MEDICAL SCHOOL UNIVERSITY OF PÉCS

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

DOCTORAL (PHD) THESIS

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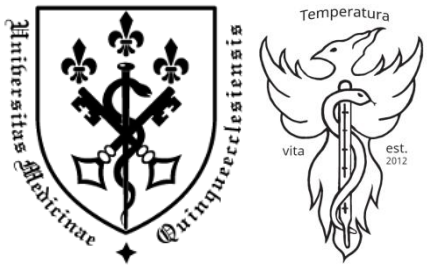

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List of abbreviations

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 ($\geq$ 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 IL-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 to 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 (ARDS), 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 receiver operating characteristic (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). 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.

4.4 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).

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 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).

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%.

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). 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 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, which demonstrates that blood MIF level measurement shows good sensitivity and specificity for the diagnosis of sepsis.

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.

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). 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. 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). 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).

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), which demonstrated that blood MIF levels were markedly higher in nonsurvivors than in survivors of sepsis.

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. 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.

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. 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 not 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, age, and survival. 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.

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. 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 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. 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 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. 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.

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. 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. 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%).
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. 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. 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 ($p = 0.230$). 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.

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. 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. 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; $p = 0.940$). 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, suggesting that the level determined on day 0 can predict its level 4 days later.

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. 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. 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, which were not statistically different between the groups even if the urine MIF/creatinine ratios were used for comparison of the groups.

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 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 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 ability 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 physician 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 could have diagnostic capability to differentiate between infectious and noninfectious systemic inflammation and could 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 prognostic 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.

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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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