OPPORTUNISTIC PATHOGENS
IN THE INTENSIVE CARE UNIT

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Opportunistic Pathogens in the Intensive Care Unit

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OPPORTUNISTIC PATHOGENS IN THE INTENSIVE CARE UNIT

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INTRODUCTION

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GENERAL INTRODUCTION

INFECTIONS IN THE INTENSIVE CARE UNIT

Infections and sepsis in the intensive care unit (ICU) contribute substantially to morbidity and mortality in critically ill patients (1). Sepsis is the systemic, deleterious host response to infection, which may lead to severe sepsis (acute organ dysfunction secondary to documented or suspected infection) and septic shock (severe sepsis plus hypotension not reversible with fluid resuscitation) (2). There is a delicate balance between microorganisms that may cause disease and the host's immune system that tries to control the initiation and the progress of an infection (3, 4). Infections may easily arise when this balance is disturbed and patients' vulnerability is increased due to the severity of illness, or due to physical interruption of natural barriers against infection by intravenous catheters or intubation. Colonization of the respiratory tract, the skin and the gastro-intestinal tract with microorganisms are important sources for subsequent hospital infections in ICU patients. Critically ill patients are not typically classified as being immunocompromised. However, the acute severity of illness of these patients may indeed have an impact on the immune system, which eventually could result in ICU-acquired infections by opportunistic pathogens (5).

OPPORTUNISTIC PATHOGENS

Opportunistic microorganisms are usually characterized as harmless microorganisms that may become pathogenic in case the host's immune system is impaired (6). These opportunistic pathogens typically emerge from commensal symbionts or from environmentally acquired microbes. Although it may be tempting to assume pathogenicity when opportunistic microorganisms are detected via regular microbiological diagnostic procedures, it remains crucial to accurately assess the disease burden due to infection that is caused by these microorganisms.

Treating opportunistic pathogens in ICU patients has been of great interest in critical care and microbiological research. However, even if a pathogen can be targeted, treatment remains unnecessary in case this pathogen only contributes marginally to an unfavorable patient outcome. Furthermore, as the clinical outcome of infection is the result of the interaction between microorganisms and the host immune response, the "pathogenicity" of a microorganism may differ in subgroups of patients.

Opportunistic pathogens can be bacteria, viruses, fungi or parasites. Examples are latently present herpes viruses that may reactivate during immunosuppressed conditions. Respiratory viruses other than the influenza virus are often considered to be harmless, but its clinical significance could very well be different in critically ill patients. Also some bacteria may be characterized as opportunistic pathogens, such as enterococci and *Pseudomonas aeruginosa*. Similarly, *Candida* species are part of the normal flora of the human gastro-intestinal tract, but may cause severe disease under certain circumstances.

ANTIMICROBIAL EXPOSURE AND RESISTANCE

Antimicrobial therapy against (opportunistic) pathogens in critically ill patients is important to prevent or clear infections. While infections by herpes viruses -in particular CMV- are thought to be due to reactivations in latent carriers of these viruses, ICU-acquired infections by *Candida* species or gram-negative bacteria are generally believed to be preceded by colonization of the host. Therefore, modulation of colonization has been pursued in ICU settings in order to prevent infections. Selective digestive decontamination (SDD) has become standard of care in the Netherlands (7, 8). Yet, there are potential drawbacks of antimicrobial exposure, such as the development of antimicrobial resistance, which may have major consequences for treatment success and subsequent patient outcome. Therefore, factors inducing resistance should be identified. SDD remains controversial in settings with high levels of antimicrobial resistance, because of the fear of inducing antimicrobial resistance by routine use of prophylactic antibiotics (9). Assessment of the various components of SDD on their effectiveness and their effects on resistance development is needed to optimize the use of this strategy.

AIMS AND OUTLINE OF THE THESIS

This thesis covers the epidemiology of infections caused by various opportunistic pathogens and their effects on clinical outcome. Furthermore, the influence of antimicrobial exposure on these pathogens is studied.

In the first part of this thesis the epidemiology and clinical impact of opportunistic viral pathogens in critically ill patients will be addressed. Currently, identification of most viral infections in ICU patients is suboptimal, because most microbiological diagnostics are primarily focused on finding bacterial agents. This may be caused by a presumed low incidence of viruses in these patients, a knowledge gap regarding the possible role viruses may have in an ICU setting, costs associated with performing viral diagnostics, and in some cases an absence of therapeutic options. Most patients admitted to the ICU have respiratory failure, and some viruses are well known to be associated with respiratory failure, e.g. influenza virus. However, the role of many other viruses could be underestimated and even missed in ICU patients.

Chapter 2 describes the current practice in respiratory virus diagnostics in two academic centers in the Netherlands and compares these practice characteristics to current available international guidelines and recommendations. In Chapter 3 we compare the incidence of respiratory syncytial virus to influenza virus as determined by polymerase chain reaction in throat swab samples of patients admitted to the mixed ICUs of two hospitals with community-acquired respiratory failure, in order to investigate whether respiratory syncytial virus plays an important role in these patients.

In contrast to respiratory viruses, diagnostics on the presence of herpes viruses in previously immunocompetent ICU patients are rarely performed. Although the disease

burden of herpes virus reactivations is well recognized in immunocompromised patients (e.g., patients with human immunodeficiency virus, hematological patients receiving chemotherapy and transplant patients), the role of systemic virus reactivation in previously immunocompetent ICU patients remains to be investigated in more detail. In Chapter 4 we explore the association between cytomegalovirus (CMV) seroprevalence and clinical outcome in ICU patients who are admitted with acute respiratory distress syndrome by comparing CMV seropositive to seronegative patients. A difference in mortality or prolonged mechanical ventilation between the two serogroups may provide a rationale for testing antiviral prophylaxis in CMV seropositive patients. In Chapter 5 we focus on CMV seropositive patients only, since only these patients are at risk for CMV reactivation and its potential negative effects. We use advanced epidemiological and statistical methodology, which accounts for confounding bias, immortal time bias and bias by the evolution of disease severity prior to reactivation, to adequately assess the attributable mortality of systemic CMV reactivation in patients with acute respiratory distress syndrome. In Chapter 6 we describe the occurrence of multiple herpes viruses in the blood of septic shock patients, and we compare the associations of these different viruses with clinical outcome.

In the second part of this thesis bacterial and fungal opportunistic pathogens in the ICU will be addressed. SDD is a prophylactic antimicrobial strategy that aims to decontaminate the digestive tract of gram-negative bacteria and yeasts in order to reduce the development of subsequent infections by these microorganisms, while it preserves anaerobic flora and enterococci. Yet, antimicrobial exposure is associated with the risk of antimicrobial resistance development. In Chapter 7 we assess the impact of different antibiotics on the development of antibiotic resistance in gram-negative bacteria with a special focus on the opportunistic pathogen *Pseudomonas aeruginosa* in a non-SDD setting. Chapter 8 evaluates the same associations but then in SDD settings. In Chapter 9 we study the effectiveness of nebulized amphotericin B as part of SDD in decolonizing the lower respiratory tract of *Candida* species. In Chapter 10 we compare the effectiveness of nystatin to amphotericin B (as part of SDD) in the prevention of *Candida* colonization or decolonization in case patients are already colonized with *Candida* at ICU admission. In clinical practice enterococci are generally assumed to be of low virulence, similar to coagulase-negative staphylococci. Nevertheless, enterococcal bacteremia is usually found in patients with the greatest severity of illness. To investigate whether ICU-acquired enterococcal bacteremia is merely a marker or a true cause of morbidity and mortality, in Chapter 11 we study the association between enterococcal bacteremia and clinical outcome, and we describe clinical management practices in response to suspected catheter-related bloodstream infections by enterococci.

Finally, we summarize and discuss our key findings that are presented throughout this thesis in Chapter 12.

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II

OPPORTUNISTIC VIRAL INFECTIONS IN THE ICU

2

CLINICAL PRACTICE OF RESPIRATORY VIRUS DIAGNOSTICS IN CRITICALLY ILL PATIENTS WITH A SUSPECTED PNEUMONIA

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ABSTRACT

Purpose

Clinical guidelines suggest testing for respiratory viruses during the influenza season, but are unclear which categories of patients should be tested. We described the clinical practice of diagnostic testing for respiratory virus infections in patients presenting to an intensive care unit (ICU) with suspected community-acquired pneumonia (CAP) or hospital-acquired pneumonia (HAP).

Methods

A prospective observational study in consecutive CAP and HAP patients with an ICU stay of more than 24 hours in two tertiary care hospitals in The Netherlands from 2011 to December 2013. The proportion of patients receiving diagnostic testing with PCR for the presence of respiratory viruses in respiratory tract specimens was determined.

Results

In total, 1452 patients were included, of which 712 patients presented with suspected CAP and 740 with suspected HAP. In CAP cases, 282 of 712 (40%) were tested for respiratory viruses (190 of 417 (46%) during the influenza season). In HAP cases, 95 of 740 (13%) were tested (50 of 372 (13%) during the influenza season). Regardless of the time of year, virus diagnostic tests were ordered significantly more often in patients with comorbidities, as well as in those presenting with elevated CRP and leucopenia. In patients who were tested during the influenza season, the prevalence of influenza was 14% in patients with CAP and 10% in those with HAP. Influenza was absent during the summer in both groups.

Conclusion

Less than half of patients admitted to the ICU with suspected pneumonia were tested for the presence of viral pathogens, either in or outside the influenza season.

INTRODUCTION

Respiratory virus infections are important causes of community-acquired pneumonia (CAP) and respiratory failure both in children and adults (1). Epidemiological studies show that the prevalence of viral respiratory tract infections can be as high as 41% in critically ill patients admitted to the intensive care unit (ICU) with a suspected CAP, and up to 34% in hospital-acquired pneumonia (HAP) (2-6). Detection of such infections in critically ill patients may have important implications for infection control measures such as isolation and, in case of (suspected) influenza, rapid initiation of antiviral medication (7, 8). These measures have an impact on ICU resource use, mandating clear assessment of patients at risk of a viral respiratory tract infection.

A recent large retrospective study indicated that influenza infections are underdiagnosed in the critically ill (9). However, current international clinical guidelines on virus diagnostics are not clear about which patients should receive testing in the ICU setting. The IDSA/ATS consensus guidelines, as well as the International Guidelines for Management of Severe Sepsis and the ERS/ESCMID guidelines, state that testing for at least influenza should be considered in adult patients admitted with suspected respiratory infection during local epidemics (10-12), but all are unclear if all patients admitted to the ICU with a suspected CAP should be tested for respiratory viruses. There are no recommendations for virus testing in patients admitted to the ICU due to HAP.

The current practice of testing for the presence of viral pathogens in critically ill patients with a suspected CAP or HAP is not known. We aimed to describe the practice of diagnostic testing for viral respiratory infections in patients admitted to the ICU with clinical symptoms suggestive for CAP or HAP. Also, the prevalence of virus infections as detected during routine care was reported.

METHODS

Study population

This study is part of a multi-center prospective cohort study, in which consecutive patients admitted to the mixed ICUs of two tertiary care hospitals in The Netherlands were enrolled between January 1st 2011 and December 31st 2013 (clinicaltrials.gov Identifier: NCT01905033). For this study patients with suspected CAP or HAP were included. Exclusion criteria were admissions with a length of ICU stay of < 24 hours and transfers from another ICU. The Ethics Committees of both participating centers approved an opt-out method of consent (protocol number 10-056C).

Study definitions

A suspected respiratory tract infection at ICU admission was defined by empiric or targeted use of systemic antibiotics for a suspected CAP or HAP initiated by the attending physicians, between seven days prior to, and two days after ICU admission.

The most likely source of each infection was determined by assessment of clinical data, radiological imaging and culture results as ordered by routine care, using strict diagnostic criteria. These criteria were based on CDC criteria as well as the International Sepsis Forum Consensus Conference definitions for CAP and HAP, which were adapted to the Dutch situation as described previously (13). All observers were trained in these definitions before the start of the study, and an electronic algorithm was used that alarmed the researchers when there were inconsistencies with other recorded clinical variables.

Respiratory virus diagnostics were defined as polymerase chain reaction (PCR) tests ordered as per discretion of attending physicians on samples from the respiratory tract, for any of the following viruses: influenza virus A and B, respiratory syncytial virus, human metapneumovirus, parainfluenza virus 1-4, human rhinovirus, coronavirus, adenovirus, enterovirus, human bocavirus and parechovirus.

Immunodeficiency was defined as a history of solid organ or stem cell transplantation, infection with the human immunodeficiency virus, hematological malignancy, use of immunosuppressive medication (prednisone >0.1 mg/kg for >3 months, prednisone >75 mg/day for >1 week, or equivalent), chemotherapy/radiotherapy in the year before ICU admission, and any known humoral or cellular immune deficiency. Leucopenia was defined as $<4x10^9$/L leucocytes.

Data analysis

Characteristics of patients who were tested were compared to patients not tested using non-parametric descriptive statistics (i.e., Chi-square and Kruskal-Wallis tests). Subgroup analyses were performed for the influenza season period and the period outside the season separately. Influenza season was defined between November 1st and April 30th. Finally, the prevalence of viral infections in those who were tested was reported. All analyses were performed using SAS 9.2 (Cary, NC, USA). P values less than 0.05 were considered to represent statistical differences.

RESULTS

During the study period, a total of 8303 patients were admitted to the ICU, of whom 2356 were excluded because of an ICU stay of less than 24 hours, 437 patients were transferred from another ICU, and 4058 did not have a suspected pneumonia. In total, 712 patients were included with a suspected CAP, and 740 patients with a suspected HAP (Figure 1).

Proportion of patients tested for respiratory viruses

In the group of patients admitted to the ICU with a suspected CAP, 282 of 712 patients (40%) were tested for respiratory viruses. Of patients admitted with a suspected HAP, 95 of 740 (13%) were tested.

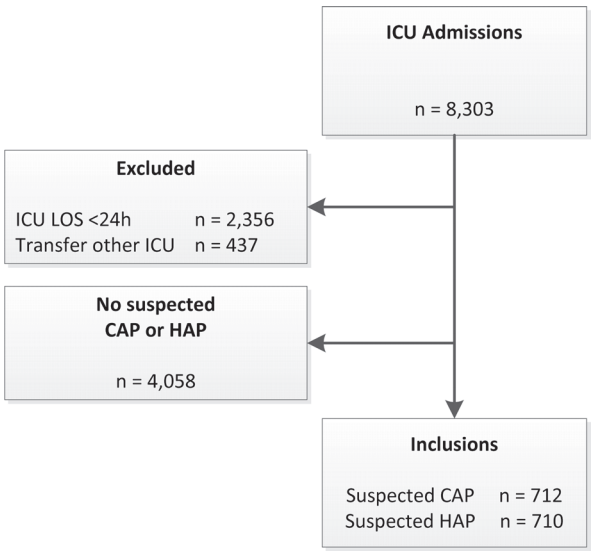

Figure 1. Flowchart of patient inclusion
CAP = community-acquired pneumonia; HAP = hospital-acquired pneumonia;

Characteristics of tested and non-tested patients with suspected CAP

Patients admitted with a suspected CAP who were tested, more often had comorbidities (including chronic obstructive pulmonary disease (COPD), chronic renal insufficiency and immune deficiency) compared to those who were not tested (Table 1). Also, within 24 hours of ICU admission, tested patients had a significantly lower acute physiology score (APS), while the Acute Physiology and Chronic Health Evaluation IV (APACHE IV) and Sequential Organ Failure Assessment (SOFA) score upon admission did not differ. Also, tested patients had higher C-reactive protein (CRP) levels, more often leucopenia, and more often acute renal failure compared to non-tested patients.

Characteristics of tested and non-tested patients with suspected HAP

Patients admitted with a suspected HAP who were tested, more often had chronic renal insufficiency and immune deficiency compared to non-tested patients (Table 1). Tested patients also had significantly higher CRP levels, more often leucopenia, acute renal failure and higher severity of all illness scores compared to patients with a suspected HAP who were not tested.

Seasonal influence on viral diagnostic testing

In the influenza season, 190 of 417 (46%) patients admitted with a suspected CAP were tested for respiratory viruses. Outside the season 92 of 295 (32%) were tested. Of the patients admitted with a suspected HAP, 50 of 372 (13%) in and 45 of 368 (12%) outside

the season were tested. In CAP patients admitted within the influenza season, baseline characteristics were compared between tested and non-tested patients, showing similar differences in characteristics as in the total group of suspected CAP patients, with the exception of the APS, which did not differ between tested and non-tested patients (Supplementary Table S1).

Table 1. Baseline characteristics of patients admitted with a suspected community-acquired pneumonia and hospital-acquired pneumonia

	Suspected community-acquired pneumonia			Suspected hospital-acquired pneumonia		
	Not tested (n=430)	Tested (n=282)	*p* value	Not tested (n=645)	Tested (n=95)	*p* value
Age (years)	61 (48-71)	62 (48-72)	0.28	65 (55-73)	59 (50-68)	<0.01
Male	289 (67%)	153 (54%)	<0.01	414 (64%)	62 (65%)	0.84
Medical admission	363 (84%)	279 (99%)	<0.01	504 (78%)	89 (94%)	<0.01
Hospital days prior to ICU admission	0 (0-1)	0 (0-1)	0.88	8 (4-17)	8 (4-18)	0.70
Chronic obstructive pulmonary disease	62 (14%)	73 (26%)	<0.01	101 (16%)	19 (20%)	0.28
Congestive heart failure	24 (6%)	17 (6%)	0.80	43 (7%)	5 (5%)	0.60
Diabetes mellitus	77 (18%)	59 (21%)	0.32	125 (19%)	17 (18%)	0.73
Chronic renal insufficiency	41 (10%)	41 (15%)	0.04	60 (9%)	17 (18%)	0.01
Malignancy	39 (9%)	24 (9%)	0.80	126 (20%)	15 (16%)	0.39
Immune deficiency	48 (11%)	84 (30%)	<0.01	71 (11%)	42 (44%)	<0.01
APACHE IV Score	76 (59-104)	76 (59-94)	0.39	75 (62-93)	87 (70-100)	<0.01
Acute Physiology Score	64 (48-92)	61 (46-77)	0.02	63 (50-80)	71 (61-84)	0.01
SOFA score on admission	7 (5-9)	7 (5-9)	0.50	7 (5-9)	9 (6-10)	<0.01
Highest central body temperature (°C) [a]	37.7 (36.8-38.5)	37.8 (37.0-38.6)	0.04	38.0 (37.3-38.7)	38.0 (37.4, 38.7)	0.90
First measured C-reactive protein (mg/L) [a]	40 (5-154)	135 (42-235)	<0.01	114.5 (51-213)	160 (92-282)	<0.01
Highest leucocytes (cells x 10^9/L) [a]	15 (10-19)	12 (8-18)	<0.01	15 (10-20)	13 (2-19)	0.01
Leucopenia [a]	43 (10%)	48 (17%)	0.01	44 (7%)	26 (27%)	<0.01
Use of vasoactive medication > 1h [a]	262 (61%)	170 (60%)	0.86	400 (62%)	65 (68%)	0.23
Acute renal failure [a]	42 (10%)	42 (15%)	0.04	77 (12%)	16 (17%)	0.18
Highest serum lactate (mmol/L) [a]	2.8 (1.7-4.8)	2.2 (1.4-3.2)	<0.01	1.9 (1.3-3.3)	1.8 (1.3-2.9)	0.74

Data are presented as median (interquartile range) or absolute number (%).
APACHE IV = Acute Physiology and Chronic Health Evaluation IV; SOFA = sequential organ failure assessment. [a] In the first 24 hours of admission.

Timing and results of diagnostic tests

The prevalence of viral respiratory tract infections in tested patients differed between suspected CAP and HAP, and between patients admitted in and outside the influenza season (Table 2). In the influenza season, viruses were found in 65 of 190 (34%) of suspected CAP patients, and in 17 of 50 (34%) of suspected HAP patients. Outside the influenza season, 17 of 92 (19%) suspected CAP patients and 7 of 45 (16%) suspected HAP patients tested positive for at least 1 virus. In the influenza season, the most prevalent pathogen was influenza virus (26 of 190 (14%) in suspected CAP and 5 of 49 (10%) in suspected HAP). Outside the influenza season, influenza virus was not found. Ordering of virus tests was mostly performed on the day of ICU admission, in both suspected CAP and HAP cases (Figure 2).

Table 2. Prevalence of viral respiratory tract infections as found by routine diagnostics

	Suspected CAP in influenza season		Suspected CAP outside influenza season		Suspected HAP in influenza season		Suspected HAP outside influenza season	
Virus	Patients tested (n)	Virus positive (n, %)	Patients tested (n)	Virus positive (n, %)	Patients tested (n)	Virus positive (n, %)	Patients tested (n)	Virus positive (n, %)
Adenovirus	173	3 (2%)	88	1 (1%)	44	0 (0%)	39	1 (3%)
Bocavirus	172	0 (0%)	88	0 (0%)	44	0 (0%)	39	0 (0%)
Coronavirus	179	13 (7%)	89	1 (1%)	44	4 (9%)	39	0 (0%)
Enterovirus	134	1 (1%)	73	0 (0%)	27	0 (0%)	24	0 (0%)
Human metapneumovirus	173	9 (5%)	88	0 (0%)	44	1 (2%)	39	2 (5%)
Influenza virus	190	26 (14%)	89	0 (0%)	49	5 (10%)	39	0 (0%)
Parechovirus	134	0 (0%)	73	0 (0%)	27	0 (0%)	24	0 (0%)
Parainfluenza virus	173	1 (1%)	88	4 (5%)	44	2 (5%)	39	3 (8%)
Rhinovirus	179	13 (7%)	89	12 (14%)	44	3 (7%)	39	2 (5%)
Respiratory syncytial virus	179	5 (3%)	89	2 (2%)	45	3 (7%)	39	0 (0%)
Total (any virus)[a]	**190**	**65 (34%)**	**92**	**17 (19%)**	**50**	**17 (34%)**	**45**	**7 (16%)**

CAP = community-acquired pneumonia; HAP = hospital-acquired pneumonia
[a] a patient with an infection with more than one virus is counted as 1 virus-positive patient.

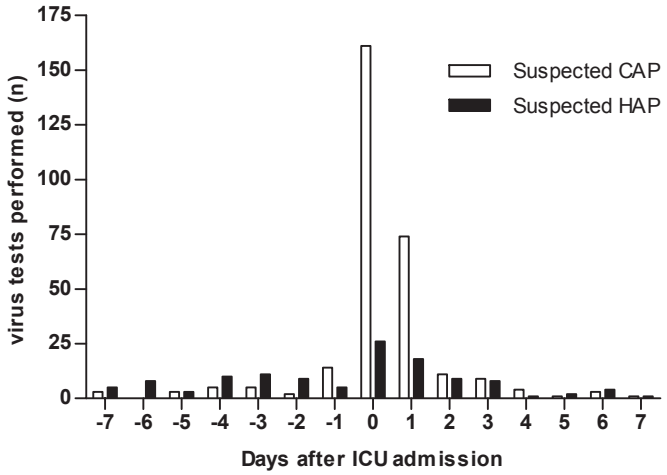

Figure 2. Timing of virus diagnostic tests, as performed by attending physicians
CAP = community-acquired pneumonia; HAP = hospital-acquired pneumonia;

DISCUSSION

Our study shows that 46% of patients admitted to the ICU with a suspected CAP during the influenza season was tested for the presence of viral pathogens, whereas 32% of CAP patients was tested outside the season. Patients admitted with a suspected HAP were tested in 13% and 12% of cases, respectively. Regardless of the season, patients with comorbidities (including COPD and immune deficiency) and inflammation biomarkers (elevated CRP and leucopenia) were tested more often. In patients who were tested, the prevalence of viral respiratory tract infections was similar in suspected CAP and HAP cases.

The results of this study show that less than half of patients admitted to the ICU with a suspected pneumonia were tested for viral infections. This may reflect a lack of awareness and clear clinical guidelines on virus diagnostics for ICU patients admitted with a suspected CAP or HAP. While international guidelines are unclear if all critically ill patients with suspected pneumonia for influenza during the winter season should be tested, the IDSA/ATS guidelines do state that "Patients with CAP should be investigated for specific pathogens that would significantly alter standard (empirical) management decisions, when the presence of such pathogens is suspected on the basis of clinical and epidemiologic clues" (10). Although, a positive virus test does not necessarily indicate virus-related or virus-induced critical illness (14, 15), and the clinical significance of non-influenza types of viruses remains controversial (16-19), detecting an influenza infection has consequences for antiviral treatment and quarantine measures (7, 8). Consequently, some experts advise testing for influenza in all patients admitted with severe pulmonary infection during the influenza season (20-22).

Alternatively, low prevalence of testing may be due to difficulties in establishing a clinical suspicion of a viral respiratory tract infection in the critically ill. As testing in our study was more often performed in patients who had more comorbidities, higher CRP and leucopenia, physicians may believe that viral infection is characterized by these characteristics. However, symptoms of influenza-like-illness may be mild or absent in hospitalized patients (23), and there is yet no clinical algorithm to distinguish viral pneumonia from bacterial pneumonia (1). Although the risk factors for more severe and complicated viral respiratory tract infections are well established, such as chronic lung- and heart disease and immunodeficiency, this does not imply that ICU patients with these characteristics are at a higher risk of having a viral infection on admittance (3). Selective testing in the ICU setting may lead to underdiagnosis of viral infections. Indeed, a large retrospective study comparing the predicted amount of influenza-related ICU admissions to the reported admissions with an influenza diagnosis, suggested that over 90% of cases are either not diagnosed or not reported (9). Small prospective observational studies show that 50-70% of detected influenza infections were unsuspected by the attending physicians (24, 25). Taken together, one could suggest that viral testing of ICU patients with a suspected respiratory tract infection should not depend on symptom severity.

Remarkably, the frequency of viral testing did not differ very much between in and outside the influenza season. Outside the influenza season, the prevalence of viral infection dropped but was still 19% in CAP and 16% in HAP cases. Whether this indicates that virus testing should also be performed outside the season cannot be concluded from our study, because only a selected group of patients were tested. Of note, influenza virus was absent outside the season.

HAP patients were tested less often for viral infection than CAP patients. However, in the tested patients, the overall prevalence of respiratory viruses was similar in CAP and HAP patients. Our data may suggest that also HAP patients have a considerable risk of having a viral infection, which is in accordance with retrospective studies on the prevalence of viral infections on the ICU that include HAP (2, 6).

Strengths of our study design are that suspected cases of CAP and HAP were prospectively assessed by a trained team of research physicians according to validated definitions, and that the attending medical staff was not aware of our study aims, which otherwise could have interfered with the actual practice of performing viral diagnostic tests. An important limitation in our study is that the prevalence of viral infections was not systematically tested in all patients with a suspected respiratory infection, which hampers the estimation of the prevalence of viral respiratory tract infections.

In conclusion, our study shows that less than half of the ICU patients admitted with a suspected pneumonia, either in or outside the influenza season, is tested for the presence of viral pathogens and that the decision to test seems primarily to depend on patient comorbidities and inflammation biomarker profile. As guidelines clearly recommend to consider testing for influenza virus during local epidemics, there is room

for increasing routine viral testing in patients with suspected pneumonia admitted to the ICU during the influenza season.

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SUPPLEMENTARY MATERIAL

Table S1. Baseline variables of a subgroup of patients admitted with a suspected community-acquired pneumonia in the influenza season

Variable	Not tested (n=227)	Tested (n=190)	P value
Age (years)	62 (48-71)	63 (49-73)	0.21
Male	162 (71%)	105 (55%)	<0.01
Medical admission	189 (83%)	188 (99%)	<0.01
Hospital days prior to ICU admission	0 (0-1)	0 (0-0)	0.54
Chronic obstructive pulmonary disease	32 (14%)	53 (28%)	<0.01
Congestive heart failure	13 (6%)	12 (6%)	0.80
Diabetes mellitus	43 (19%)	43 (23%)	0.35
Chronic renal insufficiency	17 (7%)	21 (11%)	0.21
Malignancy	22 (10%)	15 (8%)	0.52
Immune deficiency	21 (9%)	52 (27%)	<0.01
APACHE IV Score	79 (60-105)	77 (60-97)	0.61
Acute Physiology Score	65 (49-95)	63 (48-80)	0.11
SOFA score on admission	7 (5-9)	8 (5-10)	0.19
Highest central body temperature (°C) [a]	37.7 (36.9-38.4)	37.8 (37.1-38.7)	0.03
First measured C-reactive protein (mg/L) [a]	47 (6-185)	163 (50-249)	<0.01
Highest leucocytes (cells x 10^9/L) [a]	15 (11-19)	12 (7-16)	<0.01
Leucopenia [a]	22 (10%)	40 (21%)	0.01
Use of vasoactive medication > 1h [a]	139 (61%)	118 (62%)	0.86
Acute renal failure [a]	20 (9%)	33 (17%)	0.01
Highest serum lactate (mmol/L) [a]	2.6 (1.7-4.5)	2.2 (1.5-3.4)	0.01

Data are presented as median (interquartile range) or absolute number (%).
APACHE IV = Acute Physiology and Chronic Health Evaluation IV; SOFA = sequential organ failure assessment.
[a] In the first 24 hours of admission.

3

RESPIRATORY SYNCYTIAL VIRUS IN CRITICALLY ILL ADULT PATIENTS WITH COMMUNITY-ACQUIRED RESPIRATORY FAILURE

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ABSTRACT

The incidence of respiratory syncytial virus (RSV) and influenza virus infection was determined during three RSV seasons in 158 adult patients consecutively admitted to the intensive care units of two hospitals in the Netherlands with community-acquired respiratory failure. Nasopharyngeal swabs were tested for the presence of RSV and influenza virus by real-time polymerase chain reaction. Six patients (4%) were positive for RSV and all recovered. This finding was in sharp contrast to influenza (23 (15%) patients, 4 (17%) deaths). In conclusion, even in the midst of the RSV season, RSV is an infrequent cause of respiratory failure in adults admitted to the intensive care unit.

INTRODUCTION

Respiratory syncytial virus (RSV) is the most important cause of bronchiolitis and pneumonia in young children worldwide (1). Disease severity depends on the patients' age and health status, whereby premature infants are typically at greatest risk. However, RSV is not exclusively a pathogen in the pediatric population. Reported incidence proportions in the United Kingdom for adult patients presenting with influenza-like illness to the general practice have been as high as 20% (2). RSV illness in adults is often mild, but can cause significant morbidity in the elderly and in high-risk adults with mortality rates around 8% (3-6). In a population based cohort study in the United States RSV infection, independent of bacterial co-infection, was associated with poor outcome (7). In a Dutch study, seasonal mortality in the elderly was attributable to multiple viruses, including RSV (8). It has been suggested that RSV infection is a trigger for acute exacerbation of chronic obstructive pulmonary disease (COPD) (9), and thus may play an important, yet unrecognized role, in etiology and outcome of patients admitted to the intensive care unit (ICU) with respiratory failure. In contrast to the pediatric age group, routine testing for viral infections, including RSV, is not frequently performed in adult patients.

In this multi-center observational study incidences of RSV and influenza infections were determined in patients over 18 years of age admitted to the ICU with community-acquired respiratory failure.

METHODS

Participating centers in The Netherlands were the mixed ICUs at the University Medical Center Utrecht and the Medical Center Leeuwarden. All consecutive patients were prospectively included by trained research physicians in Utrecht and critical care physicians in Leeuwarden during the three winter seasons of 2010 until 2013.

Community-acquired respiratory failure was defined by symptoms of dyspnea, cough and/or wheezing present before or within 48 hours after hospital admission and the presence of respiratory failure requiring ICU admission. The Ethics Committee at the Medical Center Leeuwarden approved the study as part of a prospective cohort study. The Ethics Committee at the University Medical Center Utrecht approved this study and waived the need for informed consent.

Brushed nasopharyngeal swabs (Copan®) were taken upon ICU admission, stored in viral transportation medium, and transported to the diagnostic virology laboratory. Real-time polymerase chain reaction (PCR) was performed for the detection of RSV and influenza virus by the MagNA Pure LC extraction system (Roche Diagnostics, Mannheim, Germany) and the Taqman 7500 amplification platform (Applied Biosystems, Foster City, CA) (10). All data were analyzed with SAS 9.2 (Cary, NC).

RESULTS

A total of 158 patients were included. Data from the National Institute for Public Health and the Environment (RIVM) in The Netherlands confirmed that the study periods concurred with the RSV seasons (Figure 1). In the majority of cases, diagnosis of community-acquired pneumonia was confirmed (Table 1). There were no differences in baseline characteristics between patients with influenza and RSV infection. Six patients (4%; 95% CI 2%-8%) tested positive for RSV, all required mechanical ventilation within the first 24 hours after ICU admission and did not receive antiviral therapy before or during ICU admission. The median duration of ICU admission was 7 (IQR 6-18) days. One of the RSV infected patients was an immunocompetent 61-year-old female with severe COPD disease. Two other patients with RSV infection developed acute kidney injury of whom one required renal replacement therapy. The remaining three patients had a relatively uncomplicated course of disease. Sputum and blood cultures failed to identify bacterial or viral co-pathogens in the RSV positive patients. Twenty-three patients (15%; 95% CI 10%-21%) had influenza, of whom four (17%) died. The median duration of ICU admission was 7 (IQR 2-10) days in patients with influenza infection.

Table 1. Patient characteristics

Variable	Respiratory virus		Neither RSV nor influenza (n=129)
	RSV (n=6)	Influenza (n=23)	
Age (years)	62 (60-68)	60 (50-67)	66 (51-73)
Gender (male)	3 (50)	15 (65)	80 (62)
APACHE IV score	68 (52-81)	72 (54-95)	84 (63-105)
Mechanical ventilation within 24 hours after ICU admission	6 (100)	14 (65)	100 (78)
Corticosteroid or other immunosuppressive therapy	3 (50)	3 (13)	26 (20)
Congestive heart failure	0 (0)	0 (0)	13 (10)
Chronic Obstructive Pulmonary Disease	1 (17)	6 (26)	37 (29)
Diagnosis at time of admission			
Pneumonia	5 (83)	22 (96)	91 (71)
Congestive heart failure	0 (0)	1 (4)	11 (29)
Other / unknown	1 (17)	0 (0)	25 (20)
ICU length of stay (days)	7 (6-18)	7 (2-10)	6 (3-15)
ICU mortality	0 (0)	4 (17)	19 (15)

Data are presented in median (interquartile range) or absolute number (percentage).
APACHE = Acute Physiology and Chronic Health Evaluation; RSV = respiratory syncytial virus.

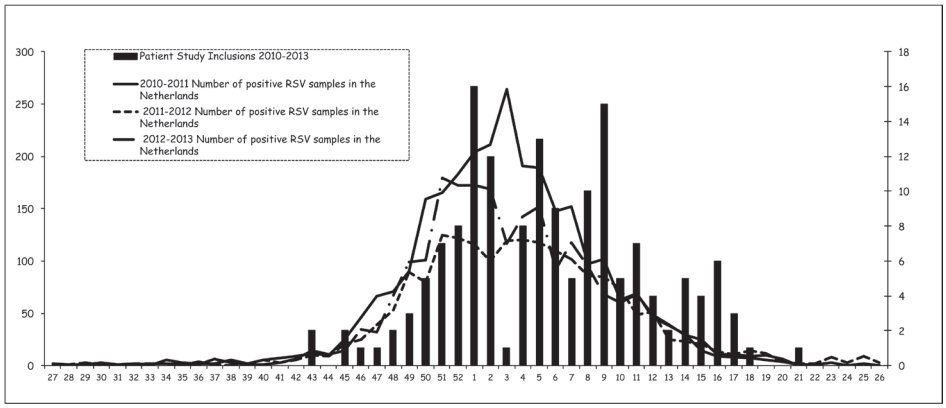

Figure 1. RSV season in the Netherlands and patient inclusion
The lines represent the number of RSV positive tests per week in the Netherlands during the three years of the study period (left vertical axis) according to The National Institute for Public Health and the Environment (RIVM). The bars represent the number of patients included each week during this study (right vertical axis).

DISCUSSION

This is the largest prospective study defining RSV incidence in adult patients with community-acquired respiratory failure admitted to either academic or non-academic ICU, including patients with or without chronic conditions. The incidence of RSV infection was 4% and lower than the observed 15% for influenza. Only three previous studies have evaluated RSV infection in ICU patients. One study included a select group of 122 ICU patients with chronic cardiac or pulmonary disorders, and found a comparable incidence proportion of 5% (9). The other study assessed 64 ICU patients with severe community-acquired and 134 ICU patients with healthcare-associated pneumonia, and found RSV incidence proportions of 11% and 2% respectively (11). The higher incidence found in the community-acquired group suggested that RSV infection is primarily found in patients with community-acquired respiratory failure. The most recent study included 70 non-immunocompromised patients with acute respiratory failure and found an incidence proportion of 6% (12). Based on the limited amount of published data, publication bias cannot be ruled out, perhaps resulting in an overestimation of RSV incidence in adult ICU patients.

Recently, Lee et al showed high morbidity and mortality in adults hospitalized for RSV infections of whom 10% required mechanical ventilation (4). Patients with RSV infection more frequently had underlying chronic lung diseases and major systemic comorbidities compared to patients with influenza, but both groups had comparable clinical outcomes. Rapid recognition of RSV as cause of infection is becoming more important because of the recent progression in development of vaccines and antiviral therapeutics of which several agents have now entered clinical trials. Antisense anti-RSV drugs, fusion inhibitors and nucleoside analogues show promising results (13, 14).

