Ventilator-associated Pneumonia and Mortality

Wilhelmina Gerrianne Melsen

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Wilhelmina Gerrianne Melsen

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Promotor: Prof. dr. M.J.M. Bonten
Co-promotor: dr. M.M. Rovers

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CHAPTER 1

General Introduction

Epidemiology of VAP

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in patients in intensive care units (ICU). ICU patients are prone to develop VAP, primarily because of intubation, but also because of their underlying diseases and the frequent use of antibiotics, although the role of antibiotics is controversial (i.e. it has been associated with both higher and lower risks). In a 1-day point–prevalence survey in 1265 ICUs worldwide in 2007, the prevalence of respiratory tract infection was 64% among all infected patients (1). Because of its obvious importance for patient care, a considerable body of clinical research has been dedicated to diagnosis, prognosis, treatment and prevention of VAP. Yet, even after more than 25 years controversies remain on important topics like defining the optimal diagnostic methods for VAP, effectiveness of different VAP prevention strategies and to what extent VAP affects patient survival.

Diagnosis of VAP

The optimal method to diagnose VAP is unknown. Various diagnostic strategies have been proposed and they can be subdivided into non-invasive techniques, based primarily on clinical features and microbiological cultures of endotracheal aspirates, and invasive techniques based on quantitative cultures of respiratory tract secretions collected by invasive techniques (e.g. bronchoalveolar lavage (BAL), plugged telescoping catheter (PTC) and protected specimen brush (PSB)). Until now there is no clinically feasible gold standard for diagnosing VAP, which precludes accurate determination of test characteristics of the different diagnostic methods. To bypass this fundamental problem of the absence of a gold standard, the consequences of using different diagnostic approaches on patient outcome have been evaluated in randomized trials(2-6). Yet, most of these studies were not able to demonstrate a beneficial effect of one strategy above the other regarding mortality. Only the study of Fagon et al (7) demonstrated a significant beneficial effect of an invasive technique regarding mortality at day 14. However no significant difference was found regarding mortality at day 28. Furthermore, most studies were too small to demonstrate a possible difference. Naturally, the lack of a gold standard for the diagnosis of VAP also complicates studies that evaluate prevention strategies for VAP or that aim to estimate the attributable mortality of VAP. Nevertheless, many studies with these specific research questions have been performed and the comparison of their results is rather difficult, as different diagnostic methods with different sensitivities and specificities have been used.

Prevention of VAP

Multiple interventions to prevent VAP have been evaluated, mostly in small single-center studies. Favourable effects on the incidence of VAP were obtained in numerous studies(8-16), yet hardly any intervention was associated with a statistically significant reduction of mortality. Probably because most of these studies were underpowered for demonstrating such a difference. Prevention methods should have the ultimate goal to improve the outcome of patients, preferably through reducing mortality. In order to adequately evaluate these prevention measures, knowledge about the attributable mortality of VAP is necessary in order to design

adequately powered trials. Moreover, quantifying attributable mortality of VAP in relevant subgroups is essential for determining the burden of disease associated with this "serious complication" of ICU treatment.

Morbidity, mortality and VAP

VAP complicates the treatment of ICU patients by increasing the duration of mechanical ventilation, length of stay in ICU and mortality. Yet, the exact attributable mortality of VAP is unknown with estimates ranging from zero to 60%(17-22). These currently available estimates are difficult to interpret and compare, because of differences in study quality, patient populations studied, diagnostic methods used and causative pathogens identified. Moreover, many studies are limited by the methodology used. As most of these studies were observational confounding could have had a major impact on study results. Typically, matching and adjustments in multivariate models have been used to address confounding. However, not all studies used these methods, and if used, large differences between studies remained in type and quantity of matching criteria and/or possible confounders. These studies are also at risk of unmeasured (i.e. potential confounders were inadequately observed or not observed at all) and/or residual (unknown) confounding. Furthermore, most studies suffered, due to small sample sizes, from a lack of statistical power, the inability to properly adjust for confounding and the evaluation of differences in mortality in subgroups of patients. Finally, in none of these studies investigators accounted for time dependent bias and competing risks. Yet, the relevance of these aspects have been clearly described in recent studies (23-25). Thus, although many attempts have been undertaken to quantify the relation between VAP and morality, the exact association remains unknown.

Aim of this thesis

The key objective of this thesis is to assess the association between VAP and mortality using various state-of-the-art statistical techniques.

More specific aims comprise:

1. The use of meta-analytical techniques:
   a. To qualitatively and quantitatively combine all available observational studies evaluating VAP and mortality.
   b. To determine the attributable mortality using summarized data out of VAP prevention trials, with a focus on limiting the role of confounding.
2. The use of survival analyses to determine the association between VAP and mortality, taking time-dependent bias and competing risks into account.
3. To evaluate the role of subgroups in the attributable mortality of VAP and prevention of nosocomial infections.

Scope and outline of this thesis

The first part of this thesis (chapters 2,3,4 and 5) focuses on the association of VAP and mortality using different meta-analytic techniques. In **chapter 2** we will estimate the attributable mortality of VAP based on all observational studies investigating this association. We will also attempt to quantitatively combine the results of all studies, including assessment of the influence of study quality, study methodology, diagnostic methods and admission diagnosis on attributable mortality. In **chapter 3**, we will determine the attributable mortality using the published data from all randomized VAP prevention trials. As patients were randomized to some preventive intervention this approach will reduce the influence of confounding. In **chapter 4** we will use the individual patient data of randomized VAP prevention trials to determine the attributable mortality of VAP in subgroups of patients. In **chapter 5** we will use the individual patient data of randomized VAP prevention trials to determine the attributable mortality of VAP caused by different pathogens.

In **chapter 6** we discuss and demonstrate the assessment of heterogeneity, the accuracy of the available methods to quantify heterogeneity and its effect on the reliability in a meta-analysis. In **chapter 7** we focus on the importance of subgroup effects and use the data of the largest study ever performed to evaluate SOD and SDD in subgroups of surgical and non-surgical patients.

In **chapter 8**, all available and applied methods when estimating the influence of nosocomial infections and mortality are discussed, in the light of known methodological challenges.

REFERENCES

(1) Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA 2009 December 2;302(21):2323-9.

(2) Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques for ventilator-associated pneumonia. N Engl J Med 2006 December 21;355(25):2619-30.

(3) Fagon JY, Chastre J, Wolff M, Gervais C, Parer-Aubas S, Stephan F et al. Invasive and noninvasive strategies for management of suspected ventilator-associated pneumonia. A randomized trial. Ann Intern Med 2000 April 18;132(8):621-30.

(4) Ruiz M, Torres A, Ewig S, Marcos MA, Alcon A, Lledo R et al. Noninvasive versus invasive microbial investigation in ventilator-associated pneumonia: evaluation of outcome. Am J Respir Crit Care Med 2000 July;162(1):119-25.

(5) Sanchez-Nieto JM, Torres A, Garcia-Cordoba F, El-Ebiary M, Carrillo A, Ruiz J et al. Impact of invasive and noninvasive quantitative culture sampling on outcome of ventilator-associated pneumonia: a pilot study. Am J Respir Crit Care Med 1998 February;157(2):371-6.

(6) Sole VJ, Fernandez JA, Benitez AB, Cardenosa Cendrero JA, Rodriguez de CF. Impact of quantitative invasive diagnostic techniques in the management and outcome of mechanically ventilated patients with suspected pneumonia. Crit Care Med 2000 August;28(8):2737-41.

(7) Fagon JY, Chastre J, Wolff M, Gervais C, Parer-Aubas S, Stephan F et al. Invasive and noninvasive strategies for management of suspected ventilator-associated pneumonia. A randomized trial. Ann Intern Med 2000 April 18;132(8):621-30.

(8) Bergmans DC, Bonten MJ, Gaillard CA, Paling JC, van-der GS, van-Tiel FH et al. Prevention of ventilator-associated pneumonia by oral decontamination: a prospective, randomized, double-blind, placebo-controlled study. Am J Respir Crit Care Med 2001;164:382-8.

(9) Camus C, Bellissant E, Sebille V, Perrotin D, Garo B, Legras A et al. Prevention of acquired infections in intubated patients with the combination of two decontamination regimens. Crit Care Med 2005;33(2):307-14.

(10) Fourrier F, Cau-Pottier E, Boutigny H, Roussel-Delvallez M, Jourdain M, Chopin C. Effects of dental plaque antiseptic decontamination on bacterial colonization and nosocomial infections in critically ill patients. Intensive Care Med 2000;26(9):1239-47.

(11) Garcia MS, Galache JAC, Diaz JL, Cerda EC, Blasco JR, Aguinaga MAG et al. Effectiveness and cost of selective decontamination of the digestive tract in critically ill intubated patients - A randomized, double-blind, placebo-controlled, multicenter trial. Am J Respir Crit Care Med 1998;158(3):908-16.

(12) Lacherade JC, De JB, Guezennec P, Debbat K, Hayon J, Monsel A et al. Intermittent Subglottic Secretion Drainage and Ventilator-associated Pneumonia: A Multicenter Trial. Am J Respir Crit Care Med 2010 June 3.

(13) Morrow LE, Kollef MH, Casale TB. Probiotic Prophylaxis of Ventilator-associated Pneumonia: A Blinded, Randomized, Controlled Trial. Am J Respir Crit Care Med 2010 June 3.

(14) Nardi G, Di Silvestre AD, De Monte A, Massarutti D, Proietti A, Grazia Troncon M et al. Reduction in gram-positive pneumonia and antibiotic consumption following the use of a SDD protocol including nasal and oral mupirocin. Eur J Emerg Med 2001;8(3):203-14.

(15) Pneumatikos I, Koulouras V, Nathanail C, Goe D, Nakos G. Selective decontamination of subglottic area in mechanically ventilated patients with multiple trauma. Intensive Care Med 2002 April;28(4):432-7.

(16) Seguin P, Tanguy M, Laviolle B, Tirel O, Mallédant Y. Effect of oropharyngeal decontamination by povidone-iodine on ventilator-associated pneumonia in patients with head trauma. Crit Care Med 2006;34:1514-9.

(17) Bercault N, Boulain T. Mortality rate attributable to ventilator-associated nosocomial pneumonia in an adult intensive care unit: a prospective case-control study. Crit Care Med 2001 December;29(12):2303-9.

(18) Erbay RH, Yalcin AN, Zencir M, Serin S, Atalay H. Costs and risk factors for ventilator-associated pneumonia in a Turkish university hospital's intensive care unit: a case-control study. BMC Pulm Med 2004 April 26;4:3.

(19) Heyland DK, Cook DJ, Griffith L, Keenan SP, Brun-Buisson C. The attributable morbidity and mortality of ventilator-associated pneumonia in the critically ill patient. The Canadian Critical Trials Group. Am J Respir Crit Care Med 1999 April;159(4 Pt 1):1249-56.

(20) Kollef MH. Ventilator-associated pneumonia. A multivariate analysis. JAMA 1993 October 27;270(16):1965-70.

(21) Kollef MH, Silver P, Murphy DM, Trovillion E. The effect of late-onset ventilator-associated pneumonia in determining patient mortality. Chest 1995 December;108(6):1655-62.

(22) Rodriguez JL, Gibbons KJ, Bitzer LG, Dechert RE, Steinberg SM, Flint LM. Pneumonia: incidence, risk factors, and outcome in injured patients. J Trauma 1991 July;31(7):907-12.

(23) Beyersmann J, Schumacher M. Time-dependent covariates in the proportional subdistribution hazards model for competing risks. Biostatistics 2008 October;9(4):765-76.

(24) Nguile-Makao M, Zahar JR, Francais A, Tabah A, Garrouste-Org, Allaouchiche B et al. Attributable mortality of ventilator-associated pneumonia: respective impact of main characteristics at ICU admission and VAP onset using conditional logistic regression and multi-state models. Intensive Care Med 2010 May;36(5):781-9.

(25) Wolkewitz M, Beyersmann J, Gastmeier P, Schumacher M. Modeling the effect of time-dependent exposure on intensive care unit mortality. Intensive Care Med 2009 May;35(5):826-32.

CHAPTER 2

Ventilator-associated Pneumonia and Mortality; A Systematic Review of Observational Studies

W.G. Melsen; M.M. Rovers and M.J.M. Bonten

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SUMMARY

Background

Ventilator-associated Pneumonia (VAP) is generally believed to increase the mortality of patients. This notion is predominantly based upon the results of observational studies.

Objective

A systematic review and meta-analysis of observational studies was performed to determine the attributable mortality of VAP.

Data sources

We performed a systematic search strategy using PubMed, Web of Science and Embase from their inception through February 2007. In addition, a reference and related article search was performed. Studies were included if they reported mortality rates of patients with and without VAP.

Results

Fifty-two studies with a total of 17.347 patients met the inclusion criteria. Pooling of all studies resulted in relative risk of 1.27 (95% CI 1.15 to 1.39), but heterogeneity was considerable (I^2statistic of 69%). The origin of heterogeneity could not be explained by differences in study design, study quality and diagnostic approach. However heterogeneity was limited for studies investigating only trauma patients (I^2=1.3%) or acute respiratory distress syndrome (ARDS) patients (I^2=0%), with estimated relative risk of 1.09 (95% CI 0.87-1.37) among trauma patients and 0.86 (95% CI 0.72-1.04) among ARDS patients.

Conclusion

There is no evidence of attributable mortality due to VAP in patients with trauma or ARDS. However, in other, non-specified, patient groups, there is evidence for attributable mortality due to VAP, but this could not be quantified due to heterogeneity in study results. More detailed studies, allowing subgroup analyses, are needed to determine the attributable mortality of VAP in these patient populations.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is one of the most frequently occurring nosocomial infections, complicating medical treatment of intensive care (ICU) patients receiving mechanical ventilation(1). In the United States, VAP has recently been proposed as a quality-of-care indicator for hospitals, since it is generally believed that VAP increases both morbidity and mortality of ICU patients(2). This belief is predominantly based on the results of observational studies using a (matched) cohort design. However, a systematic approach to quantitatively combine the results of all available studies evaluating the association between the development of VAP and mortality does not exist. The aim of this review, therefore, was to determine the attributable mortality of VAP by systematically identifying and quantitatively combining all available observational studies.

