Original Investigation | CARING FOR THE CRITICALLY ILL PATIENT

Incidence, Risk Factors, and Attributable Mortality of Secondary Infections in the Intensive Care Unit After Admission for Sepsis

Lonneke A. van Vught, MD; Peter M. C. Klein Klouwenberg, MD, PharmD, PhD; Cristian Spitoni, PhD; Brendon P. Scicluna, PhD; Maryse A. Wiewel, MD; Janneke Horn, MD, PhD; Marcus J. Schultz, MD, PhD; Peter Nürnberg, PhD; Marc J. M. Bonten, MD, PhD; Olaf L. Cremer, MD, PhD; Tom van der Poll, MD, PhD; for the MARS Consortium

IMPORTANCE Sepsis is considered to induce immune suppression, leading to increased susceptibility to secondary infections with associated late mortality.

OBJECTIVE To determine the clinical and host genomic characteristics, incidence, and attributable mortality of intensive care unit (ICU)–acquired infections in patients admitted to the ICU with or without sepsis.

DESIGN, SETTING, AND PARTICIPANTS Prospective observational study comprising consecutive admissions of more than 48 hours in 2 ICUs in the Netherlands from January 2011 to July 2013 stratified according to admission diagnosis (sepsis or noninfectious).

MAIN OUTCOMES AND MEASURES The primary outcome was ICU-acquired infection (onset >48 hours). Attributable mortality risk (fraction of mortality that can be prevented by elimination of the risk factor, acquired infection) was determined using time-to-event models accounting for competing risk. In a subset of sepsis admissions (n = 461), blood gene expression (whole-genome transcriptome in leukocytes) was analyzed at baseline and at onset of ICU-acquired infectious (n = 19) and noninfectious (n = 9) events.

RESULTS The primary cohort included 1719 sepsis admissions (1504 patients; median age, 62 years; interquartile range [IQR], 51-71 years]; 924 men [61.4%]). A comparative cohort included 1921 admissions (1825 patients, median age, 62 years; IQR, 49-71 years; 1128 men [61.8%] in whom infection was not present in the first 48 hours. Intensive care unit–acquired infections occurred in 13.5% of sepsis ICU admissions (n = 232) and 15.1% of nonsepsis ICU admissions (n = 291). Patients with sepsis who developed an ICU-acquired infection had higher disease severity scores on admission than patients with sepsis who did not develop an ICU-acquired infection (Acute Physiology and Chronic Health Evaluation IV [APACHE IV] median score, 90 [IQR, 72-107] vs 79 [IQR, 62-98]; $P < .001$) and throughout their ICU stay but did not have differences in baseline gene expression. The population attributable mortality fraction of ICU-acquired infections in patients with sepsis was 10.9% (95% CI, 0.9%-20.6%) by day 60; the estimated difference between mortality in all patients with a sepsis admission diagnosis and mortality in those without ICU-acquired infection was 2.0% (95% CI, 0.2%-3.8%; $P = .03$) by day 60. Among nonsepsis ICU admissions, ICU-acquired infections had a population attributable mortality fraction of 21.1% (95% CI, 0.6%-41.7%) by day 60. Compared with baseline, blood gene expression at the onset of ICU-acquired infections showed reduced expression of genes involved in gluconeogenesis and glycolysis.

CONCLUSIONS AND RELEVANCE Intensive care unit–acquired infections occurred more commonly in patients with sepsis with higher disease severity, but such infections contributed only modestly to overall mortality. The genomic response of patients with sepsis was consistent with immune suppression at the onset of secondary infection.

JAMA. 2016;315(14):1469-1479. doi:10.1001/jama.2016.2691
Published online March 15, 2016.

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Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: The MARS Consortium members are listed at the end of this article.

Corresponding Author: Lonneke Alette van Vught, MD, Center for Experimental and Molecular Medicine, Academic Medical Center, Meibergdreef 9, G2-130. 1105 AZ Amsterdam, the Netherlands (l.a.vanvught@amc.uva.nl).

Section Editor: Derek C. Angus, MD, MPH, Associate Editor, *JAMA* (angusdc@upmc.edu).

Sepsis is the leading cause of morbidity and mortality in hospitalized patients.[1,2] Patients with sepsis may enter a state of immune suppression due to hyporesponsiveness, exhaustion, and apoptotic depletion of immune cells and an increase in T regulatory and myeloid-derived suppressor cells.[3-5] It has been suggested that the immune suppression accompanying sepsis contributes to late sepsis mortality in the intensive care unit (ICU) caused by an increased occurrence of secondary infections.[4-6] In light of these findings, immune stimulation has been advocated as a novel treatment strategy for sepsis,[4-6] representing an approach opposite from the use of anti-inflammatory agents in many unsuccessful sepsis trials.[7,8] For the design of clinical trials with immunostimulatory therapies, knowledge of the incidence of ICU-acquired infections is important, but equally important are estimates of their attributable mortality.

The primary objective of this study was to determine the incidence, risk factors, and attributable mortality of ICU-acquired infections in patients admitted with sepsis. Additionally, in exploratory analyses, we sought to determine differences in the host response to the inciting sepsis event between patients who did and those who did not develop an ICU-acquired infection by analyses of the whole-genome transcriptome in blood leukocytes. We also assessed the incidence and attributable mortality of ICU-acquired infections in critically ill patients admitted for noninfectious disease during the same study period.

Methods

Study Design and Population

This study was part of the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) project, a prospective observational study in the mixed ICUs of 2 tertiary teaching hospitals in the Netherlands between January 2011 and January 2014 (ClinicalTrials.gov identifier NCT01905033).[9,10] For the current analysis, all consecutive patients admitted from January 2011 to July 2013 with an ICU length of stay of more than 48 hours were selected (ie, all patients at risk of an ICU-acquired infection). Patients with infection onset between 24 and 48 hours after ICU admission were excluded, for it could not be determined with certainty whether this infection was the primary reason for ICU admission or ICU-acquired. Race was determined by the researchers based on fixed categories (white, black, Asian) for future (epi)genetics studies. Patients were included via an opt-out consent method approved by the institutional review boards of both hospitals (IRB No. 10-056C); participants were informed about the study by a brochure provided at ICU admission with an opt-out card that could be completed by the patient or legal representative in case of unwillingness to participate.[9,10] During part of the current study, both ICUs were included in a cluster randomized crossover trial on the effects of selective decontamination of the digestive tract (SDD; given during 70.0% of the study period) and selective oropharyngeal decontamination (SOD; given during 30.0% of the study) (see the eMethods section in the Supplement and Oostdijk et al[11]).