However, beneficial cost-effectiveness of such new treatment and prevention modalities will most likely depend on targeting high-risk populations. Our study demonstrates that patients admitted to the ICU with community-acquired respiratory failure are not part of the high-risk population. More detailed studies are needed to provide estimates of the amount of preventable disease and health gains that could be achieved in more specific patient populations and settings.

The absence of RSV in the nasopharynx does not exclude presence of RSV in the lower respiratory tract. Although availability of lower respiratory tract specimens might have increased our diagnostic yield, it has been well established that the nasopharynx is the porte d'entrée for RSV and it is unlikely that patients with RSV infection were missed in our study (15). This study did not investigate the presence of other respiratory viruses, because of practical reasons and the specific aim of comparing RSV to influenza.

In conclusion, RSV infection is uncommon among adults with acute community-acquired respiratory failure leading to ICU admission. Further research is necessary to identify high-risk adult patients that may possibly benefit from antiviral therapy and vaccination against RSV in the future.

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4

CYTOMEGALOVIRUS SEROPREVALENCE AS A RISK FACTOR FOR POOR OUTCOME IN ACUTE RESPIRATORY DISTRESS SYNDROME

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ABSTRACT

Objective

Cytomegalovirus (CMV) reactivation may complicate critical illness in latent carriers of the virus, even in patients who were previously immunocompetent. Patients with the acute respiratory distress syndrome (ARDS) are considered to be prone for reactivation. Prophylactic antiviral therapy in immunocompetent CMV seropositive patients admitted to the ICU with ARDS has therefore been proposed. We assessed CMV seroprevalence as a risk factor for morbidity and mortality in patients with ARDS.

Design, setting and patients

In this prospective observational study we included all newly admitted patients with ARDS in the ICUs of two tertiary care hospitals in The Netherlands who received mechanical ventilation for at least 4 days. Patients with known immunocompromise and those receiving antiviral treatment prior to ICU admission were excluded. We used the number of days alive and free of mechanical ventilation on day 28 as a composite outcome measure, and used multivariable ordinal logistic regression analyses to adjust for potential confounders.

Measurements and Main Results

Over a two-year period 306 patients were included, 209 (68%) of whom were CMV seropositive. CMV reactivation occurred in 53 (26%) of these cases. One hundred patients (33%) died or continued to be mechanically ventilated by day 28. After adjustment for confounding, CMV seroprevalence was not associated with the primary outcome (crude odds ratio 1.09, 95% CI 0.70-1.70; adjusted odds ratio 1.01, 95% CI 0.64-1.59). Seroprevalence was also not associated with poor outcome in any of the prespecified subgroup analyses. However, a significant association was found in a post hoc subgroup of patients who had developed ARDS in a setting of septic shock (adjusted odds ratio 2.86, 95% CI 1.32-6.23). The time course of pulmonary markers in survivors was comparable between the two serogroups.

Conclusions

CMV seroprevalence is not associated with prolonged mechanical ventilation or increased mortality in critically ill patients with ARDS, with possible exception of patients presenting with septic shock. Therefore, a prevention strategy targeting an unselected cohort of seropositive patients with ARDS is unlikely to show any meaningful benefit.

INTRODUCTION

Cytomegalovirus (CMV) is a herpes virus that establishes a life-long infection after exposure. Seroconversion after primary infection is exclusively associated with the cumulative amount of time that has passed during which possible exposure may have occurred (i.e., age) and the presence of the virus in the environment of the host (i.e., ethnicity) (1). Seroprevalence thus gradually increases from 50% in young adults to 90% in the very elderly (2, 3).

Although the disease burden of CMV has been well-recognized for decades in immunocompromised patients (4), viremia has more recently also been described in intensive care unit (ICU) patients without known prior immune deficiency. Reactivation of latent CMV (rather than a primary infection) is thought to be the main cause of viremia in these patients, with reactivation rates ranging from 16 to 41% and typically occurring between days 4 and 12 of ICU admission (5-10). Moreover, CMV reactivation has been associated with increased durations of mechanical ventilation and/or mortality (5-7, 11-18).

Patients with acute respiratory distress syndrome (ARDS) often have a prolonged and complicated disease course, which puts them at particular risk for CMV reactivation (19). Although classical risk factors for the development of ARDS include direct triggers of lung injury, such as aspiration of gastric contents and pneumonia, as well as many indirect triggers, such as sepsis and trauma (20), CMV reactivation may play an important contributing role in non-resolving ARDS by sustaining on-going inflammation in the lung (21-23). Both ARDS itself and CMV reactivation are associated with elevations of specific pro-inflammatory cytokines (primarily IL-6 and IL-8), suggesting that CMV may be causally linked to the course of disease following the onset of ARDS (23). Furthermore, the lungs are actively involved in the immunological control of many viral infections (24). Based on previous work on CMV latency, it is very likely that lungs are also involved in the control of CMV infections (25). Hence, the pulmonary pathology associated with ARDS could promote viral reactivation. Histopathological evidence of CMV pneumonia was found in 30% of open-lung biopsies in ARDS patients with negative cultures who showed no respiratory improvement (26).

Because of these observations, it has been proposed to start prophylactic antiviral treatment with (val)ganciclovir in CMV seropositive patients with ARDS, who are generally assumed to be at high risk of reactivation (8). To assess the potential benefit of such approach, we evaluated whether CMV seroprevalence is a risk factor for mortality and a prolonged disease course in ICU patients with ARDS who are mechanically ventilated beyond day 4.

MATERIALS AND METHODS

Patients and measurements

This study was performed as part of a larger multi-center prospective cohort study focused on early diagnosis and risk stratification of patients with sepsis (27). Between January 2011 and March 2013 consecutive patients presenting with ARDS and requiring mechanical ventilation for at least 4 days were included in the mixed ICUs of two tertiary care referral centers in The Netherlands, University Medical Center Utrecht in Utrecht and the Academic Medical Center in Amsterdam. The Ethics Committee of the University Medical Center Utrecht approved an opt-out method of consent (protocol number 10-056C).

ARDS was defined according to the criteria stated by the American-European Consensus Conference on ARDS (28): that is, the diagnosis required an acute onset of symptoms, the presence of bilateral infiltrates on chest radiography, a pulmonary-artery wedge pressure <18 mmHg and/or the absence of signs of left ventricular dysfunction, and a partial pressure of oxygen in arterial blood to fraction of inspired oxygen ratio (P/F) <300. Although our study started in 2011, before the recent 'Berlin' update of the ARDS definition was published (29), we found that only 2% of the study cohort would have been excluded in case we had used the new definitions. In addition to these consensus criteria, patients that were included had to receive mechanical ventilation for at least four days, because the risk of CMV reactivation before that time was deemed negligible (9). Likewise, we excluded patients receiving drugs active against herpes viruses, including (val)ganciclovir, (val)aciclovir, cidofovir and foscarnet, in the week before ICU admission, because they were also considered not to be at risk for CMV reactivation. Furthermore, patients with a known immunodeficiency were excluded because the indication for CMV prophylaxis is well established in most of these cases. Immunodeficiency was defined as a history of solid organ or stem cell transplantation, infection with the human immunodeficiency virus, hematological malignancy, use of immunosuppressive medication (prednisone >0.1 mg/kg for >3 months, prednisone >75 mg/day for >1 week, or equivalent), chemotherapy/radiotherapy in the year before ICU admission, and any known humeral or cellular immune deficiency.

CMV serostatus was determined in a plasma sample obtained between days 5 and 14 in the ICU, using an enzyme immuno assay (Enzygnost CMV/IgG, Siemens Healthcare Diagnostic Products, Marburg, Germany), which was performed according to the instructions of the manufacturer. Subsequently, in seropositive patients a real-time Taqman CMV-DNA polymerase chain reaction was used to determine the viral load in plasma (30). Detection was performed on day 14 or at ICU discharge, whichever came first, and weekly thereafter. Viral load values were calibrated to the CMV WHO Standard (provided by the National Institute for Biological Standards and Control, United Kingdom) and were expressed in international units per milliliter

(IU/mL). CMV reactivation was defined as a load ≥ 100 IU/mL. Screening for CMV serostatus or viral load was not part of routine clinical practice in either hospital. Neither serology nor reactivation results from our study were made available to the treating physicians.

Outcomes

The number of days alive and free of mechanical ventilation on day 28 (ventilator free days; VFDs) was used as the primary outcome in our analyses. We used this composite measure, because increased mortality may falsely decrease the duration of mechanical ventilation in the most severe cases. Successful weaning was defined as liberation from mechanical ventilation for more than two consecutive days. In addition, we analyzed both 28-day mortality and the duration of mechanical ventilation separately. Finally, in the subgroup of survivors beyond day 28 only, we compared the P/F and pulmonary compliance on days 7, 10, 14, 21, and 28 between the two serogroups.

Data analysis

We used multivariable ordinal logistic regression analyses to study the relation between seroprevalence and the primary outcome. To this end, the observed VFDs were collapsed into three ordinal levels: 0, 1-18 and 19-24 days. These categories were chosen in such a way that the worst outcome level represents patients who die or remain mechanically ventilated beyond day 28, whereas the best outcome level represents patients who are successfully weaned from the ventilator within 10 days (and survive beyond day 28).

We checked for the proportional odds assumption by graphical plots, the likelihood ratio test and the score test. We decided *a priori* to adjust for age and ethnicity, because these two variables were known from the literature to be associated with CMV seroprevalence and –most likely– with the primary outcome as well. In contrast to viral reactivation, which is more likely to occur in patients who are more severely ill and/or immunocompromised, seroconversion is not thought to be related to such patient factors. Rather, CMV seroprevalence is simply determined by the cumulative probability of having encountered this virus in the past. Nonetheless, to test the robustness of our analyses, we compared our initial multivariable model to alternative models with adjustment for *a posteriori* selected covariables. We used two approaches, including: (a) covariables that changed the crude effect estimate by more than 10% (change-in-estimate method) or (b) patient characteristics that had an imbalance at baseline with a p value <0.20 (baseline difference method).

We performed prespecified subgroup analyses in patients with sepsis-related ARDS only, in patients with an initial P/F ≤200 only, and in patients with a first ICU admission only. Subsequently, we performed further post hoc analyses in subgroups where a possible signal was observed. All data were analyzed with SAS 9.2 (Cary, NC, USA) and R 2.15.1 software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

In total, 430 patients were admitted with ARDS and received mechanical ventilation for at least four days, 308 of whom fulfilled the study inclusion criteria, and 306 of whom were eventually analyzed (Figure 1). Most patients had severe hypoxemia, with 287 (94%) patients having a P/F $\leq$200 during their first four days in ICU.

CMV seroprevalence was observed in 209 (68%) out of 306 patients. Seropositive patients were older, more frequently suffered from COPD and diabetes, more commonly had a surgical reason for ICU admission, and were less likely to have a history of a prior ICU admission during their hospital stay compared to seronegative patients (Table 1). In the 209 seropositive patients CMV reactivation occurred in 53 (26%) patients. Of these 27 (51%), 15 (28%), and 11 (21%) reached maximum viral loads of 100-1,000, 1,000-10,000 and >10,000 IU/mL, respectively. Observed 28-day mortality in these patients was 28%, compared to 24% and 16% in seropositive patients without reactivation and seronegative patients, respectively.

Table 2 shows the distribution of patients across the three categories of the primary outcome by serostatus. Overall, 100 (33%) patients had a poor clinical outcome (i.e., death or mechanical ventilation beyond day 28). By crude analysis no difference was found between the serogroups for the primary outcome (Odds Ratio (OR) 1.09, 95%

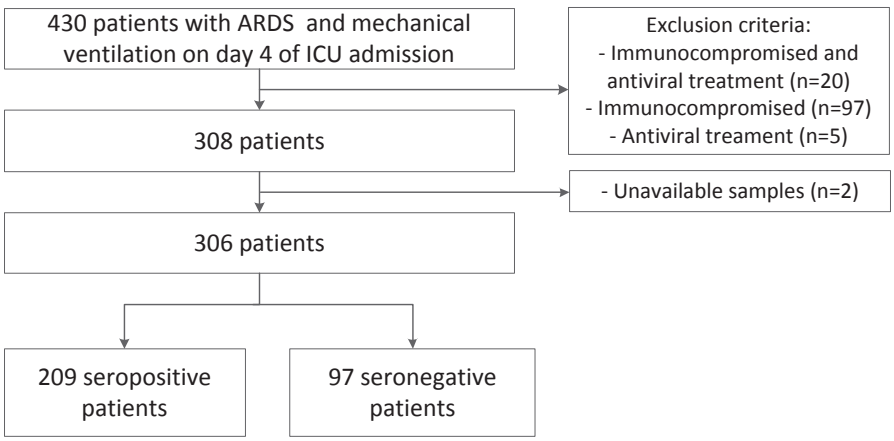

Figure 1. Patient inclusion
ARDS = acute respiratory distress syndrome; ICU = intensive care unit
* An immunocompromised state includes a history of solid organ or stem cell transplantation, human immunodeficiency virus (HIV) infection, hematological malignancy, use of immunosuppressive medication (prednisone 0.1 mg/kg for at least 3 months, prednisone 75 mg/day during at least one week, or equivalent), chemotherapy/radiotherapy in the year preceding intensive care unit admission, and a known humoral or cellular immune deficiency.
** Antiviral treatment against herpes viruses includes use of (val)ganciclovir, (val)aciclovir, cidofovir or foscarnet in the week before intensive care unit admission.

Table 1. Characteristics of patients with ARDS by CMV serostatus

	Seronegative (n=97)	Seropositive (n=209)	p-value
Patient demographics			
Age (years)	61 (51-70)	65 (56-72)	0.01
Male gender	64 (66)	132 (63)	0.63
Non-European descent	2 (2)	27 (13)	0.002
Body Mass Index	25 (23 -29)	26 (23 -29)	0.81
Prior ICU admission during hospital stay	19 (20)	26 (12)	0.10
Surgical reason for admission	26 (27)	78 (37)	0.07
Chronic comorbidities			
Chronic obstructive pulmonary disease	7 (7)	31 (15)	0.06
Congestive heart failure	3 (3)	8 (4)	1.00
Diabetes mellitus	12 (12)	26 (12)	0.99
Cancer	14 (14)	27 (13)	0.72
Renal insufficiency	7 (7)	18 (9)	0.68
Disease severity at ARDS onset [a]			
APACHE IV score	79 (63- 97)	81 (65-104)	0.42
Sequential Organ Failure Assessment score	10 (8 – 13)	10 (8 -13)	0.79
C-Reactive Protein	249 (155 – 338)	243 (142 – 310)	0.51
Lactate	3.0 (1.8 – 5.6)	3.5 (1.9 – 5.8)	0.33
Respiratory markers at ARDS onset [a]			
3 or 4 quadrants with consolidation	58 (60)	129 (62)	0.75
Pulmonary compliance (mL/cmH2O)	38 (26 – 48)	39 (28 - 47)	0.82
Positive end-expiratory pressure (cm H_tO)	12 (10 – 15)	12 (8 – 15)	0.40
PaO_2/FiO_2	111 (78 – 152)	104 (82 – 150)	0.98
Tidal volume (mL/kg bodyweight) [b]	6.2 (5.8 – 7.1)	6.4 (5.5 – 7.5)	0.98
Etiology of ARDS [c]			
Sepsis	79 (81)	165 (79)	0.66
Pneumonia	45 (46)	112 (54)	0.24
Aspiration of gastric content	14 (15)	20 (10)	0.19
Recent trauma or major surgery	16 (17)	32 (15)	0.75
Transfusion of blood products <6h before ARDS onset	3 (3)	18 (9)	0.08
Acute neurologic injury	6 (6)	11 (5)	0.72
Therapeutic interventions for ARDS			
Prone positioning	20 (21)	40 (19)	0.76
High dose corticosteroid therapy	12 (12)	29 (14)	0.72

Data are presented as medians (interquartile range) or absolute numbers (%).
APACHE = Acute Physiology and Chronic Health Evaluation; ARDS = acute respiratory distress syndrome;
[a] The numbers represent the worst observed values before day 4 in ICU (except for APACHE IV score, which represents day 1 only)
[b] Tidal volume represents the average tidal volume during the first four days in ICU
[c] Patients may have multiple concurrent risk factors for the etiology of ARDS.

confidence interval (CI) 0.70-1.70). In patients who survived beyond day 28, the median duration of mechanical ventilation was 12 versus 14 days (p=0.11) in the seropositive and seronegative groups, respectively. In addition, there were no discernable differences in P/F and pulmonary compliance on days 7, 10, 14, 21, and 28 in these patients (Table 3).

Table 2. Primary outcome by CMV serostatus

Ventilator-free days [a]	Seronegative (n=97)	Seropositive (n=209)
0 [b]	28 (29)	72 (34)
1-18	36 (37)	63 (30)
19-24	33 (34)	74 (35)

Data are presented as absolute numbers (%).
[a] The number of days alive and free of mechanical ventilation on day 28
[b] In the worst outcome category (0 ventilator-free days), there were 67 deaths and 33 survivors who were mechanically ventilated beyond day 28.

Table 3. Time course of pulmonary markers by CMV serostatus in 28-day ARDS survivors

Day	Seronegative (n=82)	Seropositive (n=157)	p-value
Patients with compliance < 60 mL/cmH_2O			
1-4	63 (77)	117 (75)	0.69
7	28 (34)	50 (32)	0.72
10	23 (28)	38 (24)	0.52
14	19 (23)	29 (18)	0.39
21	12 (15)	20 (13)	0.68
28	13 (16)	15 (10)	0.15
Patients with PaO_2/FiO_2 < 200			
1-4	78 (95)	146 (93)	0.52
7	25 (30)	45 (29)	0.77
10	17 (21)	29 (18)	0.67
14	11 (13)	18 (11)	0.66
21	3 (4)	15 (10)	0.10
28	5 (6)	6 (4)	0.43

Data are presented as absolute numbers (percentages)

After adjustment for *a priori* selected confounders CMV seroprevalence was not associated with the primary outcome (adjusted OR 1.01, 95% CI 0.64-1.59) (Table 4). When we analyzed the primary outcome by its separate components we also found non-significant results (adjusted OR 1.45, 95% CI 0.76-2.76 for death, and OR 0.70, 95% CI 0.44-1.11 for duration of mechanical ventilation, respectively). The two alternative models including *a posteriori* selected confounders (change-in-estimate method and baseline difference method) yielded similar results (adjusted OR 1.01, 95% CI 0.62-1.64 and 0.98, 95% CI 0.61-1.57, respectively). These secondary analyses additionally included APACHE IV score, SOFA score on day 4, surgical reason for admission, COPD, diabetes mellitus, presence of sepsis at admission, pneumonia, aspiration of gastric content, prior ICU admission during hospital stay, and transfusion of blood products in one of the two or both models.

In addition, we found no statistically significant effects of CMV seroprevalence in any of the pre-specified subgroup analyses, although there was a trend to worse outcome in patients with infection-related ARDS (Table 4). Post hoc analyses revealed that this effect was more prominent in patients who had developed ARDS in a setting of septic shock (adjusted odds ratio 2.86, 95% CI 1.32-6.23).

Table 4. Associations between CMV seroprevalence and the primary outcome in subgroups of ARDS patients

		N	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI) [a]
All patients		306	1.09 (0.70 – 1.70)	1.01 (0.64 – 1.59)
Infection-related ARDS		244	1.54 (0.94 – 2.52)	1.47 (0.87 – 2.46)
- Disease stage[b]	Severe sepsis	124	0.82 (0.41 – 1.64)	0.83 (0.40 – 1.70)
	Septic shock	120	3.28 (1.56 – 6.91)	2.86 (1.32 – 6.23)
- Source[b]	Pneumonia	157	1.45 (0.77 – 2.75)	1.47 (0.75 – 2.88)
	Other infections	87	1.67 (0.75 – 3.72)	1.48 (0.65 – 3.36)
ARDS with an initial PaO_2/FiO_2 <200		287	1.09 (0.69 – 1.71)	1.01 (0.63 – 1.61)
Direct ARDS (pulmonary cause) [b]		171	1.29 (0.71 – 2.34)	1.29 (0.69 – 2.42)
First ICU admissions only		261	1.07 (0.66 – 1.75)	0.98 (0.59 – 1.63)

The primary outcome in all analyses was the number of days alive and free of mechanical ventilation on day 28, collapsed into three ordinal levels.
ARDS = acute respiratory distress syndrome; ICU = intensive care unit;
[a] Adjusted for age and ethnicity; [b] Post hoc analysis

DISCUSSION

Our study showed that CMV seroprevalence is not associated with a more complicated disease course or worse clinical outcome in critically ill patients with ARDS who are immunocompetent. In addition, we did not detect an association in any of the pre-specified subgroups, with a possible exception for ARDS patients who present with septic shock. Taken together, these results suggest that CMV prophylaxis in unselected patients with ARDS who test seropositive is unlikely to be effective.

These negative findings may hint at the conclusion that CMV reactivation in ARDS patients represents an 'innocent bystander' effect, rather than a cause of harm. However, it is important to stress that –despite our explicit selection of a study population that was considered to be at very high risk– actual CMV reactivation during ICU admission occurred only in 26% of patients. This may have negated any potentially deleterious effects of viral reactivation. On the other hand, when considering prophylaxis, we should stress that (val)ganciclovir treatment may be associated with adverse effects (including pancytopenia resulting from bone marrow suppression), interaction with other drugs and high costs. Thus, before experimentally evaluating antiviral prophylaxis in ICU patients with ARDS, it is necessary to select subgroups of patients who are at even higher risk of reactivation in order to maximize the cost-benefit ratio of such therapy. ARDS patients with concurrent septic shock may possibly represent a suitable target population. However, we need to emphasize that the poor outcome that was observed in latent carriers of the virus in this particular subgroup was only a post hoc finding and thus requires confirmation by others before any intervention should be considered.

Our results in ARDS patients extend previous findings by De Vlieger et al, who demonstrated that serostatus alone is not a risk factor for morbidity and mortality in a general ICU population (31). In our current study we specifically focused on critically ill patients with ARDS who were on prolonged mechanical ventilation, as they are considered to be at highest risk of viral reactivation, and pneumonitis is a serious manifestation of CMV infection that is associated with high mortality (4). The observed overall reactivation rate of 26% in our study population falls within the range of reported incidences in other studies that have focused on a more heterogeneous group of ICU patients or those with severe sepsis (5-7). A recent systematic review reported CMV reactivation rates of up to 36% in critically ill seropositive patients with an ICU stay of more than four days (32). Our study differs from the individual studies in this review in that we included a more homogenous group of patients that was supposedly at even higher risk for viral reactivation. The reason that we found somewhat lower reactivation rates than we expected beforehand may lie in the fact that many earlier studies have reported CMV reactivation rates that included the entire hospital stay, whereas we exclusively focused on reactivations occurring during mechanical ventilation (since this would be the potential target for prophylaxis in the ICU).

Although we have identified the patients with CMV reactivation, comparing patients with and without CMV reactivation requires caution. The observed crude differences in 28-day mortality between both groups should not be causally interpreted because of the presence of time-dependent bias (i.e., CMV has less opportunity to reactivate in patients who are discharged early compared to patients who stay longer in the ICU) and many possible confounders disturbing the association between reactivation and clinical outcome.

Ours is the first study that assessed whether CMV seroprevalence (i.e., latent or active infection with CMV) contributes to the outcome in patients with ARDS. The study was nested within a large, ongoing data collection initiative that includes consecutive patients, which suggests that the potential for selection bias was low. Furthermore, all ARDS events were prospectively diagnosed by dedicated well-trained observers (27), which minimized potential information bias. However, our study was underpowered to detect very small but potentially meaningful differences between both serogroups. Based on our current results we could nonetheless exclude with 95% certainty the existence of an absolute risk difference of 9% or more between any of the three levels of the primary outcome. Based on the distribution of our crude (ordinal) outcomes, using the most favourable assumptions, we estimate that an intervention trial in a similar patient population would require at least 570 patients per group to show a risk difference of 5%.

CONCLUSIONS

CMV seroprevalence is not associated with prolonged mechanical ventilation or mortality in critically ill patients presenting to the ICU with ARDS. A prevention strategy targeting unselected seropositive patients with ARDS is therefore unlikely to show any meaningful benefit. Better identification of subgroups of patients who are either exposed to an increased risk of CMV reactivation and/or in whom reactivation will result in more detrimental effects on clinical outcomes is needed before antiviral prophylaxis should be considered in ARDS patients. Further studies may focus on ARDS patients with concurrent septic shock, in which the potential for benefit of prophylactic antiviral treatment seems to be greatest.

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5

CYTOMEGALOVIRUS REACTIVATION AND MORTALITY IN PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME

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ABSTRACT

Purpose

Cytomegalovirus (CMV) reactivation occurs frequently in patients with the acute respiratory distress syndrome (ARDS) and has been associated with increased mortality. However, it remains unknown whether this association represents an independent risk for poor outcome. We aimed to estimate the attributable effect of CMV reactivation on mortality in immunocompetent ARDS patients.

Methods

We prospectively studied immunocompetent ARDS patients who tested seropositive for CMV and remained mechanically ventilated beyond day four in two tertiary intensive care units (ICUs) in the Netherlands from 2011 to 2013. CMV loads were determined in plasma weekly. Competing risks Cox regression was used with CMV reactivation status as a time-dependent exposure variable. Subsequently, in sensitivity analyses we adjusted for the evolution of disease severity until onset of reactivation using marginal structural modeling.

Results

Of 399 ARDS patients, 271 (68%) were CMV seropositive and reactivation occurred in 74 (27%) of them. After adjustment for confounding and competing risks, CMV reactivation was associated with overall increased ICU mortality (adjusted subdistribution hazard ratio (SHR) 2.74, 95% CI 1.51-4.97), which resulted from the joint action of trends towards an increased mortality rate (direct effect; cause specific hazard ratio (HR) 1.58, 95% CI 0.86-2.90) and a reduced successful weaning rate (indirect effect; cause specific HR 0.83, 95% CI 0.58-1.18). These associations remained in sensitivity analyses. The population-attributable fraction of ICU mortality was 23% (95% CI 6-41) by day 30 (risk difference 4.4%, 95% CI 1.1-7.9).

Conclusion

CMV reactivation is independently associated with increased case fatality in immunocompetent ARDS patients who are CMV seropositive.

INTRODUCTION

Although the burden of cytomegalovirus (CMV) disease has been well established in immunocompromised patients (1), CMV viremia has also been described in intensive care unit (ICU) patients without known prior immune deficiency. This almost exclusively results from systemic viral reactivation, and incidence rates of up to 40% have been reported in critically ill CMV seropositive subjects (2-9). Furthermore, CMV reactivation in critically ill patients has been associated with a prolonged duration of mechanical ventilation (2, 4, 9-13), an increased length of stay in the ICU (3, 5, 9, 10, 13), and excess mortality (2, 4, 7-9). Nevertheless, it remains uncertain whether these findings imply that CMV reactivation is a truly independent risk factor with respect to these observed poor clinical outcomes because most studies that have assessed these associations did not adequately account for all possible sources of bias. As a consequence, CMV viremia might merely be a marker of illness severity, contributing only little to the overall burden of disease.

To achieve an accurate estimation of the true effect of CMV reactivation on clinical outcome, it is crucial in observational studies to adjust for the time-dependent occurrence of CMV reactivation and the evolution of disease severity prior to its onset. Moreover, the presence of competing events should be taken into account when follow-up time is censored (14). For instance, when ICU mortality is the outcome, then ICU discharge is a competing risk that prohibits the event of interest from occurring first.

Patients with the acute respiratory distress syndrome (ARDS) often have a long and complicated disease course in the ICU, which portends a particular risk for viral reactivations (15, 16). Despite the uncertainties regarding the clinical relevance of CMV disease in immunocompetent critically ill patients, it is etiologically plausible that virus reactivation adds to the pulmonary pathology in patients with ARDS. In experimental murine studies, CMV reactivation caused exacerbated and prolonged cytokine and chemokine expression in lung tissues, which eventually led to increased pulmonary fibrosis compared to controls (17). In a clinical study of open lung biopsies in ARDS patients with prolonged respiratory failure or in whom microbiological cultures remained negative, CMV pneumonia was found in 30% of cases (18). Both findings suggest that CMV-related pulmonary pathology may be causally linked to the clinical disease course following ARDS onset, especially in the most severely ill patients who require prolonged mechanical ventilation. If CMV reactivation does contribute to poor clinical outcome in these patients, either prophylaxis or pre-emptive therapy with (val) ganciclovir may be considered.

The aim of this study was to estimate the proportion of deaths that can be attributed to systemic reactivation of CMV in ARDS patients who are latent carriers of the virus. Some results of this study have been previously reported in the form of an abstract (19).

METHODS

Patients and measurements

The present study was conducted within the framework of the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) cohort (clinicaltrials.gov identifier: NCT01905033) for which the Institutional Review Board approved an opt-out method of informed consent (protocol number 10-056C) (20). We prospectively included consecutive adults who presented with ARDS to the mixed ICUs of two tertiary care hospitals in the Netherlands between January 2011 and December 2013 and required mechanical ventilation beyond day 4 of ICU admission. Since data collection for our study started before publication of the 'Berlin' definition in 2012, ARDS was defined according to the American-European Consensus Conference criteria (21): that is, the diagnosis required an acute onset of symptoms, the presence of bilateral infiltrates on chest radiography, a pulmonary artery occlusion pressure less than 18 mm Hg and/or the absence of left ventricular dysfunction, and PaO_2/FiO_2 ratio (P/F) less than 300. We excluded patients who had received (val)ganciclovir, (val)acyclovir, cidofovir or foscarnet in the week before ICU admission and those with known immunodeficiency (16). Immunodeficiency was defined as a history of solid organ or stem cell transplantation, infection with the human immunodeficiency virus, hematological malignancy, use of immunosuppressive medication (prednisone >0.1 mg/kg for >3 months, prednisone >75 mg/day for >1 week, or equivalent), chemotherapy/radiotherapy in the year before ICU admission, and any known humoral or cellular immune deficiency.

Leftover plasma, which was harvested from blood samples obtained daily as part of routine patient care, was stored at -80°C within 4 hours after blood draw. CMV serostatus was determined by an enzyme immunoassay (Enzygnost CMV/IgG, Siemens Healthcare Diagnostic Products, Marburg, Germany). Subsequently, in seropositive patients only, viral loads in plasma were determined by real-time Taqman CMV-DNA polymerase chain reaction (22). CMV loads were determined on a weekly basis for a maximum of 30 days following study inclusion (i.e., day 5 of ICU admission). For intermediary days, on which quantitative PCR was not performed, we estimated viral loads by log-linear imputation. CMV reactivation was defined as a viral load $\geq$100 international units per milliliter (IU/mL), as calibrated according to the CMV World Health Organization (WHO) Standard. Screening for CMV was not part of routine clinical practice in either participating hospital. Neither serology results nor viral loads measured as part of our study were made available to the treating physicians, and none of the included patients therefore received antiviral treatment directed against CMV.

Mortality was the outcome of primary interest in this study and was defined as death on mechanical ventilation before day 35 (i.e., day 30 following study inclusion). Successful weaning, which is a competing event of the primary outcome, was defined as complete liberation from mechanical ventilatory support on two or more consecutive days before day 35. We considered distal endpoints more likely to be amenable by

pre-existing comorbidities, as well as specific end-of-life practices, bed availability and other local factors. Nonetheless, in a subsequent sensitivity analysis, we used discharge and death in ICU as alternative endpoints.

Data analysis

For our primary analyses we used Cox proportional hazards modeling, in which mortality and successful weaning were considered as competing events and CMV reactivation status was fitted as a time-dependent variable. Possible confounders that were screened included all patient characteristics and therapeutic interventions listed in Table 1, and some markers of disease severity: Acute Physiology and Chronic Health Evaluation (APACHE) IV score, presence of septic shock, partial pressure of oxygen in arterial blood to fraction of inspired oxygen ratio, and positive end expiratory pressure (PEEP) setting. To account for possible confounding, we included baseline covariables that showed differences between the reactivated and non-reactivated groups at a p-value of <0.30, and changed the crude effect estimates for either mortality or weaning by >10%. We included only the strongest (possible) confounders by using these two criteria combined in order to avoid statistical overfitting (i.e., incorporating too many variables given the limited number of events).

The two possible outcomes are interrelated as increased mortality may negatively impact the duration of mechanical ventilation. A competing risks analysis accommodates for this by providing two measures of association. First, the cause-specific hazard ratio (CSHR) estimates the direct effects of CMV reactivation on each outcome of interest (i.e., mortality on the ventilator and successful weaning). Second, the subdistribution hazard ratio (SHR) estimates the risk of dying from reactivation at a given time-point, while accounting for the competing risk of successful weaning. To obtain direct estimates of cumulative risks in terms of the SHR we used the Fine and Gray model (23). Finally, to estimate the population-attributable fraction of mortality due to CMV reactivation, we used a multi-state model (Fig. S1), which accounts for the time of reactivation (24). Confidence intervals were calculated by bootstrap resampling (25, 26).

Despite these efforts to accurately assess the effect of CMV reactivation on clinical outcomes residual confounding may still remain, because markers of illness at baseline (which we included in all multivariable analyses) may no longer be representative of the disease state at the time of reactivation onset. Thus, we performed a sensitivity analysis using marginal structural modeling to adjust for the evolution of disease severity prior to the onset of CMV reactivation (see also Supplementary Material) (27, 28). Such analysis first involves estimation of the daily probabilities of CMV reactivation using a multivariable logistic regression model that includes markers of disease severity on a daily basis. These probabilities are used to calculate an inversed probability weight that is then included as a summary measure of all relevant covariables in the final Cox regression model. However, because marginal structural modeling requires

many assumptions that are difficult to be checked, we considered this a sensitivity analysis only.

Data were analyzed with SAS 9.2 (Cary, NC, USA) and R 2.15.1 software (R Foundation for Statistical Computing, Vienna, Austria; packages "etm", "mstate", "ipw").

RESULTS

We enrolled 544 patients with ARDS who required mechanical ventilation for more than four days (Fig. S2). Of these 143 were excluded because of known prior immunocompromise or antiviral treatment and two were excluded because of missing samples. Subsequently, 271 (68%) patients tested seropositive for CMV and were thus included in the study. ARDS was of primary pulmonary origin in 158 (58%) of these cases, whereas the remainder was of secondary etiology (e.g., associated with non-pulmonary sepsis, major surgery, or blood transfusion).

CMV reactivation

CMV reactivation occurred in 74 (27%) of the included patients (Table 1). These patients more frequently had –at the time of ICU-admission– concurrent septic shock, higher APACHE IV scores, and renal insufficiency compared to patients who never had CMV reactivation. In addition, a larger proportion of these patients were receiving high-dose corticosteroid therapy during the first days in ICU. The median time from ICU admission to onset of reactivation was 8.5 days (interquartile range (IQR) 4-11). Within the subgroup of patients acquiring CMV reactivation the proportion of individuals having relatively high viral loads (≥1000 IU/mL) increased over time (Fig. 1). In a patient population that is selected by an ICU stay of at least five weeks, the proportion with CMV viremia is as high as 14 of 23 patients (61%).

Clinical outcomes

On day 30 after study inclusion (this was 35 days following ICU admission) 52 (19%) patients had died, 209 (77%) were successfully weaned, and 10 (4%) remained still on mechanical ventilation (Table 2). In crude analyses, patients with CMV reactivation had both a longer duration of mechanical ventilation (15 (IQR 10-26) versus 8 (IQR 6-12) days; $p<0.01$) and higher mortality (23 of 74 (31%) versus 29 of 197 (15%) patients; $p<0.01$) compared to subjects without reactivation.

Table 3 shows the results of the various Cox survival regression analyses. Baseline variables associated with reactivation status (at $p<0.30$) which changed the crude effect estimate by more than 10% included the APACHE IV score, use of high dose corticosteroid therapy, and PEEP setting.