METHODS

We searched PubMed, Embase and Web of Science from their inception to February 2007 using the terms "ventilator associated pneumonia", "ventilator associated pneumonias", "vap", "nosocomial pneumonia", "hospital acquired pneumonia", "mortality", "outcome", "survival" and "death" to identify articles reporting on the association between VAP and mortality. In addition, a reference and related article search was performed.

Study selection and data extraction

Identified abstracts were screened without blinding to authors and journal. Potentially relevant studies were obtained and the full text examined. Studies were eligible if a group of patients with VAP was compared with a group of patients without VAP, and if patients (both in the pneumonia and reference group) were mechanically ventilated. Furthermore, in both groups determination of the total number of patients and corresponding mortality should be possible. Studies published only as abstracts or in non-english language were excluded, because sufficient data were needed for a thorough quality assessment. Studies only including cardiac surgery patients were excluded, since they compromise a distinct ICU patient population with different baseline mortality rates (0.2% and 3% (3;4)).

For each study the following characteristics were extracted: total number of patients in VAP and reference group with corresponding mortality rates, study setting, study population (e.g. trauma, surgical, medical), diagnostic criteria for VAP, matching criteria and success of matching (in case of matched cohort studies) and the statistical analyses used to evaluate the association of VAP and mortality. The study selection and data extraction were performed by W.M.

Quality assessment

The quality of the included studies was appraised by a quality scoring system quantifying both methodological quality and eligibility of the primary studies for this review. The eligibility was quantified because studies not specifically designed to determine the mortality of VAP, were

also included. The criteria of the scoring system were based upon the previously proposed criteria from Cook et al (5). We adjusted this scoring system by adding items for the assessment of diagnostic methods and matching (Box 1). The three main categories of the scoring system include comparability of study population, diagnostic methods as well as accurateness of matching in case of matched cohort studies. The sub score of each objective was, before adding up to a total score, multiplied by a factor (respectively 3, 2.4 and in the case of matching criteria with 2), in order to give each objective the same weight in the total score. The maximum score in case of cohort and matched cohort studies was 24 and 36, respectively. The studies with a total score of at least two third of the maximum score were regarded as those with the highest quality (i.e. ≥ 24 in matched cohort studies and ≥ 16 points in cohort studies).

Box 1. Assessment of Quality and Eligibility

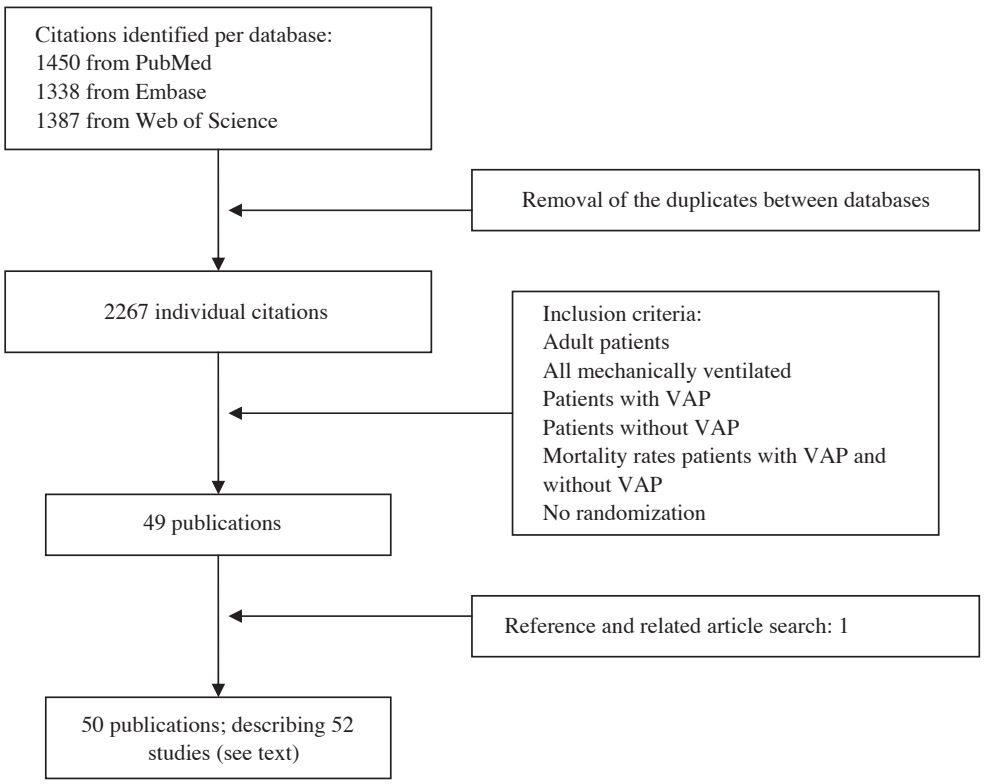
Score	Criteria
I Population	
	Patient selection
1	Case patients; ICU patients,mechanically ventilated >48 hrs, VAP
1	Control patients; ICU patients, mechanically ventilated >48hrs, no VAP
	Patient characteristics
	- Age (mean differs by <10%)
	- Sex (proportion of males in each group differs by <10%
	Diagnosis (proportion with the following differing by <10%
	- Chronic obstructive airway disease
	- Respiratory failure
	- Pneumonia at entry
2, Groups comparable on ≥6 characteristics	- Other (icu-acquired) infections
	- Tracheostomy
1, Groups comparable on 3 to 5 characteristics	- Sepsis
	- Renal failure
0, Groups comparable on ≤2 characteristics	- CNS disease/neurologic disease
	- Hepatic failure
	- Trauma
	- Surgery
	- Diabetes Mellitus
	- Malignancy
	- ARDS
	- Score for severity of illness
II Assessment of the diagnosis Ventilator-associated Pneumonia (VAP)	
	Confirmation clinical suspicion by BAL/PSB/PTC
5	- All patients
4	- Subset of patients

	Confirmation clinical suspicion by endotracheal aspirate
3	- Quantitative
2	- Semiquantative
	Clinical suspicion (CDC-criteria)
	- Radiologic evidence of new and persistant (>48hrs) pulmonary infiltrates
	+ at least two of the following features:
	- Temperature higher than 38° C and lower than 35° C
	- Peripheral leucocytosis
	- Leucocyte count lower than 4000 per mm^3
	- Purulent respiratory secretions
1	- Appearance or worsening of respiratory insufficiency
0	None of the above described methods or not clear which method was used
III Matching of the groups (in case of matched cohort studies)	
	Number of criteria
2	- >6
1	- 3-6
0	- <3
	Success of matching
2	- 95-100%
1	- 85-95%
0	- < 85% / unknown
	The following variable is part of the matching process or comparable between cases and controls:
1	- duration of mechanical ventilation prior to VAP
1	- severity of illness
Total score cohort studies: 3*I + 2.4*II (maximum score:24) Total score matched cohort studies: 3*I + 2.4*II + 2 III (maximum score: 36)	

Statistical Analysis

The meta-analysis was carried out using Review manager (version 4.2.8. The Cochrane Collaboration, Oxford). We used the random-effects model to calculate pooled relative risks and 95% confidence intervals. I^2 statistics were used to assess heterogeneity. This quantity describes the percentage of total variation across studies that is due to heterogeneity rather than chance. Larger values of I^2 show increasing heterogeneity. Heterogeneity was defined to be low, moderate or high with I^2 below 25%, between 25 and 75%, and above 75%, respectively(6).
To explain differences in outcome and to limit heterogeneity between the studies, subgroup analyses were performed, in which studies with comparable characteristics were pooled. The a priori hypotheses to explain heterogeneity were differences in study population, methods to diagnose VAP, study design and study quality. Funnel plots were used to assess publication bias.

Figure 1. Flow chart of literature search



RESULTS

Initially, 1.450 articles were identified in PubMed, 1.387 in the Web of Science and 1.338 in Embase (figure 1). Of the 2.267 individual articles, 49 met the inclusion criteria (7-55). An additional article was found by searching the reference lists(56). Most articles were excluded because no data was available on the number of patients with or without VAP, unclear information of the ventilation status of included patients and lack of the mortality rates of patients with or without VAP. The studies by Chastre(15) and Markowicz(37) were included twice since both studies provided data from general ICU patients and from patients with acute respiratory distress syndrome (ARDS). Thus in total 52 individual studies were included in our analysis. Study characteristics and mortality rates of matched cohort and cohort studies are summarized in table 1 and 2. In nine studies only trauma patients were included(9;14;16;30;36;44;45;47;56) and in four studies only patients with ARDS(15;19;37;48).

Table 1. Overview of matched-cohort studies

Author	Setting/ Datacollection	Cases	Type of mortality	Matching Criteria	Quality score Pop/Diag/Match
Baker[9] (1996)	Trauma ICU Retrospective	Traumapts who underwent bronchoscopy for culture collection. MV >?	Not specified	Age, AIS-score, gender, final discharge diagnosis and number of discharge diagnosis.	0/12/4
Bercault[10] (2001)	Medical/surgical ICU Prospective	MV> 48 hrs	ICU	Age, GCS, diagnosis on admission, indication ventilation, duration of MV prior to onset of VAP, immunologic status, cardiac status, probability of death, Secondary criteria: gender, diagnosis category, respiratory status, alcohol, preceding surgery.	12/12/12
Bonten[12] (1997)	Mixed ICU Prospective	Pts MV>72 hrs	28 days	Length of stay ICU prior VAP, diagnosis on admission, global renal and hepatic function, preceding surgery, antibiotic use and immunosuppressive therapy	9/9.6/8
Cavalcanti[14] (2006)	Trauma ICU Prospective	Traumapts MV>?	ICU	Age, APACHE II, duration of MVprior to onset of VAP.	3/9.6/6
Cocanour[16] (2005)	Trauma ICU Prospective	Traumapts MV>24 hrs	ICU	Age, Injury Severity Score.	0/2.4/2
Erbay[21] (2004)	Medical/surgical ICU Retrospective	Pts without any other infection. MV>48 hrs	ICU	Age, gender and underlying disease.	6/12/0
Fagon[22] (1993)	Mixed ICU Retrospective	Pts MV> 72 hrs	ICU	Age, SAPS score, duration of MV prior to onset of VAP, indication ventilation, date of admission.	12/12/8
Heyland[25] (1999)	Multicenter mixed ICUs. Prospective	MV> 48 hrs	ICU	APACHE, MOD 1dy prior to VAP, gender, duration of MV prior to onset of VAP, length of stay prior to VAP, diagnosis on admission, medical/surgical status, center at which treated.	9/9.6/8
Hugonnet[26] (2004)	Medical ICU Prospective	Pts MV>48 hrs	ICU	Age, gender, duration of MV prior to onset of VAP, diagnosis on admission, number discharge diagnosis, study period.	6/9.6/6
Kallel[30] (2005)	Mixed ICU Retrospective	Headtrauma patients MV> 48 hrs	Hospital	Age, GCS, Injury Severity Score, SAPS II, duration of MV prior to onset of VAP	9/7.2/10
Leone[36] (2002)	Mixed ICU Prospective	Multiple trauma pts with head trauma. MV>48hrs	ICU	Age, GCS, Injury Severity Score, APACHE II, duration of MV prior to onset of VAP.	12/12/6
Nseir[40] (2005)	Mixed ICU Prospective	COPD pts MV> 48hrs	ICU	Age, SAPS II, diagnosis on admission, duration of MV prior to onset of VAP (date of ICU admission; when more candidates)	12/7.2/6
Papazian[41] (1996)	Medical/surgical ICU Prospective	MV> 48 hrs	ICU	Age, APACHE II, gender, diagnosis on admission, indication ventilation, duration of MV prior to onset of VAP, date of admission within 1 yr	12/12/10
Rello[43] (2002)	USA database of > 100 US acute-care hospitals Retrospective	MV>48 hrs	Hospital	Age, severity of illness, duration of MV prior to onset of VAP, type of hospital admission	3/0/6
R Ferrari[44] (2004)	Neurocritical ICU Prospectively	Pts with headinjury MV>48hrs	ICU	Age, APACHE II, Injury Severity Score, Same category of Head Injury according to classification based on CT and duration of MV.	9/9.6/6
Tejerina[49] (2006)	Multicenter, international ICUs Retrospective	MV > 48hrs	Hospital	Age, SAPS, indication ventilation (coma, sepsis, ARDS), use of vasoactive drugs, use of neuromuscular blockers, plateau airway pressure, events during MV (barotrauma, ARDS, sepsis, shock, renal failure, hepatic failure, coagulopathy, metabolic acidosis and ratio PaO2 to FiO2), previous functional status.	12/2.4/8

Abbreviations: ICU= Intensive Care Unit, MV= mechanical ventilation, AIS score= abbreviated injury scale, VAP= Ventilator-associated Pneumonia, GCS= Glasgow Coma Scale, Mixed ICU= Intensive Care Unit with patients from different specialities, APACHE II= Acute Physiology And Chronic Health Evaluation Score, SAPS=simplified acute physiology score, MOD= multiple organ dysfunction score, ARDS= Acute Respiratory Distress Syndrome. Quality score: consists of the total score of respectively population, diagnosis and matching.

