.78
Urine test results*				
Total protein (mg/l)	1193 ± 642	713 ± 213	856 ± 240	0.49
Creatinine (mmol/l)	4.5 ± 1.0	5.2 ± 0.8	5.0 ± 0.6	0.61
Clinical status evaluation				
APACHE II (score)	17 ± 2	19 ± 2	18 ± 1	0.39
SAPS II (score)	40 ± 4	49 ± 4	46 ± 3	0.15
SOFA (score)	8 ± 1	10 ± 1	10 ± 1	0.16
Renal dysfunction, n (%)	13 (62)	16 (55)	29 (58)	0.77

*urine samples for the present study could not be obtained on the day of admission from 6 patients (1 survivor and 5 nonsurvivors). Data are expressed as mean ± SE, except for the sex, elderly, and renal dysfunction ratio, where number (and percentage) of patients is shown.

6.2 The levels of MIF in the serum and urine in septic patients after ICU admission

First, we investigated the median levels of serum and urine MIF in all septic patients on days 0, 2, and 4 from admission to our ICU (Figure 8). We found that the MIF levels were higher in the serum than in the urine with medians of 2500, 2255, and 3209 pg/ml in serum versus 965, 1013, and 845 pg/ml in urine, on day 0, 2, and 4, respectively. Based on previous studies (Hong *et al.* 2012; Otukesh *et al.* 2009), we normalized urine MIF levels for urine creatinine, which did meaningfully impact the observed kinetics. The medians were not statistically different between the days either in the serum or in the urine samples, even though there was a 28% increase in serum MIF from day 0 to day 4.

We also studied whether the serum and urine MIF kinetics observed in all patients remain similar when the patients are divided into subgroups based on sex (Figure 8B), age (Figure 8C), and survival (Figure 8D). We could not detect any statistical difference between males and females in serum and urine MIF levels. With regards to kinetics, the serum and urine MIF levels did not change meaningfully over time in either of the sexes. It should be noted, however, that on all days the urine MIF levels seemed somewhat higher in females than in males, but the intersex difference did not reach the level of significance. The normalization of urine MIF levels for urine creatinine did meaningfully impact the observed kinetics in either sex.

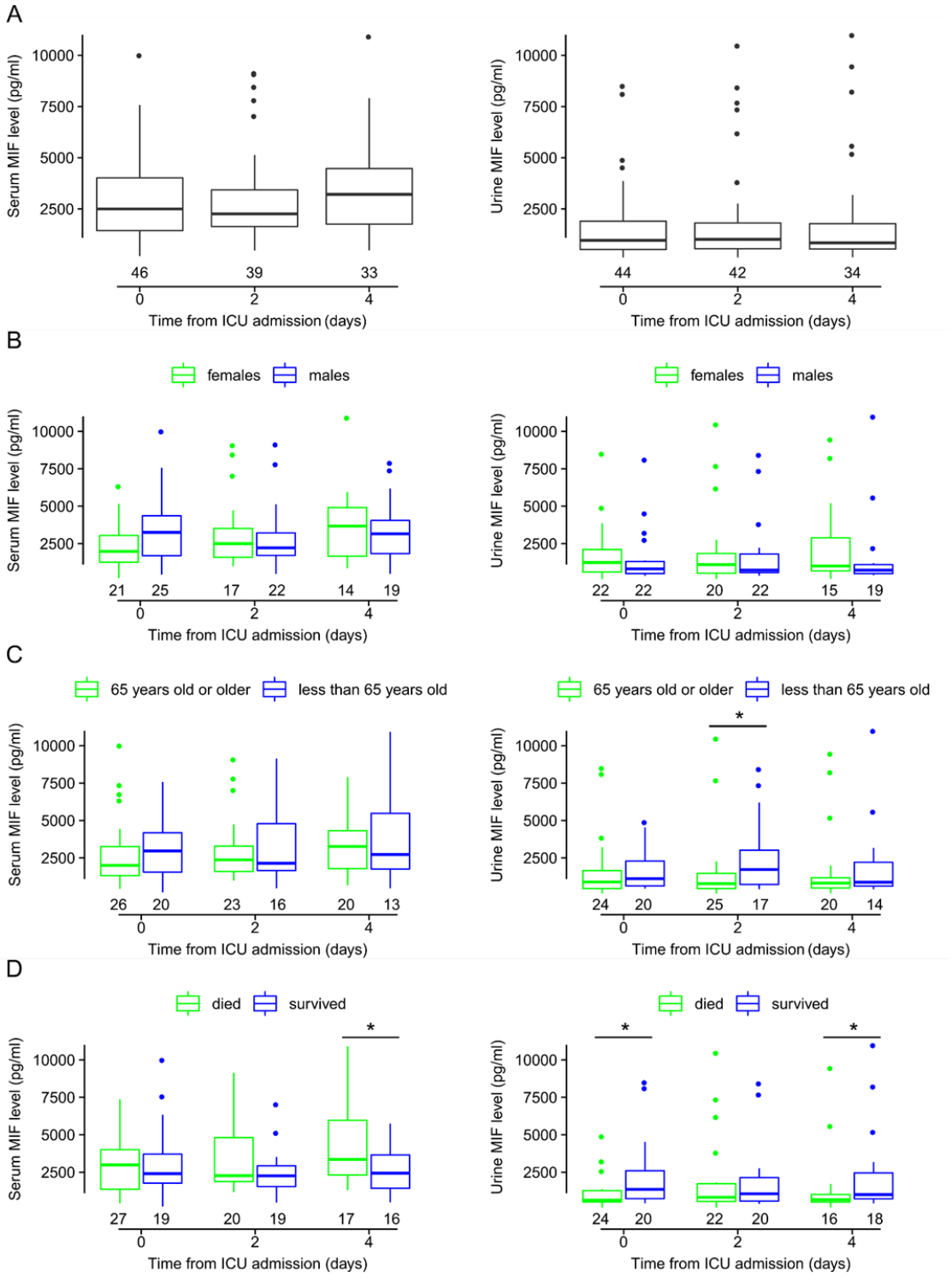

Figure 8. The serum and urine levels of MIF in septic patients on days 0, 2, and 4 from admission to the ICU (Toldi *et al.* 2023): (**A**) all patients; (**B**) females and males, (**C**) at least 65 years old and

Figure 8. (*continued*) younger than 65 years, and (**D**) deceased and survived. Here, and in Figure 12A, the horizontal line within each box represents the median, the bottom and the top of the box marks the lower and the upper quartile, respectively, which limit the interquartile range (IQR). The vertical line below and above the box shows the minimum and maximum levels, respectively. Outliers are shown with dots. The numbers below the boxes indicate the number of patients in each group. *$p < 0.05$.

When patients were divided into younger (less than 65 years old) and older groups (65 years old and above), serum MIF levels in the older patient group were 2000, 2368, and 3263 pg/ml on day 0, 2, and 4, respectively. In the younger patient group, the medians on the respective days were 2969, 2142, and 2732 pg/ml. There was no significant difference between the age groups on any of the days. The urine MIF levels did not differ meaningfully in the elderly between the days, while in the younger patients there was an increase from day 0 to day 2 reaching a median of 1722 pg/ml that was significantly different from the older age group (Figure 8C). The urine MIF/creatinine ratio was not significantly different between younger and older patients on any of the days, and it did not change markedly over time in either age group. Since the ratio was not significantly different ($p = 0.385$) between younger and older patients on day 2, these results indicate that the difference in urine MIF between the age groups on day 2 (Figure 8C) was probably due to a difference in general kidney functions and not due to a difference specifically in MIF excretion.

Finally, between survivors and nonsurvivors the median serum MIF levels did not differ statistically on days 0 and 2, however on day 4 serum MIF was significantly ($p = 0.039$) higher in patients who died than who survived with medians of 3348 and 2430 pg/ml, respectively (Figure 8D). These results already suggested that the kinetics of serum MIF

from day 0 to day 4 are different between survivors and nonsurvivors of sepsis. With regards to urine MIF, the medians did not change meaningfully over time in either of the subgroups. However, urine MIF levels were lower in patients who died than who survived on all days, which difference was significant on day 0 (638 vs 1355 pg/ml; p = 0.046) and on day 4 (672 vs 1005 pg/ml; p = 0.032). The normalization of urine MIF levels for urine creatinine did not meaningfully impact the observed kinetics in either subgroup. Importantly, similarly to urine MIF, the significant differences in the ratio were also detectable between nonsurvivors and survivors on day 0 (0.24 vs 0.50 pg/µmol; p = 0.022) and on day 4 (0.24 vs 0.80 pg/µmol; p = 0.003). These findings suggest that the observed differences in urine MIF levels between survivors and nonsurvivors were presumably caused by differences specific to renal MIF excretion and not by differences in general renal functions.

6.3 The kinetics of serum MIF levels in survivors and nonsurvivors of sepsis after ICU admission

Next, we analyzed how the serum MIF levels changed from the first until the last measurement in each enrolled individual patient, and then compared the kinetics between survivors and nonsurvivors of sepsis (Figure 9A). Only those patients were included who had a minimum of two serum MIF level values on different days during their ICU stay (N = 48). Two patients had to be excluded, because they died before a second blood sample collection could be performed. Serum MIF level increased in 15 of 27 deceased patients (~56%), while in the rest of them it did not change (N = 7) or decreased (N = 5). In contrast with the dominantly increasing pattern in the deceased patients, in the survivors the main

(~62%) trend was a decrease in serum MIF level (N = 13), while it increased only in 8 patients out of the 21.

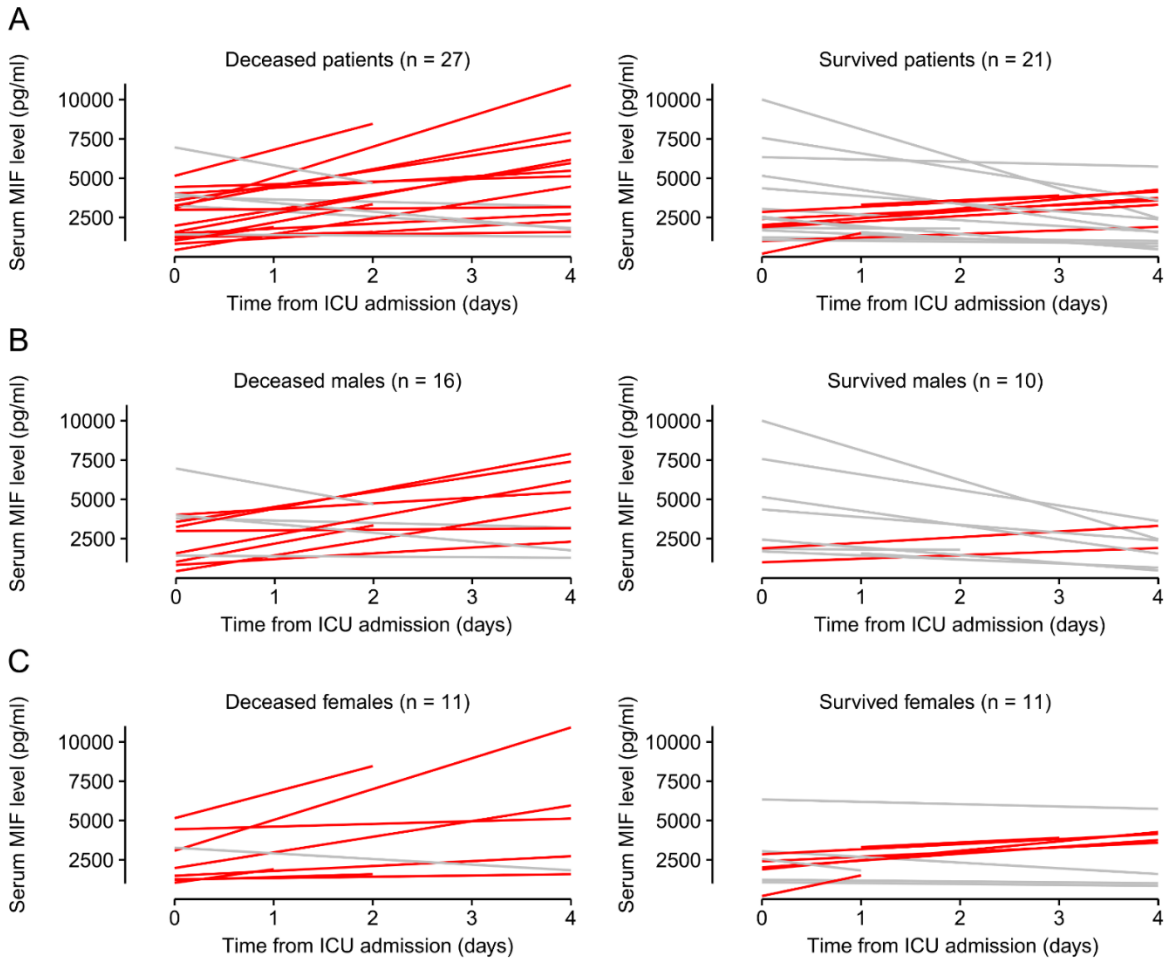

Figure 9. The individual pattern of serum MIF kinetics in each patient who had at least 2 measurements between day 0 and 4 at the ICU (Toldi *et al.* 2023). Red line indicates an increase, while gray line shows no increase in serum MIF level in deceased and survived patients based on data obtained from (**A**) both sexes, (**B**) males, and (**C**) females. The number of patients (n) is indicated in the figure in each group.

According to previous studies, an association between MIF and estrogen was indicated in experimental animal models (Ashcrof *et al.* 2003; Houdeau *et al.* 2007; Hsieh *et al.* 2007), as well as in human subjects (Aloisi *et al.* 2005). Therefore, we also studied the changes in serum MIF levels in males and females separately even at the cost of lowering the number of patients in the analyzed subgroups (Figure 9B and C). In males, similar kinetic patterns were present as in all patients: the most common (50%) trend was an increase in patients who died, while a decrease was the dominant (80%) trend in those who survived (Figure 9B). However, in females the kinetic patterns of serum MIF did not differ meaningfully between survivors and nonsurvivors: an increase was the most common (~73%) in deceased patients, as well as in the survivors (~55%) (Figure 9C).

In our next approach, we wanted to better quantify the difference between the subgroups. For that, we also compared the mean changes of serum MIF levels between days 0 and 4 in all groups (Figure 10). In patients who died, the mean (± SE) serum MIF level increased from 2997 ± 373 pg/ml on day 0 to 4394 ± 646 pg/ml by day 4, whereas in sepsis survivors serum MIF decreased from 3137 ± 576 to 2587 ± 384 pg/ml during the same time interval (Figure 10A). On a daily basis, the change in serum MIF level was significantly different between survivors and nonsurvivors, when we used the data of both sexes ($p = 0.01$) and of males ($p = 0.01$). On the contrary, there was no meaningful difference between the died and survived groups in females ($p = 0.230$) (Figure 10B). When we analyzed the changes in the respective groups on a daily basis, an overall increase versus decrease was present in all and male nonsurvivors versus survivors, respectively, while in females there was on average an increase in both outcome groups.

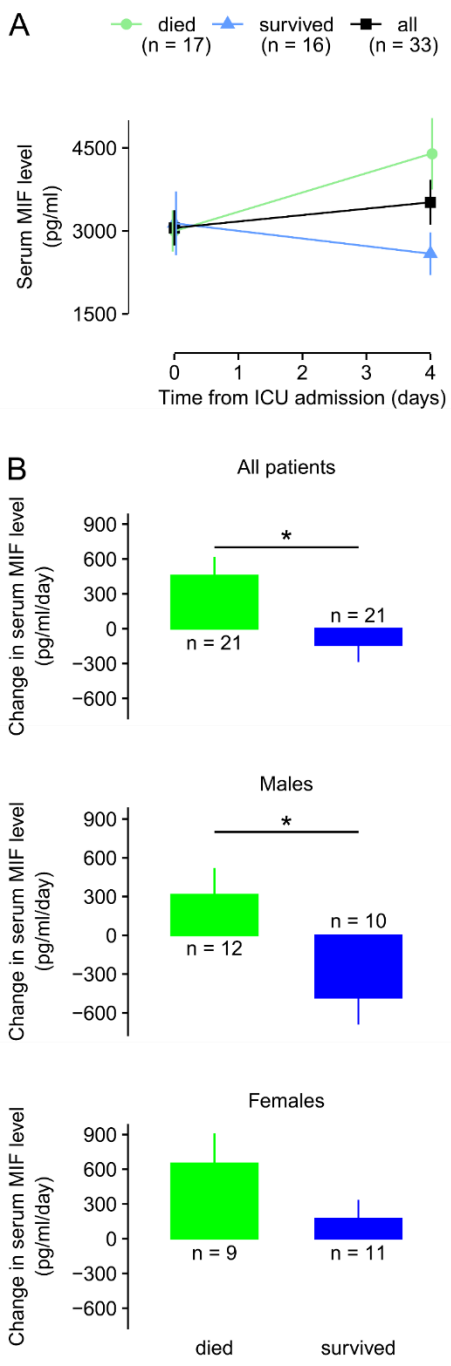

Figure 10. The kinetics of serum MIF levels in septic patients at the ICU (Toldi *et al.* 2023). (**A**) The average levels of serum MIF in all, died, and survived septic patients on day 0 and 4 from ICU admission. (**B**) The mean daily changes of serum MIF levels in died and survived patients based on data used from both sexes (top), males (middle), and females (bottom). The number of patients (n) is indicated in the figure in each group. *p < 0.05.

6.4 The kinetics of urine MIF levels in survivors and nonsurvivors of sepsis after ICU admission

After studying the kinetics of serum MIF in septic patients admitted to the ICU, we also analyzed how its levels change in the urine. As shown in Figure 8D, the urine MIF levels were significantly lower in deceased patients than in survivors on days 0 and 4. With regards to the temporal kinetics, a small and not significant increase was found in both groups from day 0 to day 4: 3021 ± 797 to 3457 ± 1016 pg/ml in survivors and 1281 ± 340 to 1629 ± 654 pg/ml in nonsurvivors (Figure 11A). Moreover, the daily change in the urine levels of MIF did also not differ significantly between survivors and nonsurvivors (109 ± 192 vs 87 ± 152 pg/ml; $p = 0.940$) (Figure 11B). When we compared males and females separately, there was still no significant difference in the daily change ($p = 0.136$ and $p = 0.228$, respectively). In our next attempt, we analyzed the data obtained from both sexes, and we found a significant positive correlation between urine MIF levels measured on day 0 and on day 4 (Figure 11C), suggesting that the level determined on day 0 can predict its level 4 days later.

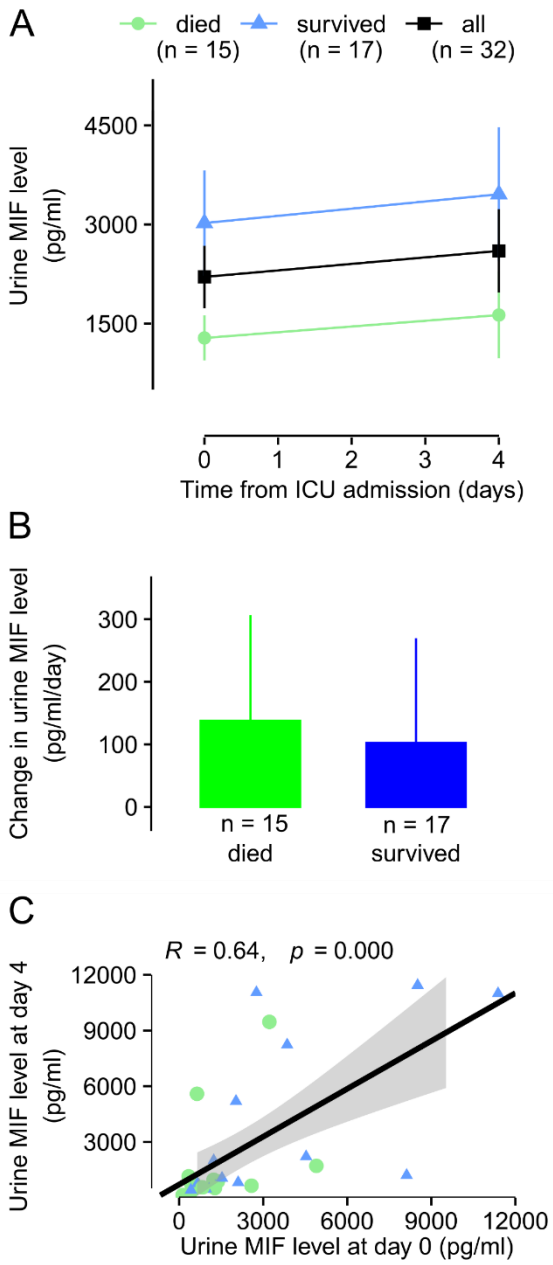

Figure 11. The kinetics of urine MIF levels in septic patients at the ICU (Toldi *et al.* 2023). (**A**) The average levels of urine MIF in all, died, and survived septic patients on day 0 and 4 from ICU admission. (**B**) The mean daily changes of urine MIF levels in died and survived patients. (**C**) The correlation between urine MIF levels measured on day 0 and on day 4 from the admission to the ICU. The number of patients (n) is indicated in the figure in each group.

6.5 The impact of kidney dysfunction on the kinetics of urine MIF levels in septic patients after ICU admission

Since urine MIF levels were suggested to be indicators of renal dysfunction associated with different nonseptic diseases (Hong *et al.* 2012; Otukesh *et al.* 2009; Brown *et al.* 2001; Brown *et al.* 2002), we compared urine MIF levels in septic patients who developed renal dysfunction and in those who did not according to the RIFLE criteria (Bellomo *et al.* 2004). Although the median urine MIF levels seemed higher in patients with healthy kidney functions than in those who had renal dysfunction on days 0, 2, and 4, the difference between the two groups did not reach the level of significance on any of the days (Figure 12A). Normalization of urine MIF levels for urine creatinine did not meaningfully impact the observed kinetics: the urine MIF/creatinine ratio seemed higher in patients without renal dysfunction on days 0 and 2, but the difference was not statistically significant between the groups on any of the days.

With regards to the kinetics, between day 0 and 4 from ICU admission, the urine MIF level changed on average from 2694 to 2534 pg/ml in patients without renal dysfunction, while from 1774 to 2658 pg/ml in patients with renal dysfunction (Figure 12B). There was no significant difference between the groups. The mean daily changes in urine MIF levels were 220 ± 157 pg/ml and -40 ± 191 pg/ml with and without renal dysfunction, respectively (Figure 12C), which were not statistically different between the groups even if the urine MIF/creatinine ratios were used for comparison of the groups.

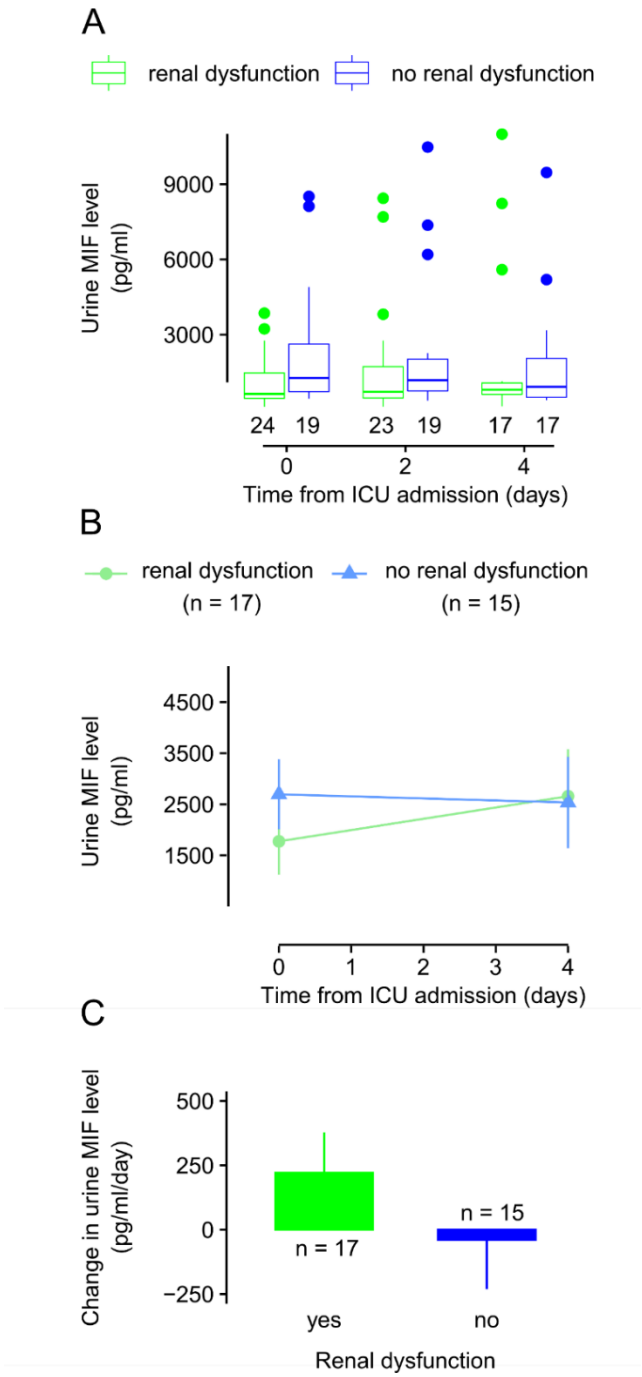

Figure 12. The kinetics of urine MIF levels in septic patients with and without renal dysfunction at the ICU (Toldi *et al.* 2023). (**A**) Box plot of urine MIF levels in septic patients with and without renal dysfunction on day 0, 2, and 4 from ICU admission. (**B**) The mean absolute urine MIF levels on day 0 and 4 in the same groups. (**C**) The mean daily changes of urine MIF in the same subgroups. The number of patients is indicated in the figure in each group.

Discussion

During my studies, we were able to convincingly support the diagnostic and prognostic biomarker value of MIF in sepsis by using a dual research approach. In the first part of my studies, we collected available human data in the literature and showed with meta-analysis that blood MIF level at hospital admission can be used for the diagnosis of sepsis and for its differentiation from noninfectious systemic inflammation. Furthermore, we also found that higher blood MIF levels at hospital admission can predict worse severity and fatal outcome in sepsis, thereby underlying the prognostic biomarker value of MIF. However, questions related to the kinetics of MIF in the blood and urine could not be studied with meta-analysis (due to the unavailability of eligible data). To fill this gap, in the second part of my studies, we conducted a prospective clinical trial, in which we assessed the kinetics of serum and urine MIF in septic patients admitted to the ICU. We showed that an increasing serum MIF pattern was characteristic for patients who died in sepsis, whereas the level was rather decreasing in those who survived. We also revealed intersex differences in the serum MIF level kinetics. Furthermore, we showed that urine MIF level was not associated with renal dysfunction, and it was lower in nonsurvivors than in survivors of sepsis.