In the primary multivariable adjusted analysis, CMV reactivation was no longer statistically associated with either increased mortality or a reduced rate of successful weaning. However, simultaneous effects on both the daily rates of death and weaning

Table 1. Characteristics of ARDS patients by CMV reactivation status

	Reactivation (n=74)	Non-reactivation (n=197)	p-value
Patient characteristics			
Age (years)	64 (56-74)	64 (54-72)	0.50
Male gender	44 (59)	120 (61)	0.83
Non-European descent	13 (18)	27 (14)	0.42
Prior ICU admission during hospital stay	16 (22)	24 (12)	0.05
Surgical reason for admission	22 (30)	73 (37)	0.26
Chronic obstructive pulmonary disease	13 (18)	30 (15)	0.64
Congestive heart failure	2 (3)	9 (5)	0.49
Diabetes mellitus	14 (19)	26 (13)	0.24
Cancer	9 (12)	28 (14)	0.66
Renal insufficiency	12 (16)	11 (6)	<0.01
Markers of disease severity			
APACHE IV score	91 (71- 113)	76 (62-99)	<0.01
Septic shock	42 (57)	81 (41)	0.02
Plasma lactate	3.6 (1.9-6.5)	3.5 (1.9-5.8)	0.47
C-Reactive Protein	244 (129-310)	241 (151-309)	0.99
≥3 quadrant consolidation	49 (66)	121 (61)	0.47
Tidal volume (mL/kg bodyweight)	6.5 (5.5-7.6)	6.5 (5.6-7.8)	0.72
PEEP setting (cm H_2O)	12 (10-15)	10 (8-15)	0.16
PaO_2/FiO_2	107 (77-150)	106 (82-149)	0.41
Pulmonary compliance (mL/cmH_2O)	35 (24-47)	39 (27-49)	0.15
Therapeutic interventions			
High dose corticosteroid therapy	48 (65)	101 (51)	0.05
Transfusion of blood products	15 (20)	18 (9)	0.18

Data are presented as medians (interquartile range) or absolute numbers (%). P-values were calculated by nonparametric tests and Chi-square tests, respectively.
Markers of disease severity represent the worst values observed during the first four days in ICU, except for tidal volume, which represents the mean value. Therapeutic interventions relate to the first four days in ICU only. High dose corticosteroid was defined as a daily dose of >250 mg hydrocortisone or equivalent.
APACHE = Acute Physiology and Chronic Health Evaluation; ARDS = acute respiratory distress syndrome; PEEP = Positive End Expiratory Pressure.

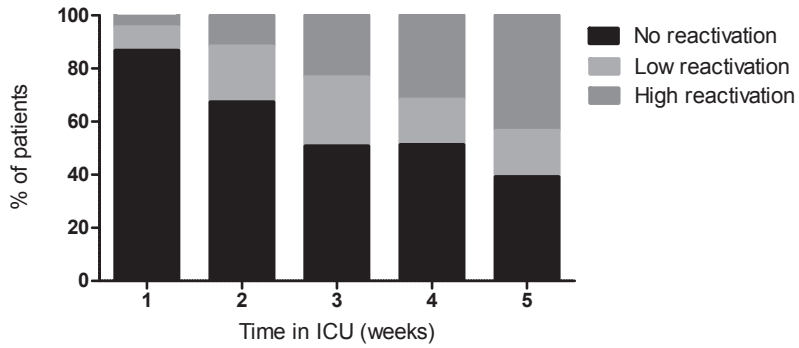

Figure 1. Viral load in CMV seropositive ARDS patients over time
The quantitative PCR results were calibrated according to the CMV WHO standard; viral loads greater than or equal to 1000 IU/mL were denoted 'high reactivation'. Viral loads of 100-999 IU/mL were denoted 'low reactivation', and undetectable loads or viral loads <100 IU/mL were denoted 'no reactivation'.

Table 2. Crude clinical outcomes of ARDS patients by CMV reactivation status

	Reactivation	Non-reactivation	p-value
Death on ventilator before day 30*	23/74 (31)	29/197 (15)	<0.01
Death in ICU#	26/76 (34)	32/195 (16)	<0.01
Death by day 90#	35/76 (46)	55/195 (28)	<0.01
Duration of mechanical ventilation (days)	15 (10-26)	8 (6-12)	<0.01
Length of stay in ICU (days)	16 (11-28)	9 (7-14)	<0.01

Data show absolute numbers (%) or medians (interquartile range).
* Primary study endpoint; # CMV reactivation occurred in two additional patients after successful weaning in the ICU; therefore, 76 instead of 74 patients were considered exposed for analyses with more distal endpoints (death in ICU, death by day 90).

did reveal a significant association with overall mortality when competing risks were accounted for (SHR 2.74, 95% CI 1.51-4.79). As a post hoc sensitivity analysis, we then used marginal structural modeling to assess potential residual confounding by differences in the evolution of disease severity prior to CMV reactivation between both groups, but found very similar results (Table 3). Changing the definitions of our primary endpoints to include all deaths in the ICU (irrespective of mechanical ventilation status) and discharge (rather than successful weaning) also did not change these findings (Table S1). Furthermore, the independent association with mortality remained among subgroups of patients receiving and not receiving high dose corticosteroid therapy; SHR 2.60 (95% CI 1.29-5.25) and 3.61 (95% CI 1.24-10.48), respectively (Table S2). Corticosteroids were mostly used for the treatment of concurrent septic shock (121 of 149 cases).

Table 3. Associations between CMV reactivation and clinical outcome

Analysis	Successful weaning (CSHR)	Death on mechanical ventilation (CSHR)	Death on mechanical ventilation (SHR)
Crude model with adjustment for - time-varying onset of CMV reactivation	0.81 (0.58-1.13)	1.75 (1.01-3.01)	3.39 (1.96-5.87)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances *	0.83 (0.58-1.18)	1.58 (0.86-2.90)	2.74 (1.51-4.97)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances * - evolution of disease prior to onset of CMV reactivation **	0.93 (0.66-1.31)	1.49 (0.78-2.85)	2.48 (1.32-4.66)

Data are presented as hazard ratios with 95% confidence intervals. The cause-specific hazard ratio (CSHR) estimates the direct effect of CMV reactivation on clinical outcome (i.e., successful weaning or death on mechanical ventilation). The subdistribution hazard ratio (SHR) is a summary measure of both separate cause-specific hazards and estimates the overall risk of dying from CMV reactivation while taking into account the competing event of successful weaning.
* APACHE IV score, use of high dose corticosteroid therapy, and PEEP setting.
** Time-dependent covariables included the Risk, Injury, Failure, Loss and End-stage kidney disease (RIFLE) score, Sequential Organ Failure Assessment (SOFA) score, presence of septic shock, and use of high dose corticosteroid therapy, which were all measured on a daily basis until 24 hours prior to reactivation onset.

Figure 2 shows the predicted mortality in a hypothetical population of ARDS patients when all CMV reactivation would be prevented, compared to true (observed) mortality in the study population. The population-attributable fraction of ICU mortality due to CMV reactivation was estimated at 23% (95% CI 6-41%) by day 30, which translates into an absolute mortality difference of 4.4% (95% CI 1.1-7.9).

In order to explore possible causal pathways for the observed association between CMV reactivation and death, we performed a post hoc descriptive analysis of the trajectories of organ dysfunction, pulmonary and inflammatory markers over time following reactivation. In short, we compared the 74 patients having CMV reactivation with 74 non-exposed patients who were matched on baseline characteristics and their length of stay in ICU at the onset of reactivation (Table S3). In summary, the total burden of organ dysfunction was slightly higher in patients at the start of CMV reactivation compared to matched non-exposed control subjects, although individual markers of pulmonary dysfunction and inflammation were similar. More importantly, there was a clear trend towards resolution of organ dysfunction over time in non-exposed subjects that was less pronounced in patients having CMV reactivation. However, it should be emphasized that these findings should be interpreted very carefully because of the presence of informative censoring (i.e., patients who die or get discharged do not further contribute to average scores on the group level).

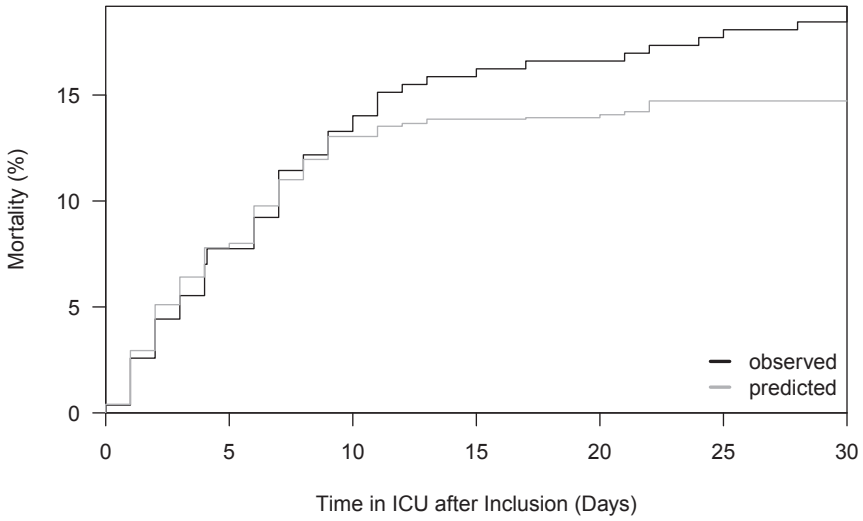

Figure 2. Observed versus predicted ICU mortality in CMV seropositive ARDS patients
The black line represents the observed ICU mortality in the study cohort of 271 CMV seropositive patients; the grey line represents the predicted ICU mortality if all cases of CMV reactivation in the cohort would have been prevented. The population-attributable fraction of ICU mortality was 23% by day 30 (absolute risk difference 4.4%).

DISCUSSION

CMV reactivation in ARDS patients increased the overall risk of death on the ventilator through the combined effect of subtle alterations in both the daily rates of death and successful weaning. After accounting for multiple sources of confounding, the absolute mortality that can be attributed to CMV reactivation was estimated to be 4.4% by day 30 following study inclusion.

Previous findings of excess mortality have triggered debate whether antiviral prophylaxis should be used (29, 30). However, a greater understanding of pathophysiology and clinical risk factors is necessary to select the optimal target population for such strategies. In our study, reactivation rates were 27% in ARDS patients overall and 34% among those with concurrent septic shock. The latter finding might be explained by the increased severity and duration of immune suppression that may be observed in patients with septic shock, including a pronounced depletion of T cells (31, 32). Indeed, a recent study investigating the potential use of antiviral prophylaxis based on the screening of ARDS patients for CMV seroprevalence found that such a strategy is unlikely to be beneficial overall, but suggested a possible benefit in a post hoc subgroup of patients with septic shock (16). As the proportion of patients with CMV reactivation increased in time, altering the minimal length of stay in the ICU as a criterion may also improve the selection of a high-risk target population. Until then, a pre-emptive treatment strategy (by which patients would be screened for CMV

and treated only if reactivation occurs) seems more attractive because the number of patients exposed to the toxicity of (val)ganciclovir would be reduced by 73%. However, the effects of pre-emptive compared to prophylactic treatment on relevant patient outcomes are most likely lower, as treatment is initiated only after reactivation has already begun. Intervention trials comparing prophylaxis, pre-emptive treatment, and wait-and-see strategies are necessary before any evidence-based recommendations regarding the clinical management of CMV reactivation in critically ill patients with ARDS can be made.

Our study has several strengths. First, observations were nested within a large prospective data collection initiative that included consecutive patients, thereby minimizing selection bias (20). All ARDS events were diagnosed by dedicated trained observers, which minimizes information bias. Moreover, we used a highly sensitive method of quantitative real-time PCR for CMV detection. Most importantly, we used advanced methodologies to account for both competing risks and time-dependent information in an attempt to produce unbiased estimates of the independent association between CMV reactivation and clinical outcome.

This methodological approach was mainly necessary because of two reasons. First, Cox regression analysis requires that censoring of survival time must be non-informative, but in our study this was clearly not the case since ARDS patients who are weaned and discharged from the ICU alive are in a better health state than those who remain on the ventilator beyond that time point (33, 34). Furthermore, when ICU mortality is the event of interest, then discharge must be regarded as a competing event as it precludes this outcome from being observed (14). The use of the subdistribution hazard model provides a general solution to this informative censoring. Second, the median time to CMV reactivation in our cohort was 8.5 (IQR 4-11) days. If ignored, such delays may cause distortion (termed immortal time bias) as non-exposed time observed before the onset of reactivation will be wrongfully attributed to the exposed time at risk, resulting in underestimation of effects associated with CMV reactivation (35, 36). Time-dependent fitting of CMV reactivation status in our regression models resolved this issue.

Our study also has several limitations. First, even the use of advanced methodology cannot rule out the possibility of unmeasured confounding in an observational study. Therefore, it remains somewhat uncertain whether the excess mortality that we observed can be fully attributed to CMV reactivation, or whether other unknown factors —including other viral reactivations (8, 37)— may also be involved. Second, the principle of multivariable analysis to adjust for confounders is to statistically 'force' exposed and non-exposed patients to be similar in all aspects of disease aside from their reactivation status. However, in a dynamic ICU setting, during which critically ill patients continuously deteriorate and improve over time, it is very difficult to verify whether such adjustment was successful. We performed marginal structural modeling as a sensitivity analysis to assess the possible impact of variations in the evolution of disease severity

between patients on our effect estimates, yet found very similar results as in our primary analysis. Third, we measured systemic CMV reactivation in plasma but did not collect information about concurrent viral loads in the lungs. This study, therefore, provides no insight into either the prevalence or relevance of pulmonary CMV reactivations. Of note, previous studies have shown that pulmonary reactivation may occur without the concurrent viremia (4, 38, 39). Furthermore, we focused exclusively on the occurrence of reactivation while patients were on mechanical ventilation (primary analysis) or in the ICU (sensitivity analysis), as we considered these to be the most relevant time windows to potentially treat or prevent CMV reactivation in the ICU. However, because of this deliberate focus we cannot provide information about possible episodes of reactivation that may have occurred later. Likewise we did not investigate the occurrence of reactivations after day 35 in the ICU. Thus, this study only provides insight into the short-term effects of systemic CMV reactivation in ARDS patients in settings in which screening or antiviral prophylaxis is not part of routine clinical practice.

In conclusion, systemic reactivation of CMV in immunocompetent ARDS patients is common and independently associated with death in the ICU. These findings support the need for future studies to better predict CMV reactivation as well as to evaluate the efficacy of treatment strategies directed against CMV reactivation in these patients.

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SUPPLEMENTARY MATERIAL

Viral load measurement

CMV load was first analyzed by PCR in a plasma sample taken on day 14 (or in the last available sample if a patient was censored earlier). If no reactivation was detected in that sample we assumed that no viral reactivation had occurred (and consequently had been cleared) before that time. However, when viral load was detectable then plasma samples taken on earlier days were also analyzed in order to determine the time of CMV reactivation more precisely. In addition, PCR's were performed weekly from day 14 onwards. Subsequently, we imputed viral loads in between sample dates using log-linear imputation, because of non-linear dynamics (1). Please see example below which illustrates this imputation process for an individual patient.

Example showing log-linear imputation of CMV loads:

Day in ICU	PCR result (IU/mL)	Imputed viral load (IU/mL)	Reactivation status (>100 IU/mL)
1	0		0
2		0	0
3		0	0
4		0	0
5 (=study entry)		0	0
6		0	0
7	0		0
8		33	0
9		54	0
10		89	0
11		148	1
12		245	1
13		405	1
14	668		1
15		721	1
16		778	1
17		839	1
18		906	1
19		978	1
20		1056	1
21	1140		1

Multi-state modeling

Because the occurrence of CMV reactivation is heavily time-dependent, a simple comparison of two survival curves (one representing patients who show ever reactivation and the other representing controls who never show reactivation) is misleading since none-exposed time before the onset of reactivation would then be falsely attributed to the exposed time at risk. Furthermore, a basic assumption of survival analysis is that censoring of follow-up time (e.g., due to the occurrence of death or discharge) must be non-informative. However, in ICU settings this condition is likely not met, as patients who are discharged alive at a certain point in time are clearly in a better health state than patients who remain in the ICU beyond that time point. A competing risk model may be used to overcome this problem, but such model does not result in a direct probability model for mortality if the variable of interest is time dependent, as in our case (2).

Therefore we had to use a multi-state model (as shown in Figure S1), in which CMV reactivation was explicitly modeled as an intermediate state, to assess attributable mortality in an unbiased way (3). Based on the transition probabilities from one state to another that were derived from this model at each time point, a hypothetical situation in case all CMV reactivation in the study population would have been prevented was constructed (Figure 3). The absolute risk difference on day 30 could then be estimated from the difference between the observed mortality in the entire study population (depicted by the solid-black line) and the predicted mortality if all CMV reactivations had been prevented (depicted by the grey line). Subsequently, the population-attributable fraction was calculated by dividing this risk difference by the observed mortality at day 30. This fraction can be interpreted as an estimation of the proportion of patients who died in the ICU by day 30 due to CMV reactivation.

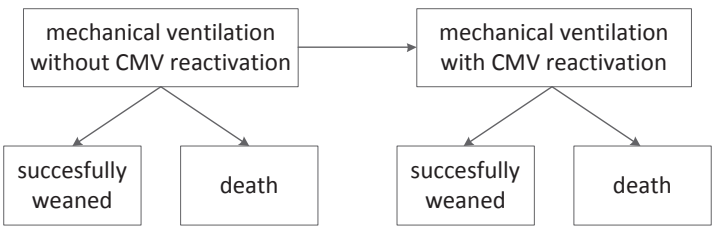

Fig S1. Multi-state model for studying the association of CMV reactivation with mortality
Patients started in the "mechanical ventilation without CMV reactivation" state and transitioned to the 'mechanical ventilation with CMV reactivation' state upon CMV reactivation. Thus, these two states were transient states, whereas the remaining states (death or successful weaning) were final (or absorbing) states.

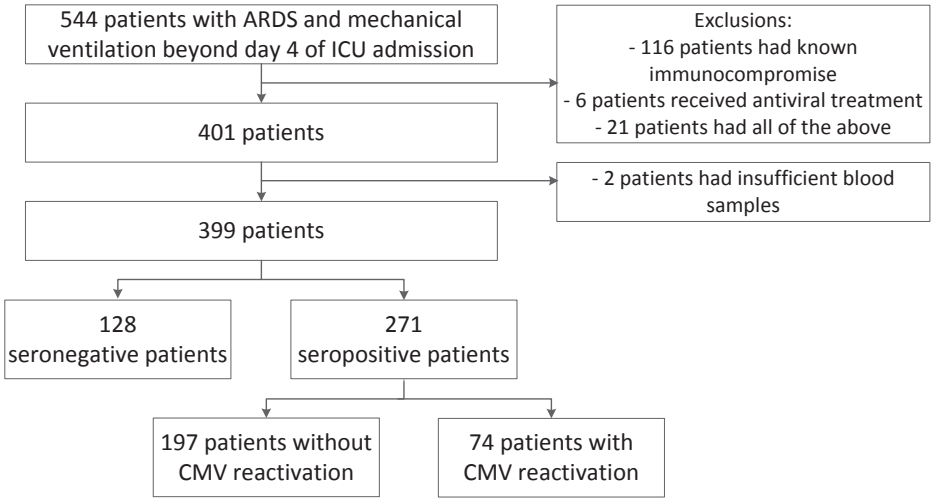

Fig S2. Flowchart of patient inclusion
Immune compromise includes a history of solid organ or stem cell transplantation, infection with the human immunodeficiency virus, hematological malignancy, use of immunosuppressive medication, chemotherapy/radiotherapy in the year before ICU admission, and any known humeral or cellular immune deficiency; antiviral treatment includes use of (val)ganciclovir, (val) aciclovir, cidofovir or foscarnet in the week before ICU admission.
ARDS = acute respiratory distress syndrome; CMV = cytomegalovirus

Marginal structural modeling

A marginal structural model analysis aims to adjust for the changes in disease severity before CMV reactivation onset, while preventing bias (4, 5). It assesses what the mortality in the intensive care unit would have been in a population in which all patients remain exempt from CMV reactivation, and is called a counterfactual analysis. For such analysis two steps are necessary. First, the daily probability of acquiring CMV reactivation in the intensive care unit must be modeled, for which we used a multivariable logistic regression analysis that included both baseline and daily patient characteristics. Based on these estimated daily probabilities, we calculated patient specific weights (inverse probability weights) representing the cumulative risk of acquiring CMV reactivation for each patient at each time point. Because adjustment for time varying variables measured at (or even after) the start of CMV reactivation may result in overcorrection and bias, we used lagged values from the preceding day to predict CMV reactivation status on each day (6). Second, we performed competing risk Cox regression analysis, using these inverse probability weights as a covariable to adjust for the differential evolution of disease severity over time, to estimate both the daily hazard and cumulative risk of death (7).

Table S1. Sensitivity analyses

A. Analysis using different endpoints (ICU discharge and mortality)			
	ICU discharge (CSHR)	ICU mortality (CSHR)	ICU mortality (SHR)
Crude model with adjustment for - time-varying onset of CMV reactivation	0.71 (0.51-0.99)	1.65 (0.97-2.83)	2.93 (1.73-4.97)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances (a)	0.74 (0.53-1.05)	1.48 (0.82-2.69)	2.42 (1.37-4.29)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances (a) - evolution of disease prior to onset of CMV reactivation (b)	0.86 (0.62-1.20)	1.29 (0.68-2.45)	2.01 (1.09-3.71)

C. Analysis using fully saturated model for covariable adjustment (d)			
	Successful weaning (CSHR)	Death on mechanical ventilation (CSHR)	Death on mechanical ventilation (SHR)
Crude model with adjustment for - time-varying onset of CMV reactivation	0.81 (0.58-1.13)	1.75 (1.01-3.01)	3.39 (1.96-5.87)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances (c)	0.82 (0.57-1.16)	1.73 (0.97-3.08)	2.83 (1.53-5.22)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances (c) - evolution of disease prior to onset of CMV reactivation (b)	0.94 (0.66-1.33)	1.60 (0.86-2.99)	2.49 (1.30-4.80)

(a) Baseline covariables included APACHE IV score, use of high dose corticosteroid therapy, and PEEP setting
(b) Time-dependent covariables included the Risk, Injury, Failure, Loss and End-stage kidney disease (RIFLE) score, Sequential Organ Failure Assessment (SOFA) score, presence of septic shock, and use of high dose corticosteroid therapy, which were all measured on a daily basis until 24 hours prior to reactivation onset.
(c) Baseline covariables included APACHE IV score, use of high dose corticosteroid therapy, prior ICU admission during hospital stay, renal insufficiency, and septic shock
(d) We selected all covariables based on their association with the exposure (CMV reactivation status) at a p-value of <0.10 only (thus not using the additional criterium of a minimal change in crude effect estimate).

Table S2. Subgroup analyses

A. Analysis in subgroup of patients receiving high-dose corticosteroids in first four days of ICU admission (n=149)			
	Successful weaning (CSHR)	Death on mechanical ventilation (CSHR)	Death on mechanical ventilation (SHR)
Crude model with adjustment for - time-varying onset of CMV reactivation	0.82 (0.53-1.27)	1.47 (0.78-2.78)	2.86 (1.51-5.41)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances (a)	0.84 (0.53-1.34)	1.54 (0.79-3.02)	2.60 (1.29-5.25)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances - evolution of disease prior to onset of CMV reactivation (b)	NA	NA	NA

B. Analysis in subgroup of patients NOT receiving high-dose corticosteroids in first four days of ICU admission (n=122)			
	Successful weaning (CSHR)	Death on mechanical ventilation (CSHR)	Death on mechanical ventilation (SHR)
Crude model with adjustment for - time-varying onset of CMV reactivation	0.89 (0.53-1.50)	2.26 (0.77-6.64)	3.91 (1.36-11.27)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances (a)	0.83 (0.48-1.43)	1.74 (0.51-5.94)	3.61 (1.24-10.48)
Multivariable model with adjustment for - time-varying onset of CMV reactivation - baseline imbalances - evolution of disease prior to onset of CMV reactivation (b)	NA	NA	NA

(a) Baseline covariables included APACHE IV score and PEEP setting
(b) Because of limited numbers in these subgroups marginal structural modeling analysis to adjust for the evolution of disease severity prior to the onset of CMV reactivation was not performed.

Table S3 Trajectories of organ failure, pulmonary and inflammation markers by time since onset of CMV reactivation

		Time since onset of CMV reactivation (days)					
		t0	t2	t4	t6	t8	t10
Exposed (n)	E	74	67	55	48	42	36
Matched non-exposed (n)	NE	74	69	53	40	27	20
SOFA total	E	8.8	8.3	7.7	8.0	8.2	8.4
	NE	7.6	7.0	5.9	5.3	4.9	5.0
respiratory component	*E*	*2.8*	*2.6*	*2.4*	*2.5*	*2.6*	*2.4*
	NE	*2.6*	*2.5*	*2.3*	*2.2*	*2.0*	*1.8*
circulatory component	*E*	*2.4*	*2.3*	*1.9*	*1.9*	*2.0*	*2.2*
	NE	*1.8*	*1.7*	*1.3*	*0.8*	*0.8*	*0.8*
renal component	*E*	*1.7*	*1.6*	*1.6*	*1.7*	*1.6*	*1.7*
	NE	*1.3*	*1.1*	*1.1*	*1.2*	*0.9*	*1.2*
Compliance (mL/cmH_2O)	E	55	54	51	48	48	51
	NE	62	63	66	63	69	76
PaO_2/FiO_2	E	221	250	258	255	263	254
	NE	232	251	254	264	285	290
PEEP (cm H_2O)	E	7.7	7.3	6.8	6.8	7.1	6.6
	NE	7.5	7.0	6.5	6.1	5.8	6.3
C-Reactive Protein	E	127	111	107	77	77	94
	NE	116	122	108	90	67	77

E = exposed; NE = non-exposed; SOFA = Sequential Organ Failure Assessment; PEEP = Positive End Expiratory Pressure.
All 74 exposed patients in whom CMV reactivation occurred were matched to non-exposed patients based on baseline characteristics and length of stay in the ICU at the time of reactivation onset. For instance, for an individual who reactivated on day 10 of the ICU admission a matched non-exposed control subject was randomly selected from the pool of patients who remained in the ICU beyond day 10, having no CMV reactivation. Exposed and non-exposed subjects were matched on the least possible difference in patient characteristics that showed significant differences at baseline (i.e., readmission status, APACHE IV score, high dose corticosteroid use, presence of renal insufficiency and presence of septic shock at admission). Data show mean values.

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6

EPIDEMIOLOGY OF MULTIPLE VIREMIA IN PREVIOUSLY IMMUNOCOMPETENT PATIENTS WITH SEPTIC SHOCK

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ABSTRACT

Background

Systemic reactivation of cytomegalovirus (CMV) has been frequently described in intensive care unit (ICU) patients, even in those without known prior immune deficiency. However, the incidence of viremia episodes by other herpes viruses has not been explored extensively. We studied the epidemiology of CMV, Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and varicella zoster virus (VZV) in the blood of patients with septic shock, and assessed their associations with clinical outcome.

Methods

From October 2011 to June 2014 we prospectively included consecutive adults who were admitted with septic shock to a mixed medical-surgical tertiary ICU in the Netherlands for more than four days. We excluded patients who had received antiviral treatment in the week preceding ICU admission and those with known immunodeficiency. Viral loads were determined in plasma weekly by polymerase chain reaction.

Results

Among 125 included patients, 77 (62%) subjects developed viremia at some time during their ICU stay. CMV, EBV, HHV-6, HSV-1, HSV-2, and VZV viremia was detected in 20 (16%), 60 (48%), 22 (18%), 33 (26%), 1 (1%) and 1 (1%) of patients, respectively. In multivariable-adjusted competing risk survival analyses, only CMV viremia was significantly associated with increased mortality (subdistribution hazard ratio 3.37, 95% CI 1.60-7.08).

Conclusions

Multiple herpes viruses may frequently be detected in the blood of previously immunocompetent patients with septic shock. Among these events, CMV viremia carries an independent risk for death.

INTRODUCTION

Viremia due to cytomegalovirus (CMV) has been frequently observed in intensive care unit (ICU) patients without known prior immune deficiency (1, 2). Systemic reactivation of latently present virus is assumed to be the main mechanism responsible for the presence of viral DNA in blood (3). These findings have led to the understanding that reactivation of herpes viruses is not exclusive to "classical" high risk groups, such as patients infected with the human immunodeficiency virus, patients receiving chemotherapy, and transplant recipients, but may also occur in immunocompetent patients when they suffer from severe critical illness.

In a previous study in patients with acute respiratory distress syndrome (ARDS), CMV reactivation was most frequently detected among subjects with concurrent septic shock (4). This finding might be explained by the increased severity and duration of immune suppression associated with septic shock as a more severe depletion of T cells occurs in patients who have the greatest severity of illness (5). Such immunosuppressive state facilitates reactivation of viral opportunistic pathogens that are latently present in their hosts. Furthermore, CMV reactivation is independently associated with increased case fatality rates in critically ill patients (6-9). However, the incidence and clinical significance of viremia due to other herpes viruses remains yet to be explored.

We hypothesized that viremia due to multiple herpes viruses may occur concurrently in patients with septic shock, and we set out to perform a comparative analysis of viremia events with respect to their epidemiology and association with patient outcome. The primary aim of our study was to describe the incidence of CMV, Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) and varicella zoster virus (VZV) viremia in patients admitted to the ICU with septic shock who survive beyond day 4. Furthermore, we assessed the associations between detection of viremia and both length of stay and mortality in the ICU.

METHODS

Patients and measurements

The present study was conducted within the framework of the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) cohort (clinicaltrials.gov Identifier: NCT01905033) for which the Institutional Review Board approved an opt-out method of informed consent (protocol number 10-056C) (10). We prospectively included consecutive adults who presented with septic shock (i.e., fulfilling the septic shock criteria within the first three days of ICU admission) to the mixed ICU of the University Medical Center Utrecht in the Netherlands between October 2011 and June 2014, and remained in the ICU beyond day four of admission (since the risk of viral reactivation before that time is negligible (1)). Septic shock was defined as sepsis complicated by hypotension refractory to fluid resuscitation (11). Sepsis and specific infection diagnoses were defined according to

CDC International Sepsis Forum Consensus Conference definitions that were translated and adapted to the Dutch situation (10). We excluded patients who had received (val) ganciclovir, (val)aciclovir, cidofovir or foscarnet in the week before ICU admission and those with known immunodeficiency. Immunodeficiency was defined as a history of solid organ or stem cell transplantation, infection with the human immunodeficiency virus, hematological malignancy, use of immunosuppressive medication (prednisone >0.1 mg/kg for >3 months, prednisone >75 mg/day for >1 week, or equivalent), chemotherapy or radiotherapy in the year before ICU admission, and any known humoral or cellular immune deficiency.

Leftover plasma, which was harvested from blood samples obtained daily as part of routine patient care, was stored within 4 hours after blood draw at -80°C until further processing. CMV and HSV serostatus were determined in plasma samples at admission by enzyme immunoassays (Enzygnost, Siemens Healthcare Diagnostic Products, Marburg, Germany). We did not determine the EBV, HHV-6 and VZV serostatus, because the seroprevalence of these viruses is known to be very high (ranging from 90% to 100%) in adult populations (12-14). Subsequently, viral loads of CMV, EBV and HHV-6 in plasma were determined weekly by quantitative real-time Taqman polymerase chain reaction (15-17), whereas HSV-1, HSV-2, and VZV were only qualitative (positive/negative) measurements. CMV and EBV was defined as a viral load >100 international units per milliliter (IU/mL) as calibrated according to the World Health Organization (WHO) Standards. HHV-6 viremia was defined as a viral load >100 copies/ml, calibrated to an electron microscopically defined standard (17). Of note, the limit of quantitation of the HHV-6 assay was 1000 copies per milliliter. Below this limit positive results could be detected, but accurate quantitation of these results was unreliable.

Screening for herpes viruses was not part of routine clinical practice. Neither serology results nor viral loads measured during our study were made available to the treating physicians.

Data analysis

The incidences of viremia and their association with patient characteristics were compared using non-parametric descriptive statistics (i.e., Chi-square and Kruskal-Wallis tests). We used survival regression analyses to assess the association between various types of viremia and clinical outcome. In these analyses, ICU discharge and ICU death are considered competing events, because in case ICU mortality is the event of interest, then discharge alive from the ICU precludes this event of interest from being observed (18). Furthermore, patients who are discharged from the ICU alive at a certain time-point are in a better health state than those who remain in the ICU beyond that same time-point and remain at risk for ICU death (19, 20). Therefore, competing risks Cox proportional hazard regressions were used to fit multivariable models with viremia status as a time-dependent exposure variable. A competing risks analysis provides two measures of association. First, the cause-specific hazard ratio (CSHR) describes the

instantaneous effect on the outcome of interest, given that the patient is still at risk. In our case, it estimates the direct effect of viremia on the rates of death and discharge in the ICU. Second, the subdistribution hazard ratio (SHR) for mortality describes the risk of dying from viremia while accounting for the competing risk of ICU discharge.

Baseline covariables that were initially evaluated as potential confounders included age, gender, ethnicity, prior ICU admission during the hospital stay, surgical reason for admission, Charlson comorbidity index, and Acute Physiology and Chronic Health Evaluation (APACHE) IV score. In subsequent multivariable analyses we accounted for possible confounding only by those baseline variables that showed a difference between viremic and non-viremic groups at a p-value of <0.30 and changed the crude effect estimate by >5%. We used these two criteria combined in order to avoid statistical overfitting due to the limited number of events.

Data were analyzed with SAS 9.2 (Cary, NC, USA) and R 2.15.1 software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We enrolled 175 patients with septic shock requiring ICU admission for more than four days. Of these, 50 were ineligible for study inclusion because of a known prior immunocompromise or previous antiviral treatment, resulting in a final inclusion of 125 patients for analysis. CMV and HSV seropositivity was found in 78 (62%) and 103 (82%) patients, respectively.

Viremia

Overall, 77 (62%) of 125 patients developed viremia with one or more herpes viruses at any time point during their ICU stay, of whom 37 (48%) had multiple viremia events simultaneously. In patients with viremia, those with CMV had the highest APACHE IV score (Table 1). CMV viremia was detected in 20 of 125 (16%) patients, 19 of whom were already CMV seropositive at ICU admission. EBV viremia was found in 60 (48%) patients. HHV-6 viremia occurred in 22 (18%) patients. HSV-1 viremia was detected in 33 of 125 (26%) patients, 32 of whom were already HSV seropositive at ICU admission. One patient developed HSV-2 viremia, but also had HSV-1 viremia during the same admission. VZV DNA was detected in only one patient (0.8%). The most frequent combination of multiple type viremia included EBV and HSV-1 (Table 2).

A viremia onset in the first week of ICU admission was observed in 11 (55%), 55 (92%), 22 (69%), and 12 (55%) of patients with CMV, EBV, HHV-6 and HSV-1 viremia, respectively.

Associations with clinical outcome

Patients with any type of viremia had a longer ICU length of stay compared to patients without viremia: 13 (IQR 10-19) versus 8 (IQR 5-11) days (p<0.01). A prolonged length

Table 1. Baseline characteristics of septic shock patients by viremia status

Patient characteristics	Never viremia (n=48)	Ever viremia				
		CMV (n=20)	EBV (n=60)	HHV-6 (n=22)	HSV-1 (n=33)	Any herpes virus (n=77)
Age (years)	65 (57-74)	69 (62-76)	68 (62-76)	68 (62-75)	69 (62-77)	68 (61-75)
Male gender	28 (58)	11 (55)	37 (62)	14 (64)	18 (55)	48 (62)
Non-European descent	3 (6)	2 (10)	2 (3)	1 (5)	2 (6)	4 (5)
Body Mass Index	26 (23-30)	27 (23-32)	24 (21-29)	27 (23-31)	27 (22-32)	25 (22-29)
Prior ICU admission	16 (33)	7 (35)	11 (18)	4 (18)	12 (36)	18 (23)
Medical admission	30 (63)	13 (65)	37 (62)	12 (55)	25 (76)	49 (64)
Charlson comorbidity index	7.1 (2.5-11.6)	6.4 (3.2-11.1)	6.4 (0-11.6)	4.7 (0-10.5)	8.1 (2.5-12.6)	6.4 (0.0-11.8)
Chronic obstructive pulmonary disease	13 (27)	2 (10)	9 (15)	3 (14)	6 (18)	12 (16)
Congestive heart failure	4 (8)	3 (15)	7 (12)	3 (14)	3 (9)	9 (12)
Diabetes mellitus	7 (15)	3 (15)	9 (15)	3 (14)	6 (18)	11 (14)
Chronic renal insufficiency	8 (17)	4 (20)	9 (15)	3 (14)	8 (24)	12 (16)
Cancer	10 (21)	6 (30)	16 (27)	5 (23)	10 (30)	20 (26)
APACHE IV score	92 (78-110)	96 (78-115)	87 (71-111)	88 (72-112)	93 (80-107)	85 (72-104)
Plasma lactate	3.5 (1.9-5.5)	3.5 (2.2-5.8)	3.4 (2.3-5.8)	3.8 (2.8-6.3)	2.4 (1.8-3.8)	3.2 (2.0-5.4)
C-reactive Protein	114 (36-253)	206 (121-269)	202 (108-287)	174 (85-330)	198 (135-306)	193 (102-285)
Source of septic shock:						
- *Pulmonary*	24 (50)	5 (25)	22 (37)	6 (27)	18 (55)	33 (43)
- *Abdominal*	14 (29)	9 (45)	13 (22)	10 (45)	3 (9)	18 (23)
- *Other*	10 (21)	6 (30)	25 (42)	6 (27)	12 (36)	26 (34)

Data are presented as medians (interquartile range) or absolute numbers (%). P-values were calculated by nonparametric tests and Chi-square tests, respectively. APACHE = Acute Physiology and Chronic Health Evaluation.

of stay was also found in the viremia group compared to the non-viremia group for each virus separately: CMV 15 (IQR 12-20) versus 9 (IQR 6-14) days; EBV 13 (IQR 10-20) versus 8 (IQR 6-14) days, HHV-6 18 (IQR 13-25) versus 10 (IQR 7-15) days; and HSV-1 15 (IQR 11-23) versus 10 (IQR 7-15) days (all $p<0.01$).