Table 2. Overview of cohort studies

Author	Setting	datacollection	Cases	Type of mortality	Quality score Population/ Diagnosis
Akca (2000)[7]	Mixed ICU	Prospective	MV>48hrs	Not specified	6/7.2
Apostolopoulou (2003)[8]	Multicenter ICUs	Prospective	MV>24hrs	Not specified	3/2.4
Bercault (2005)[11]	Medical-surgical ICU	Prospective	MV>48hrs	ICU	*/12
Boots (2004)[13]	Multicenter ICUs	Prospective	MV>48hrs	Not specified	9/9.6
Bronchard (2004)[56]	Surgical ICU	Prospective	Head trauma, MV>48hrs, <168hrs	ICU	6/12
Chastre (1998)[15]	Mixed ICU	Prospective	MV>48hrs, ARDS	ICU	9/12
Chastre II (1998)[15]	Mixed ICU	Prospective	MV>48hrs, without ARDS	ICU	9/12
Cook (1998)[17†]	Multicenter ICUs	Prospective	MV>48hrs	ICU	9/9.6
Craven (1986)[18]	Mixed ICUs	Prospective	MV>48hrs	Hospital	*/12
Delclaux (1997)[19]	Medical ICU	Prospective	ARDS,MV>?	Not specified	3/12
Ensminger (2006)[20]	Coronary Care Unit (CCU)	Retrospective	CCUpatients, MV >48hrs	Hospital	9/2.4
Georges (2000)[23]	Mixed ICU	Retrospective	Tracheotomy, MV >?	Not specified	0/12
Guimaraes (2006)[24]	Mixed ICU	Prospective	MV>24hrs	ICU	0/9.6
Ibrahim (2001)[27]	Medical-surgical ICU	Prospective	MV>?	Hospital	3/2.4
Jaimes (2006)[28]	Mixed ICUs	Prospective	MV>48hrs	ICU	*/9.6
Jimenez (1989)[29]	Respiratory ICU	Prospective	MV>48hrs	ICU	6/9.6
Kanafani (2003)[31]	Mixed ICUs	Prospective	MV>48hrs	Not specified	6/9.6
Kappstein (1992)[32]	Anesthesiological ICU	Prospective	MV>24hrs	ICU	*/2.4
Kollef (1993)[33]	Mixed ICUs	Prospective	MV>24hrs	ICU	3/2.4
Kollef (1995)[34]	Mixed ICUs (two centers)	Prospective	MV>120hrs, only late onset VAP	Hospital	9 /2.4

Table 2 (Continued)

Kollef (1997)[35]	Mixed ICUs (two centers)	Prospective	MV>12hrs	Hospital	3/2.4
Kooi (2006)[52]	Multicenter ICUs	Prospective	MV started on day before or day of infection	ICU	0/0
Markowicz (2000)[37]	Multicenter ICUs	Prospective	MV>48hrs, ARDS	ICU	9/12
Markowicz (2000)[37]	Multicenter ICUs	Prospective	MV>48hrs, without ARDS	ICU	*/12
Myny (2005)[38]	Medical-surgical ICU	Prospective	MV>48hrs	ICU	6/4.8
Noor (2005)[39]	Medical-surgical ICU	Prospective	MV>48hrs	Not specified	6/7.2
Rello (1991)[42]	Medical-surgical ICU	Prospective	MV>48hrs	ICU	6/12
Rodriguez (1991)[45]	Trauma Center	Prospective	Trauma patients, MV>?	Not specified	3/2.4
Sofianou (2000)[46]	Mixed ICU	Prospective	MV>48hrs	Not specified	0/7.2
Sole Violan (1998)[53]	Mixed ICU	Prospective	MV>?	ICU	0/12
Stephan (2006)[47]	Trauma ICU	Prospective	MV>24hrs	ICU	3/12
Sutherland (1995)[48]	Mixed and Trauma ICU	Prospective	MV>48hrs, ARDS	Not specified	*/12
Timsit (1996)[50]	Medical-surgical ICU	Prospective	MV>48hrs	ICU	9/12
Torres (1990)[51]	Mixed ICU	Prospective	MV>48hrs	Not specified	9/2.4
Warren (2003)[54]	Medical-surgical ICU	Prospective	MV>24hrs	Hospital	3/2.4
Woske (2001)[55]	Surgical ICU	Prospective	MV>48hrs	ICU	6/12

Abbreviations: Mixed ICU= Intensive Care Unit with patients from different specialities, MV= mechanical ventilation, ICU=Intensive Care Unit, ARDS=Acute Respiratory Distress Syndrome.

*unknown; study was designed for another research question/no information on baselinecharacteristics of those with and without VAP

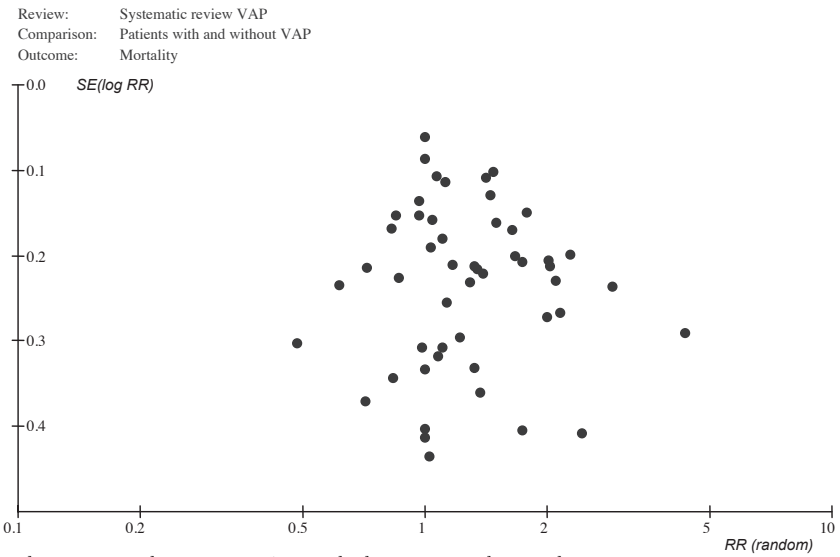
†This study used the same database as the study from Heyland, but evaluated more patients because matching was not performed. We only included this study in the analysis when the cohort studies were evaluated seperately.

Quality score: consists of the total score of respectively population and diagnosis.

Quality assessment

All studies were qualified using the previous described criteria, resulting in the scores as provided in table 1 and 2. Baseline characteristics of patients with and without VAP were missing in some studies. Comparability of study groups was reached for less than two baseline characteristics in 4 matched cohort and 12 cohort studies. The diagnostic approach of the studies consisted of quantitative cultures obtained by PSB, BAL or PTC (n=33) or endotracheal aspirates (n=6) to confirm the clinical suspicion of VAP, but for some studies no uniform approach could be determined, as data from multiple centres with different approaches were used. Among the matched cohort studies different criteria were used for matching and the numbers of criteria ranged from 2 to 13. The success of matching (when provided) ranged from around 74%(9) to 100% (10;30;36). The studies with a total score of at least two third of the maximum score were regarded as those with the highest quality (i.e. ≥ 24 in matched cohort studies (n=9) and ≥ 16 points in cohort studies (n=9)).
The funnel plot for all the studies evaluating VAP was symmetrical, indicating that publication bias was limited (figure 2).

Figure 2: Funnelplot

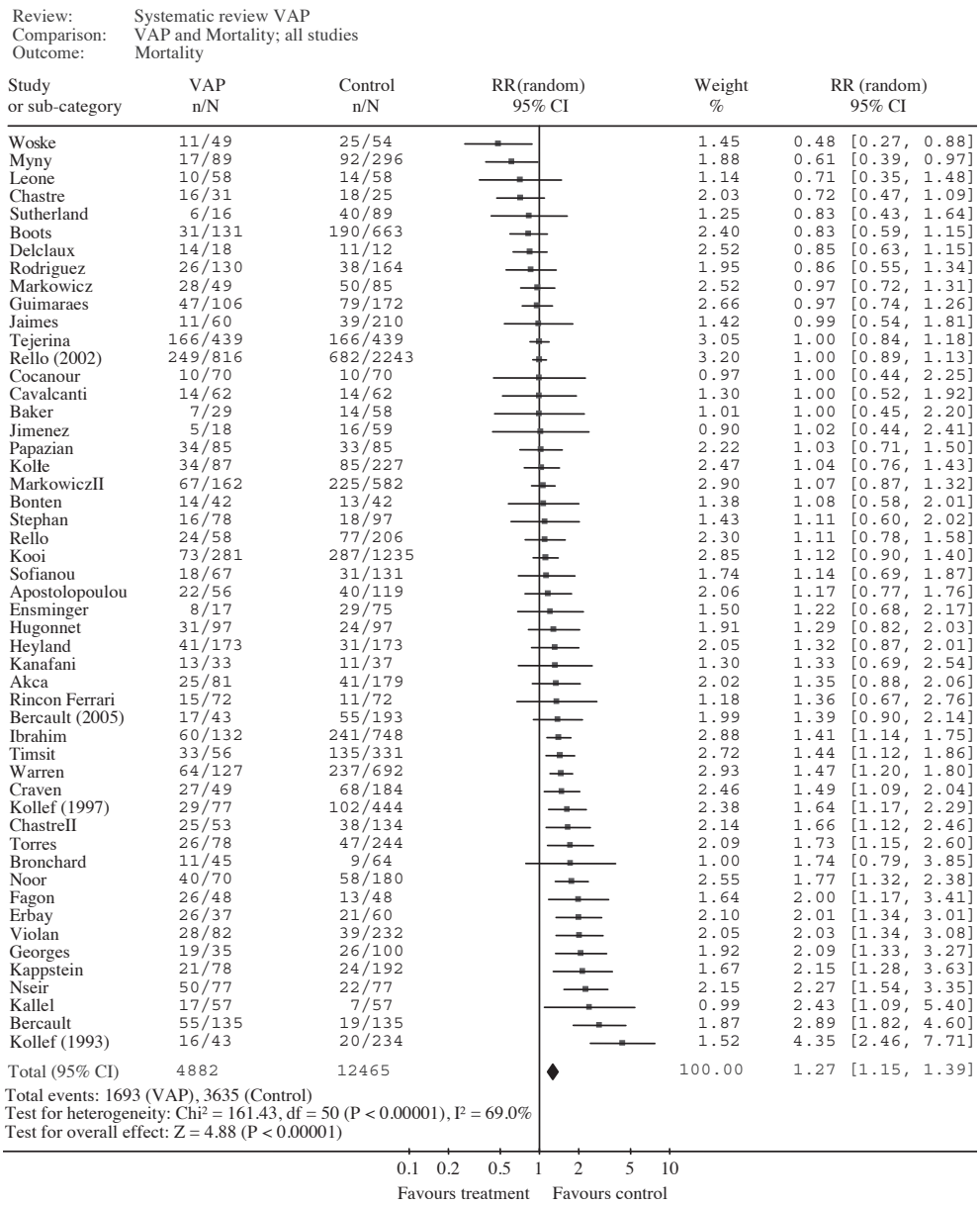


VAP= Ventilator-associated Pneumonia, SE=standard error, RR= relative risk

Mortality

In total, 52 studies including 4882 VAP patients and 12465 control patients were eligible for our review. A considerable variation in mortality rates of patients with VAP was found; 14 to 70% in the matched cohort studies and 16 to 78% in the other observational studies. The pooled relative risk of the association of VAP and mortality was 1.27 (95% CI 1.15 to 1.39), but with an I^2 statistic of 69%, indicating considerable heterogeneity among studies (figure 3).

Figure 3. Relative risk of Ventilator-associated Pneumonia on mortality

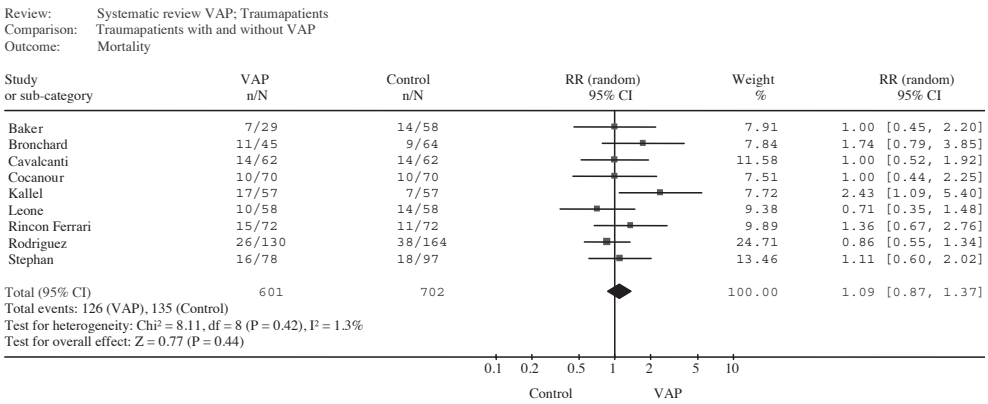


Graph sorted by study design (matched-cohort versus cohort studies).

VAP= Ventilator-associated Pneumonia, n=total number of patients who died, N= total number of patients, RR= relative risk, CI= confidence interval.