Definitions

The likelihood of infection for which the clinical team initiated antibiotics was classified for each infectious source as none, possible, probable, or definite by research physicians using Centers for Disease Control and Prevention[12] and International Sepsis Forum consensus definitions,[13] as described.[9] Sepsis was defined as suspected infection (with likelihood possible, probable, or definite) diagnosed within 24 hours after ICU admission accompanied by at least 1 additional parameter described in the 2001 International Sepsis Definitions Conference (eTable 1 in the Supplement).[14] In patients with a noninfectious admission diagnosis (including those admitted with suspected infection but with a post hoc infection likelihood of none) enrolled during the same period, the Acute Physiology and Chronic Health Evaluation IV (APACHE IV) primary admission diagnosis was used to classify patients into broad diagnostic categories.[15]

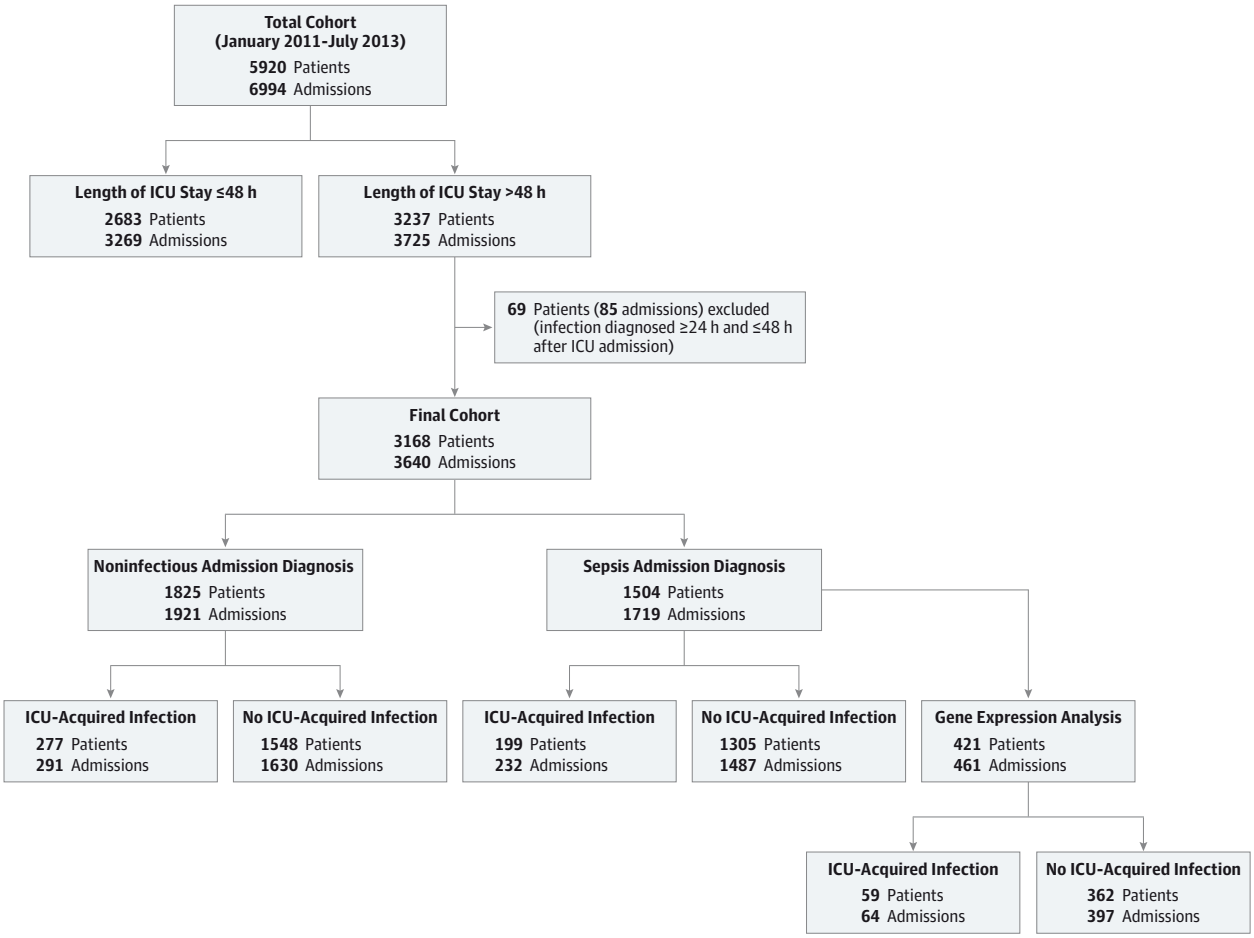
The primary outcome was the first occurrence of an ICU-acquired infection, defined as any new-onset infection (with likelihood possible, probable, or definite)[9,12,13] starting more than 48 hours after ICU admission for which the clinical team started a new antibiotic regimen. The most likely causative microorganisms were classified based on all microbiology results.

Organ failures were defined as a score of 3 or higher on the Sequential Organ Failure Assessment (SOFA) score, except for cardiovascular failure for which a score of 1 or higher was used.[16] The SOFA score for the central nervous system subgroup was not included. Severe sepsis was defined as sepsis plus failure of 1 or more organs. Shock was defined by the use of noradrenaline for hypotension in a dose of more than 0.1 μg/kg/min during at least 50% of the ICU day. Comorbidity was defined as described in the eMethods section of the Supplement.

Microarray Analysis and Bioinformatics

Whole blood was collected in PAXgene tubes (Becton-Dickinson) within 24 hours after the ICU admissions of 421 patients with sepsis (461 admissions) (**Figure 1**), defined as a definite or probable infection[9] accompanied by at least 1 additional parameter described in the 2001 International Sepsis Definitions Conference (eTable 1 in the Supplement),[14] enrolled between January 2011 and July 2012. Paired comparisons of PAXgene blood samples collected at ICU admission and at the onset of an ICU-acquired complication were performed in both ICU-acquired infection (n = 19) and no ICU-acquired infection (n = 9) patient groups; the latter group comprised patients who developed ICU-acquired acute lung injury (n = 2), acute kidney injury (n = 6), and acute myocardial infarction (n = 1). PAXgene blood samples were also obtained from 42 healthy controls. RNA was hybridized to the Human Genome U219 96-array plate. After preprocessing, 24 646 expressed probes were recovered that were assessed for differential abundance by means of the limma method.[17-19] Throughout, Benjamini-Hochberg (BH)[20] multiple comparison-adjusted probabilities (false-discovery rate <5%) defined significance. Ingenuity Pathway Analysis (Ingenuity Systems IPA, http://www.ingenuity.com) was used to evaluate the association with canonical signaling pathways. Association significance was measured by Fisher exact test BH-adjusted *P* values.

Figure 1. Flowchart of Included Patients



When a patient was readmitted for the alternative admission diagnosis within the study time frame (eg, first admission for sepsis, second admission for a noninfectious condition), this readmission was seen as the first admission for that specific admission diagnosis, explaining the discrepancy in the patient numbers of the final cohort and the following boxes downstream from the final cohort. ICU indicates intensive care unit.

Statistical Analysis

Baseline characteristics age, sex, race, and comorbidities were measured for each first admission. Survival status was calculated from the first ICU admission with the same admission reason (ie, sepsis or noninfectious), except for ICU mortality, for which all ICU admissions were used. For all other parameters as well as for the competing risk and population attributable mortality fraction analyses, readmissions for the same admission reason were included as unique admissions.