Mortality was 50% in patients with CMV viremia compared to 24% in those without (ever) CMV viremia ($p=0.02$). None of the other herpes viruses were significantly associated with ICU mortality, although trend effects were present: EBV (35% versus 22%, $p=0.09$), HHV-6 (36% versus 26%, $p=0.34$), and HSV-1 (36% versus 25%, $p=0.21$).

Table 2. Viremia

Type(s) of viremia	Frequencies
CMV + EBV + HHV-6 + HSV-1 (no VZV)	3
CMV + EBV + HSV-1 (no HHV-6, no VZV)	6
EBV + HHV-6 + HSV-1 (no CMV, no VZV)	5
CMV + EBV + HHV-6 (no HSV-1, no VZV)	3
CMV + HHV-6 + HSV-1 (no EBV, no VZV)	2
EBV + HSV-1 only	7
EBV + HHV-6 only	6
CMV + EBV only	3
CMV + HHV-6 only	1
CMV + HSV-1 only	1
CMV only	1
EBV only	27
HHV-6 only	2
HSV-1 only	9
VZV only	1
No viremia	48

After adjustment for baseline imbalances and taking the time-dependent nature of viremia and competing risks into account, we found that CMV viremia remained significantly associated with ICU mortality (subdistribution hazard ratio 3.37, 95% CI 1.60-7.08) (Table 3). In contrast, other types of viremia were not independently associated with mortality. In the multivariable-adjusted cause-specific analyses, only EBV viremia was associated with a decreased rate to ICU discharge (cause-specific hazard ratio 0.56, 95% CI 0.36-0.87).

Table 3. Associations between viremia and clinical outcome

Virus	Cox model	ICU discharge (CSHR)	Death in ICU (CSHR)	Death in ICU (SHR)
CMV	Crude	0.71 (0.36-1.38)	1.66 (0.74-3.73)	3.21 (1.54-6.69)
	Adjusted [a]	0.74 (0.38-1.43)	1.91 (0.85-4.28)	3.37 (1.60-7.08)
EBV	Crude	0.54 (0.35-0.82)	0.93 (0.46-1.90)	1.79 (0.91-3.52)
	Adjusted [a]	0.56 (0.36-0.87)	0.78 (0.37-1.64)	1.67 (0.83-3.33)
HHV-6	Crude	0.62 (0.35-1.12)	0.96 (0.42-2.17)	1.73 (0.78-3.83)
	Adjusted [a]	0.60 (0.33-1.08)	0.77 (0.33-1.82)	1.75 (0.79-3.88)
HSV-1	Crude	0.69 (0.42-1.13)	1.17 (0.57-2.43)	1.76 (0.87-3.54)
	Adjusted [a]	0.67 (0.41-1.12)	1.32 (0.64-2.75)	1.80 (0.89-3.64)
HSV-2	NA	NA	NA	NA
VZV	NA	NA	NA	NA

Data are presented as hazard ratios with 95% confidence intervals. The cause-specific hazard ratio (CSHR) estimates the direct effect of viremia on clinical outcome (i.e., ICU discharge or death). The subdistribution hazard ratio (SHR) is a summary measure of both separate cause-specific hazards and estimates the overall risk of dying from viremia while taking into account the competing event of discharge alive from the ICU. Due to the limited numbers no further analyses were performed for HSV-2 and VZV.
[a] Adjusted for Acute Physiology and Chronic Health Evaluation (APACHE) IV score and prior ICU admission during hospital stay. These variables were selected because of a difference at baseline between the any viremia and non-viremia group at a p-value of <0.30 and a change in the crude effect estimate by >5%.

DISCUSSION

Viremia occurred in up to 62% of previously immunocompetent subjects who were admitted to the ICU with septic shock. Among the various herpes virus types that were tested, viremia due to EBV was observed most commonly, followed by HSV-1, CMV, and HHV-6. HSV-2 and VZV viremia was each detected in only one patient. With the exception of two cases, all CMV and HSV viremia events seemed to be caused by viral reactivation rather than primary infections. After adjustment for confounding, only CMV remained associated with increased mortality.

Currently, critically ill patients are not routinely screened on the presence of viremia in contrast to for example transplant patients or patients with hematological malignancies. Most previous studies have exclusively focused on CMV, but our study underlines that viremia by other herpes viruses is also frequent in previously immunocompetent critically ill patients, although the clinical consequence of these events may not be self-evident. The CMV reactivation rate of 24% in CMV seropositive patients with septic shock observed in our study was comparable to the previously reported reactivation rate in ARDS patients (27%), but lower than the 34% reactivation rate reported in a subgroup of patients with both ARDS and septic shock (4). The largest study published to date looking at multiple virus reactivations was performed in unselected septic patients

irrespective of their severity of disease (2). This study also reported high rates of viremia due to EBV, CMV, HSV and HHV-6, and of these only CMV was crudely associated with increased mortality. In contrast to our study, however, these investigators also analyzed whole blood (in addition to plasma) for the presence of virus particles, which may have resulted in the detection of non-replicating latent viral DNA present within blood cells as well. Furthermore, in our study we used a higher cut-off (mostly >100 IU/mL) to define viremia in order to reduce the likelihood of false positive results, because very low levels of viremia might also be the result of non-replicating viral DNA that is redistributed from lytic cells to plasma (21).

In a prospective observational study including immunocompetent patients with an ICU stay of more than four days EBV was detected in two-thirds of patients, and was associated with increased morbidity and mortality in crude analyses (22). This association with clinical outcome was not demonstrated in our study, nor in the study by *Walton et al* (2). The apparently weaker or lack of association found for herpes viruses other than CMV, is supported by other studies. Although HHV-6 viremia was detected in peripheral blood leucocytes in a remarkably high proportion of critically ill patients (53%), HHV-6 viremia was not associated with increased disease severity and outcome (23). Another retrospective study showed that the mortality rate in HSV-viremic patients was not significantly increased in comparison to the overall ICU mortality rate (24).

Our study has several strengths. First, our study differs from previous studies in that we have assessed the burden of disease while accounting for different kinds of bias, including adjustments for potential confounders and the time-dependent nature of viremia (2). The delay in the time to onset of viremia after ICU admission causes bias when days before the onset of viremia are wrongfully attributed to the viremic group. Time-dependent fitting of viremia status in our Cox regression models resolved this issue. Second, our study was nested within a large prospective data collection initiative that included consecutive patients, thereby minimizing selection bias (10). Moreover, we used a highly sensitive method of real-time PCR for viral load detection. However, our study has some important limitations that need to be acknowledged. First, it remains undetermined whether the trends to increased mortality is attributable to these viruses, as our observational study cannot prove causality. Even after adjustment for baseline imbalances, in a dynamic ICU setting, during which critically ill patients continuously deteriorate and improve over time, it is very difficult to verify whether such adjustment is sufficient. Second, this study only provides insight into the occurrence of viremia during ICU admission. We cannot elaborate on viremia that may have started after ICU discharge.

In conclusion, critically ill patients admitted to the ICU with septic shock are prone to develop viremia due to various types of herpes viruses, even if they were previously immunocompetent. A clear and independent association with increased mortality was found for patients following CMV reactivation, whereas this association was less clear for the other herpes viruses that were tested. The clinical implications of these findings need to be explored in more detail.

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III

OPPORTUNISTIC BACTERIAL AND FUNGAL PATHOGENS IN THE ICU

7

ANTIBIOTIC EXPOSURE AND RESISTANCE DEVELOPMENT IN *PSEUDOMONAS AERUGINOSA* AND *ENTEROBACTER* SPECIES IN INTENSIVE CARE UNITS

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ABSTRACT

Objective

We quantified the association between antibiotic exposure and acquisition of antibiotic resistance in *Pseudomonas aeruginosa* and *Enterobacter* species in intensive care unit patients.

Design

Prospective cohort study.

Setting and Patients

In 1201 patients respiratory tract colonization was determined through regular screening on admission, twice weekly and on discharge. Primary outcome was the acquisition of antibiotic resistance in prior antibiotic sensitive *Pseudomonas aeruginosa* and *Enterobacter* species, with acquisition due to cross-transmission excluded based on genotyping and epidemiological linkage. Cox regression analysis, adjusted for covariables, was performed to calculate hazard ratios of antibiotic exposed compared to non-exposed patients.

Measurements and Main Results

In all, 194 and 171 patients were colonized with *Pseudomonas aeruginosa* and *Enterobacter* species, respectively. Two or more cultures per episode were available for 126 and 108 patients. For *Pseudomonas aeruginosa* ceftazidime exposure was associated with 6.3 acquired antibiotic resistance events per 100 days of exposure, whereas incidence rates were lower for ciprofloxacin, meropenem and piperacillin-tazobactam. In multivariable analysis, meropenem, ciprofloxacin and ceftazidime were significantly associated with risk of resistance development in *Pseudomonas aeruginosa* (adjusted hazard ratio [HR] 11.1, 95% CI 2.4-51.5 for meropenem; adjusted HR 4.1, 95% CI 1.1-16.2 for ciprofloxacin; adjusted HR 2.5, 95% CI 1.1-5.5 for ceftazidime). For *Enterobacter*, ceftriaxone and ciprofloxacin exposure were associated with most antibiotic resistance acquisitions. No significant associations were found in multivariable analysis.

Conclusions

Meropenem exposure is associated with the highest risk of resistance development in *Pseudomonas aeruginosa*. Increasing carbapenem use due to emergence of Gram-negative bacteria producing extended-spectrum beta-lactamases will enhance antibiotic resistance in *Pseudomonas aeruginosa*.

INTRODUCTION

Nosocomial infections are associated with increased morbidity and mortality in patients treated in intensive care units (ICUs) (1). To treat these infections, many patients need antibiotic treatment, but this is also considered an important cause of emerging antibiotic resistance (2, 3). In ICUs the problem of antibiotic resistance is even more urgent, due to high vulnerability of patients, many invasive procedures, high antibiotic selective pressure and high prevalence of resistant bacteria (2). When infections are caused by antibiotic resistant bacteria, in-hospital mortality rates and length of hospital stay are higher as compared to infections caused by antibiotic-susceptible bacteria (4).

Infections caused by antibiotic-resistant bacteria in ICU are almost always preceded by colonization, which may result from either endogenous or exogenous acquisition (5). In case of endogenous acquisition, a patient is already colonized with, initially, undetectable bacterial numbers, which ranks increase above detection limits, for instance because of selective antibiotic pressure. Yet, it is also possible that antibiotic-susceptible bacteria acquire resistance mechanisms (or start to express resistance traits), changing their phenotype from susceptible to resistant (6). Again, antibiotic exposure is believed to be critical for this process.

Exogenous acquisition is caused by microorganisms from the ICU environment, either inanimate or animate. Resistant bacteria may be transferred from patient to patient, most frequently through temporarily contaminated hands of health care workers (7). Although antibiotic selective pressure may facilitate such events of cross-transmission, lapses in adherence to basic hygiene measures must be considered crucial for this mode of transmission of antibiotic resistance.

Few studies have quantified the effects of antibiotic exposure on the endogenous selection of antibiotic resistance in *Pseudomonas aeruginosa* (8-10). However, in these studies the role of exogenous acquisition as a cause for resistance acquisition has not been ruled out, thereby obscuring direct effects of antibiotic exposure on endogenous acquisition of antibiotic resistance. Furthermore, other Gram-negative bacteria like *Enterobacter* species have not been rigorously investigated on this specific topic. In this study we, therefore, aimed to quantify the occurrence of a phenotype switch from susceptible to resistant in *Pseudomonas aeruginosa* and *Enterobacter* species in colonized ICU patients.

MATERIALS AND METHODS

Study design and patient population

From January 2007 through February 2008, a prospective cohort study was performed among patients admitted to the ICU for at least 48 hours and colonized with *Pseudomonas aeruginosa* and *Enterobacter* species. Four ICUs participated: two units (10 and 8 beds, respectively) in the University Medical Center Utrecht and two units (each 8 beds) in St

Elisabeth Hospital in Tilburg, a large teaching hospital. Patients readmitted to ICU after initially being discharged from ICU were assigned as new patients in this study. All ICUs had a mixed population of adult patients including surgical and non-surgical patients. This cohort study was embedded within a crossover trial evaluating the effects of open and closed endotracheal suctioning on cross-transmission (11). The institutional review board of both hospitals waived the requirement for informed consent, since cultures were part of the surveillance program.

Outcome

The primary outcome was the incidence of acquired antibiotic resistance, which was defined as the conversion from carriage with antibiotic susceptible to antibiotic resistant bacteria in subsequent respiratory tract cultures. The effects of the following antibiotics on antibiotic resistance were assessed: ciprofloxacin, ceftazidime, meropenem, piperacillin-tazobactam (for *Pseudomonas aeruginosa* and *Enterobacter*); cotrimoxazol, gentamicin, ceftriaxone, tobramycin (for *Enterobacter*).

To quantify antibiotic use in our study population, the number of defined daily doses (DDDs) per 100 patient-days was calculated, according to the Anatomical Therapeutic Chemical / Defined Daily Doses (ATC/DDD) Index 2010 from the WHO Collaborating Centre for Drug Statistics Methodology (12). The number of acquired antibiotic resistance events was expressed per 100 days of antibiotic exposure in which patients were at risk for developing antibiotic resistance. Antibiotic treatment was only considered if it had been prescribed before the date of onset of resistance.

Bacterial sampling

All patients admitted to ICU were screened on admission, subsequent twice weekly (Monday, Thursday) and on discharge for bacterial colonization of the respiratory tract. All cultures (endotracheal aspirate in mechanically ventilated patients, oropharyngeal swabs in non-ventilated patients) were analysed according to hospital protocol. The following minimum inhibitory concentrations for determining resistant categories for the different antibiotics were used, according to the Clinical Laboratory Standards Institute: ciprofloxacin ≥4 mg/L, ceftazidime ≥32 mg/L, meropenem ≥16 mg/L; piperacillin-tazobactam ≥128/4 mg/L, cotrimoxazol ≥4/76 mg/L, gentamicin ≥16 mg/L, ceftriaxone ≥64 mg/L, tobramycin ≥16 mg/L (13).

To exclude the occurrence of possible cross-transmission, genotyping was conducted for *Pseudomonas aeruginosa* and *Enterobacter* species isolates. From patients colonized with one or both species the first isolate (per pathogen) was genotyped, as were subsequent isolates in case of a change in antibiogram, morphologic differences or when ≥ 10 cultures with identical antibiograms had been obtained. Cross-transmission was defined as acquired colonization with a genetically identical pathogen and with overlapping time periods to a potential source patient. Genotyping was performed after the trial was finished, therefore medical staff was not aware of the results during

the trial. *Pseudomonas aeruginosa* isolates were genotyped with Multiple-Locus Variable-number tandem-repeats Analysis (MLVA) (14), and *Enterobacter* species were genotyped with DiversiLab (15). MLVA patterns were analyzed with BioNumerics software version 5.10 (Applied Maths, St-Martens-Latem, Belgium), and single locus variants (where the profile varies at one locus) were used as cut-off point for genetic relatedness. For *Enterobacter* species analysis was performed with DiversiLab software (version 3.4) using 95% similarity as cut-off point for genetic relatedness.

Data analysis

To determine acquisition of antibiotic resistance, only patients of whom at least two microbial cultures were available were included in analysis; in patients with only one culture it was not retrievable whether possible antibiotic resistance was acquired.

To assess the effect of antibiotics administered during ICU admission, Cox proportional hazards models were used. The following covariables were considered for our multivariable models: age, gender, Acute Physiology and Chronic Health Evaluation (APACHE) II score, simultaneous use of other antibiotics, previous use of antibiotics before ICU admission, ICU day of first colonization, and surgical or non-surgical patient. For every multivariable model each covariable was tested for confounding by adding it to an univariable model containing the antibiotic exposure variable and examining its effect on the beta coefficient of the antibiotic exposure variable. Variables that caused substantial confounding (a change in the beta coefficient of greater than 10%) were included in the final model. The time interval between first positive culture and the occurrence of resistance acquisition was used as time variable. The date of acquisition was determined as the date on which the first resistant isolate was obtained from the patient.

Bivariable analyses with Spearman correlation coefficients (rho) were carried out to rule out multicollinearity among variables entered in multivariable analysis.

Differences in antibiotic resistance acquisitions between patients exposed to antibiotic treatment and patients not exposed to antibiotic treatment were expressed by hazard ratios with corresponding 95% confidence intervals. Data were analysed with the Statistical Package for Social Sciences version 16.0 for Mac.

RESULTS

In all, 1201 patients were admitted to one of the ICUs for at least 48 hours and among them 316 patients were colonized with *Pseudomonas aeruginosa* or *Enterobacter* species (Figure 1). In 111 of the colonized patients, only one positive microbial culture with *Pseudomonas aeruginosa* or *Enterobacter* species was acquired, leaving 205 patients with two or more isolates, corresponding to 126 and 108 patients with *Pseudomonas aeruginosa* and *Enterobacter* species, respectively. In total 29 patients had combined colonization.

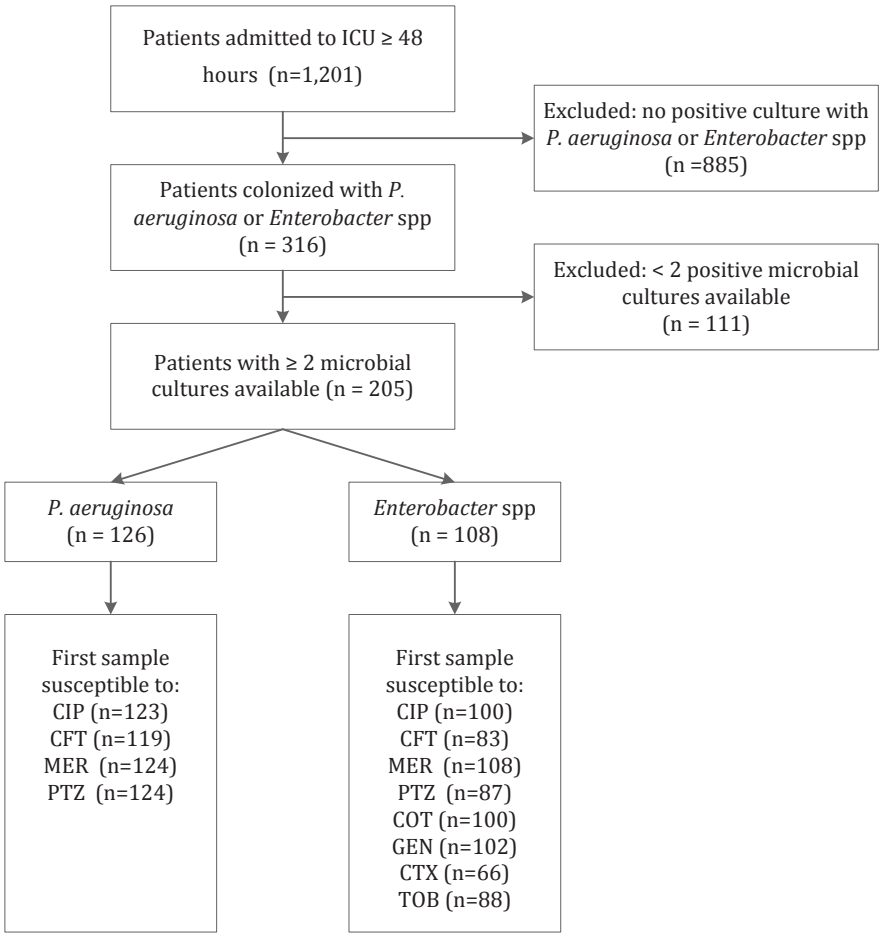

Figure 1. Patient inclusion
n = number of patients
CIP = ciprofloxacin, CFT = ceftazidime, MER = meropenem, PTZ = piperacillin-tazobactam, COT = cotrimoxazol, GEN = gentamicin, CTX = ceftriaxone, TOB = tobramycin

Patients in the *Pseudomonas aeruginosa* group were colonized later, and had a longer length of stay as compared to those colonized with *Enterobacter* species (Table 1). Trauma was more frequently the reason for admission in patients colonized with *Enterobacter* species, whereas a respiratory cause was the most frequent reason in *Pseudomonas aeruginosa*. The mortality rate in ICU was 17.5% and 16.7% for patients colonized with *Pseudomonas aeruginosa* and *Enterobacter* species, respectively (Table 1). Antibiotic exposure was highest to ciprofloxacin in both groups, being 25.9 and 29.7 DDDs per 100 patient days (Table 2). None of the included patients received

aerosolized antibiotics, nor did they receive selective decontamination of the digestive tract or oral antiseptics like chlorhexidine.

Table 1. Patient characteristics

Characteristic	Pseudomonas	Enterobacter
Number of patients	126	108
Age in years - median (IQR)	59 (44-72)	62 (41 – 75)
Gender - % (female)	25%	26%
APACHE II score - mean (SD)	20.3 (6.5)	19.6 (6.8)
Previous antibiotic use before ICU admission - %	19.5	17.6
Surgical versus not-surgical patient - % surgical	35	37
ICU day of first colonization - median (IQR)	5 (1-15)	4 (1-8)
Ventilation route - number of patients (%)		
Tracheostomy	25 (20)	13 (12)
Endotracheal tube	41 (33)	52 (48)
Endotracheal tube and tracheostomy	59 (47)	43 (40)
No mechanical ventilation	1 (1)	0 (0)
Duration of mechanical ventilation - median days (IQR)	19 (9-29)	16 (9-28)
Suctioning method - number of patients (%)		
Open	66 (52)	47 (44)
Closed	49 (39)	44 (41)
Open and Closed	11 (9)	17 (16)
Length of stay - median (IQR)	26 (14-40)	20 (11-36)
Mortality on ICU - %	17.5	16.7
Reason for ICU admission		
Cardiovascular/vascular/circulatory - %	10.3	15.7
Gastro-intestinal - %	14.3	11.1
Neurologic - %	4.0	3.7
Neurosurgical - %	4.8	7.4
Pulmonary/respiratory - %	35.7	21.3
Sepsis - %	11.1	8.3
Thorax surgical - %	2.4	0.9
Trauma - %	15.1	28.7
Other - %	2.3	2.9

IQR = Interquartile range; APACHE = Acute Physiology and Chronic Health Evaluation; ICU = intensive care unit ; SD = standard deviation

Table 2. Antibiotic use and incidences of acquired antibiotic resistance in *Pseudomonas aeruginosa* and *Enterobacter* species

Antibiotic	Number of susceptible episodes [A]	Events [B] (%)	Patient days	Events [B] per 100 patient days (95% CI)	Number of antibiotic exposed episodes (days of exposure)	DDDs per 100 patient days (95% CI)	Events [C] in antibiotic exposed episodes	Events [C] per 100 days of antibiotic exposure (95% CI)
Pseudomonas (n =126)								
Ciprofloxacin	123	11 (8.9)	1803	0.6 (0.3-1.0)	42 (315)	25.9 (23.8-27.9)	8	2.5 (0.8-4.3)
Ceftazidime	119	29 (24.4)	1427	2.0 (1.3-2.8)	45 (268)	19.0 (17.0-21.0)	17	6.3 (3.4-9.3)
Meropenem	124	12 (9.7)	1713	0.7 (0.3-1.1)	24 (221)	14.4 (12.8-16.1)	5	2.3 (0.3-4.2)
Piperacillin-tazobactam	124	18 (14.5)	1594	1.1 (0.6 -1.7)	23 (229)	13.6 (11.9-15.3)	6	2.6 (0.6-4.7)
Enterbacter (n = 108)								
Ciprofloxacin	100	13 (13.0)	1058	1.2 (0.6 -1.9)	31 (220)	29.7 (27.0-32.5)	11	5.0 (2.1-7.9)
Ceftazidime	83	14 (16.9)	920	1.5 (0.7-2.3)	18 (121)	7.9 (6.1-9.6)	2	1.7 (-0.6-3.9)
Meropenem	108	0 (0.0)	1305	0	22 (262)	24.7 (22.3-27.0)	0	0
Piperacillin-tazobactam	87	11 (12.6)	1080	1.0 (0.4-1.6)	14 (89)	6.1 (4.7-7.6)	2	2.2 (-0.8-5.3)
Cotrimoxazol	100	8 (8.0)	1271	0.6 (0.2-1.1)	36 (293)	18.5 (16.4-20.6)	6	2.0 (0.4-3.7)
Gentamicin	102	9 (8.8)	1106	0.8 (0.3 -1.3)	4 (11)	0.6 (0.2-1.1)	1	9.1 (-7.9-26.1)
Ceftriaxone	66	12 (18.2)	643	1.9 (0.8-2.9)	17 (104)	12.8 (10.2-15.3)	5	4.8 (0.7-8.9)
Tobramycin	88	7 (8.0)	979	0.7 (0.2-1.2)	16 (130)	6.7 (5.1-8.2)	1	0.8 (-0.7-2.3)

[A] First isolate susceptible for antibiotic
[B] Acquired resistance events in both antibiotic exposed and non-exposed episodes
[C] Acquired resistance events in antibiotic exposed episodes

Pseudomonas aeruginosa

Of 126 patients colonized with *Pseudomonas aeruginosa,* 546 cultures were available (median number of follow-up cultures 3; IQR 1-7). 189 Isolates were selected for genotyping, yielding 81 different *Pseudomonas* MLVA-types.

A phenotype switch from susceptible to resistant for one or more antibiotics occurred in 41 patients. Acquisition of resistance to ceftazidime occurred in 29 of 119 episodes (24%) of ceftazidime-susceptible *Pseudomonas aeruginosa* colonization, corresponding to an acquisition rate of 2.0 (95% CI 1.3-2.8) per 100 patient days at risk. Seventeen of 29 patients had been exposed to ceftazidime for a total of 268 days, which yields an acquisition rate of 6.3 (95% CI 3.4-9.3) per 100 days of antibiotic exposure. Incidence rates were lower for ciprofloxacin, meropenem and piperacillin-tazobactam, with number of events per 100 days of antibiotic exposure ranging from 2.3 to 2.6 (Table 2).

Five patients (4.0%) had a genotypic match in MLVA type and epidemiological linkage, suggesting cross-transmission, and were, therefore, excluded in multivariable analysis. Patients who had meropenem prescribed had the highest risk of developing meropenem resistance, with an adjusted hazard ratio (HR) of 11.1 (95% CI 2.4-51.5) (Table 3). The adjusted HRs for ciprofloxacin and ceftazidime were 4.1 (95% CI 1.1-16.2) and 2.5 (95% CI 1.1-5.5), respectively. There appeared no additional risk of piperacillin-tazobactam exposure (adjusted HR 0.8; 95% CI 0.2-3.2). Analysis of exposure to any cephalosporin (ceftazidime, cefotaxime, ceftriaxone, cefuroxime, cefazolin) and the development of ceftazidime resistance showed an adjusted HR of 5.9 (95% CI 1.4-12.5). In this analysis cross-transmission was defined as genotypical matching and overlapping time periods in ICU for presumed donor and acceptor. Expanding the time window to nine days in the definition of cross-transmission resulted in two additional patients with a genotypic match. Excluding these patients in multivariable analyses did not alter the results.

Enterobacter species

Of 108 patients colonized with *Enterobacter* species, 313 cultures were available (median number of follow-up cultures 2; IQR 1-4). Of these, 135 isolates were selected for genotyping, yielding 63 different types.

A phenotype switch from susceptible to resistant for one or more antibiotics occurred in 46 patients. Acquisition of resistance to ciprofloxacin occurred in 13 of 100 episodes (13%) of ciprofloxacin-susceptible *Enterobacter* colonization, corresponding to an acquisition rate of 1.2 (95% CI 0.6-1.9) per 100 patient days at risk. Eleven of 13 patients had been exposed to ciprofloxacin for a total of 220 days, which yields an acquisition rate of 5.0 (95% CI 2.1-7.9) per 100 days of antibiotic exposure. A similar incidence rate was observed for ceftriaxone (4.8 per 100 days of exposure; 95% CI 0.7-8.9), whereas the incidence rate for cotrimoxazol was lower (2.0 per 100 days of exposure; 95% CI 0.4-3.7). Incidence rates could not be reliably calculated for other antibiotics because of limited numbers of events (Table 2).

In three patients (2.8%) a genotypic match in Diversilab typing was found. These patients were excluded in multivariable analyses for the reason of possible cross-transmission. Patients with antibiotic exposure were not associated with higher risks for acquiring antibiotic resistance compared to patients without exposure (Table 3). Exposure to any cephalosporin was also not significantly associated with development of ceftazidime resistance (adjusted HR 1.9; 95 % CI 0.4-2.5).

Table 3. Cox regression analysis in patients with *Pseudomonas aeruginosa* and *Enterobacter* species

	Pseudomonas (n=121)*		*Enterobacter* (n=105)*	
	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
Ciprofloxacin vs no ciprofloxacin	2.8 (0.7-10.9)	4.1 (1.1-16.2) [A]	1.7 (0.6-4.7)	1.5 (0.5-4.3) [B]
Ceftazidime vs no ceftazidime	2.8 (1.3-6.1)	2.5 (1.1-5.5) [C]	1.0 (0.3-3.4)	0.8 (0.2-3.1) [D]
Meropenem vs no meropenem	8.7 (2.2-33.9)	11.1 (2.4-51.5) [E]	-	-
Piperacillin-tazobactam vs no piperacillin-tazobactam	2.0 (0.7-5.6)	0.8 (0.2-3.2) [F]	1.1 (0.2-5.3)	1.3 (0.3-6.5) [G]
Cotrimoxazol vs no cotrimoxazol	n/a	n/a	3.1 (0.6-15.8)	3.1 (0.6-15.8) [H]
Gentamicin vs no gentamicin	n/a	n/a	2.5 (0.3-20.0)	4.8 (0.5-45.4) [I]
Ceftriaxone vs no ceftriaxone	n/a	n/a	1.6 (0.5-4.7)	2.4 (0.7-8.9) [J]
Tobramycin vs no tobramycin	n/a	n/a	0.6 (0.1-5.4)	0.4 (0.04-4.7) [K]

n/a: Not applicable
* Number of episodes, excluding episodes with possible cross-transmission
[A] Adjusted for gender, previous use of antibiotics, ICU day of first colonization
[B] Adjusted for simultaneous use of other antibiotics, previous use of antibiotics, ICU day of first colonization, surgical or not-surgical patient
[C] Adjusted for ICU day of first colonization
[D] Adjusted for age, gender, simultaneous use of other antibiotics, previous use of antibiotics, surgical or not-surgical patient
[E] Adjusted for APACHE II score
[F] Adjusted for age, gender, APACHE II score, simultaneous use of other antibiotics, previous use of antibiotics, ICU day of first colonization, surgical or not-surgical patient
[G] Adjusted for age, gender, simultaneous use of other antibiotics, ICU day of first colonization, surgical or not-surgical patient
[H] No adjustment required
[I] Adjusted for age, APACHE II score
[J] Adjusted for age, APACHE II score simultaneous use of other antibiotics, previous use of antibiotics
[K] Adjusted for age, gender, APACHE II score, ICU day of first colonization, surgical or not-surgical patient

DISCUSSION

In this study a phenotypical switch from susceptible to resistant for at least one antibiotic occurred in 41 ICU patients colonized with *Pseudomonas aeruginosa* and 46 colonized with *Enterobacter* species. For respiratory tract colonization with *Pseudomonas aeruginosa*, exposure to meropenem was, after adjustment for covariables, associated with the highest risk of resistance development (adjusted HR 11.1; 95% CI 2.4-51.5). Among 124 patients colonized with meropenem-susceptible *Pseudomonas aeruginosa,* meropenem exposure was 14.4 DDD/100 patient days, yielding 2.3 resistance acquisition events per 100 days of antibiotic exposure. In contrast, no single event of meropenem resistance acquisition was documented among 108 patients colonized with meropenem-susceptible *Enterobacter* species, despite meropenem exposure of 24.7 DDD/100 patient days.

Few studies have assessed the effects of individual patient antibiotic exposure on the acquisition of antibiotic resistance in *Pseudomonas aeruginosa* by using time dependent variables. In a retrospective study in a single tertiary care hospital in the Unites States, quinolones, third-generation cephalosporins and imipenem were all associated with acquisition of antibiotic resistance among *Enterobacteriaceae* and *Pseudomonas aeruginosa*, when analysed at the individual-patient level. Among these antibiotics, imipenem was associated with the highest risk (8). In another tertiary care U.S. hospital, emergence of resistance to imipenem and ciprofloxacin among *Pseudomonas aeruginosa*, after exposure to these antibiotics, was considerably higher than the risk of ceftazidime resistance after ceftazidime exposure (9). In a French study of ICU patients, the risk of *Pseudomonas aeruginosa* resistance to imipenem (and piperacillin-tazobactam to a lesser extent) was strongly linked to imipenem exposure and no such risk could be demonstrated for ceftazidime use (10).

Our study differs from these studies in that we investigated a specific patient population (i.e., ICU patients only) instead of a hospital-wide population (8, 9), that we used colonization data from protocolized surveillance instead of culture results from samples submitted to the microbiology lab for clinical indication (8-10), that we meticulously ruled out possible events of cross-transmission through genotyping and epidemiological linkage, and that we included *Enterobacter* species as a separate group in our analysis. Quantifying the occurrence of cross-transmission is important as such events may create nonlinear dynamics, obscuring the direct effects of antibiotic exposure. The standardized surveillance as used in our study minimizes the risk of selection bias, as obtaining cultures for clinical reasons is more likely to be performed in the more severely ill patients.

Our findings, together with those from previous studies (8-10), strongly suggest that carbapenems pose a more serious risk on inducing antibiotic resistance in *Pseudomonas aeruginosa* than other beta-lactam antibiotics and fluoroquinolones. Nevertheless, the confidence intervals around the risk estimates were large, which can be attributed to

the small number of events. This underscores the difficulties of accurately determining the direct associations between antibiotic use and resistance. Even after inclusion of 1201 consecutive ICU patients and analyzing 1093 microbiological cultures in 205 patients with either *Pseudomonas aeruginosa* or *Enterobacter* colonization confidence intervals of hazard ratios were overlapping and we were unable to quantify increased risks for *Enterobacter* species. Naturally, similar studies in settings with higher levels of antibiotic use and higher acquisition rates would have more power to accurately quantify risk associations. Of note, the difficulties to determine these associations on an individual patient level should not be embraced by using aggregated data instead, as this might lead to wrong interpretations (8).

Although the baseline prevalence of antibiotic resistance for *Pseudomonas aeruginosa* in this study population (1-6%) was lower as compared to other ICU populations (5-37%) (9, 16, 17), it does not affect our findings and ability to extrapolate to other ICU populations, since we here focus on the direct effect of antibiotic exposure on the process of antimicrobial resistance development in previous sensitive bacteria within a single patient.

Our study had a few limitations. First, the date of phenotype switch to antibiotic resistance was determined as the date of the first resistant isolate. Although extensive and regular culturing was conducted in this study, the exact number of days at risk would be slightly lower when the exact day of resistance switch was known, and thereby increasing the incidence rates per 100 patient days at risk or per 100 days of antibiotic exposure. However, this would not have altered our hazard ratios significantly, since both exposed and not exposed group of patients would have been equally influenced. Second, inherent to the observational design of our study, results may have been influenced by confounding variables. We attempted to minimize this by adjusting for confounding variables, such as previous and simultaneous antibiotic use, in multivariable analysis. Furthermore, resistance development may not only result from the type of antibiotic or the number of antibiotic exposure days, but also from the actual dosing of antibiotics. The latter variable, though, was not explicitly included, although dosages are to some extent incorporated in the calculation of DDDs. Finally, we did not investigate the development of multiple antibiotic resistance. Combined resistance acquisition in *Pseudomonas aeruginosa* was observed in half of meropenem resistance acquisitions and in half of ceftazidime acquired resistances. In ciprofloxacin and piperacillin-tazobactam about 80% to 90% concerned the development of combined resistance. It is difficult to include multiple resistances in time-dependent analyses, since resistance development for multiple antibiotics did not always occur simultaneously. Moreover, the numbers of combined resistance development were too low for statistical analysis.

Our findings underscore the potential detrimental consequences of increased usage of carbapenems in ICU patients. With the global emergence of ESBL-producing *Enterobacteriaceae* it will be challenging to balance the increasing need to treat patients with carbapenems against the risks of creating antibiotic resistance.

CONCLUSION

Meropenem use in ICU patients with *Pseudomonas aeruginosa* was associated with antibiotic resistance development to meropenem. The association was stronger for meropenem than for other antibiotics. These findings indicate that an increase of carbapenem use as a result of the global emergence of Gram-negative bacteria producing extended-spectrum beta-lactamases (ESBL) creates a serious risk for rapid emergence of carbapenem resistance among *Pseudomonas aeruginosa*. Therefore, antibiotic stewardship to optimize carbapenem use (i.e., to minimize its unnecessary use) is recommended.

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8

ANTIBIOTIC-INDUCED WITHIN-HOST RESISTANCE DEVELOPMENT OF GRAM-NEGATIVE BACTERIA IN PATIENTS RECEIVING SELECTIVE DECONTAMINATION OR STANDARD CARE

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ABSTRACT

Objective

To quantify antibiotic-associated within-host antibiotic resistance acquisition rates in *Pseudomonas aeruginosa* (PA), *Klebsiella* species (KS) and *Enterobacter* species (ES) from lower respiratory tract samples of Intensive Care Unit (ICU) patients receiving selective digestive decontamination (SDD), selective oropharyngeal decontamination (SOD) or standard care.