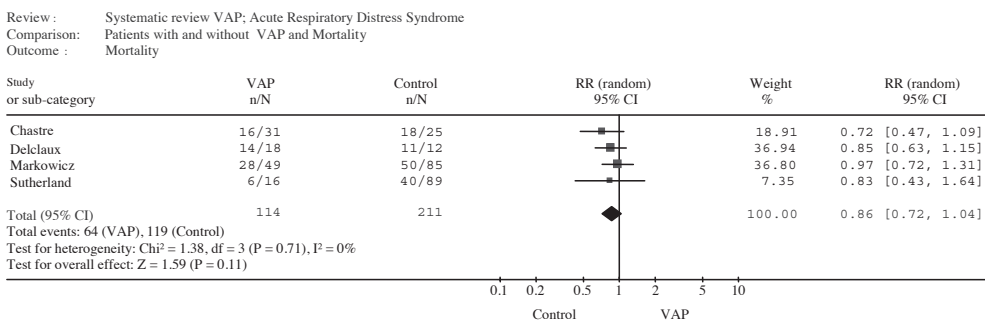
This heterogeneity remained high when we subdivided the matched cohort and cohort studies (71.5% and 67.3%, respectively; table 3), and when we evaluated studies only including patients mechanically ventilated for more than 48 hours (data not shown). The association between VAP and mortality was further analysed by pooling studies with comparable characteristics, such as diagnostic approaches, quality score and patient populations. Although these analyses demonstrated pooled relative risks ranging from 1.21 to 1.35, heterogeneity was >60% in each analysis (table 3). Little heterogeneity (I^2=1.3%), though, was found after pooling the results of the studies evaluating trauma patients, yielding a RR of 1.09 (95% CI 0.87 to 1.37) (figure 4). Pooling the results of patients with acute respiratory distress syndrome (ARDS) also yielded a low level of heterogeneity (I^2 = 0%) and a RR of 0.86 (95% CI 0.72 to 1.04) (figure 5).

Figure 4. Relative risk of Ventilator-associated Pneumonia on mortality among trauma patients



VAP= Ventilator-associated Pneumonia, n=total number of patients who died, N= total number of patients, RR= relative risk, CI= confidence interval.

Figure 5. Relative risk of Ventilator-associated Pneumonia on mortality among patients with Acute Respiratory Distress Syndrome (ARDS)



VAP= Ventilator-associated Pneumonia, n=total number of patients who died, N= total number of patients, RR= relative risk, CI= confidence interval.

In the studies that did not include only trauma patients or patients with ARDS (n=38), 31 showed a RR above 1 and 16 studies were able to demonstrate a significant higher mortality rate of VAP (pooled RR 1.34 95% CI (1.21 to 1.49) (data not shown)). Nine of these studies also performed multivariate analyses to adjust for possible confounders. In five of these studies (18;21;27;33;53) the association between VAP and mortality disappeared, whereas in the other four (10;35;40;50) the association remained. In one of these studies(10), though, only for episodes of VAP caused by multiresistant pathogens.

Table 3. Overview of subgroup analyses

	VAP No of deaths/ Total pts	Control No of deaths/ Total pts	RR 95% CI	I^2-statistic
All studies (n=51)	1693/4882	3635/12465	1.27 [1.15 to 1.39]	69.0%
Study Design				
Cohort studies (n=36)	970/2762	2706/9526	1.23 [1.11 to 1.38]	67.3%
Matched cohort studies (n=16)	765/2297	1094/3776	1.35 [1.12 to 1.64]	71.5%
Diagnostic Approach				
BAL/PSB/PTC (n=31)	722/2010	1376/4419	1.21 [1.07 to 1.37]	62.3%
Clinical Criteria (n=20)	971/2872	2259/8046	1.35 [1.16 to 1.56]	76.9%
Studypopulation				
General ICU patients[#] (n=35)	1427/4038	3305/11300	1.31 [1.18 to 1.46]	72.1%
Trauma Patients (n=9)	126/601	135/702	1.09 [0.87 to 1.37]	1.3%
ARDS (n=4)	64/114	119/211	0.86 [0.72 to 1.04]	0%
Study Quality[*]				
Highest score (n=18)	483/1396	870/3146	1.26 [1.04 to 1.52]	72.0%
Lowest score (n=28)	1103/3255	2479/8706	1.27 [1.12 to 1.43]	70.3%

[#] General ICU patients: mix of patients consisting of variable proportions of medical, surgical and trauma patients.
[*] Highest score; the studies with a total score above 24 (matched cohort studies) and 16(cohort studies).

DISCUSSION

This systematic review of all studies evaluating the association between VAP and mortality provides very contradictory results. When pooling all available evidence the relative risk of the association of VAP and mortality was estimated to be 1.27 (95% CI 1.15 to 1.39), but with an amount of heterogeneity among study outcomes being as high as 69%. This high level of heterogeneity could not be reduced by pooling studies with similar methodology, clinical characteristics and quality. Only for studies including trauma patients or patients with ARDS the variation among study results was low (with I^2 of 1.3% and 0%, respectively). The pooled analysis of these studies failed to demonstrate attributable mortality due to VAP (RR of 1.09 (95% CI 0.87 to 1.37) for trauma patients and a RR of 0.86 (95% CI 0.72 to 1.04) for patients with ARDS

Since there is currently no consensus on how to deal with heterogeneity, and many systematic reviews calculate pooled estimates even when heterogeneity is high(6;57-59), we decided to provide pooled estimates for all analyses independent from the level of heterogeneity (table 3). However, a high level of heterogeneity indicates that there are genuine differences underlying the results of the studies(6), which makes the overall effect estimates unreliable. We, therefore, strongly feel that providing pooled estimates independently from the level of heterogeneity is not appropriate and would be very conservative in accepting the results of pooled effect estimates when I^2 is above 25%. Nevertheless, despite extensive heterogeneity, pooled estimates of the studies that were not restricted to trauma or ARDS patients (RR 1.34 95%CI (1.21 to 1.49)) suggest that VAP may be associated with increased mortality. Most of these studies (31 of 38) had a RR above 1. Therefore, it seems very likely that there is attributable mortality of VAP in these populations, although the amount of this effect remains uncertain.

To the best of our knowledge, this is the first analysis of all available evidence regarding the association between VAP and mortality. Heyland et al(25) and Safdar et al(60) performed systematic reviews of matched cohort studies only, including six and nine studies, respectively. Heyland et al did not calculate a pooled estimate but demonstrated the differences in mortality rates of VAP among these studies. Safdar et al provided a pooled OR for ICU mortality (2.03 95% CI 1.16-3.56) although significant statistical heterogeneity was found (p=.05).
The absence of attributable mortality of VAP in trauma patients is supported by the results of several VAP prevention trials. In three studies(61-63) evaluating trauma patients only, selective decontamination was associated with relative risk reductions ranging from 0.51 to 0.71 without any obvious effect on mortality. An absence of attributable mortality due to VAP for trauma and ARDS patients should be taken into account when using mortality as an outcome of VAP prevention studies. Moreover, patient population specific differences in attributable mortality of VAP might explain why so many VAP prevention trials failed to reduce patient outcome, despite impressive reductions in VAP incidence.

Although we have provided a complete overview of all observational studies identified by means of a thorough systematic search, there are some issues that should be addressed. Most of these issues result from the sparsity of patient specific data available for analysis. First, we were limited in our subgroup analyses to data reported in the original papers and could, therefore, not evaluate the influence of items like disease severity, early versus late onset disease, causative pathogens and adequacy of treatment. Some authors have suggested that severity of illness could influence the association of VAP and mortality(64;65). We have demonstrated that in patients with ARDS ("extremely severe illness") and in trauma patients ("less severe illness") VAP is not associated with a higher mortality rate. However, the exact role of severity of illness regarding the mortality of VAP remains to be determined.

Second, although most studies provided information about the causative pathogens, with *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii* and *Haemophilus influenzae* being most common, the influence of different pathogens on attributable mortality was only specifically assessed in some studies. Eight of these studies(25;38;40;41;44;45;51;53) concluded that mortality rates are not influenced by different causative (high risk) pathogens, whereas

others(10;13;22;30;34;50) reported associations of higher mortality and VAP due to high risk (antimicrobial resistant) pathogens. We were not able to provide a pooled estimate due to summarized data and differences in definition of high risk pathogens.

Third, the (initial) adequacy of antibiotic treatment has been shown to be an important determinant of mortality. A minority (n=11) of the studies described the adequacy of treatment (range 64%-98%), and five studies (10;13;25;38;40) addressed this issue in their analysis. These studies failed to demonstrate a higher mortality rate or an independent role of VAP in mortality among patients who were treated inadequately.

Fourth, (residual) confounding may play a role in the observed associations between VAP and mortality in the studies included in this review. Only a minority of the studies performed multivariable analyses to control for possible confounders. The heterogeneity in the study results may, among other things, be the result of uncontrolled confounding within these studies.

In conclusion, we have demonstrated that the current available evidence is heterogeneous and to a large extent limited by the lack of adjustments for possible confounders and estimations of attributable mortality in subgroups of patients. For those subgroups with low heterogeneity, i.e. trauma patients and patients with ARDS, no association was found between VAP and mortality. However, our analyses do suggest an association between VAP and mortality for non specified patients groups. Analyses which adequately control for potential confounders and allow appropriate subgroup analyses are needed to determine the attributable mortality of VAP in these patient populations.

REFERENCES

(1) Chastre J, Fagon JY. Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002 April 1;165(7):867-903.

(2) Klompas M, Platt R. Ventilator-associated pneumonia—the wrong quality measure for benchmarking. Ann Intern Med 2007 December 4;147(11):803-5.

(3) Bouza E, Perez A, Munoz P, Jesus PM, Rincon C, Sanchez C et al. Ventilator-associated pneumonia after heart surgery: a prospective analysis and the value of surveillance. Crit Care Med 2003 July;31(7):1964-70.

(4) Pawar M, Mehta Y, Khurana P, Chaudhary A, Kulkarni V, Trehan N. Ventilator-associated pneumonia: Incidence, risk factors, outcome, and microbiology. J Cardiothorac Vasc Anesth 2003 February;17(1):22-8.

(5) Cook DJ, Laine LA, Guyatt GH, Raffin TA. Nosocomial pneumonia and the role of gastric pH. A meta-analysis. Chest 1991 July;100(1):7-13.

(6) Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003 September 6;327(7414):557-60.

(7) Akca O, Koltka K, Uzel S, Cakar N, Pembeci K, Sayan MA et al. Risk factors for early-onset, ventilator-associated pneumonia in critical care patients: selected multiresistant versus nonresistant bacteria. Anesthesiology 2000 September;93(3):638-45.

(8) Apostolopoulou E, Bakakos P, Katostaras T, Gregorakos L. Incidence and risk factors for ventilator-associated pneumonia in 4 multidisciplinary intensive care units in Athens, Greece. Respir Care 2003 July;48(7):681-8.

(9) Baker AM, Meredith JW, Haponik EF. Pneumonia in intubated trauma patients. Microbiology and outcomes. Am J Respir Crit Care Med 1996 January;153(1):343-9.

(10) Bercault N, Boulain T. Mortality rate attributable to ventilator-associated nosocomial pneumonia in an adult intensive care unit: a prospective case-control study. Crit Care Med 2001 December;29(12):2303-9.

(11) Bercault N, Wolf M, Runge I, Fleury JC, Boulain T. Intrahospital transport of critically ill ventilated patients: a risk factor for ventilator-associated pneumonia—a matched cohort study. Crit Care Med 2005 November;33(11):2471-8.

(12) Bonten MJ, Froon AH, Gaillard CA, Greve JW, de Leeuw PW, Drent M et al. The systemic inflammatory response in the development of ventilator-associated pneumonia. Am J Respir Crit Care Med 1997 October;156(4 Pt 1):1105-13.

(13) Boots RJ, Lipman J, Bellomo R, Stephens D, Heller RF. Disease risk and mortality prediction in intensive care patients with pneumonia. Australian and New Zealand practice in intensive care (ANZPIC II). Anaesth Intensive Care 2005 February;33(1):101-11.

(14) Cavalcanti M, Ferrer M, Ferrer R, Morforte R, Garnacho A, Torres A. Risk and prognostic factors of ventilator-associated pneumonia in trauma patients. Crit Care Med 2006 April;34(4):1067-72.

(15) Chastre J, Trouillet JL, Vuagnat A, Joly-Guillou ML, Clavier H, Dombret MC et al. Nosocomial pneumonia in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 1998 April;157(4 Pt 1):1165-72.

(16) Cocanour CS, Ostrosky-Zeichner L, Peninger M, Garbade D, Tidemann T, Domonoske BD et al. Cost of a ventilator-associated pneumonia in a shock trauma intensive care unit. Surg Infect (Larchmt) 2005;6(1):65-72.

(17) Cook DJ, Walter SD, Cook RJ, Griffith LE, Guyatt GH, Leasa D et al. Incidence of and risk factors for ventilator-associated pneumonia in critically ill patients. Ann Intern Med 1998 September 15;129(6):433-40.

(18) Craven DE, Kunches LM, Kilinsky V, Lichtenberg DA, Make BJ, McCabe WR. Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am Rev Respir Dis 1986 May;133(5):792-6.

(19) Delclaux C, Roupie E, Blot F, Brochard L, Lemaire F, Brun-Buisson C. Lower respiratory tract colonization and infection during severe acute respiratory distress syndrome: incidence and diagnosis. Am J Respir Crit Care Med 1997 October;156(4 Pt 1):1092-8.

(20) Ensminger SA, Wright RS, Baddour LM, Afessa B. Suspected ventilator-associated pneumonia in cardiac patients admitted to the coronary care unit. Mayo Clin Proc 2006 January;81(1):32-5.

(21) Erbay RH, Yalcin AN, Zencir M, Serin S, Atalay H. Costs and risk factors for ventilator-associated pneumonia in a Turkish university hospital's intensive care unit: a case-control study. BMC Pulm Med 2004 April 26;4:3.

(22) Fagon JY, Chastre J, Vuagnat A, Trouillet JL, Novara A, Gibert C. Nosocomial pneumonia and mortality among patients in intensive care units. JAMA 1996 March 20;275(11):866-9.

(23) Georges H, Leroy O, Guery B, Alfandari S, Beaucaire G. Predisposing factors for nosocomial pneumonia in patients receiving mechanical ventilation and requiring tracheotomy. Chest 2000 September;118(3):767-74.

(24) Guimaraes MM, Rocco JR. Prevalence of ventilator-associated pneumonia in a university hospital and prognosis for the patients affected. J Bras Pneumol 2006 July;32(4):339-46.

