Simultaneously Mounted Pro- and Anti-inflammatory Host Response Relates to the Development of Secondary Infections in Patients with Sepsis

In this issue of the *Journal*, van Vught and colleagues (pp. 458–470) report that hyperinflammation, apart from the previously reported immune suppression, contributes to the susceptibility to develop secondary infections in patients with sepsis admitted to the intensive care unit (ICU) (1). In this large cohort (>1,000 patients), the patients who developed secondary infections had higher disease severity scores and were more likely to suffer from septic shock on ICU admission. As a more dysregulated host response to the primary infection is likely related to disease severity, this is not surprising, but it may increase the chances of residual confounding. However, the authors nicely show that the differences between groups remain statistically significant after propensity score matching for Acute Physiology and Chronic Health Evaluation–IV score, Sequential Organ Failure Assessment score, source of infection, and presence of shock, as well as additional sensitivity analyses related to immune-suppressive underlying diseases and treatment. As more pronounced hyperinflammation persisted to relate to the development of secondary infections, even when controlled for clinical variables related to disease severity and immunity, this observation indicates that development of a secondary infection is intrinsically related to the more dysregulated host response and is not merely the result of an epiphenomenon.

These results appear to contradict their mRNA expression data reported earlier (2). In the same cohort of patients, no differences in whole blood inflammatory gene expression were found between patients with sepsis who did develop secondary infections compared with those who did not. This apparent discrepancy may illustrate the difference between mRNA expression in circulating immune cells and biomarker protein levels that are released not only by circulating cells but also by extracellular resident cells. Indeed, several animal studies have demonstrated that tissue macrophages are crucial for the *in vivo* cytokine response (3–5). Also in humans, experimental endotoxemia experiments have demonstrated that the *in vitro* LPS-stimulated cytokine response does not correlate at all with the *in vivo* cytokine response after intravenous administration of LPS (6) and that immunoparalysis recovers within hours in circulating cells *in vitro*, whereas it is still present *in vivo* weeks after endotoxemia (7). These findings illustrate that whole blood leukocyte genome transcriptome changes may not reflect what is really happening in a patient's body.

Naturally, the observational nature of the study of van Vught and colleagues does not allow conclusions concerning the cause–effect relationship between hyperinflammation and the development of secondary infections (1). Although it was previously believed that, after an infection, first a proinflammatory response is mounted, followed by an anti-inflammatory counteracting response to bring the immune system back to homeostasis again, it has become clear over the last few years that the pro- and anti-inflammatory response are actually mounted simultaneously (8). Although the study of van Vught and colleagues predominantly focuses on proinflammatory biomarkers, the authors do report that the archetypical anti-inflammatory cytokine IL-10 was also higher during the first days in the ICU in the patients who eventually developed a secondary infection (1). This indeed indicates that the patients may both be hyperinflamed and immune suppressed at the same time.

Currently, it appears that monocytic human leukocyte antigen–antigen D related (HLA-DR) expression is the optimal method to determine immunoparalysis. Of interest, especially the change in HLA-DR expression appears to be best associated with the development of secondary infections (9). Unfortunately, the presence of immunoparalysis was not determined by HLA-DR expression or LPS-stimulated cytokine responses in the study of van Vught and colleagues (1). As a consequence, the present study does not allow direct relation of the development of secondary infections to immune suppression. It appears plausible that more hyperinflammation may be related to a more pronounced immune suppression and that this immune suppression is eventually most relevant for the observed increase in the susceptibility to secondary infections. The simultaneous presence of hyperinflammation and immune suppression may hamper the possibilities to therapeutically intervene. One might argue that concurrent hyperinflammation may pose a risk for these therapies aimed to stimulate the immune response. The only randomized trial to date using immunotherapy used only suppressed HLA-DR expression, and not signs of hyperinflammation, as an inclusion criterion. This trial demonstrated that administration of granulocyte macrophage–colony-stimulating factor is able to restore HLA-DR expression and *in vitro* LPS-stimulated cytokine responses in patients with sepsis (10). In this proof-of-principle study, clinical endpoints were also positively influenced, illustrating that it is feasible and safe to stimulate the innate immune response. A case series showed that the immunostimulatory IFN-γ is able to increase HLA-DR expression in patients with suppressed values, whereas it did not influence the HLA-DR expression in patients without suppressed HLA-DR levels (11). Again, no detrimental effects of immune stimulation were observed. Trials with other immune stimulating compounds, including IL-7 and anti–programmed cell death protein 1 antibodies, are currently being designed and conducted in patients with sepsis. Side effects that may be related to enhanced cytokine release need to be monitored, but so far no safety concerns were reported.

The present work of van Vught and colleagues illustrates that a proinflammatory immune response does not prevent secondary infections but that patients with a more pronounced hyperinflammatory response are actually more likely to develop a secondary infection (1). A limitation of the article is that it mainly focuses on innate immunity biomarkers. Previous work has indicated that not only is the innate immune response attenuated in patients with sepsis but also the adaptive immune response (12). To what extent the early innate immune response influences, for example, lymphopenia and T-cell diversity is unknown and could also be of paramount importance for the development of secondary infections in patients with sepsis.

The results of the study clearly increase our pathophysiological insight. However, as is illustrated by the figures, the statistically

significant differences between groups are relatively small, and the overlap in biomarker concentrations between the patients who develop a secondary infection and those who do not is huge. It is important to realize that, as a consequence of this heterogeneity, the predictive value and clinical relevance of a certain value for a specific patient will be very limited. In other words, for the individual patient with sepsis who is admitted to the ICU, the level of the measured biomarkers has insufficient sensitivity and specificity to use its concentration to guide additional diagnostic or therapeutic actions related to the development of secondary infections. Greater appreciation for the role of the dysregulated immune response is represented in the new definition of sepsis (13), defining sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to infection. The study of van Vught and colleagues confirms that this is correct (1). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Early Lung Function Decline in Cystic Fibrosis
Can Registry Data Explain Divergent Phenotypes?

During the last decade, the cystic fibrosis (CF) population has evolved such that the majority of those living with the disease are adults (1). Earlier diagnosis, novel interventions to treat CF lung disease and comorbidities, and the spread of rigorous quality improvement methods across care centers have contributed to these overall improvements in outcomes (2). Despite these advances, CF is still a life-limiting condition, with progressive respiratory disease as the leading cause of early mortality. Phenotypic variability is commonly observed, and differences in clinical course, even among individuals within the same family, are often unexplained.

Originally Published in Press as DOI: 10.1164/rccm.201704-0813ED on May 15, 2017

Data from large databases, such as the U.S. CF Foundation Patient Registry, enable clinicians and researchers to observe the natural history of CF and identify patient risk factors for critical health outcomes (3). Prior epidemiologic studies have provided important insight into risk factors for lung function decline and mortality in CF (4, 5). In this issue of the *Journal*, Szczesniak and colleagues (pp. 471–478) extend and expand on these prior epidemiologic analyses (6). They present a rigorous analysis of 16 years of CF Foundation Patient Registry data to determine the characteristics of patients with more rapidly deteriorating lung function. Using a novel statistical methodology, the authors evaluate risk factors for overall lung function decline and identify clusters of the population based on the age at onset of lung function decline. Their analysis confirms that lung function decline initiates during adolescence for many individuals,

The Host Response in Patients with Sepsis Developing Intensive Care Unit–acquired Secondary Infections

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Abstract

Rationale: Sepsis can be complicated by secondary infections. We explored the possibility that patients with sepsis developing a secondary infection while in the intensive care unit (ICU) display sustained inflammatory, vascular, and procoagulant responses.

Objectives: To compare systemic proinflammatory host responses in patients with sepsis who acquire a new infection with those who do not.

Methods: Consecutive patients with sepsis with a length of ICU stay greater than 48 hours were prospectively analyzed for the development of ICU-acquired infections. Twenty host response biomarkers reflective of key pathways implicated in sepsis pathogenesis were measured during the first 4 days after ICU admission and at the day of an ICU-acquired infection or noninfectious complication.

Measurements and Main Results: Of 1,237 admissions for sepsis (1,089 patients), 178 (14.4%) admissions were complicated by ICU-acquired infections (at Day 10 [6–13], median with interquartile range). Patients who developed a secondary infection showed higher disease severity scores and higher mortality up to 1 year than those who did not. Analyses of biomarkers in patients who later went on to develop secondary infections revealed a more dysregulated host response during the first 4 days after admission, as reflected by enhanced inflammation, stronger endothelial cell activation, a more disturbed vascular integrity, and evidence for enhanced coagulation activation. Host response reactions were similar at the time of ICU-acquired infectious or noninfectious complications.

Conclusions: Patients with sepsis who developed an ICU-acquired infection showed a more dysregulated proinflammatory and vascular host response during the first 4 days of ICU admission than those who did not develop a secondary infection.