Sepsis affects tens of millions of patients annually and it constitutes an ongoing challenge for the healthcare system due to its high mortality and economic burden, especially in its severe forms (Angus *et al.* 2001). In the ICU, hospital-acquired sepsis is frequent and accounts for a high (over 40%) mortality rate (Markwart *et al.* 2020). In order to improve

the outcomes, it is required to further develop the approaches for early diagnosis and implementation of adequate treatment of sepsis. The successful use of biomarker molecules could greatly help to achieve these goals. Not surprisingly, a plethora of potential biomarkers was evaluated for the diagnosis and prognosis of sepsis (Pierrakos *et al.* 2020). Already at the initiation of systemic inflammation, the activation of innate immune cells leads to the production of various inflammatory cytokines (Garami *et al.* 2018). The protein in the focus of my studies, MIF is one of these proinflammatory cytokines (Garai *et al.* 2017). In humans, several studies showed that blood MIF level is increased in different forms of systemic inflammation (Beishuizen *et al.* 2001; Calandra *et al.* 2000; Merk *et al.* 2011), therefore, MIF was proposed as a potential diagnostic and prognostic biomarker in sepsis (Pierrakos *et al.* 2020; Grieb *et al.* 2010; Hertelendy *et al.* 2018). However, it remained unclear whether septic and nonseptic systemic inflammation can be distinguished based on the different extent of elevation in blood MIF levels. Some authors found that MIF levels were higher in sepsis than in noninfectious systemic inflammation (Beishuizen *et al.* 2001; Brenner *et al.* 2010; Meawed *et al.* 2015; Pohl *et al.* 2020), whereas others did not find a significant difference in MIF levels between the two forms of systemic inflammation (Lehmann *et al.* 2001; Lehmann *et al.* 2008). In our analysis (Toldi *et al.* 2021), we compared MIF levels in 257 septic patients and in 134 patients with noninfectious inflammation, and showed that blood MIF concentration is markedly increased in case of sepsis compared to nonseptic systemic inflammation. Our results suggest that MIF can be used as a diagnostic tool to distinguish sepsis from other systemic inflammatory diseases. It can be assumed that the production of MIF is more enhanced when the triggering agent of the inflammatory reaction

is a microbial pathogen than when it is a damage-associated molecular pattern (DAMP). Indeed, it has been shown that DAMPs and pathogen associated molecular patterns (PAMPs) activate the immune system differently. In particular, DAMPs produce weaker innate immune activation than PAMPs, which also involves more pronounced production of inflammatory cytokines in case of PAMPs (Eppensteiner *et al.* 2019). Moreover, the already increased MIF levels in multiple trauma patients were further elevated when an infection developed, suggesting that MIF may be an indicator of secondary infection (Cho *et al.* 2017; Joshi *et al.* 2000).

The potential prognostic value of MIF was also a controversial issue. The levels of MIF tended to be higher in septic shock patients who developed ARDS than in those who did not ($p=0.115$) (Beishuizen et al. 2001), and MIF levels also seemed to be higher in septic shock than in severe sepsis, again, without a clear statistical difference between the groups (Calandra et al. 2000). Furthermore, MIF levels did not differ between survivors and nonsurvivors of severe sepsis (Lehmann et al. 2008), contradicting earlier reports about higher circulating MIF levels in nonsurvivor sepsis patients (Beishuizen et al. 2001; Brenner et al. 2010; Gando et al. 2001). In our work (Toldi et al. 2021), we showed that MIF levels were significantly higher in the groups with worse prognosis, indicating that MIF can be a useful biomarker to predict the severity and the outcome of the disease. It can be assumed that in severe forms of sepsis an overt inflammatory reaction develops, which also involves a pronounced cytokine storm and excessive production of MIF. Hence, the pro- and anti-inflammatory processes become unbalanced, the inflammatory response loses its adaptive biological function, and turns into a dysregulated, destructive process, which is no longer

beneficial, but instead, harmful for the host. Since it is well documented that MIF counter-regulates the anti-inflammatory and immunosuppressive effects of glucocorticoids (Calandra et al. 1995; Daun & Cannon 2000; Mitchell et al. 1999), it can be crucial in the disruption of the pro- and anti-inflammatory balance. With the help of this hypothesis, it can be also explained why the neutralization of MIF with antibodies improved the outcome in animal models of severe systemic inflammation (Bernhagen et al. 1993; Calandra et al. 1995; Kobayashi et al. 1999).

Some limitations of our meta-analysis should be noted. Due to the nature of the method, we have studied the reported mean MIF levels in patient groups, instead of MIF levels in individual patients. The latter approach would certainly allow one to draw firmer conclusions about the association between MIF and the diagnosis and prognosis of sepsis, but that would require access to the original data of the analyzed articles, which was not feasible. Due to lack of data, we could not perform a network meta-analysis to compare the performance of MIF with other frequently used inflammatory biomarkers, hence we cannot make any comment on its real value compared to others. In our study, we compared blood MIF level in septic patients to that of either healthy controls or patients with nonseptic systemic inflammation. This method can be useful to identify potential diagnostic biomarkers, but it cannot be used to determine the diagnostic performance of MIF. An ideal study would include patients who were clinically suspected of sepsis and compare their MIF levels with confirmed diagnosis of sepsis. Unfortunately, the analyzed studies did not have such an ideal design. However, in one of the studies, MIF levels between septic patients and healthy

volunteers were compared and ROC curve analysis was performed, which indicated excellent sensitivity and specificity for MIF (AUC of 0.99) (Merk *et al*. 2011). As an attempt to perform ROC curve analysis, we extracted individual patient data from eligible papers (Leaver *et al.* 2010; Merk *et al*. 2011; Wiersinga *et al.* 2010), and then showed that blood MIF level has good diagnostic performance to distinguish septic patients from healthy controls. However, we could not collect sufficient data to perform the ROC curve analysis for the diagnostic value of MIF between infectious and noninfectious systemic inflammation and for its prognostic performance. Therefore, to exclude the possibility that mean levels of MIF simply differed significantly between the cohorts examined, in future studies additional ROC curve analyses are warranted to support our findings about the diagnostic and prognostic capability of MIF. The studied population of patients was quite diverse and statistical, methodological, and medical differences in study design could all contribute to the considerably high between-study heterogeneity (indicated by an I^2 of 70–90%), as observed in our analysis. To account for the presence of heterogeneity, we used the random-effects model in all forest plots of our meta-analyses. In the analyzed studies, blood MIF levels between patients' groups were compared within the same study and the difference was included in the forest plot. Since the reported MIF values differed substantially among the analyzed studies, ranging between 121 ng/l (Kofoed *et al.* 2006) and 46,829 ng/l (Lehmann *et al.* 2008) in healthy controls, SMDs had to be used to mitigate methodological differences in MIF level measurements. Consequently, in the present analysis we could not determine a specific cut-off MIF level which would be a diagnostic or prognostic threshold in sepsis. Lastly, we could not extract data to determine the kinetics of MIF in the serum and urine

after admission of septic patients to the ICU, therefore, to compare the temporal kinetic changes between survivor and nonsurvivor groups. This latter issue was investigated in the second part of my studies.

Using data obtained from our prospective clinical study (Toldi *et al.* 2023), we presented the kinetics of serum and urine MIF levels in septic patients on the initial days from ICU admission. We showed that the patterns of serum MIF kinetics are different between patients who survived and who died in sepsis. We also reported that serum MIF level increased after ICU admission in those patients who died in sepsis, whereas it decreased in the survivors of the disease. We demonstrated sex-dependent differences in the kinetics of serum MIF in sepsis: the decreasing trend in the survivors was present only in males, but not in females. Moreover, we showed that urine MIF level can be a valuable prognostic marker of mortality in sepsis, as it was markedly lower in nonsurvivors than in survivors, and it did not change significantly over time in either of the groups. We did not find a difference in the urine MIF levels in association with the presence or absence of renal dysfunction.

The serum MIF kinetics clearly differed between sepsis survivors and nonsurvivors after ICU admission, since in the nonsurvivors serum MIF increased, whereas in survivors it decreased. Considering that statistically significant difference between the outcome groups could not always be detected based on single measurements, the new finding about the distinct kinetics indicates that repeated serum MIF level measurements in the same patient can be better predictors of the outcome than single time-point measurement at the ICU. In accordance with our proposal, the significant prognostic value of MIF was not found in some

previous studies, in which the authors performed only one measurement of its serum level (see above).

Interestingly, in survivor and deceased females the patterns of serum MIF kinetics were somewhat different from males. In women, the serum MIF level increased in both groups, though the extent tended to be greater in nonsurvivors than in survivors ($p = 0.13$). Moreover, in the survivors there was an increase in females instead of the decrease observed in males. The observed intersex difference can be due to the influence of sex hormones. Indicating a suppressive role of estrogen on MIF, its levels in the plasma were lower in healthy women than in men (Aloisi *et al.* 2005; Mizue *et al.* 2000). It should be noted, however, that the difference in MIF levels between males and females was only present in the younger population (<55 years old) (Aloisi *et al.* 2005). In our study, the average age of the patients was 66 ± 2 years, and the youngest woman was 47 years old. It can be assumed that the majority of the included females were already in the postmenopausal period, therefore had low estrogen levels. In fact, the plasma estradiol concentration in males was shown to be significantly higher than in postmenopausal women (Vermeulen *et al.* 2002). Therefore, the decreased estrogen levels in postmenopause can serve as a hypothetical reason why the MIF levels increased in both survivor and nonsurvivor septic females to a greater extent than in males in our study. Interestingly, different prognosis between septic males and females was reported earlier (Schroder *et al.* 1998), which might be explained, at least in part, by the intersex differences in serum MIF levels in sepsis as shown in our study.

Besides serum MIF, we also studied the value of urine MIF level as a biomarker in sepsis. We showed that urine MIF remained relatively constant on the initial days after ICU admission in both survivors and nonsurvivors. However, in the deceased patients it was markedly lower than in survivors. Our results indicate that urine MIF can be an easily measurable prognostic biomarker of the outcome in sepsis. Due to its relatively stable levels over time, a random measurement on any day could be possibly used in practice. This is also supported by the strong correlation between the first and last measured urine MIF levels shown in our study. Importantly, the urine MIF levels were similar in patients with and without renal dysfunction. Our results suggest that urine MIF can be used as a predictive biomarker in sepsis independently from the kidney function, however, it does not indicate the development of sepsis-associated acute kidney injury.

The lower urine versus increasing serum MIF level paradox in patients who died in sepsis, can be possibly resolved by taking into account the diverse source and complex role of MIF in inflammation. MIF is synthetized in many cells in the kidney, including tubular cells, podocytes, mesangial, and endothelial cells (Kong *et al. 2022).* While it is constantly produced in the kidney to some extent, in kidney inflammation it is markedly upregulated (Lan 2008). Not surprisingly, the level of urine MIF showed an inferior correlation with serum MIF (Xing *et al.* 2018), indicating that its concentration in the urine is not only influenced by clearance of serum MIF, but also by its renal production and glomerular and tubular processing (Matsumoto *et al.* 2002). The described lack of correlation between serum and urine levels of MIF may also explain why higher serum levels were not accompanied by increased urine levels in nonsurvivors in our clinical study. Renal MIF was shown to possess

a renoprotective function in different kidney diseases (Djudjaj *et al.* 2017; Ochi *et al.* 2017; Stoppe *et al.* 2018), thus it can be speculated that the endogenous renoprotective effect of renal MIF was attenuated in the nonsurvivor group, thereby indicating the increased severity of the disease. This hypothesis might explain our findings, but it should be mentioned that MIF rather caused than prevented the development of kidney injury according to some studies (Chen *et al.* 2015; Lan *et al.* 1997; Leng *et al.* 2011). The nature of the disease, the different sources and roles of MIF in the pathomechanisms were suggested as the causes for the contradictory (i.e., renoprotective versus harmful) roles (Djudjaj *et al.* 2017).

Limitations of our clinical study must be also mentioned. Our sample size was relatively small, which resulted in low number of patients after dividing the population into multiple subgroups (e.g., survivor men and women). The patients were enrolled at a single clinical center in our study, thus further clinical trials at multiple (preferably international) centers are needed to improve diversity of the patients and allow for conclusions in broader population. We focused on patients admitted to the ICU, however, it would be also important to see how MIF kinetics develop in septic patients before the ICU admission (see our meta-analysis), which could help physicians to get an insight about the prognosis at an earlier stage of the disease. Last, we did not correlate the kinetics of MIF levels with other biomarkers, therefore the prognostic performance of MIF could not be compared with other markers.

Conclusions

In conclusion, by using a complex approach (consisting of meta-analysis and clinical study), we provided evidence for the real clinical biomarker value of MIF in sepsis. In our meta-analysis, we concluded that blood MIF levels have the diagnostic capability to differentiate between infectious and noninfectious systemic inflammation and have prognostic value for the outcome of sepsis. In our clinical study, we reported the kinetics of serum and urine MIF in septic patients admitted to the ICU, for the first time to the best of our knowledge. In summary, we showed that an increasing serum MIF pattern was characteristic for patients who died in sepsis, whereas the level was rather decreasing in those who survived. Intersex differences in the serum MIF level kinetics were also revealed. Last, we showed that urine MIF level was not associated with renal dysfunction, and it was lower in nonsurvivors than in survivors of sepsis. Despite of their limitations, together our studies highlight the biomarker value of serum and urine MIF values and kinetics for the diagnosis and for the prediction of the outcome of sepsis. Our results can also serve as an encouraging basis for designing future studies at multinational level, which are required to determine the real biomarker value and clinical feasibility of repeated MIF level measurements in septic patients.

Appendix

Publications related to the subject of the thesis

- Number of publications related to the subject of the thesis: 3
- Number of publications not related to the subject of the thesis: 5
- Number of book chapters: 1
- Sum of all impact factors: 13.470
- Sum of impact factors from publications related to the topic of PhD thesis: 9.596

Publications related to the topic of the PhD thesis

Garai J., Kanizsai P., Rumbus Z., **Toldi J**., Garami A., Az akut szisztémás gyulladás kórélettana az alapkutatásoktól a klinikai vonatkozásokig. *Aneszteziológia és Intenzív Terápia*, 47. évfolyam 4. szám, 5-21, 16 p. (2017).

Toldi J., Nemeth D., Hegyi P., Molnar Zs., Solymar M., Farkas N., Hussain A., Rumbus Z., Pakai E., Garami A., Macrophage migration inhibitory factor as a diagnostic and predictive biomarker in sepsis: meta – analysis of clinical trials. *Scientific Reports* 11:1 Paper: 8051, 12 p. (2021). **Impact factor: 4.996; SJR rank: Q1/D1**

Toldi J., Kelava L., Marton S., Muhl D., Kustan P., Feher Zs., Maar K., Garai J., Pakai E., Garami G. Distinct patterns of serum and urine macrophage migration inhibitory factor kinetics predict death in sepsis: a prospective, observational clinical study. *Scientific Reports* 13:1 Paper: 588, 15 p. (2023). **Impact factor: 4.600; SJR rank: Q1/D1 (2022)**

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Ferencz A, **Toldi J**, Fehér Zs, Gasz B, Benkő L, Jancsó G, Rőth E. NF-kB activation after intestinal preconditioning. International Proceedings, 11th Congress of the European Shock Society, Monduzzi, Editore Press: Bologna 2005, 85-88.

Ferencz A, **Toldi J**, Fehér Zs, Gasz B, Benkő L, Jancsó G, Rőth E. Detection of oxidative stress and NF-kB activation in preconditioned and autotransplanted small bowel. Shock 2005, 23:52.

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International oral and poster presentations

Ferencz A, **Toldi J**, Fehér Zs, Rőth E. Detection oxidative injury with or without small bowel ischemic preconditioning prior to autotransplantation. 5th European Transplant Fellow Workshop, 8-10 October, 2004. Malmö, Sweden.

Ferencz A, **Toldi J**, Fehér Zs, Gasz B, Jancsó G, Rőth E. Detection of oxidative stress and NF-kB activation in preconditioned and autotransplanted small bowel. 11th Congress of the European Shock Society, 8. Vienna Shock Forum, 27-30 January, 2005. Vienna, Ausztria

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Toldi J, Toldiné Beck M, Pető A, Tóth K, Márton S. A posztoperatív fájdalomcsillapítás lehetőségi TLH műtétet követően 2017. Siófok

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Az akut szisztémás gyulladás kórélettana az alapkutatásoktól a klinikai vonatkozásokig

The pathophysiology of acute systemic inflammation: from basic research to clinical aspects

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Összefoglalás: Az akut szisztémás gyulladás etiológiájában és klinikai megjelenésében egyaránt komplex betegségcsoportot foglal magába. Kialakulhat fertőző vagy nem-fertőző ágensek hatására egyaránt és megjelenhet, mint betegség viselkedés/szindróma, szisztémás gyulladásos válasz szindróma, szepszis, szeptikus sokk, vagy akár, mint több-szervi funkciózavar szindróma. Gyakori előfordulása és nagy mortalitása miatt a szisztémás gyulladás napjainkban is az intenzív terápia egyik fontos területét képezi.

Jelen referátumban rövid áttekintést nyújtunk a szisztémás gyulladás klinikai jelentőségéről hazai viszonylatokban, majd az annak diagnosztikájában, prognosztikájában említhető biomarkerekről.

A szisztémás gyulladásban betöltött kiemelt szerepe miatt külön fejezetben tárgyaljuk a makrofág migráció inhibítor faktort. A klinikai vonatkozások után ismertetjük a szisztémás gyulladás alapkutatásokból ismert mechanizmusait, említést teszünk annak vizsgálati lehetőségeiről állatmodellekben, valamint kitérünk néhány nagyrészt még felderítetlen terület bemutatására is. Következtetéseinkben megállapítjuk, hogy a szisztémás gyulladás sikeres felismerése és kezelése kizárólag az abban részt vevő folyamatok pontos ismerete révén lehetséges.
Ennek eléréséhez elengedhetetlen az alapkutatási és klinikai vizsgálatok transzlációs jelleggel való összehangolása, valamint az egyes orvostudományi diszciplínák koordinált összefogása.

Kulcsszavak: szepszis, SIRS, MIF, láz, hipotermia

Summary: Acute systemic inflammation constitutes a complex group of diseases both in their etiology and clinical manifestation. It can develop due to infectious or non-infectious causes and it can occur as sickness behavior/syndrome, systemic inflammatory response syndrome, sepsis, septic shock or as multiple organ dysfunction syndrome. Because of its frequent occurrence and high mortality rates, as of today, the systemic inflammation represents a highly important field in intensive therapy.

In the current review, we give a short overview about the clinical importance of systemic inflammation in Hungary, and then about biomarkers, which can be mentioned in association with its diagnosis and prognosis.

Because of its crucial role in systemic inflammation, we dedicate a separate chapter to the discussion of the macrophage migration inhibitory factor. After the clinical aspects, we present the mechanisms of systemic inflammation, which have been already discovered with the help of basic research, we mention its investigation possibilities in animal models, and we give examples of some associated research areas, which have not been explored yet. In our conclusions, we affirm that the successful diagnosis and therapy of systemic inflammation can be achieved exclusively through the exact knowledge of the participating mechanisms.

In order to reach such goal, it is inevitable to harmonize basic research with clinical investigations in a translational manner and to jointly coordinate all medicinal disciplines.

Keywords: sepsis, SIRS, MIF, fever, hypothermia

Bevezetés

A szisztémás gyulladás témaköre a szervezetet érő fertőző és nem-fertőző faktorok által kiváltott teljes testünket érintő reakciók egész sorát magában foglalja, úgymint „betegség viselkedés/szindróma" (sickness behavior/syndrome), „szisztémás gyulladásos válasz szindróma" (systemic inflammatory response syndrome, SIRS), „szepszis szindróma" (septic syndrome), szepszis, szeptikus sokk és „többszervi működészavar szindróma" (multiple organ dysfunction syndrome, MODS). Amint azt a felsorolás is mutatja, az esetek többségében szindrómákról beszélhetünk, ami már önmagában is jelzi, hogy etiológiájában és kórélettanában többszörösen összetett folyamatokról van szó, amelyeknek – egymással többé-kevésbé átfedést mutató tünettanuk ellenére – egyelőre még nem teljesen ismert a pontos mechanizmusa, így lényegében gyűjtőfogalomként használatosak hasonló klinikai kimenetellel járó nem-specifikus kórképek leírására.

A szepszis, mint fogalom már az ókori görögök körében ismert volt, akkoriban elsősorban a rothadásra, hanyatlásra, mint életet veszélyeztető, fertőzéssel kapcsolatos, magas mortalitású állapot leírására használták. Az orvostudomány fejlődésével párhuzamosan a szepszis definíciója is folyamatosan változott, míg 1991-ben konszenzus nem született a SIRS, szepszis, súlyos szepszis, szeptikus sokk és MODS definícióira vonatkozóan (1).

Később, 2001-ben a második nemzetközi konszenzus konferencián a szepszis definícióját illetően megerősítették a SIRS kritériumainak használhatóságát a szepszis diagnózisának felállításában (2).

A jelenség aktualitását mutatja, hogy 2014-2015-ben újra felülvizsgálták az addig használatos definíciókat, változtattak a szepszis és a szeptikus sokk diagnózisának kritériumain, a SIRS pedig törlésre került a fogalmak közül (3). A SIRS eliminálásának oka, hogy a SIRS, melyet okozhat fertőző vagy nem-fertőző ágens egyaránt, kritériumai alapján az intenzív terápiás ellátásra szoruló betegek csaknem 90%-nál diagnosztizálható. Továbbá, a SIRS jelenlegi definíciója nem teszi lehetővé a szervezet számára előnyös válaszreakció elkülönítését a patológiás, szervkárosodáshoz vezető folyamatoktól. Végül, bizonyos SIRS esetekben a fertőző eredet kimutatása különösen nehézkes, mert a steril gyulladás (pl. súlyos trauma, égés, pankreatitisz esetén) és a kiterjedt fertőzés egyaránt képes az akut szisztémás gyulladás klinikai tüneteinek kiváltására (4,5). A szepszist újabban a szervezet olyan fertőzésre adott válaszreakciójaként említik, ami már károsítja a szervezet saját szöveteit és szerveit is (6). Azért, hogy a jelen referátumban az előbbiekben bemutatott fogalmazásbeli nehézségeket áthidaljuk, a betegség viselkedés/szindróma, SIRS és szepszis kategóriákat egyaránt a szervezet által – etiológiától függetlenül – adott akut szisztémás gyulladásnak tekintjük.

Írásunkban először áttekintjük az akut szisztémás gyulladás klinikai jelentőségét és intenzív terápiás ellátás szempontjából leggyakoribb megjelenési formáit, azok alapvető jellemzőit. Az adatok ismertetése során elsősorban a hazánkban tapasztalt eredményekre fókuszálunk. Ezután összefoglaljuk az akut szisztémás gyulladás diagnosztikájában és prognózisának megítélésében kiemelt jelentőségű biomarkerek használatának előnyeit és nehézségeit. Külön fejezetben tárgyaljuk a makrofág migráció inhibitor faktor (MIF) kapcsán felmerülő diagnosztikai, prognosztikai és terápiás lehetőségeket szisztémás gyulladásban. Végül, áttekintést nyújtunk, néhány, az akut szisztémás gyulladás alapkutatásában használt kísérletes vizsgálati lehetőségeiről és eredményeiről állatmodellekben.

Annak érdekében, hogy utóbbiak fontosságát példákkal is alátámasszuk, kitérünk bizonyos, a patomechanizmus szempontjából érdekes, friss alapkutatási eredmény ismertetésére is.

Az akut szisztémás gyulladás klinikai jelentősége és manifesztációja

A szeptikus betegségek megjelenése egyidős az emberrel. Az orvostudomány egészéhez hasonlóan, a szeptikus betegek ellátása is óriási fejlődésen ment keresztül az évszázadok folyamán.
A szepszis mindig mikroorganizmusok inváziójával, szövetkárosodással van összefüggésben, felismerése, kezelése alapvető orvosi feladat.

Világviszonylatban a tizedik leggyakoribb halálok, naponta nagyjából 1400 áldozatot követel, az estek harmadában a diagnózis felállítását követő egy hónapon belül. Magyarországon a szepszis az intenzív osztályokon történő mortalitás és morbiditás legfőbb oka. Incidenciája évről évre növekszik, a betegek 30-60%-át elveszítjük.

Országunkban évente körülbelül 9000 szepszisben szenvedő beteget kezelnek intenzív osztályokon (7). Az Országos Egészségbiztosítási Pénztár egy régebbi adata szerint a többszervi elégtelenséggel intenzív osztályra kerülők halálozási aránya 80%; amit erősen indokolt lenne 50-60%-ra csökkenteni. Nemzetközi viszonylatban az Európai Intenzív Terápiás Társaság tíz éve tűzte ki célul, hogy az európai átlag 50%-ról 30%-ra kellene leszorítani ezt a számot (8).
Megjegyzendő, hogy a 2015-ben (2005 és 2010 után újra) a november 1. és 14. között intenzív osztályról távozott betegek néhány adatát feljegyezték az országos szepszis regiszterbe. Ezáltal a Magyar Aneszteziológiai és Intenzív Terápiás Társaság szakmai oldalán nagyjából 350 beteg adata gyűlt össze elemzés céljából.

A multidiszciplináris összefogás fontosságát Prof. Dr. Molnár Zsolt a Seps-East konferenciák tudományos bizottságának elnöke is több alkalommal hangsúlyozta és elengedhetetlennek tartja azt a szeptikus betegek mortalitásának csökkentése érdekében, mivel a betegek rosszulléte prehospitálisan/otthonukban kezdődik, kórházi körülmények közt

pedig leggyakrabban sebészeti, belgyógyászati osztályokon infektálódnak. A prehospitális területen a családorvostant, esetleg oxiológusokat, hospitálisan pedig a medicina szinte minden területét érinti a betegség, ezért minden gyakorló orvosnak ismernie kell az aktuális ellátási irányelveket. Fertőzésre sokszor általános tünetek hívhatják fel a figyelmet (pl. rossz általános állapot, zavartság, irritabilitás, étvágytalanság, inkontinencia), különösen idős korban. Fontos az egyes tünetek időben való változásának nyomon követése, ismételt értékelése (lehetőleg ugyanazon személy által). Láz esetén egyértelmű fokális panaszok, tünetek hiányában gondolni kell szepszisre. Kiemelten fontos az első ellátó szerepe, hogy felismeri-e a veszélyeztető állapotot és megfelelően intézkedik-e a továbbiakban (pl. intenzív osztályra helyezés), de ehhez elengedhetetlen az új szepszis kritériumok ismerete, amelyek a Sepsis-Related Organ Failure Assessment (SOFA) pontrendszeren alapulnak (3). Ezek szerint szepszisről beszélünk ha a SOFA érték 2-vel emelkedik, vagy qSOFA alapján a három kritériumból kettő teljesül (szisztémás vérnyomás <100 Hgmm, Glasgow coma score <15, légzésszám >22/min). Szeptikus sokkról akkor beszélünk ha megfelelő folyadékpótlás ellenére vazopresszor terápia szükséges és szérum laktát magasabb, mint 2 mmol/l. A sürgősségi orvostan és az intenzív terápia gyakran már csak súlyos, előrehaladott problémák esetén jut szerephez. A probléma javításának kulcsa viszont ezen szakterületek kezében van. Ők adhatnak ugyanis reanimációs készenlétet a fekvőbeteg intézményekben és megfelelő létszám esetén lehetőségük lenne „Medical Emergency Team" és „Rapid Response Team" üzemeltetésére, akik az osztályokon elhelyezett betegek állapotát mérnék fel napi több alkalommal, észlelve ezzel a szepszis (vagy bármilyen más akut/szubakut kórkép) előjeleit.