(25) Heyland DK, Cook DJ, Griffith L, Keenan SP, Brun-Buisson C. The attributable morbidity and mortality of ventilator-associated pneumonia in the critically ill patient. The Canadian Critical Trials Group. Am J Respir Crit Care Med 1999 April;159(4 Pt 1):1249-56.

(26) Hugonnet S, Eggimann P, Borst F, Maricot P, Chevrolet JC, Pittet D. Impact of ventilator-associated pneumonia on resource utilization and patient outcome. Infect Control Hosp Epidemiol 2004 December;25(12):1090-6.

(27) Ibrahim EH, Tracy L, Hill C, Fraser VJ, Kollef MH. The occurrence of ventilator-associated pneumonia in a community hospital: risk factors and clinical outcomes. Chest 2001 August;120(2):555-61.

(28) Jaimes F, De La RG, Gomez E, Munera P, Ramirez J, Castrillon S. Incidence and risk factors for ventilator-associated pneumonia in a developing country: Where is the difference? Respir Med 2007 April;101(4):762-7.

(29) Jimenez P, Torres A, Rodriguez-Roisin R, de la Bellacasa JP, Aznar R, Gatell JM et al. Incidence and etiology of pneumonia acquired during mechanical ventilation. Crit Care Med 1989 September;17(9):882-5.

(30) Kallel H, Chelly H, Bahloul M, Ksibi H, Dammak H, Chaari A et al. The effect of ventilator-associated pneumonia on the prognosis of head trauma patients. J Trauma 2005 September;59(3):705-10.

(31) Kanafani ZA, Kara L, Hayek S, Kanj SS. Ventilator-associated pneumonia at a tertiary-care center in a developing country: incidence, microbiology, and susceptibility patterns of isolated microorganisms. Infect Control Hosp Epidemiol 2003 November;24(11):864-9.

(32) Kappstein I, Schulgen G, Beyer U, Geiger K, Schumacher M, Daschner FD. Prolongation of hospital stay and extra costs due to ventilator-associated pneumonia in an intensive care unit. Eur J Clin Microbiol Infect Dis 1992 June;11(6):504-8.

(33) Kollef MH. Ventilator-associated pneumonia. A multivariate analysis. JAMA 1993 October 27;270(16):1965-70.

(34) Kollef MH, Silver P, Murphy DM, Trovillion E. The effect of late-onset ventilator-associated pneumonia in determining patient mortality. Chest 1995 December;108(6):1655-62.

(35) Kollef MH, Von HB, Prentice D, Shapiro SD, Silver P, St JR et al. Patient transport from intensive care increases the risk of developing ventilator-associated pneumonia. Chest 1997 September;112(3):765-73.

(36) Leone M, Bourgoin A, Giuly E, Antonini F, Dubuc M, Viviand X et al. Influence on outcome of ventilator-associated pneumonia in multiple trauma patients with head trauma treated with selected digestive decontamination. Crit Care Med 2002 August;30(8):1741-6.

(37) Markowicz P, Wolff M, Djedaini K, Cohen Y, Chastre J, Delclaux C et al. Multicenter prospective study of ventilator-associated pneumonia during acute respiratory distress syndrome. Incidence, prognosis, and risk factors. ARDS Study Group. Am J Respir Crit Care Med 2000 June;161(6):1942-8.

(38) Myny D, Depuydt P, Colardyn F, Blot S. Ventilator-associated pneumonia in a tertiary care ICU: analysis of risk factors for acquisition and mortality. Acta Clin Belg 2005 May;60(3):114-21.

(39) Noor A, Hussain SF. Risk factors associated with development of ventilator associated pneumonia. J Coll Physicians Surg Pak 2005 February;15(2):92-5.

(40) Nseir S, Di PC, Soubrier S, Cavestri B, Jozefowicz E, Saulnier F et al. Impact of ventilator-associated pneumonia on outcome in patients with COPD. Chest 2005 September;128(3):1650-6.

(41) Papazian L, Bregeon F, Thirion X, Gregoire R, Saux P, Denis JP et al. Effect of ventilator-associated pneumonia on mortality and morbidity. Am J Respir Crit Care Med 1996 July;154(1):91-7.

(42) Rello J, Quintana E, Ausina V, Castella J, Luquin M, Net A et al. Incidence, etiology, and outcome of nosocomial pneumonia in mechanically ventilated patients. Chest 1991 August;100(2):439-44.

(43) Rello J, Ollendorf DA, Oster G, Vera-Llonch M, Bellm L, Redman R et al. Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. Chest 2002 December;122(6):2115-21.

(44) Rincon-Ferrari MD, Flores-Cordero JM, Leal-Noval SR, Murillo-Cabezas F, Cayuelas A, Munoz-Sanchez MA et al. Impact of ventilator-associated pneumonia in patients with severe head injury. J Trauma 2004 December;57(6):1234-40.

(45) Rodriguez JL, Gibbons KJ, Bitzer LG, Dechert RE, Steinberg SM, Flint LM. Pneumonia: incidence, risk factors, and outcome in injured patients. J Trauma 1991 July;31(7):907-12.

(46) Sofianou DC, Constandinidis TC, Yannacou M, Anastasiou H, Sofianos E. Analysis of risk factors for ventilator-associated pneumonia in a multidisciplinary intensive care unit. Eur J Clin Microbiol Infect Dis 2000 June;19(6):460-3.

(47) Stephan F, Mabrouk N, Decailliot F, Delclaux C, Legrand P. Ventilator-associated pneumonia leading to acute lung injury after trauma: importance of Haemophilus influenzae. Anesthesiology 2006 February;104(2):235-41.

(48) Sutherland KR, Steinberg KP, Maunder RJ, Milberg JA, Allen DL, Hudson LD. Pulmonary infection during the acute respiratory distress syndrome. Am J Respir Crit Care Med 1995 August;152(2):550-6.

(49) Tejerina E, Frutos-Vivar F, Restrepo MI, Anzueto A, Abroug F, Palizas F et al. Incidence, risk factors, and outcome of ventilator-associated pneumonia. J Crit Care 2006 March;21(1):56-65.

(50) Timsit JF, Chevret S, Valcke J, Misset B, Renaud B, Goldstein FW et al. Mortality of nosocomial pneumonia in ventilated patients: influence of diagnostic tools. Am J Respir Crit Care Med 1996 July;154(1):116-23.

(51) Torres A, Aznar R, Gatell JM, Jimenez P, Gonzalez J, Ferrer A et al. Incidence, risk, and prognosis factors of nosocomial pneumonia in mechanically ventilated patients. Am Rev Respir Dis 1990 September;142(3):523-8.

(52) van der Kooi TI, de Boer AS, Mannien J, Wille JC, Beaumont MT, Mooi BW et al. Incidence and risk factors of device-associated infections and associated mortality at the intensive care in the Dutch surveillance system. Intensive Care Med 2007 February;33(2):271-8.

(53) Violan JS, Sanchez-Ramirez C, Mujica AP, Cendrero JC, Fernandez JA, de Castro FR. Impact of nosocomial pneumonia on the outcome of mechanically-ventilated patients. Crit Care (Lond) 1998;2(1):19-23.

(54) Warren DK, Shukla SJ, Olsen MA, Kollef MH, Hollenbeak CS, Cox MJ et al. Outcome and attributable cost of ventilator-associated pneumonia among intensive care unit patients in a suburban medical center. Crit Care Med 2003 May;31(5):1312-7.

(55) Woske HJ, Roding T, Schulz I, Lode H. Ventilator-associated pneumonia in a surgical intensive care unit: epidemiology, etiology and comparison of three bronchoscopic methods for microbiological specimen sampling. Crit Care 2001;5(3):167-73.

(56) Bronchard R, Albaladejo P, Brezac G, Geffroy A, Seince PF, Morris W et al. Early onset pneumonia: risk factors and consequences in head trauma patients. Anesthesiology 2004 February;100(2):234-9.

(57) Chan EY, Ruest A, Meade MO, Cook DJ. Oral decontamination for prevention of pneumonia in mechanically ventilated adults: systematic review and meta-analysis. BMJ 2007 April 28;334(7599):889.

(58) Griffiths J, Barber VS, Morgan L, Young JD. Systematic review and meta-analysis of studies of the timing of tracheostomy in adult patients undergoing artificial ventilation. BMJ 2005 May 28;330(7502):1243.

(59) Turgeon AF, Hutton B, Fergusson DA, McIntyre L, Tinmouth AA, Cameron DW et al. Meta-analysis: intravenous immunoglobulin in critically ill adult patients with sepsis. Ann Intern Med 2007 February 6;146(3):193-203.

(60) Safdar N, Dezfulian C, Collard HR, Saint S. Clinical and economic consequences of ventilator-associated pneumonia: a systematic review. Crit Care Med 2005 October;33(10):2184-93.

(61) Pneumatikos I, Koulouras V, Nathanail C, Goe D, Nakos G. Selective decontamination of subglottic area in mechanically ventilated patients with multiple trauma. Intensive Care Med 2002 April;28(4):432-7.

(62) Quinio B, Albanese J, Bues-Charbit M, Viviand X, Martin C. Selective decontamination of the digestive tract in multiple trauma patients. A prospective double-blind, randomized, placebo-controlled study. Chest 1996 March;109(3):765-72.

(63) Stoutenbeek CP, van Saene HK, Little RA, Whitehead A. The effect of selective decontamination of the digestive tract on mortality in multiple trauma patients: a multicenter randomized controlled trial. Intensive Care Med 2007 February;33(2):261-70.

(64) Krueger WA, Lenhart FP, Neeser G, Ruckdeschel G, Schreckhase H, Eissner HJ et al. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. Am J Respir Crit Care Med 2002 October 15;166(8):1029-37.

(65) Rello J, Rue M, Jubert P, Muses G, Sonora R, Valles J et al. Survival in patients with nosocomial pneumonia: impact of the severity of illness and the etiologic agent. Crit Care Med 1997 November;25(11):1862-7.

CHAPTER 3

Estimating the attributable mortality of ventilator-associated pneumonia from randomized prevention studies

W.G.Melsen; M.M. Rovers;M. Koeman and M.J.M. Bonten

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SUMMARY

Objective

To assess the attributable mortality of Ventilator-Associated Pneumonia (VAP) using results from randomized controlled trials on VAP prevention.

Data Sources

A systematic search was performed in PubMed, Embase, Web of Science and Cochrane Library from their inception until July 2010. In addition a reference and related article search was performed. Randomized VAP prevention studies in which all patients were mechanically ventilated, and from which VAP and mortality rates of intervention and control group could be extracted, were included.

Results

Fifty-three papers were identified, describing 58 comparisons. Statistical significant reductions in VAP incidences were reported in 20 of the 58 comparisons, whereas none of these trials reported a significant reduction of mortality. Pooled estimates of the relative risk reductions (RRR) of both VAP and mortality were calculated and the attributable mortality was estimated as the ratio between the RRRs of mortality and VAP. Effects of study quality, diagnostic methods used and effectiveness of preventing VAP on the mortality rate of VAP were assessed in subgroup analyses. The overall attributable mortality of VAP was estimated as 9%. In subgroup-analyses the attributable mortality varied between 3% and 17%.

Conclusion

Based on the results of 58 randomized studies on VAP prevention, the attributable mortality rate of VAP was estimated to be 9%, and ranged between 3% and 17% in subgroup analyses. Together with the results of other recent studies there is cumulative evidence that the attributable mortality due to VAP is around 10%

INTRODUCTION

Ventilator-associated Pneumonia (VAP) is a frequently occurring nosocomial infection complicating medical treatment of patients admitted to the intensive care unit (ICU). Although it is widely believed that VAP increases mortality, accurate determination of this so-called attributable mortality is difficult, but critically important for estimating the potential benefits of VAP prevention.

Different approaches have been used to quantify this attributable mortality of VAP, such as a systematic review of observational studies (1) and cohort analyses using sophisticated statistical methods (2) (3). All these methods suffer from their observational nature, i.e. uncontrolled confounding cannot be precluded. Ideally, patients should be randomized to "receive" VAP or not, which of course is highly unethical. The opposite reasoning is that all patients run a certain risk of developing VAP and that this is– at random – prevented. Based on this we aimed to determine attributable mortality of VAP using the results from randomized trials on VAP prevention. If the attributable mortality due to VAP would be 100%, a 50% relative risk reduction (RRR) of VAP incidence due to a randomly applied intervention should lead to a 50% RRR of ICU mortality. The ratio of the RRR of mortality and the RRR of VAP will, therefore, provide an estimate of the attributable mortality.

There are many randomized trials on different preventive measures for VAP and these were retrieved in a systematic approach in order to quantify attributable mortality. Of note, we do not intend to identify the most effective prevention measure, as this has been done by others (4-6), but to determine the attributable mortality of VAP (=RRR mortality/RRR VAP).

METHODS

Study selection

We performed a comprehensive search strategy through PubMed, Embase, the Cochrane Library and Web of Science from their inception until July 2010 to identify all eligible studies, using the following keywords and synonyms "ventilator-associated pneumonia" and "randomisation". We only selected randomized VAP prevention studies in which all patients were 1) mechanically ventilated and 2) VAP and mortality rates of prevention and control group could be extracted. Studies only evaluating specific patient populations (i.e. cardiac surgery, liver transplant or failure, esophageal resection, comateus patients, paediatric patients and tracheotomised patients) or studies evaluating the following interventions were excluded; tracheostomy (as these are usually only investigated in patients with an expected stay in the ICU of several days) and circuit changes of mechanical ventilation (as these studies were mostly interested in applying the intervention without increasing the incidence VAP, rather than preventing VAP). Studies diagnosing VAP using "inadequate" methods (i.e. methods in which either chest X-rays were not part of the VAP diagnosis or in which the diagnosis was based on chest X-ray interpretation with only two clinical signs) or studies that did not describe their methods adequately were excluded.