Keywords: ICU-acquired infection; intensive care unit; sepsis; host response; biomarker

Sepsis is characterized by an injurious host response to an infection, and a leading cause of hospitalization, morbidity, and mortality (1, 2). Most research seeking to obtain insight into mechanisms contributing to sepsis mortality focused on early lethality, presumably caused by an overzealous activation of the innate immune system in response to acute infection (3, 4). However, most deaths in sepsis occur more than 1 week after admission to the intensive care unit (ICU) (5–8). This relatively late sepsis

(Received in original form June 19, 2016; accepted in final form January 18, 2017)

Supported by the Center for Translational Molecular Medicine (http://www.ctmm.nl), project MARS (grant 04I-201).

Author Contributions: L.A.v.V. and T.v.d.P. contributed to the design of the study. L.A.v.V., M.A.W., A.J.H., B.P.S., J.F.F., J.H., P.M.C.K.K., O.L.C., M.J.S., M.M.J.B., and T.v.d.P. acquired the data. A.H.Z. provided statistical guidance and R.L. guided biomarker measurements. L.A.v.V. had full access to all the data in the study and takes full responsibility for the integrity of the data and the accuracy of the data analysis. L.A.v.V. and T.v.d.P. were involved in the interpretation of the data. L.A.v.V. and T.v.d.P. drafted the manuscript, and all authors revised it critically for important intellectual content. All authors gave final approval of this version and declare that they have no conflicts of interest.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 196, Iss 4, pp 458–470, Aug 15, 2017

Originally Published in Press as DOI: 10.1164/rccm.201606-1225OC on January 20, 2017
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Recent observational studies have found that patients with sepsis show signs of prolonged immune suppression, which has been postulated to enhance susceptibility toward secondary infections, thereby contributing to late sepsis mortality. Indeed, several investigators have documented a variety of immune defects in patients with sepsis, such as hyporesponsiveness and a profound loss of innate and adaptive immune cells. However, a systemic hyperinflammatory reaction is not captured by the assays used to study immune suppression in previous investigations.

What This Study Adds to the Field: Patients with sepsis who went on to develop an intensive care unit (ICU)-acquired infection demonstrated a more dysregulated host response during the first 4 days after admission, as reflected by enhanced inflammation, stronger endothelial cell activation, a more disturbed vascular integrity, and evidence for enhanced coagulation activation. This enhanced hyperinflammation was sustained up to the day of ICU-acquired infection development, and no differences were found between the host response during ICU-acquired infection and noninfectious ICU-acquired complications (acute kidney injury or acute respiratory distress syndrome). Although this study does not contradict earlier investigations reporting immune suppression in patients with sepsis, it indicates that patients with sepsis who develop secondary infections while in the ICU also demonstrate hyperinflammation.

mortality has received much attention in recent years, and it has been suggested that immune suppression and, as consequence thereof, ICU-acquired infections are key causative denominators herein (3, 6, 9–11). Indeed, a variety of immune defects have been documented in ICU patients with sepsis, most notably impaired responsiveness of immune cells to bacterial antigens and a profound loss of T and B cells because of apoptotic cell death (3, 10, 11).

We recently reported on the incidence, risk factors, and attributable mortality of ICU-acquired infections in patients admitted to the ICU with sepsis (12). In a prospectively enrolled cohort consisting of 1,719 consecutive sepsis admissions, ICU-acquired infections occurred in 13.5% of cases, bearing a population-attributable mortality fraction of 10.9% by Day 60 (12). Although earlier studies on susceptibility to ICU-acquired infections focused on immune suppression (3, 4, 9–11, 13), we here explored the possibility that the more dysregulated host response in patients with sepsis who acquire an infection while in the ICU is also reflected by a systemic hyperinflammatory reaction that is not captured by the assays used to delineate immune suppression. As such, the primary objective of this study was to compare systemic proinflammatory host responses in patients with sepsis who during their ICU stay acquire a new infection with those who do not. For this we performed a substudy in the previously described cohort (12), and report the levels of 20 host response biomarkers reflective of key pathways implicated in sepsis pathogenesis, measured during the first 4 days after ICU admission and at onset of ICU-acquired infection.

Methods

Study Design and Population

This was an explorative substudy of a previously reported cohort used to

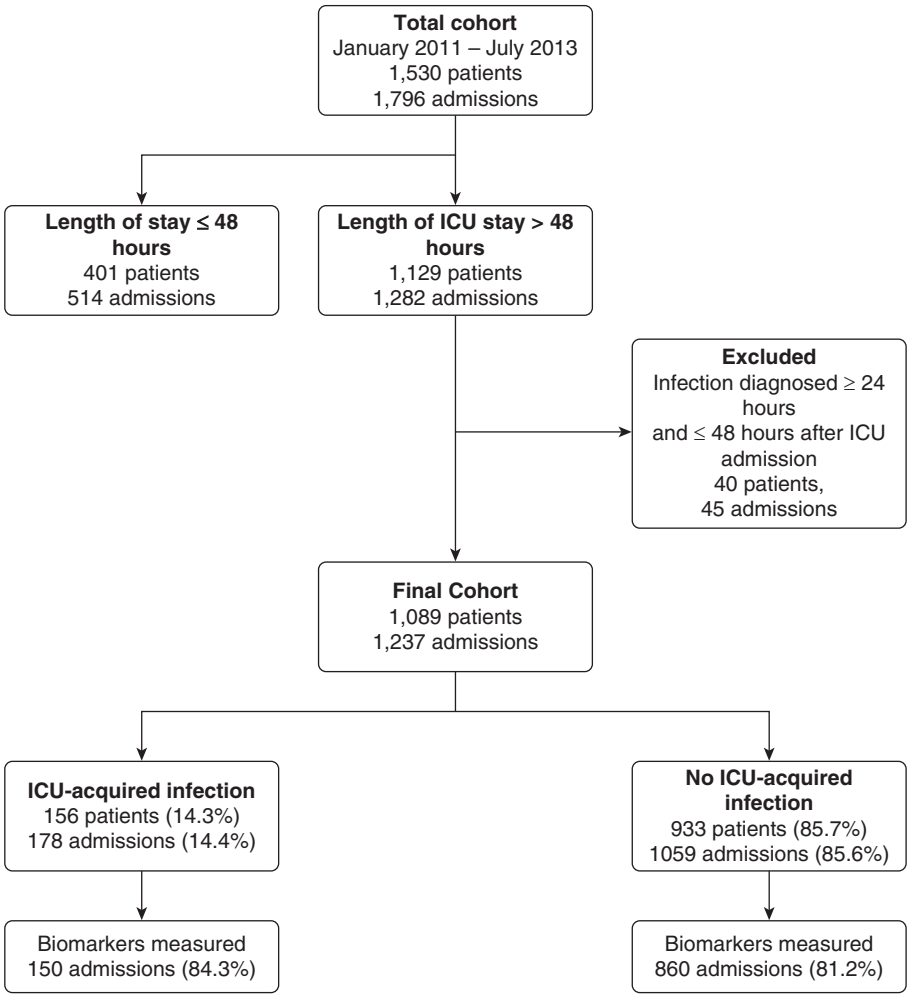

Figure 1. Flowchart of patient inclusion. ICU = intensive care unit.

determine the incidence and attributable mortality of ICU-acquired infections in critically ill patients with sepsis (12). The study was conducted as part of the MARS (Molecular Diagnosis and Risk Stratification of Sepsis) project, a prospective observational cohort study in the mixed ICUs of two tertiary teaching hospitals in the Netherlands (12, 14, 15). Consecutive patients older than 18 years of age admitted to the two ICUs were included via an opt-out method approved by the medical ethical committees of the participating hospitals, the Academic Medical Center in Amsterdam and the University Medical Center in Utrecht. Both ICUs used protocolized care, including selective decontamination of the digestive tract (12, 16).

For every admitted patient the plausibility of an infection was assessed daily using a four-point scale (ascending from none, possible, probable, to definite) (14). Sepsis was defined as the presence of infection diagnosed within 24 hours after ICU admission with a probable or definite likelihood, accompanied by at least one additional parameter as described in the 2001 International Sepsis Definitions Conference (17). ICU-acquired infection was defined as any new-onset infection (with likelihood possible, probable, or definite) starting greater than 48 hours after ICU admittance for which the clinical team started a new antibiotic regimen. Organ failures, shock, and comorbidities were defined as described in the online supplement; acute kidney injury (AKI) and acute respiratory distress syndrome (ARDS) (18, 19) were deemed ICU-acquired when starting greater than 48 hours after ICU admittance.

For the current study consecutive patients with sepsis admitted to the ICU from January 2011 until July 2013 with a length of ICU stay greater than 48 hours were analyzed. Patients with an infection diagnosed greater than or equal to 24 hours but less than or equal to 48 hours after ICU admission were excluded because their infection could not with certainty be deemed the reason for admission or ICU-acquired. Readmissions, defined as any second admission within the 2.5-year study period, were analyzed as new unique admissions. For patients who were readmitted to the ICU demographic and long-term follow-up data (≥30 d) are shown for the first ICU admission only.