Design

Prospective cohort.

Setting

This study was nested within a cluster-randomized crossover study of SDD and SOD in 16 ICUs in the Netherlands.

Patients

Eligible patients were those colonized in the respiratory tract with PA, KS or ES susceptible to one of the marker antibiotics and with at least two subsequent microbiological culture results from respiratory tract samples available.

Measurements

Antibiotic resistance acquisition rates were defined as the number of conversions from susceptible to resistant for a specific antibiotic per 100 patient days or 100 days of antibiotic exposure within an individual patient. The hazard of antibiotic use for resistance development in PA was based on time-dependent Cox regression analysis. Findings were compared to a previous cohort of patients not receiving SDD/SOD.

Main results

Numbers of eligible patients were 277 for PA, 174 for KS and 106 for ES. Resistance acquisition rates per 100 patient days ranged from 0.2 (for colistin and ceftazidime in PA and for carbapenems in KS), to 3.0 (for piperacillin-tazobactam in PA and ES). For PA the acquisition rates per 100 days of antibiotic exposure ranged from 1.4 for colistin to 4.9 for piperacillin-tazobactam. Acquisition rates were comparable for patients receiving SDD/SOD and those receiving standard care. Carbapenem exposure had the strongest association with resistance development (adjusted hazard ratio 4.2; 95% confidence interval 1.1-15.6).

Conclusion

Within-host antibiotic resistance acquisition rates for systemically administered antibiotics were comparable between patients receiving selective decontamination and those receiving standard care, and were highest during carbapenem use.

INTRODUCTION

Patients in the intensive care unit (ICU) are vulnerable to opportunistic nosocomial infections, which are frequently caused by antibiotic-resistant bacteria (1). Such infections are associated with a prolonged hospital stay and increased morbidity and mortality (2, 3). Although antibiotic use is associated with higher antibiotic resistance rates, some antibiotics are associated with the risk of resistance development stronger than other antibiotics (4-7).

Selective Digestive Decontamination (SDD) and Selective Oropharyngeal Decontamination (SOD) are two preventive strategies that are associated with improved patient outcomes in settings with low levels of antibiotic resistance (8, 9). During ICU stay SDD treated patients receive, four times a day, non-absorbable topical antibiotics (usually consisting of tobramycin, colistin and amphotericin B) in the oropharynx and intestinal tract. Moreover, a third-generation cephalosporin is intravenously administered every 6 hours, during the first four days (10). SOD treated patients only receive topical antibiotics in the oropharynx. Both SDD and SOD reduce colonization and infection rates with Gram-negative bacteria in the lower respiratory tract (11-14). Although not confirmed in a large meta-analysis (15), concerns related to the ecological safety of these interventions remain (16, 17).

Emergence of antibiotic resistance can arise from within-host selection, usually facilitated through antibiotic selective pressure, and through transmission of resistant bacteria to a new host (18, 19). Few studies have attempted to quantify the rate of within-host resistance development (4-7). In a previous study the association between systemic antibiotic exposure and resistance development in *Pseudomonas aeruginosa* (PA) was assessed in a non-SDD/SOD setting (7). It is unknown whether these associations are similar in SDD/SOD settings. Moreover, there is inconsistence on the influence of host characteristics on this association (4-7, 20). This study assessed antibiotic-induced within-host resistance development rates in PA, *Enterobacter* species (ES) and *Klebsiella* species (KS) colonizing the lower respiratory tract of patients receiving SDD/SOD and compared these to the rates in non-SDD/SOD settings that were previously reported (7).

MATERIALS AND METHODS

Study Design and Patients

This study was part of a multicenter prospective cluster-randomized crossover study on the effects of SDD and SOD on antibiotic resistance that was performed in 16 ICUs in the Netherlands between 2009 and 2013 (21). Four university hospitals, six teaching hospitals and six non-teaching hospitals were included. The Institutional Review Boards of the participating hospitals waived the need for informed consent as SDD and SOD were considered to be standard care. Lower respiratory tract samples were collected at ICU admission and twice weekly thereafter until ICU discharge.

Adult patients with an expected stay of at least 48 hours were included. A patient who was readmitted to the ICU after 48h was considered as a new patient in the analysis. Patients without lower respiratory tract colonization with PA, KS or ES or with only one positive culture were excluded.

Study definitions

The primary outcome was acquired antibiotic resistance in PA, KS and ES in the lower respiratory tract of ICU patients. Antibiotic resistance was based on minimum inhibitory concentration (MIC) breakpoints according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or Clinical and Laboratory Standards Institute (CLSI) method, depending on hospital guidelines. Intermediate resistance was considered as resistant. Antibiotic resistance development was defined as the conversion from susceptible to resistant in consecutively obtained cultures for one of the following antibiotics: meropenem/imipenem, gentamicin/tobramycin, ceftazidime, other cephalosporins (ceftriaxone, cefotaxime, cefuroxime, cefoxitine, cefazoline and cefpodoxim), piperacillin-tazobactam, colistin, ciprofloxacin and cotrimoxazol. Results were presented per pathogen-antibiotic combination. If antibiotic resistance was documented within the first 48 hours after admission, these antibiotic-bug associations were excluded from analysis.

Antibiotic use was defined as the number of defined daily doses (DDDs) per 100 patient days, based on the Anatomical Therapeutic Chemical/Defined Daily Doses Index 2013 (ATC/DDD) of the World Health Organization Collaboration Centre for Drug Statistics Methodology. All days from the start of colonization until the day that resistance was documented or until the end of a colonization episode contributed to the total number of patient days. The number of antibiotic resistance events was expressed per 100 patient days at risk and per 100 days of antibiotic exposure. Patient days at risk were defined as all ICU days from the first day of documented carriage until resistance acquisition or until ICU-discharge, and could, thus, differ per bug-drug combination in individual patients. These methods are identical to those used in the previous cohort from 2007-2008 in which no SDD or SOD was used (7).

Data analysis

The effect of antibiotic use on the emergence of antibiotic resistance was analyzed with a Cox proportional hazard model, in which antibiotic use was a time-varying variable. We decided to perform two multivariable models. The first model included *a priori* the co-variables SDD or SOD treatment and the use of other antibiotics in the multivariable model. Patient related covariables including age, gender, Acute Physiology and Chronic Health Evaluation IV score (APACHE IV), Chronic Obstructive Pulmonary Disease (COPD) and immune status were separately tested in bivariable analyses for its potential confounding influence. If the β–coefficient of the antibiotic exposure variable changed more than 10% after adding the co-variable to the model,

this variable was also included in a second, fully adjusted model. Presence of multi-collinearity was tested. Data were analyzed with SAS 9.2 (Cary, NC) for Windows.

RESULTS

Among 8067 patients with an expected stay of at least 48 hours and who received SDD or SOD, 1058 (13%) patients were colonized in the lower respiratory tract with at least one of the three pathogens of interest. After exclusion of 503 patients with only one positive culture result 277 patients with two or more samples growing PA, 172 with two or more samples growing KS and 106 patients with two or more samples growing ES remained (Figure 1). Of these, 17 patients were colonized with both PA and KS, 9 with PA and ES, 9 with KS and ES, and 3 were colonized with all three pathogens.

Antibiotic resistance acquisition in SDD/SOD settings

Resistance acquisition to at least one of the tested antibiotics occurred in 99/277 (36%), 23/172 (13%) and 15/106 (14%) in PA, KS en ES, respectively. Median time to first resistance acquisition (against any antibiotic) was 7 (IQR 4-13), 9 (IQR 5-26) and 8 (IQR 6-14) days for PA, KS en ES, respectively.

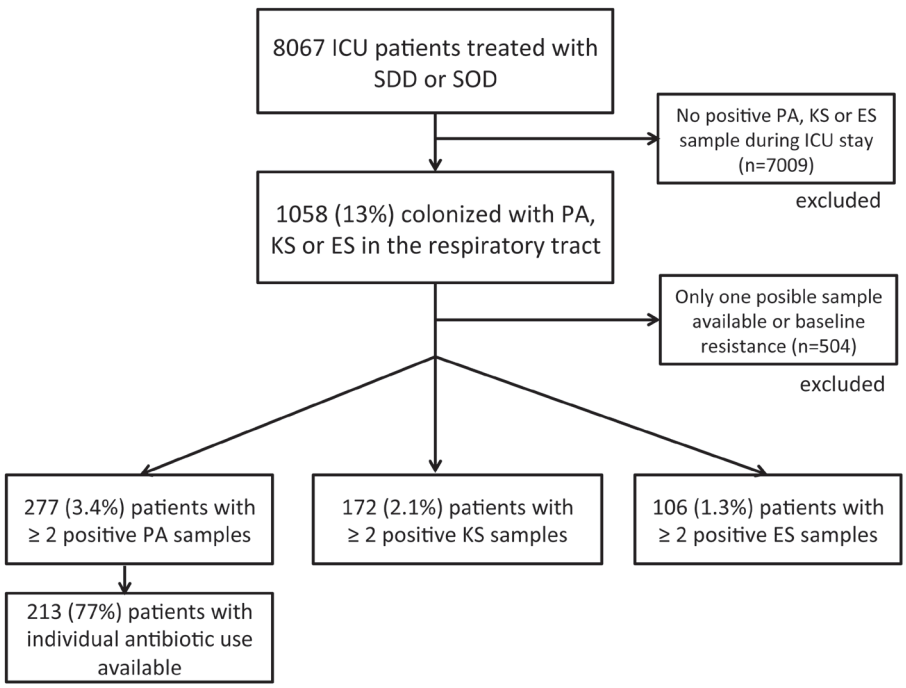

Figure 1. Flowchart of patient in- and exclusion

Table 1. Acquisition rates of antibiotic resistance in *Pseudomonas aeruginosa*, *Klebsiella* species and *Enterobacter* species (16 hospitals)

Antibiotic	First isolate susceptible (%)	Resistance acquisition events (%)	Total patient-days	Events per 100 patient-days (95% CI)
Pseudomonas aeruginosa (n=277)				
Carbapenem	194 (78)	13 (7)	1653	0.8 (0.5-1.4)
Aminoglycoside	221 (81)	22 (10)	2019	1.1 (0.7-1.7)
Ceftazidime	173 (82)	32 (19)	1310	2.4 (1.7-3.5)
Ciprofloxacin	230 (84)	33 (14)	2047	1.6 (1.1-2.3)
Piperacilline-tazobactam	175 (74)	41 (23)	1375	3.0 (2.2-4.0)
Colistin	195 (99)	9 (5)	1900	0.5 (0.2-0.9)
Any of the above antibiotics	277 (100)	99 (35)	2233	4.4 (3.6-5.4)
Two or more of the above	235 (85)	40 (17)	2987	1.3 (1.0-1.8)
Klebsiella species (n=172)				
Aminoglycoside	134 (96)	5 (4)	1161	0.4 (0.2-1.0)
Ceftazidime	106 (98)	2 (2)	953	0.2 (0.1-0.8)
Other cephalosporin*	105 (72)	9 (9)	899	1.0 (0.5-1.9)
Ciprofloxacin	139 (95)	7 (5)	1246	0.6 (0.3-1.2)
Cotrimoxazol	104 (96)	4 (4)	856	0.5 (0.2-1.2)
Piperacilline-tazobactam	75 (95)	4 (5)	450	0.9 (0.3-2.4)
Colistin	111 (98)	2 (2)	976	0.2 (0.1-0.8)
Any of the above antibiotics	172 (100)	23 (13)	1555	1.5 (1.0-2.2)
Two or more of the above	160 (93)	11 (7)	1559	0.7 (0.4-1.3)
Enterobacter species (n=106)				
Carbapenem	90 (99)	1 (1)	538	0.2 (0.0-1.3)
Aminoglycoside	89 (84)	3 (3)	489	0.6 (0.2-1.9)
Ceftazidime	40 (95)	2 (5)	219	0.9 (0.2-3.7)
Other cephalosporin**	12 (24)	1 (8)	157	0.6 (0.1-4.5)
Ciprofloxacin	97 (95)	5 (5)	502	1.0 (0.4-2.4)
Cotrimoxazol	63 (84)	2 (3)	383	0.5 (0.1-2.1)
Piperacilline-tazobactam	51 (89)	6 (12)	201	3.0 (1.3-6.6)
Colistin	72 (97)	2 (3)	351	0.6 (0.1-2.3)
Any of the above antibiotics	106 (100)	15 (14)	654	2.3 (1.4-3.8)
Two or more of the above	99 (93)	6 (6)	670	0.9 (0.4-2.0)

* other cephalosporins included: ceftriaxone, cefotaxim, cefuroxim, cefpodoxim, cefepime, cefoxitine and cefazoline
** other cephalosporins included: ceftriaxone, cefotaxim, cefuroxim, cefpodoxim and cefepime

Acquisition of resistance occurred most frequently against piperacillin-tazobactam with rates of 3.0 (95% CI 2.2-4.0), 0.9 (95% CI 0.3-2.4) and 3.0 (95% CI 1.3-6.6) conversions per 100 patients days for PA, KS and ES, respectively (Table 1). Other antibiotics with high resistance development were ceftazidime in PA (19%, 2.4 per 100 patient days), other cephalosporins in KS (9%, 1.0 per 100 patients days) and ciprofloxacin in ES (5%, 1.0 per 100 patient days).

Resistance acquisition in antibiotic exposed Pseudomonas aeruginosa

Antibiotic exposure data on individual patient level was available from four academic hospitals and two large teaching hospitals, and, therefore, only patients from these ICUs were analyzed in multivariable models. As there were only six and seven resistance acquisition events for KS and ES, this analysis was only performed for 213 (77%) patients colonized with PA in which 34 acquisition events occurred. In these patients, carbapenems were prescribed most frequently (25.1 DDDs per 100 patient days) followed by ceftazidime (20.7 DDDs per 100 patient days), but resistance acquisition rates were highest for the use of piperacillin-tazobactam (4.9 conversions per 100 days of exposure) and ceftazidime (4.3 per 100 days of exposure) (Table 2). In multivariable analysis, with adjustment for other determinants of resistance development, carbapenem use had the strongest association with resistance development (full adjusted hazard ratio (HR) 4.2; 95% CI 1.1-15.6). For ciprofloxacin use there was a non-significant trend for resistance development (full adjusted HR, 2.3; 95% CI, 0.8-6.0) (Table 3).

Comparison with non-SDD/SOD setting

As compared to patients with PA colonization in the previously studied cohort, patients in the current SDD/SOD cohort were older (median 64 years, IQR 54-73, versus 59, IQR 44-72, $p<0.01$), less frequently male (63% versus 75% male, $p=0.02$), and had a shorter median ICU length of stay of 16 days (IQR 8-30) versus 26 (IQR 14-40), respectively ($p<0.01$). Proportions of mechanically ventilated patients and of surgical admissions were similar in both study populations.

Resistance acquisition rates (per 100 days of antibiotic exposure) of antibiotic exposed PA were comparable in the current SDD/SOD cohort and the previous cohort without SDD/SOD. Carbapenem exposures had the lowest rates: 2.6 (95% CI 1.0-7.0) versus 2.3 (95% CI 0.3-4.2) in the current and previous cohort, whereas ceftazidime exposures had the highest rates: 4.3 (95% CI 2.3-7.9) versus 6.3 (95% CI 3.4-9.3), respectively (Table 2).

Median colonization durations were also similar, 7 (IQR 3-13) versus 9 (IQR 4-22) in the current and previous cohort, respectively. Median days to resistance were not different in both studies: for ciprofloxacin 9 (IQR 5-19) versus 9 (IQR 3-15), for ceftazidime 11 (IQR 7-17) versus 6 (IQR 4-15), for carbapenems 11 (IQR 7-21) versus 11 (IQR 5-21), and for piperacillin-tazobactam 10 (IQR 7-15) versus 12 (IQR 5-20), respectively. However, in this PA colonized patient group, ICU length of stay was shorter in the current cohort compared to the previous cohort: 16 (IQR 8-31) versus 26 (IQR 14-40) days, respectively.

Table 2. Resistance acquisition and antibiotic use in *Pseudomonas aeruginosa* colonized patients (6 hospitals)

Antibiotic	First isolate susceptible (%)	Resistance acquisition events	Resistance acquisition rates per 100 patient days (95% CI)	DDDs	DDDs per 100 patient-days (95% CI)	Resistance acquisition events in antibiotic exposed group (%)	Resistance acquisitions per 100 days of antibiotic exposure (95% CI)	Resistance acquisition rates per 100 patient days (95% CI) *[A]*	Resistance acquisitions per 100 days of antibiotic exposure (95% CI) *[A]*
Carbapenem (n=190)	143 (75)	12	0.9 (0.5-1.7)	710	25.1 (23.2-26.9)	4 (33)	2.6 (1.0-7.0)	0.7 (0.3-1.1)	2.3 (0.3-4.2)
Aminoglycoside (n=212)	163 (77)	21	1.3 (0.8-2.0)	583	16.9 (15.5-18.3)	6 (29)	1.8 (0.8-3.9)	n.a.	n.a.
Ceftazidime (n=150)	121 (81)	26	2.8 (1.9-4.1)	496	20.7 (18.9-22.5)	10 (39)	4.3 (2.3-7.9)	2.0 (1.3-2.8)	6.3 (3.4-9.3)
Ciprofloxacin (n=211)	174 (82)	26	1.5 (1.0-2.2)	754	17.8 (16.6-19.1)	11 (42)	3.0 (1.7-5.5)	0.6 (0.3-1.0)	2.5 (0.8-4.3)
Piperacillin-tazobactam (n=180)	126 (70)	34	3.1 (2.2-4.3)	392	13.4 (12.1-14.7)	8 (24)	4.9 (2.5-9.8)	1.1 (0.6-1.7)	2.6 (0.6-4.7)
Colistin (n=168)	163 (99)	10	0.6 (0.3-1.1)	347	8.5 (7.6-9.4)	3 (30)	1.4 (0.5-4.5)	n.a.	n.a.

DDDs= Defined daily doses. N.A. = not available
[A] Ong et al, Crit Care Med 2011 [7].

Table 3. Time-dependent Cox regression analyses on antibiotic resistance development in patients colonized with *Pseudomonas aeruginosa*

Antibiotic	Crude HR (95% CI)	Antibiotics adjusted HR (95% CI)	Patient and antibiotics adjusted HR (95% CI)
Ceftazidime	1.7 (0.7-3.8)	1.7 (0.8-3.9)[a]	1.5 (0.6-3.5)[b]
Ciprofloxacin	2.4 (1.1-5.3)	2.7 (1.2-6.2)[a]	2.3 (0.8-6.0)[c]
Colistin	2.5 (0.6-10.1)	3.3 (0.8-14.1)[a]	2.1 (0.4-10.1)[d]
Aminoglycoside	1.4 (0.5-3.7)	1.7 (0.6-4.5)[a]	1.5 (0.5-4.1)[e]
Carbapenem	2.7 (0.8-9.4)	3.5 (1.0-12.7)[a]	4.2(1.1-15.6)[f]
Piperacillin-tazobactam	1.6 (0.7-3.7)	1.7 (0.7-2.8)[a]	1.0 (0.1-15.6)[g]

a= adjusted for use of other antibiotics and SDD/SOD treatment
b= adjusted for use of other antibiotics (HR 1.3, 95% CI 0.5-3.3), SDD/SOD treatment (HR 0.8, 95% CI 0.3-1.8), APACHE IV score (HR 1.01, 95% CI 0.99-1.03) and COPD (HR 0.2, 95% CI 0.0 -1.5)
c= adjusted for use of other antibiotics (HR 0.8, 95% CI 0.3-2.1), SDD/SOD treatment (HR 1.9, 95% CI 0.7-4.8) and APACHE IV score (HR 0.99, 95% CI 0.98-1.01).
d= adjusted for use of other antibiotics (HR 0.5, 95% CI 0.1-2.4), SDD/SOD treatment (HR 1.2, 95% CI 0.3-4.6), age (HR 0.98, 95% CI 0.94-1.02) and gender (HR 1.2, 95% CI 0.3-5.3).
e= adjusted for use of other antibiotics (HR 0.3, 95% CI 0.1-0.9), SDD/SOD treatment (HR 0.6, 95% CI 0.2-1.6), gender (HR 1.1, 95% CI 0.4-2.8) and immune status (HR 2.6, 95% CI 1.0-7.0).
f= adjusted for use of other antibiotics (HR 0.4, 95% CI 0.1-1.6), SDD/SOD treatment (HR 1.5, 95% CI 0.4-4.9) and age (HR 0.97, 95% CI 0.93-1.02).
g= adjusted for use of other antibiotics (HR 1.4, 95% CI 0.6-3.5), SDD/SOD treatment (HR 0.5, 95% CI 0.2-1.2), APACHE IV score (HR 1.00, 95% CI 0.98-1.02), COPD (HR 0.4, 95% CI 0.1-2.1) and immune status (HR 1.3, 95% CI 0.3-4.8).

In general, resistance acquisition rates (per 100 patient days) in both cohorts were highest for ceftazidime: 4.3 (95% CI 2.3-7.9) versus 6.3 (95% CI 3.4-9.3) for the current and previous cohort, respectively. Rates were lowest for carbapenem resistance: 0.9 (95% CI 0.5-1.7) versus 0.7 (95% CI 0.3-1.1), respectively (Table 2). Nevertheless, exposure to carbapenems was in both cohorts associated with higher resistance development when compared to non-exposed patients.

DISCUSSION

Antibiotic-associated resistance acquisition rates of Gram-negative bacteria in the lower respiratory tract of ICU patients did not differ between patients receiving SDD, SOD or standard care.

Previous studies investigating associations between antibiotic exposure and resistance development in individual patients were inconclusive in establishing whether patient characteristics were potential confounders (i.e., related to both antibiotic exposure and resistance acquisition in these microorganisms) or that patients are merely hosts for bacterial colonization and subsequent resistance development (20). Therefore, we presented two adjusted models, one with only *a priori* selected antibiotic related factors and one with additional inclusion of host characteristics. Both models

yielded comparable results, which suggest that patient characteristics in comparison to antibiotic factors play a minor role in antibiotic resistance development, which was supported by absence of statistically significant associations between several patient characteristics and antibiotic resistance development in the final multivariable models.

Resistance development against piperacillin-tazobactam occurred most frequently in the current study, but rates were similar in exposed and non-exposed patients, suggesting that preceding piperacillin-tazobactam exposure did not influence acquisitions rates. This finding might reflect the observed increasing trend of piperacillin-tazobactam resistant PA in the Netherlands over the past years (22-24). Our results suggest that there is an ability of PA for resistance development to piperacillin-tazobactam without direct antibiotic pressure.

Although carbapenem resistance acquisition rates were low, carbapenem exposure had the strongest association with resistance development in *Pseudomonas aeruginosa*. In a large cluster-randomized crossover study in 13 Dutch ICUs SDD and SOD were associated with decreased use of systemic antibiotics, with reductions of 45.7% and 25.4% of carbapenem use (9). Therefore, implementation of selective decontamination might be beneficial in reducing carbapenem resistance by lowering total carbapenem use.

This was the first study on within-host resistance acquisition in SDD or SOD treated patients. The large multicenter design of this study favors the generalizability of our results, especially for low-endemic antibiotic resistance settings, but also to other settings as we studied within-host processes that are not influenced by external factors, such as other colonized patients. Others have advocated measuring acquisition of antibiotic resistance not only on a group level but also at the individual level because of ecological bias (6). We, therefore, analyzed acquisition on an individual level in six hospitals (corresponding to 213 (77%) of 277 *Pseudomonas* colonization episodes) where individual antibiotic treatment data were available.

The study also has some limitations. First, findings in patients receiving SDD/SOD were compared to a historic comparator. As SDD/SOD had become standard of care in the Netherlands it was impossible to include a control group without SDD/SOD via a randomized controlled design. Yet, sampling frequency and resistance determination was similar and standardized in both cohorts, preventing sampling bias. Furthermore, both studies included Dutch ICUs from academic and non-academic hospitals. Nevertheless, there were some differences in age, gender and length of stay in the ICU in the two patient cohorts. Second, genotyping of PA isolates was not performed, and, therefore, the occurrence of cross-transmission cannot be ruled out completely. However, the probability of cross-transmission is considered low as patients with acquired antibiotic resistance were widely distributed over different hospitals and time periods, without epidemiological linkage.

CONCLUSION

Within-host antibiotic resistance acquisition rates of Gram-negative bacteria in the lower respiratory tract of patients receiving SDD/SOD ranged from 0.2 to 3.0 per 100 patient days. SDD/SOD did not enhance resistance development associated with systemic antimicrobial exposure. PA was associated with higher resistance acquisition rates than ES and KS, and carbapenem use was associated with the highest rate of antibiotic resistance development.

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NEBULIZED AMPHOTERICIN B TO ERADICATE *CANDIDA* COLONIZATION FROM THE RESPIRATORY TRACT IN CRITICALLY ILL PATIENTS RECEIVING SELECTIVE DIGESTIVE DECONTAMINATION

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ABSTRACT

Introduction

Colonization of the lower respiratory tract with *Candida* species occurs in 25% of mechanically ventilated critically ill patients, and is associated with increased morbidity. Nebulized amphotericin B has been used to eradicate *Candida* as part of selective decontamination of the digestive tract (SDD) protocols, but its effectiveness is unknown. We aimed to determine the effectiveness of nebulized amphotericin B in eradicating *Candida* respiratory tract colonization in patients receiving SDD.

Methods

We included consecutive mechanically ventilated patients during a four-year period. Microbiological screening was performed upon admission and twice weekly thereafter according to a standardized protocol. A colonization episode was defined as the presence of *Candida* species in two consecutive sputum samples taken at least one day apart. To correct for time-varying bias and possible confounding, we used a multistate approach and performed time-varying Cox regression with adjustment for age, disease severity, *Candida* load at baseline and concurrent corticosteroid use.

Results

Among 1,819 patients, colonization with *Candida* occurred 401 times in 363 patients; 333 of these events were included for analysis. Decolonization occurred in 51 of 59 episodes (86%) and in 170 of 274 episodes (62%) in patients receiving and not receiving nebulized amphotericin B, respectively. Nebulized amphotericin B was associated with an increased rate of *Candida* eradication (crude HR 2.0; 95% CI 1.4-2.7, adjusted HR 2.2; 95% CI 1.6-3.0). Median times to decolonization were 6 and 9 days, respectively. The incidence rate of ventilator-associated pneumonia, length of stay and mortality did not differ between both groups.

Conclusions

Nebulized amphotericin B reduces the duration of *Candida* colonization in the lower respiratory tracts of mechanically ventilated critically ill patients receiving SDD, but data remain lacking that this is associated with a meaningful improvement in clinical outcomes. Until more evidence becomes available, nebulized amphotericin B should not be used routinely as part of the SDD protocol.

INTRODUCTION

Candida species are opportunistic pathogens that ordinarily inhabit the human gastro-intestinal tract. Colonization of the lower respiratory tract (LRT) by *Candida* occurs in 25% of critically ill patients receiving mechanical ventilation and in 50% of patients suspected of ventilator-associated pneumonia (VAP), and has been associated with longer intensive care unit (ICU) stay, a prolonged duration of mechanical ventilation, an increased risk of bacterial VAP, and possibly increased in-hospital mortality (1-4). Whether the presence of *Candida* is a cause or merely a marker of a more severe clinical course is uncertain.

Furthermore, it remains unclear how to differentiate between colonization and infection of the LRT. In general, *Candida* species are not assumed to be primary causative pathogens in VAP patients (5). In a post-mortem study in patients with evidence of pneumonia at autopsy, none of the subjects with a tracheal aspirate or bronchoalveolar lavage culture positive for *Candida* species had histopathological evidence of invasive *Candida* growth (6). However, there is evidence that colonization of the LRT by *Candida* species promotes the development of pneumonia by creating biofilms that are capable of holding other micro-organisms (7). Moreover, *Candida* is assumed to have an indirect effect by decreasing the immune defence and favouring bacterial development (8). Experimental and clinical studies have shown that *Candida* colonization was associated with an increased risk for VAP by *Pseudomonas aeruginosa*, and that systemic antifungal treatment decreased the risk for *Pseudomonas aeruginosa* infection in colonized patients (1, 9, 10).

Side effects associated with systemic antifungal treatment have limited the use of pre-emptive strategies for *Candida* colonization to patients who are at high-risk for invasive candidiasis, such as patients with severe and multiple-site colonization (11). However, local use of antifungal medication may provide a potentially attractive alternative approach. Nebulized amphotericin B (NAB) has been used to eradicate *Candida* species from the LRT as part of various selective decontamination of the digestive tract (SDD) protocols in patients with persistent *Candida* colonization despite topical use of amphotericin B (12). However, the clinical effectiveness of this approach in reducing the burden of *Candida* colonization in ICU patients is not known.

MATERIALS AND METHODS

Patients and measurements

This study was performed in a 32-bed mixed ICU of the University Medical Center Utrecht in the Netherlands between April 2008 and February 2012. The Ethics Committee of the University Medical Center Utrecht approved this study and waived the need for informed consent. We analyzed all mechanically ventilated adults who had been admitted to the ICU for >72 hours. Microbiological screening for the presence of *Candida* was prospectively carried out on admission and twice weekly thereafter

according to a standardized protocol that was part of a SDD/Selective Oropharyngeal Decontamination (SOD) trial (12). In brief, samples were inoculated on malt extract agar plates, and *Candida* load was semi-quantitatively determined (i.e. classified as <10, 10-100, and >100 colony forming units). Colonization was defined as the presence of *Candida* species in two or more consecutive bronchoalveolar lavage or sputum samples obtained on different days in the ICU, and the colonization start date as the first positive sample. Decolonization was defined as the absence of *Candida* in two consecutive samples on different days, or as the absence of *Candida* in the last available sample. We excluded episodes during which patients had received systemic antifungal treatment, as well as episodes during which NAB was initiated within the first two days after colonization start date (before results of microbiological surveillance cultures had become available), because the reason for use of amphotericin B in these cases was likely to be different.

Decontamination protocol

All patients received either SDD (from April 2008 to August 2009 and from June 2011 to February 2012) or SOD (from September 2009 to May 2011). During SDD (but not during SOD) NAB, four times five milligram daily, was recommended in case of *Candida* colonization of the LRT (12). To this end, 50 milligram of conventional amphotericin B for intravenous use was dissolved in 10 mL water for injection. For each nebulization session 2 mL of water for injection was added to 1 mL (=5 mg amphotericin B) of the prepared solution and aerosolized with a jet nebulizer system (Covidien, Mansfield, MA, USA). Air or oxygen under high pressure with a flow rate of 5 to 8 L per minute was used to generate aerosols with a mass median aerodynamic diameter of <5 micrometer. Each session lasted until all medication was inhaled (about 15 to 30 minutes). The nebulizer was attached to the ventilator circuit with a T-piece adaptor positioned between the endotracheal tube and the humidification filter. The nebulization protocol did not recommend specific ventilator settings. In our clinical practice, however, patients remained on a pressure-controlled ventilator with unchanged PEEP and inspiratory pressure settings. Depending on the mechanical ventilator type nebulization was continuously administered during both inspiration and expiration in half of the NAB treatments, whereas in the remaining cases nebulization was only during inspiration. Allocation of a ventilator depended on availability and was independent of patient characteristics. The humidification filter in the mechanical ventilation circuit was changed after each nebulization session to avoid obstruction.

Data analysis

Because the delay in the initiation of NAB treatment (relative to the start of colonization) may vary between patients, it is important to correct for time-varying bias, which occurs when the exposure variable is categorized to its final status rather than considering the timing of the change in status (13, 14). Furthermore, patients who spontaneously

decolonize early have less opportunity to receive NAB. These cases contribute better outcomes to the non-exposed group and therefore may lead to an underestimation of the treatment effect. To deal with these issues, we used time-varying Cox proportional hazards regression with adjustment for age, Sequential Organ Failure Assessment (SOFA) score, *Candida* load at baseline, and concurrent use of corticosteroids.

In order to graphically represent the results of our time-varying analysis and correctly estimate the median time to decolonization relative to the start of NAB (15), we used the multistate model shown in Figure 1 (16).

As a secondary study outcome, we compared the incidence rates of VAP occurring after the onset of the *Candida* colonization episode in both groups. For the period 2008-2010, we retrospectively assessed the medical records for the occurrence of VAP, according to established CDC criteria (17). For the period 2011-2012, we prospectively recorded the incidences of VAP as part of an ongoing cohort study that is aimed at finding early diagnostic and prognostic markers of sepsis (18). Furthermore, we compared ICU mortality and remaining length of stay in ICU following the onset of *Candida* colonization in patients receiving NAB compared to patients not receiving NAB.

To assess possible effect modification by the timing of treatment, we performed a secondary analysis on the overall treatment effect by comparing patients who had received NAB treatment early (within 5 days) versus late (after 5 days) following the colonization start date. Because of a reduced number of decolonization events in this subgroup analysis of early and late NAB starters, we tested possible confounders by adding each of them separately to a univariable Cox regression model containing only

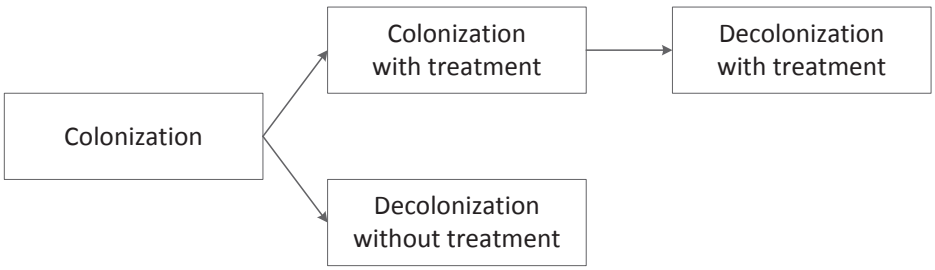

Figure 1. Multistate model
This multistate model was used for estimating the median time to decolonization relative to the start of nebulized amphotericin B, and for graphically representing colonization persistence probability curves in figure 4. A patient with colonization remained in this state as long as no treatment was given. Patients transitioned to the "colonization with treatment" state upon receiving treatment, and subsequently transitioned to "decolonization with treatment" state when decolonization occurred. The patient underwent transition to "decolonization without treatment" state in case decolonization occurred without treatment or before treatment was started. Thus, two states were transitional states, whereas the remaining two states were final states in our model (meaning that no further data were included in the model beyond this point in time).

NAB as an explanatory variable and examining its effect on the beta coefficient of the NAB variable on decolonization. Covariables that caused substantial confounding (i.e. a change in effect estimate greater than 10%) were included in the final multivariable models. In addition, to assess possible indication bias we performed a per-protocol analysis of patients treated according to the SDD and SOD arms of the trial. Data were analyzed with SAS 9.2 and R 2.15.1 software (package mstate).

RESULTS

Out of a total of 1,819 patients who had been admitted to the ICU for at least 72 hours and received mechanical ventilation, colonization with *Candida* occurred 401 times in 363 patients (Figure 2). Colonization rates did not differ between the SDD and SOD periods (21% versus 19% respectively, p=0.44). After exclusion of colonization episodes in which concurrent systemic antifungal treatment was administered (n=59) and episodes in which NAB were started within the first two days after the start of colonization (n=9), 333 episodes remained for analysis.

Almost half of the patients (48%) were already colonized upon ICU admission. These patients did not have significantly more colonization episodes compared to

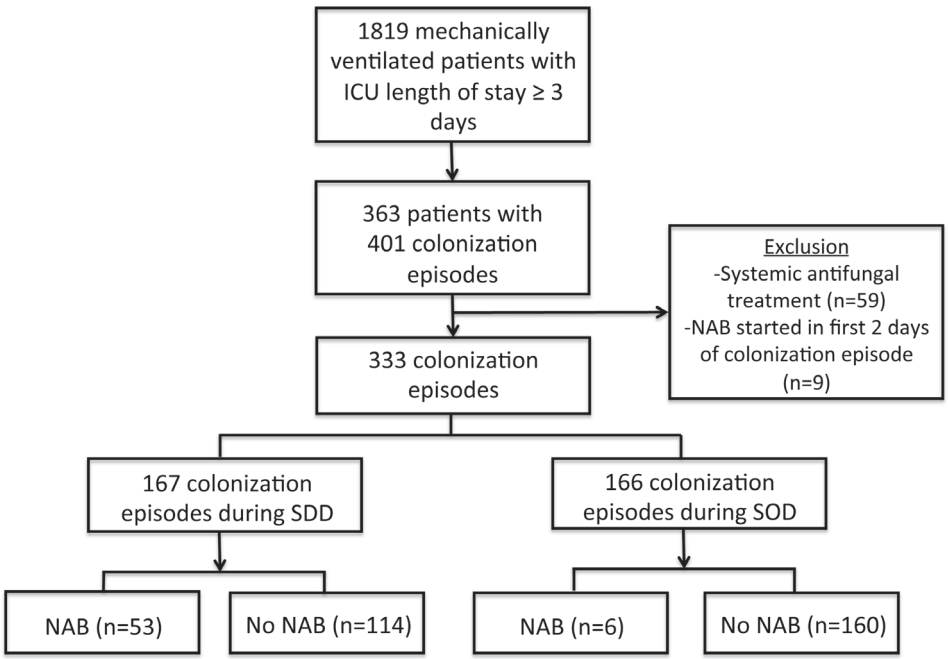

Figure 2. Patient inclusion

patients who acquired colonization during the ICU admission (p=0.09). Overall, 44% of positive cultures were classified as *Candida albicans*, 11% as *Candida glabrata*, and 8% *as non-albicans/non-glabrata/non-krusei* species (37% of cultures were not further subtyped). Concurrent oropharyngeal *Candida* co-colonization was present in 247 out of the 317 (78%) initial colonization episodes, and in 10 out of the 16 (63%) recolonization episodes.

