MAJOR ARTICLE

Association between High Levels of Blood Macrophage Migration Inhibitory Factor, Inappropriate Adrenal Response, and Early Death in Patients with Severe Sepsis

Marieke Emonts,[1] Fred C. G. J. Sweep,[2] Nicolai Grebenchtchikov,[2] Anneke Geurts-Moespot,[2] Marlies Knaup,[4] Anne Laure Chanson,[4] Veronique Erard,[6] Pascal Renner,[4] Peter W. M. Hermans,[3] Jan A. Hazelzet,[1] and Thierry Calandra[4,5]

[1]Department of Pediatrics, Erasmus MC–Sophia Children's Hospital University Medical Center, Rotterdam, and Departments of [2]Chemical Endocrinology and [3]Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [4]Infectious Diseases Service, Department of Medicine, Centre Hospitalier Universitaire Vaudois and [5]University of Lausanne, Lausanne, Switzerland; and [6]Division of Clinical Research, Fred Hutchinson Cancer Research Center, Program in Infectious Diseases, Seattle, Washington

Background. Identification of new therapeutic targets remains an imperative goal to improve the morbidity and mortality associated with severe sepsis and septic shock. Macrophage migration inhibitory factor (MIF), a proinflammatory cytokine and counterregulator of glucocorticoids, has recently emerged as a critical mediator of innate immunity and experimental sepsis, and it is an attractive new target for the treatment of sepsis.

Methods. Circulating concentrations of MIF were measured in 2 clinical trial cohorts of 145 pediatric and adult patients who had severe sepsis or septic shock caused predominantly by infection with *Neisseria meningitidis* or other gram-negative bacteria, to study the kinetics of MIF during sepsis, to analyze the interplay between MIF and other mediators of sepsis or stress hormones (adrenocorticotropic hormone and cortisol), and to determine whether MIF is associated with patient outcome.

Results. Circulating concentrations of MIF were markedly elevated in 96% of children and adults who had severe sepsis or septic shock, and they remained elevated for several days. MIF levels were correlated with sepsis severity scores, presence of shock, disseminated intravascular coagulation, urine output, blood pH, and lactate and cytokine levels. High levels of MIF were associated with a rapidly fatal outcome. Moreover, in meningococcal sepsis, concentrations of MIF were positively correlated with adrenocorticotropic hormone levels and negatively correlated with cortisol levels and the cortisol:adrenocorticotropic hormone ratio, suggesting an inappropriate adrenal response to sepsis.

Conclusions. MIF is markedly and persistently up-regulated in children and adults with gram-negative sepsis and is associated with parameters of disease severity, with dysregulated pituitary-adrenal function in meningococcal sepsis, and with early death.

Numerous adjunctive therapies for patients with severe sepsis and septic shock have been tested in clinical trials. Until recently, most antisepsis therapies yielded disappointing results. However, the use of drotrecogin alfa (activated), corticosteroid therapy, and early goal–directed therapy has recently given encouraging results in adult patients [1–3]. However, identification of new therapeutic targets to further improve the morbidity and mortality associated with severe sepsis and septic shock remains an imperative goal.

Discovered 40 years ago as a T cell cytokine that inhibited macrophage motility [4, 5], macrophage migration inhibitory factor (MIF) remained an enigmatic molecule until its rediscovery in the early 1990s as a neuroendocrine peptide and an effector molecule of innate immunity [6]. Constitutively expressed by endocrine and immune cells, MIF is released in a hor-

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Reprints or correspondence: Dr. Thierry Calandra, Infectious Diseases Service, Dept. of Medicine, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland (Thierry.Calandra@chuv.ch).

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mone-like fashion by the anterior pituitary gland and the adrenal cortex after exposure to endotoxin (lipopolysaccharide [LPS]), corticotropin-releasing hormone, or physiological stress [6–9]. MIF is also released by innate immune cells that are exposed to proinflammatory mediators and microbial products, and it acts to promote inflammatory and immune responses [10, 11].