Sampling and Assays

Daily (on admission and at 6 A.M. thereafter) ethylenediaminetetraacetic acid anticoagulated plasma harvested from blood obtained for regular patient care was stored within 4 hours after blood draw at −80°C. For assays, *see* the online supplement.

Table 1. Baseline Characteristics and Outcome of Patients Admitted with Sepsis Stratified according to Development of ICU-acquired Infection or Not

	ICU-acquired Infection	No ICU-acquired Infection	*P* Value
Patients	156	933	
Demographics			
Age, yr, mean (SD)	60 (13.9)	60 (14.9)	0.96
Male sex, n (%)	100 (64.1)	542 (58.1)	0.17
Chronic comorbidity			
Any comorbidity, n (%)	118 (75.6)	682 (73.1)	0.53
Immunocompromised state, n (%)	44 (28.2)	219 (23.5)	0.22
Charlson comorbidity index, median (IQR)	4 (3–6)	4 (2–6)	0.93
Admissions	178	1,059	
Source of sepsis admission diagnosis, n (%)			
Pulmonary tract	73 (41.0)	431 (40.7)	>0.99
Abdomen	40 (22.5)	206 (19.5)	0.34
Bloodstream infection	11 (6.2)	24 (2.3)	0.01
Catheter-related bloodstream infection	4 (2.2)	18 (1.7)	0.76
Neurologic	3 (1.7)	61 (5.8)	0.02
Urinary tract	9 (5.1)	72 (6.8)	0.41
Soft tissue infection	2 (1.1)	51 (4.8)	0.02
Other*	36 (20.2)	196 (18.5)	0.60
Admission type, n (%)			
Medical	138 (77.5)	801 (75.6)	0.65
Readmission	22 (12.4)	126 (11.9)	0.91
Severity of disease			
APACHE IV score, mean (SD)	92 (27.5)	82 (27.9)	<0.0001
SOFA score, median (IQR)	8 (6–11)	7 (5–9)	<0.0001
Shock, n (%)	82 (46.1)	371 (35.0)	<0.01
Corticosteroid treatment in the first 4 d after ICU admission, n (%)			
Any hydrocortisone use†	118 (66.3)	605 (57.1)	0.03
Hydrocortisone >200 mg/d†	100 (56.2)	463 (43.7)	<0.01
SDD use‡	123 (69.1)	705 (66.6)	0.54
Outcome			
Length of ICU stay, d, median (IQR)	24 (15–34)	6 (4–10)	<0.0001
Length of hospital stay, d, median (IQR)	37 (22–66)	20 (10–39)	<0.0001
Mortality, n (%)			
ICU§	69 (38.8)	174 (16.4)	0.001
Hospital‖	81 (51.9)	267 (28.6)	<0.0001
30 d‖	49 (31.4)	237 (25.4)	0.13
60 d‖	73 (46.8)	281 (30.1)	<0.0001
90 d‖	82 (52.6)	308 (33.0)	<0.0001
1 yr‖¶	91 (58.3)	406 (43.4)	<0.0001

Definition of abbreviations: APACHE IV = Acute Physiology and Chronic Health Evaluation IV; ICU = intensive care unit; IQR = interquartile range; SDD = selective decontamination of the digestive tract; SOFA = Sequential Organ Failure Assessment.

*Other infections include lung abscess, sinusitis, pharyngitis, tracheobronchitis, endocarditis, mediastinitis, myocarditis, postoperative wound infection, bone and joint infection, oral infection, eye infection, and viral infections.

†Use of hydrocortisone or its equivalent (hydrocortisone dose = 4 × prednisolone dose, 5 × methylprednisolone dose, 25 × dexamethasone dose).

‡Patients not on SDD received selective oropharyngeal decontamination.

§ICU mortality was calculated using all ICU admissions for sepsis.

‖Follow-up data were calculated using the first ICU admission for sepsis for each patient during the study period; readmissions were not included in this analysis.

¶Twenty-six patients were lost to 1-year follow-up (3.8% in patients with sepsis developing an ICU-acquired infection and 2.1% in patients with sepsis with no ICU-acquired infection; $P = 0.25$).

Statistical Analysis

Biomarker measurements were analyzed using all unique admissions, and readmitted patients were not excluded. Biomarkers were transformed to their 10 log scale for plotting purposes. Biomarker distribution over time was analyzed using a general mixed model analysis in which a linear regression model was fitted on logarithmically transformed biomarker data using the different data time points (i.e., admission, Day 2, and Day 4). Different mixed models were fitted taking the group (ICU-acquired infection vs. no ICU-acquired infection), time, and their interaction as fixed effects and patient-specific intercept and slope of time as random effects. The model with the best fit was regarded most appropriate. The overall *P* value reported in the figures and the tables is derived from the fixed-effect model in which group + time was used in addition to the random effects model, unless otherwise specified. The rate of change in biomarker levels over time was analyzed using the mixed effects model using the regression coefficient of time alone or in combination with the interaction between group and time when significant. In addition, this regression coefficient was transformed into percentage change over time. Biomarker distribution at a single time point was compared using a nonparametric Mann-Whitney *U* test. Multiple-comparison–adjusted (Benjamini-Hochberg) *P* value less than 0.05 defined significance of plasma biomarkers. For more details, *see* the online supplement.

Propensity Score Matching

Considering that the release of host response biomarkers in sepsis often is proportional to disease severity (20), propensity score matching was used in patients with biomarkers to account for disease severity on ICU admission and other baseline differences between patients who did and those who did not develop an ICU-acquired infection. A logistic regression implemented in the R library MatchIt version 2.4–21 (http://gking.harvard.edu/matchit) (21) was used, including variables associated with disease severity and baseline variables that were different between groups. The propensity score included Acute Physiology and Chronic Health Evaluation (APACHE)-IV score, Sequential Organ Failure Assessment (SOFA) score, source of infection, and shock, all on ICU admission. Patients developing an ICU-acquired infection were matched 1:3 to patients without the development of ICU-acquired infection, using nearest matching with a caliper of 0.20 SD of the normally distributed propensity score. If less than three control subjects could be matched, fewer matches were allowed, making optimal use of the control subjects. The individual time points (i.e., admission, Day 2, and Day 4) were analyzed separately taking clustering of matching into account by including match-pair identifiers in a mixed model analysis. Both the mixed model analysis and the Mann-Whitney *U* test showed similar results and for consistency the Mann-Whitney *U* test is reported.

Results

Patients

We studied 1,237 admissions for sepsis with a length of ICU stay greater than 48 hours (1,089 patients) (Figure 1). Of these, 178 admissions (14.4%), concerning 156 patients, were complicated by one or more ICU-acquired infections, involving a total of 262 ICU-acquired infections. Patients developing an ICU-acquired infection were more often admitted for primary bacteremia and less often for neurologic and soft tissue infection (Table 1). Patients with sepsis who developed a secondary infection while in the ICU were more severely ill on admission than those who did not, as reflected by higher APACHE IV and SOFA scores, and a higher proportion of shock (Table 1).

ICU-acquired Infections and Outcome

The first ICU-acquired infections occurred at a median of Day 10 (interquartile range, 6–13). The most common ICU-acquired infections were catheter-related bloodstream infections (n = 73; 27.9%), pneumonia (n = 64; 24.4%), and abdominal infection (n = 42; 16.0%) (Table 2). The

Table 2. Characteristics of ICU-acquired Infections

Number and timing of infections	
Admissions associated with an ICU-acquired infection, n (%)	178 (14.4)
ICU-acquired infections	262
Admissions associated with multiple ICU-acquired infections, n (%)	61 (34.3)
Day of first ICU-acquired infection, median (IQR)	10 (6–13)
Source of infection, n (%)	
Pulmonary	64 (24.4)
Hospital-acquired pneumonia	18 (6.9)
Ventilator-associated pneumonia	46 (17.6)
Cardiovascular	85 (32.4)
Bacteremia	12 (4.6)
Catheter-related bloodstream infection	73 (27.9)
Abdomen	42 (16.0)
Abdominal infection	41 (15.6)
Gastrointestinal infection	1 (0.4)
Neurologic	3 (1.1)
Primary meningitis	1 (0.4)
Secondary meningitis	2 (0.8)
Soft tissue infection	11 (4.2)
Urinary tract	3 (1.1)
Other*	54 (20.6)
Causative pathogen, n (%)	
Gram-positive bacteria	118 (45.0)†
Gram-negative bacteria	74 (28.2)
Fungi	24 (9.2)
Viral (including reactivation)	27 (10.3)
Other	8 (3.1)
Unknown	64 (24.4)

Definition of abbreviations: ICU = intensive care unit; IQR = interquartile range.
*Other infections include lung abscess, sinusitis, pharyngitis, tracheobronchitis, endocarditis, mediastinitis, myocarditis, postoperative wound infection, bone and joint infection, oral infection, eye infection, and viral infections.
†Percentages depict the portion of ICU-acquired infections (total n = 262) caused by the pathogen group indicated. In total 251 pathogens were assigned to 262 ICU-acquired infections; in 51 (19.5%) of all ICU-acquired infections more than one pathogen was assigned as causative.

most common causative pathogens were gram-positive bacteria (n = 118; 45.0%), followed by gram-negative bacteria (n = 74; 28.2%), fungi (n = 24; 9.2%), and viruses (n = 27; 10.3%) (Table 2).