The initial *Candida* load in the sputum was significantly higher in patients receiving amphotericin B compared to those not receiving therapy (Table 1). However, the number of bacterial co-colonizations was similar in both groups at baseline. There was also considerable variation in the time delay between the start of *Candida* colonization and start of therapy, with 46% of patients starting treatment after more than 5 days (Figure 3).

Table 1. Baseline characteristics

Characteristic	Nebulized Amphotericin B (n=59)	Standard Care (n=274)	p-value
Age	63 (43-76)	63 (52-72)	0.78
Gender male	43 (73)	189 (69)	0.55
APACHE IV score	77 (60-96)	75 (58-92)	0.42
Corticosteroid use [(a)]	18 (31)	75 (27)	0.63
SOFA score [(b)]	6 (3-8)	5 (3-8)	0.51
Days in ICU before onset of the colonization episode	0 (0-2)	1 (0-3)	0.24
Candida load in sputum (CFUs) [(d)]			<0.01
<10	9 (15)	117 (43)	
10-100	32 (54)	102 (37)	
>100	18 (31)	55 (20)	
Candida colonization of the oropharynx	50 (85)	213 (78)	0.23
Candida colonization of the rectum	23 (39)	75 (27)	0.08
Delay between onset of *Candida* colonization in sputum and NAB start	5 (4-7)	N.A.	N.A.
Bacterial co-colonization in sputum:			
Gram-negative rods [(e)]	13 (22)	83 (30)	0.20
Pseudomonas species	1 (2)	20 (7)	0.11
Gram-positive cocci	13 (22)	63 (23)	0.87

Data are presented as medians (IQR) or absolute numbers (%).
APACHE = Acute Physiology and Chronic Health Evaluation; CFU = colony forming units; N.A.= not applicable; SDD = selective digestive decontamination; SOD = selective oral decontamination; SOFA = Sequential Organ Failure Assessment;
[a] Corticosteroid use was defined as a daily dose > 100 mg hydrocortisone or equivalent;
[b] We used a modified sum score, excluding points for the central nervous system;
[c] SDD period from April 2008 to August 2009 and from June 2011 to February 2012, SOD period from September 2009 to May 2011;
[d] *Candida* load at baseline was determined by semi-quantitative culture;
[e] Gram-negative rods including *Pseudomonas* species

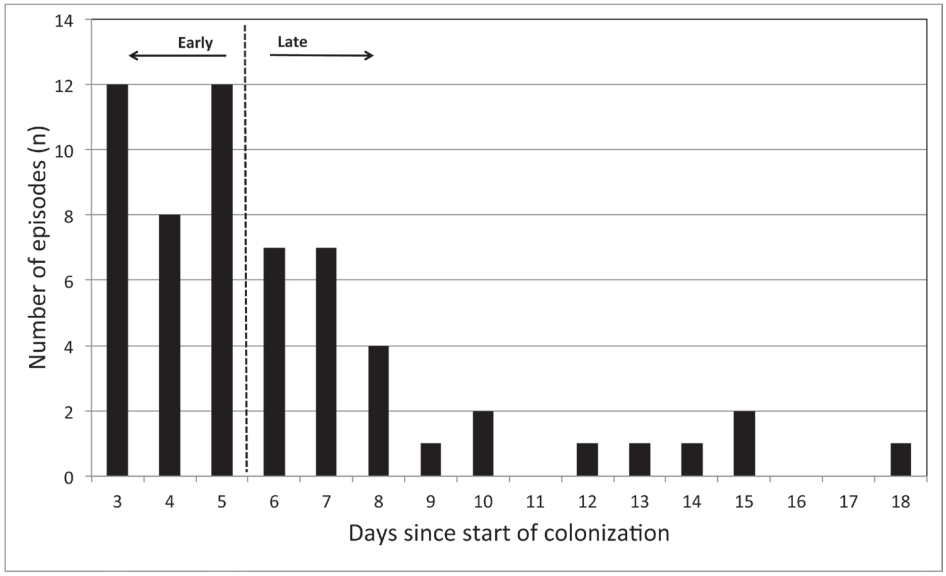

Figure 3. Time delay between start *Candida* colonization and treatment initiation
This figure depicts the observed time delay between the start of the *Candida* colonization episode and the initiation of nebulized amphotericin B treatment. Episodes during which treatment was initiated within the first two days after start of colonization (before results of microbiological surveillance cultures had become available) were excluded from the study, because the reason for antifungal treatment in these cases is likely to be different.

Decolonization occurred in 51 of 59 (86%) and 170 of 274 (62%) episodes in patients receiving and not receiving NAB, respectively. Following the start of NAB treatment the proportion of sputum cultures growing *Candida* decreased over time (Table 2), suggesting true decolonization rather than in vitro suppression of growth by amphotericin. Recolonization occurred in 2% and 6% of patients receiving and not receiving NAB, respectively (Table 3).

Table 2. Proportion of consecutive sputum cultures showing *Candida* growth following the start of NAB treatment

Consecutive sputum cultures following the start of NAB	Proportion of patients with positive culture (%)
1	100
2	67
3	25
4	19
5	9

Table 3. Univariable analysis of secondary outcomes

Characteristic	NAB (n=59)	Standard Care (n=274)	p-value
VAP incidence after the onset of *Candida* colonization (per 1000 ICU days)	6.5	5.5	0.64
Number of *Candida* recolonization episodes *	1 (2)	15 (6)	0.32
Length of stay in ICU after onset of *Candida* colonization	23 (12 – 30)	14 (10 – 23)	0.004
Length of stay in ICU after NAB start	14 (7-25)	N.A.	N.A.
ICU Mortality	10 (17)	54 (20)	0.62

Data are presented as medians (IQR) or absolute numbers (%), except for the ventilator-associated pneumonia rate that is presented as the number per 1000 ICU days.
N.A. = not applicable; NAB = nebulized amphotericin B; VAP = ventilator-associated pneumonia;
* The denominators for the calculation of the numbers of *Candida* recolonization events in the NAB and standard care group are 58 and 259 initial colonization episodes, respectively.

Figure 4 shows the persistence probability of colonization as analyzed using a multistate approach. Both curves are conditioned on colonization being present until at least day 4. Median times to elimination of *Candida* were estimated as 6 (IQR 5-7) days and 9 (IQR 6-20) days in patients receiving and not receiving NAB, respectively. In time-varying analyses, NAB treatment was associated with an approximately two-fold increase in the rate of decolonization (crude HR 2.0; 95% CI 1.4-2.7, adjusted HR 2.2; 95% CI 1.6-3.0).

The incidence rate of VAP and ICU mortality did not differ between both groups (Table 3). The length of stay in ICU after NAB initiation was comparable to the length of stay in ICU after onset of *Candida* colonization in the control group, 14 (IQR 7-25) and 14 (10-23) days, respectively. In 5 of 59 episodes NAB treatment was discontinued before successful decolonization was achieved, because of extubation (n=2), clinical decision to stop all antimicrobial therapy (n=1) or unclear reasons (n=2). No adverse effects (such as cough or bronchospasm) were documented in the medical records for any of the 59 cases.

Secondary analysis

There were 27 / 31 (87%) and 24 / 28 (86%) decolonization events in early and late NAB starters, respectively, resulting in an adjusted HR of 2.0 (95% CI 1.3-3.1), and 2.0 (95% CI 1.3-3.2), respectively. During the SOD period, in which NAB treatment was not recommended in the protocol, NAB was applied in 6 of 166 (4%) cases, whereas during the SDD period 114 of 167 (68%) cases did not receive NAB while being colonized. However, after exclusion of episodes during SDD in which NAB was not started as well as episodes during SOD in which NAB was administrated (per-protocol analysis), the effect estimates remained similar (crude HR 2.1; 95% CI 1.4-3.0, adjusted HR 2.2; 95% CI 1.5-3.3).

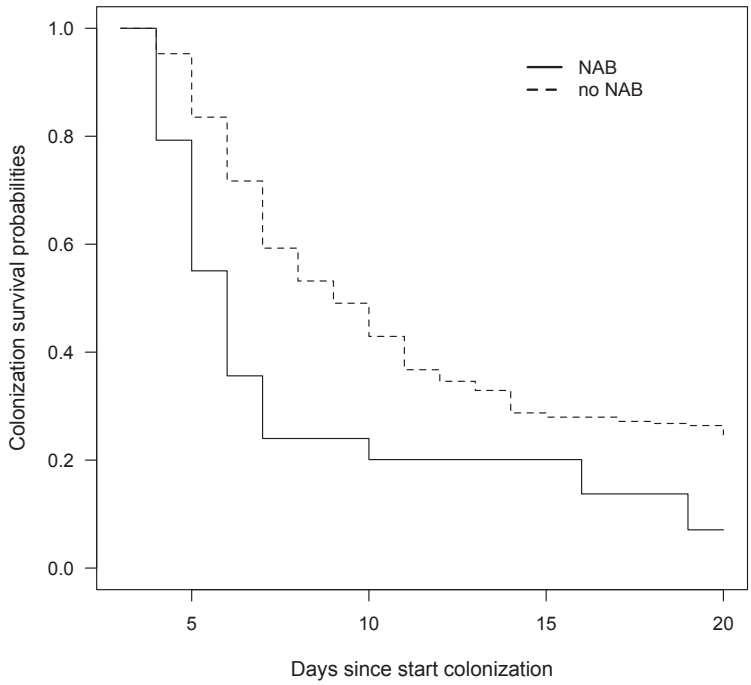

Figure 4. Time to *Candida* eradication of the lower respiratory tract
Colonization persistence probability curves of the nebulized amphotericin B (NAB) group and the no NAB group were plotted with 95% confidence intervals using the multistate approach. Both curves were conditioned on being colonized at day 4 in order to have a meaningful comparison between the treated and untreated patients. One any given day, one curve represented episodes in which NAB treatment was given and the other represented those without treatment.

DISCUSSION

Inhalation therapy with NAB resulted in a three-day reduction in the duration of *Candida* colonization in the LRT of mechanically ventilated patients who are (also) receiving topical applications of amphotericin B as part of a SDD protocol. This effect remained after adjustment for potential confounders and was approximately constant irrespective of the duration of colonization prior to the start of treatment.

Although NAB has been an essential component in SDD protocols for many years (12, 19), this is the first study to assess the effectiveness of inhalation treatment with NAB for eradication of *Candida* in colonized ICU patients. Previous studies have focused on lung transplant recipients in whom NAB was used as prophylactic treatment for, in particular, Aspergillus infection (20-22). Inhalation treatment seems attractive, because high drug concentrations can be reached at the site of interest while reducing nephrotoxicity and drug interactions (23, 24). However, inhalation of amphotericin B has thus far been restricted to off-label use, due to a lack of effectiveness data

and standardized methods for nebulized administration. Although inhalation of NAB is generally well-tolerated and considered to be safe (25), various reports of adverse effects, including cough, bronchospasm, dyspnea, nausea, and an unpleasant aftertaste, warrant prudency (26-29). In our study we observed only a few cases in which NAB treatment was discontinued before successful decolonization was achieved and we did not find adverse effects documented in the medical records.

Our study has some limitations. First, the study was performed in an SDD/SOD setting exclusively. This included the daily application of a topical paste containing polymyxin E, tobramycin and amphotericin B to prevent colonization with gram-negative bacteria and yeasts in the mouth. In addition, SDD (but not SOD) includes the use of a suspension to selectively decontaminate the digestive tract, and the systemic administration of cefotaxime during the first four days of ICU admission (12). Oropharyngeal decontamination by oral paste might influence *Candida* colonization in the lower respiratory tract over time by aspiration of topically applied amphotericin in the oropharynx. However, because in both groups the topical application was entirely the same, we assume that the estimation of the efficacy of NAB in combination with oral paste in comparison to oral paste alone is valid within the SDD setting. Both SDD and SOD have been shown to effectively reduce the incidence of VAP (19, 30), therefore it is possible that the clinical relevance of reducing the burden of *Candida* colonization by NAB treatment will differ between settings with and without SDD.

Moreover, during SDD NAB was not started in all patients eligible for this intervention according to protocol recommendations. This probably reflects indication bias. It is plausible that patients with a longer expected ICU stay or greater disease severity were more likely to receive NAB than patients in better health conditions. However, we reduced the possible effects of indication bias by excluding patients who received NAB before microbiological results had become available and by adjusting for potential confounding variables, such as disease severity and *Candida* load. Furthermore, we performed a per-protocol analysis that yielded similar results. Another source of bias may be related to the large variation in the timing of start of treatment that was observed, also known as immortal time bias (31). Again, by incorporating a time-varying approach into our analysis, we aimed to minimize such bias.

Although we did not find a trend towards a reduced incidence rate of VAP in patients receiving NAB, we stress that the interpretation of this apparent lack of clinical effectiveness requires caution, because our study lacks the statistical power to detect potentially meaningful differences in VAP rates and because of the presence of some concurrent interventions during the use of SDD for which we did not control.

CONCLUSIONS

Inhalation therapy with NAB is associated with a two-fold increase in the rate of *Candida* decolonization of the LRT in mechanically ventilated patients receiving SDD,

resulting in an average reduction of the colonization time by approximately three days. However, we found no evidence that effective decolonization translates into a clinically meaningful reduction in VAP rates, ICU length of stay or mortality. The routine use of NAB in current SDD protocols, therefore, cannot be recommended.

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10

NYSTATIN VERSUS AMPHOTERICIN B TO PREVENT AND ERADICATE *CANDIDA* COLONIZATION DURING SELECTIVE DIGESTIVE TRACT DECONTAMINATION IN CRITICALLY ILL PATIENTS

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ABSTRACT

Purpose

To compare the effectiveness of topical application of nystatin and amphotericin for prevention of *Candida* colonization in the intestinal and respiratory tract of critically ill patients.

Methods

In a before-after study in a 32-bed mixed ICU in a tertiary care center, we determined colonization with *Candida* species in the intestinal and respiratory tracts of patients receiving one of three regimens of Selective Digestive tract Decontamination (SDD): SDD with amphotericin B as antifungal component in both oropharyngeal paste and enteral suspension (Am/Am, 16 months); amphotericin B in oropharyngeal paste and nystatin in enteral suspension (Am/Nys, 17 months); and nystatin in both oropharyngeal paste and enteral suspension (Nys/Nys, 10 months). Acquisition of *Candida* colonization in the rectum and respiratory tract, and decolonization of *Candida* from the rectum was based on rectum and respiratory tract surveillance cultures.

Results

During the three periods we enrolled 867, 849 and 489 patients, respectively. Rectal colonization rates were 1.9 (95% CI 1.5-2.4), 1.3 (95% CI 1.0-1.7) and 1.0 (95% CI 0.6-1.3) events per 100 days for subsequent periods. Nys/Nys was independently associated with less rectal acquisition of *Candida* compared to Am/Am (adjusted hazard ratio (HR) 0.52, 95% CI 0.33-0.83) and associated with faster rectal decolonization (adjusted HR 1.70, 95% CI 1.18-2.45) in 373 patients who were already colonized at admission. Acquisition rates in the respiratory tract were not significantly different in the three study periods.

Conclusion

Nystatin was more effective than amphotericin in both preventing and eradicating *Candida* colonization of the rectum during SDD, and had comparable effects in preventing respiratory tract colonization with *Candida*.

INTRODUCTION

The use of selective digestive tract decontamination (SDD) and selective oropharyngeal decontamination (SOD) in intensive care units (ICUs) with low levels of antibiotic resistance has been associated with absolute reductions in day-28 mortality of 2.9%-3.5% compared to standard care, corresponding to a number needed to treat of 29-34 (1). SDD has been shown to reduce ICU length of stay, ICU mortality and hospital mortality (2, 3). Both strategies aim to eliminate potential pathogenic microorganisms, such as *Staphylococcus aureus*, Gram-negative bacteria and yeasts from the oropharynx and digestive tracts of ICU patients while preserving the anaerobic flora to prevent colonization, overgrowth and subsequent infections such as ventilator-associated pneumonia.

Fungal colonization, most often by *Candida* species, occurs frequently in ICU patients who are admitted for 7 days or more (4). In these patients *Candida* colonization was present in 21-29% of rectal surveillance samples and 18-26% of bronchial aspirate surveillance samples (4). Moreover, *Candida* colonization was an independent risk factor for subsequent yeast infection (5).

The antimicrobial regimen most often used in SDD consists of three components: a topical mouth paste, gastro enteral suspension, and systemic antimicrobial prophylaxis with a broad-spectrum antimicrobial agent, such as a third generation cephalosporin. The mouth paste and suspension usually contain colistin, tobramycin and amphotericin B. The latter is to eradicate and prevent carriage and subsequent yeast infections. In some studies nystatin has been used instead of amphotericin B (6-8). Nystatin and amphotericin B are both effective against a broad range of fungi and are not absorbed from the digestive tract (9). The effects of amphotericin B and nystatin, as used in SDD regimens, on *Candida* colonization and eradication have never been determined. Yet, amphotericin B is currently more expensive than nystatin and periodically has been difficult to acquire due to scarcity of its raw materials, fuelling initiatives to substitute one drug for the other. We, therefore, prospectively compared the efficacy of nystatin and amphotericin B as intestinal and oropharyngeal components of SDD with respect to *Candida* colonization.

METHODS

We performed a before-after study evaluating subsequent changes to an SDD regimen in a 32-bed medical-surgical ICU of a tertiary care hospital in the Netherlands from June 2011 to December 2014. All patients who were admitted for 48 hours or longer (and thus eligible to receive SDD until discharge from the ICU) were included in the study. We excluded patients who received concurrent systemic antifungal treatment during their ICU stay. The local ethics committee waived the need for informed consent.

SDD treatment

There were three study periods: (1) from June 2011 till October 1st 2012 (16 months) SDD treatment included daily (q.d.s.) application of a mouth paste containing 2% polymyxin E, 2% tobramycin and 2% amphotericin B, administration (q.d.s) of a suspension with the same components (100 mg polymyxin E, 80 mg tobramycin and 500 mg amphotericin B) through the nasogastric tube, and the systemic administration (q.d.s.) of cefotaxime during the first four days of ICU admission (Am/Am); (2) from October 2012 till March 1st 2014 (17 months) nystatin (2 x 10^6 units per dose) replaced amphotericin B in the enteral solution only (Am/Nys); (3) from March 2014 till January 1st 2014 (10 months) nystatin replaced amphotericin B in both the oropharyngeal paste and enteral solution (Nys/Nys). All other components of the SDD protocol, including infection surveillance methods, isolation measures and indications for treatment of infections remained unchanged during the three study periods.

Microbiological cultures

Microbiological screening samples, including rectal swabs and respiratory tract samples (endotracheal aspirate or bronchoalveolar lavage (BAL) samples when available), were routinely collected in all patients on admission and twice weekly thereafter as part of the SDD protocol. For *Candida* detection, samples were inoculated on malt extract agar plates, and *Candida* load was semi-quantitatively determined (i.e. classified as <10, 10-100, and >100 colony forming units). Culture results were used to modify the SDD regimen, for instance by adding nebulization with amphotericin B in case of persistent carriage of *Candida* in the lower respiratory tract despite SDD (10).

Outcome

The primary outcome measures were the acquisition rates of *Candida* colonization in the rectum and respiratory tract in each study period. The secondary outcomes were the prevalence of *Candida* in the rectum and respiratory tract during the three study periods and eradication of rectal carriage with *Candida* in patients who were colonized with *Candida* at the time of ICU admission.

Colonization was defined as the presence of *Candida* species in two or more consecutive samples from one body site (rectal or respiratory tract) obtained on different days in the ICU or the presence of *Candida* in the last available sample of that body site. Patients were decolonized if two or more consecutive rectal samples showed no growth of *Candida* in a patient previously identified as colonized, or the absence of *Candida* in the last available rectum sample.

The effect of nystatin and amphotericin B on decolonization of the respiratory tract was not studied, as we hypothesized that the SDD mouth paste would not eradicate *Candida* from the lower respiratory tract, but rather prevent colonization.

Data analysis

Cox survival regression was used to study the associations between the SDD regimens and acquisition of *Candida* colonization in the rectum and respiratory tract. For this analysis we considered patients who had at least two respiratory or two rectal samples available and were not colonized by *Candida* in the rectum or respiratory tract at admission, respectively, based on the first two days since admission. We calculated the proportion of patients who acquired *Candida* colonization and the corresponding acquisition rates in the rectum and respiratory tract. In our multivariable models we adjusted for all co-variables that changed the crude effect estimate of either one of the SDD regimens by more than 10% as well as all patient characteristics that were imbalanced between the groups at baseline (p value <0.20).

For the secondary analysis on decolonization of rectal carriage Cox survival regression was used. We included patients who had at least two rectal samples available and had documented rectal colonization at admission. All data were analyzed with SAS 9.2 (Cary, NC).

RESULTS

A total of 2456 patients were admitted for 48 hours or longer during the entire study period. After exclusion of patients who received concurrent systemic antifungal treatment, 2205 patients remained. The median length of ICU stay was 5.6 (IQR 3.3-10.0), 5.8 (IQR 3.2-10.8) and 5.8 days (IQR 3.4-11.7) for the Am/Am, Am/Nys and Nys/Nys periods, respectively (p=0.71). A median of 2 (IQR 1-4) rectum surveillance and 2 (IQR 1-4) respiratory surveillance cultures were available per patient (Table 1). *Candida* was present in 23%, 21% and 18% of rectum surveillance cultures and in 35%, 42% and 33% of respiratory surveillance cultures, for study period 1 to 3 respectively.

Of the 2205 included patients, 1468 patients had at least two rectum surveillance cultures available, and 1378 patients had at least two sputum surveillance cultures available.

Acquisition of Candida colonization in the rectum

Of the 1468 patients who had at least two rectum surveillance cultures available, 1095 cases (75%) were not colonized at the start of ICU admission and included in the analysis on *Candida* acquisition in the rectum.

Intestinal *Candida* colonization occurred in 80 of 441 (18%), 62 of 415 (15%), and 24 of 239 (10%) patients during period 1 (Am/Am), period 2 (Am/Nys) and period 3 (Nys/Nys) (p=0.02), respectively. Acquisition rates were 1.9 (95% CI 1.5-2.4), 1.3 (95% CI 1.0-1.7) and 1.0 (95% CI 0.6-1.3) events per 100 patient days, respectively. The time until colonization was comparable: 6 (IQR 4-10), 6 (IQR 4-12) and 7 (IQR 3-12) days (p=0.58), respectively. Compared to the reference period (i.e., Am/Am) and after adjustment for baseline imbalances (Table 2), no significant difference was found for Am/Nys (adjusted

Table 1. Timeline of the study

Period	1 (Am/Am)	2 (Am/Nys)	3 (Nys/Nys)
Follow up (months)	16	17	10
Date	June 2011 - October 2012	October 2012 - March 2014	March 2014 - January 2015
Mouthpaste [a]	Amphotericin B	Amphotericin B	Nystatin
Gastro-enteral suspension [a]	Amphotericin B	Nystatin	Nystatin
Number of patients [b]	867	849	489
Number of: Rectum surveillance cultures	2277	2584	1486
Candida positive rectum cultures (%)	531 (23%)	548 (21%)	274 (18%)
Median number of cultures / patient (IQR)	2 (1-3)	2 (1-4)	2 (2-4)
Number of Respiratory surveillance cultures [c]	2430	2671	1569
Candida positive respiratory cultures (%)	844 (34%)	1133 (42%)	518 (33%)
Median number of cultures / patient (IQR)	2 (1-4)	2 (1-4)	2 (1-4)

[a] in combination with colistin and tobramycin
[b] all patients with stay >48 hours (excluding patients with systemic antifungal therapy)
[c] sputum, endotracheal aspirate or broncho-alveolar lavage (BAL) samples

HR 0.74, 95% CI 0.53-1.03), whereas Nys/Nys was associated with prevention of *Candida* colonization in the intestinal tract (adjusted HR 0.52, 95% CI 0.33-0.83) (Table 3).

Acquisition of Candida colonization in lower respiratory tract

Of 1378 patients from whom at least two sputum cultures were available, 702 had no evidence of respiratory tract colonization with *Candida* at admission. Acquired respiratory tract colonization was demonstrated in 61 of 295 (21%), 79 of 215 (31%), and 31 of 156 (20%) patients during period 1 to period 3, respectively (p<0.01). Acquisition rates were 2.5 (95% CI 1.8-3.1), 2.7 (95% CI 2.1-3.3) and 2.1 (95% CI 1.3-2.8) events per 100 patient days, respectively. After adjustment for baseline imbalances (Table 2), neither Am/Nys nor Nys/Nys was associated with altered acquisition rates of *Candida* in the lower respiratory tract (Table 3).

Candida decolonization in the intestinal tract

Among the 373 patients with rectal carriage with *Candida* at admission, decolonization occurred in 59 of 127 (46%), 84 of 150 (56%), and 57 of 96 (59%) patients during period 1 to period 3 (p=0.12), respectively. Decolonization rates were 6.7 (95% CI 5.0-8.4), 8.2 (95% CI 6.5-10.0) and 10.8 (95% CI 8.0-13.6) events per 100 patient days, respectively. After adjustment for baseline imbalances, Nys/Nys was associated with faster decolonization of *Candida* in the rectum (adjusted HR 1.70, 95% CI 1.18-2.45) (Table 3).

Table 2. Patient characteristics

Characteristic	Rectum analysis (n=1095) [a] Period			Respiratory tract analysis (n=702) [a] Period		
	1 (n=441)	2 (n=415)	3 (n=239)	1 (n=295)	2 (n=251)	3 (n=156)
Age - years	59 (48-71)	61 (50-70)	61 (48-72)	58 (48-69)	60 (49-69)	58 (40-73)
Male sex	302 (68)	291 (70)	159 (67)	189 (64)	168 (67)	104 (67)
Body Mass Index	25 (23-28)	26 (23-30)	26 (23-29)	25 (23-28)	26 (23-30)	26 (23-30)
Chronic obstructive pulmonary disease	50 (11)	46 (11)	26 (11)	29 (10)	27 (11)	12 (8)
Diabetes mellitus	74 (17)	66 (16)	23 (10)	34 (12)	32 (13)	21 (13)
Hypertension	157 (36)	142 (34)	64 (27)	94 (32)	83 (33)	45 (29)
Chronic dialysis	4 (1)	7 (2)	1 (0)	3 (1)	6 (2)	1 (1)
Cancer	60 (14)	66 (16)	31 (13)	38 (13)	35 (14)	23 (15)
Immune deficiency [b]	29 (7)	41 (10)	25 (10)	20 (7)	19 (8)	15 (10)
Corticosteroids [c]	37 (8)	31 (7)	15 (6)	23 (8)	13 (5)	10 (6)
Medical reason for admission	13 (3)	13 (3)	10 (4)	11 (4)	9 (4)	5 (3)
Other immunosuppressive medication	246 (56)	233 (56)	135 (56)	165 (56)	132 (53)	95 (16)
Prior ICU admission during hospital stay	59 (13)	58 (14)	28 (12)	39 (13)	34 (14)	14 (9)
APACHE IV score	73 (58-90)	73 (60-96)	76 (67, 89)	73 (58-91)	71 (60-97)	76 (63-88)
ICU length of stay	8 (5-13)	9 (5-15)	9 (5-14)	8 (5-13)	10 (6-17)	10 (6-16)
ICU mortality	52 (12)	51 (12)	39 (16)	44 (15)	37 (15)	25 (16)

Data are presented as medians (IQR) or absolute numbers (%).
[a] Patients with 2 or more culture results available, not colonized on admission
[b] Immune deficiency was defined as use of immunosuppressive medication (prednisone 0.1 mg/kg for at least 3 months, prednisone 75 mg/day during at least one week, or equivalent), chemotherapy/radiotherapy in the year preceding intensive care unit admission, and a known humoral or cellular immune deficiency
[c] Corticosteroid use was defined as a daily dose > 100 mg hydrocortisone or equivalent;

Table 3. Effectiveness of nystatin versus amphotericin B in preventing *Candida* acquisition and achieving decolonization

Outcome	Crude Hazard Ratio [95% CI]	Adjusted Hazard Ratio [95% CI]
Candida acquisition in rectum		
Am/Am (period 1 = reference)	1	1
Am/Nys (period 2)	0.75 (0.54-1.05)	0.74 (0.53-1.03) [a]
Nys/Nys (period 3)	0.53 (0.33-0.83)	0.52 (0.33-0.83) [a]
Candida acquisition in sputum		
Am/Am (period 1 = reference)	1	1
Am/Nys (period 2)	1.23 (0.88-1.72)	1.21 (0.86-1.70) [b]
Nys/Nys (period 3)	0.87 (0.57-1.33)	0.85 (0.55-1.31) [b]
Candida decolonization in rectum		
Am/Am (period 1 = reference)	1	1
Am/Nys (period 2)	1.23 (0.88-1.72)	1.22 (0.87-1.70) [c]
Nys/Nys (period 3)	1.71 (1.19-2.48)	1.70 (1.18-2.45) [c]

[a] Covariables selected for multivariable analysis were APACHE IV score, diabetes mellitus, hypertension, immune deficiency and body mass index.
[b] Covariables selected for multivariable analysis were body mass index and COPD.
[c] Covariables selected for multivariable analysis were gender, body mass index and corticosteroid use (a daily dose > 100 mg hydrocortisone or equivalent).

DISCUSSION

This study evaluated the microbiological effects of amphotericin and nystatin during SDD. Fewer patients acquired intestinal *Candida* colonization when nystatin was used in the topical components of SDD, compared to amphotericin. Nystatin and amphotericin were equally effective in preventing acquisition of *Candida* in the lower respiratory tract.

Although amphotericin B and nystatin use for decolonization purposes have never been compared head to head on clinical outcome parameters, nystatin has been used in previous SDD regimens (6-8). Although the optimal dosage of nystatin is unknown, most studies have used 1-2 x 10^6 units per dose, similar to the dose used in this study. A recent biopharmaceutical study in 5 healthy volunteers and 10 ICU patients showed that amphotericin B is poorly released from the mouth paste, as opposed to nystatin, yielding higher nystatin concentrations in saliva compared to amphotericin B (11).

In our study, SDD could not prevent intestinal acquisition of *Candida* in 10-18% of patients. In a previous Dutch multicenter study, SDD with amphotericin B led to increased acquisition of *Candida* and other yeasts in the respiratory tract, compared

to standard care without SDD (Odds Ratio (OR) 1.59; 95% CI 1.31-1.93) (12). In contrast, in the same study the odds of candidemia with *Candida* or other yeasts were lower in SDD compared to standard care (OR 0.33; 95% CI 0.13-0.82) (12). Furthermore, two meta analyses found a beneficial effect of SDD with amphotericin B or nystatin on the odds of yeast colonization in general (OR 0.12; 95% CI 0.05-0.29 and OR 0.32; 95% CI 0.19-0.53) (13, 14) and fungal infections (OR 0.29; 95% CI 0.18-0.45 and OR 0.30; 95% CI 0.17-0.53) (13).

SDD intends to change the ICU ecology (1, 2). The treatment effect could be reduced if patients admitted to the same ward would be randomized to different decolonization strategies simultaneously. Therefore, unit-wide application of the intervention is the most appropriate design, allowing for unbiased evaluation of ICU ecology. The three phases of the study with sequential changes of antifungal components allowed an estimation of the effect per component change, during periods of at least 10 months. All patients were subject to protocolized surveillance of respiratory tract and rectal samples obtained on admission and twice weekly thereafter, which minimizes the risk for information bias. Treatment indications and surveillance for *Candida* did not change during the study period.

This study has some limitations. We did not evaluate resistance of fungal species against amphotericin or nystatin. However, resistance to nystatin among *Candida* species is rare (9). Furthermore, we did not evaluate whether there was any effect of the intervention on the bacterial colonization status. This could be subject of further research, as could be the optimal dose of nystatin and frequency of application.

Amphotericin B and nystatin are similar, as both belong to the class of polyene antifungal agents. Yet, in our hospital amphotericin B is nowadays five times more expensive than nystatin. This is largely due to a global-wide shortness of raw materials of amphotericin. Since nystatin seems to be at least as effective as amphotericin B in prevention of colonization, nystatin will improve the cost-effectiveness of SDD.

CONCLUSION

SDD suspension with nystatin is more effective than amphotericin B in preventing rectal *Candida* colonization in ICU patients and it is not inferior to amphotericin in preventing respiratory tract colonization when applied in a mouthpaste. Given the current cost developments of amphotericin B, nystatin use instead of amphotericin B will improve cost-effectiveness of SDD.

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EPIDEMIOLOGY, MANAGEMENT AND RISK-ADJUSTED MORTALITY OF ICU-ACQUIRED ENTEROCOCCAL BACTEREMIA

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ABSTRACT

Background

Enterococcal bacteremia has been associated with high case fatality, but it remains unknown to what extent death is caused by these infections. We, therefore, quantified attributable mortality of intensive care unit (ICU)-acquired bacteremia caused by enterococci.

Methods

From 2011 to 2013 we studied consecutive patients who stayed >48 hours in two tertiary ICUs in the Netherlands, using competing risk survival regression and marginal structural modeling to estimate ICU mortality caused by enterococcal bacteremia.

Results

Among 3,080 admissions, 266 events of ICU-acquired bacteremia occurred in 218 (7.1%) patients, of which 76 were caused by enterococci (incidence rate 3.0 per 1000 patient days at risk, 95% CI 2.3-3.7). A catheter related bloodstream infection (CRBSI) was suspected in 44 (58%) of these, prompting removal of 68% of indwelling catheters and initiation of antibiotic treatment for a median duration of 3 (interquartile range 1-7) days. Enterococcal bacteremia (of any type) was independently associated with an increased case fatality rate in the ICU (adjusted subdistribution hazard ratio (SHR) 2.68, 95% CI 1.44-4.98). However, for patients with CRBSI, case fatality was similar for infections caused by enterococci and coagulase-negative staphylococci (CoNS) (adjusted SHR 0.91, 95% CI 0.50-1.67). Population-attributable fraction of mortality was 4.9% (95% CI 2.9%-6.9%) by day 90, reflecting a population-attributable risk of 0.8% (95% CI 0.4-1.1%).

Conclusions

ICU-acquired enterococcal bacteremia is associated with increased case fatality, but the mortality attributable to these infections is low from a population perspective. The virulence of enterococci and CoNS in a setting of CRBSI seems comparable.

INTRODUCTION

The clinical management of bacteremia depends on the presumed source of infection. Bacteremia that occurs more than 48 hours after intensive care unit (ICU) admission, and is thus assumed to be newly acquired in the ICU, can often be related to catheter-related bloodstream infections (CRBSI) or ventilator-associated pneumonias (1), but may frequently also be of unknown source and significance. As critically ill patients frequently have indwelling central venous and arterial catheters, critical care physicians often face questions regarding optimal catheter management and antimicrobial treatment in patients with ICU-acquired bacteremia (2, 3). Heterogeneity of the underlying conditions and clinical presentation of infection, uncertainty about the interpretation of blood culture results (i.e., pathogen or contaminant), and various assumptions about the virulence of the cultured microorganism all contribute to variability in the clinical management of ICU-acquired bacteremia (4, 5). A more aggressive clinical response will typically be triggered in case of a highly virulent pathogen as compared to microorganisms with presumed low virulence. These differences are also reflected by international guidelines (6, 7).

Enterococci are frequent causes of ICU-acquired bacteremia (8), but their clinical significance is not self-evident. Although enterococcal bacteremia most frequently occurs in severely ill patients and has been associated with mortality rates ranging from 23% to 48% (9-11), many physicians consider this pathogen to be of low virulence. In fact, enterococci are natural colonizers of the human gastrointestinal tract and have been used as a probiotic for decades (12). It therefore remains uncertain whether enterococcal bacteremia is a true cause of mortality or merely a marker of morbidity associated with higher risk of dying. Therefore, we described the epidemiology and clinical management of ICU-acquired enterococcal bacteremia, and estimated the mortality that is caused by this infection from both the individual patient and the population perspective.

METHODS

Patients and measurements

This project was performed as part of an ongoing prospective observational cohort study in the mixed ICUs of two tertiary care hospitals in the Netherlands (13). Selective digestive decontamination was part of standard care in both centers (14). The Institutional Review Board approved an opt-out consent method by which participants and family members were notified of the study by a brochure provided at ICU admission with an attached opt-out card that could be completed by the patient or by his/her legal representative in case they declined to participate (protocol number 10-056C).

For the current study, we included consecutive patients having an ICU length of stay of more than two days between January 2011 and March 2013. From this cohort patients with enterococcal bacteremia occurring during the first two days in ICU were excluded.