Interestingly, MIF and corticosteroids together function as a homeostatic counterregulatory dyad modulating inflammatory and immune responses [7]. Low doses of corticosteroids were found to induce MIF release, which, in turn, counterbalanced the immunosuppressive and antiinflammatory effects of glucocorticoids. Cytoplasmic phospholipase A2 and mitogen-activated protein kinase phosphatase 1 have been identified as key molecular targets of MIF-glucocorticoid crosstalk [12, 13]. The expression of Toll-like receptor 4, the signal transducing molecule of the LPS receptor complex, is up-regulated by MIF, thereby facilitating the detection of gram-negative bacteria [14]. Moreover, MIF has been shown to play an important role in experimental sepsis [6, 7, 11, 15, 16], acute respiratory distress syndrome, and several inflammatory and autoimmune diseases (reviewed in [17]). Thus, many lines of evidence indicate that MIF is an interesting candidate target for therapeutic intervention in patients who have severe sepsis.

In the present study, we measured circulating concentrations of MIF in 2 clinical trial cohorts of patients with severe sepsis or septic shock comprising children with meningococcal sepsis and adults with gram-negative sepsis. We studied the kinetics of MIF release in the systemic circulation during sepsis; the interplay between MIF, mediators of sepsis, and stress hormones (adrenocorticotropic hormone [ACTH] and cortisol); and whether MIF was associated with patient outcome, and if so, whether it might help to identify patients who may benefit from anti-MIF treatment strategies.

METHODS

Adult patients. The adult study population consisted of 68 patients with sepsis, severe sepsis, or septic shock who were part of a prospective, double-blind study that investigated the efficacy of 2 IgG preparations for the treatment of gram-negative severe sepsis and septic shock [18]. Severe sepsis and septic shock were defined as previously reported [18, 19]. Clinical and laboratory parameters were recorded at study entry (i.e., near the onset of severe sepsis or septic shock) and during the course of disease. The evaluation of patient outcome was made prospectively at the time of the study [18, 19]. Patients were classified as having survived ("survivors"); as having died of fulminant, irreversible septic shock ("early death"); or as having died of an indirect consequence or of a relapse of shock after transient reversal of shock, defined as a normalization of blood pressure and discontinuation of supportive vasopressor therapy ("late death"). The severity of underlying diseases was reported according to the classification proposed by McCabe and Jackson [20]. Concentrations of MIF were also measured in 196 healthy subjects. The study was approved by the ethics committees of the participating centers, and informed consent was obtained from patients or from their relatives [19].

Pediatric patients. The pediatric study population consisted of 77 patients who were admitted to the pediatric intensive care unit of the Erasmus MC–Sophia (Rotterdam, The Netherlands) who had been enrolled in meningococcal sepsis studies [21–25]. All studies were approved by the ethical committee of the Erasmus Medical Center, and informed consent was obtained from the parents or guardians of patients. Inclusion criteria (tachycardia, tachypnea, rectal body temperature <36°C or >38.5°C, and petechiae) were in accordance with the recommendations of the International Pediatric Sepsis Consensus Conference [26]. Clinical data, severity scores, and laboratory parameters were collected at study entry and during the course of disease, as has been previously defined [27, 28]. *Neisseria meningitidis* serogroups were determined at The Netherlands Reference Laboratory for Bacterial Meningitis (Academic Medical Center Amsterdam, Amsterdam, The Netherlands).