Egyértelmű jelek a SIRS kritériumok megjelenései, részleges szervelégtelenségek (vizeletprodukció beszűkülése), de a tudatállapot romlása – tudatzavar, delírium – is jelezheti a kórfolyamat kezdetét. Sajnos országunkban az ilyen kezdeményezések száma jelenleg még elenyésző.

Gyors, hatékony és pontos diagnózist kell felállítani a Sepsis Guidelines követésével klinikai jelek és labor paraméterek alapján, meg kell előzni a súlyos oxigénadósság kialakulását, azaz el kell kerülni a célszervek hipoperfúzióját időben történő észleléssel és az oxigénszállítás javításával. A SIRS felismerése nem szokott nehézségekbe ütközni egyértelmű kritérium rendszere miatt, ami 2 vagy több tünet fennállása a következők közül: testhőmérséklet <36°C vagy >38°C; szívfrekvencia >90/perc; légzésszám >20/perc vagy CO_2 tenzió <32 Hgmm; fehérvérsejtszám <4.000/mm^3 vagy >12.000/mm^3 vagy >10% éretlen alak (1). A szepszis igazolása viszont csak infekció bizonyításával lehetséges.

Évek óta folyamatosan keressük azt a biomarkert, ami a legtökéletesebb biztonságot nyújthatná diagnosztikai szempontból, de ilyen rutinszerűen mérhető,

nagy szenzitivitású és specificitású marker még nem áll rendelkezésre (ld. következő fejezet).

A primer fertőzések vagy a másodlagos, kórházi infekciók (légúti, húgyúti, savós hártyákat érintő, lágyszöveti, idegrendszeri vagy véráramfertőzések) változatos módon jelennek meg, és az egyes betegekben nagyon eltérő tüneteket produkálnak. Ezek között a SIRS-t, a szepszist és a szeptikus sokkot kell megemlítenünk, amikor nagy eséllyel vazodilatációs és/vagy redisztribúciós sokkal állunk szemben, ahonnan már csak egy lépés a MODS. Ha a szervezetet súlyos behatás éri (sokk, vérzés, égés, politrauma) ez irányban tett elhárító lépéseink hatékonysága, sikeressége javíthat a kimenetelen. A primer infekció – mint azt már említettük – lehet kórházon kívül vagy azon belül szerzett, illetve más súlyos vagy életveszélyes állapotot okozó betegség szövődménye. A folyamat előrehaladása többszervi elégtelenséget eredményezhet. Szervkárosodás felmérésekor a következő szervrendszereket vesszük sorra: központi idegrendszer, kardiovaszkuláris rendszer, légzőrendszer, kiválasztórendszer, gasztrointesztinum, vérképző rendszer.

Értékelő rendszerünk a Sepsis-Related Organ Failure Assessment (SOFA) pontrendszer alapján ezt számszerűsíthetjük is (3), az állapot súlyosságának megítélésére használt eljárás a Simplified Acute Physiology (SAPS II) pontrendszer (9). A progressziót a folyamat súlyossága és a szervezet válaszkészsége tudja befolyásolni. Ezek kezelési elveit az utóbbi években több nemzetközi szakmai testület egységesíteni kívánta, amely törekvésekhez a magyarországi intenzív terápiás gyakorlatnak is fel kell zárkóznia (10).

A sürgető szükség oka az, hogy a szepszisnek (feltételezett vagy igazolt infekció) és az abból származó további, nagyon súlyos állapotoknak tulajdonítható a legtöbb intenzív osztályos és kórházi halálozás. Ez a patológiás folyamat adja a tartós intenzív osztályos kezelés legnagyobb orvosi és ápolói kihívását, legnagyobb feladatmennyiségét. Végül, de nem utolsó sorban a szepszis és következményei jelentősen terhelik a kórházi költségvetést is. A kezelési stratégia magában foglalja az alapbetegség, mint kiváltó ok folyamatos kontrollját, kezelését (diagnosztikus vizsgálatok, műtétek, antibiotikumok), a non-invazív és invazív monitorozást, a bázisterápiát és a szervpótló kezeléseket.

Az egészségügyi ellátás bármely területén ápolt betegnél nagy figyelmet kell fordítanunk a megelőzésre (aszepszis), az alap probléma megfelelő kezelésére, és egy felismert góc mielőbbi eltávolítására. Nagyjából 90%-uk bakteriális eredetű (Gram negatívok és pozitívok egyenlő arányban), 10%-uk gombákkal hozható összefüggésbe. Azonban a jelenlévő kórokozót igazoló mikrobiológiai eredmények érkezését, esetleges zavaró álnegativitást meg kell előznie a diagnózisnak. Nem szorulhat háttérbe az alapos fizikális vizsgálat, amely modern világunkban sem hanyagolható el.

A fertőző góc felderítése érdekében vegyünk korán mikrobiológiai mintát, ha lehetséges, hemokulturát is, kezdjük el az antibiotikum adását amilyen gyorsan csak lehet hemokultúra vétele után (ideálisan 1 órán belül), identifikáljuk a forrást, mutassuk ki a kórokozót, javítsuk az oxigénszállítást, csökkentsük a hipoperfúziót. A felsoroltak mellett költségesebb technikát is segítségül hívhatunk (pl. IgM). Az ellátás első lépcsője a fluid challenge (500-1000 ml krisztalloid), azaz, a folyadék mielőbbi szervezetbe juttatása (akár túlnyomással is), a változásokat a betegágy mellett figyelve.

A Frank-Starling törvény okán célunk javítani növekvő preloadunk segítségével a verőtérfogatot. A redisztribúciós sokkot kezeljük vazopresszor terápiával (noradrenalin). Mérjük fel a perfúziót klinikai jelek (tudat, bőrhőmérséklet), haemodinamikai változók (centrális vénás nyomás) és vérvizsgálatok (laktát, centrális vénás O_2 szaturáció) segítségével. A fentiekkel próbáljuk meg elérni az aktuális irányelvek (2012 Surviving Sepsis Campaign Guidelines) által meghatározott reszuszcitációs célértékeket.

Folytassunk alacsony légzési térfogattal történő lélegeztetést, sokk esetén alkalmazzunk szteroidot. Ellenőrizzük folyamatosan a vércukor értékeket, folytassunk stressz fekély- és thromboprofilaxist, valamint fordítsunk nagy figyelmet a szükséges szedáció értékelésére és annak monitorozására.

Új lehetőségek a szisztémás gyulladás diagnózisában és prognózisának megítélésében

A szisztémás gyulladás, mint önálló entitás, meglehetősen nehezen értelmezhető, diagnózisként nem is kezelhető tünetegyüttes, mely gyakran nehezíti meg az akut ellátók feladatát. Ugyan a gyulladás általános jeleit már évszázadok óta ismerjük, a SIRS patomechanizmusát csak néhány évtizede kezdték behatóan tanulmányozni. A 90-es évek előtt úgy gondolták, hogy a gyulladásos válaszreakció egy egyirányú, előremenő folyamat, melyben a lépések szigorúan egymást követik térben és időben is.

A későbbiekben írták le a pro- és antiinflammatorikus válaszok párhuzamos működését (5), melyek akár proinflammatorikus, akár antiinflammatorikus (CARS – kompenzatorikus antiinflammatorikus válasz) utak aktiválásával végső soron a SIRS, illetve a többszervi elégtelenség kialakuláshoz vezetnek.

A szisztémás gyulladás és a szepszis nehezen választhatók el egymástól. Ugyan a 2016-ban napvilágot látott SEPSIS-3 iránymutatás nem javasolja a SIRS használatát (ld. Bevezetés), szemet mégsem hunyhatunk afelett, hogy eddig a SIRS, illetve valamilyen kórokozó megléte és/vagy gyanúja jelentette a szepszis diagnózisát (3). A diagnosztikai nehézségeket csak fokozza, hogy az úgynevezett általános változók sem specifikusak. Ennél fogva hosszú ideje próbálják megtalálni azt a markert, ami a gyulla-

dásos, vagy akár a szeptikus folyamat prognózisában, lefolyásában, annak kimenetelében megfelelő prediktív értékkel bír és megfelelően specifikus is.

A szisztémás gyulladás okozta számos kórfolyamat végső soron dezintegrációhoz vezet, melyben többek közt a kapilláris membrán áteresztőképességének fokozódása, a leukotriéneikozanoid rendszer nem megfelelő működése, a véralvadási kaszkád károsodása, a fokozott NO szintézis csupán részjelenségei a gyulladásos válasznak, de ha bármi oknál fogva nem megfelelő a restitúció, akkor ezek összessége a magas mortalitással járó többszervi elégtelenség irányába terelik a folyamatot. Azért sincs könnyű dolgunk a SIRS markereivel kapcsolatban, mert ugyan számos, a patomechanizmus kialakulásáért felelős molekulát ismerünk, de ezek nem bonthatók „csak jó" és „csak rossz" csoportokra.

A felszabaduló interleukinok (IL), tumor nekrózis faktor (TNF), leukotriének, prosztaglandinok (PG), NO szintje ugyan mérhető, de a SIRS folyamatának bonyolult kórtana nem tisztázott még teljes részletességgel, így egy kiragadott időpillanatban csupán kvantitatív analízis végezhető, kvalitatív nem. Az alábbiakban kiemeltünk néhány az akut szisztémás gyulladás kapcsán kiemelt jelentőségűnek számító markert.

A *C-reaktív protein* egy olyan akut fázis fehérje, melyet régóta használunk a klinikai gyakorlatban a gyulladásos folyamat súlyosságának, prognózisának megállapítására, de annak nyomon követésére is. Sajnos 68%-os specificitása alacsony, szenzitivitása is csupán 75% szeptikus betegekben (11). További hátránya, hogy szintje minden gyulladásos folyamatban, így pl. autoimmun megbetegedésekben is emelkedik, így diagnosztikus értéke ebben, illetve egyéb gyulladásos kórképekben szenvedő betegekben alacsony.

Szeptikus betegekben sokkal célravezetőbb a *procalcitonin (PCT)* szint mérése. A PCT egy 116 aminosavból álló fehérje, szintje normál körülmények közt nagyon alacsony (<0,05 ng/ml), azonban bakteriális fertőzés hatására a CALC-1 gén expressziójának fokozásával, valamint a parenchimás szövetekből történő mobilizáció segítségével gyorsan emelkedik. Ezt a kezdeti emelkedést nevezzük PCT indukciónak, mely rövidebb időt vesz igénybe, mint a CRP termelődésének megindulása, így a PCT SIRS diagnosztikában betöltött szerepe alapvetően a bakteriális fertőzések detektálása, azok nyomon követése, a megkezdett antibiotikus kezelés sikerességének ellenőrzése, a kórfolyamat kimenetelének vizsgálata (11).

IL-6 és PCT együttes alkalmazása során azt találták, hogy a PCT inkább a SIRS-szepszis differenciáldiagnosztikájában segített, az IL-6 pedig a mortalitás megítélésében (12).

A SIRS és szepszis elkülönítésében próbálkoztak a *preszepszin*, más néven szolubilis CD14-altípus fehérjének a vizsgálatával (13). Eredményeik szerint a preszepszin sem értelmezhető, mint op-

timális differenciáldiagnosztikai marker. Hasonló eredményekre jutottak a *D-dimer, a lipopoliszacharid (LPS)-kötő fehérje* és a *keringő leukociták felszíni markereinek* vonatkozásában is (14).

Egy proprotein konvertáz enzimmel, a *furin*nal is végeztek hasonló vizsgálatokat, mivel immunaktiváció során annak szintje emelkedik. Meggyőző eredményt a SIRS-szepszis differenciáldiagnosztikában itt sem tudtak felmutatni (15).

A veleszületett humorális immunitás szabályozásában játszanak szerepet a *pentraxinok*.
A pentraxin-3 jelentőségével kapcsolatban végzett vizsgálatok azt találták, hogy ugyan diagnosztikus értéke alacsony szeptikus betegekben, de a SIRS és szepszis prognózisában jól használható (16).

A molekuláris genetika fejlődésével egyre szélesebb teret nyernek a *genomika, a proteomika és metabolomika* tudományterületei. Mivel a genotípusnak fontos szerepe van a veleszületett immunitás meghatározásában, ezért valószínűsíthető, hogy az egyéni variabilitást is kódoló génállomány mélyebb megismerése segítségünkre lehet a gyulladásos és fertőzéses folyamatok differenciáldiagnosztikájában és prognózisában. Jelentős különbségeket találtak SIRS-ben szenvedő, szeptikus és egészséges betegek metabolikus profilját összehasonlítva. A vizsgált metabolitok közül hétnek is szerepet tulajdonítottak a szepszis és SIRS differenciáldiagnosztikájának kapcsán (17).

A SIRS és szepszis diagnosztikája és prognosztizálása versenyfutás az idővel. A korai felismerés és korai célzott kezelés a kimenetellel egyértelműen pozitív korrelációt mutat, így nem véletlen, hogy a kutatások célkeresztjében egy olyan pontos, megbízható diagnosztikus és prognosztikus rendszer megteremtése áll, mellyel biztonsággal felismerhető, illetve elkülöníthető a SIRS és a szepszis. Úgy tűnik, hogy a XXI. század technikai lehetőségei mellett a multimarkeres megközelítés jelentheti a megoldást, hiszen ezáltal lehetőség nyílik több patomechanizmus egyidejű vizsgálatára, így a térben és időben együtt zajló, de egymást is befolyásoló folyamatok nyomon követésére, mint ahogy az SIRS-ben, vagy szepszisben történik.

A makrofág migráció inhibítor faktora (MIF) alias a mindennél izgalmasabb faktor

A MIF-t a legelső potens proinflammatorikus citokinek között fedezték fel az 1960-as évek elején, mely fontos mediátora az immunrendszernek. Kezdetben termelődését kizárólag a T-limfociták aktivizálódásához kötötték, mely hatására gátlódik a makrofágok migrációja a gyulladásos folyamat helyszínén. Napjainkban ismét a klinikai kutatások előterébe került, mint stressz-indukálta citokin, amelynek egyik legfontosabb termelődési helye a hipofízis elülső lebenye, és fontos szerepe van a glükokorti-

koidok gyulladáscsökkentő hatásának szabályozásában (18).

Kulcsszerepét kísérletes és klinikai munkák is megerősítették számos gyulladásos kórfolyamat progressziójában. Ide tartozik a reumatoid artritisz, a szisztémás lupusz eritematózusz, az asztma bronchiale, az ARDS, a szepszis, a pankreatitisz, a kolitisz ulcerosa, az endometriózis és az ateroszklerózis is. A MIF expressziója a legtöbb tumortípusban is fokozott, úgy tűnik a gyulladás és tumorfejlődés közötti kapcsolatban van szerepe.

Kimutatták, hogy előmozdítja a malignus sejttranszformációt, gátolja a tumorsejt-specifikus immun-citolitikus választ és elősegíti a neovaszkularizációt (19).

A stressz indukálta MIF szekréció az adenohipofizisből elsősorban proinflammatorikus stimulus, mint pl. endotoxin, TNF-α, valamint glükokortikoidok hatására fokozódik (18). Klinikai tanulmányok kimutatták, hogy központi szerepet játszik az endotoxinémia okozta toxikus reakciókban és szepszisben, valamint ARDS-ben is (20).
Egereknek intraperitoneális LPS adását követően a hipofízis MIF szintjének nagymértékű csökkenését találták, majd fokozatos emelkedését a MIF mRNS szintézisének, mely mellett a szérum koncentráció progresszív emelkedése volt megfigyelhető.

Állatkísérletekben a MIF potenciálta az endotoxin okozta mortalitást, míg MIF ellenes antitestek adását követően a mortalitás csökkenését találták (21). Calandra és munkatársai hipofízis eltávolított egereknél a bakteriális infekcióra létrejövő MIF termelését elsősorban a makrofágoknak tulajdonították (18).
A makrofágok intracelluláris raktárral rendelkeznek, melyből ez a citokin proinflammatorikus stimulus hatására hasonló módon szabadul fel, mint a hipofízisből. Szintén állatkísérletekben észlelték, hogy bakteriális stimulus hatására MIF termelődés indult meg nem csak a hipofízisben, makrofágokban, hanem a tüdőben, mellékvesében, lépben és a májban is, a beadást követő hat órán belül (22).
Kritikus állapotú, szeptikus sokkban lévő betegeknél szintén emelkedett MIF szintet találtak, mely a túlélő csoportban szignifikánsan alacsonyabb volt, mint a nem túlélő csoportban (23). A sebészi trauma hatására kialakuló stressz reakció emelkedett ACTH és kortizol termeléssel jár, mely mellett májrezekción átesett betegeknél a kortizol szinttel párhuzamos MIF szekréció emelkedést észleltünk (24).
Az utóbbi időben megkülönböztetett figyelem övezi a MIF-t különleges enzimatikus tautomeráz aktivitásának köszönhetően is, mely szubsztrátjaként dopachrome vagy fenilpiruvát ill. OH-fenilpiruvát is szolgálhat, továbbá thiolprotein oxidoreduktáz aktivitását is leírták (25). Egyes elképzelések szerint a MIF jelátvitel egyes részei magukba foglalhatják a célfehérjék és/vagy kis molekulájú szubsztrátok enzimatikus módosítását.

Ezen citokin rejtélyes enzimatikus aktivitásának pontos biológiai szerepe rész-

leteiben mind a mai napig feltáratlan. Ennek ellenére a MIF tautomerázt már ma is tucatnyi gyulladásos állapot jövőbeni terápiájának egyik legígéretesebb farmakológiai célpontjaként tartják számon, és világszerte intenzív gyógyszerfejlesztés is zajlik ezirányban (26).

Saját eredményeink alapján egyes növényi eredetű gyulladásgátló hatású polifenol vegyületek figyelemre méltóan potens gátlószernek bizonyultak a MIF tautomerázra in vitro, csakúgy, mint a ketontestek, valamint az acetaminophen, ill. annak metabolitja.

Legfrissebb, szintetikus molekulacsaládokkal nyert enzimgátlási eredményeink is bíztatóak, és további erőfeszítésekre sarkallnak, hogy jobb vezérmolekulákhoz jussunk a MIF gátláshoz.

Richard Bucala szavait idézve e „Mindennél Izgalmasabb Faktor" (27) proinflammatórikus, angiogenikus és tumornövekedést elősegítő aktivitásainak hatásmechanizmusát vizsgáló kutatások a közeljövőben bizonyára szolgáltatnak még meglepetéseket és további talányokat is.

A szisztémás gyulladás kísérletes vizsgálata az alapkutatásban

Az akut szisztémás gyulladás olyan mértékű összefüggésben áll a testhőmérséklet változásaival, hogy annak mindenfajta klinikai diagnózisa magában foglalja az abnormális testhőmérséklet változásokat. A szisztémás gyulladásban szenvedő betegek többsége (kb. 90%-a) lázas, a maradék (kb. 10%) testhőmérséklete viszont alacsonyabb a normálisnál (28). Az alapkutatásban a leginkább elterjedt állatmodell a szisztémás gyulladás vizsgálatára a bakteriális LPS adása. Ezekben a kísérletes modellekben a testhőmérséklet változása a környezeti hőmérséklettől és a beadott LPS dózisától függ. Neutrális vagy szupraneutrális (meleg) környezeti hőmérsékleten láz alakul ki, ami kisdózisú LPS adása esetén monofázisos, de polifázisossá válik a LPS dózisának növelésével.
Előbbiekkel ellentétben szubneutrális (hideg) környezeti hőmérsékleten LPS hatására hipotermia jön létre, amelynek mértéke a beadott LPS dózisától függ(28).

Mind a láz, mind a hipotermia része az akut szisztémás gyulladás tünettanának. Az utóbbi évtizedekben a láz biológiai jelentőségét illetően megállapításra került, hogy a láz a szervezet szempontjából előnyös, mert kísérletesen kimutatták, hogy például a gyíkok viselkedésileg fokozzák testhőmérsékletüket, amikor patogén baktériumokkal fertőzöttek, így képesek túlélni egyébként halálos betegségeket. Továbbá, evolúciós szempontból, ha a láz nem rendelkezne adaptív, előnyös funkciókkal, akkor ez az energetikailag költséges mechanizmus nem maradt volna meg az élőlények nagy részében az évmilliók során. A láz szervezetre kifejtett jótékony biológiai hatásával szemben a szisztémás gyulladás során időnként kialakuló hipotermia szerepe szinte teljesen elhanyagolt

maradt. Ennek oka egyrészt, hogy a hipotermia megjelenése sokkal kevésbé gyakori, másrészt, hogy annak diagnózisára (részben a klinikai gyakorlatban használt hőmérők kialakítása miatt) ritkábban kerül sor, harmadrészt, hogy magának a hipotermiának a megléte kevésbé jelentős a gyakorló orvos számára, hiszen olyankor általában a beteg állapota nagyon súlyos, gyakran preterminális (29). A szepszis kapcsán fellépő testhőmérsékleti eltérések halálozási esélyt előjelző szerepét nemrég igazoltuk nagyszámú (10000 feletti) beteg adatainak metaanalízisével, amelynek során kimutattuk, hogy a láz kisebb, a hipotermia viszont nagyobb halálozási kockázattal jár szeptikus betegekben (30). A hipotermia megléte azonban korántsem feltétlenül káros. Ezt bizonyítja például, hogy amikor altatott kutyákban szeptikus sokkot hoztak létre fekália intraperitoneális adásával és a kutyák egyik csoportjában külső hűtést alkalmaztak, hat órával később a kontroll csoportban 100% volt a mortalitás, míg a hűtésnek kitett kutyák esetén csak 36%. Egerekben endotoxin sokk kiváltása után öreg egerek lázzal, míg fiatalok hipotermiával reagáltak. Az utóbbiak túlélési aránya lényegesen jobb volt. A hipotermia jótékony hatását szisztémás gyulladásban klinikai vizsgálatokkal is igazolták: azok a kritikus állapotú betegek, akiknél a megfelelő intenzív terápia mellett hűtést is alkalmaztak a hipotermia életmentőnek bizonyult. Az előbbiekben említett kutatásokat Romanovsky és Székely foglalták össze (29).

A láz és a hipotermia a szervezet két különböző védekező stratégiáját jellemzi szisztémás gyulladásban. A láz a betegség viselkedés/szindróma korai fázisára jellemző és hiperalgézia, motoros túlérzékenység jellemzi. Időnként hipertenzió és fokozott éberség is kíséri. A késői fázisa a betegség szindrómának ezzel szemben csökkent motoros aktivitással, hipoalgéziával, alacsony (vagy normál) vérnyomással, aluszékonysággal és hipotermiával jellemezhető (29). A klinikai gyakorlatban az előbbi fázisok gyakran nem teljesen elkülöníthetők, hiszen az állatkísérletekben használt bólusban adott endotoxinnal szemben a lázkeltő ágens lassan, fokozatosan kerül a szervezetbe, így a fázisok egymással gyakran átfedésben jelennek meg.

A fertőzés kezdetén megjelenő, lázzal kísért, korai fázis az egészséges szervezet válaszreakcióját jellemzi a kialakuló betegséggel szemben. Tényezői mind arra irányulnak, hogy harcba szálljanak a fertőző ágenssel szemben. Klinikailag az ebben a fázisban lévő betegek lázasak, nyugtalanok, túlérzékenyek fénynyel, zajokkal szemben. A szisztémás gyulladás késői fázisa az előbbiekkel szemben azt az állapotot jellemzi, amikor a fertőző betegség már előrehaladott. Ilyenkor a beteg szervezet a fertőző ágens legyőzésére használt energia-igényes folyamatok (pl. láz, hipermetabolizmus) helyett, sokkal inkább a meglévő energiaraktárak megőrzésére összpontosít (29).

Az akut szisztémás gyulladás alapkutatásokból ismert molekuláris mechanizmusai

A fertőzés során az immunrendszer többféle mechanizmuson keresztül aktiválódik. Több immunsejt rendelkezik gyakori makromolekulákat felismerő re-ceptorral, így például a CD14 képes a LPS megkötésére, ami Toll-like receptor 4 aktiválásán keresztül intracelluláris jelátviteli útvonalak és immunválasz kiváltását eredményezi. Ez a veleszületett immunválasz az aktivált sejtekből hormonok és citokinek felszabadulását eredményezi, mint például interleukin IL-1ß, IL-6 és TNF-α. Bizonyos citokinek további immunsejtek odahívását segítik, míg mások a perifériás szövetek gyulladásos mediátorainak felszabadulását idézik elő. Utóbbiak közé tartoznak a PG-k is.

A PG-k az arachidonsav származékai, amelyből ciklooxigenáz (COX) hatására alakul ki a PGH_2. A COX-nak két formája ismert a COX-1, ami állandóan jelen van több szövettípusban és a COX-2, ami többnyire gyulladás által indukálható. A COX-2 folyamatosan expresszálódik az agy neuronjaiban, de szisztémásan adott LPS hatására elsősorban az agy kis vénáinak perivaszkuláris és endotél sejtjeiben indukálható. Ezek a venulák legsűrűbben a hipotalamusz preoptikus areájában, a ventrolaterális medullában és a nukleusz szolitariuszban találhatók. A PGH_2 aztán tovább metabolizálódik PG-k, prosztaciklin és IL-k formájába, amelyek közül a szisztémás gyulladás szempontjából különösen fontos a PGE_2 és a PGD_2.

A PGE_2-t a mikroszómális PGE szintáz-1 hozza létre és négy különböző receptora van EP1-4, amelyek az agy több különböző részén expresszálódnak. A PGD_2 a lipokalin PGD szintáz terméke és elsősorban DP1 receptoron keresztül hat, amely az agyhártyában található, emellett a hipotalamusztól ventrálisan fekvő régióban. A DP2 receptor kismértékben található csak meg az agyban, szerepe egyelőre kérdéses (31).
A betegség szindrómában a PGE_2 szerepe igazolt a szisztémás gyulladás korai fázisának létrehozásáért (ld. alább), míg a PGD_2 elsősorban a késői fázis tüneteinek kialakításáért, így az aluszékonyság, étvágytalanság, analgézia és feltételezhetően a hipotermia létrejöttéért (32).

Sokáig kérdéses volt, hogy az endogén pirogéneknek is nevezett gyulladásos citokinek (IL-1, IL-6, TNF- α) közvetlenül az agyba jutva fejtik ki hatásukat, vagy köztes mediátorokon keresztül, mint például a PG-k. Ezek a molekulák ugyanis túl nagyok ahhoz, hogy a véragy gáton nagy mennyiségben átjussanak. Igaz, hogy centrális injektálásuk esetén lázválasz kiváltható, ez sokkal inkább enkefalitiszre jellemző tünetekkel jár, mintsem szisztémás gyulladással. Kisebb mennyiségben azonban a gyulladásos citokinek átjuthatnak a véragy gáton, különösen a cirkumventrikuláris régiókban. Emellett, a citokinek aktív transzporttal is képesek bejutni a központi idegrendszerbe, hogy aztán ki-

váltsák a szisztémás gyulladásra jellemző tüneteket (31).