Trained observers prospectively collected baseline patient characteristics and markers of disease severity, as well as daily clinical events and illness characteristics during the ICU admission. ICU-acquired bacteremia was defined as a first positive blood culture occurring more than two days after ICU admission (without a prior positive blood culture with the same pathogen for at least 30 days). Recurrent bacteremia was defined as one or more consecutive positive blood culture(s) with the same pathogen taken more than 3 days after the first positive sample. The most likely source of each bacteremia was determined by post-hoc physician assessment, using strict diagnostic criteria (13). In addition, the clinical management of indwelling catheters, the initiation of antimicrobial therapy, and various clinical outcomes related to the bacteremia event were recorded.

Descriptive analyses

The incidence of ICU-acquired enterococcal bacteremia and associated patient characteristics are reported in comparison to other commonly isolated blood stream pathogens using non-parametric descriptive statistics (i.e., Chi-square and Kruskal-Wallis tests). Because decisions regarding catheter management and antibiotic therapy much depend on the presumed source of bacteremia, we restricted the reporting of management practices to cases of bacteremia that were attributed to CRBSI only.

Association with outcome

Cox proportional hazards models were used to assess the association between enterococcal bacteremia and mortality. For this analysis ICU discharge and death were considered as competing events (15), and the occurrence of enterococcal bacteremia was fitted as a time-dependent variable (16). A competing risks analysis provides two measures of association. First, the cause-specific hazard ratio (CSHR) describes the instantaneous effect on the outcome of interest, given that the patient is still at risk. In our case, it estimates the direct effect of enterococcal bacteremia on the rate of death in the ICU. Second, the subdistribution hazard ratio (SHR) describes the risk of dying from enterococcal bacteremia while accounting for the competing risk of ICU discharge. To correct for confounding, we decided *a priori* to adjust for age, gender, non-European ethnicity, Charlson comorbidity index, surgical reason for admission, previous ICU admission, and Acute Physiology and Chronic Health Evaluation (APACHE) IV score, which were considered to be likely associated with both enterococcal bacteremia as well as mortality based on careful consideration of the literature (8, 17, 18).

As confounding may remain because markers of illness at the time of ICU admission may not be fully representative of the disease state at the time of bacteremia (which typically does not occur until the second week of admission), marginal structural modeling was subsequently performed to adjust for the evolution of disease severity prior to the onset of bacteremia (19, 20). First, such analysis involves the estimation of daily probabilities of acquiring enterococcal bacteremia in the ICU using a multivariable logistic regression model that includes markers of disease severity on a daily basis. In a

second analysis step, these probabilities are used to calculate patient-specific inversed probability weights, which are then included as a summary measure of all relevant covariables in the final Cox regression model.

Comparative analysis of enterococci versus coagulase-negative staphylococci (CoNS)

To further evaluate the impact of ICU-acquired enterococcal bacteremia on patient outcome, we compared mortality rates between patients with CRBSI due to either enterococci or CoNS. We restricted this analysis to CRBSI cases in order to reduce confounding. We chose for a direct comparison to CoNS because this group of microorganisms represents the most frequent cause of ICU-acquired bacteremia and is widely considered to be of low virulence (21). As for the primary analysis, competing risk survival regression was used, with the follow up time starting at the time of bacteremia and ending at either death or discharge from the ICU (15). We adjusted for both baseline patient characteristics (age, surgical reason for ICU admission and Charlson comorbidity index) as well as illness characteristics at the time of bacteremia (Sequential Organ Failure Assessment (SOFA) score from the day before bacteremia, immunosuppression and presence of intravascular or orthopedic implants).

Population-attributable mortality

Finally, we used a multi-state model to estimate the fraction of ICU mortality that is attributable to enterococcal bacteremia on the population level, again taking both the time-dependent nature of bacteremia occurrence and competing events into account (22). For formal definitions of risk difference, population-attributable risk and population-attributable fraction of ICU mortality we refer to the online supplementary. Bootstrapping was used to estimate the confidence intervals (CI) for the population-attributable risk and population-attributable fraction of mortality (23).

All analyses were performed using SAS 9.2 (Cary, NC, USA) and R 2.15.1 software (R Foundation for Statistical Computing, Vienna, Austria; package 'mstate'). P values less than 0.05 were considered to be statistically significant.

RESULTS

Incidence

Among 3080 included patients, 218 (7.1%) individuals had 266 episodes of ICU-acquired bloodstream infection (Figure 1). Enterococci were responsible for 76 of these episodes, yielding an incidence rate of 3.0 (95% CI 2.3-3.7) events per 1000 patient days at risk (Table 1). Median time from ICU admission to bacteremia onset was 9.5 (interquartile range (IQR) 7.0-17.5) days. Thirty-two patients acquired bacteremia with two different pathogens and eight patients acquired three different pathogens at any time during their ICU admission.

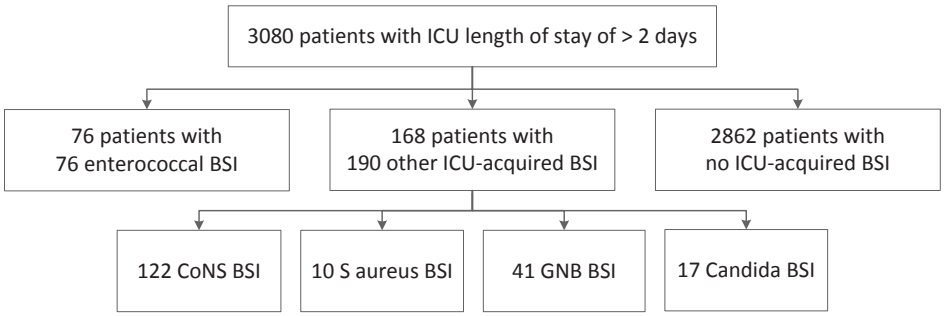

Figure 1. Patient inclusion
BSI = bloodstream infection; CoNS = coagulase negative staphylococci; GNB = gram-negative bacteria; ICU = intensive care unit;

Patient characteristics

Patients with ICU-acquired enterococcal bacteremia tended to have more chronic comorbidities (median Charlson comorbidity score 7.4 versus 5.5, p=0.08) and higher severity of disease at baseline (median APACHE IV score 91 versus 86, p=0.13), compared to patients who had ICU-acquired bacteremia caused by other pathogens (Table 1). Furthermore, these patients had more often been previously admitted to the ICU (22% versus 13%, p=0.05). On the day that bacteremia was acquired, those with enterococcal bacteremia also had higher median SOFA scores (10 versus 7, p<0.01) and more frequently had intravascular or orthopedic implants (41% versus 24%, p<0.01) compared to those with bacteremia caused by other pathogens (Table 2).

Crude associations with mortality

Observed ICU mortality was 47% in patients with enterococcal bacteremia versus 15% in those without enterococcal bacteremia (p<0.01), yielding a crude risk difference of 32% (95% CI 21-44%). The mortality associated with enterococci was also higher than the 33% crude mortality in patients with ICU-acquired bacteremia caused by other pathogens combined (p=0.03). In 55 of 76 patients with enterococcal bacteremia more than 50% of all blood cultures taken on the first day of bacteremia grew enterococci (indicating a high bacterial load). ICU mortality in these patients was 53%, compared to 33% in patients having fewer positive blood cultures (p=0.12). A second (concurrent) pathogen was cultured in 33 of 76 (43%) patients with enterococcal bacteremia. ICU mortality was 52% and 44% in patients with polymicrobial versus enterococcal bacteremia, respectively (p=0.53).

Risk-adjusted mortality

Associations between enterococcal bacteremia, ICU discharge, and ICU death are listed in Table 3 and eTable 1. After adjustment for imbalances in patient characteristics at baseline, accounting for the time-dependent nature of the exposure, and including

Table 1. Patient characteristics at baseline

	ICU-acquired bacteremia					Reference
	Enterococci (n=76)	Coagulase negative staphylococci (n=122)	Staphylo-coccus aureus (n=10)	Gram-negative rods (n=41) [a]	Candida species (n=17)	No bacteremia (n=2862)
Age - years	66 (54-73)	59 (49-69)	57 (42-78)	64 (52-71)	52 (43-65)	62 (50-71)
Male sex	50 (66)	83 (68)	6 (60)	21 (51)	11 (65)	1743 (61)
Non-European ethnicity	9 (12)	24 (20)	4 (40)	6 (15)	3 (18)	300 (10)
Charlson comorbidity index	7.4 (3.0-12.4)	5.6 (2.0-12.6)	5.7 (0.0-8.1)	6.4 (2.5-10.4)	3.9 (2.0-6.4)	4.6 (1.0-10.9)
Surgical reason for admission	27 (36)	47 (39)	1 (10)	18 (44)	7 (41)	1234 (43)
Previous ICU admission	17 (22)	18 (15)	0 (0)	3 (7)	3 (18)	373 (13)
APACHE IV score	91 (71-114)	86 (66-103)	86 (60-115)	86 (65-100)	78 (69-97)	72 (54-92)
ICU mortality	36 (47)	37 (30)	4 (40)	16 (39)	6 (35)	425 (15)

Data are presented in median (interquartile range) or in absolute number (percentage). Some patients had ICU-acquired bacteremia caused by multiple pathogens, therefore, the total number of bacteremia is more than the total number of patients in this study.
APACHE = Acute Physiology and Chronic Health Evaluation.
[a] Among gram-negative rods *Pseudomonas aeruginosa* (n=10) and *Escherichia coli* (n=9) were the most frequently isolated pathogens

Table 2. Patient characteristics at the day of ICU-acquired bacteremia

	Entero-coccus (n=76)	Coagulase negative Staphylococcus (n=122)	Staphylo-coccus aureus (n=10)	Gram-negative rod (n=41)	Candida species (n=17)
Clinical characteristics:					
Day of ICU admission	10 (7-18)	12 (7-21)	12 (4-35)	12 (8-24)	8 (6-13)
SOFA score from the previous day	10 (6-13)	7 (4-10)	5 (4-9)	8 (4-12)	12 (10-15)
Use of renal replacement therapy	29 (38)	25 (20)	2 (20)	13 (32)	10 (59)
Use of mechanical ventilation	66 (87)	109 (89)	10 (100)	36 (88)	15 (88)
Immunosuppression [a]	33 (43)	38 (31)	2 (20)	16 (39)	12 (71)
Presence of intravascular or orthopedic foreign bodies	31 (41)	27 (22)	0 (0)	15 (37)	4 (24)

Table 2. Patient characteristics at the day of ICU-acquired bacteremia (*Continued*)

	Entero-coccus (n=76)	Coagulase negative Staphylococcus (n=122)	Staphylo-coccus aureus (n=10)	Gram-negative rod (n=41)	Candida species (n=17)
Indwelling catheters:					
Arterial	70 (92)	113 (93)	8 (80)	38 (93)	17 (100)
Pulmonary artery	4 (5)	3 (2)	0 (0)	1 (2)	0 (0)
Short term CVC (non-RRT)	52 (68)	79 (65)	4 (40)	28 (68)	16 (94)
RRT	26 (34)	30 (25)	1 (10)	14 (34)	12 (71)
Long term CVC	2 (3)	3 (2)	0 (0)	1 (2)	1 (6)
Other type	2 (3)	8 (7)	0 (0)	0 (0)	1 (6)
Prior antibiotic exposure during ICU stay:					
Vancomycin	3 (4)	7 (6)	0 (0)	8 (20)	3 (18)
Gentamicin	2 (3)	1 (1)	0 (0)	0 (0)	0 (0)
Amoxicillin	1 (1)	4 (3)	0 (0)	5 (12)	2 (12)
Flucloxacillin/clindamycin	7 (9)	5 (4)	0 (0)	1 (2)	3 (18)
Cephalosporin	12 (16)	16 (13)	0 (0)	4 (10)	4 (24)
Most likely source of bacteremia:					
Catheter infection	44 (58)	76 (62)	4 (40)	11 (27)	11 (65)
Abdominal	16 (21)	6 (5)	0 (0)	8 (20)	1 (6)
Pulmonary	2 (3)	0 (0)	4 (40)	11 (27)	0 (0)
Urinary tract	0 (0)	0 (0)	0 (0)	3 (7)	0 (0)
Endocarditis	2 (3)	0 (0)	2 (20)	1 (2)	0 (0)
Wound	2 (3)	4 (3)	0 (0)	2 (5)	1 (6)
Other	2 (3)	10 (8)	1 (10)	1 (2)	0 (0)
Unknown	15 (20)	36 (30)	1 (10)	7 (17)	6 (35)
Contamination	2 (3)	20 (16)	1 (10)	0 (0)	0 (0)
Severity of bacteremia:					
High bacterial load [b]	55 (72)	72 (59)	8 (80)	35 (83)	11 (65)
Bacteremia recurrence [c]	11 (14)	20 (16)	1 (10)	5 (12)	5 (29)

Data are presented in median (interquartile range) or in absolute number (percentage). Some bacteremia cases were allocated to two likely sources of infection.
SOFA = Sequential Organ Failure Assessment; CVC = central venous catheter; RRT = renal replacement therapy
[a] Immunosuppression was defined as solid organ transplantation, stem cell transplantation or chemo-radiotherapy during current hospital admission, neutropenia (absolute neutrophil count lower than 1.0 x 109/L) or corticosteroid use (hydrocortisone >100 mg or equivalent) at the time of bacteremia.
[b] High bacterial load was presumed if more than 50% of blood culture bottles obtained on the first day of the bacteremia episode showed growth for the indicated pathogen.
[c] Bacteremia recurrence was defined by the presence of one or more consecutive blood cultures (obtained more than 3 days after the first positive sample) showing growth for the same pathogen.

Table 3. Associations between enterococcal bacteremia and clinical outcome

Cox proportional model	ICU discharge CSHR (95% CI)	ICU mortality CSHR (95% CI)	ICU mortality SHR (95% CI)
Crude model with adjustment for: - time-varying onset of bacteremia	0.51 (0.40-0.66)	2.27 (1.63-3.16)	7.99 (5.63-11.32)
Multivariable model with adjustment for: - time-varying onset of bacteremia - baseline covariables [a]	0.57 (0.44-0.74)	1.70 (1.14-2.52)	5.29 (3.51-7.96)
Multivariable model with adjustment for: - time-varying onset of bacteremia - baseline covariables [a] - evolution of disease prior to onset of bacteremia [b]	0.83 (0.62-1.08)	1.10 (0.61-1.98)	2.68 (1.44-4.98)

The cause-specific hazard ratio (CSHR) estimates the direct effect of enterococcal bacteremia on clinical outcome (i.e., discharge alive or death). The subdistribution hazard ratio (SHR) is a summary measure of both separate cause-specific hazards and estimates the overall risk of dying from enterococcal bacteremia while taking into account the competing event of discharge alive.
[a] Baseline covariables were age, sex, non-European ethnicity, prior ICU admission during the hospital stay, surgical reason for admission, Charlson comorbidity index, and Acute Physiology and Chronic Health Evaluation (APACHE) IV score.
[b] To predict enterococcal bacteremia on each day we included the Sequential Organ Failure Assessment (SOFA) score, prior amoxicillin or vancomycin administration, and abdominal perforation or surgery as time-dependent covariables. For SOFA and amoxicillin/vancomycin administration we explicitly used lagged daily values from two days before to avoid possible over-adjustment as the scores measured within 24 hours before the onset of bacteremia may have been influenced by an insidious onset of bacteremia.

ICU discharge as a competing risk for mortality, ICU-acquired enterococcal bacteremia was associated with an increased overall risk of death in the ICU (SHR 5.29, 95% CI 3.51-7.96). After further adjustment for potentially remaining confounders by accounting for the evolution of disease severity prior to bacteremia onset using a marginal structural model analysis, an association with mortality remained (adjusted SHR 2.68, 95% CI 1.44-4.98). Cause-specific analyses showed that this was the combined result of both (a trend to) a reduced rate of ICU discharge (adjusted CSHR 0.83, 95% CI 0.62-1.08) and (a trend to) an increased daily risk of death (adjusted CSHR 1.10, 95% CI 0.61-1.98).

Comparative analysis of enterococci versus CoNS

44 of 76 episodes (58%) of enterococcal ICU-acquired bacteremia were considered catheter-related, as were 76 of 122 (62%) episodes of CoNS bacteremia events. In patients with CRBSI, the rate of replacement or removal of indwelling catheters was similar during episodes caused by enterococci and CoNS (Table 4). However, patients with CRBSI caused by enterococci more frequently received targeted antimicrobial treatment, with a trend for longer durations. The risk of ICU death was similar in patients with CRBSI due to enterococci and CoNS, both by crude analysis (observed mortality 43% versus 37%, p=0.49; CSHR 1.32, 95% CI 0.73-2.40) as well as in multivariable-adjusted analyses (SHR

Table 4. Intravascular catheters and antimicrobial management in the subgroup of catheter-related bloodstream infections

Clinical management	Enterococci (n=44)	Coagulase negative Staphylococci (n=76)	p-value
Number (%) of indwelling catheters that were removed within 3 days	63/92 (68)	95/161 (59)	0.14
- Peripheral arterial catheter [a]	22/40 (53)	35/73 (48)	NA
- Short term CVC (non-RRT catheter) [a]	28/36 (75)	36/52 (69)	NA
- RRT catheter [a]	11/14 (73)	18/25 (66)	NA
Number (%) of (appropriate) antibiotic treatment against pathogen [b] within 3 days	36/44 (82) [c]	47/76 (62)	0.02
Duration of antibiotic treatment [d]	3 (1-7)	2 (1-4)	0.09

Data are presented in absolute number (percentage) unless otherwise specified.
CVC = central venous catheter; NA = not applicable; RRT = renal replacement therapy
[a] Percentages were calculated for the three most frequent catheters. The denominators for each type of catheter and pathogen group were different.
[b] Appropriate treatment was defined as vancomycin for amoxicillin resistant enterococci, vancomycin or amoxicillin for amoxicillin sensitive enterococci, and vancomycin for coagulase negative staphylococci.
[c] Appropriate treatment in the first three days after onset of bacteremia consisted of 92% vancomycin monotherapy, 3% amoxicillin monotherapy, and 5% vancomycin-amoxicillin switch therapy.
[d] Duration of antibiotic treatment was only calculated for patients in whom treatment was stopped before the last day of ICU (92%), because registration of antibiotic treatment after ICU discharge was not performed. Thus, for 8% of cases it remained uncertain whether antibiotic treatment was continued after ICU discharge.

0.91, 95% CI 0.49-1.67). The cumulative incidence of recurrent bacteremia was 14% and 17% for CRBSIs caused by enterococci and CoNS, respectively (p=0.62).

Comparative analysis of CRBSI versus non-CRBSI

Enterococcal bacteremia due to CRBSI appeared to be associated with lower case fatality than enterococcal bacteremia due to other causes (crude mortality 43% versus 53%, p=0.39), although this difference did not reach statistical significance. After extensive correction for time-dependent confounding and competing events, comparable effect estimates were found in both subgroups (eTable 2 and eTable 3).

Population-attributable mortality

The cumulative incidence of death in the observed cohort (n=3080) and the mortality that would be expected if all episodes of enterococcal bacteremia could be prevented is shown in Figure 2. The population-attributable fraction of mortality associated with ICU-acquired enterococcal bacteremia was 4.9% (95% CI 2.9%-6.9%) by day 90 (eFigure 1), which translates into a population-attributable risk of 0.8% (95% CI 0.4%-1.1%).

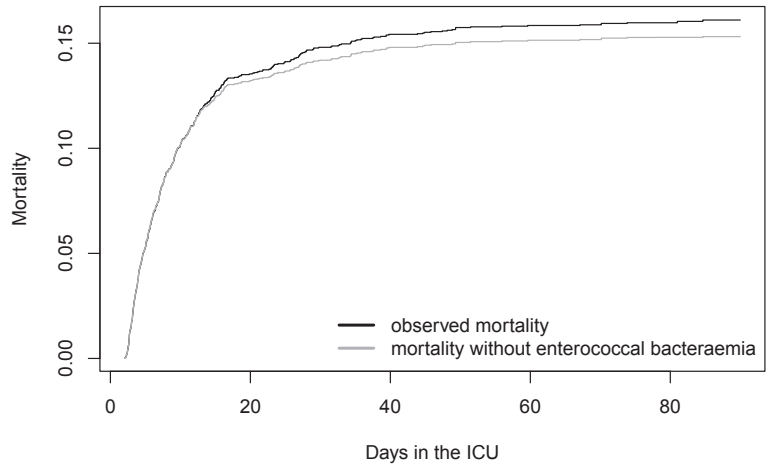

Figure 2. Observed ICU mortality versus expected ICU mortality without enterococcal bacteremia

This figure presents the observed ICU mortality in the patient cohort (black line) and the expected mortality in case all ICU-acquired enterococcal bacteremia in the same ICU patient population were prevented (light grey line). Thus, the light grey line represents mortality both in patients who never acquired enterococcal infection as well as in those who did acquire bacteremia, but then in the (hypothetical) situation by which all exposure time to enterococcal bacteremia would be annihilated.

DISCUSSION

In the present study ICU-acquired enterococcal bacteremia occurred at a rate of 3.0 events per 1000 days at risk. These events presented with a peak incidence approximately ten days after ICU admission and occurred in the most severely ill and in those with chronic illnesses. As a result, comorbidities and time-dependent bias largely accounted for the high crude ICU case fatality rates that were observed in patients with enterococcal bacteremia. However, despite the use of advanced methodologies to adjust for potential confounding by disease severity prior to bacteremia onset, a significant independent association with mortality remained. From a population perspective, the excess mortality that could be attributed to enterococcal bacteremia in the ICU was low nonetheless (0.8% by day 90). This modest impact may have resulted from the relatively low incidence of these events, limited virulence of the bug, or both. In any respect, direct comparisons suggested that the virulence of enterococci and CoNS in a setting of CRBSI is similar.

Although Infectious Diseases Society of America (IDSA) guidelines recommend that all catheters be removed and antimicrobial treatment initiated for 7 to 14 days in cases of suspected CRBSI due to enterococci (7), in the current setting only 68% of indwelling catheters were pulled and just 82% of patients received appropriate antimicrobial treatment for a median duration of only 3 days. In part, this observation might be

explained by a lenient attitude of attending clinicians regarding the presumed virulence of enterococci and the focus of infection. Abdominal infections were the second most frequent source of enterococcal bacteremia in our study, and the joint IDSA and Surgical Infection Society guidelines currently recommend antibiotic treatment for 4 to 7 days in the presence of adequate source control, and depending on clinical response (24). In our study appropriate systemic antibiotics were given for 6.5 (IQR 3.5-8.5) days for enterococcal abdominal infections. Although evidence regarding optimal duration of antibiotic treatment for enterococcal infections is lacking, it is conceivable that the increased risk of death that remained despite optimal adjustment for confounding was caused –in part– by clinical management that was insufficiently aggressive. Nonetheless, the observed case fatality rate in our study cohort was compatible with other reported mortality rates of bloodstream infections in the ICU varying from 35% to 50% (8). Furthermore, results from a recent meta-analysis suggest that antibiotic therapy for shorter periods than recommended by the IDSA guideline seem to be as effective as longer-duration therapy in achieving clinical cure, microbiologic cure, and survival in critically ill patients with bacteremia (25). In particular, a large randomized controlled trial in patients with complicated intra-abdominal infections recently showed that antibiotic therapy for a fixed duration of 4 days was as effective as traditional, longer treatment (26). Nevertheless, we cannot exclude the possibility that in our study population some subgroups would have benefited from more prolonged antibiotic treatment.

Strengths of our study include the use of advanced methodologies to adjust for various sources of potential confounding. Moreover, because data were prospectively collected using validated protocols (13) the risk of information bias was minimized. Nonetheless, we cannot completely exclude the presence of residual confounding or bias, and some limitations with regard to the interpretation of our results must be considered. First, our study was performed in two centers in the Netherlands only and thus may not reflect ICU practice in all settings. As both hospitals used selective digestive decontamination protocols, the incidence of ICU-acquired bacteremia caused by Gram-negative bacteria and *Candida* species is probably lower than in settings not using these strategies (27). Yet, there is no evidence that these interventions influence either the incidences or outcomes of ICU-acquired bacteremia caused by enterococci and CoNS. Indeed, in a multi-center cluster randomized controlled trial in which selective digestive decontamination was compared to standard care the incidence of ICU-acquired enterococcal bacteremia was similar in both groups (27). Furthermore, the incidence that was observed in our study (i.e., 76 episodes among 3080 patients, or 2.5%) lies well within the 0.9% to 5.4% range of previously documented occurrence rates for enterococcal bacteremia (17, 28, 29). However, it is important to consider that our study exclusively focused on infections that were newly acquired in the ICU, and that the total incidence of bacteremia events due to enterococci in an ICU setting (or in the hospital at large) may thus be higher. In fact, we observed 108 patients having at least a single

blood culture growing enterococci during ICU admission, but only 76 of these events were considered ICU-acquired based on the definitions used in our study. Second, as all cultured enterococci in our study were susceptible to vancomycin, our findings may not apply to settings with a high prevalence of infections caused by vancomycin resistant enterococci. Third, it is plausible that some events that we considered to represent enterococcal bacteremia were –in fact– clinically irrelevant and should have been considered as merely contaminated blood draws. However, case fatality seemed to be increased in cases of higher bacterial loads, which underlines the clinical relevance of finding a blood culture positive for enterococci. Furthermore, from a clinical perspective it is frequently very difficult to distinguish true infection from contamination, even in retrospect. Case fatality also appeared higher in polybacterial versus monobacterial blood stream infections involving enterococci. Although interesting, it is important to stress that these findings are limited by small numbers of observations and that we did not perform multivariable modeling in these subgroups.

In conclusion, our findings show that ICU-acquired enterococcal bacteremia is independently associated with increased case fatality, although the population-attributable mortality of these infections remains low. The high crude mortality observed in cases of enterococcal bacteremia in general, and in cases of non-CRBSI in particular, can be largely explained by concomitant patient and disease characteristics, suggesting that these bloodstream infections may –for the most part– be markers of impending complications that carry a high risk of death in the ICU, rather than being causes of death out of their own. This hypothesis is supported by our observation that patients with CRBSI caused by enterococci had similar outcomes as CRBSI caused by CoNS, indicating that the virulence of enterococci in this setting is indeed low.

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SUPPLEMENTARY MATERIAL

Formal definitions regarding measures of association

Risk difference

= Observed mortality in patients with ICU-acquired enterococcal bacteremia minus observed mortality in patients without (ever) acquiring enterococcal bacteremia

Population-attributable risk

= Observed mortality in the study population minus expected mortality if all cases of ICU-acquired enterococcal bacteremia would be prevented

Population-attributable fraction of mortality

= Fraction of population-attributable risk and observed mortality in the study population (i.e., observed mortality in the study population minus expected mortality if all cases of ICU-acquired enterococcal bacteremia would be prevented, divided by the observed mortality in the study population)

eTable 1. Multivariable models for the association between enterococcal bacteremia and clinical outcome

A. Multivariable model with adjustment for: - time-varying onset of bacteremia - baseline covariables	ICU discharge CSHR (95% CI)	ICU mortality CSHR (95% CI)	ICU mortality SHR (95% CI)
Enterococcal bacteremia	0.57 (0.44-0.74)	1.70 (1.14-2.52)	5.29 (3.51-7.96)
Age (per year)	1.01 (1.00-1.01)	1.01 (1.00-1.01)	1.00 (1.00-1.01)
Male sex	0.95 (0.87-1.03)	0.93 (0.77-1.11)	0.97 (0.80-1.16)
Non-European ethnicity	1.01 (0.89-1.15)	0.86 (0.63-1.18)	0.86 (0.64-1.17)
Previous ICU admission	0.93 (0.82-1.05)	0.79 (0.60-1.04)	0.84 (0.63-1.11)
Surgical reason for admission	1.00 (0.92-1.08)	0.95 (0.78-1.15)	0.95 (0.78-1.15)
Charlson comorbidity index (per point)	1.00 (0.99-1.00)	0.99 (0.98-1.01)	1.00 (0.99-1.02)
Acute Physiology and Chronic Health Evaluation IV score (per point)	0.99 (0.99-0.99)	1.01 (1.01-1.02)	1.02 (1.02-1.02)

B. Multivariable model with adjustment for: - time-varying onset of bacteremia - baseline covariables - evolution of disease prior to onset of bacteremia	ICU discharge CSHR (95% CI)	ICU mortality CSHR (95% CI)	ICU mortality SHR (95% CI)
Enterococcal bacteremia	0.83 (0.62-1.08)	1.10 (0.61-1.98)	2.68 (1.44-4.98)
Age (per year)	1.00 (1.00-1.01)	1.01 (1.00-1.01)	1.00 (0.99-1.01)
Male sex	0.95 (0.87-1.03)	0.93 (0.77-1.12)	0.98 (0.81-1.18)
Non-European ethnicity	1.02 (0.90-1.16)	0.87 (0.64-1.20)	0.87 (0.63-1.18)
Previous ICU admission	0.94 (0.83-1.06)	0.79 (0.60-1.05)	0.83 (0.63-1.11)
Surgical reason for admission	1.00 (0.92-1.08)	0.94 (0.77-1.14)	0.95 (0.78-1.16)
Charlson comorbidity index (per point)	0.99 (0.99-1.00)	1.00 (0.98-1.01)	1.00 (0.98-1.02)
Acute Physiology and Chronic Health Evaluation IV score (per point)	0.99 (0.99-0.99)	1.01 (1.01-1.02)	1.02 (1.02-1.02)
Time-dependent covariables *	NA	NA	NA

This table provides a full description of the multivariable models used to estimate the association between enterococcal bacteremia and clinical outcomes (discharge and mortality). The results are equivalent to those presented in Table 3 of the main manuscript.
CSHR = cause-specific hazard ratio; SHR = subdistribution hazard ratio.
* Time-dependent variables included the Sequential Organ Failure Assessment (SOFA) score, prior amoxicillin or vancomycin administration, and abdominal perforation or surgery. No CSHR and SHR can be presented for these time-dependent variables. The daily (changing) information was used to calculate patient specific weights (inverse probability weights) representing the cumulative risk of acquiring enterococcal bacteremia for each patient at each time point, which were subsequently used in Cox regression analysis to adjust for the differential evolution of disease severity over time.

To investigate whether effect modification was present, we performed additional risk-adjusted analyses for enterococcal bacteremia of CRBSI and non-CRBSI origin separately. In these analyses the alternative cause of enterococcal bacteremia was considered as an additional competing event (beside ICU discharge and death). The subdistribution hazard ratio (SHR) presented in eTable 1 and eTable 2 cannot be directly compared to the SHR presented in Table 3 of the manuscript, because these models used three instead of two competing events.

Enterococcal bacteremia due to CRBSI appeared to be associated with lower case fatality than enterococcal bacteremia due to other causes (adjusted cause specific hazard ratio 0.95 versus 1.35). Similarly, when competing events were taken into account, mortality was lower for CRBSI bacteremia compared to non-CRBSI enterococcal bacteremia (adjusted SHR 2.87 versus 3.23).

eTable 2. Associations between enterococcal bacteremia of catheter-related origin and clinical outcome

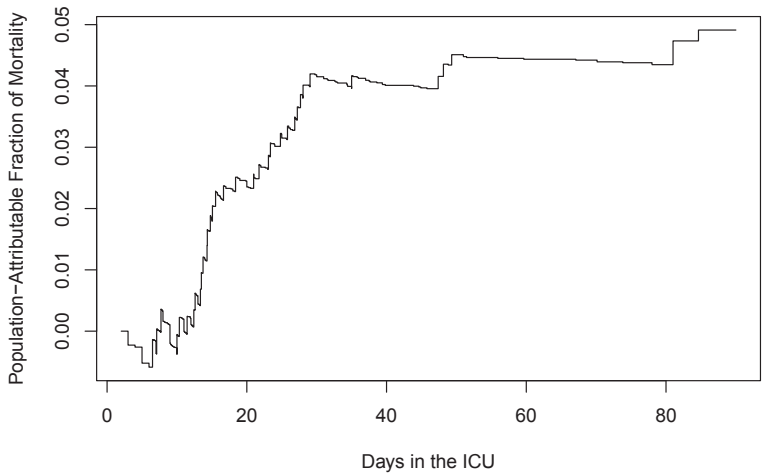
Cox proportional model	ICU discharge CSHR (95% CI)	ICU mortality CSHR (95% CI)	ICU mortality SHR (95% CI)
Crude model with adjustment for: - time-varying onset of bacteremia	0.53 (0.40-0.69)	1.97 (1.21-3.22)	7.77 (4.71-12.81)
Multivariable model with adjustment for: - time-varying onset of bacteremia - baseline covariables *	0.57 (0.42-0.77)	1.42 (0.77-2.59)	4.73 (2.60-8.59)
Multivariable model with adjustment for: - time-varying onset of bacteremia - baseline covariables * - evolution of disease prior to onset of bacteremia **	0.79 (0.57-1.10)	0.95 (0.42-2.16)	2.87 (1.32-6.26)

* Baseline covariables were age, sex, non-European ethnicity, prior ICU admission during the hospital stay, surgical reason for admission, Charlson comorbidity index, and Acute Physiology and Chronic Health Evaluation (APACHE) IV score.
** To predict enterococcal bacteremia on each day we included the Sequential Organ Failure Assessment (SOFA) score, prior amoxicillin or vancomycin administration, and abdominal perforation or surgery as time-dependent covariables.

eTable 3. Associations between enterococcal bacteremia of non-catheter related origin and clinical outcome

Cox proportional model	ICU discharge CSHR (95% CI)	ICU mortality CSHR (95% CI)	ICU mortality SHR (95% CI)
Crude model with adjustment for: - time-varying onset of bacteremia	0.49 (0.31-0.79)	2.76 (1.94-3.94)	8.61 (5.44-13.62)
Multivariable model with adjustment for: - time-varying onset of bacteremia - baseline covariables *	0.57 (0.36-0.90)	2.13 (1.43-3.18)	6.29 (3.79-10.44)
Multivariable model with adjustment for: - time-varying onset of bacteremia - baseline covariables * - evolution of disease prior to onset of bacteremia **	0.93 (0.55-1.60)	1.35 (0.60-3.05)	3.23 (1.19-8.78)

* Baseline covariables were age, sex, non-European ethnicity, prior ICU admission during the hospital stay, surgical reason for admission, Charlson comorbidity index, and Acute Physiology and Chronic Health Evaluation (APACHE) IV score.
** To predict enterococcal bacteremia on each day we included the Sequential Organ Failure Assessment (SOFA) score, prior amoxicillin or vancomycin administration, and abdominal perforation or surgery as time-dependent covariables.



eFigure 1. Attributable mortality over time
This figure shows the population-attributable fraction of ICU mortality as a function of time. The line represents the proportion of deaths in the intensive care unit that could be attributable to ICU-acquired enterococcal bacteremia or the percentage of the observed ICU deaths that could be avoided by preventing ICU-acquired enterococcal bacteremia. The population-attributable fraction of ICU mortality increased in time, and reached 4.9% by day 90.

IV

DISCUSSION

12

SUMMARY AND GENERAL DISCUSSION

The aim of this thesis was to evaluate the role of several opportunistic pathogens in critically ill patients, and to assess the impact of antimicrobial therapy and strategies on colonization and resistance development in these opportunistic pathogens. Most studies presented in this thesis were performed as part of the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) consortium, which aimed to generate tools that provide rapid and accurate information about the pathogen(s) and the immune response of critically ill patients with sepsis. Accurate assessments of opportunistic pathogens and risk stratification in patients are important, because of the heterogeneity in virulence of different microorganisms as well as the heterogeneity of patients admitted to the intensive care. In this chapter the main clinical findings and methodological aspects that were important during the conduct of our studies are discussed.

As for all infectious diseases the interaction between the insulting pathogen and the patient (i.e., the host) will eventually determine the clinical outcome. Microorganisms are mostly labeled as opportunistic in case pathogenicity is primarily restricted to hosts with an impaired immune system. Nevertheless, the definition of an impaired immune system is not straightforward. Most intensive care unit (ICU) patients are usually not considered as being immune compromised, in contrast to for example patients receiving exogenous immunosuppressive therapy (e.g., transplant patients) or patients with an innate immune deficiency. However, the immune system of ICU patients can be acutely impaired due to the sudden and intense severity of illness (1). Therefore, ICU patients could be considered immunocompromised by endogenous (instead of exogenous) nature. Clearly, in a vulnerable patient population in which infections contribute substantially to morbidity and mortality (2), it is worthwhile to address the effects of opportunistic pathogens in this critically ill patient population specifically.

EPIDEMIOLOGY OF RESPIRATORY VIRUSES AND DIAGNOSTIC MANAGEMENT

Respiratory tract infections caused by viral pathogens, such as the influenza virus, may cause respiratory failure leading to ICU admission (3). Detection of influenza in critically ill patients has important implications for infection control measures such as isolation and initiation of antiviral medication (4, 5).