Continuous nonparametric data were analyzed using a Mann-Whitney *U* or Kruskal-Wallis test; categorical data were analyzed using a 2-tailed χ^2 or Fisher exact test. All continuous parametric data were analyzed using a *t* test or analysis of variance. All data were analyzed using R studio built under R version 3.0.2 (R Core Team 2013).[21] For calculation of the population attributable mortality fraction, the R-package "mstate" was used. For the analysis of SOFA scores during ICU stay, a mixed-effects model was executed. $P < .05$ (2-sided) was considered statistically significant.

To identify risk factors for the development of an ICU-acquired infection, we used a multivariable competing risk survival model.[22] In patients with multiple ICU-acquired infections, only the first ICU-acquired infection was used. A multivariable competing risk survival analysis provides 2 measures of association: the cause-specific hazard ratio (HR), which estimates the direct effect of the exposure of interest (ie, severity of disease) on the various outcomes (ie, ICU discharge, ICU mortality, and the development of an ICU-acquired infection), and the subdistribution HR, which describes the risk of the development of an ICU-acquired infection while accounting for the competing events. Admission variables included in this model are listed in the eMethods of the Supplement.

A multistate approach was used to estimate the population attributable mortality fraction.[23,24] The population attributable mortality fraction represents the fraction of mortality that can be prevented by eliminating the exposure (ie, ICU-acquired infection). A progressive disability model was fitted to account for the time dependency of the risk factor,

the presence of competing risks (ie, discharge or mortality without an ICU-acquired infection) at each time point, and the heterogeneity of the study population. The multistate model used is derived from a Markov model.[25] Quartiles of APACHE IV scores and of age per specific patient population (ie, sepsis and noninfectious) were used as covariables. In this model, an estimator for the population attributable mortality fraction can be derived in terms of the transition probabilities. The transition probabilities of a time-inhomogeneous Markov multistage model were estimated with the Aalen-Johansen estimator. The arrival time in a state (the past) was tested for significance for a given transition (the future) by including it as a covariate in a Cox model for the transition hazards. Because the test was not significant, the Markov assumption was reasonable for our data set. For this multivariable analysis only the first ICU-acquired infection was used. The population attributable mortality fraction was expressed as the percentage of ICU mortality caused by the ICU-acquired infections.

Sensitivity analyses were conducted including only first admissions for sepsis and including only patients with sepsis with 1 ICU admission.

Results

ICU-Acquired Infections in Patients With a Sepsis Admission Diagnosis

We studied 5920 patients during 6994 ICU episodes, of which 3725 admissions (53.3%) had a duration of more than 48 hours. We excluded 85 admissions (2.3%) because infection was diagnosed between 24 and 48 hours after ICU admission. Almost half of the remaining 3640 admissions involved patients admitted with sepsis (n = 1719, 47.2%) (Figure 1). **Table 1** shows baseline characteristics of patients with a sepsis admission diagnosis stratified according to development (or not) of ICU-acquired infection.

Of all admissions for sepsis, 232 (13.5%) were complicated by a total of 334 ICU-acquired infections (Table 1 and **Table 2**). The median interval from admission for sepsis to the first ICU-acquired infection was 9 days (interquartile range [IQR], 6-13 days) (Table 2). The incidence of ICU-acquired infections per week was stable after the first week of ICU stay (eFigure 1 in the Supplement). The most common ICU-acquired infections were catheter-related bloodstream infections (n = 88, 26.3%), pneumonia (n = 85, 25.4%), and abdominal infections (n = 53, 15.9%) (Table 2). The most common causative pathogens were gram-positive bacteria (n = 151, 45.2%), followed by gram-negative bacteria (n = 89, 26.6%), fungi (n = 32, 9.6%), and viruses (n = 33, 9.9%) (Table 2; eTable 2, eFigure 2 in the Supplement).

Risk Factors for ICU-Acquired Infections in Patients With a Sepsis Admission Diagnosis

Patients with sepsis who did or did not develop an ICU-acquired infection were not different with regard to age, sex, race, and Charlson comorbidity index (Table 1). Use of SDD and SOD did not differ between groups. Patients with sepsis with primary bacteremia on admission more often developed ICU-acquired infections (12 patients [5.2%] vs 19 patients [1.3%], respectively, $P = .001$; difference, 3.9%; 95% CI, 1.6%-7.6%); all other admission diagnoses were equally distributed between patients who did and those who did not develop an ICU-acquired infection (eTable 3 in the Supplement).

Patients with sepsis who developed an ICU-acquired infection were more severely ill on admission than those who did not, as indicated by higher APACHE IV score (90 [IQR, 72-107] vs 79 [IQR, 62-98], respectively, $P < .001$) and SOFA scores (8 [IQR, 6-11] vs 7 [IQR, 4-9], respectively, $P < .001$) and had more shock (104 patients [44.8%] vs 479 patients [32.2%]; $P < .001$; difference, 12.6%; 95% CI, 5.9%-19.5%) (Table 1). Multivariable competing risks survival analysis supported the association between prior disease severity and development of an ICU-acquired infection. Using the lowest APACHE IV quartile as the reference, the fourth APACHE IV quartile was independently associated with an increased risk of ICU-acquired infection (subdistribution HR, 1.86; 95% CI, 1.18-2.92) (**Table 3**; eTable 4 in the Supplement). Moreover, patients with sepsis who developed an ICU-acquired infection had higher SOFA scores up to 2 days before the event than did those who did not ($P \le .001$) (eFigure 3 in the Supplement). Other independent risk factors for ICU-acquired infection were respiratory insufficiency as comorbid condition (subdistribution HR, 1.44; 95% CI, 1.05-2.99), the use of a central venous catheter (subdistribution HR, 2.63; 95% CI, 1.53-4.53), and mechanical ventilation (subdistribution HR, 6.22; 95% CI, 1.54-25.17) (Table 3). In a sensitivity analysis including only first admissions (n = 1504), the third and fourth APACHE IV quartile were independently associated with an increased risk of ICU-acquired infection with a subdistribution HR of 1.70 (95% CI, 1.05-2.75) and 2.05 (95% CI, 1.26-3.34), respectively (eTable 5A in the Supplement). The same was true for a sensitivity analysis in which only patients with 1 admission (n = 1333) were included with subdistribution HRs of 1.86 (95% CI, 1.12-3.09) and 2.20 (95% CI, 1.31-3.69), respectively (eTable 5B in the Supplement).

Outcome and Population Attributable Mortality Fraction of ICU-Acquired Infections in Patients With a Sepsis Admission Diagnosis

Relative to patients with sepsis who did not develop an infection while in the ICU, those who did had a longer ICU length of stay (22 days [IQR, 15-33] vs 5 days [IQR, 3-9], $P < .001$)), more complications and a higher mortality (day 60, 88 patients [44.2%] vs 381 patients [29.1%], $P < .001$; difference, 15.0%; 95% CI, 7.8%-22.4%) (Table 1).