A szisztémás gyulladás tünetei közül az egyik legfontosabb a testhőmérséklet változása, leggyakrabban láz. Az, hogy a lázválasz hiányzik COX gátlók adása esetén és olyan egerekben, amelyekben genetikusan hiányzik a mikroszómális PGE szintáz-1, azt mutatja, hogy a lázat PGE_2 hozza létre. A láz különböző fázisait mediáló PGE_2 azonban több helyről is származhat a szervezetben. Az első (korai) fázis létrehozásáért például a periférián termelődő PGE_2 felelős, amit az is bizonyít, hogy a vér-agy gáton át nem jutó PGE_2 ellenes antitest adásával a láz első fázisának kialakulása kivédhető. A LPS a máj Kuppfer sejtjei és pulmonáris makrofágok által expresszált Toll-like receptor 4-hez kötődve a foszfolipáz A_2, COX-2 és mikroszómális PGE szintáz-1 enzimek mRNS- és fehérjeszintű termelődésének fokozódását váltja ki, ami az artériás és vénás vér PGE_2 szintjének emelkedéséhez vezet (33). Ez a PGE_2 albuminhoz kötődik, ami megvédi az enzimatikus lebomlástól, majd a vér-agy gáthoz jutva az albuminról disszociál és a hipotalamuszba jut, ahol kifejti hatását. A láz későbbi fázisait (kb. 1 órával a LPS adást követően) már a perivaszkuláris és endotél sejtekben fokozott COX-2 aktivitás által termelt PGE_2 tartja fenn. A PGE_2 hatásának legfőbb mediátora az EP3 receptor, amely leginkább a medián preoptikus nukleuszban expresszálódik. Ennek bizonyítéka, hogy az itteni EP3 receptorok lokalizált kiiktatása a szisztémásan adott LPS és agykamrába adott PGE_2 lázkeltő hatását is kivédi (34).

A preoptikus EP3 receptort expresszáló neuronok GABA-t termelnek, így gátolják azokat az idegi elemeket, amelyek a testhőmérséklet fokozását hoznák létre. Amikor PGE_2 kötődik hozzájuk aktivitásuk csökken, így a testhőmérséklet fokozódást létrehozó mechanizmusok gátlásuk alól felszabadulnak és láz alakul ki. A láz létrejöttében szerepet játszik egyrészt a bőrerek konstrikciója, amelyért a medián preoptikus nukleuszból a rostrális medulláris raphe magokba, majd onnan a szimpatikus preganglionáris neuronokba való idegpálya aktiválása felelős. Másrészt, a medián preoptikus nukleuszban található neuronok másik csoportja a dorso-mediális hipotalamusszal áll összeköttetésben szintén a rostrális medulláris raphe magokon keresztül és a barnazsírszöveti hőtermelés szabályozásáért felelős.
Előbbiek alapján tehát PGE_2 hatására rágcsálókban az autonóm hidegellenes effektorok (fokozott hőkonzerválás és hőtermelés) aktiválása jön létre a preoptikus area GABA-ergicitású, EP3 receptort kifejező neuronjainak csökkent aktivitásán keresztül, aminek eredménye a bőrér konstrikcióra és barnazsírszöveti hőtermelésre ható szimpatikus aktivitás gátlásának feloldása (31).

Természetesen a láz kialakulásában az előbbiekben ismertetett autonóm mechanizmusok mellett viselkedési hőszabályozási mechanizmusok is szerepelnek (pl. meleg környezet keresése, betakarózás), de az ezekben szereplő köz-

ponti idegrendszeri elemek még nagyrészt tisztázatlanok.

Meg kell említeni, hogy az előbbiekben tárgyalt kísérletek nagy részében a szisztémás gyulladást LPS adása után vizsgálták, ami klasszikus értelemben szeptikus állapotnak kevésbé, SIRS-nek sokkal inkább felel meg. Igazi szepszis rágcsálókban legelterjedtebben a cökális ligatúra és punkció módszerével hozható létre. Az ily módon kialakult szisztémás gyulladás bizonyos tulajdonságait illetően különbözik a LPS-indukálta gyulladástól, például ilyen esetekben a LPS szint alacsony marad, ezek alapján nem meglepő, hogy LPS-antitest adása nem javította a szeptikus betegek prognózisát (35). További különbségek a LPS és cökális ligatúra és punkció állatmodellek között fellelhetőek a tranziens receptor potenciál vanilloid-1 ioncsatorna pro- és antiinflammatórikus szerepét illetően is (36).

Következtetések

Következtetésként levonhatjuk, hogy annak ellenére, hogy az akut szisztémás gyulladást már évezredekkel ezelőtt felismerték, klinikai megjelenési formáinak pontos meghatározása, így azok diagnosztikai kritériumainak megállapítása többször is változott és feltehetően a jövőben is változni fog.

A SIRS a legutóbbi ajánlások alapján kikerült a szisztémás gyulladásos kórképek közül, annak ellenére, hogy kritériumrendszere a szepszis diagnosztikájában (is) alkalmazhatónak bizonyult.
A pontos definiálás és klinikai diagnosztikai, prognosztikai és terápiás szempontok egységesítése és az irányelvek betartása a szisztémás gyulladás esetén kulcsfontosságú, hiszen nemzetközi és magyarországi szinteken is kiemelt gyakoriságú betegségcsoportról van szó, amelynek halálozási aránya magas, továbbá jelentős alap- és kórházi ellátási, valamint gazdasági terheket ró az egészségügyre. A szisztémás gyulladás sikeres intenzív terápiás ellátásának és a mortalitás csökkentésének érdekében elengedhetetlen az abban szereplő élettani, kórélettani, molekuláris, neuroendokrinológiai és egyéb mechanizmusok alapos felderítése, ezek nagy része azonban jelenleg is kutatások tárgyát képezi. Előbbiek sikeres kivitelezése segíthetne új, megbízható (azaz specifikus és szenzitív) diagnosztikus és prognosztikai biomarkerek identifikálásában. Talán ezek között, de akár terápiás szempontból is kiemelt jelentőségű lehet a MIF, szisztémás gyulladásban betöltött szerepének és pontos hatásmechanizmusának tisztázása azonban várat még magára. Az alapkutatások segíthetnek az előbbiekben említett kérdések megválaszolásában, valójában több folyamat (pl. a PGE_2 hatásmechanizmusa) már részletes feltérképezésre került állatmodellekben, de továbbra is sok a nagy fontosságú és jelenleg még felderítetlen terület, ezáltal kiaknázatlan lehetőség (pl. a viselkedési hőszabályozó mechanizmusok idegpályái nagy-

részt ismeretlenek, de a szisztémás gyulladás során kialakuló hipotermia molekuláris mediátora is kérdéses).

Az alapkutatási eredmények igazi értékkel pedig csak akkor bírnak, ha transzlációs jelleggel, azok humán vizsgálatokban is igazolásra kerülnek. A leglényegesebb élettani mechanizmusok evolúciós szempontból konzerváltak, így emlősökben csak kismértékű fajok közötti különbségeket mutatnak (pl. normál vérnyomás vagy testhőmérséklet fenntartása), de az azokban szereplő tényezők különbözőek lehetnek, példaként említhető erre, hogy míg rágcsálókban a hőleadás legfontosabb lehetősége a farokbőr vértartalmának növelése (verejtékezni nem képesek), addig emberben a hőleadás fokozása elsősorban evaporatív hővesztéssel, vagyis verejtékezéssel jön létre, habár a fokozott bőrér dilatáció a test bizonyos részein emberben is fontos szerepet játszik. Vannak azonban olyan folyamatok is, amelyek lényeges különbségeket mutatnak az alapkutatásokban gyakran használt rágcsálók és emberek között: emberek például nem képesek a gyulladásos válaszokban is szerepet játszó C-vitamin szintézisére, míg patkányok májában megtalálhatók azok az enzimek, amelyekkel C-vitamin szintézise glükózból lehetséges. Az említett nehézségek áthidalása kizárólag az alapkutatás és a klinikai vizsgálatok összehangolásával, vagyis transzlációs jelleggel oldható meg. A szisztémás gyulladás komplexitása, változatos etiológiája és klinikai manifesztációja továbbá szükségessé teszi a legtöbb orvostudományi diszciplína bevonását is az alapellátástól a különféle klinikai szakágakon át egészen az intenzív terápiás ellátásig.

Támogatás

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Macrophage migration inhibitory factor as a diagnostic and predictive biomarker in sepsis: meta-analysis of clinical trials

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The hunt for useful sepsis biomarkers is ongoing. Macrophage migration inhibitory factor (MIF) was implicated as a biomarker in sepsis, but its diagnostic and prognostic value has remained unclear in human studies. Here, we aimed at clarifying the value of MIF as a sepsis biomarker with the meta-analysis of clinical trials. PubMed, EMBASE, and Cochrane Central Register of Controlled Trials databases were searched until December 2019. From the included studies, blood MIF levels and indicators of disease severity were extracted in septic and control patient groups. Twenty-one eligible studies were identified, including data from 1876 subjects (of which 1206 had sepsis). In the septic patients, blood MIF levels were significantly higher than in healthy controls with a standardized mean difference (SMD) of 1.47 (95% confidence interval, CI: 0.96–1.97; $p < 0.001$) and also higher than in patient groups with nonseptic systemic inflammation (SMD = 0.94; CI: 0.51–1.38; $p < 0.001$). Markedly greater elevation in blood MIF level was found in the more severe forms of sepsis and in nonsurvivors than in less severe forms and in survivors with SMDs of 0.84 (CI: 0.45–1.24) and 0.75 (CI: 0.40–1.11), respectively ($p < 0.001$ for both). In conclusion, blood MIF level is more elevated in systemic inflammation caused by infection (i.e., sepsis) compared to noninfectious causes. In more severe forms of sepsis, including fatal outcome, MIF levels are higher than in less severe forms. These results suggest that MIF can be a valuable diagnostic and prognostic biomarker in sepsis given that well-designed clinical trials validate our findings.

Sepsis, a form of systemic inflammation, is defined as life-threatening organ dysfunction caused by dysregulated host response to infection[1]. Even nowadays, sepsis and related diseases represent a major challenge for the healthcare system. According to a novel analysis of cause-of-death data from 109 million records in the Global Burden of Diseases, Injuries, and Risk Factors Study, nearly 49 million incident cases of sepsis could be estimated worldwide and 11 million sepsis-related deaths were reported[2]. In a cohort from 6 hospitals in the US, sepsis was present in more than half of the hospitalizations and accounted for the highest ratio (35%) among the causes of death[3]. While there was some evidence of a trend towards decreasing mortality rates in septic patients over the last decade, a continuous decline in mortality was not observed among patients with sepsis or septic shock in a recent systematic review[4]. These data warrant for the need of better sepsis management, which could be facilitated by improved diagnostic and prognostic tools.

In spite of the desperate need for reliable biomarkers in sepsis, according to the Sepsis-3 definition consensus, the novel candidates require further validation before they can be incorporated into the clinical practice[1]. In 2010, an electronic search identified 178 sepsis-related biomarkers, but none of them was found eligible for routine use in clinical practice[5]. According to a current review by the same group[6], the list of potential biomarkers in sepsis has expanded, and in 2020 it included more than 250 substances, but only a few of them were evaluated in a large patient population or in repeated studies, which still limits their clinical usability.

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Macrophage migration inhibitory factor (MIF), a mediator of the innate immune system, is involved in many inflammatory processes and related disorders, including obesity and diabetes mellitus[7,8], autoimmune disorders[7,9], and cancer[7,10]. Besides the role of MIF in chronic inflammation, as a proinflammatory cytokine, it is rapidly released into the bloodstream in different forms of acute systemic inflammation[11]. The causes of acute systemic inflammation can be diverse, including diseases induced by microbial pathogens (e.g., sepsis, septic shock), as well as, noninfectious illnesses due to stress, autoimmune reaction, trauma, surgery, burns, etc. Increased blood MIF levels were reported in forms of acute systemic inflammation originating from both infectious and noninfectious etiologies[7], but it has remained questionable whether the magnitude of the increase is similar or different in the two forms, therefore, if MIF can be used as a diagnostic tool in sepsis. A similar increase in MIF levels was observed in patients with systemic inflammation of septic and nonseptic (i.e., caused by major surgery) origin compared to the healthy controls[12], suggesting that MIF may serve as a biomarker for critical illness without the ability to differentiate between infectious and noninfectious causes. However, in other studies, MIF levels were markedly higher in sepsis than in patients with other forms of systemic inflammation[13–15], indicating that MIF can be used as a diagnostic biomarker in sepsis.

The prognostic value of MIF has also remained controversial. On the one hand, high serum levels of MIF were found in septic patients and even higher MIF levels in patients with septic shock, though the difference was not statistically significant ($p = 0.3$)[16]. Not significantly higher MIF levels were also reported in septic patients with lung complications compared to those without it[13]. On the other hand, no significant correlation was found between serum MIF levels and sepsis severity or mortality[17]. Further complicating the issue, circulating MIF levels did not differ between sepsis survivors and nonsurvivors in one study[18], whereas nonsurvivors had significantly higher MIF levels in another study[13].

In the present meta-analysis, we aimed at studying the diagnostic and prognostic value of blood MIF levels in sepsis by analyzing the currently available published data in humans.

Methods

Our meta-analysis was conducted in accordance with the guidelines of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) statement[19] (Supplementary Table S1). The question of our analysis was defined in the PICO [Patients, Indicator, Comparison, Outcome] format: in adult septic patients, we aimed at assessing the biomarker role of MIF in the diagnosis and prognosis of the disease. This meta-analysis has been registered with PROSPERO (CRD42020139137).

Search strategy. We searched the PubMed, EMBASE, and CENTRAL (Cochrane Central Register of Controlled Trials) databases for original human studies without time period limitations. The following search term was used: ("macrophage migration inhibitory factor" OR MIF) AND (sepsis OR septic). As in our previous meta-analysis of sepsis[20], publications reporting immunosuppressive conditions (e.g., transplantation, HIV infection) were not included in the current analysis. Similarly to our past studies[20,21], the search was conducted separately by two authors (JT, AG), who also assessed study eligibility and extracted data from the selected studies independently. Disagreements were resolved by consensus, with the help of a third party (ZR).

Study selection, data extraction, and risk of bias assessment. The titles and abstracts of the publications identified by the literature search were screened, and the full texts of potentially eligible articles were obtained. We included studies which reported blood MIF levels in two or more different patient groups, at least one of which groups consisted of septic patients. For analysis of the prognostic value, an indication of disease severity or outcome (e.g., mortality rate) was also required for the groups. From all included articles we extracted the country of origin, characteristics of the patient populations (sample size, sex ratio, age, severity score, mortality), and the reported blood MIF level values of the patient groups with the corresponding indicator of standard deviation (SD). The extracted values were converted to mean and SD unless specified otherwise. Different patient groups within a study (e.g., survivor vs. nonsurvivor, septic vs. nonseptic systemic inflammation) were extracted separately.

We assessed the quality of each study included in the meta-analysis by using the Newcastle–Ottawa Scale[22] (Supplementary Table S2).

Statistical analysis. For each included study, we calculated the difference between the blood MIF level of a septic patient group and that of another septic group or a control group. For all groups, the means were standardized (based on variances) to obtain standardized mean differences (SMDs). For standardization, the means were divided by their corresponding SD values, which was required because the different MIF measuring methods could result in different variances among the study groups and influence the results. The SMDs with 95% confidence intervals (CIs) were calculated by using the random effect model by DerSimonian and Laird[23], and then compared using standard meta-analysis tools (i.e., forest plot).

In accordance with the Cochrane Handbook for Systematic Reviews[24], between-study heterogeneity was tested with I^2 statistical test, where I^2 is the proportion of total variation attributable to between-study variability (an I^2 value of more than 50% was considered as an indication of substantial heterogeneity). The presence of publication bias was determined by visual inspection of funnel plots (Supplementary Figs. S1-S4) for the lack of asymmetry and evaluated quantitatively by Egger's test ($p < 0.1$ indicating publication bias). Sensitivity analysis (i.e., iteratively omitting one study from the analyses and recalculating SMD to investigate the impact of the individual study on the summary estimate) was performed to test the impact of the individual studies. The meta-analyses were performed with Comprehensive Meta-Analysis (version 3.3; Biostat, Engelwood, MJ, USA) software.

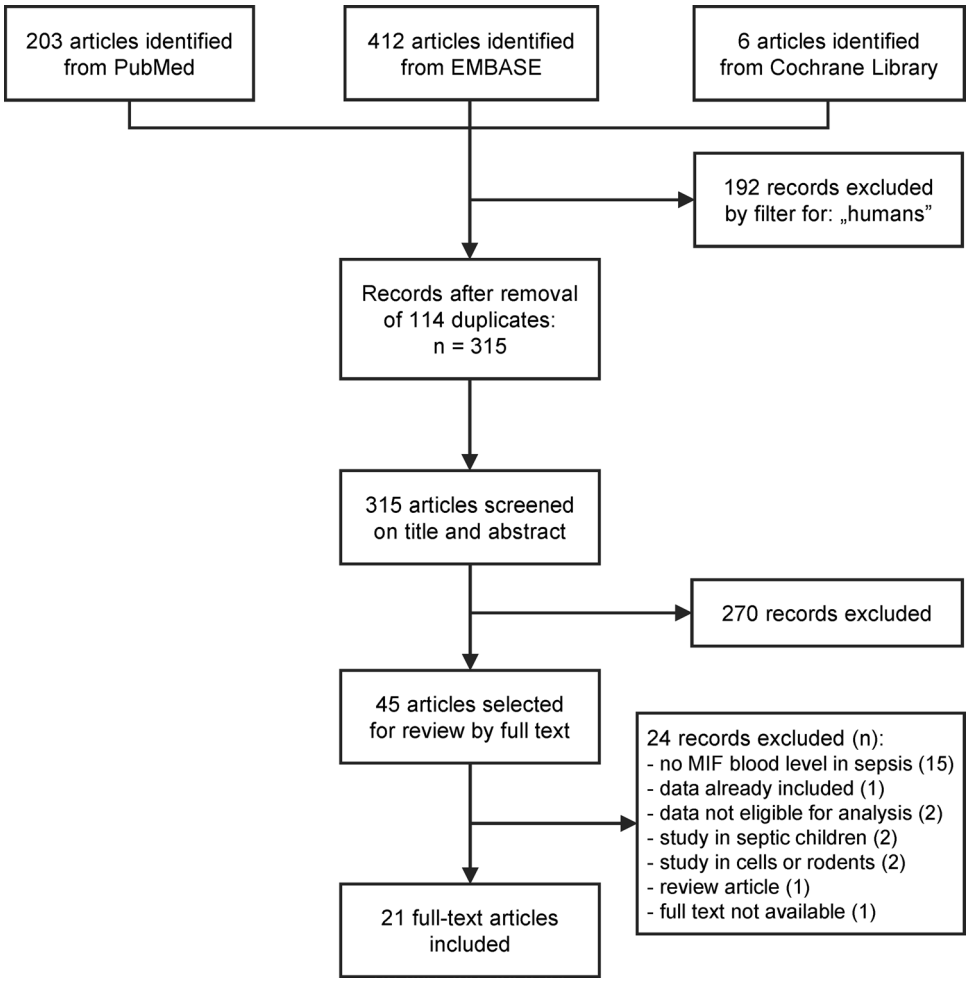

Figure 1. Flowchart of study selection and inclusion.

A receiver operating characteristic (ROC) curve was constructed to evaluate the diagnostic performance of blood MIF levels in sepsis. For that, individual blood MIF level data of septic patients and healthy controls were extracted with WebPlotDigitizer application from eligible papers[25–27], which presented the data in figures with linear scales. The area under the ROC curve (AUC) was calculated to assess the accuracy of blood MIF level measurement as a diagnostic test in sepsis. Within the range of 0.5 (no diagnostic ability) to 1.0 (perfect diagnostic ability), a higher AUC indicates better performance of a test. ROC curve analysis was performed using IBM SPSS Statistics for Windows, version 26 (IBM Corporation, Armonk, NY, USA).

Results

Study selection, characteristics, and quality. The flow chart of the study selection is presented in Fig. 1. Until December 2019, the electronic literature search identified altogether 621 studies from the PubMed, EMBASE, and CENTRAL databases. After enabling filters for human studies and removal of duplicates, 315 articles remained, which were screened on title and abstract for inclusion criteria. As a result, the full texts of 45 articles were obtained, out of which 21 publications were found eligible for statistical analysis[12–18,25–38], including data from a total of 1876 human subjects. The studied groups consisted of 1206 septic patients, 134 patients with noninfectious systemic inflammation, and 536 healthy controls (i.e., subjects without known systemic inflammation). The study characteristics are presented in Table 1.

According to our quality assessment, 16 studies were considered as high quality, while 5 studies as moderate quality (Supplementary Table S2). Based on visual inspection of the funnel plots (Supplementary Figs. S1–S4), some asymmetry could be present, indicating the possible existence of publication bias, which was confirmed by the results of Egger's test ($p < 0.1$) in one of the analyses (Supplementary Fig. S4). Sensitivity analysis was performed for overall SMD presented in the forest plots. The overall SMDs did not vary substantially after excluding any individual study, indicating that the results were not driven by one of the analyzed individual studies (Tables S3–S6).

Blood levels of MIF in sepsis, noninfectious systemic inflammation, and healthy control groups. First, we investigated the change in blood MIF levels in response to sepsis compared to healthy

Study report [number in list of references]	Country	Study population	Population subgroups	N (males)	Mean years of age (SD)	Mean severity score (SD)	Deaths N (%)
Ameen et al.[37]	Kingdom of Saudi Arabia	Severe sepsis and septic shock	Survivor	22 (12)	59 (5)	APACHE = 25 (4)	0
			Nonsurvivor	17 (9)	64 (4)	APACHE = 21 (2)	17 (100)
Beishuizen et al.[13]	The Netherlands	Healthy control		41 (23)	62 (9)	NA	0
		Multiple trauma		8 (7)	52 (17)	APACHE II = 10 (2)	0
		Septic shock		32 (20)	64 (13)	APACHE II = 15 (6)	11 (34)
			Survivor	21 (NR)	61 (11)	APACHE II = 12 (5)	0
			Nonsurvivor	11 (NR)	67 (14)	APACHE II = 18 (5)	11 (100)
			Without ARDS	24 (NR)	59 (13)	APACHE II = 11 (6)	NR
			With ARDS	8 (NR)	64 (12)	APACHE II = 19 (4)	NR
Bozza et al.[29]	Brazil	Healthy control		11 (NR)	NR	NA	NR
		Sepsis		17 (10)	59 (23)	APACHE II = 17 (6)	3 (18)
		Septic shock		25 (15)	59 (27)	APACHE II = 21 (7)	13 (52)
Brenner et al.[14]	Germany	Healthy control		18 (10)	35 (9)	NA	0
		Major surgery		28 (12)	62 (14)	NR	0
		Severe sepsis and septic shock	Survivor and nonsurvivor	87 (51)	69 (12)	NR	44 (51)
Calandra et al.[16]	Switzerland	Healthy control		6 (NR)	Median = 40	NA	NR
		Sepsis	Severe sepsis and septic shock	16 (13)	52 (18)	SAPS II = 45 (14)	6 (38)
Chuang et al.[38]	Taiwan	Severe sepsis	Survivor	81 (44)	67 (23)	APACHE II = 23 (8)	0
			Nonsurvivor	31 (24)			31 (100)
Chuang et al.[33]	Taiwan	Severe sepsis and septic shock	Survivor	109 (68)	71 (15)	APACHE II = 22 (8)	0
			Died in 48 h	12 (6)	68 (18)	APACHE II = 27 (7)	12 (100)
			Died after 48 h	32 (21)	74 (12)	APACHE II = 25 (8)	32 (100)
de Mendonca-Filho et al.[35]	Brazil	Sepsis	Negative microbiology	24 (16)	70 (2)	APACHE II = 15 (1)	5 (21)
			Positive microbiology	25 (17)	71 (2)	APACHE II = 16 (1)	12 (48)
Emonts et al.[31]	Switzerland and The Netherlands	Healthy control		196 (NR)	NR	NA	NR
		Sepsis, severe sepsis, and septic shock	Survivor	36 (18)	47 (17)	NR	0
			Early death	20 (17)	53 (14)	NR	20 (100)
			Late death	12 (9)	61 (13)	NR	12 (100)
Gando et al.[28]	Japan	Healthy control		10 (NR)	NR	NA	NR
		SIRS and sepsis	Without DIC	28 (17)	56 (3)	APACHE II = 17 (1)	1 (4)
			With DIC	20 (8)	51 (5)	APACHE II = 27 (2)	12 (60)
Gao et al.[17]	USA	Healthy control		53 (NR)	NR	NA	NR
		Sepsis		36 (NR)	NR	NR	NR
		Sepsis-induced acute lung injury		53 (NR)	NR	NR	19 (36)
Kofoed et al.[32]	Denmark	Healthy control		10 (NR)	NR	NA	NR
		Sepsis		10 (NR)	NR	NR	NR
Leaver et al.[25]	UK	Healthy control		20 (10)	NR	NA	NR
		Severe sepsis and septic shock		35 (22)	62 (22)	19 (6)	10 (29)
Lehmann et al.[12]	Germany	Healthy control		10 (NR)	NR	NA	NR
		Nonseptic critically ill		18 (17)	60 (18)	SOFA = 2 (1)	NR
		Severe sepsis		19 (14)	44 (16)	SOFA = 10 (2)	NR
Lehmann et al.[18]	Germany	Healthy control		34 (NR)	NR	NA	NR
		Nonseptic critically ill		10 (7)	61 (17)	SOFA = 3 (1)	0
		Severe sepsis	Survivor	23 (NR)	55 (11)	SOFA = 9 (3)	0
			Nonsurvivor	14 (NR)		SOFA = 16 (3)	14 (100)
Meawed et al.[15]	Egypt	Nonseptic systemic inflammation		28 (19)	50 (5)	NR	NR
		Sepsis	Survivor and nonsurvivor	25 (15)	53 (6)	APACHE II = 17 (3)	4 (16)
		Severe sepsis		27 (16)	63 (7)	APACHE II = 20 (3)	15 (56)
Merk et al.[26]	Canada	Healthy control		85 (NR)	NR	NA	NR
		Severe sepsis and septic shock		37 (22)	60 (17)	APACHE II = 22 (7)	10 (27)

Continued

Study report [number in list of references]	Country	Study population	Population subgroups	N (males)	Mean years of age (SD)	Mean severity score (SD)	Deaths N (%)
Miyauchi et al.[34]	Japan	Sepsis	Normal adrenal response	22 (14)	63 (17)	APACHE II = 26 (6)	6 (27)
			Adrenal insufficiency	19 (16)	66 (15)	APACHE II = 26 (10)	6 (32)
Payen et al.[36]	France	Severe sepsis and septic shock	Without acute kidney injury	47 (30)	Median = 60	Median SOFA = 5	6 (12)
			Mild acute kidney injury	75 (47)	Median = 61	Median SOFA = 7	20 (26)
			Severe acute kidney injury	54 (34)	Median = 63	Median SOFA = 10	22 (41)
Pohl et al.[30]	Germany	Healthy control		10 (NR)	NR	NA	NR
		Nonseptic critically ill		42 (28)	69 (13)	APACHE II = 24 (9)	35 (83)
		Severe sepsis and septic shock		30 (19)	69 (11)	APACHE II = 26 (9)	13 (43)
Wiersinga et al.[27]	Thailand	Healthy controls		32 (23)	41 (9)	NA	NR
		Sepsis	Survivor and nonsurvivor	34* (17)	52 (16)	NR	15* (44)

Table 1. Characteristics of participants in the studies included in the meta-analysis. *MIF levels were reported for 29 septic and 10 survivor patients. *ARDS* adult respiratory distress syndrome, *APACHE* acute physiology and chronic health evaluation score, *DIC* disseminated intravascular coagulation, *NA* not applicable, *NR* not reported, *SAPS* simplified acute physiology score, *SIRS* systemic inflammatory response syndrome, *SOFA* sequential (sepsis-related) organ failure assessment score.

control subjects. We found 14 studies, reporting data from 579 septic patients and 536 healthy participants that could be included in our analysis (Fig. 2). The relative weight of the studies was similar, ranging between 5 and 8%. As it could be expected based on the function of MIF as a proinflammatory cytokine[11], the blood levels of MIF were higher in septic patient groups than in controls in the analyzed studies with SMDs ranging from 0.23 to 3.51 between the groups. Overall, in septic patient groups blood MIF levels were significantly ($p < 0.001$) higher than in healthy controls with an SMD of 1.47 (95% CI: 0.96–1.97) (Fig. 2).