To identify additional relevant studies, a related article and reference list search as well as screening of relevant meta-analyses was performed. Studies that were only published as abstracts, conference summaries or written in a non-english language were excluded because thorough quality assessment is not possible for these studies.

Assessment of methodological quality

The methodological quality of each study was determined by a scoring system that was adapted from earlier systems used by Cook et al(7) and Van Nieuwenhoven et al(8) (table 1). Zero, 1 or 2 points were given for each of the 6 criteria: patient selection, patient characteristics, allocation sequence, concealment of allocation, blinding and for the criteria of diagnosing VAP, summing to a maximum of 12 points. Studies were rated as high quality when the total score was equal or higher than 9.

Table 1: Criteria for the Assessment of Methodological Quality

Criteria	Score
Population	
Patient Selection	
Consecutive eligible consenting patients at random series (<10% dropout)	2
Attempt made to enroll as such, with failure due to reasons outlined explicitly	1
Selected patients (not consecutive or random) or not described	0
Patient characteristics	
Age (mean differs <10%)	
Sex (proportion of men in each group differs by <10%)	
Acute Physiology and Chronic Health Evaluation, Simplified Acute Physiology Score or Injury Severity Score (mean differs by <10%)	
Diagnosis (proportion of the following differs by <10%)	2 Groups comparable on ≥ 6 characteristics
Chronic obstructive airway disease	
Respiratory failure	
Pneumonia at admission	
Other (icu-acquired) infections	1 Groups comparable on 3 to 5 characteristics
Tracheostomy	
Sepsis	
Renal failure	
Central nervous system/neurologic disease	0 Groups comparable on ≤ 2 characteristics
Hepatic failure	
Trauma	
Surgery	
Diabetes Mellitus	
Malignancy	
ARDS	

Table 1 (Continued)

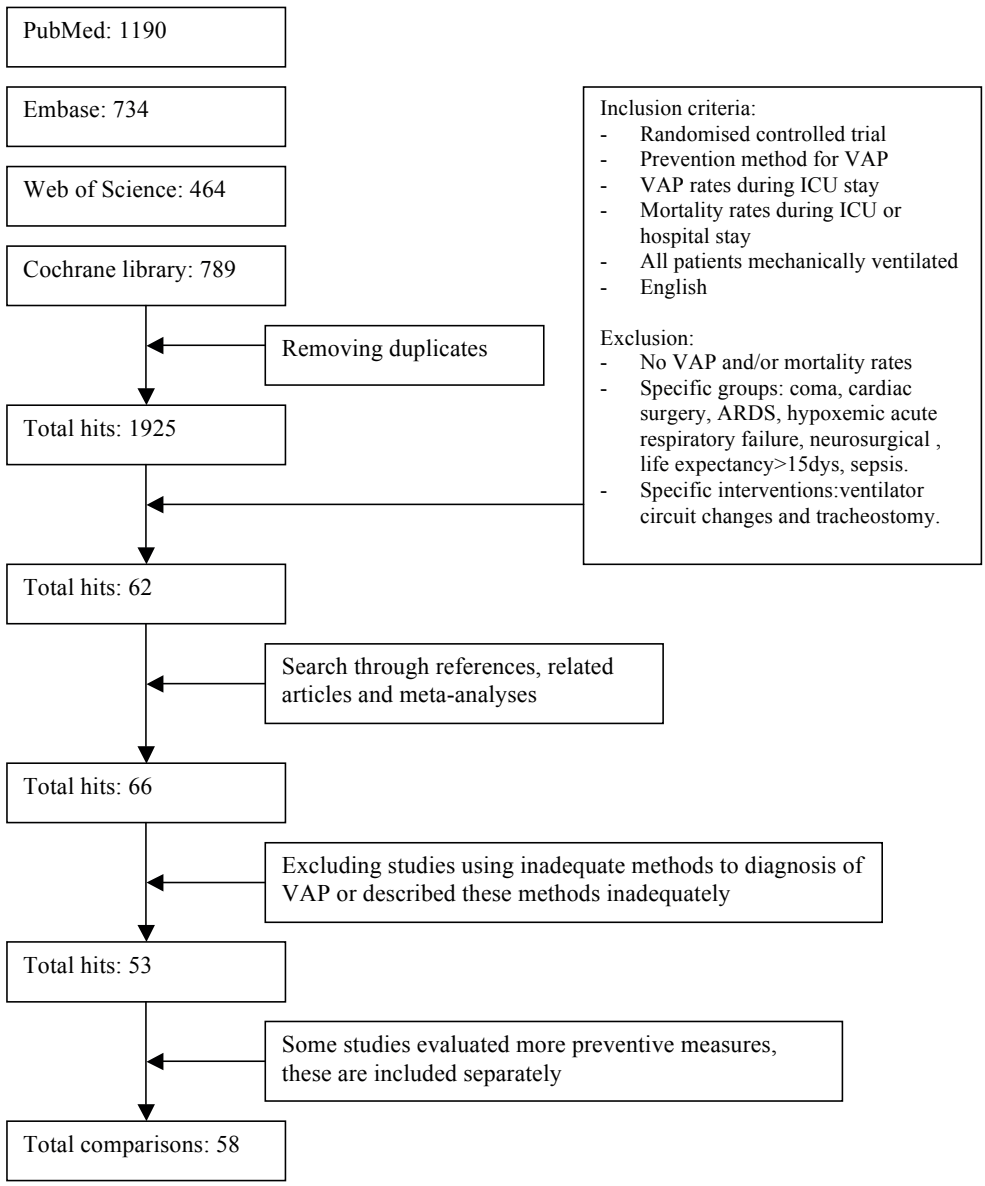
Criteria	Score
Intervention	
Allocation sequence	
Computerized generated allocation, random number table	2
No more information	1
Quasi-randomization (hospital identification, date)	0
Concealment of allocation	
Nonmanipulable (call to data coordinating center, masking drug packages)	2
Potentially manipulable (sealed envelope, computer-generated random number table) or randomisation without further information.	1
Open label	0
Blinding	
Blinding of radiologist to treatment group and blinding physicians to clinical endpoint.	2
Blinding of radiologist to treatment group or blinding physicians to clinical endpoint.	1
Potentially unblinded, unblinded , or cannot tell.	0
Definition of ventilator-associated pneumonia	
Probable pneumonia: Roentgenographic criterion and at least 2 other criteria (ie. Fever, leukocytosis, purulent sputum, isolation of pathogenic bacteria from sputum or blood, or decreased alveolar-arterial oxygenation difference), and significant growth from samples obtained from lungs by bronchoscopic techniques (PSB, BAL, blinded or not blinded) or by quantitative cultures of endotracheal aspirates.	2
Possible pneumonia: Roentographic criterion and at least 3 other criteria (ie Fever, leukocytosis, purulent sputum, isolation of pathogenic bacteria from sputum or blood, or decreased alveolar-arterial oxygenation difference) or CPIS score >6.	1

Statistical analyses

The analyses were performed using Review manager (version 5. Cochrane Collaboration, Oxford). We used random effect models to calculate pooled relative risks. We estimated the relative risk reductions (RRR) (defined as 1-RR) of VAP and mortality and their corresponding 95% confidence intervals by pooling all studies, studies with a statistically significant reduction of VAP, studies with comparable RRR of VAP (0-0.33; 0.34-0.66; 0.67-1), studies with methodological quality scores equal or above 9, studies with comparable reliability of methods used for diagnosing VAP, studies with a high and low incidence of VAP, and studies with those interventions that are recommended in published guidelines (i.e., aspiration of subglottic secretions, kinetic bed therapy, semirecumbent positioning, oropharyngeal decontamination with antiseptics(4-6)). The attributable mortality was defined as the ratio between the RRR of

mortality and VAP. Heterogeneity was assessed by calculating the I^2 statistics, and was low if below 25%, moderate when between 25%-75%, and high when above 75%(9). To determine 95% confidence intervals for estimates of attributable mortality we used bootstrap analyses (n=100.000) using the effect measures from the original studies.

Figure 1: Flow chart of search



RESULTS

Fifty-three eligible trials were identified(10-62)(figure I). As four trials evaluated different intervention groups (16;29;50;61) there were 58 group comparisons. In total, 6304 patients received preventive measures for VAP and 6526 patients did not. RRR of VAP and mortality of all comparisons grouped by intervention method are listed in Table 2. Accurate allocation was used in 42 of 58 (72%) trials, whereas two studies used quasi-randomization (hospital identification or date) (Table 1). Concealment of allocation was considered not manipulable in eleven (19%) and potentially manipulable in 45 (78%) of 58 trials. In 19 (33%) trials both the radiologists and physicians were blinded to the preventive measure given to patients. Twenty-six studies used bronchoscopic techniques or quantitative cultures of tracheal aspirates to confirm a clinical suspicion of VAP.

Relative risk reduction of VAP

Statistically significant reductions in VAP incidence were reported in 20 of 58 comparisons. The pooled RRR for VAP of all studies was 0.33 (95% CI 0.23 to 0.41), fairly similar to the pooled estimate of interventions included in guideline recommendations (RRR VAP of 0.29 (95% CI 0.04 to 0.48)) and the 21 studies with high methodological quality (RRR VAP of 0.37 (95% CI 0.24 to 0.47)), with moderate levels of heterogeneity for all three analyses (Table 3). Naturally, the pooled RRR was higher (0.57; 95% CI 0.51 to 0.63) for those studies reporting statistically significant reduction of VAP due to intervention, with a low level of heterogeneity (I^2=0%). Based upon the individual preventive effects on VAP we created three groups of studies: "highly effective VAP prevention" which resulted in a pooled RRR of 0.74 (95% CI 0.64-0.81), "effective prevention" with a pooled RRR of 0.50 (95% CI 0.43-0.57) and "moderately effective" prevention with a pooled RRR of 0.20 (95% CI 0.08-0.30), each with absence of heterogeneity (I^2=0%).

Table 2: Overview of included studies

		Prevention			Control				
Author	Year	Total patients	Total VAP	Total Mort	Total patients	Total VAP	Total Mort	RRR VAP	RRR mortality
Selective digestive decontamination (SDD)									
Aerdts	1991	17	0	2	39	10	6	0.89(-0.71 to 0.99)	0.24(-2.41 to 0.83)
Ferrer	1994	39	7	12	41	10	11	0.26(-0.74 to 0.69)	-0.15(-1.29 to 0.43)
Gastinne	1992	220	26	75	225	33	67	0.19(-0.30 to 0.50)	-0.14 (-0.50 to 0.13)
Hammond	1992	114	8	14	125	8	15	-0.10(-1.83 to 0.57)	-0.02(-1.03 to 0.46)
Nardi	2001	119	9	25	104	20	26	0.61 (0.17 to 0.81)	0.16(-0.36 to 0.48)
PalomarA	1997	41	7	10	42	21	13	0.66(0.28 to 0.84)	0.21(-0.59 to 0.61)
PalomarB	1997	46	14	10	42	21	13	0.39(-0.04 to 0.64)	0.30(-0.43 to 0.65)
Quinio	1996	76	19	13	72	37	10	0.51 (0.24 to 0.69)	-0.23(-1.63 to 0.42)
SanchezGarcia	1998	131	15	51	140	41	66	0.61 (0.33 to 0.77)	0.17(-0.09 to 0.37)
Stoutenbeek	2007	201	19	42	200	46	44	0.59 (0.32 to 0.75)	0.05(-0.38 to 0.35)
VerwaestA	1997	193	22	34	185	40	31	0.47 (0.15 to 0.67)	-0.05(-0.64 to 0.32)
VerwaestB	1997	200	31	31	185	40	31	0.28(-0.10 to 0.53)	0.07(-0.46 to 0.41)
Wiener	1995	30	8	11	31	8	15	-0.03(-1.40 to 0.55)	0.24(-0.37 to 0.58)
Stress ulcer prophylaxis									
Bonten	1995	67	15	26	74	16	24	-0.04 (-0.93 to 0.44)	-0.20(-0.87 to 0.23)
Cook	1998	604	98	138	596	114	140	0.15 (-0.08 to 0.34)	0.03(-0.20 to 0.21)
Driks	1987	61	7	18	69	16	32	0.51 (-0.12 to 0.78)	0.36(-0.01 to 0.6)
Eddleston	1991	30	3	8	30	10	7	0.70 (0.02 to 0.91)	-0.14(-1.75 to 0.53)
Fabian	1993	99	29	16	179	52	34	-0.01(-0.48 to 0.31)	0.15 (-0.46 to 0.50)
HanischA	1998	57	10	7	57	12	12	0.17 (-0.77 to 0.61)	0.42(-0.37 to 0.75)
HanischB	1998	44	10	12	57	12	12	-0.08(-1.27 to 0.49)	-0.30(-1.60 to 0.35)
Laggner	1989	16	0	8	16	2	8	0.80(-2.86 to 0.99)	0.00(-1.00 to 0.50)
Maier	1994	47	10	6	51	14	11	0.22(-0.57 to 0.62)	0.41(-0.47 to 0.76)
O'Keefe	1998	47	10	6	49	14	11	0.26(-0.51 to 0.63)	0.43(-0.41 to 0.77)
Pickworth	1993	39	6	2	44	5	4	-0.35(-3.09 to 0.55)	0.44(-1.91 to 0.89)
Ryan	1993	58	8	22	56	7	19	-0.10(-1.84 to 0.57)	-0.12(-0.83 to 0.32)
Thomason	1996	80	30	10	162	57	27	-0.07(-0.52 to 0.25)	0.25(-0.47 to 0.62)
Selective oral decontamination with antibiotics (SOD)									
Abele Horn	1992	58	13	11	30	23	5	0.71 (0.51 to 0.83)	-0.14(-1.97 to 0.56)
Bergmans	2001	87	9	25	139	38	53	0.62 (0.26 to 0.81)	0.25(-0.12 to 0.49)
CamusB	2005	130	8	39	126	20	41	0.61 (0.15 to 0.82)	0.08(-0.33 to 0.36)
CamusC	2005	129	4	28	126	20	41	0.80 (0.44 to 0.93)	0.33(-0.01 to 0.56)
Pneumatikos	2002	31	5	5	30	16	7	0.70 (0.28 to 0.87)	0.31(-0.94 to 0.75)