The population attributable mortality fraction caused by ICU-acquired infection gradually increased over time (**Figure 2**). The population attributable mortality fraction was 5.5% (95% CI, −0.3% to 11.3%; $P = .06$) by day 30 and 10.9% (95% CI, 0.9%-20.9%; $P = .03$) by day 60; the difference between mortality in the entire cohort and mortality in the subgroup of patients without ICU-acquired infection was 0.9% (95% CI, −0.1% to 1.9%; $P = .08$) by day 30 and 2.0% (95% CI, 0.2% to 3.8%; $P = .03$) by day 60.

Sensitivity and Subgroup Analyses in Patients With a Sepsis Admission Diagnosis

Sensitivity and subgroup analyses are shown in eTable 6 in the Supplement. When the analysis was restricted to only patients

Table 1. Baseline Characteristics and Outcome of Patients Admitted With Sepsis Stratified According to Development of ICU-Acquired Infection or Not[a]

	ICU-Acquired Infection, No. (%) (n=199)	No ICU-Acquired Infection, No. (%) (n=1305)	*P* Value
Demographics			
Age, mean (SD), y	58.9 (14.9)	60.1 (15.4)	.31
Men	128 (64.3)	796 (61.0)	.38
White race	178 (89.4)	1161 (89.0)	.93
Chronic comorbidity			
None	52 (26.1)	356 (27.3)	.79
Immunocompromised state	65 (28.0)	311 (20.9)	.02
Cardiovascular insufficiency	58 (25.0)	370 (24.9)	>.99
Malignancy	46 (19.8)	322 (21.7)	.55
Renal insufficiency	38 (16.4)	186 (12.5)	.11
Respiratory insufficiency	53 (22.8)	269 (18.1)	.09
Charlson comorbidity index, median (IQR)	4 (2-5)	4 (2-6)	.16
Admissions			
Total	232 (13.5)	1487 (86.5)	
Medical	179 (77.2)	1147 (77.1)	.71
Surgical	53 (22.8)	340 (22.9)	
Readmission	33 (14.2)	182 (12.2)	.45
Severity of disease			
APACHE IV score			
Overall, median (IQR)	90 (72-107)	79 (62-98)	<.001
Quartile			
1 (<64)	38 (16.4)	413 (27.8)	<.001
2 (64-80)	49 (21.1)	376 (25.3)	
3 (81-99)	63 (27.2)	361 (24.3)	
4 (100-205)	82 (35.3)	337 (22.7)	
SOFA score, median (IQR)	8 (6-11)	7 (4-9)	<.001
Severe sepsis	218 (94.0)	1312 (88.2)	.10
Septic shock	104 (44.8)	479 (32.2)	<.001
No. of organs failing			
None	35 (15.1)	378 (25.4)	<.001
1	86 (37.1)	600 (40.3)	
2	69 (29.7)	305 (20.5)	
3	32 (13.8)	116 (7.8)	
4	6 (2.6)	22 (1.5)	
5	0	1 (0.1)	
Unknown	4 (1.7)	65 (4.4)	
Treatment Interventions Before ICU-Acquired Infectious Event			
Urinary catheter	226 (97.4)	1405 (94.5)	.08
Central venous catheter	217 (93.5)	1180 (79.4)	<.001
Surgical drain	30 (12.9)	154 (10.4)	.24
Mechanical ventilation	230 (99.1)	1368 (92.0)	<.001
Renal replacement therapy	73 (31.5)	260 (17.5)	<.001
Corticosteroid use			
Any hydrocortisone use[b]	164 (70.7)	828 (55.7)	.001
Hydrocortisone >200 mg[c]	138 (59.5)	631 (42.4)	<.001
SDD use[c]	162 (69.8)	1007 (67.7)	.55

(continued)

Table 1. Baseline Characteristics and Outcome of Patients Admitted With Sepsis Stratified According to Development of ICU-Acquired Infection or Not (continued)[a]

	ICU-Acquired Infection, No. (%) (n=199)	No ICU-Acquired Infection, No. (%) (n=1305)	*P* Value
Outcome			
Length of stay, median (IQR), d			
ICU	22 (15-33)	5 (3-9)	<.001
Hospital	46 (28-76)	26 (13-49)	<.001
Complications			
None	98 (42.2)	1241 (83.5)	<.001
Acute kidney injury	58 (25.0)	129 (8.7)	<.001
Acute lung injury	29 (12.5)	61 (4.1)	<.001
Discharge location			
Clinical ward	143 (61.6)	1195 (80.4)	.001
Deceased	83 (35.8)	231 (15.5)	
Home	0	6 (0.4)	
Other/unknown	6 (2.6)	55 (3.7)	
Mortality			
ICU[d]	83 (35.8)	231 (15.5)	<.001
Hospital[e]	98 (49.2)	351 (26.9)	<.001
30 d[e]	63 (31.7)	320 (24.5)	.04
60 d[e]	88 (44.2)	381 (29.2)	<.001
90 d[e]	99 (49.7)	420 (32.2)	<.001
1 y[f]	109 (54.8)	544 (41.7)	<.001

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

[a] The Charlson comorbidity index consists of points for multiple preexisting comorbid diseases combined with points for age.[34] An increase in Charlson comorbidity index represents more (or more severe) preexisting comorbid diseases, increased age, or both. The APACHE IV score is calculated using preexisting comorbidities, acute diagnosis, and acute physiology variables.[15] Increases in the APACHE IV score are associated with increased risk of mortality. SOFA scores can range from 0 to 20 and consist of a 0- to 4-point scale for SOFA circulation, coagulation, liver, renal, and respiration. An increase in SOFA score represents more severe organ failure.

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

[c] Patients not using SDD received selective oropharyngeal decontamination.

[d] Intensive care unit mortality was calculated using all ICU admissions for sepsis.

[e] 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.

[f] Forty patients were lost to 1-year follow-up (4.5% in patients with sepsis developing an ICU-acquired infection and 2.4% in patients with sepsis with no ICU-acquired infection).

with severe sepsis on admission or only patients with septic shock on admission, the incidence of ICU-acquired infections was 14.2% (n = 218) and 17.8% (n = 104), respectively. The population attributable mortality fractions were 10.1% (95% CI, −1.0% to 21.1%; P = .07) and 7.7% (95% CI, −11.6% to 27.1%; P = .44) by day 60, respectively. When patients with only definite and probable infection likelihoods at admission were included in the analysis, the incidence of ICU-acquired infections was 14.4% (n = 178) with a population attributable

Table 2. Characteristics of ICU-Acquired Infections After Admission for Sepsis

Timing of Infections	
Admissions associated with an ICU-acquired infection, No. (%)	232 (13.5)
Total ICU-acquired infections, No.	334
Admissions associated with multiple ICU-acquired infections, No. (%)	71 (30.6)
Day of first ICU-acquired infection, median (IQR)	9 (6-13)
Source of Infection, No. (%)	
Pulmonary	85 (25.4)
Hospital-acquired pneumonia	27 (8.1)
Ventilator-associated pneumonia	58 (17.4)
Cardiovascular	106 (35.3)
Bacteremia	18 (5.4)
Catheter-related bloodstream infection	88 (26.3)
Abdomen	53 (15.9)
Abdominal infection	51 (15.3)
Gastrointestinal infection	2 (0.6)
Neurological	9 (2.7)
Brain abscess	1 (0.3)
Primary meningitis	2 (0.6)
Secondary meningitis	6 (1.8)
Skin	13 (3.9)
Urinary tract	4 (1.2)
Other[a]	64 (19.2)
Causative Pathogen, No. (%)	
Gram-positive bacteria	151 (45.2)[b]
Gram-negative bacteria	89 (26.6)
Fungi	32 (9.6)
Viral (including reactivation)	33 (9.9)
Other	8 (2.4)
Unknown	82 (24.5)

Abbreviations: ICU, intensive care unit; IQR, interquartile range.