Blood sampling and cytokine, coagulation factor, and stress hormone measurements. In adult patients, serum samples were collected at study entry (i.e., before the infusion of immunoglobulins), 2 h thereafter (for 61 patients), at day 1 (for 57 patients), and at day 10 (for 41 patients). In children, citrate plasma samples were obtained at baseline (i.e., within 4 h of pediatric intensive care unit admission for 71 patients and at later time-points for 6 patients) and 12 h (for 23 patients) and 24 h (for 62 patients) thereafter. Convalescent blood samples, obtained 3 months after hospital admission, was obtained from 13 patients. Serum and plasma samples were stored at −80°C and underwent, at most, 2 freeze and thaw cycles before being assayed blindly. Concentrations of MIF were measured using ELISA, as described elsewhere [29]. The analytic sensitivity of the human MIF ELISA was 39 pg/mL. Intrarun and interrun coefficients of variation were 6% and 12%, respectively. Concentrations of cholesterol, ACTH, cortisol, C-reactive protein, procalcitonin TNF, IL-1β, IL-1ra, IL-6, IL-8, IL-10, macrophage inflammatory protein–1β, activated protein C, thrombin-antithrombin III complexes, and plasminogen activator inhibitor–1 were determined as previously described [19, 22–25, 30, 31].

Statistical analysis. Comparison among groups was assessed using the Kruskall-Wallis test for continuous variables and Pearson's χ^2 or Fisher's exact test for categorical variables, as appropriate. Spearman's correlation coefficient was used to evaluate the correlation between concentrations of MIF and other laboratory parameters. The interday differences for cytokine levels were evaluated using the Wilcoxon signed-rank

Table 1. Characteristics of adult patients in the study population, by outcome.

Characteristic	Survivors (n = 36)	Early death group (n = 20)	Late death group (n = 12)
Age, years	52 (7–78)	58.5 (21–74)	67 (34–76)
Male sex, no. (%) of patients	18 (50)	17 (85)	9 (75)
Severity of underlying diseases, no. of patients			
Rapidly fatal	0	2	3
Ultimately fatal	7	5	5
Nonfatal	29	13	4
Corticosteroid use, no. (%) of patients			
None	27 (75)	13 (65)	6 (50)
Chronic use	2 (6)	4 (20)	1 (8)
Acute use	7 (19)	3 (15)	5 (42)
Site of infection, no. of patients			
Intraabdominal	11	9	6
Respiratory tract	9	6	2
Genitourinary tract	6	2	0
Skin and soft-tissue	1	2	1
CNS	3	1	0
Other site	0	0	1
No site identified	6	0	2
Pathogen, no. of patients[a]			
Gram-negative bacteria	19	13	5
Gram-positive bacteria	3	0	1
Fungi	3	0	0
Mixed infections	6	7	5
Not documented	5	0	1
Temperature, °C	38.2 (35.0–40.4)	37.3 (35.6–40.4)	38.1 (35.9–39.4)
Mean arterial pressure, mm Hg	63 (17–115)	60 (23–87)	62.5 (47–85)
Duration of hypotension, h	12 (2–36)	11.5 (0–96)	10 (3–144)
Urine output, mL/min	30 (0–200)	0 (0–170)	27.5 (0–60)
Arterial pH	7.39 (7.27–7.57)	7.30 (7.17–7.47)	7.38 (7.26–7.52)
Leukocyte count, G/L	10.25 (0.2–42.0)	6.2 (0.1–100)	6.95 (0.8–13.7)
Thrombocyte count, G/L	89.5 (17–516)	73 (22–352)	109 (12–400)

NOTE. Data are median (range), unless otherwise indicated. Adult patients were classified as having survived (survivors); as having died of fulminant, irreversible septic shock (early death group); or as having died of an indirect consequence or of a relapse of shock after transient reversal of shock, defined as a normalization of blood pressure and discontinuation of supportive vasopressor therapy (late death group). The 3 groups of patients differed in sex ratio (P = .02), severity of the underlying disease (P = .01), age (P = .09), urine output (P < .001), arterial pH (P = .02), and time to death (P < .001).

[a] Some patients had mixed infections.

test. The risk of death associated with blood MIF levels was assessed using logistic regression analyses. Two-sided P values <.05 were considered to indicate statistical significance. Analyses were performed using SPSS software, version 14.0 (SPSS) and STATA software, version 9.0 (Statacorp).