Next, we studied whether blood MIF levels are increased to a similar or to a different extent in sepsis and in noninfectious systemic inflammation. We could include 6 studies in the quantitative analyses, which reported data from 257 septic patients and 134 patients with nonseptic systemic inflammation (Fig. 3). In the latter group, the cause of systemic inflammation was surgical intervention[12,14,18], multiple trauma[13], and not sepsis-related fever[15] or critical illness[30] (see also Table 1). The relative weight of the studies ranged from 11 to 20%. Blood MIF levels were higher in septic patient groups than in patient groups with nonseptic systemic inflammation in all of the analyzed studies. The overall SMD was 0.94 (95% CI: 0.51–1.38) between the groups ($p < 0.001$) (Fig. 3).

From three studies which presented blood MIF level values of individual participants[25–27], we could extract the data of 101 septic patients and 141 healthy controls. ROC curve analysis of these data revealed an AUC of 0.850 (Fig. 4), indicating that blood MIF level measurement shows good sensitivity and specificity for the diagnosis of sepsis.

Blood levels of MIF in septic patient groups with different severities of the disease. After studying MIF as a potential diagnostic biomarker in sepsis, we also wanted to analyze whether the elevation in blood MIF levels can predict the severity of the disease. We found eligible data to address this question from two approaches: (1) by comparing patient groups with less and more severe sepsis (e.g., based on the presence of organ dysfunction) within the same study; and (2) by comparing survivor and nonsurvivor septic patient groups within the same study.

We found 11 studies, in which blood MIF levels were reported in different severity groups of sepsis. The groups with more severe form of the disease were categorized based on different criteria in the different studies, which included the presence of one of the following conditions: organ damage (viz., pulmonary, kidney or adrenal gland dysfunction)[13,17,34,36], septic shock[16,29], early fatality[31,33], severe sepsis[15], disseminated intravascular coagulopathy[28], and positive blood culture[35] (for details, see Table 1). In the majority of the studies, higher clinical severity scores were also reported in the patient groups with more severe disease. In total, 347 patients were included in the more severe and 274 patients in the less severe septic groups. The relative weight of the studies was between 7 and 11%. Our meta-analysis revealed that blood MIF level was significantly ($p < 0.001$) higher in the more severe forms of sepsis with an overall SMD of 0.84 (95% CI: 0.45–1.24) (Fig. 5).

Blood MIF levels were compared between survivors and nonsurvivors of sepsis in 11 studies, including 447 and 257 patients in the groups, respectively. The studies had similar relative weights, ranging from 7 to 11%. For the meta-analysis, SMD was calculated by subtracting the mean blood MIF level of sepsis survivors from that of sepsis nonsurvivors. We found that the overall SMD was significantly ($p < 0.001$) higher than zero (0.75, 95% CI: 0.40–1.11) (Fig. 6), indicating that blood MIF levels were markedly higher in nonsurvivors than in survivors of sepsis.

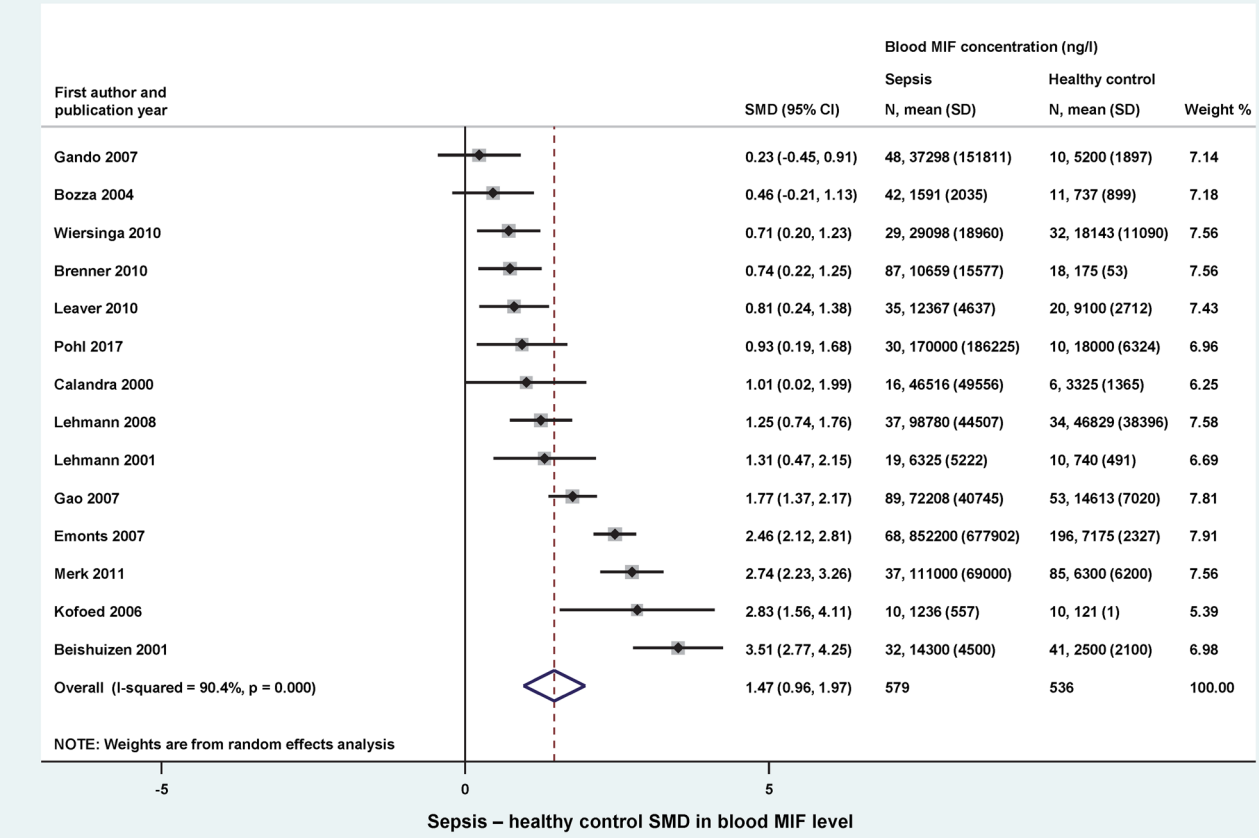

Figure 2. Forest plot of standardized mean differences (SMDs) in blood levels of macrophage migration inhibitory factor (MIF) between septic patients and healthy controls. Here, and in Figs. 3, 5, and 6 black diamonds represent the SMD for each study, while the left and right horizontal arms of the diamonds indicate the corresponding 95% confidence intervals (CIs). The size of the gray box surrounding the diamond is proportional to the relative weight of the study. The open rhombus on the bottom represents the average SMD calculated from the SMDs of all individual studies. The left and right vertices of the rhombus represent the CIs of the average SMD, while the vertical diagonal and the dashed line indicate the average SMD of all studies in the forest plot. A negative SMD indicates higher MIF levels in healthy controls, whereas an SMD greater than zero indicates increased MIF levels in sepsis. *SD* standard deviation.

Discussion

In the present study, we show that blood MIF level can be a useful biomarker in sepsis for both diagnostic and prognostic purposes, to the best of our knowledge for the first time, with the meta-analysis of the available data in the literature. The main new findings of our meta-analyses are that blood MIF levels are increased to a greater extent in sepsis than in systemic inflammation of noninfectious origins and that MIF levels are higher in the more severe forms of sepsis and in nonsurvivors than in less severe forms and survivors, respectively.

Sepsis affects tens of millions of patients annually and it constitutes an ongoing challenge for the healthcare system due to its high mortality and economic burden, especially in its severe forms[39]. A recent analysis showed that in intensive care units, hospital-acquired sepsis is frequent and accounts for a high (over 40%) mortality rate[40]. In order to improve outcomes, it is required to further develop the approaches for early diagnosis and implementation of adequate treatment of sepsis. The use of biomarkers can help to achieve these goals. As a consequence, a plethora of potential biomarkers was evaluated for the diagnosis and prognosis of sepsis (for a recent review, see[6]).

As an early step in the development of systemic inflammation, the activation of innate immune cells leads to the production of inflammatory cytokines[41]. MIF is one of these proinflammatory cytokines, which was originally thought to be produced in the pituitary gland and T lymphocytes, but later it was found to be expressed in a variety of cells, including endothelial cells, eosinophils, and macrophages[42]. Upon stimulation by endotoxins and cytokines, macrophages release MIF, which acts in concert with other cytokines (e.g., tumor necrosis factor-α) and promotes the acute inflammatory response[43]. In humans, high MIF concentrations were first found in the alveolar airspaces of patients with acute respiratory distress syndrome[44], which is a frequent complication in severe (often fatal) forms of sepsis[45]. Since then, several studies showed that blood MIF level is increased in different forms of systemic inflammation[13,16,26]. As a consequence, MIF was considered amongst the potential diagnostic and prognostic biomarkers in sepsis[6,7,46].

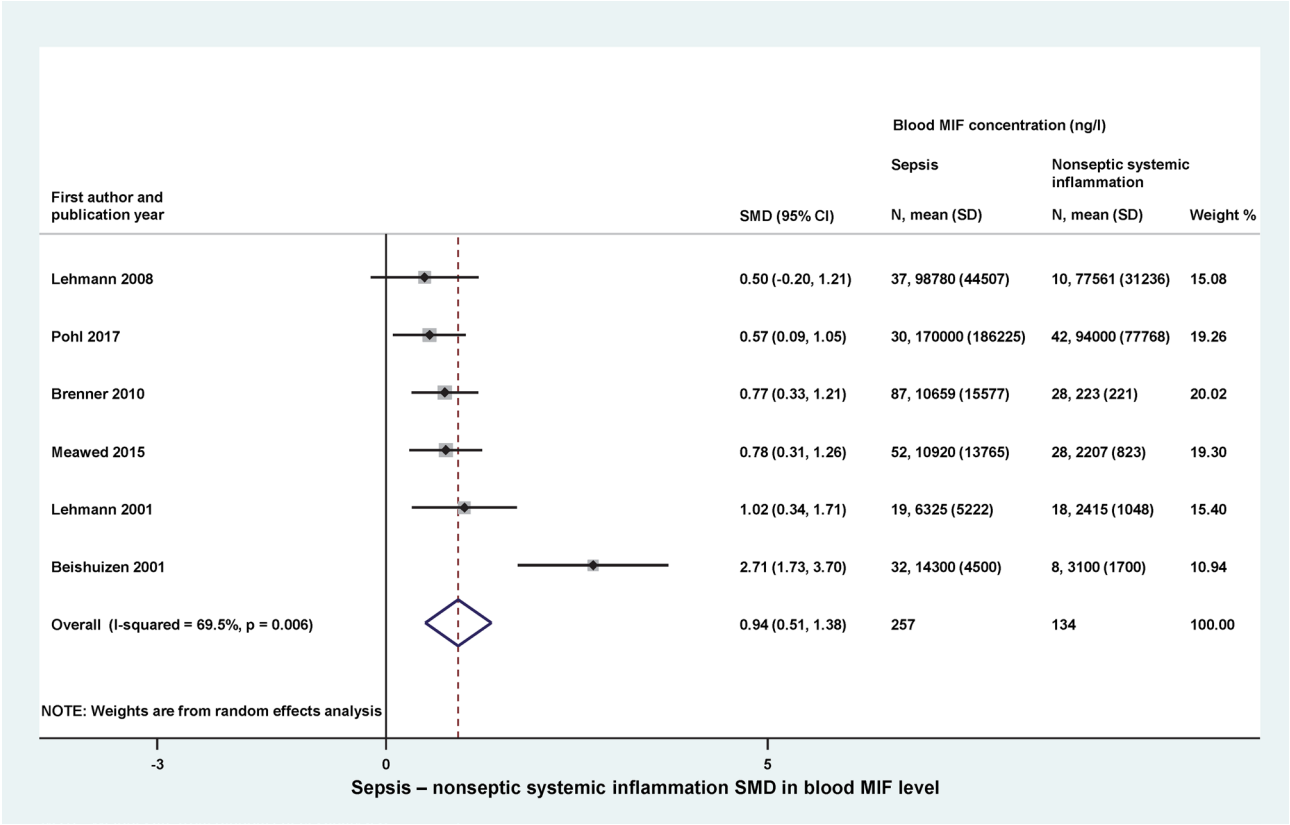

Figure 3. Forest plot of standardized mean differences (SMDs) in blood levels of macrophage migration inhibitory factor (MIF) between septic patients and patients with systemic inflammation due to noninfectious causes. *CI* confidence interval, *SD* standard deviation.

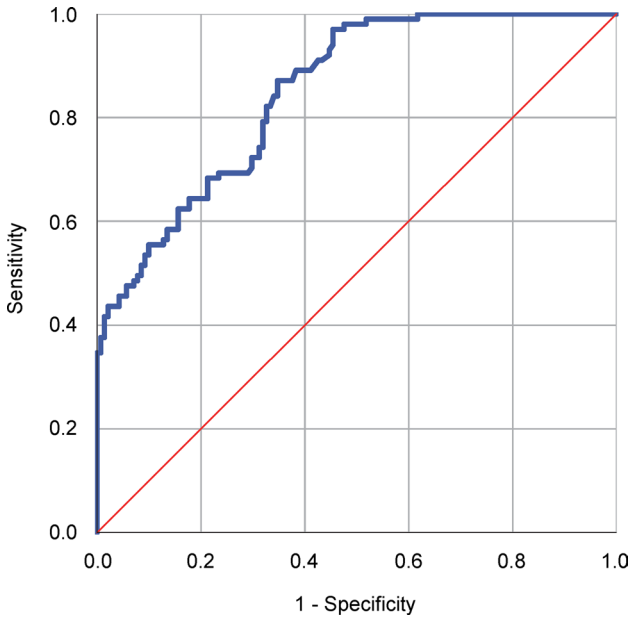

Figure 4. Receiver operating characteristic (ROC) curve analysis of the diagnostic performance of blood macrophage migration inhibitory factor (MIF) levels in sepsis. The individual data of septic patients (N = 101) and healthy controls (N = 141) were extracted from previously published studies[25–27]. The area under the blue ROC curve was 0.850. The diagonal red line serves as a reference line corresponding to the ROC curve of a diagnostic test that randomly classifies the condition (i.e., a test that has no diagnostic ability).

It has not been fully clarified, however, whether septic and nonseptic systemic inflammation can be

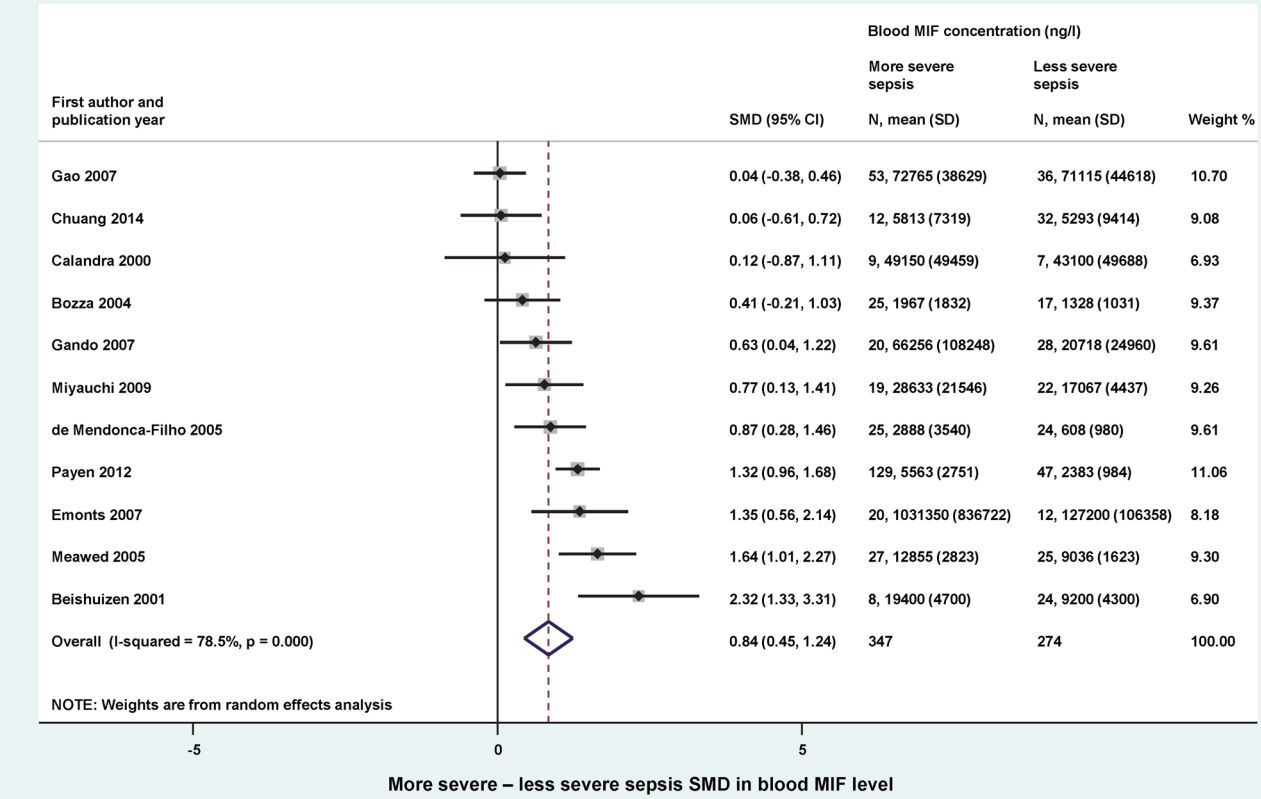

Figure 5. Forest plot of standardized mean differences (SMDs) in blood levels of macrophage migration inhibitory factor (MIF) between patients with more severe and less severe forms of sepsis. *CI* confidence interval, *SD* standard deviation.

distinguished based on the different extent of elevation in blood MIF levels. Some authors found that MIF levels were higher in sepsis than in noninfectious systemic inflammation[13–15,30], whereas others did not find a significant difference in MIF levels between the two forms of systemic inflammation[12,18]. In the present study, we compared MIF levels in sepsis and in noninfectious inflammation of different origins (see Table 1, for details) in 257 and 134 patients, respectively, and showed that blood MIF concentration is markedly increased in case of sepsis compared to nonseptic systemic inflammation. These findings suggest that MIF can be used as a diagnostic tool to distinguish sepsis from other systemic inflammatory diseases. It can be assumed that the production of MIF is more enhanced when the triggering agent of the inflammatory reaction is a microbial pathogen than when it is a damage-associated molecular pattern (DAMP). Indeed, it has been shown that DAMPs and pathogen-associated molecular patterns (PAMPs) activate the immune system differently, in particular, DAMPs produce weaker innate immune activation than PAMPs, which also involves more pronounced production of inflammatory cytokines in case of PAMPs[47]. In line with these findings in experimental models, the increased MIF levels in multiple trauma patients were further elevated when an infection developed, suggesting that MIF may be an indicator of secondary infection[48,49].

The prognostic value of MIF is also a controversial issue. In the study by Beishuizen et al.[13], MIF levels tended to be higher in septic shock patients who developed acute respiratory distress syndrome than in those who did not ($p = 0.115$). MIF levels seemed higher in septic shock than in severe sepsis in the fundamental study by Calandra et al.[16], but the difference between the groups was not significant. Furthermore, MIF levels did not differ between survivors and nonsurvivors of severe sepsis[18], contradicting earlier reports about higher circulating MIF levels in nonsurvivor sepsis patients[13,14,50], and about its association with fatal outcome in sepsis[29]. In the present work, we showed that MIF levels were significantly higher in the groups with worse prognosis, indicating that MIF can be a useful biomarker to predict the severity and the outcome of the disease. It can be assumed that in severe forms of sepsis an overt inflammatory reaction develops, which also involves a pronounced cytokine storm and excessive production of MIF. As a result, the pro- and anti-inflammatory processes become unbalanced, the inflammatory response loses its adaptive biological function, and turns into an unregulated, destructive process, which is no longer beneficial, but instead harmful for the host. The role of MIF can be crucial in the disruption of the pro- and anti-inflammatory balance, because MIF counter-regulates the anti-inflammatory and immunosuppressive effects of glucocorticoids[51–53]. Based on this scenario, it can be also understood, why neutralization of MIF with antibodies improved the outcome in animal models of severe systemic inflammation[16,54,55]. Whether MIF can be used as a therapeutic target and marker in septic patients, as proposed by different authors[16,56], remains subject for future research.

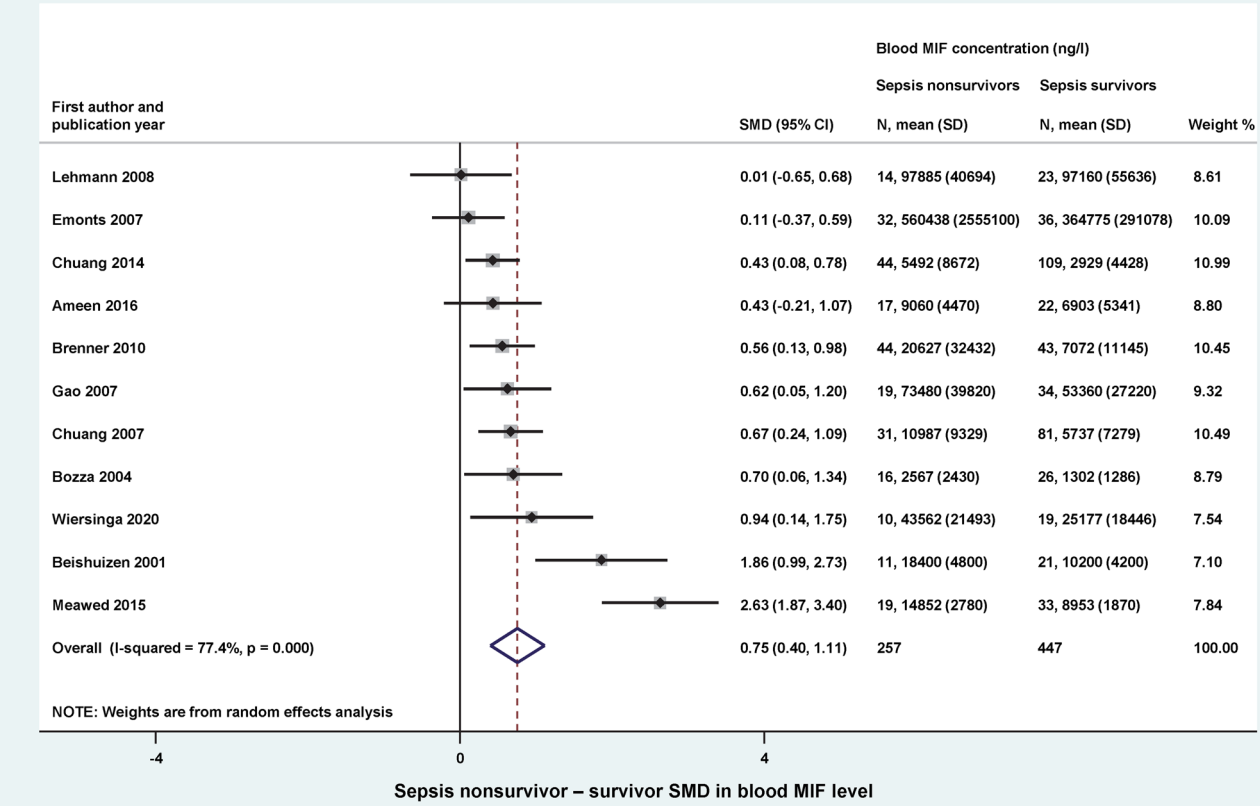

Figure 6. Forest plot of standardized mean differences (SMDs) in blood levels of macrophage migration inhibitory factor (MIF) between sepsis nonsurvivors and survivors. *CI* confidence interval, *SD* standard deviation.

Some limitations of our study should be noted. Due to the nature of the meta-analysis method, we have studied the reported mean MIF levels in patient groups, instead of MIF levels in individual patients. The latter approach would certainly allow one to draw firmer conclusions about the association between MIF and the diagnosis and prognosis of sepsis, but that would require access to the original data of the analyzed articles, which was not feasible. Due to lack of data, we could not perform a network meta-analysis to compare the performance of MIF with other frequently used inflammatory biomarkers, hence we cannot make any comment on its real value compared to others.

In our study, we compared blood MIF level in septic patients to that of either healthy controls or patients with nonseptic systemic inflammation. This method can be useful to identify potential diagnostic biomarkers, but it cannot be used to determine the diagnostic performance of MIF. To evaluate diagnostic test performance, the pre- and post-test probabilities are required, but "pre-test probability" amongst healthy controls is 0 (and thus "post-test probability" is also 0). The diagnostic performance of MIF is likely to be lower when distinguishing noninfectious systemic inflammation from sepsis, because of the smaller sample size (391 vs. 1115) and the lower SMD (0.94 vs. 1.47) compared to the analysis of healthy controls and septic patients.

An ideal study would include patients who were clinically suspected of sepsis, and compare their MIF levels with confirmed diagnosis of sepsis as this would allow assessment of the post-test probability of this test. Unfortunately, the analyzed studies did not have such ideal design. There were only two studies which included patients with suspicion of sepsis[29,30], but those did not report the diagnostic performance of MIF only its good performance for the prediction of mortality. In another study, MIF levels between septic patients and healthy volunteers were compared and ROC curve analysis was performed, which indicated excellent sensitivity and specificity for MIF (AUC of 0.99)[26]. Further, in patients with clinical diagnosis of sepsis, MIF levels showed good performance in the prediction of positive bacterial cultures (AUC of 0.823)[35].