[a] Other infections include lung abscess, sinusitis, pharyngitis, tracheobronchitis, endocarditis, mediastinitis, myocarditis, postoperative wound infection, bone and joint infection, oral infection, eye infection, reproductive tract infection.

[b] Percentages depict the portion of ICU-acquired infections (total N = 334) caused by the pathogen group indicated. In total, 313 pathogens were assigned to 334 ICU-acquired infections. In 49 (14.7%) of all ICU-acquired infections, more than 1 pathogen was assigned as causative.

mortality fraction of 12.7% (95% CI, 0.3%-25.1%; $P < .05$) by day 60. When only first admissions were included (ie, exclusion of all readmissions), the incidence of ICU-acquired infections was 13.2% (n = 199) and the population attributable mortality fraction 9.7% (95% CI, −1.1% to 20.5%; $P = .08$) by day 60. When only patients with a single admission were included (ie, exclusion of all patients with >1 admission), the incidence of ICU-acquired infections was 13.5% (n = 180) and the population attributable mortality fraction, 9.1% (95% CI, −1.9% to 20.1%; $P = .11$) by day 60.

The 3 most common ICU-acquired infections—catheter-related bloodstream infection, ventilator-associated pneumonia, and abdominal infection—were also analyzed separately (eTable 7 in the Supplement). The incidence of these infections was 4.7% (n = 74), 3.5% (n = 54), and 2.9% (n = 44), respectively; their population attributable mortality fraction 4.7% (95% CI, −4.5% to 13.9%; $P = .32$), 4.6% (95% CI, −13.9% to 23.2%, $P = .63$), and 0.3% (95% CI, −2.9% to 3.6%; $P = .86$) respectively at 60 days.

Host Response in Patients With a Sepsis Admission Diagnosis

In exploratory analyses, we determined whether the host response to the inciting sepsis event differed between patients who did and those who did not develop an ICU-acquired infection. To this end, we analyzed the blood leukocyte whole-genome transcriptome in patients with sepsis enrolled during the first 1.5 years of this study with an admission infection likelihood of definite or probable (eTable 8 in the Supplement). Both patients who did and those who did not develop an ICU-acquired infection presented changes in admission gene expression profiles compared with healthy participants; however, there were no differences between the 2 groups (**Figure 3**A). Common overexpressed genes were associated with typical proinflammatory and anti-inflammatory pathways, such as toll-like receptors, interleukin 1 (IL-1), IL-6, IL-8, and IL-10 signaling, as well as with mitochondrial (dys) function; common underexpressed genes associated with energy metabolism, antigen presentation, and T-cell functions (eTable 9 in the Supplement).

In paired analyses comparing global gene expression profiles on ICU admission with gene expression profiles at the time of ICU-acquired infection, ICU-acquired infection was associated with a reduced expression of genes involved in cellular gluconeogenesis and glycolysis (Figure 3C and D; eFigure 4 in the Supplement). In contrast, paired analyses of leukocyte gene expression profiles determined on admission and at the time of a noninfectious ICU-acquired complication did not reveal differences (Figure 3B).

ICU-Acquired Infections in Patients With a Noninfectious Admission Diagnosis

We also analyzed the incidence and population attributable mortality fraction of ICU-acquired infections in patients with a noninfectious admission diagnosis during the same period (1921 admissions, involving 1825 patients; eTable 10 in the Supplement). Of these, 291 admissions (15.1%) were complicated by 366 ICU-acquired infections. The median interval from admission to the first ICU-acquired infection was 4 days (IQR, 3-7 days) (eTable 11 in the Supplement). The incidence of ICU-acquired infections per week is shown in eFigure 5 in the Supplement. Pneumonia was the most common ICU-acquired infection (117 patients [48.4%]; eTable 11 in the Supplement). Gram-positive and gram-negative bacteria were identified as causative pathogen in similar proportions (124 patients [33.9%] and 122 [33.3%], respectively; eTables 11 and 12 and eFigure 6 in the Supplement). Patients who developed an ICU-acquired infection were less often admitted for respiratory failure (21 patients [7.2%] vs 201 [12.3%], $P = .01$; difference, 5.1%; 95% CI, 1.2%-8.1%);

Table 3. Multivariable Competing Risk Analysis for Acquiring an Infection While in the ICU in Patients With a Sepsis Admission Diagnosis[a]