RESULTS

Adult patients. Among the 68 adult patients, 15 (22%) presented with severe sepsis and 53 (78%) presented with septic shock. Thirty-two patients (47%) died. Nonsurvivors had been prospectively subdivided into 2 groups: 20 patients (29%) who rapidly died of fulminant, irreversible septic shock (early death; median time to death, 2.5 days) and 12 patients (18%) who experienced late death (median time to death, 14.5 days). Table 1 lists the demographic, clinical, and laboratory characteristics of the survivor group, the early death group, and the late death group. Sepsis was caused by gram-negative bacteria in 54 patients (80%), by gram-positive bacteria in 4 patients (6%), and by *Candida albicans* in 3 patients (4%). In 7 patients (10%), all sample cultures had negative results. Blood cultures had positive results in 43 patients (63%) (table 2). Polymicrobial bacteremia occurred in 8 patients (12%).

MIF was detected in the serum samples of all patients who were enrolled in the study. MIF levels were much higher among patients with sepsis (median level at study entry, 103.7 ng/mL; range, 1.4–3200 ng/mL) than among healthy subjects (median

Table 2. Microorganisms isolated from blood samples obtained from adult study patients.

Microorganism	No. of isolates
Gram-negative bacteria	
Escherichia coli	14
Pseudomonas species	7
Klebsiella species	4
Enterobacter species	3
Neisseria meningitidis	3
Serratia species	2
Citrobacter species	1
Proteus species	1
Bacteroides species	1
Other	2
Gram-positive bacteria	
Staphylococcus aureus	1
Enterococcus faecalis	1
Coagulase-negative staphylococci	1
Fungi	
Candida albicans	2

level at study entry, 5.2 ng/mL; range, 2.8–15.5 ng/mL; $P < .001$). Serum concentrations of MIF continued to increase within 2 h after study entry (median, 131.5 ng/mL; range, 2.5–3200 ng/mL) and progressively decreased thereafter (median level at day 1, 73.7 ng/mL; range, 5.2–1772 ng/mL; median [range] at day 10, 50.3 ng/mL; range, 0–388 ng/mL). In 71% of the patients, peak levels of MIF were reached within 2 h after enrollment. Only 3 patients (4%) had peak MIF levels (1.4, 5.9, and 6.5 ng/mL) that were within the range for healthy subjects, probably because of chemotherapy-induced neutropenia (defined as an absolute neutrophil count <100 cells/mm^3) in 1 patient and because of treatment with high doses of hydrocortisone or methylprednisolone in the other 2 patients. Twenty-two patients (32%) were receiving corticosteroid therapy at study entry, which had a significant impact on the circulating MIF levels that were measured at study entry ($P = .001$). Interestingly, patients who had been receiving long-term prednisone therapy ($n = 7$) had higher MIF levels (median level, 350.3 ng/mL; range, 52.5–525 ng/mL) than those who had not received corticosteroids (median level, 109.1 ng/mL; range, 4.2–1585.5 ng/mL; $P = .07$), whereas patients who were treated shortly before enrollment with high doses of hydrocortisone, methyprednisolone, or dexamethasone ($n = 15$) for the treatment of sepsis had much lower MIF levels (median level, 37.7 ng/mL; range, 1.4–3200 ng/mL; $P = .003$).

Peak MIF serum concentrations (defined as the highest MIF level within the first 2 h after study entry) were inversely correlated with urine output ($R = -.37$; $P = .002$) and, to a lesser extent, with arterial pH ($R = -.22$; $P = .07$). There was a trend toward a positive correlation between MIF levels and leukocyte counts ($R = .21$; $P = .09$), a finding that was in agreement with the observation of low MIF levels in a neutropenic patient. Serum concentrations of MIF were found to correlate with those of macrophage inflammatory protein–1β ($P = .001$), IL-1β ($P = .05$) and, to a lesser extent, with those of plasminogen activator inhibitor–1 ($P = .06$), IL-8 ($P = .09$), and IL-6 ($P = .10$), but not with those of TNF ($P = .25$)

Pediatric patients with meningococcal sepsis. The demographic, clinical, and microbiological characteristics of the 77 pediatric patients are shown in table 3. Seventy-one patients (92%) presented with septic shock and 6 (8%) presented with with severe sepsis, and 10 patients (13%) died. *N. meningitidis* was isolated from blood samples by culture in 65 patients and

Table 3. Characteristics of pediatric patients with meningococcal sepsis, by outcome.