Selective oral decontamination with antiseptics									
CamusA	2005	130	14	36	126	20	41	0.32(-0.28 to 0.64)	0.15(-0.24 to 0.41)
Fourrier	2000	30	5	3	30	18	7	0.72 (0.35 to 0.88)	0.57(-0.50 to 0.88)
Seguin	2006	36	3	6	62	25	16	0.79 (0.36 to 0.93)	0.35(-0.50 to 0.72)
Ventilator circuit management									
Boots	1997	42	6	6	41	7	4	0.16(-1.28 to 0.69)	-0.46(-3.81 to 0.55)
Boots	2006	190	32	29	191	27	34	-0.19(-0.91 to 0.26)	0.14(-0.35 to 0.45)
Lacherade	2005	185	47	60	184	53	63	0.12(-0.23 to 0.37)	0.05(-0.26 to 0.29)
Lorente	2006	53	21	13	51	8	12	-1.53(-4.18 to -0.23)	-0.04(-1.07 to 0.47)
Memish	2001	123	14	40	120	19	30	0.28 (-0.37 to 0.62)	-0.30(-0.94 to 0.13)
Closed suction									
Combes	2000	50	4	13	54	9	15	0.52(-0.46 to 0.84)	0.06(-0.77 to 0.5)
Deppe	1990	46	12	12	38	11	11	0.10(-0.81 to 0.55)	0.10(-0.81 to 0.55)
Lorente	2005	210	42	52	233	41	50	-0.14(-0.68 to 0.23)	-0.15(-0.62 to 0.18)
Lorente	2006	236	32	31	221	30	30	0.00(-0.59 to 0.37)	0.03(-0.54 to 0.39)
Gastric vs small intestinal feeding									
Kortbeek	1999	37	10	4	43	18	3	0.35(-0.22 to 0.66)	-0.55(-5.48 to 0.63)
Subglottic secretion suctioning									
Lacherade	2010	169	25	71	164	42	65	0.42(0.10 to 0.63)	-0.06(-0.37 to 0.18)
Valles	1995	76	14	30	77	25	28	0.43(-0.01 to 0.68)	-0.09(-0.63 to 0.28)
Probiotics									
Klarin	2008	23	1	5	21	3	4	0.70(-1.70 to 0.97)	-0.14(-2.69 to 0.65)
Knight	2009	130	12	28	129	17	35	0.30(-0.41 to 0.65)	0.21(-0.22 to 0.49)
Morrow	2010	68	17	12	70	33	15	0.47(0.14 to 0.67)	0.18(-0.63 to 0.58)
Endotracheal tube									
Kollef	2008	766	37	233	743	56	198	0.36(0.04 to 0.57)	-0.14(-0.34 to 0.03)
Lorente	2007	140	11	26	140	31	32	0.65(0.32 to 0.81)	0.19(-0.29 to 0.49)
Body position: semirecumbent									
Drakulovic	1999	39	2	7	47	11	13	0.78(0.07 to 0.95)	0.35(-0.47 to 0.71)
Nieuwenhoven	2006	112	16	33	109	20	33	0.22(-0.42 to 0.57)	0.03(-0.46 to 0.35)
Automatic control tracheal tube cuff pressure									
Valencia	2007	73	11	20	69	10	16	-0.04(-1.29 to 0.53)	-0.18(-1.09 to 0.33)
Bacterial filters									
Lorente	2003	114	29	37	116	28	28	-0.05(-0.65 to 0.33)	-0.34(-1.04 to 0.11)
Chest physiotherapy									
Ntoumenopoulos	2002	24	2	6	36	14	3	0.79(0.14 to 0.95)	-2.00(-9.86 to 0.17)
Small bore nasogastric tubes									
Ibanez	2000	16	4	4	14	3	4	-0.17(-3.34 to 0.69)	0.12(-1.86 to 0.73)
Enteral feeding: early vs late									
Ibrahim	2002	75	37	15	75	23	20	-0.61(-1.42 to -0.07)	0.25(-0.35 to 0.58)

VAP=ventilator associated pneumonia; Mort= mortality; RRR= relative risk reduction

Table 3: Overview of the pooled relative risk reductions for both VAP and mortality and the estimates of attributable mortality

	Total pts	RRR vap (95%CI)	I^2	RRR mortality (95%CI)	I^2	AM	95% CI*
All studies (n=58)	12830	0.33 (0.23 to 0.41)	59%	0.03 (-0.03 to 0.08)	0%	0.09	-0.09 to 0.28
Significant VAP studies (n=20)	5014	0.57 (0.51 to 0.63)	0%	0.05 (-0.05 to 0.13)	3%	0.09	-0.09 to 0.28
Guidelines (n=11)	2130	0.29 (0.04 to 0.48)	52%	0.03 (-0.12 to 0.15)	0%	0.10	-0.33 to 0.41
High Quality (n= 21)	6528	0.37 (0.24 to 0.47)	53%	0.02 (-0.07 to 0.10)	7%	0.05	-0.22 to 0.26
Studies with RR VAP 0-0.33 (n=11)	900	0.74 (0.64 to 0.81)	0%	0.19 (-0.03 to 0.37)	0%	0.26	-0.11 to 0.41
Studies with RR VAP 0.33-0.66 (n=17)	4801	0.50 (0.43 to 0.57)	0%	0.03 (-0.06 to 0.11)	0%	0.06	-0.13 to 0.29
Studies with RR VAP 0.66-1 (n=14)	3933	0.20 (0.08 to 0.30)	0%	0.03 (-0.08 to 0.13)	0%	0.15	-0.25 to 0.55
Possible pneumonia (n=32)	5461	0.30 (0.16 to 0.41)	55%	0.05 (-0.04 to 0.14)	0%	0.17	-0.11 to 0.57
Probable pneumonia (n=26)	7369	0.36 (0.23 to 0.48)	64%	0.01 (-0.06 to 0.09)	0%	0.03	-0.19 to 0.26
Incidence of VAP <25% (n=34)#	9449	0.25 (0.12 to 0.35)	46%	-0.01(-0.18 to 0.06)	0%	0	-0.40 to 0.28
Incidence of VAP >25% (n=24)#	3381	0.42 (0.28 to 0.54)	69%	0.11(0.01 to 0.20)	0%	0.26	0.08 to 0.52

n=number of comparisons included, AM: attributable mortality, 95%CI: 95% confidence interval
*95% confidence interval attributable mortality estimated with bootstrap analyses
#incidence VAP in the control group of these studies
See Supplemental table 1 for an overview of studies included in each subgroup analysis.

Relative risk reduction of mortality

A statistically significant RRR of both VAP and mortality was not reported in any of the 58 comparisons. The pooled RRR for mortality of all studies was 0.03 (95% CI -0.03 to 0.08), which was comparable to the pooled RRR from studies on interventions recommended in guidelines (0.03; 95% CI -0.12 to 0.15), and studies with high methodological quality (0.02; 95% CI -0.07 to 0.10), all with low levels of heterogeneity (Table 3). Statistically significant RRRs of mortality were also not obtained from the pooled analyses of studies reporting statistically significant reductions of VAP (RRR of 0.05; 95% CI -0.05 to 0.13), or when studies were grouped upon the individual preventive effects on VAP; pooled RRRs were 0.19 (95% CI -0.03 to 0.37), 0.03 (95% CI -0.06 to 0.11) and 0.03 (95% CI -0.08 to 0.13) for the studies being "highly effective", "effective" and "moderately effective" in preventing VAP. A statistically significant reduction of mortality was found in studies with a VAP incidence of more than 25% (RRR 0.11 (95% CI 0.01 to 0.20). Finally, pooled RRR for mortality were estimated to be 0.01 and 0.05, with overlapping confidence intervals, for studies grouped on the reliability of methods used for diagnosing VAP. No heterogeneity was found in any of the analyses of the relative risk reductions of mortality

Attributable mortality

The attributable mortality of VAP was estimated as 0.09 (RRRmortality/RRRVAP = 0.03/0.33, Table 3), which was similar to the estimates based on the results of studies with significant reductions of VAP or on guideline recommended interventions (being 0.09 and 0.10, respectively). In the other subgroup analyses attributable mortality rates varied between 3% and 17%, with two apparent outliers of 26% for studies being "highly effective" in preventing VAP and studies with a high incidence of VAP.

DISCUSSION

Based upon the data of 58 randomized comparisons of preventive measures for VAP the attributable mortality of VAP was estimated to be 9%. Subgroup analyses, of studies grouped on methodological quality of study design, reliability of diagnostic criteria for VAP and preventive effects for VAP yielded comparable estimates.

Levels of heterogeneity were high in almost all analyses estimating the RRR of VAP and absent in the analyses of the RRR of mortality. It is important to note that the level of heterogeneity represented by the I-square reflects differences at the outcome level (statistical heterogeneity) and not specifically in-between study differences, like differences in patient population, prevention regimes, patient characteristics etc. However for our purpose, i.e. to assess the attributable mortality of VAP using results from randomized controlled prevention trials, these mentioned differences are not relevant. The estimates of the RRRs should be seen as a summary estimate in order to estimate the attributable mortality instead of a reliable efficacy measure for the intervention. The high level of heterogeneity in the estimates of the RRR of

VAP reflects the differences in effectiveness of the various intervention measures evaluated. To limit heterogeneity, studies with comparable preventive effects on VAP incidence were pooled. Based on the results from studies reporting the highest efficacy in preventing VAP (RRR of VAP between 0.67 and 1) the highest attributable mortality of VAP (26%) was found. However, the 95% confidence interval is very broad, limiting the precision of this estimate. Furthermore, the analysis of studies with reported incidences of VAP >25% yielded an attributable mortality of 26%. However, we do think that this results from the fact that the incidence of VAP is, among other things, dependent on patient population and quality of care. Yet, both parameters (patient population and quality of care) also determine mortality due to VAP. In such an event, when both a higher incidence of VAP as well as higher mortality due to VAP occur simultaneously, the resulting estimate of attributable mortality will be biased with the methods used in this manuscript. As, with our methods, we assume a linear relationship between the reduction of VAP and mortality

As far as we are aware, this is the first attempt to estimate the attributable mortality of VAP using data from randomized trials on VAP prevention. The findings of our study further improve our understanding of the influence of the relationship between VAP and mortality. In a previous meta-analysis of observational studies (1) only estimates of attributable mortality were rather heterogeneous, with estimates of attributable mortality rates ranging from 14% to 70%. These analyses were limited by small study populations and lack of adjustments for possible confounders. Recent studies large scale observational studies (2;3), using more sophisticated analyses (i.e. controlling for competing risks and time-dependent nature of VAP) and including more patients, yielded attributable mortality rates of 8.1% (95% CI 3.1% to 13.1%)(2), 10.4% (95%CI 5.6% to 24.5%)(2) and 10.6% (3), which is much lower than could be expected from previous studies (including our meta-analysis of observational studies). However, all studies performed so far had methodological limitations. Our new and original approach, in which we were able to include large numbers of patients and avoid confounding (as the preventive intervention was randomly allocated in all studies), also resulted in an estimated attributable mortality of 9%. Yet, our approach does not allow determination of attributable mortality in subgroups of patients as we only had access to the published data. Moreover, adequacy of VAP treatment could have been a confounder in our analyses if there would have been differences in adequacy of treatment between patients randomized to intervention and control arms. Only an analysis with individual patient data can provide more accurate estimates of the attributable mortality of VAP in subgroup of patients.

The lower estimates of attributable mortality are of critical importance for interpreting the findings of so-called "negative" intervention studies, as many were hugely underpowered to demonstrate improvements in patient outcome. Furthermore, considering the difficulties in diagnosing VAP, it has been stated that VAP prevention studies should focus on demonstrating effects on solid outcomes rather than on the incidence of VAP(63;64). The new knowledge on attributable mortality due to VAP underpins the need of large studies (>1,000 patients per study group) to demonstrate whether VAP prevention improves patient outcomes.

SUPPLEMENT

Table 1: Overview studies per subgroup analysis.