Risk Factors	Cause-Specific Hazard Ratio (95% CI)			ICU-Acquired Infection, Subdistribution Hazard Ratio (95% CI)
	Discharge	Mortality	ICU-Acquired Infection	
Admission				
APACHE IV score by quartile				
1 (<64)	1 [Reference]	1 [Reference]	1 [Reference]	1 [Reference]
2 (64-80)	0.86 (0.74-1.01)	1.19 (0.71-2.00)	0.95 (0.61-1.47)	1.24 (0.80-1.93)
3 (81-99)	0.82 (0.69-0.97)	1.58 (0.95-2.62)	1.16 (0.74-1.82)	1.53 (0.99-2.37)
4 (100-205)	0.69 (0.57-0.83)	2.06 (1.24-3.43)	1.37 (0.87-2.16)	1.86 (1.18-2.92)
Men	0.96 (0.85-1.07)	0.79 (0.61-1.04)	1.25 (0.95-1.65)	1.31 (1.00-1.73)
Age (per 1 y)	1.01 (1.00-1.01)	1.01 (1.00-1.02)	1.00 (0.99-1.00)	0.99 (0.98-0.997)
Surgical admission	1.10 (0.96-1.26)	0.94 (0.67-1.33)	1.15 (0.83-1.59)	1.06 (0.77-1.45)
Organ failure (per No. of organs failing ranging from 0-5)	0.97 (0.90-1.05)	1.46 (1.24-1.71)	1.11 (0.93-1.31)	1.00 (0.86-1.17)
SOD vs SDD	1.12 (0.99-1.27)	1.03 (0.77-1.37)	0.97 (0.73-1.30)	0.89 (0.67-1.19)
Chronic comorbidity				
Immunocompromised state	1.04 (0.89-1.21)	0.98 (0.70-1.36)	1.17 (0.85-1.61)	1.15 (0.83-1.58)
Cardiovascular insufficiency	0.97 (0.85-1.12)	0.83 (0.60-1.14)	0.85 (0.62-1.17)	0.89 (0.64-1.23)
Malignancy	1.04 (0.90-1.20)	1.22 (0.89-1.67)	0.76 (0.54-1.07)	0.70 (0.50-0.99)
Renal insufficiency	1.23 (1.01-1.49)	0.77 (0.50-1.18)	1.29 (0.87-1.92)	1.16 (0.79-1.71)
Respiratory insufficiency	0.99 (0.85-1.15)	1.00 (0.70-1.43)	1.31 (0.95-1.80)	1.44 (1.05-2.99)
Interventions during admission[b]				
Urinary catheter	0.70 (0.55-0.89)	1.33 (0.59-3.04)	1.30 (0.57-2.96)	1.79 (0.79-4.06)
Central venous catheter	0.66 (0.56-0.76)	0.87 (0.53-1.42)	1.43 (0.82-2.50)	2.63 (1.53-4.53)
Surgical drain	1.07 (0.88-1.30)	0.83 (0.54-1.29)	1.19 (0.80-1.77)	1.21 (0.81-1.80)
Mechanical ventilation	0.23 (0.18-0.29)	1.02 (0.37-2.79)	1.11 (0.27-4.59)	6.22 (1.54-25.17)
Renal replacement therapy	0.53 (0.44-0.64)	1.01 (0.71-1.42)	0.84 (0.59-1.19)	1.32 (0.94-1.87)
Hydrocortisone >200 mg	0.77 (0.67-0.87)	1.03 (0.75-1.40)	1.06 (0.79-1.43)	1.34 (0.998-1.80)

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; SDD, selective decontamination of the digestive tract; SOD, selective oropharyngeal decontamination.

[a] A multivariable competing risk analysis provides 2 measures of association: the cause-specific hazard ratio, which estimates the direct effect of the exposure of interest (ie, severity of disease) on the various outcomes (ie, ICU discharge, ICU mortality, and the development of an ICU-acquired infection) and the subdistribution hazard ratio, which describes the risk for the development of an ICU-acquired infection while accounting for the competing events. A lower cause-specific hazard ratio for discharge means that there is a lower hazard for discharge, in other words a higher likelihood for a longer ICU stay. Admission variables included in this model were quartiles of the APACHE IV score, sex, age, admission type (medical vs surgical), the absolute number of organs failing at admission (ranging from 0-5), the use of either SDD or SOD, the comorbidities; a history of immunocompromised state, cardiovascular insufficiency, malignancy, renal and respiratory insufficiency, and the use of urinary catheter, central venous catheter, surgical drain, mechanical ventilation, renal replacement therapy; and the use of corticosteroids before the event (ICU-acquired infection, mortality, or discharge). In patients with multiple ICU-acquired infections, only the first ICU-acquired infection was used.

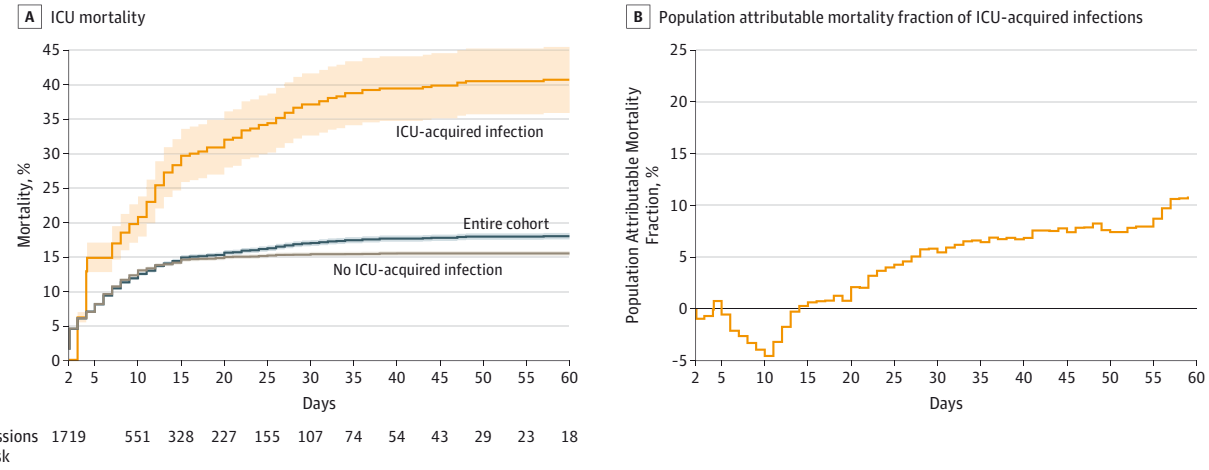
[b] All interventions were included until the onset of the event.

eTable 13 in the Supplement). Use of SDD was slightly less common in patients who developed an ICU-acquired infection (201 patients [69.1%]) than in those who did not (1227 patients [75.3%]); $P = .03$; difference, 6.2%; 95% CI, 0.7%-12.1%; eTable 10 in the Supplement). There was an association between prior severity of disease and the development of an ICU-acquired infection: patients who developed an ICU-acquired infection had higher APACHE IV and SOFA scores and more shock on admission (eTable 10 in the Supplement) and had higher SOFA scores up to 2 days before the event (eFigure 7 in the Supplement). Patients with an ICU-acquired infection had higher mortality rates than those who did not develop an infection while in the ICU (eTable 10 in the Supplement). The population attributable mortality fraction of ICU-acquired infection was 16.8% (95% CI, 7.1%-26.4%; $P < .001$) by day 30 and was 21.1% (95% CI, 0.6%-41.7%; $P = .04$) by day 60 (eFigure 8 in the Supplement); the difference between mortality in the entire cohort and mortality in the subgroup of patients without ICU-acquired infection was 2.2% (95% CI, 0.9%-3.4%; $P < .001$) by day 30 and was 2.8% (95% CI, 0.1%-5.6%; $P < .05$) by day 60.