Characteristic	Survivors ($n = 67$)	Nonsurvivors ($n = 10$)
Age, median years (range)	4.38 (0.12–16.11)	1.10 (0.46–9.43)
Male sex	41 (61)	8 (80)
Shock	61 (92)	10 (100)
Receipt of mechanical ventilation	41 (62)	10 (100)
Disseminated intravascular coagulation	34 (51)	10 (100)
PRISM score within first 6 h, median score (range)	20.0 (4–37)	31.5 (23–43)
Predicted mortality based on the Rotterdam score, % (range)	5.9 (0.0–96.3)	95.9 (55.1–99.6)
Documentation of *Neisseria meningitidis* infection		
Blood culture	55 (82)	10 (100)
PCR	2 (3)	0
Neisseria meningitidis serogroup		
B	39 (75)	7 (87.5)
C	13 (25)	1 (12.5)

NOTE. Data are no. (%) of patients, unless otherwise indicated. The 2 groups of patients differed in age ($P = .002$), need for mechanical ventilation ($P = .03$), presence of disseminated intravascular coagulation ($P = .004$), PRISM score ($P = .001$), and predicted mortality ($P < .001$). PRISM, pediatric risk of mortality.

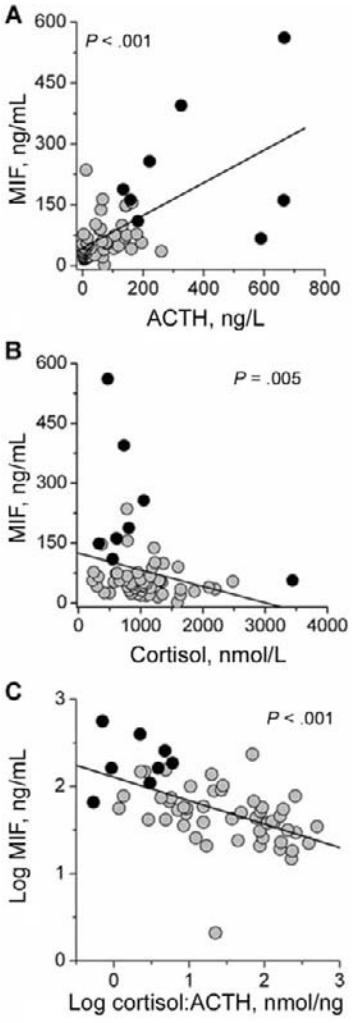

Figure 1. Correlations between levels of macrophage migration inhibitory factor (MIF) and adrenocorticotropic hormone (ACTH; *A*), MIF and cortisol levels *(B),* and MIF level and the cortisol:ACTH ratio *(C),* measured at admission to the hospital, in children with meningococcal severe sepsis and septic shock. *Black circles,* nonsurvivors; *gray circles,* survivors. Spearman's correlation coefficients: $R = .597$ *(A),* $R = -.355$ *(B),* and $R = -.627$ *(C).*