The median ICU length of stay was longer in patients who acquired a secondary infection than in those who did not (24 [15–24] vs. 6 [4–10] d, respectively; $P < 0.001$) (Table 1). ICU mortality was higher in patients developing a secondary infection than in patients who did not (38.8% vs. 16.4%; $P < 0.001$); the mortality difference between groups remained until 1 year after ICU admission (Table 1).

Host Response Biomarkers in Patients with Sepsis Who Did and Those Who Did Not Develop an ICU-acquired Infection

In a subset of patients (n = 1,010; 81.6%), biomarkers indicative of the host response during sepsis were measured. Patients with sepsis displayed a profound systemic inflammatory reaction (elevated plasma levels of interleukin (IL)-6, IL-8, IL-10, and matrix metalloproteinase-8) (Figure 2; *see* Figure E1 in the online supplement), activation of the vascular endothelium (elevated plasma concentrations of soluble E-selectin, soluble intercellular adhesion molecule-1 [ICAM-1], and fractalkine), increased loss of vascular integrity (increased levels of angiopoietin-2 and reduced levels of angiopoietin-1) (Figure 3), and a net procoagulant state (elevated plasma levels of D dimer, reduced levels of the anticoagulants antithrombin and protein C, and prolonged activated partial thromboplastin time [aPTT] and prothrombin time) (Figure 4). Most of these characteristic sepsis responses were exaggerated in patients who developed an ICU-acquired infection relative to those who did not, significantly so for IL-6, IL-8, IL-10, soluble ICAM-1, fractalkine, angiopoietin-2, the angiopoietin 2:1 ratio, and aPTT (all $P < 0.01$). Platelet counts were significantly lower in patients who developed an ICU-acquired infection ($P < 0.001$ vs. those who did not). This more disturbed host response remained after exclusion of readmissions (*see* Table E1). Plasma levels of tumor necrosis factor-α, IL-1β, IL-13, granulocyte–macrophage colony–stimulating factor, and IFN-γ were undetectable in most patients and not different between groups (data not shown). The rate of biomarker change in the first 4 days was comparable between patients developing ICU-acquired infections and patients that did not except for fractalkine and platelets (higher in the former group) (*see* Table E2).

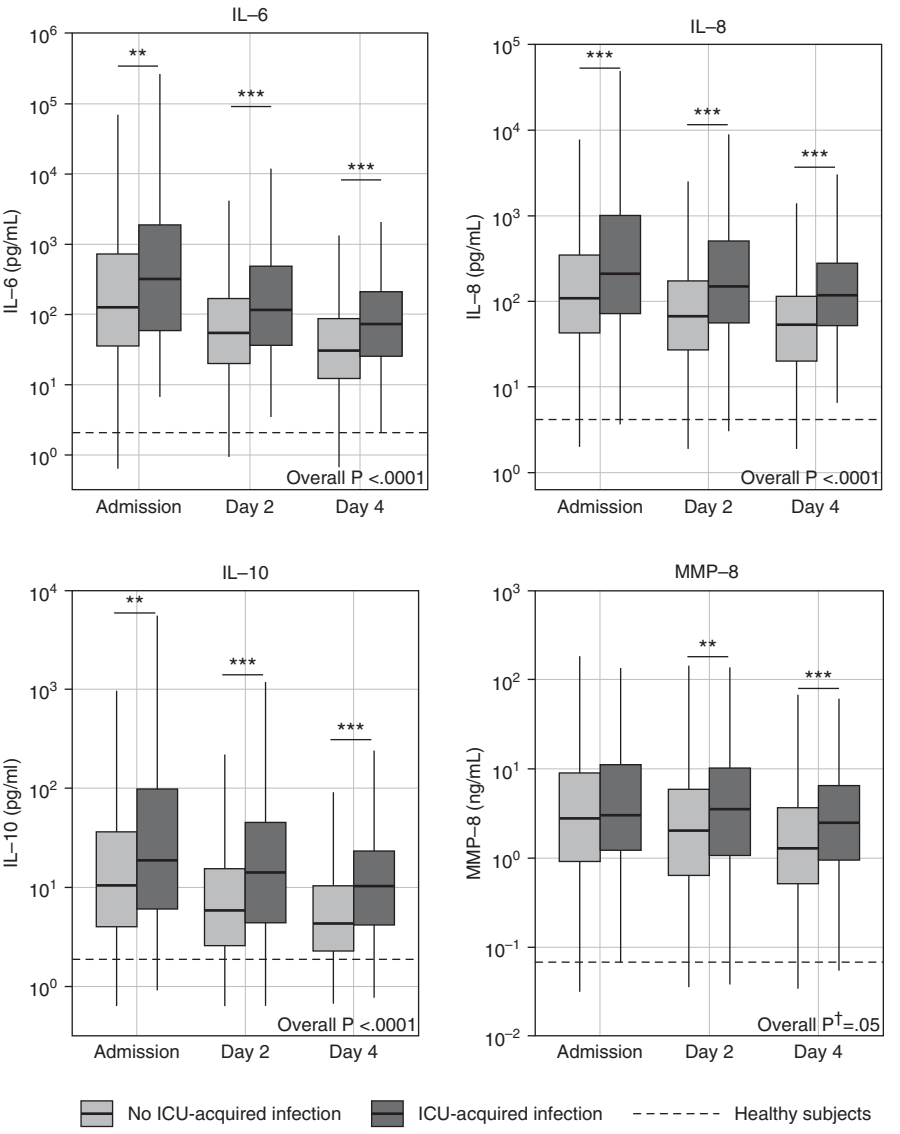

Figure 2. Inflammatory responses in patients with sepsis during the first 4 days of ICU admission stratified according to the development of an ICU-acquired infection or not. Data are expressed as *box-and-whisker* diagrams depicting the median and lower quartile, upper quartile, and their respective 1.5 interquartile range as *whiskers* (as specified by Tukey; *see* online supplement). *Dashed lines* indicate median values obtained in 27 healthy age-matched subjects. Overall *P* values depicted in the figure are derived from the fixed-effect model in which group + time was used in addition to the random effects model, except for biomarkers indicated with a dagger (i.e., MMP-8), in which no fixed-effect model could be fitted so the model with merely random intercept and slope of time was used. Differences between groups at specific days are indicated as multiple-comparison–adjusted (Benjamini-Hochberg) *P* value: $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ (by Mann-Whitney *U* test). ICU = intensive care unit; MMP = matrix metalloproteinase.

Considering that patients who went on to acquire a secondary infection while on the ICU had higher baseline APACHE IV and SOFA scores than those who did not, and considering that the levels of host response biomarkers in sepsis often are proportional to disease severity (20), we matched patients who did and those who did not develop an ICU-acquired infection for disease severity on ICU admission. A total of 133 admissions complicated by