Discussion

In this prospectively enrolled ICU cohort, 13.5% of sepsis admissions was complicated by 1 or more ICU-acquired infections, which was associated with a population attributable mortality fraction of 10.9% (95% CI, 0.9%-20.9%) by

Figure 2. ICU Mortality and Population Attributable Mortality Fraction of ICU-Acquired Infections in Patients With a Sepsis Admission Diagnosis



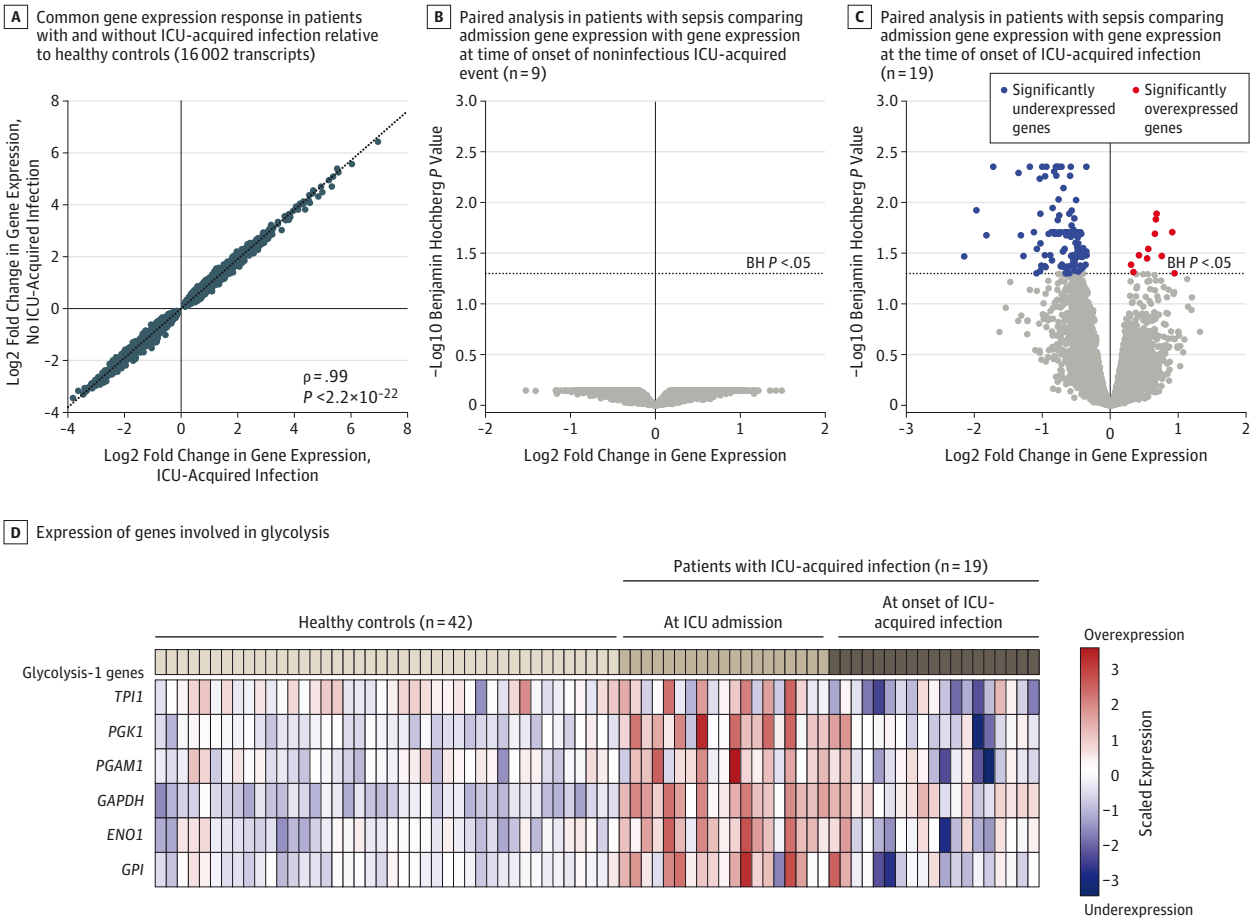
A, Estimated intensive care unit (ICU)–mortality calculated using the multistate model (see the Methods section in the Supplement). Shaded areas represent 95% CIs. B, Population attributable ICU mortality fraction over time adjusted for quartiles of Acute Physiology and Chronic Health Evaluation IV score and quartiles of age. The population attributable mortality fraction was expressed as the percentage of ICU mortality caused by the ICU-acquired infections. The negative values of attributable mortality fraction in the first 14 days after ICU admission are most likely driven by the most severely ill patients encountering the competing event (death without ICU-acquired infection) before being able to develop an ICU-acquired infection[24]; additionally, the discharge hazard for patients with an ICU-acquired infection is smaller than the discharge hazard for patients without an ICU-acquired infection, resulting in a higher chance of dying (longer ICU stay) and a delay in deaths in the former group, leading to a negative attributable mortality fraction in the earlier days.

day 60. The difference between mortality in all patients with sepsis and mortality in patients with sepsis who did not develop an ICU-acquired infection was 2.0% (95% CI, 0.2%-3.8%) at day 60. The results on population attributable mortality fraction appeared robust in several sensitivity and subgroup analyses. This relatively low population attributable mortality fraction is informative for future clinical trials seeking to prevent ICU-acquired infections with mortality as an end point. Besides previously described risk factors, such as the use of central venous lines and mechanical ventilation,[26,27] patients with more severe disease were at increased risk of acquiring an infection while in the ICU.

In a subgroup of patients with sepsis, we performed exploratory analyses of whole-genome expression profiles in blood leukocytes obtained on ICU admission in an effort to disclose immunological signs of immune suppression in those who went on to acquire an infection while in the ICU. However, the leukocyte genomic response to the inciting sepsis event was not different between patients who did and those who did not develop an ICU-acquired infection. The common genomic response was characterized by concurrent up-regulation of multiple proinflammatory and anti-inflammatory pathways, and down-regulation of adaptive immunity pathways, corroborating previous studies.[28] Paired follow-up samples revealed no alterations in the leukocyte transcriptome in patients who developed a noninfectious ICU-acquired complication. In contrast, patients with an ICU-acquired infection developed a diminished expression of genes involved in leukocyte glucose metabolism. Glycolysis is an essential energy source for immune cells and their capacity to mount an inflammatory response, a phenomenon known as the Warburg effect in malignant cells.[29,30] Thus, the transcriptome data in our study suggest a role for impaired glycolysis in immune suppression in patients with sepsis and their susceptibility for ICU-acquired infections.[3-6] The common genomic response, showing concurrent proinflammatory and immune suppressive alterations, remained unchanged during follow-up of patients with sepsis. Such sustained concurrent proinflammatory and immune suppressive responses have also been described in patients after major noninfectious injury.[31] The data in our study support the notion that the same pathophysiological mechanisms apply to patients with sepsis.[32]

When analyzing the incidence and outcome of ICU-acquired infections in patients admitted for a noninfectious condition during the same study period, we did not directly compare these patients with those with a sepsis admission diagnosis because of the large differences between these 2 groups, including different exposure to antibiotic therapy and different lengths of ICU stay. Nevertheless, taking these limitations into account, a few findings deserve attention. First, the incidence of ICU-acquired infections after the first week of ICU stay was comparable in both admission groups. Second, a higher proportion of patients with a sepsis admission diagnosis acquired more than 1 infection in the ICU compared with patients with a noninfectious admission diagnosis. Third, patients with sepsis on admission developed more ICU-acquired infections with opportunistic pathogens like enterococci, *Pseudomonas aeruginosa*, and viruses, hinting at possible immune suppression. Fourth, the population attributable mortality fraction of ICU-acquired infection does not seem to be higher in patients