by PCR in 2. The other 10 patients had possible meningococcal infections on the basis of clinical criteria [27]. At study entry, concentrations of MIF were found to be negatively correlated with patient age ($P < .001$) and positively correlated with pediatric risk of mortality score ($P < .001$) and predicted mortality ($P < .001$) on the basis of Rotterdam score. MIF levels were also significantly higher in patients who had disseminated intravascular coagulation (median level, 74 ng/mL; range, 22–560 ng/mL) than in those who did not (median level, 36 ng/mL; range, 8–235 ng/mL; $P < .001$) and higher in patients with shock (median level, 57 ng/mL; range, 18–560 ng/mL) than in those who did not experience shock (median level, 20 ng/mL; range, 2–35 ng/mL; $P < .001$). Moreover, MIF levels were positively correlated with lactate and procalcitonin levels ($P = .006$ and $P = .016$, respectively), whereas they were negatively correlated with levels of C-reactive protein ($P = .02$) and cholesterol ($P = .001$). Serum concentrations of MIF were positively correlated with levels of IL-1β ($P < .001$), IL-1ra ($P = .002$), IL-6 ($P = .001$), IL-8 ($P = .001$), and soluble TNF receptor ($P = .01$) and, to a lesser extent, with levels of TNF ($P = .08$) and IL-10 ($P = .08$). In addition, MIF was correlated with thrombin-antithrombin III complexes ($P = .003$), plasminogen activator inhibitor–1 ($P = .006$), and activated protein C ($P = .02$).

MIF and the hypothalamo-pituitary-adrenal (HPA) function. The relationship between circulating levels of MIF and stress hormones (ACTH and cortisol) was examined in children with meningococcal sepsis. Interestingly, circulating concentrations of MIF were found to be positively correlated with ACTH levels ($P < .001$) (figure 1*A*) and negatively correlated with cortisol levels ($P = .005$) (figure 1*B*) and with the cortisol:ACTH ratio ($P < .001$) (figure 1*C*). Although ACTH stimulation tests were not performed, the stress hormone pro-

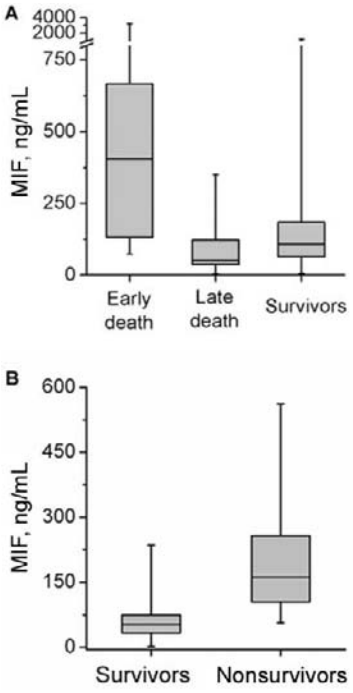

Figure 2. Box plots of macrophage migration inhibitory factor (MIF) levels in adults *(A)* and in children *(B).* The bottom, median, and top lines of the box mark the 25th, 50th, and 75th percentiles, respectively. The vertical line with whiskers shows the range of values. *A,* Survivors and nonsurvivors subdivided into those who experienced late death and those who experienced early death. Global *P* value, $P < .001$; those who experienced early death versus survivors, $P = .001$; and those who experienced early death versus those who experienced late death, $P < .001$; survivors versus those who experienced late death, $P = .23$. *B,* survivors and nonsurvivors, $P < .001$.

Table 4. Kinetics of blood macrophage migration inhibitory factor (MIF) concentrations in adult and pediatric study patients, by outcome.

MIF concentration	Survivors	Nonsurvivors	Early death group	Late death group
Adult patients				
At study entry	71 (4–717)	…	165 (124–369)	51 (14–350)
After 2 h	75 (3–1131)	…	447 (92–743)	106 (27–299)
On day 1	108 (5–862)	…	670 (110–1772)	87 (23–317)
On day 10	36 (0–273)	…	231 (118–388)	55 (4–191)
Pediatric patients				
At study entry	53 (2–235)	161 (56–561)	…	…
After 12 h	43 (18–155)	207 (202–735)	…	…
After 24 h	32 (2–386)	347 (213–481)	…	…

NOTE. Values are median (range). Adult patients were classified as having survived (survivors); as having died of fulminant, irreversible septic shock (early death group); or as having died of an indirect consequence or of a relapse of shock after transient reversal of shock, defined as a normalization of blood pressure and discontinuation of supportive vasopressor therapy (late death group). Pediatric patients were classified as having survived (survivors) or as having died (nonsurvivors).

file showing an inverse correlation between ACTH and cortisol levels ($P < .001$) was very suggestive of an inappropriate adrenal response to ACTH. This was particularly obvious in nonsurvivors in whom blood profiles exhibited a typical pattern of high MIF levels and a low cortisol:ACTH ratio pathognomonic of a dysregulated MIF-glucocorticoid balance with an exuberant proinflammatory response (figure 1*C*).