For the assessment of diagnostic performance, the separation between positive and negative cases is important as it indicates the potential for false positive and false negative results. This is best assessed by ROC curve analysis, which requires individual patient data. As an attempt to perform ROC curve analysis, we extracted individual patient data from eligible papers[25–27], and showed that blood MIF level has good diagnostic performance to distinguish septic patients from healthy controls. However, we could not collect sufficient data to perform the ROC curve analysis for the diagnostic value of MIF between infectious and noninfectious systemic inflammation and for its prognostic performance. Therefore, to exclude the possibility that mean levels of MIF simply differed

significantly between the cohorts examined, in future studies additional ROC curve analyses are warranted to support our findings about the diagnostic and prognostic ability of MIF.

Another important issue with the comparison between sepsis and nonseptic systemic inflammation is that in 3 of the analyzed studies[12,13,18] the clinical severity scores were significantly higher in septic than in nonseptic patients. Since we also showed that blood MIF levels are higher in more severe forms of sepsis than in less severe forms (Fig. 5), it cannot be excluded that the difference in MIF levels between septic and nonseptic patients was also influenced by the higher severity scores in the septic patients in some of the studies.

The studied population of patients was quite diverse and statistical, methodological, and medical differences in study design could all contribute to the considerably high between-study heterogeneity (indicated by an I^2 of 70–90%), as observed in our analysis (Figs. 2, 3, 5, 6). To account for the presence of heterogeneity, we used the random-effects model in all forest plots of our meta-analyses.

In the analyzed studies, blood MIF levels between patients' groups were compared within the same study and the difference was included in the forest plot. Since the reported MIF values differed substantially among the analyzed studies, ranging between 121 ng/l[32] and 46,829 ng/l[18] in healthy controls (Fig. 2), SMDs had to be used to mitigate methodological differences in MIF level measurements. Consequently, in the present analysis we could not determine a specific cut-off MIF level which would be a diagnostic or prognostic threshold in sepsis. The most convincing method to obtain direct evidence for the diagnostic and prognostic performance of MIF in sepsis would be to conduct high-quality, targeted clinical trials in a broad population of patients who are clinically suspected of sepsis. Until such or similar trials are conducted, we are restricted to use different (not so direct) approaches, e.g., meta-analyses. In the design of future studies, other classical and novel biomarkers, perhaps in combination with MIF, may be also considered, for example, neutrophil CD64, which was superior to procalcitonin for the identification of sepsis according to a recent meta-analysis[57].

Despite the mentioned limitations, we believe that the size of the analyzed sample (N = 1876) was big enough to mitigate the methodological differences among the studies, therefore we may draw, at least some, conclusions about the potential diagnostic and prognostic value of MIF in septic patients.

Conclusions

To the best of our knowledge, this is the first meta-analysis to show that blood MIF levels could have diagnostic ability to differentiate between infectious and noninfectious systemic inflammation and could have prognostic value for the outcome of sepsis. Our results can also serve as an encouraging basis for the design of high-quality, targeted clinical studies aiming to determine the real diagnostic and prognostic performance of MIF level measurements in sepsis.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information.

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Author contributions
J.T. and A.G. conceived the study, participated in the design, collected and interpreted the data, and wrote the manuscript. D.N. and N.F. performed statistical analyses, interpretation of data, and helped to draft the manuscript. P.H., Z.M., M.S., Z.R., and E.P. participated in study design and helped to edit and review the manuscript. Z.R. and E.P. contributed to interpretation of the data and editing of the manuscript. Z.M. and H.A. contributed to the development of the review concept and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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SUPPLEMENTARY INFORMATION

Macrophage migration inhibitory factor as a diagnostic and predictive biomarker in sepsis: meta-analysis of clinical trials

Janos Toldi, David Nemeth, Peter Hegyi, Zsolt Molnar, Margit Solymar, Nelli Farkas, Hussain Alizadeh, Zoltan Rumbus, Eszter Pakai, and Andras Garami

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TABLE S1

PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	1-2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (*e.g.*, Web address), and, if available, provide registration information, including registration number.	2
Eligibility criteria	6	Specify study characteristics (*e.g.*, PICOS, length of follow-up) and report characteristics (*e.g.*, years considered, language, publication status) used as criteria for eligibility, giving rationale.	2
Information sources	7	Describe all information sources (*e.g.*, databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	2
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	2
Study selection	9	State the process for selecting studies (*i.e.*, screening) and list the inclusion and exclusion criteria.	2
Data collection process	10	Describe method of data extraction from reports (*e.g.*, piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	2
Data items	11	List and define all variables for which data were sought (*e.g.*, PICOS, funding sources) and any assumptions and simplifications made.	2
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	2
Summary measures	13	State the principal summary measures (*e.g.*, risk ratio, difference in means).	2
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency for each meta-analysis.	2
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (*e.g.*, publication bias, selective reporting within studies).	2
Additional analyses	16	Describe methods of additional analyses (*e.g.*, sensitivity or subgroup analyses, meta-regression), if done, indicating which were prespecified.	2-3

RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusion at each stage, ideally with a flow diagram.	3; Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (*e.g.*, study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table S2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a "forest plot".	5; Figs. 2, 3, 5, and 6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	5; Figs. 2, 3, 5, and 6
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Figs. S1-S4
Additional analysis	23	Give results of additional analyses, if done [(*e.g.*, sensitivity or subgroup analyses, meta-regression (see Item 16)].	5; Fig. 4; Tables S3-S6
DISCUSSION			
Summary of evidence	24	Summarize the main findings, including the strength of evidence for each main outcome; consider their relevance to key groups (*e.g.*, healthcare providers, users, and policy makers).	6-10
Limitations	25	Discuss limitations at study and outcome level (*e.g.*, risk of bias), and at review-level (*e.g.*, incomplete retrieval of identified research, reporting bias).	9-10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (*e.g.*, supply of data); role of funders for the systematic review.	12

TABLE S2

Quality assessment of the studies included in the meta-analysis using the Newcastle-Ottawa Scale

Study report	Selection	Comparability	Outcome / Exposure	Total score
Ameen et al. 2016	****	*	**	7
Beishuizen et al. 2001	***	**	**	7
Bozza et al. 2004	****	**	***	9
Brenner et al. 2010	***	**	**	7
Calandra et al. 2000	***	*	**	6
Chuang et al. 2007	****	*	**	7
Chuang et al. 2014	****	*	**	7
de Mendonca-Filho et al. 2005	****	**	**	8
Emonts et al. 2007	****	*	***	8
Gando et al. 2007	***	*	**	6
Gao et al. 2007	***	*	***	7
Kofoed et al. 2006	***	**	**	7
Leaver et al. 2010	****	**	**	8
Lehmann et al. 2001	***	**	**	7
Lehmann et al. 2008	***	**	**	7
Meawed et al. 2015	***	*	**	6
Merk et al. 2011	***	*	**	6
Miayuchi et al. 2009	****	*	**	7
Payen et al. 2012	***	**	**	7
Pohl et al. 2017	***	*	**	6
Wiersinga et al. 2010	***	**	**	7

A score of 7 to 9 indicates a good, a score of 4 to 6 a fair, and a score of 0 to 3 a low methodological quality.

TABLE S3

Sensitivity analysis of standardized mean difference (SMD) of blood macrophage migration inhibitory factor (MIF) levels between septic patients and healthy controls

Study omitted	Estimated SMD	95% Confidence interval	
Beishuizen et al. 2001	**1.3131386**	**0.83256066**	**1.7937164**
Bozza et al. 2004	**1.542878**	**1.0238485**	**2.0619075**
Brenner et al. 2010	**1.5268586**	**0.9980076**	**2.0557096**
Calandra et al. 2000	**1.4980285**	**0.97095335**	**2.0251036**
Emonts et al. 2007	**1.3799106**	**0.8725971**	**1.8872242**
Gando et al. 2007	**1.5613711**	**1.0528249**	**2.0699174**
Gao et al. 2007	**1.4433948**	**0.87894446**	**2.0078452**
Kofoed et al. 2006	**1.3892726**	**0.87214762**	**1.9063975**
Leaver et al. 2010	**1.5203255**	**0.98886555**	**2.0517855**
Lehmann et al. 2001	**1.4789149**	**0.94641781**	**2.0114119**
Lehmann et al. 2008	**1.4859716**	**0.93801826**	**2.0339251**
Merk et al. 2011	**1.3613919**	**0.85557604**	**1.8672078**
Pohl et al. 2017	**1.5072438**	**0.97631407**	**2.0381734**
Wiersinga et al. 2010	**1.5301822**	**1.0035641**	**2.0568004**
None	**1.4670072**	**0.9625479**	**1.9714665**

TABLE S4

Sensitivity analysis of standardized mean difference (SMD) of blood macrophage migration inhibitory factor (MIF) levels between septic patients and patients with systemic inflammation due to noninfectious causes

Study omitted	Estimated SMD	95% Confidence interval	
Beishuizen et al. 2001	**0.72372264**	**0.4889625**	**0.95848274**
Brenner et al. 2010	**1.0167089**	**0.4488349**	**1.5845827**
Lehmann et al. 2001	**0.94416475**	**0.43312234**	**1.4552072**
Lehmann et al. 2008	**1.0349311**	**0.5354175**	**1.5344447**
Meawed et al. 2015	**1.0095743**	**0.45152059**	**1.567628**
Pohl et al. 2017	**1.0511286**	**0.51861775**	**1.5836395**
None	**0.94433918**	**0.5105363**	**1.3781421**

TABLE S5

Sensitivity analysis of standardized mean difference (SMD) of blood macrophage migration inhibitory factor (MIF) levels between patients with more severe and less severe forms of sepsis

Study omitted	Estimated SMD	95% Confidence interval	
Beishuizen et al. 2001	**0.73298991**	**0.35420334**	**1.1117765**
Bozza et al. 2004	**0.88733292**	**0.45857477**	**1.3160911**
Calandra et al. 2000	**0.89526081**	**0.48256442**	**1.3079573**
Chuang et al. 2014	**0.91881663**	**0.50963479**	**1.3279985**
de Mendonca-Filho et al. 2005	**0.84071708**	**0.40133503**	**1.280099**
Emonts et al. 2007	**0.79637277**	**0.3768**	**1.2159455**
Gando et al. 2007	**0.8657974**	**0.42823938**	**1.3033555**
Gao et al. 2007	**0.93599939**	**0.56038755**	**1.3116113**
Meawed et al. 2015	**0.75772506**	**0.35516757**	**1.1602826**
Miayuchi et al. 2009	**0.85059726**	**0.41473499**	**1.2864596**
Payen et al. 2012	**0.78183091**	**0.36327559**	**1.2003863**
None	**0.84135154**	**0.44536898**	**1.2373341**

TABLE S6

Sensitivity analysis of standardized mean difference (SMD) of blood macrophage migration inhibitory factor (MIF) levels between sepsis nonsurvivors and survivors

Study omitted	Estimated SMD	95% Confidence interval	
Ameen et al. 2016	**0.79046631**	**0.4045704**	**1.1763623**
Beishuizen et al. 2001	**0.66525865**	**0.32198629**	**1.0085311**
Bozza et al. 2004	**0.76476264**	**0.37764108**	**1.1518843**
Brenner et al. 2010	**0.78628105**	**0.38203526**	**1.1905268**
Chuang et al. 2007	**0.77411079**	**0.36889201**	**1.1793295**
Chuang et al. 2014	**0.80348003**	**0.39395151**	**1.2130086**
Emonts et al. 2007	**0.82790214**	**0.45119721**	**1.2046071**
Gao et al. 2007	**0.77398223**	**0.3826614**	**1.1653031**
Lehmann et al. 2008	**0.82480216**	**0.45337233**	**1.196232**
Meawed et al. 2015	**0.55956095**	**0.3251034**	**0.79401851**
Wiersinga et al. 2010	**0.74225289**	**0.36428884**	**1.120217**
None	**0.75497058**	**0.40006185**	**1.1098793**

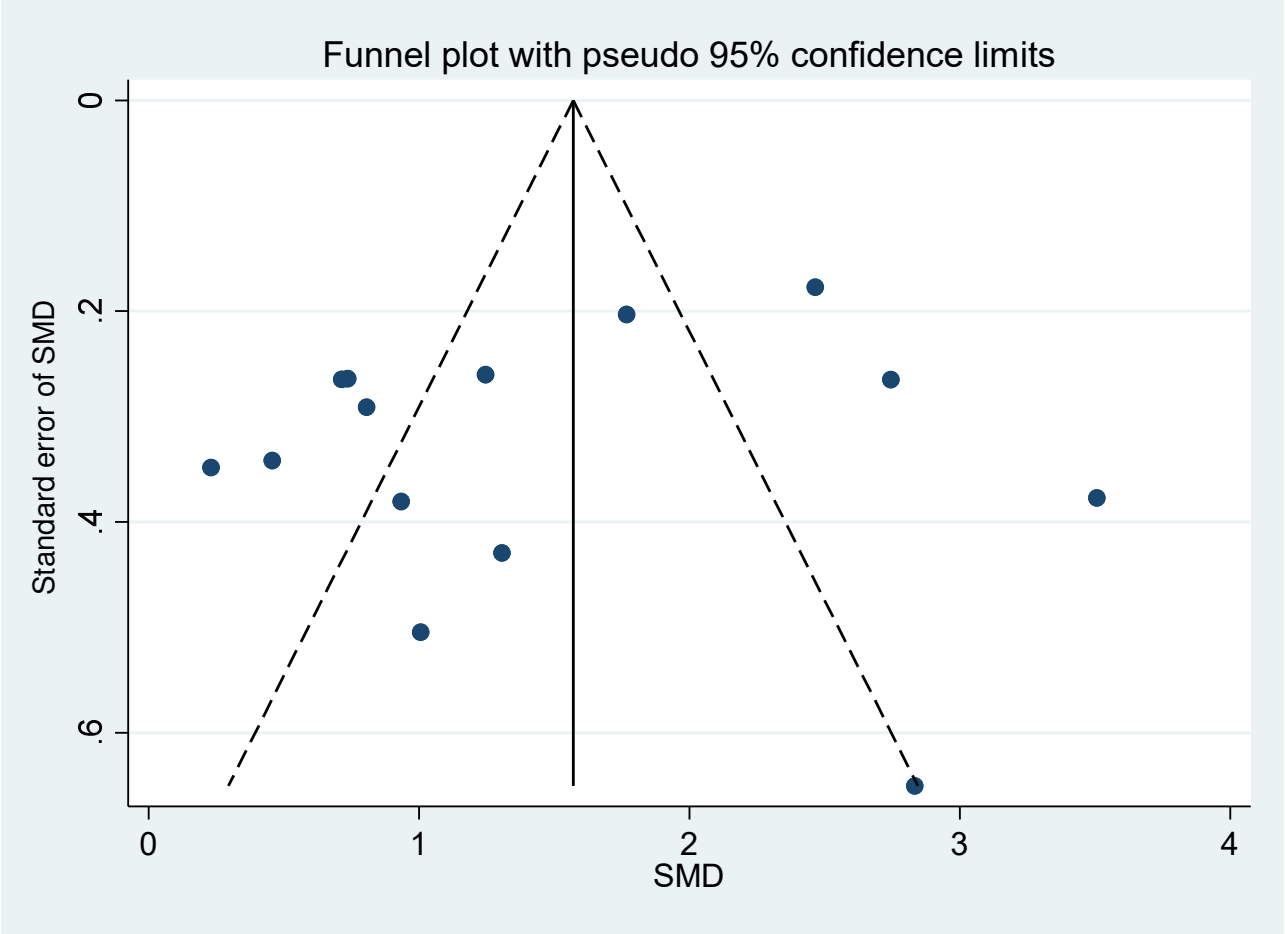

FIGURE S1. Funnel plot of the studies that were included in the comparison of blood macrophage migration inhibitory factor (MIF) levels between septic patients and healthy controls (n = 14, Egger's test: p = 0.407). Here, and in Figures S2-S4, the dots represent results from studies included in the corresponding forest plot. The triangular area is expected to harbor 95% of data in the absence of publication bias. The average standardized mean difference (SMD) for all studies corresponds to the "funnel axis" (vertical black line). A high degree of asymmetry in the distribution of data relative to the funnel axis and significant Egger's test (p < 0.1) indicate that the results are likely to be affected by a bias.

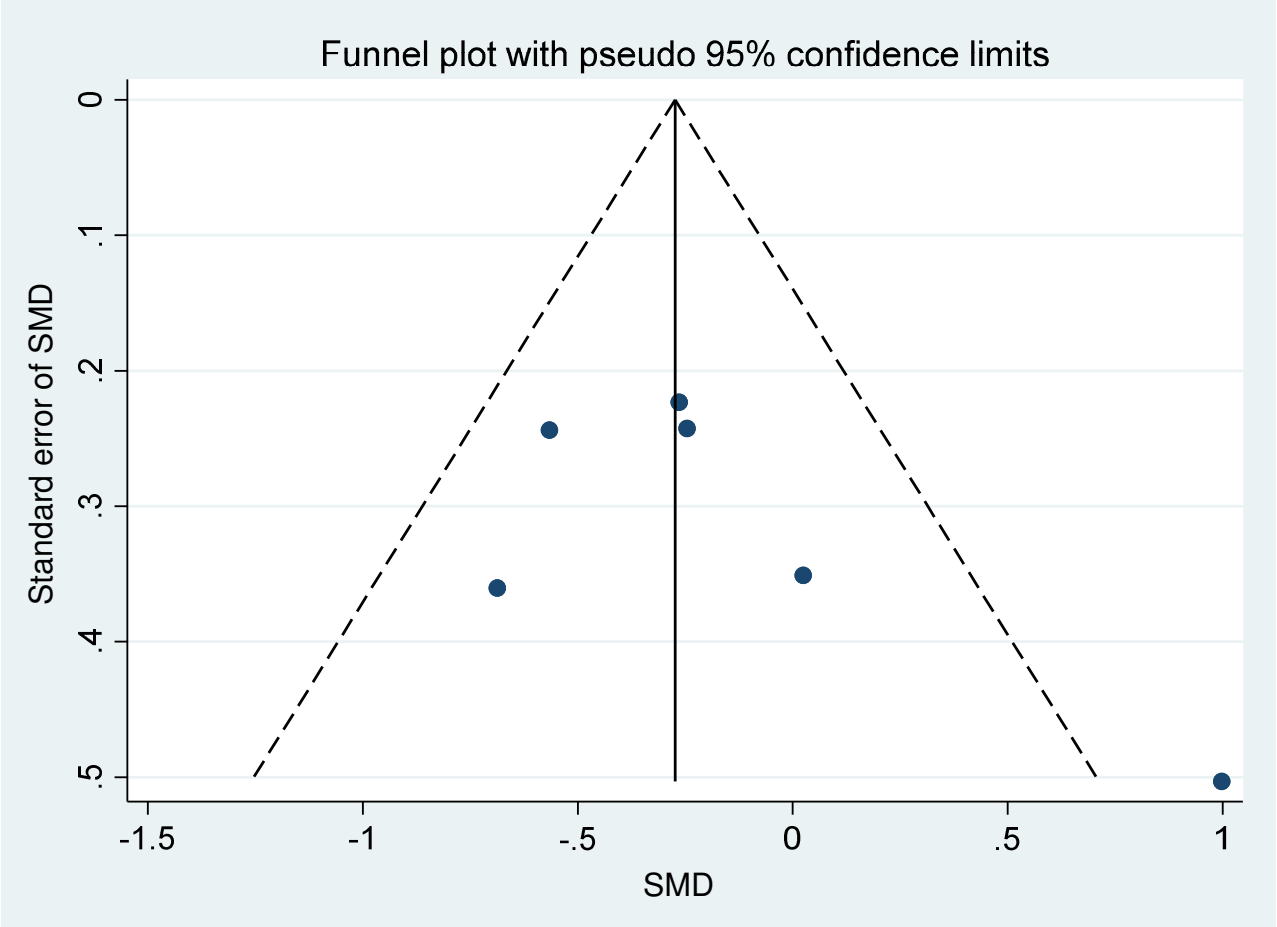

FIGURE S2. Funnel plot of the studies that were included in the comparison of blood macrophage migration inhibitory factor (MIF) levels between septic patients and patients with systemic inflammation due to noninfectious causes (n = 6).

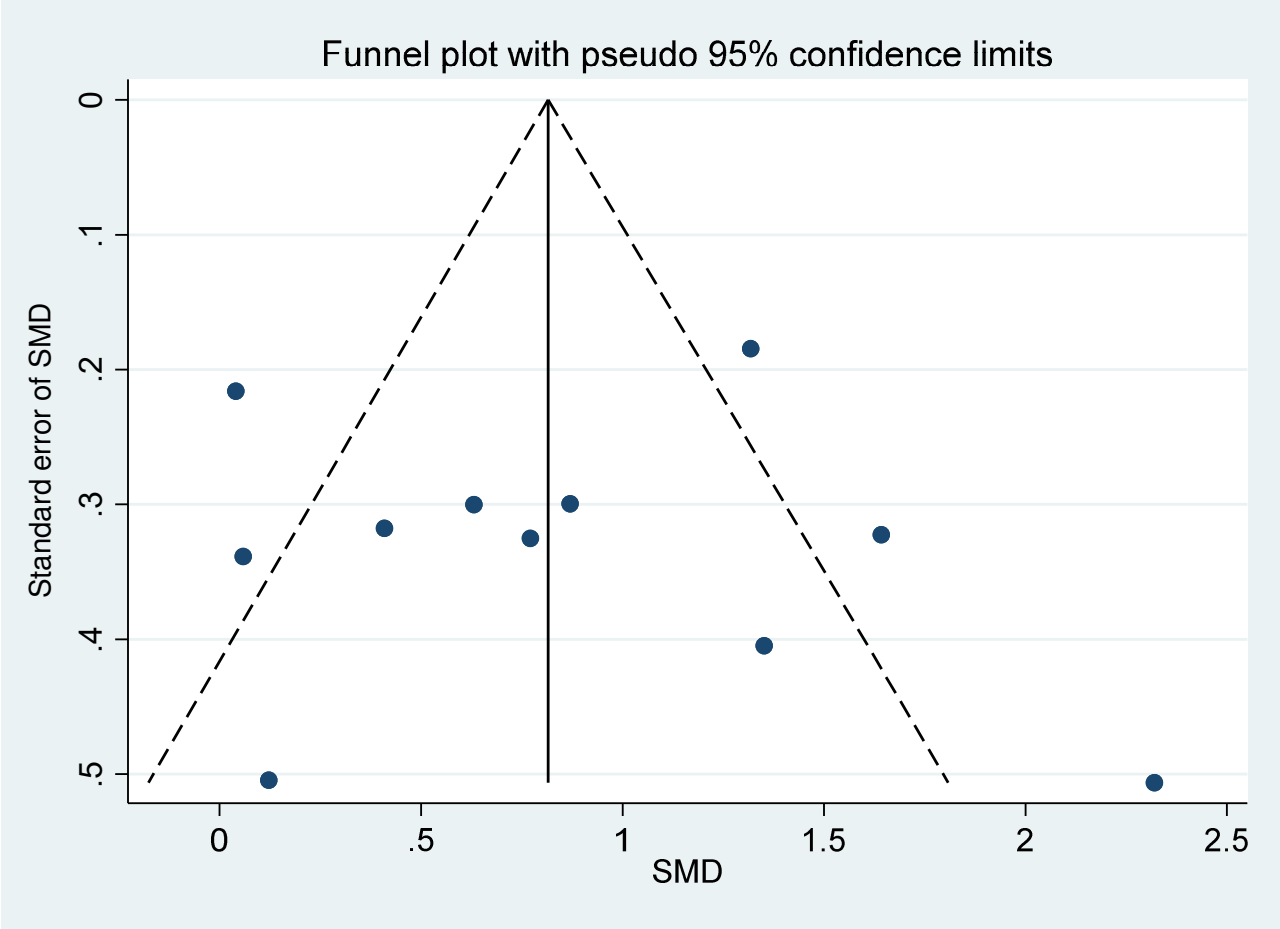

FIGURE S3. Funnel plot of the studies that were included in the comparison of blood macrophage migration inhibitory factor (MIF) levels between patients with more severe and less severe forms of sepsis (n = 11, Egger's test: $p = 0.815$).

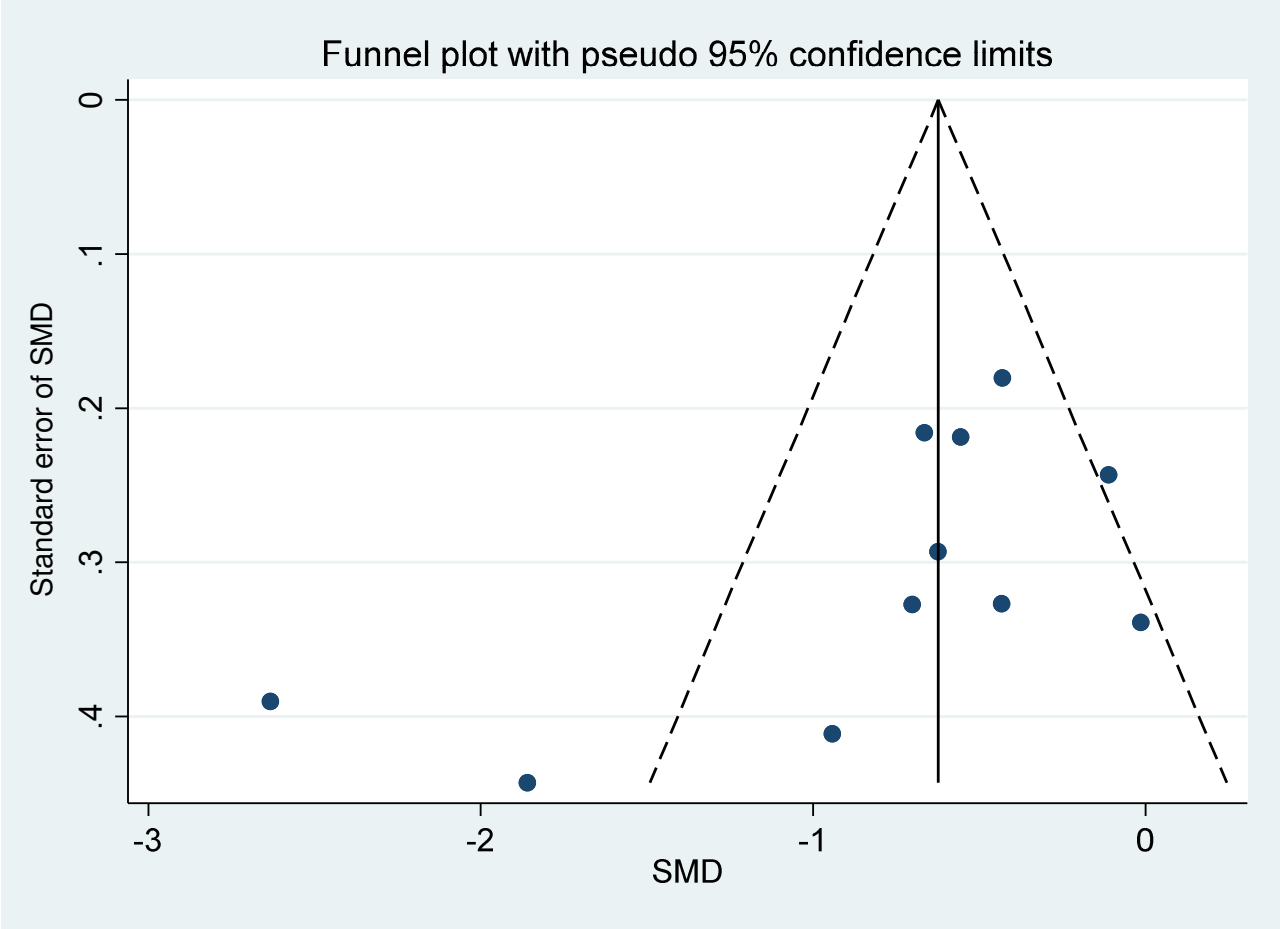

FIGURE S4. Funnel plot of the studies that were included in the comparison of blood macrophage migration inhibitory factor (MIF) levels between sepsis nonsurvivors and survivors (n = 11, Egger's test: $p = 0.086$).