Figure 3. Genome-Wide Transcriptional Analysis of Blood Leukocytes From Patients With Sepsis Who Did or Did Not Acquire an Infection While in the ICU



A, Genome-wide blood gene expression profiles of 64 patients who developed an intensive care unit (ICU)–acquired infection and 398 patients who did not were each compared to 42 healthy controls. This analysis revealed 97% of the significantly altered gene transcripts in patients with and without acquired infection were common. Log2 fold changes relate to differences in gene expression between patients and healthy controls. Spearman correlation analysis of the resultant log2-transformed fold changes of standard biotin-fluorescence intensities per gene transcript between patients and healthy subjects (intensity $gene_{i,patients}$ − intensity $gene_{i,health}$) showed a strongly correlating gene expression response. No differences in blood transcriptional profiles were uncovered when directly comparing patients with and without ICU-acquired infections with samples from patients with no ICU-acquired infection. ρ Indicates Spearman ρ. Each dot represents a specific gene transcript. B and C, Volcano plot representation (integrating log2 fold changes and −log10 Benjamin Hochberg [BH] *P* values) of genome-wide blood transcriptional profiles of paired ICU admission and event (follow-up) samples from 9 patients with no and 19 patients with ICU-acquired infections. Log2 fold changes relate to the difference in gene expression between (paired) admission and follow-up samples. Each dot represents a specific gene transcript. B, Gene expression profiles of patients without ICU-acquired infections sampled at ICU admission were compared with those sampled at the onset of a noninfectious ICU-acquired complication (2 acute lung injuries, 6 acute kidney injuries; 1 acute myocardial infarction). No differences were identified. C, Gene expression profiles of patients with ICU-acquired infections sampled at ICU admission were compared with samples taken at the onset of an ICU-acquired infectious complication. A total of 128 significantly different genes were identified in the paired samples of patients with ICU-acquired infections. Gray dots denote nonsignificant genes; red dots, significantly overexpressed genes (n=11); blue dots, significantly underexpressed genes (n=117). D, Heat map representation of samples from 19 patients with ICU-acquired infections collected at both ICU admission and at the onset of the infectious complication (paired analysis). Glycolysis-I gene expression values (log2-transformed intensities) were scaled and depicted in color code format, with red denoting overexpression and blue denoting underexpression. *GPI* indicates glucose-6-phosphate isomerase; *TPI1*, triosephosphate isomerase 1; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *PGK1*, phosphoglycerate kinase 1; *PGAM1*, phosphoglycerate mutase 1; and *ENO1*, enolase 1.

with a sepsis admission diagnosis than in patients admitted with a noninfectious condition.

A strength of this study was that it prospectively followed up all patients admitted to 2 mixed ICUs who were at risk of developing an ICU-acquired infection during a 2.5-year period, during which clinical and microbiological data were collected by researchers, resulting in detailed and validated documentation of daily ICU events in clinical practice. However, study limitations need to be considered. First, gene expression analyses of blood leukocytes only provide insight in immune pathways regulated at mRNA level in circulating cells. Second, the 2 ICUs contributing to the current cohort participated in a cluster randomized trial evaluating SDD vs SOD[11] during approximately 50% of this observa-

tional study; otherwise, patients received SDD, the standard of care in the Netherlands. Although our study was not designed to compare SDD with SOD, subgroup analyses are concordant with data from 2 large clinical trials on this topic,[11,33] ie, relative to SDD, SOD had no effect on mortality and was associated with a modest increase in the incidence of ICU-acquired infections. This latter finding only was demonstrable in patients with a noninfectious admission diagnosis, which may be related to the fact that patients with sepsis are almost invariably treated with systemic broad-spectrum antibiotics during the first days after ICU admission. The use of SDD may influence generalization of our results to ICUs without SDD.

Conclusions

Intensive care unit–acquired infections occurred more commonly in patients with sepsis with higher disease severity, but such infections contributed only modestly to overall mortality. The blood genomic response of patients with sepsis was consistent with immune suppression at the onset of secondary infection.

ARTICLE INFORMATION

Published Online: March 15, 2016. doi:10.1001/jama.2016.2691.

Author Affiliations: Center for Experimental and Molecular Medicine, Division of Infectious Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands (van Vught, Scicluna, Wiewel, van der Poll); Department of Intensive Care Medicine, University Medical Center Utrecht, Utrecht, the Netherlands (Klein Klouwenberg, Cremer); Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands (Klein Klouwenberg, Bonten); Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands (Klein Klouwenberg, Bonten); Department of Mathematics, Utrecht University, Utrecht, the Netherlands (Spitoni); Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands (Scicluna); Department of Intensive Care, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands (Horn, Schultz); Cologne Center for Genomics, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, and Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany (Nürnberg).

Author Contributions: Drs van Vught and Klein Klouwenberg had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: van Vught, Klein Klouwenberg, van der Poll.
Acquisition, analysis, or interpretation of data: van Vught, Klein Klouwenberg, Spitoni, Scicluna, Wiewel, Horn, Schultz, Nürnberg, Bonten, Cremer.
Drafting of the manuscript: van Vught, Klein Klouwenberg, van der Poll.
Critical revision of the manuscript for important intellectual content: van Vught, Klein Klouwenberg, Spitoni, Scicluna, Wiewel, Schultz, Nürnberg, Bonten, Cremer, van der Poll.
Statistical analysis: van Vught, Klein Klouwenberg, Spitoni, Scicluna.
Obtained funding: van der Poll.
Administrative, technical, or material support: Nürnberg.
Study supervision: van der Poll.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: This work was supported by grant 041-201 from the Center for Translational Molecular Medicine (CTMM) (http://www.ctmm.nl), project MARS.

Role of the Funder/Sponsor: The sponsor CTMM had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Group Information: Members of the MARS consortium: Friso M. de Beer, MD, Lieuwe D. J. Bos, MD (Department of Intensive Care, Academic Medical Center, University of Amsterdam), Jos F. Frencken, MD (Department of Intensive Care Medicine and Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands), Gerie J. Glas, MD, Roosmarijn T. M. van Hooijdonk, MD (Department of Intensive Care, Academic Medical Center, University of Amsterdam), Arie J. Hoogendijk, PhD, Mischa A. Huson, MD (Center for Experimental and Molecular Medicine, Academic Medical Center, University of Amsterdam), David S. Y. Ong, MD (Department of Intensive Care Medicine and Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands), Laura R. A. Schouten, MD (Department of Intensive Care, Academic Medical Center, University of Amsterdam), Marleen Straat, MD, Esther Witteveen, MD, and Luuk Wieske, MD (Department of Intensive Care, Academic Medical Center, University of Amsterdam).

Additional Contributions: The authors thank all members of the MARS consortium for the participation in data collection and Marak Franitza, PhD, and Mohammed R. Toliat, PhD (Cologne Center for Genomics, University of Cologne, Cologne, Germany), for assisting in the array analyses, none of whom received financial compensation for this study.

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