MIF and survival. Peak serum levels of MIF differed markedly between the 3 groups of adult patients ($P < .001$) (figure 2*A*). Levels were markedly higher in the 20 patients in the early death group (median level, 425.9 ng/mL; range, 73.6– 3200 ng/mL)) than in the 36 survivors (median level, 108.2 ng/mL; range, 5.9–1236.8 ng/mL; $P = .001$) or in the 12 patients in the late death group (median level, 78.0 ng/mL; range, 2.5–350.3 ng/mL; $P < .001$) ($P = .23$ for survivors vs. late death group). At study entry, each incremental elevation of 100 ng/mL of MIF increased the risk of early death by a factor of 1.67 (95% CI, 1.17–2.41; $P = .005$). This finding remained significant (OR, 1.49; 95 % CI, 1.02–2.19; $P = .03$) after adjustments for sex and urine output, 2 variables that were significantly associated with MIF levels and the risk of death. Table 4 shows the kinetics of MIF levels in survivors and nonsurvivors over a 10-day period.

Circulating concentrations of MIF were also associated with outcome in children with meningococcal sepsis. MIF levels were significantly higher in nonsurvivors than in survivors (at study entry, $P < .001$; at 12 h, $P = .005$; and at 24 h, $P = .01$) (figure 2*B* and table 4). Similar to the observation made in adults, each incremental elevation of 100 ng/mL of MIF increased the risk of death by a factor of 7.4 (95% CI, 2.1–26; $P = .002$). Levels of MIF measured in convalescent children 3 months after admission were significantly lower than those measured at admission ($P = .009$).

DISCUSSION

Analyses of the kinetics of blood MIF in 2 cohorts of 145 patients with gram-negative severe sepsis and septic shock caused by *N. meningitidis* infection in children and predominantly caused by Enterobacteriaceae and *Pseudomonas* species infection in adults revealed that 96% of the pediatric and adult patients with severe sepsis or septic shock had elevated MIF levels that were 10 times above the normal range of MIF concentrations in healthy subjects. These results confirm and extend previous findings of 4 series of patients who had either systemic inflammatory response syndrome, severe sepsis, or septic shock [32–35]. MIF levels remained elevated for at least 10 days after the onset of severe sepsis and septic shock. This rather unique kinetic profile offers a wide window for therapeutic interventions that is likely to be a major advantage for the design of future clinical trials with anti-MIF therapies that are currently under development.

MIF levels were correlated with sepsis severity scores (pediatric risk of mortality score and predicted mortality rates in children with meningococcal sepsis), morbidity (presence of shock, disseminated intravascular coagulation, low urine output and arterial pH, and high lactate levels), and, importantly, with mortality. Indeed, very high MIF levels at the onset of sepsis were associated with fulminant and rapidly fatal disease. In contrast, adults in the late death group of patients who initially recovered from shock but who died later from complications of shock or from a relapse of sepsis had MIF levels that were in the range of those of survivors. Therefore, measurement of blood MIF may help to identify patients with poor prognosis and in whom anti-MIF treatment strategies might improve survival.

Consistent with its proinflammatory activities, MIF level was found to be correlated with markers of inflammation or sepsis