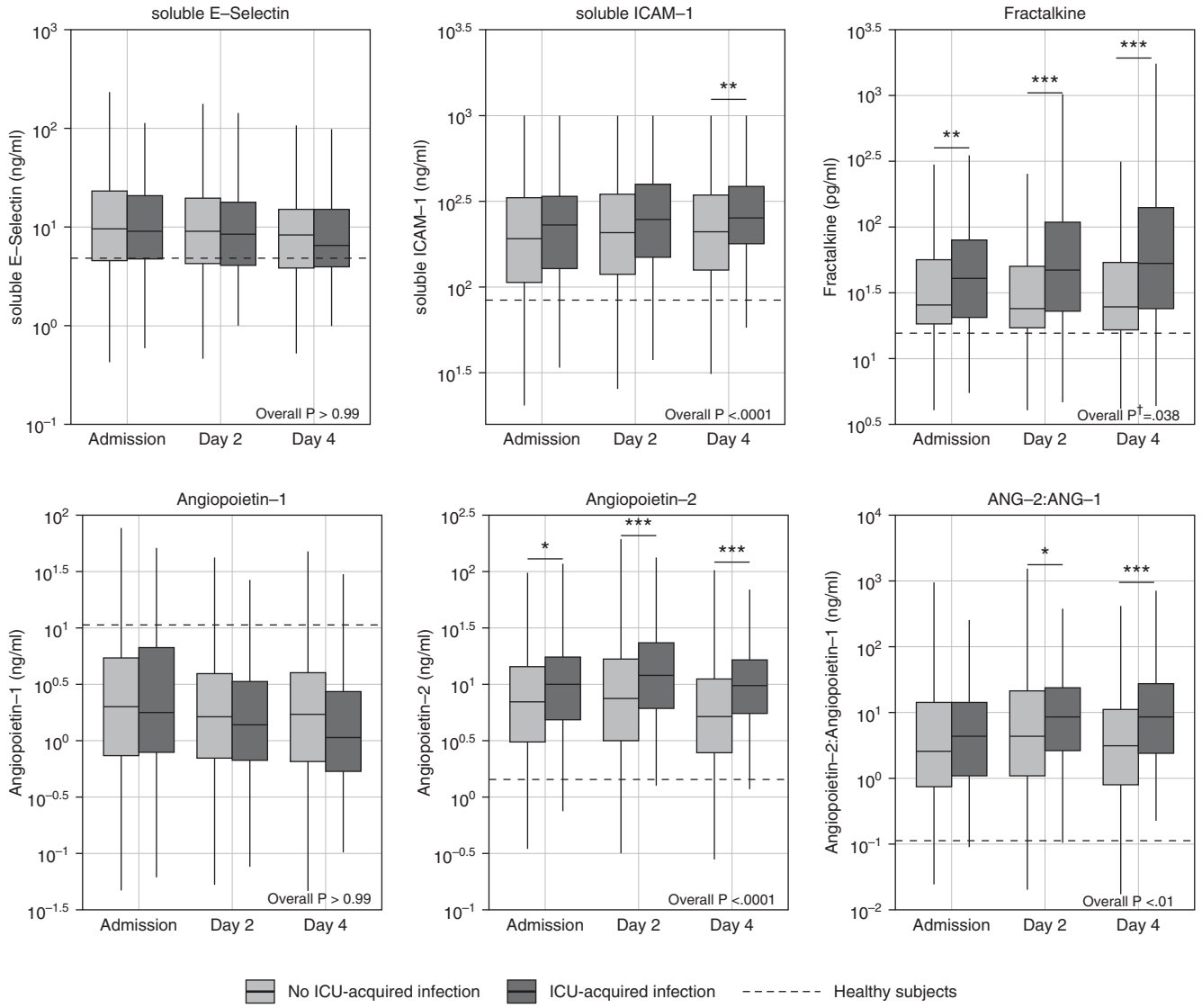

Figure 3. Biomarkers reflecting endothelial cell activation in patients with sepsis during the first 4 days of ICU admission stratified according to the development of an ICU-acquired infection or not. Data are expressed as *box-and-whisker* diagrams depicting the median and lower quartile, upper quartile, and their respective 1.5 interquartile range as *whiskers* (as specified by Tukey; *see* online supplement). *Dashed lines* indicate median values obtained in 27 healthy age-matched subjects. Overall *P* values depicted in the figure are derived from the fixed-effect model in which group + time was used in addition to the random effects model, except for biomarkers indicated with a dagger (i.e., fractalkine), in which the interaction between ICU-acquired infection and time was significant, suggesting that biomarker distribution over time was significantly different. Differences between groups at specific days are indicated as multiple-comparison–adjusted (Benjamini-Hochberg) *P* value: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ (by Mann-Whitney *U* test). ANG = angiopoietin; ICAM = intercellular adhesion molecule; ICU = intensive care unit.

ICU-acquired infection were matched to 322 admissions without ICU-acquired infection with comparable disease severity and source of infection on ICU admission (Table 3). In this matched cohort, many sepsis host response biomarkers remained more aberrant in patients who developed an ICU-acquired infection (Table 4), significantly so for IL-6, IL-8, and IL-10. This outcome was consistent in sensitivity analyses including immunocompromised state in the matching procedure or including immunocompromised state and corticosteroid treatment during the first 4 days of ICU stay in the matching procedure (*see* Tables E3–E6).

Host Response Biomarkers at the Time of ICU-acquired Infection

To obtain insight into the host response at the time of ICU-acquired infection, we first compared biomarkers measured in samples obtained within 24 hours after the diagnosis of an ICU-acquired infection (n = 104) or a noninfectious ICU-acquired complication (i.e., AKI, n = 71; ARDS, n = 34) (Table 5). Most host response parameters were not different between groups (Figure 5).

In a final analysis, biomarker distribution at the last standardized sampling moment (i.e., Day 4) was compared with biomarker distribution at

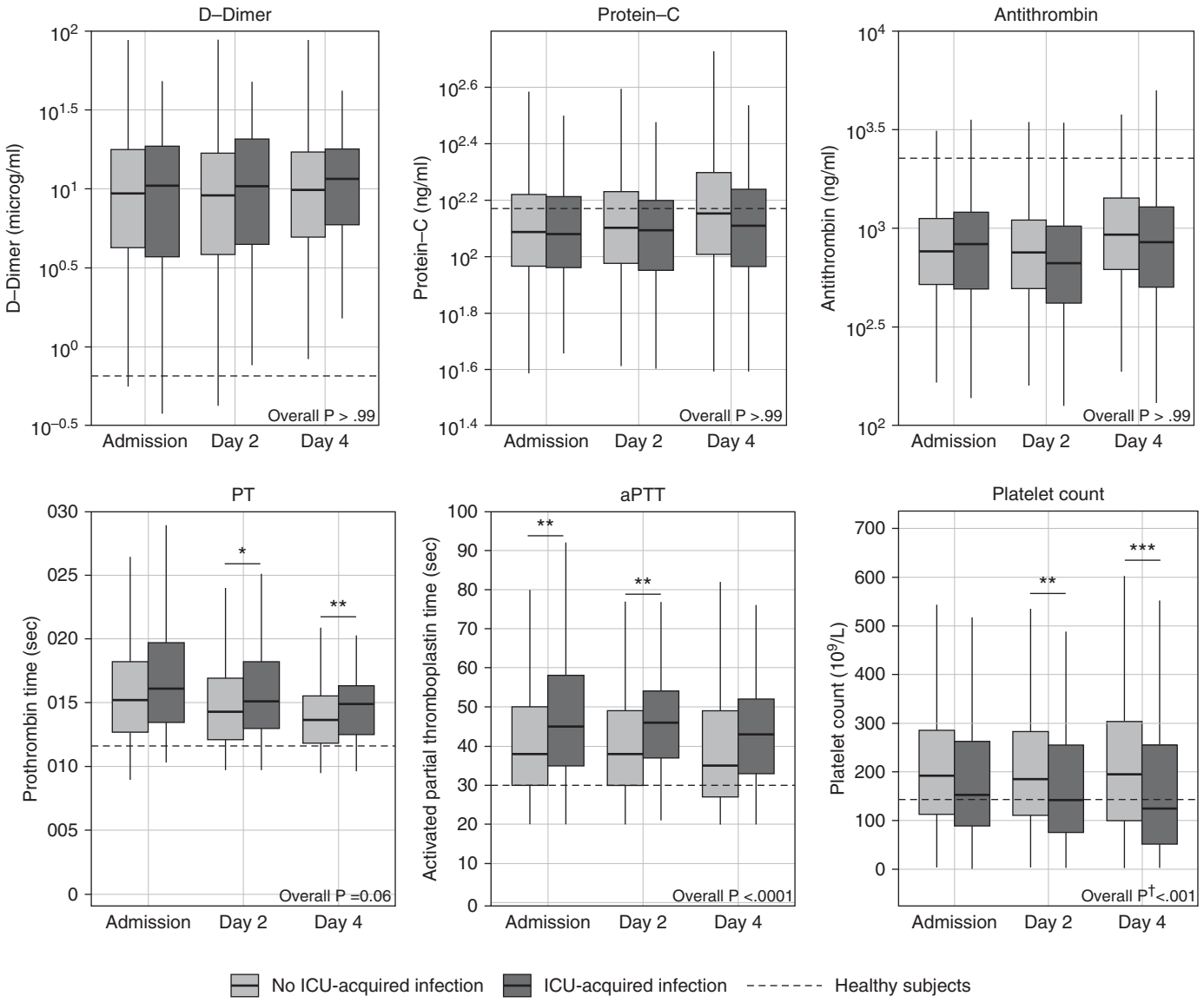

Figure 4. Biomarkers reflecting coagulation activation in patients with sepsis during the first 4 days of ICU admission stratified according to the development of an ICU-acquired infection or not. Data are expressed as *box-and-whisker* diagrams depicting the median and lower quartile, upper quartile, and their respective 1.5 interquartile range as *whiskers* (as specified by Tukey; *see* online supplement). *Dashed lines* indicate median values obtained in 27 healthy age-matched subjects. Overall *P* values depicted in the figure are derived from the fixed-effect model in which group + time was used in addition to the random effects model, except for biomarkers indicated with a dagger (i.e., platelets), in which the interaction between ICU-acquired infection and time was significant, suggesting that biomarker distribution over time was significantly different. Differences between groups at specific days are indicated as multiple-comparison–adjusted (Benjamini-Hochberg) *P* value: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ (by Mann-Whitney *U* test). aPTT = activated partial thromboplastin time; ICU = intensive care unit; PT = prothrombin time.

the time of ICU-acquired infection in all patients from whom paired samples were available (n = 84), revealing no differences (Table 6).