In **Chapter 2** of this thesis the practice of diagnostic testing for viral respiratory tract infections was described in patients admitted to the ICU with clinical symptoms suggestive for community-acquired pneumonia (CAP) or hospital-acquired pneumonia (HAP). Testing for any respiratory virus, including influenza, was only performed in 46% of the patients with a suspected CAP during the influenza season. It is impossible to distinguish bacterial from viral pneumonia by clinical symptoms alone and symptoms of influenza-like-illness may be mild, making the diagnosis of a viral pneumonia on clinical grounds only impossible (6). The joined Infectious Diseases Society of America (IDSA) and American Thoracic Society (ATS) guidelines recommend testing for specific

pathogens that would significantly alter management decisions on the basis of clinical and epidemiologic clues (7). This implies that testing for the presence of respiratory viruses is desired as it may have significant consequences for clinical management (especially for influenza because of the possibility of treatment). Based on these observations there is room for improvement in performing respiratory virus diagnostics.

Although an infection with influenza virus is associated with significant morbidity and mortality in critically ill patients (8), the virulence and clinical significance of other respiratory viruses are more controversial. Respiratory syncytial virus (RSV) illness in adults is often mild, but can cause significant morbidity in the elderly and high-risk adults, and is associated with substantial mortality (9). High morbidity and mortality rates were found for adults hospitalized for RSV infections, of whom 10% required mechanical ventilation (10). Thus it has been suggested that RSV infection may also play an important role in the etiology and outcome of patients admitted to the ICU with respiratory failure (11).

To investigate the epidemiology of RSV in the ICU, we included consecutive critically ill patients who were admitted to the mixed ICUs of two hospitals with community-acquired respiratory failure in a prospective observational study during three consecutive RSV seasons (**Chapter 3**). We observed a much lower incidence of RSV as compared to influenza virus (4% versus 15%). Moreover, all patients who were positive for RSV recovered, which was in contrast to influenza virus that was associated with 17% mortality.

Only a few studies have evaluated RSV infections in adult ICU patients. Our findings correspond to incidence rates reported in these earlier studies (11-13), and are in line with the general observation that in adults RSV occurs less frequently in comparison to influenza virus. Although rapid recognition of RSV as cause of infection may become more important in the future because of the recent developments of vaccines and antiviral therapeutics (14), the beneficial cost-effectiveness of new treatment and prevention modalities will depend on targeting high-risk populations. Patients admitted to the ICU with community-acquired respiratory failure are apparently not at high-risk for having RSV infections.

SYSTEMIC REACTIVATION OF HERPES VIRUSES

The occurrence of viremia in ICU patients without known prior immune deficiency has been described in several reports (15-17). Most research focused on cytomegalovirus (CMV) viremia, of which the main pathophysiological mechanism is assumed to be reactivation of latent CMV (18). Patients with acute respiratory distress syndrome (ARDS) are thought to be at an increased risk for CMV reactivation, and according to small observational studies CMV reactivation in critically ill patients is associated with a prolonged duration of mechanical ventilation (19, 20), an increased length of stay in the ICU (21-23), and excess mortality (16, 19, 20, 23, 24). It has been therefore

suggested that the use of prophylactic antiviral treatment in CMV seropositive patients (i.e., patients who are prone for virus reactivation) might be beneficial.

In **Chapter 4** we compared CMV seropositive to seronegative patients, who were admitted to the ICU with ARDS, and assessed the association between seroprevalence and mortality or prolonged disease course. We found no association between CMV seroprevalence and outcome after adjustment for potential confounders. This implies that giving antiviral prophylaxis to all CMV seropositive patients with the aim to favorably modify patient outcome, by prevention of possible CMV reactivations during ICU admission, is unlikely to be effective. This finding is in line with a previous study performed in a more general and heterogeneous group of ICU patients that also showed no difference in clinical outcome between CMV seropositive and seronegative patients (25). In our cohort of CMV seropositive ARDS patients who remain beyond day four of ICU admission, only 26% of patients eventually showed reactivation even without prophylactic treatment. A greater understanding of the underlying pathophysiological mechanisms as well as clinical predictors of CMV reactivation is thus needed to improve the selection of patients that may benefit from antiviral prophylaxis. Post hoc analyses showed that the subgroup of CMV seropositive patients with septic shock was independently associated with prolonged weaning or increased mortality compared to their seronegative counterparts.

In **Chapter 5** we extended our study cohort to focus in more detail on latent carriers of the virus only (i.e., CMV seropositive patients) and to determine the attributable mortality that is caused by CMV reactivation. Although a possible association with increased case fatality was found in some studies, it remains uncertain whether CMV reactivation is a true independent risk factor for poor clinical outcome, because most studies did not adequately account for immortal time bias caused by the delayed onset of reactivation and the evolution of disease severity prior to onset of reactivation, and have ignored informative censoring by competing risks (e.g., death and discharge). As a consequence, CMV viremia could still be merely a marker of illness severity, contributing only little to the overall burden of disease. Despite extensive adjustment for confounding in our study, CMV reactivation was independently associated with increased ICU mortality, of which the absolute mortality due to CMV reactivation was estimated to be 4.4% (95% CI 1.1-7.9) by day 35 in the ICU.

Based on our findings in **Chapter 4** and **Chapter 5** a pre-emptive treatment strategy (by which patients would be screened for CMV and treated only if reactivation occurs) seems more attractive compared to a prophylactic strategy, because the number of patients exposed to the toxicity of (val)ganciclovir would be reduced by almost 75%. However, a pre-emptive approach compared to prophylactic treatment is likely less effective, as treatment is initiated only after reactivation has already begun.

As our results in **Chapter 4** suggest that the subgroup of septic shock patients are most prone for CMV reactivation and its detrimental effects, we continued our research with a special focus on previously immunocompetent ICU patients with septic shock,

and expanded our scope of research to other herpes viruses as well (**Chapter 6**). We observed viremia in up to 62% of patients with septic shock. Epstein-Barr virus was most frequently found (48%), followed by herpes simplex virus (26%), human herpesvirus 6 (18%), CMV (16%), and varicella zoster virus (1%). An association with mortality was found for patients with CMV, but not for patients with other herpes viruses. This could imply that CMV is more virulent in comparison to other herpes viruses, and also supports our finding in **Chapter 5** that CMV is indeed a true pathogen. Currently, critically ill patients are not routinely screened on the presence of any virus in blood.

ANTIBIOTIC EXPOSURE AND RESISTANCE DEVELOPMENT

Many ICU patients need antibiotic treatment, especially because of their increased risk of acquiring infections and their increased vulnerability to detrimental effects of infections (2). However, antibiotic exposure is an important cause of emerging antibiotic resistance (26). Acquisition of resistance can be either endogenous or exogenous. The latter is usually caused through temporarily contaminated hands of healthcare workers (27).

In **Chapter 7** and **Chapter 8** we quantified the antibiotic-associated within-host antibiotic resistance acquisition rates in gram-negative bacteria that colonize the lower respiratory tract of ICU patients. In a setting without selective digestive decontamination (SDD) we found that meropenem exposure was associated with the highest increased risk of subsequent resistance development for the exposed antibiotic in previously antibiotic-sensitive *Pseudomonas aeruginosa* in the lower respiratory tract. This study was different compared to other studies, as a specific patient population (i.e., ICU patients) and only endogenous acquisition rates were studied (i.e., we ruled out possible events of cross-transmission through genotyping and epidemiologic linkage). Subsequently, we examined whether these associations between antibiotic exposure and resistance development were similar in settings in which SDD is routinely applied in the ICU (**Chapter 8**) and we compared these results to the findings described in **Chapter 7**. We found that antibiotic-associated resistance acquisition rates of gram-negative bacteria in the lower respiratory tract of ICU patients did not differ much between patients receiving SDD and those not receiving SDD. Similar to non-SDD settings, carbapenem use was associated with the highest rate of antibiotic resistance development in *Pseudomonas aeruginosa*. We did not find independent associations between antibiotic exposure and resistance development for *Enterobacter* and *Klebsiella* species. Our findings, together with those from previous studies (28-30), strongly indicate that carbapenems pose a more serious risk than other antibiotics in inducing antibiotic resistance in *Pseudomonas aeruginosa*. It remains challenging in clinical practice to balance the increasing need to treat patients with carbapenems (because of the emergence of Gram-negative bacteria producing extended-spectrum beta-lactamases) against the risks of creating antibiotic resistance.

SDD AND CANDIDA COLONIZATION

SDD and selective oropharyngeal decontamination (SOD) are controversial antimicrobial interventions in the ICU because of the possibility of inducing antimicrobial resistance by this prophylactic antibiotic exposure. However, their use is associated with absolute reductions in mortality compared to standard care in settings with low levels of antibiotic resistance (31, 32).

Candida normally inhabits the gastrointestinal tract, but can also colonize the lower respiratory tract in critically ill patients receiving mechanical ventilation. Therefore, SDD includes an antifungal component, usually topically administered amphotericin B, to prevent colonization or infection with *Candida*. Despite this prophylaxis some mechanically ventilated patients may still acquire *Candida* colonization of the lower respiratory tract. Inhalation therapy with nebulized amphotericin B is commonly used to treat persistent colonization in the respiratory tract as part of SDD/SOD protocols. However, the effectiveness of this practice is unknown. In **Chapter 9** we thus studied the effectiveness of nebulized amphotericin B in decolonizing the respiratory tract of *Candida* species. We found that nebulized amphotericin B reduced the duration of *Candida* colonization of the lower respiratory tract by approximately three days. There is some evidence that colonization of the lower respiratory tract by *Candida* species could promote the development of pneumonia by creating biofilms that are capable of holding other micro-organisms, and colonization might decrease the immune defense favoring bacterial development (33, 34). Some studies support the association between *Candida* colonization and subsequent *Pseudomonas* ventilator-associated pneumonia development (35, 36). However, in our study the incidence rate of ventilator-associated pneumonias was not different between the treatment and control group. An important consideration is the lack of standardized methods for nebulized administration of amphotericin B. In our setting jet-nebulizers were used, but probably the use of other methods such as vibrating mesh nebulizers may result in a higher deposit of the drug at the target place (37), which could mean that the effectiveness of nebulized amphotericin B treatment may be improved. Nonetheless, the clinical significance of *Candida* in the lower respiratory tract remains controversial. Therefore, nebulized amphotericin B in its current practice should not be routinely used as part of SDD, at least until evidence supporting its effectiveness becomes available.

There is a global-wide shortness of raw materials that are needed to produce amphotericin B, which increases the costs of amphotericin B. This has led to a search for alternative antifungal components in SDD. In **Chapter 10** we compared the effectiveness of nystatin to amphotericin B. When amphotericin B was replaced by nystatin in the topical mouth paste and gastro enteral suspension of the SDD regimen, fewer patients acquired intestinal *Candida* colonization in the intestinal tract. No difference was observed in respiratory tract colonization. This suggests that amphotericin B can be replaced by nystatin in SDD without losing effectiveness, while making this strategy more cost-effective.

ICU-ACQUIRED ENTEROCOCCAL BACTEREMIA

Enterococci are natural colonizers of the human gastrointestinal tract and have been used as a probiotic for decades (38). Some suggest the pathogenicity of enterococci is low and resembling the virulence of coagulase-negative staphylococci. Nevertheless, enterococcal bacteremia is found in patients with the highest severity of illness and is associated with mortality rates ranging from 23 to 48% (39-41). However, this strong association with morbidity and mortality could also be based on a marker phenomenon instead of a causal relation.

In Chapter 11 we addressed the association between ICU-acquired enterococcal bacteremia and clinical outcome. Although crude differences in mortality were apparent, severity of illness, comorbidities and time-dependent bias largely accounted for the high case fatality rates that were observed in patients with enterococcal bacteremia. Nonetheless, ICU-acquired enterococcal bacteremia remained independently associated with increased case fatality even after taking into account many sources of bias. Yet, the virulence of enterococci seemed comparable to that of coagulase-negative staphylococcal bacteremia. From a population perspective the excess mortality attributable to enterococcal bloodstream infections was low. This modest impact may have resulted from both the relatively low incidence of these events and limited virulence of enterococci. It is important to emphasize that our findings on attributable mortality only represent vancomycin-susceptible enterococci. Whether this conclusion would be different for vancomycin-resistant enterococci remains to be determined. Furthermore, we cannot exclude that some enterococcal bacteremia events are clinically more relevant than others, and that a proportion of positive cultures was caused by contaminated blood draws, diluting the association with poor outcome.

Another important observation from this study was the difference between the observed clinical practice on catheter replacement and antibiotic therapy in our study and existing international guidelines. Antibiotic course durations were shorter in comparison to suggested durations from the Infectious Diseases Society of America (IDSA) guidelines, which recommend that all catheters be removed and antimicrobial treatment given for 7 to 14 days in cases of suspected CRBSI due to enterococci. Of note, evidence supporting the longer durations of treatment as suggested by these guidelines is actually lacking.

METHODOLOGICAL CONSIDERATIONS

This thesis describes some important associations between opportunistic pathogens, antimicrobial strategies and different outcome measures. Several methodological challenges were considered during the conduct of our studies: (a) time-dependent bias, (b) confounding at baseline, (c) confounding due to the evolution of disease severity prior to the onset of a time-varying variable of interest, and (d) competing events.

To assess the association between a variable of interest and outcome, it is important to consider the timing of the variable of interest. In some situations the variable of interest is an entity that remains fixed during the entire study follow-up, for example the gender of a patient. In contrast, the occurrence of virus reactivations (**Chapter 5** and **Chapter 6**), administration of nebulized amphotericin B (**Chapter 9**), and occurrence of enterococcal bacteremia (**Chapter 11**) are variables that may change during the follow-up period. As a result time-dependent bias or immortal time bias may occur, which is crucial in observational studies to recognize and adjust for. Otherwise, it may cause distortion as non-exposed time observed before the onset of the variable of interest will be wrongfully attributed to the exposed time at risk, usually resulting in an underestimation of effects associated with the variable of interest (42, 43).

Differences in patient characteristics may contribute to the observed differences in outcome when two groups of patients are compared. Therefore, multivariable models are used in order to adjust for baseline imbalances between the groups that are compared. In almost all models presented in this thesis we have adjusted for patient characteristics, which are assumed to be potential confounders. Of note, when antibiotic resistance acquisition was studied, the inclusion of patient characteristics did not influence the effect estimate. As illustrated in **Chapter 8** both models (one with and one without patient characteristics) yielded comparable results, which suggest that patient characteristics, in contrast to antibiotic factors, play a minor role in antibiotic resistance development. This was also supported by the absence of statistically significant associations between patient characteristics and antibiotic resistance development in multivariable models.

The principle of multivariable analysis to adjust for confounders is to statistically force exposed and non-exposed patients to be similar in all aspects of disease aside from their variable of interest. However, in a dynamic ICU setting, during which critically ill patients continuously deteriorate and improve over time, it is very difficult to verify whether such adjustment was successful. From the start of ICU admission until the occurrence of, for example, enterococcal bacteremia on day 10 in the ICU, the patients' condition at admission may have been completely changed. There is a need to assess the possible impact of variations in the evolution of disease severity between patients on the effect estimates that are studied. With marginal structural modeling we tried to adjust for the evolution of disease severity prior to the onset of the variable of interest. In the CMV analysis, we found similar results as in the analysis not using marginal structural modeling (**Chapter 5**). On the contrary, in the ICU-acquired enterococcal analysis we observed a greater decrease in effect estimate following more rigorous adjustments (**Chapter 11**). The latter case illustrates that for ICU-acquired enterococcal bacteremia there is a large component of 'marker of disease severity' contributing to the observed association with mortality.

Finally, the presence of competing events should be taken into account when follow-up time is censored (44). In several chapters presented in this thesis, time-dependent

analyses were performed. Cox regression analysis requires that censoring of survival time must be non-informative, but in an ICU setting this is clearly not the case since ICU patients who are discharged from the ICU alive are in a better health state than those who remain in the ICU beyond that time-point (45, 46). Furthermore, when ICU mortality is the event of interest, then discharge must be regarded as a competing event as it precludes this event of interest from being observed (44). The use of the subdistribution hazard model provides a general solution to overcome this informative censoring. However, the interpretation of a subdistribution hazard might not be straightforward. While the cause-specific hazard directly relates to the incidence rate of the event of interest, the subdistribution hazard represents a less intuitive measure of the association between the exposure and the cumulative risk of the event when taking competing events into account. In the setting of a time-dependent variable (such as CMV reactivation) it remains difficult to conceptually define exposure on the interval from the observed failure time to any (potential) future censoring. Nonetheless, it remains possible to define and interpret the subdistribution hazard in a valid manner (47, 48).

Although we applied advanced and up-to-date statistical methodology and tried to adjust for most sources of bias to achieve an accurate estimation, we cannot completely rule out the presence of residual confounding. This is inherent to observational clinical studies. One might suggest that testing pharmacological prophylaxis against an opportunistic pathogen in a randomized controlled trial will provide a definite answer on the etiologic role of an opportunistic pathogen. For example ganciclovir to prevent CMV reactivation: if ganciclovir turns out to be effective then CMV is a true causative pathogen, but if not effective then CMV is only a marker of disease severity. Unfortunately, in real life it is much more complicated and a trial will not provide a definite answer with respect to the causative role of CMV, because observed clinical endpoints will be influenced by both the effectiveness of the antiviral drug to prevent reactivation as well as any negative (side) effects caused by the drug. More importantly, we are currently not able to accurately predict patients who will acquire CMV reactivation, resulting in unnecessary exposure to a drug in three-quarters of patients when selection is based on clinical criteria only. Translational research, including in vitro and in vivo experimental studies, could provide more insight in the pathophysiology of an opportunistic pathogen. However, the challenge is that these results may be hampered by the validity of translating findings from pre-clinical models to clinical reality (49). One type of study design on itself will, therefore, not be the magic bullet. Ultimately, a combination of all types of research is necessary, including translational research to increase our understanding of the problem, large clinical observational studies to identify within the large group of heterogeneous ICU patients subgroups who are most at risk or where a possible intervention is likely to be effective, and randomized controlled trials to test the efficacy of interventions in an ICU setting.

FUTURE PERSPECTIVES

We have assessed the clinical significance of several opportunistic pathogens and some frequently used antimicrobial strategies in critically ill patients. Although we have increased our understanding, many new questions remain to be answered. We need to further enhance our understanding and explore possibilities that could favorably modify patient outcome, and improve current (prophylactic) treatment.

For respiratory viruses in particular, larger prospective observational studies containing granular data of daily clinical parameters and microbiological specimens are necessary to achieve an accurate estimation of the incidence of different types of respiratory viruses in general ICU patients as well as particular subgroups of critically ill patients.

Identification should be improved of patients who either have an increased risk of systemic virus reactivation or in whom reactivation will result in more detrimental effects on clinical outcomes. The host response should also be studied as it may provide additional insight on the virulence of opportunistic pathogens. Alongside clinical parameters, genome-wide microarray analysis may lead to finding new biomarkers that may serve as predictors of virus reactivation.

The findings in this thesis provide support for the future testing of pre-emptive strategies using (val)ganciclovir, as CMV is independently associated with increased case fatality despite extensive adjustments for many kinds of bias. Intervention trials comparing pre-emptive treatment and wait-and-see strategies are necessary before any evidence-based recommendations regarding the clinical management of CMV reactivation in critically ill patients should be formulated.

The clinical implications of the observed high incidences of (multiple) viremia in septic shock patients need to be explored in more detail. Future studies should focus on underlying pathophysiological mechanisms of viremia, including an investigation whether low levels of viremia could also be the result of non-replicating viral DNA that is redistributed from lytic cells to plasma (due to the high severity of illness), instead of reactivation. Also a greater understanding on the underlying effects on a cellular and molecular level of each of these viruses on clinical outcomes is required. Other herpes viruses seemed to be less virulent compared to CMV. These other opportunistic viral pathogens may be used as markers of immune suppression, thereby providing a tool for the physician to monitor the condition of the immune system.

New studies assessing the concurrent presence of viruses in the respiratory tract and blood will possibly provide more insight into the possible relevance of pulmonary virus reactivation only, systemic virus reactivation only, and the combination of both.

In our studies we especially focused on short-term effects of opportunistic pathogens. Future studies should also investigate the impact on long-term endpoints. Moreover, the occurrence of virus reactivation after ICU discharge and the interaction between bacterial and viral infections are interesting to elucidate. For this, large

observational studies with sufficient statistical power to detect small but potentially meaningful differences are needed to obtain valuable information.

Physicians need to be aware of risk factors that induce the development of antibiotic resistance, because this may influence the success of treatment. Durations of antimicrobial treatment as suggested by current guidelines require further investigation and optimization. In all of the frequently used interventions in the ICU, cost-effectiveness should be continuously evaluated. As an example, the individual components of SDD have to be critically evaluated to determine whether improvements can be made in the regimen composition. We have evaluated the effects of changing amphotericin B to nystatin on *Candida* colonization outcomes, but did not study the effects on subsequent ICU-acquired infections.

CONCLUDING REMARKS

Accurate assessment of the effects of opportunistic pathogens in the ICU is important, because it determines to what extent diagnostic and therapeutic measures should be performed. Some pathogens are overestimated in their pathogenicity, whereas others are underestimated for their role. Among respiratory viruses, the influenza virus seems to be the most important pathogen to consider in critically ill patients, whereas the role of other respiratory viruses remains controversial. ICU patients are at an increased risk for systemic virus reactivation. Cytomegalovirus reactivation was independently associated with increased ICU mortality. The role of *Candida* colonization remains controversial. There are however effective decolonization strategies available. The virulence of enterococci seems comparable to that of coagulase-negative staphylococcal bacteremia.

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APPENDICES

DUTCH SUMMARY (NEDERLANDSE SAMENVATTING)

Het doel van dit proefschrift is om de rol van verschillende opportunistische pathogenen (ziekteverwekkers) in intensive care (IC) patiënten in kaart te brengen en de effecten van antimicrobiële therapie op kolonisatie met deze pathogenen en resistentie ontwikkeling te bestuderen.

De gevolgen van een infectie zijn het resultaat van de interactie tussen een pathogeen en een patiënt. Pathogenen worden meestal als opportunistisch bestempeld indien de virulentie voornamelijk beperkt is tot personen waarbij het immuunsysteem duidelijk verminderd is. Deze definitie is echter niet strikt. De meerderheid van IC patiënten wordt doorgaans niet als immuungecompromitteerd beschouwd, in tegenstelling tot bijvoorbeeld transplantatie patiënten die zware immunosuppressieve medicatie gebruiken. Toch kan het immuunsysteem van sommige IC patiënten ook als verminderd beschouwd worden door de acute ernstige ziekte-episode die deze patiënten ondergaan.

Risicostratificatie is belangrijk, omdat er heterogeniteit is in de virulentie (oftewel het ziekteverwekkende vermogen) van pathogenen en hun mogelijke effecten in verschillende subgroepen van IC patiënten.

EPIDEMIOLOGIE EN DIAGNOSTIEK VAN RESPIRATOIRE VIRUSSEN

Respiratoire (luchtweg) infecties veroorzaakt door virussen zoals het influenza virus kunnen ademhalingsproblemen veroorzaken die een IC opname noodzakelijk maken. De detectie van respiratoire virussen heeft consequenties voor infectiecontrole (bijvoorbeeld isolatiemaatregelen) en een eventuele start van antivirale middelen.

In hoofdstuk 2 van dit proefschrift bleek dat slechts 46% van de IC patiënten, die verdacht werden op het hebben van een community-acquired pneumonie (buiten het ziekenhuis opgelopen longontsteking), op respiratoire virussen werd getest gedurende het influenza seizoen. Op basis van klinische verschijnselen alleen is het onmogelijk om bacteriële en virale infecties van elkaar te onderscheiden. Internationale richtlijnen adviseren daarom om op virussen te testen op basis van zowel klinische als epidemiologische gegevens; in ieder geval indien het aantonen van de aanwezigheid van dergelijke virussen zou leiden tot een verandering in het medisch beleid. Op basis van onze bevindingen is er ruimte om de virale diagnostiek in de IC te verbeteren.

In tegenstelling tot het influenzavirus is de virulentie van andere respiratoire virussen onduidelijk. Uit eerder onderzoek is gebleken dat respiratoir syncytieel virus (RSV) ziekte in volwassenen meestal mild verloopt. Echter RSV kan ook ziekte veroorzaken in hoog-risico patiënten, zoals oudere patiënten met bepaalde longziekten. In hoofdstuk 3 werd een lagere incidentie van RSV (4%) in vergelijking met influenza (15%) gevonden in patiënten die opgenomen werden op de IC met ademhalingsproblemen. Alle patiënten met RSV herstelden zich uiteindelijk, terwijl 17% van de patiënten met

influenza uiteindelijk op de IC kwamen te overlijden. Hieruit blijkt dat RSV ogenschijnlijk geen prominente rol speelt in volwassen IC patiënten.

Systemische reactivatie van herpes virussen

Het optreden van een viremie (oftewel virus in het bloed) in IC patiënten blijkt regelmatig voor te komen, zelfs in voorheen immuuncompetente patiënten. Tot op heden hebben onderzoekers zich vooral geconcentreerd op reactivatie van het cytomegalovirus (CMV). Patiënten met een 'acute respiratory distress syndrome' (ARDS) lopen een hoger risico op CMV reactivatie. Uit eerdere studies is gebleken dat CMV reactivatie geassocieerd is met een toegenomen beademingsduur, IC ligduur en mortaliteit. Dit heeft geleid tot het idee dat profylactische antivirale behandeling in CMV seropositieve patiënten mogelijk van toegevoegde waarde kan zijn.

In **hoofdstuk 4** hebben we CMV seropositieve en seronegatieve ARDS patiënten met elkaar vergeleken. We vonden geen associatie tussen CMV seroprevalentie en klinische uitkomst na correctie voor confounding. Dit betekent dat een profylactische strategie gericht op alle CMV seropositieve patiënten met ARDS hoogstwaarschijnlijk niet effectief zal zijn. CMV reactivatie trad op in 26% van de seropositieve patiënten. Alleen in de subgroep van patiënten met een septische shock werd een langere beademingsduur of hogere mortaliteit waargenomen in CMV seropositieve patiënten ten opzichte van seronegatieve patiënten.

Alhoewel eerdere studies een mogelijke associatie hebben aangetoond tussen CMV reactivatie in het bloed en een slechtere klinische uitkomst, was het onzeker of CMV reactivatie daadwerkelijk de oorzaak of slechts een marker van ziekte-ernst is, omdat in de meeste studies niet volledig is gecorrigeerd voor factoren die de beoordeling van deze associatie kunnen verstoren. In de meeste studies is er namelijk niet gecorrigeerd voor tijdsafhankelijke bias en is er geen rekening gehouden met het feit dat de ziekte-ernst vanaf IC opname tot aan het optreden van CMV reactivatie totaal veranderd kan zijn, waardoor correctie voor alleen baseline verschillen onvoldoende is. Tevens is het belangrijk om rekening te houden met bias die ontstaat door informatieve censoring. In **hoofdstuk 5** toonden wij aan dat ondanks correctie voor alle bovengenoemde factoren een significante associatie met een toegenomen mortaliteit bleef bestaan.

Op basis van eerdere bevindingen dat de effecten van CMV in de subgroep van septische shock mogelijk het grootst zijn (**hoofdstuk 4**), vervolgden wij ons onderzoek in deze specifieke groep van patiënten en breidden we onze virologische diagnostiek uit naar andere herpesvirussen (**hoofdstuk 6**). In deze studie liet 62% van de patiënten een viremie zien, waarvan 48% met Epstein-Barr virus, 26% met herpes simplex virus, 18% met humane herpesvirus 6, 16% met CMV en 1% met varicella zoster virus. Een associatie met mortaliteit werd gevonden voor CMV, maar niet voor de overige herpes virussen, wat de hypothese ondersteunt dat CMV een oorzakelijke rol heeft en niet slechts een marker is van ziekte-ernst.

ANTIBIOTISCHE BLOOTSTELLING EN RESISTENTIE ONTWIKKELING

In hoofdstuk 7 en hoofdstuk 8 bestudeerden wij antibiotica resistentie acquisities in gramnegatieve bacteriën die de lagere luchtwegen van IC patiënten koloniseren. Onder de verschillende antibiotica waren carbapenems (zoals meropenem) geassocieerd met de grootste toename in antibiotica resistentie acquisitie in *Pseudomonas aeruginosa*. In een situatie waar profylactisch antibiotica gegeven werd in het kader van selectieve darm decontaminatie (SDD) (hoofdstuk 8) waren de geobserveerde associaties vergelijkbaar met de situatie waarin geen SDD was gegeven (hoofdstuk 7). Voor *Enterobacter* en *Klebsiella* species werden geen significante associaties gevonden. Onze resultaten wijzen erop dat carbapenems een hoger risico vormen op het induceren van antibiotica resistentie in *Pseudomonas aeruginosa* in vergelijking met andere antibiotica. De uitdaging in de klinische praktijk is om steeds een balans te vinden tussen de behoefte aan carbapenems bij toenemende antibiotica resistentie enerzijds en het risico voor het induceren van nieuwe antibiotica resistentie anderzijds.

SDD EN CANDIDA KOLONISATIE

Candida species kunnen de darmen en de luchtwegen van IC patiënten koloniseren. Om die reden zit in SDD profylaxe een antifungale component om kolonisatie en infectie met *Candida* te voorkomen. Ondanks deze profylaxe kunnen vooral mechanisch beademde patiënten toch *Candida* krijgen in de lagere luchtwegen. Inhalatie therapie met vernevelde amfotericine B wordt regelmatig gebruikt om de lagere luchtwegen te dekoloniseren in vooral degenen met persisterende *Candida* kolonisatie. In hoofdstuk 9 lieten wij zien dat vernevelde amfotericine B de duur van kolonisatie in de lagere luchtwegen reduceerde met ongeveer drie dagen. Echter de klinische significantie van *Candida* in de luchtwegen blijft controversieel, aangezien het bewijs voor effectiviteit van amfotericine B verneveling op klinische eindpunten ontbreekt en in onze studie geen verschil in de incidentie van ventilator-associated (beademing-gerelateerde) pneumonie tussen de behandelde en controle groep werd gevonden.

In hoofdstuk 10 werd de effectiviteit van nystatine met amfotericine B vergeleken, waarbij minder patiënten met *Candida* gekoloniseerd raakten in het rectum tijdens nystatine gebruik. Er werd geen verschil aangetoond in effectiviteit tussen nystatine en amfotericine B op het voorkomen van kolonisatie in de lagere luchtwegen.

IC VERWORVEN ENTEROCOCCEN BACTERIEMIE

In hoofdstuk 11 bestudeerden wij de associatie tussen IC verworven enterokokken bacteriemie en klinische uitkomst. Alhoewel de mortaliteit duidelijk hoger was in patiënten met enterokokken bacteriemie, bleek de ziekte-ernst, comorbiditeit en tijdsafhankelijk bias een groot deel van de verhoogde mortaliteit te verklaren. Ondanks

correctie voor verschillende soorten van bias bleef IC verworven enterokokken bacteriemie onafhankelijk geassocieerd met toegenomen mortaliteit. Desalniettemin was de virulentie van een enterokokken bacteriemie vergelijkbaar met die van een coagulase-negatieve stafylokokken bacteriemie (die als laag virulent beschouwd kan worden) wanneer de meest waarschijnlijke oorzaak een lijninfectie betrof. De IC mortaliteit die toegeschreven kon worden aan een enterokokken bacteriemie was laag. Dit kan hoogstwaarschijnlijk verklaard worden door zowel een relatief lage incidentie van enterokokken bacteriemie als de beperkte virulentie van het pathogeen. Een andere belangrijke observatie was het verschil in beleid op het gebied van intraveneuze lijnen en antibiotische therapie bij lijninfecties in de klinische praktijk en de aanbevelingen genoemd in internationale richtlijnen. De antibiotica duur was opvallend korter dan bijvoorbeeld wat de Infectious Diseases Society of America (IDSA) richtlijnen adviseren. Het bewijs voor de noodzaak van een langere behandelingsduur ontbreekt echter tot op heden.

METHODOLOGISCHE OVERWEGINGEN

Tijdens het uitvoeren van de studies waren er verschillende methodologische uitdagingen: (a) tijdsafhankelijke bias, (b) confounding bij baseline (oftewel verstorende factoren aanwezig bij het begin van de studie follow-up), (c) confounding door de continue verandering van ziekte-ernst vanaf de baseline tot het moment dat de status van een tijdsafhankelijke variabele verandert en (d) competitieve eindpunten.

Om de associatie tussen een determinant en een uitkomst adequaat te beoordelen, is het belangrijk om rekening te houden met de timing van de statusverandering van de determinant. In sommige situaties blijft de status van de determinant onveranderd gedurende de studieduur. Maar in een aantal situaties verandert de status gedurende de IC opname, bijvoorbeeld virus reactivatie (**hoofdstuk 5** en **hoofdstuk 6**), verneveling van amfotericine B (**hoofdstuk 9**) en enterokokken bacteriemie (**hoofdstuk 11**). Hierdoor kan er tijdsafhankelijke bias ontstaan, waarmee in de analyse rekening mee gehouden moet worden om betrouwbare conclusies te kunnen trekken.

Verschillen in patiëntkarakteristieken kunnen bijdragen aan geobserveerde verschillen in uitkomsten wanneer groepen met elkaar vergeleken worden. Daarom worden multivariabele modellen gebruikt om te corrigeren voor baseline verschillen tussen de groepen die met elkaar vergeleken worden. Het principe van multivariabele analyse is om de groepen vergelijkbaar te maken in alle aspecten behalve in de determinant door te corrigeren voor de invloeden van confounders. Echter in een dynamische IC situatie, waarbij patiënten voortdurend verbeteren of verslechteren qua klinische toestand, is het moeilijk te verifiëren of correctie voldoende gelukt is. Bijvoorbeeld, vanaf IC opname tot aan het optreden van een enterokokken bacteriemie op dag 10 van de IC, kan de situatie van de patiënt volledig veranderd zijn ten opzichte van de baseline. Met behulp van een techniek genaamd 'marginal structural modeling'

kan geprobeerd worden om te corrigeren voor de evolutie van ziekte-ernst tot het moment van statusverandering van de determinant.

Tenslotte moet er ook rekening gehouden worden met de aanwezigheid van competitieve eindpunten. In een IC situatie is er vrijwel altijd sprake van informatieve censoring tijdens de studie follow-up, aangezien patiënten die de IC levend verlaten meestal in een betere conditie zijn dan patiënten die op de IC achterblijven.

Alhoewel we geavanceerde en moderne methodologie hebben gebruikt om te corrigeren voor de meeste vormen van bias kunnen we de aanwezigheid van residuele confounding niet uitsluiten. Uiteindelijk is een combinatie van verschillende typen onderzoek nodig: translationeel onderzoek om ons begrip van het probleem te vergroten, observationele studies om subgroepen binnen de grotere groep van heterogene IC patiënten te identificeren waarin een probleem het grootst is en klinische gerandomiseerde studies die de effectiviteit van bepaalde interventies tegen deze opportunistische pathogenen onderzoekt.

Concluderend, een nauwkeurige inschatting van de betekenis van opportunistische pathogenen is belangrijk, omdat het de noodzaak en mate van diagnostische en therapeutische maatregelen beïnvloedt.

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CURRICULUM VITAE

David Ong was born in The Hague, the Netherlands, on the 11th of October 1984. He grew up in Zoetermeer, where he completed his secondary school (*cum laude*) at Alfrink College in 2003. He studied Pharmacy at Utrecht University, where he received his Bachelor of Sciences degree (*cum laude*) in 2006 and his Master of Sciences / PharmD degree in 2010. In 2004 he also decided to study Medicine at Utrecht University, where he received his 'propedeuse' (*cum laude*) in 2005 and his Medical Doctor degree in 2010. Furthermore, he finished a subsidiary subject "Languages and Cultures of China" at Leiden University. During his studies David was active in several committees, including Department Council Pharmaceutical Sciences (Departementsraad), Council of Medical Interns (Co-raad), and Faculty Council Medicine (Faculteitsraad). He also did executive work as Treasurer and Secretary at the National Assembly of Medical Interns Foundation (Stichting Landelijk Overleg Co-Assistenten (LOCA)).

His particular interest in infectious diseases research began at the Julius Center (University Medical Center Utrecht) during his six months scientific research internship on antibiotic use in general practice in 2007 under supervision of Dr. M.M. Kuyvenhoven and Prof. dr. T.J.M. Verheij. Subsequently, he continued with research on antibiotic exposure and resistance development in intensive care units under supervision of Dr. Irene Jongerden and Prof. dr. M.J.M. Bonten.

After finishing his studies David started to work as a research physician and a PhD student for the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) study, which is a multicenter CTMM project that aims to develop and validate new technologies for early diagnosis and prognostication in sepsis, under supervision of Dr. O.L. Cremer (Intensive Care Medicine, UMC Utrecht) and Prof. dr. M.J.M. Bonten (Medical Microbiology and Julius Center, UMC Utrecht). In 2011 he also worked in the intensive care units as a clinical resident during three months. He represented the interests of PhD students in the Scientific Committee of the Division of Anesthesiology, Intensive Care and Emergency Medicine from 2012 to 2015. During his PhD project, he completed a postgraduate Master in Clinical Epidemiology at Utrecht University.

David was admitted to the five-year residency program for Clinical Microbiology in 2012 at UMC Utrecht, under supervision of Dr. A.M. Wensing (UMC Utrecht) and Dr. B.M. de Jongh (St Antonius Hospital, Nieuwegein).