(C-reactive protein and procalcitonin levels in children) and with proinflammatory cytokine levels. However, some discrepancies were observed between children and adults with respect to correlations between MIF and proinflammatory cytokine (TNF, IL-6, and IL-8) levels. Several factors may account for these discrepancies, such as the timing of blood sampling; the patient's age, which might influence MIF responses (a hypothesis that deserves further investigation); the type of infections (community-acquired infections in children vs. a mixture of community-acquired and health care–related infections in adults); the underlying risk factors (e.g., meningococcal sepsis typically occurs in young, otherwise healthy subjects with low bactericidal antibody titers, whereas gram-negative sepsis in adults occurs in the context of comorbidities likely to affect the magnitude of cytokine responses); and, of course, the etiology of sepsis (*N. meningitidis* infection in children vs. Enterobacteriaceae and *Pseudomonas* species infection in adults). Indeed, in contrast to other types of gram-negative sepsis, meningococcal sepsis usually follows a fulminant course characterized by a rapid invasion of the bloodstream, very high concentrations of endotoxin in the systemic circulation, and the induction of a vigorous cytokine response and powerful activation of the complement and coagulation systems [36]. Consistent with these observations and with previous findings of an association between increased plasminogen activator inhibitor–1 levels and increased mortality associated with meningococcal sepsis or gram-negative septic shock, as well as with findings regarding the impact of activated protein C on LPS-induced MIF release [30, 37], MIF levels were positively correlated with disseminated intravascular coagulation and with levels of plasminogen activator inhibitor–1, thrombin-antithrombin III complexes, and activated protein C.

Activation of the HPA axis is an essential feature of the systemic stress response to infection, resulting in the release of glucocorticoids, which play an essential role in the regulation of the host inflammatory and immune responses [38]. One of the intriguing features of MIF has been its abundant expression in the pituitary and adrenal glands and its circulation in the bloodstream with a circadian rhythm synchronous with that of glucocorticoids [6, 8, 9, 39]. Moreover, low doses of glucocorticoids have previously been shown to induce MIF release, whereas high doses were found to suppress MIF expression [7, 40, 41]. In turn, MIF overrides the immunosuppressive and antiinflammatory effects of glucocorticoids [7], leading to the concept that MIF and glucocorticoids function as a physiological counterregulatory dyad that modulates inflammatory and immune responses. In line with these observations, treatment with long-term corticosteroids was associated with a 3.5-fold up-regulation of MIF levels at study entry, whereas treatment with short-term high-dose corticosteroids caused a 3.0-fold down-regulation of MIF levels. The positive correlation noted between MIF and ACTH levels measured at admission in children with meningococcal septic shock are well in agreement with the fact that corticotropin-releasing hormone and LPS are powerful MIF secretagogues in the pituitary gland [8]. In contrast to what is observed in adults with sepsis [35], MIF levels were inversely correlated with cortisol levels in septic children and, thus, with the cortisol:ACTH ratio, which is consistent with previous observations that high doses of corticosteroids inhibit MIF production [7, 40, 41]. Cholesterol, the starting compound of cortisol synthesis, is a major lipid constituent of high-density lipoproteins, which are very potent inhibitors of LPS activity in vivo and a prognostic factor of susceptibility to and outcome of sepsis [24, 42]. When forming complexes with high-density lipoproteins and LPS, cholesterol may no longer be used for cortisol synthesis, providing a possible explanation for low cortisol levels in patients with meningococcal sepsis. The observation of high ACTH levels but low cortisol and glucose levels was highly suggestive of relative adrenal insufficiency, commonly observed in severe meningococcal sepsis [21, 25]. Taken together with the observation of very high circulating concentrations of MIF in nonsurvivors, the stress hormone blood profile indicated that the immunoregulatory balancing act played by MIF and glucocorticoids was clearly leaning towards an overwhelming proinflammatory response likely to contribute to multiple-organ dysfunction and death.

In summary, the present data showed that MIF was markedly and persistently up-regulated in children and adults who had gram-negative sepsis. High MIF levels were associated with disease severity scores, proinflammatory markers of sepsis, and disregulated pituitary-adrenal function and early death, highlighting the presence of a marked imbalance between proinflammatory (MIF) and antiinflammatory (glucocorticoid) regulatory systems in gram-negative sepsis. Given the important position of MIF in innate immune responses to microbial pathogens, these results provide a strong rationale for the development of anti-MIF treatment strategies for the treatment of patients with severe sepsis and septic shock.

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