Discussion

Sepsis is associated with prolonged immune suppression and it has been suggested that immune suppression renders patients with sepsis susceptible to secondary infections (3, 6, 9–11, 13, 22). We here examined the possibility that patients with sepsis who, during their ICU stay acquire a secondary infection, besides immune-suppressive features, also display more profound "hyperinflammatory" responses when compared with those who do not develop a secondary infection. For this we measured 20 host response biomarkers reflective of typical proinflammatory sepsis responses, including cytokine release and activation of the vascular endothelium and the coagulation system, in a large cohort of patients with sepsis during the first 4 days after ICU admission and at the time of an ICU-acquired complication (infectious or noninfectious). Our main findings are (*1*)

Table 3. Baseline Characteristics of Patients Admitted with Sepsis Who Did and Those Who Did Not Develop an ICU-acquired Infection Propensity Matched for APACHE IV Score, SOFA Score, Source of Infection, and Shock on ICU Admission

	ICU-acquired Infection	No ICU-acquired Infection	*P* Value
Patients	123	301	
Demographics			
Age, yr, mean (SD)	60.9 (14.0)	61.1 (14.0)	0.93
Sex, male, n (%)	74 (60.2)	166 (55.1)	0.38
Chronic comorbidity			
Any comorbidity, n (%)	90 (73.2)	237 (78.7)	0.25
Immunocompromised state, n (%)	33 (26.8)	74 (24.6)	0.70
Charlson comorbidity index, median (IQR)	4 (3–6)	4 (3–6)	0.21
Admissions	133	322	
Source of infection, n (%)			
Pulmonary tract	56 (42.1)	145 (45.0)	0.61
Abdomen	33 (24.8)	77 (23.9)	0.90
Cardiovascular	5 (4.0)	13 (4.0)	>0.99
Neurologic	3 (2.3)	9 (2.8)	0.78
Urinary tract	7 (5.3)	20 (6.2)	0.83
Skin sepsis	2 (1.5)	5 (1.6)	>0.99
Other*	27 (20.3)	53 (16.5)	0.33
Admission type, n (%)			
Medical	102 (76.7)	246 (76.4)	>0.99
Readmission	10 (7.5)	21 (6.5)	0.70
Severity of disease			
APACHE IV score, mean (SD)	89 (26.7)	85 (26.0)	0.10
SOFA score, median (IQR)	8 (6–10)	8 (6–10)	0.33
Shock, n (%)	55 (41.4)	128 (39.8)	0.76
Corticosteroid treatment in the first 4 d after ICU admission, n (%)			
Any hydrocortisone use†	89 (66.9)	189 (58.7)	0.12
Hydrocortisone >200 mg/d†	73 (54.9)	147 (45.6)	0.08
SDD use‡	91 (68.4)	225 (69.9)	0.82
Outcome			
Length of ICU stay, d, median (IQR)	24 (15–35)	7 (4–10)	<0.0001
Length of hospital stay, d, median (IQR)	35 (22–65)	20 (10–43)	<0.0001
Mortality, n (%)			
ICU§	54 (39.4)	71 (21.6)	<0.001
Hospital‖	70 (53.8)	100 (33.0)	<0.001
30 d‖	44 (33.8)	86 (28.4)	0.30
60 d‖	64 (49.2)	102 (33.7)	<0.01
90 d‖	70 (53.8)	107 (35.3)	<0.001
1 yr‖¶	80 (61.5)	148 (48.8)	<0.01

Definition of abbreviations: APACHE IV = Acute Physiology and Chronic Health Evaluation IV; ICU = intensive care unit; IQR = interquartile range; SDD = selective decontamination of the digestive tract; SOFA = Sequential Organ Failure Assessment.

*Other infections include lung abscess, sinusitis, pharyngitis, tracheobronchitis, endocarditis, mediastinitis, myocarditis, postoperative wound infection, bone and joint infection, oral infection, eye infection, and viral infections.

†Use of hydrocortisone or its equivalent (hydrocortisone dose = 4 × prednisolone dose, 5 × methylprednisolone dose, 25 × dexamethasone dose).

‡Patients not on SDD received selective oropharyngeal decontamination.

§ICU mortality was calculated using all ICU admissions for sepsis.

‖Follow-up data were calculated using the first ICU admission for sepsis for each patient during the study period; readmissions were not included in this analysis.

¶Twelve patients were lost to 1-year follow-up (4.6% in patients with sepsis developing an ICU-acquired infection and 2.0% in patients with sepsis with no ICU-acquired infection; $P = 0.19$).

patients with sepsis who went on to develop an ICU-acquired infection demonstrated enhanced cytokine release and stronger endothelial cell and coagulation activation than those who did not develop a secondary infection, (*2*) hyperinflammation was sustained up to the day of occurrence of the secondary infection, and (*3*) the hyperinflammatory host response detected at the time of an ICU-acquired infection was not different from that measured in patients with a noninfectious ICU-acquired complication (i.e., AKI or ARDS).

Our current data do not contradict previous investigations reporting immune suppression in patients with sepsis (3, 6, 9–11, 13, 22–27). These studies focused on mononuclear cells, particularly their responsiveness to bacterial products, antigen presentation capacity, and features of apoptosis, and in some an association was demonstrated between the extent of immune suppression in patients with sepsis and the subsequent development of a secondary infection (23–27). Our measurements reveal proinflammatory responses generated at least in part by host mediator systems not captured in the studies on immune suppression cited previously (3, 6, 9–11, 13, 22–27), especially with regard to activation of the endothelium. In line with our finding, one earlier study, performed in 98 patients with septic shock, showed elevated plasma midregional-proadrenomedulin levels in those who developed a secondary infection (28).

We argue that patients with sepsis who develop secondary infections while in the ICU demonstrate *concurrent* immune suppression and hyperinflammation, and both to a larger extent than patients with sepsis who do not develop an ICU-acquired infection. This overall more disturbed host response is in accordance with our previous finding that patients with sepsis who develop a secondary infection are more severely ill than those who do not (12), which we confirmed in the present subgroup analysis. Likewise, earlier studies in trauma patients have reported a strong association between injury severity and an increased susceptibility to nosocomial infection (29–31). Biomarker concentrations showed a large overlap between patient groups, which precludes firm conclusions on the clinical relevance of the differences detected, yet confirms the heterogeneity of the sepsis population and the accompanying host response. The relative hyperinflammation detected in patients with sepsis who went on to develop a secondary infection partially remained detectable after correction for disease severity.

Table 4. Plasma Biomarkers in Patients Admitted with Sepsis Who Did and Did Not Develop an ICU-acquired Infection Propensity Matched for APACHE IV Score, SOFA Score, Source of Infection, and Shock on ICU Admission