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Distinct patterns of serum and urine macrophage migration inhibitory factor kinetics predict death in sepsis: a prospective, observational clinical study

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Macrophage migration inhibitory factor (MIF) has been considered as a biomarker in sepsis, however the predictive value of the pattern of its kinetics in the serum and in the urine has remained unclarified. It is also unclear whether the kinetics of MIF are different between males and females. We conducted a single-center prospective, observational study with repeated measurements of MIF in serum and urine on days 0, 2, and 4 from admission to the intensive care unit (ICU) in 50 adult septic patients. We found that in patients who died within 90 days, there was an increase in serum MIF level from day 0 to 4, whereas in the survivors there was rather a decrease ($p = 0.018$). The kinetics were sex-dependent as the same difference in the pattern was present in males ($p = 0.014$), but not in females ($p = 0.418$). We also found that urine MIF was markedly lower in patients who died than in survivors of sepsis ($p < 0.050$). Urine MIF levels did not show temporal changes: there was no meaningful difference between day 0 and 4. These results suggest that kinetics of serum MIF during the initial days from ICU admission can predict death, especially in male patients. Additionally, lower urine MIF levels can also indicate death without showing meaningful temporal kinetics.

Sepsis is a life-threating disease that develops when the host immune response to an infection becomes dysregulated, thereby it damages its own tissues and organs[1]. The global burden of sepsis constitutes a challenge for the patients and healthcare personnel, which is also indicated by the high incidence of hospital-treated sepsis cases across all regions (189/100,000 person years) reported in 2020[2]. Moreover, the estimated death rate in septic patients was as high as 26.7%, which was further increased to 41.9% when the patients were treated at the intensive care unit (ICU)[2]. In the same year, another study concluded that the estimated burden of sepsis worldwide is twice as much as what was thought previously[3]. Further increasing its burdens, sepsis was also associated with greater rehospitalization rates and higher healthcare costs compared to matched hospitalized controls according to a recent study[4]. The early diagnosis and assessment of severity could reduce the burdens of sepsis, which can be achieved through the discovery of reliable biomarkers.

In our recent meta-analysis, we showed that the blood level of macrophage migration inhibitory factor (MIF), a pro-inflammatory and immunoregulatory cytokine[5,6], can be a valuable diagnostic and prognostic biomarker in sepsis[7]. We found that blood MIF levels were higher in septic patients who had more advanced severity and did not survive the disease. However, in most of the analyzed studies, the blood MIF level was measured only once on a single day in the patients, which did not allow us to assess the temporal kinetics of blood MIF level during the progression of sepsis and its association with the outcome of the disease. We identified only three studies, in which blood MIF levels were measured and reported on at least two days in sepsis survivors and nonsurvivors[8–10],

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which were not suitable for proper meta-analysis. Importantly, their results were controversial. In one of those papers, there was no meaningful difference in blood MIF levels between days 0, 2, and 5 in sepsis survivors ($p = 0.196$) and nonsurvivors ($p = 0.105$)[8], whereas in another study, high incremental increases in blood MIF levels between days 1 and 2 were associated with higher mortality in severely septic patients[9]. In the third study, serum MIF level seemed to increase on days 0 and 1 of the study, then it decreased by day 10 in both survivors and nonsurvivors, however the statistical analysis of the temporal changes was not reported[10].

The kinetics of a biomarker incorporates the time-dependent changes in the synthesis, metabolism, and elimination of the substance, which can show variations during the progression of a disease. In accordance, the importance of biomarker kinetics has been recognized in sepsis, although its low investigation rate compared to single time-point measurements was also noted[11,12]. For instance, the kinetics of plasma procalcitonin was superior to a single measurement for the prediction of death in septic patients[13]. Similarly, the time-dependent change in blood heparin-binding protein level was more accurate than its initial value for prediction of the fatal outcome in sepsis[14].

In addition to the blood levels of MIF, urine MIF may also serve as a useful biomarker in inflammatory diseases[6], but to our knowledge its potential value as a predictor of the outcome in sepsis has not been investigated. Some studies showed a correlation between urine MIF levels and kidney injury in infectious acute pyelonephritis[15,16], in glomerulonephritis[17], and in renal transplant rejection[18], which may suggest that urine MIF could be a useful predictive parameter of renal dysfunction, but data on urine MIF kinetics in septic patients could not be found in the literature.

In the present study, we aimed at determining the kinetics of blood and urine MIF levels in septic patients during the initial days from their admission to the ICU at the University of Pecs, Hungary.

Methods

Patients. Between January 2012 and May 2015, we enrolled 51 septic patients into this prospective, observational study from our ICU (Department of Anesthesiology and Intensive Therapy, University of Pecs, Pecs, Hungary). Our study protocol was approved by the Regional Research Ethical Committee of the University of Pecs (registration no.: 2406/2005; full date of first registration: 01/04/2005) and the study was performed in accordance with the ethical standards in the 2008 Declaration of Helsinki. Due to the pure observational nature of our study, further registration was not required according to the recommendations of the International Committee of Medical Journal Editors (ICMJE). Following the detailed explanation of the study procedure, written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

Inclusion and exclusion criteria. Sepsis was defined according to the criteria of the 2001 International Sepsis Definitions Conference[19]. Septic patients with elevated serum procalcitonin level at admission to the ICU were enrolled in the study. Patients were excluded if they were under 18 years or above 85 years of age or if they refused to participate in the study. Except for the measurements of MIF levels, the diagnostic and treatment procedures were conducted according to the sepsis guidelines in the patients.

Data collection. Demographic data on age and sex were collected from all enrolled patients. Mortality was followed up for 90 days from ICU admission. Laboratory parameters including serum concentrations of C-reactive protein, procalcitonin, lactate, urea, and creatinine, as well as blood cell counts were measured on days 0, 2, and 4 from ICU admission. On the same days, the urine concentrations of creatinine and total protein, as well as the estimated glomerular filtration rate were also determined. The Sequential Organ Failure Assessment (SOFA) score[20], the Simplified Acute Physiology Score (SAPS) II[21], and the Acute Physiology and Chronic Health Evaluation (APACHE) II score[22] was calculated on admission to the ICU. Renal dysfunction was defined as more than 50% increase in serum creatinine levels above the baseline according to the RIFLE (acronym indicating Risk of renal dysfunction; Injury to the kidney; Failure of kidney function, Loss of kidney function, and End-stage kidney disease) criteria[23].

Measurement of MIF concentration. Urine and venous blood samples were collected for the measurements of MIF levels on days 0, 2, and 4 from ICU admission. Blood was collected in Vacutainer serum tubes with silicon coating as clot accelerator (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and it was kept in the tubes at room temperature to clot for at least 60 min. Serum was collected after centrifugation at 1300 g for 10 min at room temperature, then it was aliquoted and stored at −70°C until the analysis. The levels of MIF were measured in urine and serum by using standard enzyme-linked immunosorbent assay (ELISA) kits (catalog number: DY289; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's recommendations as in a previous study[24]. All measurements were performed in duplicates. The plates were read at 450 nm by using an iEMS MF microphotometer (Thermo Labsystem, Beverly, MA, USA).

The rational for using serum samples was that serum MIF measurements were successfully used to investigate the biomarker role of MIF in septic patients by different authors before patient recruitment started into our study[9,25]. Although the use of serum (instead of plasma) MIF was later criticized[26], another study showed no significant difference between plasma and serum cytokine levels, including MIF[27], while more recently the use of serum rather than plasma samples for MIF detection was recommended in clinical studies to prevent interference from anticoagulants and maintain the consistency of research[28]. Nevertheless, since we used the same sample type in all patients in the present study, the quantitative comparisons of the patient groups should be appropriate based on prior recommendations[29].

When studying renal dysfunction, the levels of urine MIF were also calculated as ratios relative to the urine creatinine level based on earlier studies[15,16].

Statistical analysis. The statistical analysis of the collected data was performed with the R software (version 3.6.1; R Development Core Team, Vienna, Austria). The Mann-Whitney test was used to detect significant differences in urine and serum MIF levels between survivors and nonsurvivors. In subgroup analysis, repeated measures ANOVA was performed with either serum MIF or urine MIF as a dependent variable, while time and either sex or age were the independent variables. To analyze whether temporal changes of MIF levels during sepsis can have significant predictive value, the change in serum MIF from day 0 to day 4 was calculated, and then the change was compared between survivors and nonsurvivors with the Mann-Whitney test. Frequency tables for deaths were generated in groups with different patterns of MIF kinetics, and then the number of deaths were compared with the Fisher test between the groups. The data are reported in the mean ± standard error (SE) format, unless specified otherwise.

Results

Patient characteristics. During the study period, 59 patients were eligible for the study according to the inclusion criteria, but only 51 patients could be enrolled, because 8 of them refused to participate after they received detailed information about the study. One patient had to be excluded, because the outcome could not be recorded at the end of the 90-day follow up. We included data from 50 patients in the final analysis. The flow diagram of the study is shown in Fig. 1. In the included patient population, sepsis was diagnosed post-surgically in 33 cases (25 after acute and 8 after elective surgical interventions), while in the remaining cases without a preceding surgery, pneumonia (n = 10), pancreatitis (n = 2), urosepsis (n = 1), erysipelas (n = 1), and unidentified initial infections (n = 3) were associated with sepsis.

The baseline characteristics of the 50 patients analyzed in the study are summarized in Table 1. The statistical comparison of all parameters between survivor (n = 21) and nonsurvivor (n = 29) groups is also included in the table. The 90-day mortality rate was 58% in this study population, which is comparable with recent data reported in the literature[30]. The sex and age distribution of the patients were similar in the two groups, so was the number of cases with renal dysfunction as assessed by the RIFLE criteria[23]. Importantly, on the day of admission to the ICU, we did not detect a significant difference in any parameters between the two groups, although, the SAPS II and SOFA scores tended to be higher in nonsurvivors than in survivors ($p = 0.15$ and 0.16, respectively), as it could be expected.

Serum and urine MIF levels in sepsis on the initial days from ICU admission. Figure 2A shows the median levels of serum and urine MIF in all septic patients on days 0, 2, and 4 from admission to our ICU. On all days, the MIF levels were higher in the serum than in the urine with medians (and interquartile range, IQR) of 2500 (1441–4015), 2255 (1638–3432), and 3209 (1761–4470) pg/ml in serum versus 965 (520–1905), 1013 (561–1813), and 845 (541–1783) pg/ml in urine, on day 0, 2, and 4, respectively. As in earlier studies[15,16], we also normalized urine MIF levels to urine creatinine, which did meaningfully impact the observed kinet-

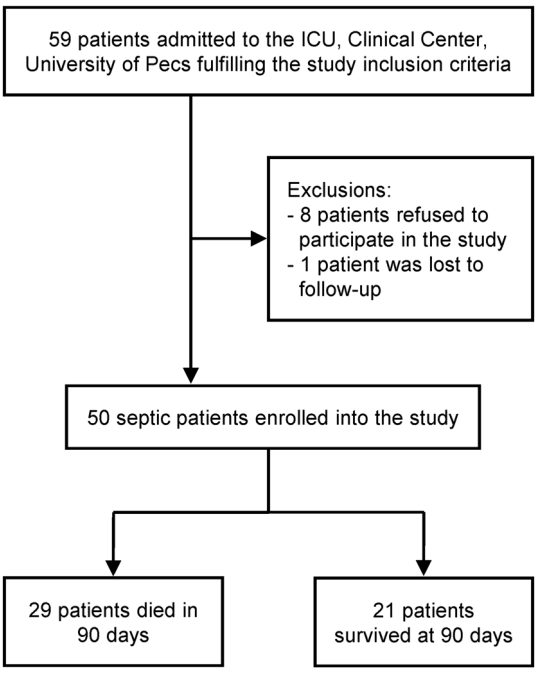

Figure 1. Flow diagram of the study.

Parameters (unit)	Survivors (n = 21)	Nonsurvivors (n = 29)	All (n = 50)	*p* value
Demographic characteristics				
Age (years)	67 ± 3	66 ± 3	66 ± 2	0.78
65 years old or older, n (%)	12 (57)	17 (59)	29 (58)	1.00
Female, n (%)	12 (57)	11 (38)	23 (46)	0.57
Blood test results				
Red blood cell count (10^{12}/l)	3.7 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	0.17
White blood cell count (10^9/l)	13.6 ± 0.2	15.1 ± 2.1	14.5 ± 1.5	0.63
Neutrophil percentage (%)	12 ± 2	14 ± 2	13 ± 1	0.68
C-reactive protein (mg/l)	232.4 ± 28.1	253.5 ± 21.8	244.7 ± 17.2	0.56
Procalcitonin (ng/ml)	23.62 ± 10.99	40.22 ± 10.19	33.86 ± 7.60	0.27
Lactate (mmol/l)	4.4 ± 1.3	4.0 ± 1.3	4.2 ± 0.9	0.81
Creatinine (µmol/l)	180.2 ± 31.0	173.8 ± 23.1	176.5 ± 18.5	0.87
Urea (mmol/l)	13.7 ± 1.8	15.6 ± 1.7	14.8 ± 1.2	0.47
Estimated glomerular filtration rate (ml/min/1.73 m^2)	37.6 ± 5.1	39.6 ± 4.4	38.8 ± 3.3	0.78
Urine test results*				
Total protein (mg/l)	1193 ± 642	713 ± 213	856 ± 240	0.49
Creatinine (mmol/l)	4.5 ± 1.0	5.2 ± 0.8	5.0 ± 0.6	0.61
Clinical status evaluation				
APACHE II (score)	17 ± 2	19 ± 2	18 ± 1	0.39
SAPS II (score)	40 ± 4	49 ± 4	46 ± 3	0.15
SOFA (score)	8 ± 1	10 ± 1	10 ± 1	0.16
Renal dysfunction, n (%)	13 (62)	16 (55)	29 (58)	0.77

Table 1. Basic demographic data, laboratory parameters, and clinical scores of the survivor and nonsurvivor septic patients on the admission day to the intensive care unit. *urine samples for the present study could not be obtained on the day of admission from 6 patients (1 survivor and 5 nonsurvivors). Data are expressed as mean ± standard error, except for the sex, elderly, and renal dysfunction ratio, where number (and percentage) of patients is shown. *APACHE*, acute physiology and chronic health evaluation score; *SAPS*, simplified acute physiology score; *SOFA*, sequential (sepsis-related) organ failure assessment score.

ics: the medians and (IQRs) of the urine MIF/creatinine ratios on day 0, 2, and 4 were 0.30 (0.15–1.34), 0.54 (0.19–1.28), and 0.29 (0.16–0.80) pg/µmol, respectively. The medians were not statistically different between the days either in the serum or in the urine samples, even though there was a 28% increase in serum MIF from day 0 to day 4. The minimum and maximum serum MIF levels were also the highest on day 4 (478 and 7902 pg/ml, respectively).

The influence of sex and age on the progression of sepsis was proposed in previous studies[31–33]. Therefore, next we studied whether the serum and urine MIF kinetics observed in all patients remain similar when the patients are divided into subgroups based on sex (Fig. 2B), age (Fig. 2C), and survival (Fig. 2D). We did not find statistical difference between males and females in serum and urine MIF levels on any of the days. In females, the median serum MIF levels were 1979, 2495, and 3676 pg/ml on day 0, 2, and 4, respectively, while in males the medians were 3252, 2217, and 3163 pg/ml on the respective days (Fig. 2B). Urine MIF levels did not change meaningfully over time in either of the sexes. On all days the levels seemed somewhat higher in females than in males, but the intersex difference did not reach the level of significance. The urine MIF/creatinine ratio did also not change meaningfully over time in either sex, and it was not significantly different between females and males on any of the days.

When patients were divided into younger (less than 65 years old) and older groups (65 years old and above), serum MIF levels in the older patient group were 2000, 2368, and 3263 pg/ml on day 0, 2, and 4, respectively. In the younger patient group, the medians on the respective days were 2969, 2142, and 2732 pg/ml. There was no significant difference between the age groups on any of the days. The urine MIF levels did not differ meaningfully in the elderly between the days, while in the younger patients there was an increase from day 0 to day 2 reaching a median of 1722 pg/ml (vs. 782 pg/ml in the elderly; $p = 0.028$), then it decreased to similar median (871 pg/ml) as in the elderly (819 pg/ml) on day 4 (Fig. 2C). The urine MIF/creatinine ratio was not significantly different between younger and older patients on any of the days, and it did not change markedly over time in either age group. Since the ratio was not significantly different ($p = 0.385$) between younger and older patients on day 2 with respective medians (and IQRs) of 0.56 (0.35–1.22) pg/µmol and 0.32 (0.19–1.28) pg/µmol, these results indicate that the difference in urine MIF between the age groups on day 2 (Fig. 2C) was probably due to a difference in general kidney functions and not due to a difference specifically in MIF excretion.

The median serum MIF levels did not differ statistically between survivors and nonsurvivors on days 0 and 2, however on day 4 serum MIF was significantly ($p = 0.039$) higher in patients who died than who survived with medians (and IQRs) of 3348 (2313–5961) and 2430 (1284–3691) pg/ml, respectively (Fig. 2D). These results suggested different kinetics of serum MIF from day 0 to day 4 between survivors and nonsurvivors of sepsis. With

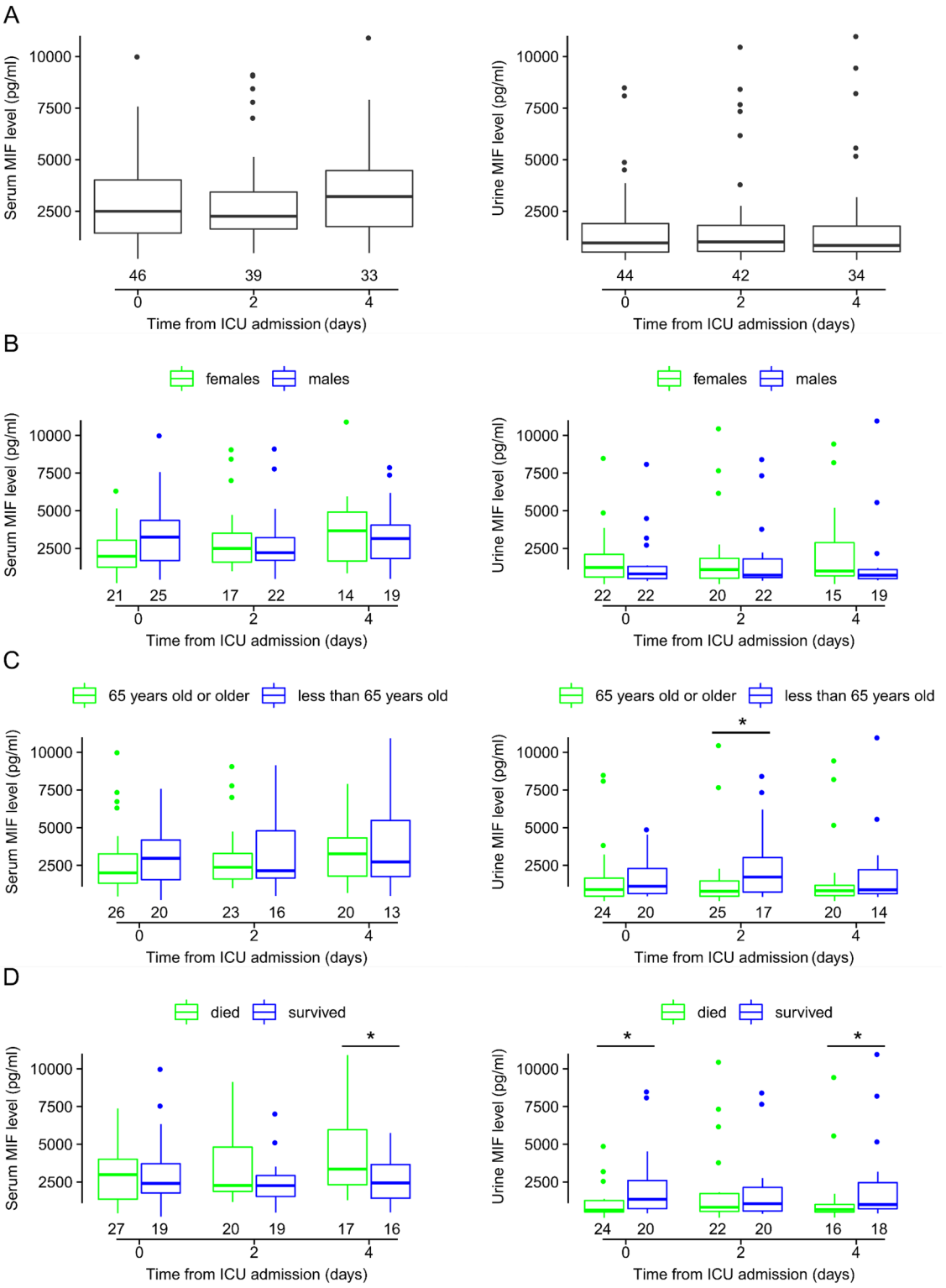

Figure 2. The serum and urine levels of macrophage migration inhibitory factor (MIF) in septic patients on days 0, 2, and 4 from admission to the intensive care unit (ICU). The MIF levels are shown in (**A**) all patients and in patient subgroups of (**B**) females and males, (**C**) at least 65 years old and younger than 65 years, and (**D**) deceased and survived. Here, and in Fig. 6A, the horizontal line within each box represents the median, the bottom and the top of the box marks the lower and the upper quartile, respectively, which limit the interquartile range (IQR). The vertical line below and above the box shows the minimum and maximum levels, respectively. Outliers are shown with dots. The numbers below the boxes indicate the number of patients in each group. Note that on day 0, serum MIF level could not be determined in 4 patients and urine MIF level in 6 patients due to technical issues. $^{*}p < 0.05$.

regards to urine MIF, the medians did not change meaningfully over time in either of the subgroups. However, urine MIF levels were lower in patients who died than who survived on all days, which difference was significant on day 0 (638 vs. 1355 pg/ml; $p = 0.046$) and on day 4 (672 vs. 1005 pg/ml; $p = 0.032$). Similar to urine MIF, the urine MIF/creatinine ratio was not significantly different between the days in either subgroup. More importantly, as in the case of urine MIF, the significant differences in the ratio were also detectable between nonsurvivors and survivors on day 0 (0.24 vs. 0.50 pg/μmol; $p = 0.022$) and on day 4 (0.24 vs. 0.80 pg/μmol; $p = 0.003$). These findings suggest that the observed differences in urine MIF levels between survivors and nonsurvivors were presumably caused by differences specific to renal MIF excretion and not by differences in general renal functions.

The kinetics of serum MIF levels in survivors and nonsurvivors of sepsis after ICU admission. Serum MIF levels were significantly higher in nonsurvivors than in survivors on day 4, but they did not differ on the day of ICU admission (Fig. 2D). Thus, we analyzed how the serum MIF levels changed from the first until the last measurement in each individual patient, and then compared the kinetics between those who survived and who deceased in sepsis (Fig. 3A). We included only those patients who had at least two serum MIF level values on different days during their stay at the ICU (n = 48), while 2 patients could not be included, because they died before a second blood sample could be collected from them. We found that serum MIF level increased in 15 of 27 deceased patients (~ 56%), while in the rest it did not change (n = 7) or decreased (n = 5). In contrast with the dominance of the increasing pattern in the deceased patients, in the survivors the most common (~ 62%) trend was a decrease in serum MIF level (n = 13), while it increased only in 8 out of the 21 patients.

In previous studies, an association between MIF and estrogen was indicated in inflammatory conditions, since estrogen inhibited endotoxin-induced MIF production in murine macrophages[34], and it decreased MIF production in rat models of colitis[35] and trauma-hemorrhage-induced lung injury[36]. Furthermore, MIF plasma levels were positively correlated with testosterone and negatively with estradiol in human patients[37]. Therefore, we also studied the changes in serum MIF levels in males and females separately even though the subgrouping lowered the number of patients in the analyzed groups (Fig. 3B,C). In males, similar kinetic patterns were present as in all patients: the most common (50%) trend was an increase in deceased patients, while a decrease was the

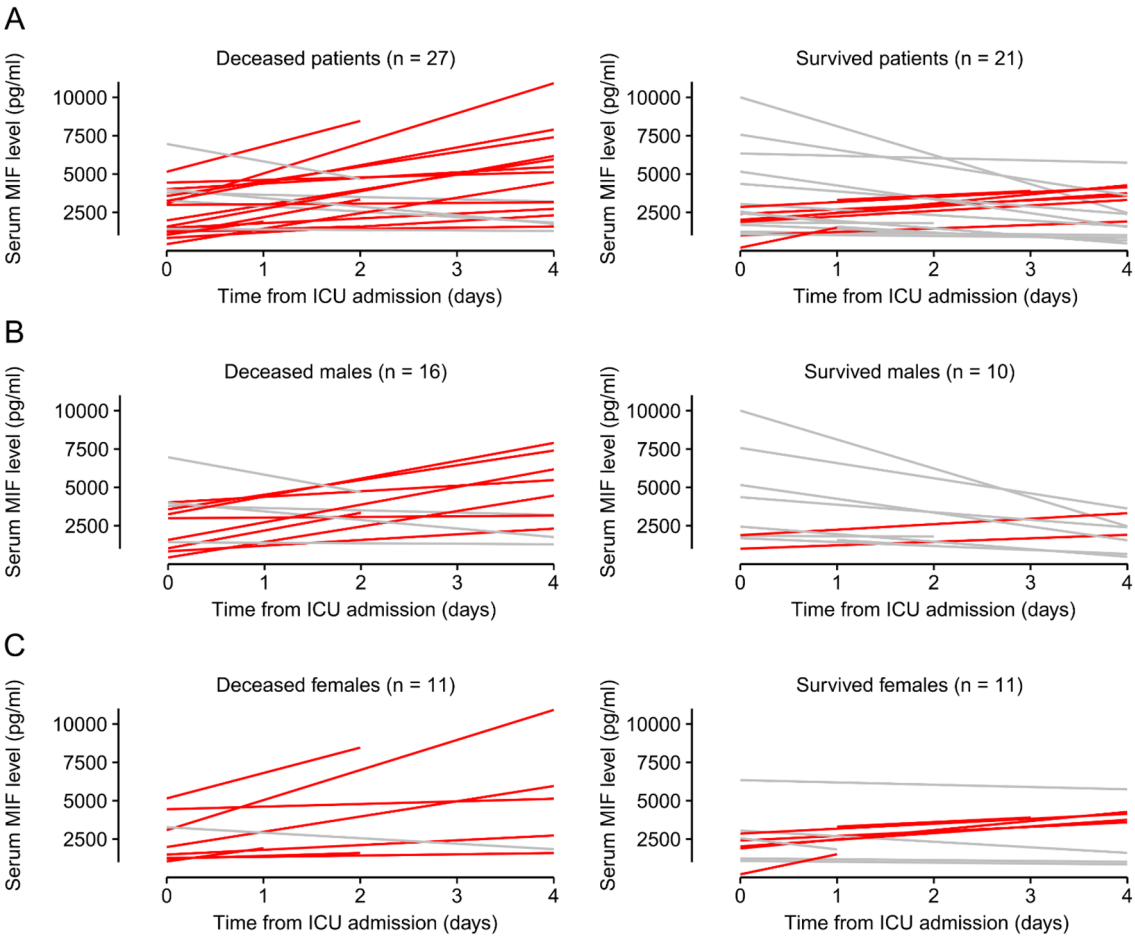

Figure 3. The individual pattern of serum macrophage migration inhibitory factor (MIF) kinetics in each patient who had at least 2 measurements between day 0 and 4 at the intensive care unit (ICU). Red line indicates an increase, while gray line shows no increase in serum MIF level in deceased and survived patients based on data obtained from (**A**) both sexes, (**B**) females, and (**C**) males. The number of patients (n) is indicated in the figure in each group.

dominant (80%) trend in the survivors (Fig. 3B). In contrast with males, in females the kinetic patterns did not differ meaningfully between survivors and nonsurvivors. In females, an increase in serum MIF was the most frequent (~73%) in deceased patients, as well as in the survivors (~55%) (Fig. 3C).