	Admission		Day 2		Day 4		
	ICU-acquired Infection (*n* = 133)	No ICU-acquired Infection (*n* = 322)	ICU-acquired Infection (*n* = 127)	No ICU-acquired Infection (*n* = 308)	ICU-acquired Infection (*n* = 126)	No ICU-acquired Infection (*n* = 234)	Overall *P* Value
Inflammation							
IL-6, pg/ml	337.25 (63.8–1940.83)	146.77 (41.78–770.55)	113.38 (35.2–485.08)*	69.82 (22.38–233.34)	68.92 (25.02–200.65)†	33.39 (15.24–92.77)	<0.0001
IL-8, pg/ml	203.8 (71.89–1003.74)	133.17 (62.27–401.55)	149.92 (55.7–542.58)†	79.10 (33.34–190.68)	119.61 (53.99–276.66)†	62.80 (23.45–131.59)	<0.0001
IL-10, pg/ml	19.64 (5.98–96.05)	11.80 (4.46–31.33)	13.65 (4.1–46.19)†	6.56 (2.46–15.43)	10.40 (4.34–21.04)†	3.78 (2.29–8.97)	<0.0001
MMP-8, ng/ml	2.93 (1.22–10.45)	3.07 (0.96–9.62)	3.12 (0.93–11.26)	2.23 (0.7–6.39)	2.16 (0.95–5.74)*	1.32 (0.45–3.55)	0.73
Endothelial cell activation							
sE-selectin, ng/ml	8.95 (4.76–23.12)	9.33 (4.14–23.62)	8.75 (4.66–18.74)	9.25 (4.58–19.45)	6.49 (4.05–17.88)	7.83 (4.18–15.47)	>0.99
sICAM-1, ng/ml	221.00 (128.12–333.49)	188.46 (106.75–337.02)	249.84 (157.06–400.87)	218.94 (136.4–376.7)	264.69 (181.95–379.76)	237.18 (144.76–355.63)	>0.99
Fractalkine, pg/ml	36.81 (19.87–82.73)	26.96 (17.9–58.7)	41.01 (22.57–87.25)†	25.03 (15.88–55.58)	50.51 (24.07–123.79)†	24.07 (16.55–59.12)	0.01
ANG-1, ng/ml	1.92 (0.8–6.85)	1.78 (0.71–5.09)	1.49 (0.67–3.57)	1.36 (0.66–3.72)	1.16 (0.54–3.01)	1.62 (0.62–4.33)	>0.99‡
ANG-2, ng/ml	9.01 (4.73–17.07)	7.41 (3.75–15.23)	11.59 (6.39–21.58)	8.25 (3.73–17.68)	9.54 (5.16–16.15)§	6.03 (3.08–11.03)	0.09
ANG-2:ANG-1	3.96 (1.08–13.29)	3.23 (0.93–16.64)	7.67 (2.21–21.53)	5.44 (1.34–23.97)	7.69 (2.02–24.22)*	3.57 (0.89–12.03)	0.99‡
Coagulation activation							
D dimer, μg/ml	10.12 (3.63–17.8)	9.20 (4.56–17.09)	10.39 (4.44–19)	9.25 (3.99–17.51)	11.47 (5.84–17.63)	9.66 (4.96–16.41)	>0.99‡
PT, s	15.55 (12.78–18.52)	15.15 (12.78–18.4)	15 (12.6–17.2)	14.90 (12.4–17.12)	14.70 (12.5–16.3)	13.70 (11.8–15.38)	>0.99
aPTT, s	42 (32.5–54.5)	38 (31–51)	45.50 (35–52)	40.00 (31.25–50)	40.00 (32–51)	43.00 (29–54)	>0.99
Protein C, ng/ml	119.99 (95.45–163.1)	122.14 (94.46–159.19)	123.95 (88.92–157.85)	125.96 (94.64–163.81)	132.44 (98.17–171.16)	138.23 (98.63–189.29)	>0.99
Antithrombin, ng/ml	807.28 (490.91–1198.3)	757.55 (527.75–1154.03)	702.62 (417.08–1027.7)	739.21 (457.62–1078.2)	849.68 (500.09–1325.9)	895.16 (595.66–1373.3)	>0.99
Platelets, 10^9/L	209 (125–292)	228 (133–318)	147 (76–259)	179 (103–287)	140 (55–268)*	198 (103–312)	>0.99

Definition of abbreviations: ANG = angiopoietin; APACHE IV = Acute Physiology and Chronic Health Evaluation IV; aPTT = activated partial thromboplastin time; ICAM = intercellular adhesion molecule; ICU = intensive care unit; MMP = matrix metalloproteinase; PT = prothrombin time; sE-selectin = soluble E-selectin; sICAM-1 = soluble ICAM-1; SOFA = Sequential Organ Failure Assessment.
Patients who did and did not develop an ICU-acquired infection were matched for APACHE-IV score, SOFA score, source of infection, and shock (all on ICU admission). Data are presented as median (interquartile range). Overall *P* values represent the fixed-effect model in which group + time was used in the random effects model.
*$P < 0.05$, multiple-comparison adjusted (Benjamini-Hochberg).
†$P < 0.001$, multiple-comparison adjusted (Benjamini-Hochberg).
‡For biomarkers (i.e., ANG-1, ANG-1:ANG-2, and D dimer) in which no fixed-effect model could be fitted, the model with merely random intercept of time was used.
§$P < 0.01$, multiple-comparison adjusted (Benjamini-Hochberg).

In accordance, in a recent investigation in patients with trauma matched for injury characteristics and severity, multiple proinflammatory mediators were elevated within the first 24 hours after trauma in patients who subsequently developed a nosocomial infection (31).

Whole-genome expression profiles of blood leukocytes harvested from patients with sepsis (12) and trauma (32) showed sustained and concurrent activation of

Table 5. Baseline Characteristics and Outcome of Patients Developing ICU-acquired Infection, Acute Kidney Injury, and Acute Respiratory Distress Syndrome

	ICU-acquired Infection	Acute Kidney Injury	Acute Respiratory Distress Syndrome	*P* Value
Patients	102	70	34	
Demographics				
Age, yr, mean (SD)	59.5 (14.0)	62.6 (13.3)	59.6 (14.1)	0.30
Sex male, n (%)	63 (61.8)	44 (62.9)	23 (67.6)	0.85
Chronic comorbidity				
Any comorbidity, n (%)	82 (80.4)	55 (78.6)	27 (79.4)	0.98
Charlson comorbidity index, median (IQR)	4 (3–5)	4 (3–6)	5 (3–7)	0.19
Admissions	104	71	34	
Time of event, d, median (IQR)	10 (7–15)	4 (2–7)	3 (2–4)	<0.0001
Severity of disease during event				
SOFA score, median (IQR)	7 (5–10)	7 (5–9)	7 (5–9)	0.45
Shock, n (%)	26 (25.0)	25 (35.2)	8 (23.5)	0.28
Outcome				
Length of ICU stay, d, median (IQR)	24 (14–33)	11 (7–19)	11 (6–16)	<0.0001
Length of hospital stay, d, median (IQR)	35 (21–64)	20 (11–50)	22 (12–41)	<0.001
Mortality, n (%)				
ICU*	43 (41.3)	23 (32.4)	8 (23.5)	0.15
Hospital†	58 (56.9)	36 (51.4)	12 (35.3)	0.08
30 d†	35 (34.3)	29 (41.4)	10 (29.4)	0.48
60 d†	51 (50.0)	33 (47.1)	12 (35.3)	0.36
90 d†	59 (57.8)	36 (51.4)	12 (35.3)	0.05
1 yr†‡	66 (64.7)	42 (60.0)	16 (47.1)	0.13

Definition of abbreviations: ICU = intensive care unit; IQR = interquartile range; SOFA = Sequential Organ Failure Assessment.
*ICU mortality was calculated using all ICU admissions for sepsis.
†Follow-up data were calculated using the first ICU admission for sepsis for each patient during the study period; readmissions were not included in this analysis.
‡Five patients were lost to 1-year follow-up (3.9% in patients with sepsis developing an ICU-acquired infection and 2.9% in patients with sepsis developing an ICU-acquired acute respiratory distress syndrome).

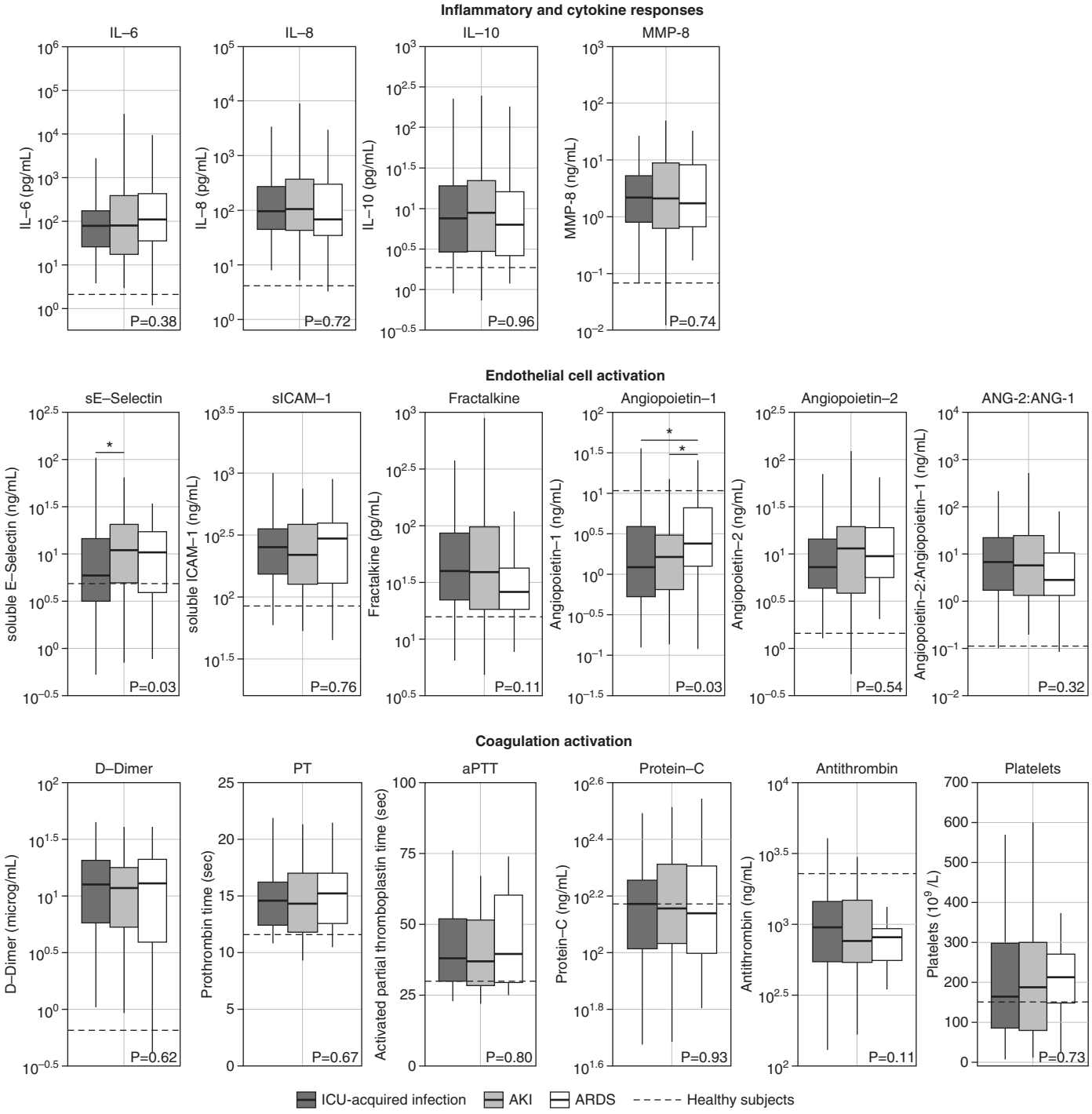

Figure 5. Biomarker distribution in patients with sepsis <24 hours after developing an infectious or noninfectious ICU-acquired complication (AKI/ARDS). Data are expressed as *box-and-whisker* diagrams depicting the median and lower quartile, upper quartile, and their respective 1.5 interquartile range as *whiskers* (as specified by Tukey; *see* online supplement). *Dashed lines* indicate median values obtained in 27 healthy age-matched subjects. Overall *P* values depicted in the figure represent differences between groups determined by Kruskal-Wallis test; specific *P* values are calculated using a Dunn test of multiple comparisons using rank sums. *$P \leq 0.05$. Multiple-comparison–adjusted (Benjamini-Hochberg) *P* value for all >0.99 except for ANG-1, $P = 0.634$. AKI = acute kidney injury; ANG = angiopoietin; aPTT = activated partial thromboplastin time; ARDS = acute respiratory distress syndrome; ICAM = intercellular adhesion molecule; ICU = intensive care unit; MMP = matrix metalloproteinase; PT = prothrombin time.

Table 6. Host Response Biomarkers at Day 4 after Admission and at the Time of an ICU-acquired Infection

	Day 4 (*n* = 84)	Time of ICU-acquired Infection (*n* = 84)	*P* Value*
Inflammation			
IL-6, pg/ml	68.92 (24.45–194.38)	57.72 (23.3–133.07)	0.44
IL-8, pg/ml	132.28 (54.99–246.13)	93.71 (41.73–224.73)	0.18
IL-10, pg/ml	10.67 (4.56–26.02)	7.00 (2.89–16.05)	0.07
MMP-8, ng/ml	2.39 (1.04–5.79)	2.17 (0.82–4.72)	0.31
Endothelial cell activation			
sE-selectin, ng/ml	6.29 (3.82–12.95)	5.58 (2.91–12.71)	0.23
sICAM-1, ng/ml	238.05 (168.21–359.23)	241.76 (140.37–344.83)	0.60
Fractalkine, pg/ml	55.07 (25.98–151.59)	38.50 (20.92–80.33)	0.11
ANG-1, ng/ml	1.18 (0.57–3.11)	1.18 (0.51–4.48)	0.77
ANG-2, ng/ml	9.14 (5.54–16.19)	6.85 (4.3–13.73)	0.11
ANG-2:ANG-1	8.15 (2.56–28.06)	8.27 (1.66–21.85)	0.43
Coagulation activation			
D dimer, μg/ml	12.47 (7.11–21.09)	12.09 (5.58–23.2)	0.64
PT, s	14.80 (12.3–16.1)	14.60 (12.55–16)	0.93
aPTT, s	41 (31–47)	38 (30–53)	0.96
Protein C, ng/ml	138.11 (90.38–185.55)	148.67 (111.57–187.56)	0.30
Antithrombin, ng/ml	883.85 (513.08–1358.3)	949.65 (558.14–1415.43)	0.40
Platelets, 10^9/L	111 (49.5–243.5)	168 (93–323)	0.02

Definition of abbreviations: ANG = angiopoietin; aPTT = activated partial thromboplastin time; ICAM = intercellular adhesion molecule; ICU = intensive care unit; MMP = matrix metalloproteinase; PT = prothrombin time; sE-selectin = soluble E-selectin; sICAM-1 = soluble ICAM-1.
Data are presented as median (interquartile range).
*Multiple-comparison–adjusted (Benjamini-Hochberg) *P* value for all >0.99, except for platelets *P* = 0.44.

multiple proinflammatory, antiinflammatory, and immune-suppressive pathways. In the trauma literature these findings have led to the concept of the so-called persistent inflammation, immunosuppression and catabolism syndrome (33). The present results indicate that sepsis can also lead to persistent inflammation, immunosuppression, and catabolism syndrome, further suggesting that the host response to sepsis and severe noninfectious injury is not fundamentally different (34). As such, we argue that patients who remain critically ill for prolonged periods of time enter a state of sustained hyperinflammation and immune suppression irrespective of the inciting event (sepsis or noninfectious injury), which together with invasive procedures and devices, such as mechanical ventilation and intravenous catheters (12, 35), render patients more susceptible to ICU-acquired complications. Although this observational study does not prove a causal link between enhanced inflammatory responses during the first 4 days of ICU stay and subsequent development of ICU-acquired infections, we consider hyperinflammation and disturbed barrier integrity part of a syndrome that has been named a "failure of homeostasis" (34), resulting in dysfunction of immune and other cells, at least in part caused by mitochondrial damage and impaired cellular oxygen use, which together with a lengthy requirement of invasive care, are main drivers of the occurrence of both noninfectious and infectious complications on the ICU.

Of note, the previously reported whole-blood leukocyte genome transcriptome changes were not different between patients who did and those who did not develop an ICU-acquired infection in this cohort (12), which at least in part can be explained by the fact that gene expression analyses of blood leukocytes only provide insight in immune pathways regulated at mRNA level in circulating cells, whereas the protein biomarkers reported here mostly are derived from extravascular cells. Furthermore, although the blood genomic response was measured at a single time point within 24 hours after ICU admission, plasma protein biomarkers were measured at multiple time points.

Biomarker analyses at the time of an ICU-acquired infection versus a noninfectious ICU-acquired complication (AKI or ARDS) showed comparable host response reactions. In paired analyses, no differences were found between Day 4 and the day of the ICU-acquired infection (Table 4) or AKI/ARDS (data not shown). These results indicate that the dysregulation of key host mediator systems as measured here is sustained and not different in patients with either one of these major ICU-acquired complications.

Our study has strengths and limitations. We provide information on a large, well-defined, prospectively collected cohort with extensive information on ICU-acquired complications. Although we measured 20 host response biomarkers reflecting activation of key pathways implicated in sepsis pathogenesis, we did not perform functional and/or flow cytometry measurements that would have provided information on the extent of immune suppression. Hence, we cannot examine potential correlations between hyperinflammatory, procoagulant, and immune-suppressive responses in individual patients. In addition, most measurements were confined to the first 4 days after ICU admission; however, paired analyses of biomarker levels at Day 4 and the day of an ICU-acquired infection did not show differences with the single exception of platelet counts. In this study, statistical methods are used to adjust for differences between groups; however, we cannot exclude the effect of unmeasured covariables residually confounding our outcome. In addition, in some cases persistent infections may be difficult to distinguish from new-onset infections, especially in abdominal sepsis. We can therefore not exclude the possibility that occasionally an ongoing infection was deemed ICU acquired.

Conclusions

Patients with sepsis developing secondary infections during ICU stay showed a more dysregulated proinflammatory and vascular host response in the first 4 days of ICU admission than patients with sepsis who did not develop an ICU-acquired infection. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors acknowledge all members of the MARS consortium for the

participation in data collection and especially acknowledge Friso M. de Beer, M.D., Lieuwe D. J. Bos, M.D., Ph.D., Gerie J. Glas, M.D., and Roosmarijn T. M. van Hooijdonk, M.D., Ph.D. (Department of Intensive Care, Academic Medical Center, University of Amsterdam); Michaëla A. M. Huson, M.D., Ph.D. (Center for Experimental and Molecular Medicine, Academic Medical Center, University of Amsterdam); David S. Y. Ong, M.D., Ph.D. (Department of Intensive Care Medicine and Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands); and Laura R. A. Schouten, M.D., Marleen Straat, M.D., Esther Witteveen, M.D., and Luuk Wieske, M.D., Ph.D. (Department of Intensive Care, Academic Medical Center, University of Amsterdam).

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