For a more quantitative analysis of the serum MIF kinetics, in our next approach we also compared the mean changes of serum MIF levels between day 0 and 4 in all groups (Fig. 4). In accordance with our observations regarding the patterns of the kinetics, in deceased patients, the mean (± SE) serum MIF level increased from 2997 ± 373 pg/ml on day 0 to 4394 ± 646 pg/ml on day 4, whereas in the sepsis survivors serum MIF decreased from 3137 ± 576 to 2587 ± 384 pg/ml during the same time interval (Fig. 4A). The daily change in serum MIF level was significantly different between survivors and nonsurvivors, when we analyzed the data obtained from both sexes ($p = 0.01$) and from males ($p = 0.01$), whereas there was no marked difference between the deceased and survived groups in females ($p = 0.230$) (Fig. 4B). The previously observed patterns were also reflected by the mean daily changes in serum MIF, since an overall increase versus decrease was present in all and male nonsurvivors versus survivors, respectively, while in females there was on average an increase in both outcome groups.

The kinetics of urine MIF levels in survivors and nonsurvivors of sepsis after ICU admission. In addition to serum levels of MIF, we also studied how the levels of MIF change in the urine after the admission of the septic patients to the ICU. As mentioned before, the urine MIF levels were significantly lower in deceased patients than in survivors on days 0 and 4 (see Fig. 2D). When we looked at the kinetics within the groups, we found a small, not significant increase in both groups from day 0 to day 4: from 3021 ± 797 to 3457 ± 1016 pg/ml in survivors and from 1281 ± 340 to 1629 ± 654 pg/ml in nonsurvivors (Fig. 5A). Importantly, the daily change in the urine levels of MIF did not differ significantly between survivors and nonsurvivors (109 ± 192 vs. 87 ± 152 pg/ml; $p = 0.940$) (Fig. 5B). There was also no significant difference in the daily change of urine MIF levels between the outcome groups when we compared males and females separately ($p = 0.136$ and $p = 0.228$, respectively). By analyzing the data obtained from both sexes, we found a strong positive correlation between urine MIF levels measured on day 0 and on day 4 (Fig. 5C), suggesting that the level determined on day 0 can predict the value on day 4.

The kinetics of urine MIF levels in septic patients with and without renal dysfunction after ICU admission. Some studies showed that urine MIF can be an indicator of renal dysfunction associated with different diseases[15–18], but whether it has a similar indicator role in sepsis has remained unclear. Therefore, in our next approach we compared urine MIF levels in septic patients who developed renal dysfunction and in those who did not according to the RIFLE criteria[23].

The median urine MIF levels seemed higher in patients with healthy kidney functions than in those who had renal dysfunction on days 0, 2, and 4, which difference was the biggest on day 0 with medians (and IQRs) of 1268 (725–2626) pg/ml and 638 (461–1467) pg/ml, respectively (Fig. 6A). Importantly, however, the difference between the groups did not reach the level of significance on any of the days. Normalization of urine MIF levels to urine creatinine did not meaningfully impact the observed kinetics: the urine MIF/creatinine ratio seemed higher in patients without renal dysfunction on days 0 and 2 with the biggest difference in the medians (and IQRs) between patients with and without renal dysfunction on day 2: 0.29 (0.16–0.87) pg/μmol and 0.65 (0.28–1.88) pg/μmol. However, the difference was not statistically significant between the groups on any of the days.

Between day 0 and 4 from ICU admission, the urine MIF level changed on average from 2694 ± 686 to 2534 ± 893 pg/ml in patients without renal dysfunction, while from 1774 ± 653 to 2658 ± 918 pg/ml in patients with renal dysfunction (Fig. 6B). There was no significant difference between the groups. The mean daily changes in urine MIF levels were 220 ± 157 pg/ml and − 40 ± 191 pg/ml with and without renal dysfunction, respectively (Fig. 6C), which were not statistically different between the groups even if the urine MIF/creatinine ratios were used for comparison of the groups (0.01 ± 0.04 vs. − 0.01 ± 0.13 pg/μmol/day, respectively).

Discussion

Here, we present the kinetics of serum and urine MIF levels in septic patients on the initial days from ICU admission. We show that the patterns of serum MIF kinetics are different between patients who survived and who died in sepsis. We report, to the best of our knowledge for the first time, that serum MIF level increased after ICU admission in those patients who died in sepsis, whereas it decreased in the survivors of the disease. With subgroup analysis, we detected intersex difference in the kinetics of serum MIF in sepsis, since the decreasing trend in the survivors was present in males, but not in females. Moreover, we show that urine MIF level can be a valuable prognostic marker of mortality in sepsis, as it was markedly lower in nonsurvivors than in survivors, and it did not change significantly over time in either of the groups. We did not find a difference in the urine MIF levels in association with the presence or absence of renal dysfunction.

Sepsis continues to constitute a serious burden for patients and a significant challenge for the healthcare system even nowadays due to its high incidence, potentially fatal outcome, and substantial costs of care[2,4]. One way to mitigate the burdens of sepsis is to discover biomarkers, which can be used for the diagnosis and for the prediction of the outcome of the disease. In accordance with that approach, a plethora of sepsis biomarker candidates were proposed (for reviews, see[38,39]), which also included MIF as a promising biomarker[6]. MIF is a multifaceted cytokine playing diverse roles in the host immune response to infectious and non-infectious stimuli[40]. It underlines the importance of MIF biology in sepsis that variant MIF alleles have been linked to altered MIF expression and Gram-negative bacteremia[41], and that MIF levels in sepsis have previously been shown to correlate with APACHE II scores[42]. Our recent meta-analysis suggested that serum MIF level can serve as a valuable diagnostic and predictive biomarker in sepsis[7]. However, previous studies about its kinetics were scarce and reported controversial results[8–10], even though the importance of some other biomarkers' kinetics in

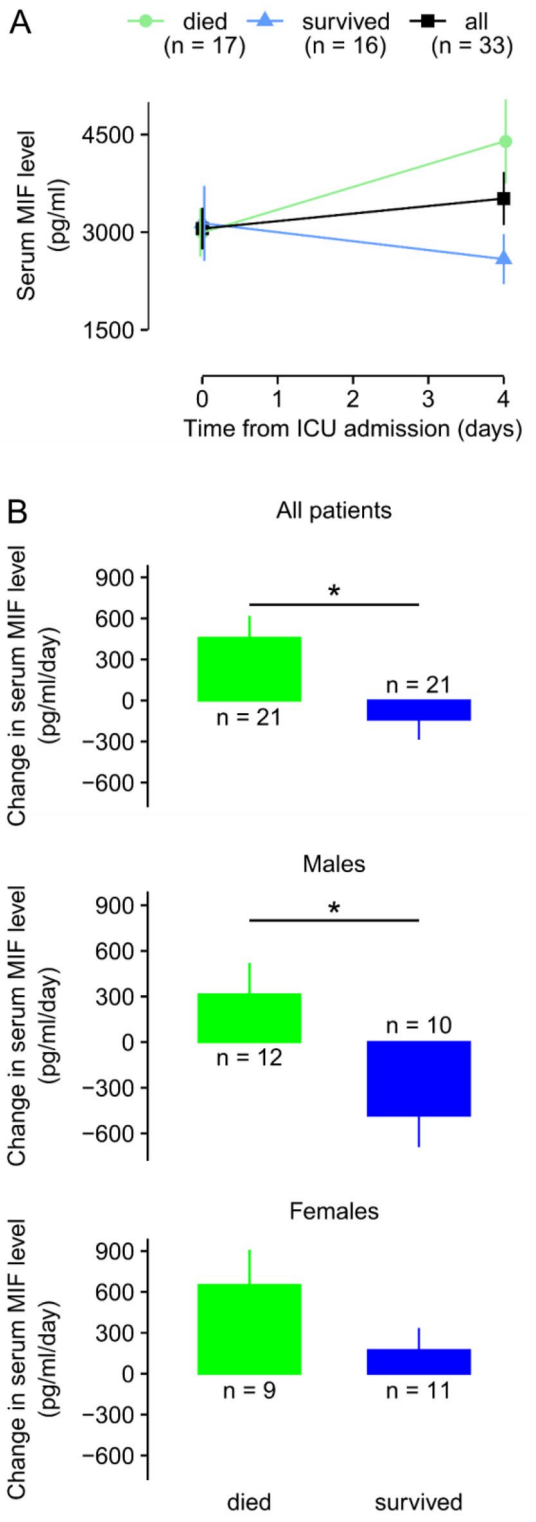

Figure 4. The kinetics of serum macrophage migration inhibitory factor (MIF) levels in septic patients at the intensive care unit (ICU). (**A**) The mean absolute serum levels of MIF in all, deceased, and survived septic patients on day 0 and 4 from admission to the ICU. (**B**) The mean daily changes of serum MIF levels in deceased and survived patients based on data obtained from both sexes (top), males (middle), and females (bottom). The number of patients (n) is indicated in the figure in each group. *$p < 0.05$.

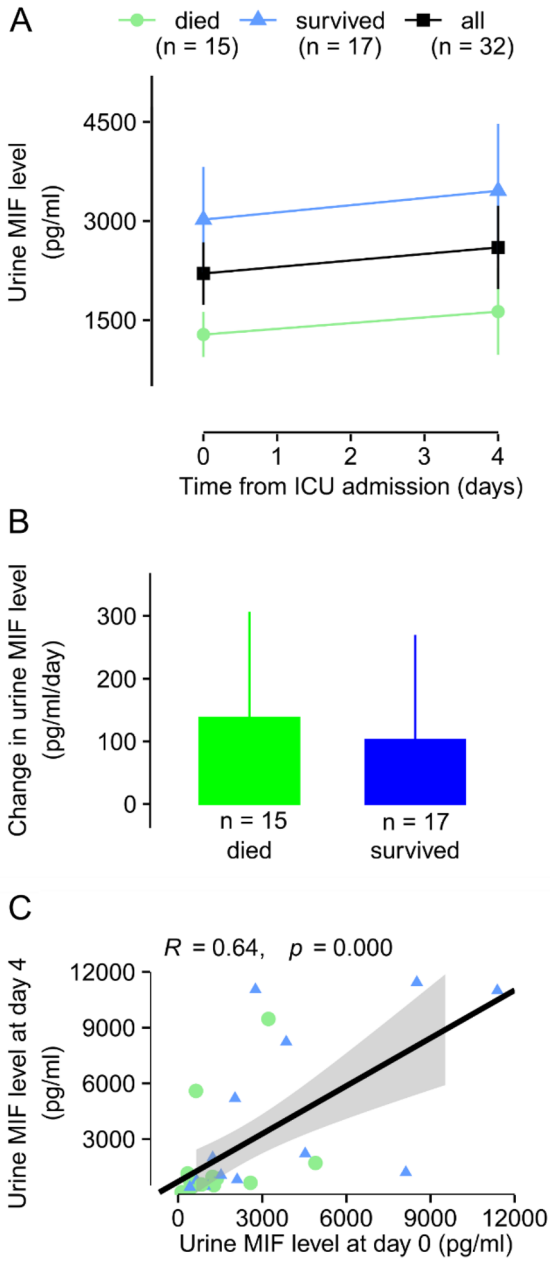

Figure 5. The kinetics of urine macrophage migration inhibitory factor (MIF) levels in septic patients at the intensive care unit (ICU). (**A**) The mean absolute urine levels of MIF in all, deceased, and survived septic patients on day 0 and 4 from admission to the ICU. (**B**) The mean daily changes of urine MIF levels in deceased and survived patients. (**C**) The correlation between urine MIF levels measured on day 0 and on day 4 from the admission to the ICU. The number of patients (n) is indicated in the figure in each group.

sepsis has been recognized and investigated in recent studies[14,43,44]. To shed more light on the temporal changes of serum MIF in sepsis, in the present study we report its absolute levels on the initial days after ICU admission in septic patients, who were also divided into subgroups based on age, sex, and survival.

The serum MIF kinetics clearly differed between sepsis survivors and nonsurvivors after ICU admission, since in the nonsurvivors serum MIF increased, whereas in survivors it decreased. Considering that we did not always detect statistically significant difference between the outcome groups when only single measurements were compared, the novel finding about the distinct kinetics indicates that repeated serum MIF level measurements in the same patient can be better predictors of the outcome than single time-point measurement at the ICU. Indeed, the significant prognostic value of MIF was not found in some previous studies, in which the authors performed only one measurement of its serum level[45–47]. The increasing levels of serum MIF associated with the fatal outcome can be assumed to be related to the progression of the disease. MIF is a proinflammatory cytokine that promotes the immune response to defeat the pathogen[5], which can explain why higher levels were

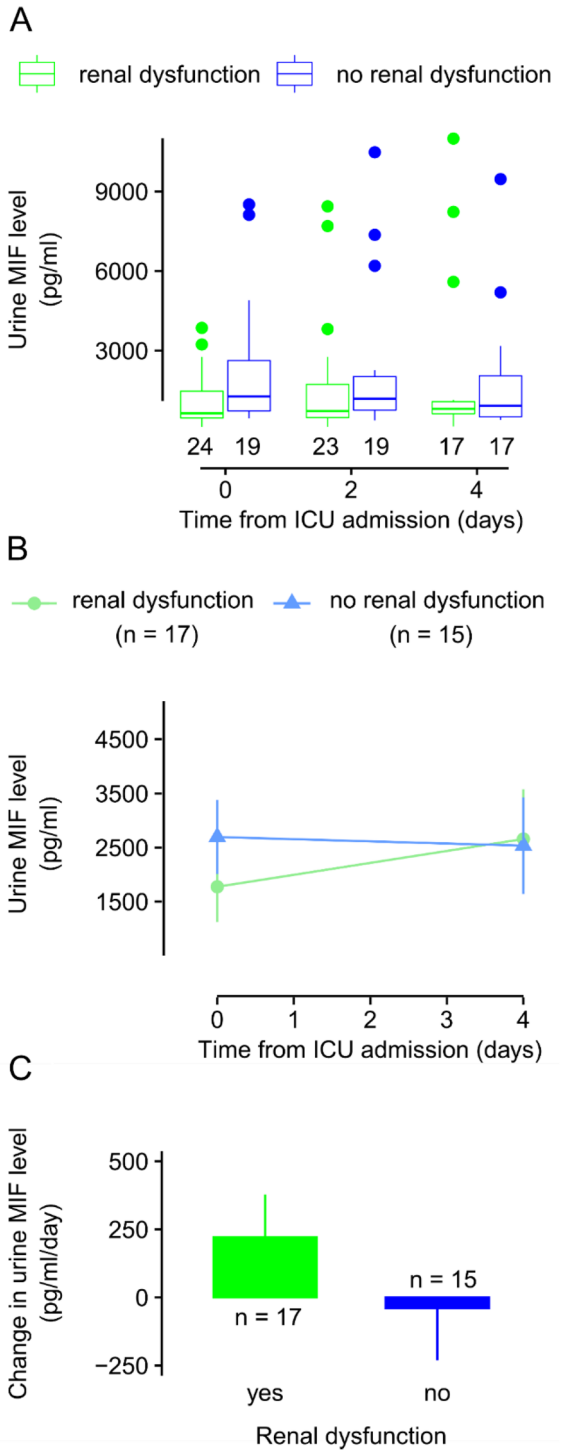

Figure 6. The kinetics of urine macrophage migration inhibitory factor (MIF) levels in septic patients with and without renal dysfunction at the intensive care unit (ICU). (**A**) Box plot of urine levels of MIF in septic patients with and without renal dysfunction on day 0, 2, and 4 from admission to the ICU (for explanation of symbols, see Fig. 2). (**B**) The mean absolute urine levels of MIF in septic patients with and without renal dysfunction on day 0 and 4 from admission to the ICU. (**C**) The mean daily changes of urine MIF levels in septic patients with and without renal dysfunction. The number of patients is indicated in the figure in each group.

found in patients with systemic inflammation in several studies (for review, see[7]). However, when the pathogen load is excessive or the anti-inflammatory response is depleted, the proinflammatory response can be overtly

activated and become harmful for the host. The gradually increasing serum MIF level may serve as a marker for the excessively intensifying proinflammatory activity, which can be an early warning sign for healthcare personnel to initiate more aggressive treatments before fatal consequences develop. It should be noted also that MIF is present in different cell types in pre-formed, intracytoplasmic pools[47–52], thus its increasing levels may also reflect escalating tissue damage and necrosis.

Interestingly, in survivor and deceased females the patterns of serum MIF kinetics were somewhat different from males. In females, the level of MIF increased in both groups, though the extent of the increase tended to be markedly greater in nonsurvivors than in survivors ($p = 0.13$). Similar to males, the bigger increase also developed in the deceased patients, which was, however, more pronounced in deceased females than males (651 ± 258 vs. 313 ± 207 pg/ml) (for details, see Fig. 3B). Furthermore, in the survivors there was an increase in females instead of the decrease observed in males. The intersex difference in the serum levels of MIF in the septic patients can be due to the influence of sex hormones. In experimental models of inflammation, estrogen reduced the production of MIF[34–36,53]. In accordance, MIF levels in the plasma were lower in female than in male healthy human subjects[37,54]. It should be noted, however, that estrogens were inactive when MIF was abundantly present in one of the models[53], and that the difference in MIF levels between males and females was only present in the younger than 55 years old age group in the study by Aliosi et al.[37]. As part of the inflammatory response, MIF is rapidly produced and released into the bloodstream in sepsis[5], thus its concentrations can be high enough to overcome the suppressive effect of estrogen on its production. With regards to age, in our study the average age of the patients was 66 ± 2 years and the youngest woman was 47 years old. It can be assumed that the majority of the included females were already in the postmenopausal period, therefore had low estrogen levels. Indeed, in a previous study the plasma concentration of estradiol in males were significantly higher than in postmenopausal women[55]. Taken together, the abundance of MIF in the bloodstream in sepsis and the decreased estrogen levels in postmenopause can serve as a hypothetical reason why the MIF levels increased in both survivor and nonsurvivor septic females to a greater extent than in males in our study. The intersex differences in serum MIF levels in sepsis can be a contributing factor to the previously reported different prognosis between septic males and females[33]. It should be mentioned also that the prognostic discrepancy between MIF levels in males and females may have a genetics basis, which is supported by differences in the statistical association between variant MIF alleles and sex in the inflammatory disease multiple sclerosis[56].

Besides serum MIF, urine MIF level was also proposed as a disease biomarker[6]. Accordingly, its role was studied in kidney injury due to a variety causes[15–18,57], but, to our surprise, we could not find data in the literature about the kinetics of urine MIF in sepsis and its association with sepsis-related kidney injury. In the present study, we show that urine MIF remains relatively constant on the initial days after ICU admission in both survivors and nonsurvivors. Importantly, however, in the deceased patients it was markedly lower than in survivors. These findings suggest that urine MIF can be an easily accessible biomarker for prediction of the outcome in sepsis. Due to its relatively stable levels over time, a random measurement on any days could be possibly used in practice. This is also supported by the finding that there was a strong correlation between the first and last measured levels of urine MIF in the present study. An obvious question related to urine MIF is how its levels are influenced by acute kidney injury, which is a common complication in critically ill patients at the ICU[58]. When we compared urine MIF levels between patients with and without renal dysfunction, urine MIF levels were similar in the two groups on all days and there was no difference in the kinetics and overall change in its level over time. This is in harmony with the results of an earlier study showing that the progression of renal injury was independent from renal MIF expression in a mouse model of nephropathy[59]. Our results suggest that urine MIF can be used as a predictive biomarker in sepsis independently from the kidney function, however, it does not indicate the development of sepsis-associated acute kidney injury.

The lower urine MIF level in the nonsurvivors was an unexpected new finding, which requires discussion. The increasing serum levels of MIF seem to contradict the lower urine MIF levels in patients who died in sepsis, but it can be explained by the diverse source and complex role of MIF in inflammation. Besides immune cells, MIF is produced in most cells in the kidney, e.g., tubular cells, podocytes, mesangial and endothelial cells (for recent review, see[60]). MIF is constitutively expressed in kidney tissues at low levels, but it is markedly upregulated in disease conditions such as kidney inflammation[61]. Urine MIF level showed an inferior correlation with serum MIF in a previous study[62], indicating that its concentration in the urine is not only influenced by clearance of serum MIF, but also by its renal synthesis. Furthermore, it should be mentioned that differences in protein permeability through the glomerular basement membrane and in protein reabsorption by tubular epithelial cells may be also associated with urinary excretion of MIF in nephropathy[63]. The lack of correlation between serum and urine levels of MIF can also explain why higher serum levels were not accompanied by increased urine levels in nonsurvivors in the present study. Renal MIF was shown to possess a renoprotective function in different kidney diseases, also including acute kidney injury[64–66]. Since the urine MIF level in sepsis survivors was higher than that of deceased patients in the present study, it can be speculated that the endogenous renoprotective effect of renal MIF was attenuated in the nonsurvivor group, thereby indicating the increased severity of the disease. While the described scenario might be a possible explanation for our current findings, it should be mentioned that a causative role for MIF in the development of kidney injury was also proposed by previous studies[67–69]. The disease context and the different roles of MIF in disease pathogenesis were suggested as the causes for the contradictory (i.e., renoprotective vs. detrimental) roles in the different studies[64]. Future studies are warranted to reveal the exact function of renal MIF in sepsis.

Limitations of our study must be also mentioned. Our sample size was relatively small, which resulted in low number of patients after dividing the population into multiple subgroups (e.g., survivor males and females). The patients were enrolled at a single clinical center in the present study, thus further studies at multiple (preferably international) centers are needed to improve diversity of the patients and allow for conclusions in broader population. In the present study, we focused on patients admitted to the ICU, however, it would be also important

to see how MIF kinetics develop in septic patients before the ICU admission, which could help physicians to get an insight about the prognosis at an earlier stage of the disease. Lastly, we did not correlate the kinetics of MIF levels with other biomarkers, therefore the prognostic performance of MIF could not be compared with other markers. However, Kofoed et al.[70] showed that MIF performed similarly as procalcitonin, C-reactive protein, and neutrophil count in the detection of a bacterial cause in systemic inflammation as indicated by the areas under the receiver operating characteristics curve (AUROC) of 0.63, 0.72, 0.81, and 0.74, respectively[70]. In the same study, the measurement of combination of all these four with two other biomarkers (suPAR and sTREM-1) was found to be more useful (with AUROC of 0.88) than that of the single markers. In another report, plasma levels of MIF, procalcitonin, interleukin (IL)-6, -8, -10, and thioredoxin were elevated in patients with systemic inflammation, however, in neutropenic sepsis, MIF and thioredoxin levels were lower, whereas IL-8 and procalcitonin levels were higher compared to sepsis without neutropenia[71]. Since no correlation was found between MIF and leukocyte cell counts in that study[71], the authors concluded that the severely reduced leukocyte number was unlikely to cause decreased MIF levels in the neutropenic patients. In contrast, there was a trend toward a positive correlation between MIF levels and leukocyte counts in another study, which finding was in agreement with the authors' observation of low MIF levels in a neutropenic patient[10]. With regards to the prediction performance of fatal outcome in sepsis, the AUROC was found to be 0.79 for MIF and 0.68 for IL-6[72]. Significant correlations were shown between MIF and IL-6 levels and disease severity scores in septic patients, whereas no relation was found between MIF and markers of the acute phase response (procalcitonin, C- reactive protein, and lipopolysaccharide-binding protein)[73]. Finally, in certain infections, the serum level of MIF was a better biomarker than C-reactive protein or IL-6 for predicting death[74]. Taken together, the investigation of the exact correlation of serum and urine MIF level kinetics with those of other biomarkers remains subject for future studies. Nevertheless, based on the present and previous findings, the changes in MIF levels alone or in combination with other biomarkers can be useful in the diagnosis of sepsis and in prediction of the outcome. For example, it was proposed that the continuous and combined monitoring of MIF and procalcitonin levels may be useful to distinguish patients suffering from post-burn inflammation from those that will develop fatal systemic inflammation or sepsis[75].

To the best of our knowledge, this is the first study that reports the kinetics of serum and urine MIF in septic patients admitted to the ICU. In summary, we showed that an increasing serum MIF pattern was characteristic for patients who died in sepsis, whereas the level was rather decreasing in those who survived. Intersex differences in the serum MIF level kinetics were also revealed. Last, we showed that urine MIF level was not associated with renal dysfunction and it was lower in nonsurvivors than in survivors of sepsis. Despite of its limitations, our study highlights the biomarker value of serum and urine MIF kinetics for the prediction of the outcome of sepsis. Our results can also serve as an encouraging basis for designing future studies at multinational level, which are required to determine the real prognostic value and clinical feasibility of repeated MIF level measurements in septic patients. The aims of such desirous studies could be also extended to investigate the role of the MIF congener MIF-2, which signals through the same cognate receptor (CD74), and measures of sCD74, both of which have been measured in clinical studies of other conditions[42,76,77].

Data availability

All data generated or analyzed during this study are included in this published article.

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Author contributions

J.T. and A.G. conceived the study, participated in the design, interpreted the data, and wrote the manuscript. J.T., S.M., D.M., P.K., and Z.F. recruited the patients and collected the data. J.T., J.G., and A.G. supervised the MIF level measurements. J.T., L.K., K.M., and A.G. performed statistical analyses, interpretation of data, and helped to draft the manuscript. S.M., D.M., E.P., and J.G. participated in study design and helped to edit and review the manuscript. L.K., Z.F., and E.P. contributed to interpretation of the data and editing of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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