Subgroup analyses	
All studies (n=58)	Abele Horn 1992, Aerdts 1991, Bergmans 2001, Bonten 1995, Boots 1997, Boots 2006, Camus A+B+C 2005, Combes 2000, Cook 1998, Deppe 1990, Drakulovic 1999, Driks 1987, Eddleston 1991, Fabian 1993, Ferrer 1994, Fourrier 2000, Gastinne 1992, Hammond 1992, Hanisch A+B 1998, Ibanez 2000, Ibrahim 2002, Klarin 2008, Knight 2009, Kollef 2008, Kortbeek 1999, Lacherade 2005, Lacherade 2010, Laggner 1989, Lorente 2003, Lorente 2005, Lorente 2006, Lorente 2006, Lorente 2007, Maier 1994, Memish 2001, Morrow 2010, Nardi 2001, Ntoumenopoulos 2002, O'Keefe 1998, Palomar A+B 1997, Pickworth 1993, Pneumatikos 2002, Quinio 1996, Ryan 1993, Sanchez Garcia 1998, Seguin 2006, Stoutenbeek 2007, Thomason 1996, Valencia 2007, Valles 1995, Van Nieuwenhoven 2006, Verwaest A+B 1997, Wiener 1995.
Significant VAP studies (n=20)	Abele Horn 1992, Bergmans 2001, Camus B+C 2005, Drakulovic 1999, Eddleston 1991, Fourrier 2000, Kollef 2008, Lacherade 2010, Lorente 2007, Morrow 2010, Nardi 2001, Ntoumenopoulos 2002, Palomar A 1997, Pneumatikos 2002, Quinio 1996, Sanchez Garcia 1998, Seguin 2006, Stoutenbeek 2007, Verwaest A 1997.
Guidelines (n=11)	Camus A 2005, Combes 2000, Deppe 1990, Drakulovic 1999, Fourrier 2000, Lacherade 2010, Lorente 2005, Lorente 2006, Seguin 2006, Valles 1995, Van Nieuwenhoven 2006.
High Quality (n= 21)	Bergmans 2001, Bonten 1995, Camus A+B+C 2005, Cook 1998, Drakulovic 1999, Ferrer 1994, Fourrier 2000, Gastinne 1992, Hanisch A+B, 1998, Kollef 2008, Lacherade 2005, Lorente 2003, Lorente 2007, Morrow 2010, Quinio 1996, Sanchez Garcia 1998, Valencia 2007, Van Nieuwenhoven 2006.
Studies with RR VAP 0-0.33 (n=11)	Abele Horn 1992, Aerdts 1991, Camus C, 2005, Drakulovic 1999, Eddleston 1991, Fourrier 2000, Klarin 2008, Laggner 1989, Ntoumenopoulos 2002, Pneumatikos 2002, Seguin 2006.
Studies with RR VAP 0.33-0.66 (n=17)	Bergmans 2001, Camus B 2005, Combes 2000, Driks 1987, Kollef 2008, Kortbeek 1999, Lacherade 2010, Lorente 2007, Morrow 2010, Nardi 2001, Palomar A+B, 1997, Quinio 1996, Sanchez Garcia 1998, Stoutenbeek 2007, Valles 1995, Verwaest A 1997.

Table 1 (Continued)

Subgroup analyses	
Studies with RR VAP 0.66-1 (n=14)	Boots 1997, Camus A 2005, Cook 1998, Deppe 1990, Ferrer 1994, Gastinne 1992, Hanisch A 1998, Knight 2009, Lacherade 2005, Maier 1994, Memish 2001, O'Keefe 1998, Van Nieuwenhoven 2006, Verwaest B 1997.
Possible pneumonia (n=32)	Abele Horn 1992, Aerdts 1991, Boots 1997, Boots 2006, Combes 2000, Deppe 1990, Driks 1987, Eddleston 1991, Fabian 1993, Gastinne 1992, Hammond 1992, Hanisch A+B 1998, Ibrahim 2002, Klarin 2008, Knight 2009, Kortbeek 1999, Laggner 1989, Maier 1994, Memish 2001, Ntoumenopoulos 2002, O'Keefe 1998, Pickworth 1993, Quinio 1996, Ryan 1993, Sanchez Garcia 1998, Stoutenbeek 2007, Thomason 1996, Valles 1995, Verwaest A+B 1997, Wiener 1995.
Probable pneumonia (n=26)	Bergmans 2001, Bonten 1995, Camus A+B+C 2005, Cook 1998, Drakulovic 1999, Ferrer 1994, Fourrier 2000, Ibanez 2000, Kollef 2008, Lacherade 2005, Lacherade 2010, Lorente 2003, Lorente 2005, Lorente 2006, Lorente 2006, Lorente 2007, Morrow 2010, Nardi 2001, Palomar A+B, 1997, Pneumatikos 2002, Seguin 2007, Valencia 2007, Van Nieuwenhoven 2006.
Incidence of VAP <25% (n=34)[#]	Bonten 1995, Boots 1997, Boots 2006, Camus A+B+C 2005, Combes 2000, Cook 1998, Drakulovic 1999, Driks 1987, Ferrer 1994, Gastinne 1992, Hammond 1992, Hanisch A+B 1998, Ibanez 2000, Klarin 2008, Knight 2009, Kollef 2008, Laggner 1989, Lorente 2003, Lorente 2005, Lorente 2006, Lorente 2006, Lorente 2007, Memish 2001, Nardi 2001, Pickworth 1993, Ryan 1993, Stoutenbeek 2007, Valencia 2007, Van Nieuwenhoven 2006, Verwaest A+B 1997.
Incidence of VAP >25% (n=24)[#]	Abele Horn 1992, Aerdts 1991, Bergmans 2001, Deppe 1990, Eddleston 1991, Fabian 1993, Fourrier 2000, Ibrahim 2002, Kortbeek 1999, Lacherade 2005, Lacherade 2010, Maier 1994, Morrow 2010, Ntoumenopoulos 2002, O'Keefe 1998, Palomar A+B 1997, Pneumatikos 2002, Quinio 1996, Sanchez Garcia 1998, Seguin 2006, Thomason 1996, Valles 1995, Wiener 1995.

REFERENCES

(1) Melsen WG, Rovers MM, Bonten MJ. Ventilator-associated pneumonia and mortality: a systematic review of observational studies. Crit Care Med 2009 October;37(10):2709-18.

(2) Nguile-Makao M, Zahar JR, Francais A, Tabah A, Garrouste-Org, Allaouchiche B et al. Attributable mortality of ventilator-associated pneumonia: respective impact of main characteristics at ICU admission and VAP onset using conditional logistic regression and multi-state models. Intensive Care Med 2010 May;36(5):781-9.

(3) Schumacher M, Wangler M, Wolkewitz M, Beyersmann J. Attributable mortality due to nosocomial infections. A simple and useful application of multistate models. Methods Inf Med 2007;46(5):595-600.

(4) Bouza E, Burillo A. Advances in the prevention and management of ventilator-associated pneumonia. Curr Opin Infect Dis 2009 August;22(4):345-51.

(5) Muscedere J, Dodek P, Keenan S, Fowler R, Cook D, Heyland D. Comprehensive evidence-based clinical practice guidelines for ventilator-associated pneumonia: prevention. J Crit Care 2008 March;23(1):126-37.

(6) Valencia M, Torres A. Ventilator-associated pneumonia. Curr Opin Crit Care 2009 February;15(1):30-5.

(7) Cook DJ, Laine LA, Guyatt GH, Raffin TA. Nosocomial pneumonia and the role of gastric pH. A meta-analysis. Chest 1991 July;100(1):7-13.

(8) Van Nieuwenhoven CA, Buskens E, Van Tiel FH, Bonten MJ. Relationship between methodological trial quality and the effects of selective digestive decontamination on pneumonia and mortality in critically ill patients. JAMA 2001 July 18;286(3):335-40.

(9) Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003 September 6;327(7414):557-60.

(10) Abele Horn M, Dauber A, Bauernfeind A, Russwurm W, Seyfarth M, I, Gleich P et al. Decrease in nosocomial pneumonia in ventilated patients by selective oropharyngeal decontamination (SOD). Intensive Care Med 1997;23:187-95.

(11) Aerdts SJA, Vandalen R, Clasener HAL, Festen J, Vanlier HJJ, Vollaard EJH. Antibiotic-Prophylaxis of Respiratory-Tract Infection in Mechanically Ventilated Patients - A Prospective, Blinded, Randomized Trial of the Effect of A Novel Regimen. Chest 1991;100(3):783-91.

(12) Bergmans DC, Bonten MJ, Gaillard CA, Paling JC, van-der GS, van-Tiel FH et al. Prevention of ventilator-associated pneumonia by oral decontamination: a prospective, randomized, double-blind, placebo-controlled study. Am J Respir Crit Care Med 2001;164:382-8.

(13) Bonten MJ, Gaillard CA, van der GS, Van Tiel FH, Beysens AJ, Smeets HG et al. The role of intragastric acidity and stress ulcus prophylaxis on colonization and infection in mechanically ventilated ICU patients. A stratified, randomized, double-blind study of sucralfate versus antacids. Am J Respir Crit Care Med 1995 December;152(6 Pt 1):1825-34.

(14) Boots RJ, Howe S, George N, Harris FM, Faoagali J. Clinical utility of hygroscopic heat and moisture exchangers in intensive care patients. Crit Care Med 1997;25(10):1707-12.

(15) Boots RJ, George N, Faoagali JL, Druery J, Dean K, Heller RF. Double-heater-wire circuits and heat-and-moisture exchangers and the risk of ventilator-associated pneumonia. Crit Care Med 2006;34(3):687-93.

(16) Camus C, Bellissant E, Sebille V, Perrotin D, Garo B, Legras A et al. Prevention of acquired infections in intubated patients with the combination of two decontamination regimens. Crit Care Med 2005;33(2):307-14.

(17) Combes P, Fauvage B, Oleyer C. Nosocomial pneumonia in mechanically ventilated patients, a prospective randomised evaluation of the Stericath closed suctioning system. Intensive Care Med 2000 July;26(7):878-82.

(18) Cook D, Guyatt G, Marshall J, Leasa D, Fuller H, Hall R et al. A comparison of sucralfate and ranitidine for the prevention of upper gastrointestinal bleeding in patients requiring mechanical ventilation. New Engl J Med 1998;338(12):791-7.

(19) Deppe SA, Kelly JW, Thoi LL, Chudy JH, Longfield RN, Ducey JP et al. Incidence of colonization, nosocomial pneumonia, and mortality in critically ill patients using a Trach Care closed-suction system versus an open-suction system: prospective, randomized study. Crit Care Med 1990 December;18(12):1389-93.

(20) Drakulovic MB, Torres A, Bauer TT, Nicolas JM, Nogue S, Ferrer M. Supine body position as a risk factor for nosocomial pneumonia in mechanically ventilated patients: a randomised trial. Lancet 1999 November 27;354(9193):1851-8.

(21) Driks MR, Craven DE, Celli BR, Manning M, Burke RA, Garvin GM et al. Nosocomial pneumonia in intubated patients given sucralfate as compared with antacids or histamine type 2 blockers. The role of gastric colonization. N Engl J Med 1987 November 26;317(22):1376-82.

(22) Eddleston JM, Vohra A, Scott P, Tooth JA, Pearson RC, McCloy RF et al. A comparison of the frequency of stress ulceration and secondary pneumonia in sucralfate- or ranitidine-treated intensive care unit patients. Crit Care Med 1991 December;19(12):1491-6.

(23) Fabian TC, Boucher BA, Croce MA, Kuhl DA, Janning SW, Coffey BC et al. Pneumonia and stress ulceration in severely injured patients. A prospective evaluation of the effects of stress ulcer prophylaxis. Arch Surg 1993 February;128(2):185-91.

(24) Ferrer M, Torres A, Gonzalez J, Delabellacasa JP, ElEbiary M, Roca M et al. Utility of Selective Digestive Decontamination in Mechanically Ventilated Patients. Ann Intern Med 1994;120(5):389-95.

(25) Fourrier F, Cau-Pottier E, Boutigny H, Roussel-Delvallez M, Jourdain M, Chopin C. Effects of dental plaque antiseptic decontamination on bacterial colonization and nosocomial infections in critically ill patients. Intensive Care Med 2000;26(9):1239-47.

(26) Garcia MS, Galache JAC, Diaz JL, Cerda EC, Blasco JR, Aguinaga MAG et al. Effectiveness and cost of selective decontamination of the digestive tract in critically ill intubated patients - A randomized, double-blind, placebo-controlled, multicenter trial. Am J Respir Crit Care Med 1998;158(3):908-16.

(27) Gastinne H, Wolff M, Delatour F, Faurisson F, Chevret S. A controlled trial in intensive care units of selective decontamination of the digestive tract with nonabsorbable antibiotics. The French Study Group on Selective Decontamination of the Digestive Tract. N Engl J Med 1992 February 27;326(9):594-9.

(28) Hammond JM, Potgieter PD, Saunders GL, Forder AA. Double-blind study of selective decontamination of the digestive tract in intensive care. Lancet 1992 July 4;340(8810):5-9.

(29) Hanisch EW, Encke A, Naujoks F, Windolf J. A randomized, double-blind trial for stress ulcer prophylaxis shows no evidence of increased pneumonia. Am J Surg 1998 November;176(5):453-7.

(30) Ibanez J, Penafiel A, Marse P, Jorda R, Raurich JM, Mata F. Incidence of gastroesophageal reflux and aspiration in mechanically ventilated patients using small-bore nasogastric tubes. JPEN J Parenter Enteral Nutr 2000 March;24(2):103-6.

(31) Ibrahim EH, Mehringer L, Prentice D, Sherman G, Schaiff R, Fraser V et al. Early versus late enteral feeding of mechanically ventilated patients: results of a clinical trial. JPEN J Parenter Enteral Nutr 2002 May;26(3):174-81.

(32) Klarin B, Molin G, Jeppsson B, Larsson A. Use of the probiotic Lactobacillus plantarum 299 to reduce pathogenic bacteria in the oropharynx of intubated patients: a randomised controlled open pilot study. Crit Care 2008;12(6):R136.

(33) Knight DJW, Gardiner D, Banks A, Snape SE, Weston VC, Bengmark S et al. Effect of synbiotic therapy on the incidence of ventilator associated pneumonia in critically ill patients: A randomised, double-blind, placebo-controlled trial. Intensive Care Med 2009;35(5):854-61.

(34) Kollef MH, Afessa B, Anzueto A, Veremakis C, Kerr KM, Margolis BD et al. Silver-coated endotracheal tubes and incidence of ventilator-associated pneumonia: the NASCENT randomized trial. JAMA 2008 August 20;300(7):805-13.

(35) Kortbeek JB, Haigh PI, Doig C. Duodenal versus gastric feeding in ventilated blunt trauma patients: a randomized controlled trial. J TRAUMA 1999 June;46(6):992-6.

(36) Lacherade JC, Auburtin M, Cerf C, Van de LA, Soufir L, Rebufat Y et al. Impact of humidification systems on ventilator-associated pneumonia: a randomized multicenter trial. Am J Respir Crit Care Med 2005 November 15;172(10):1276-82.

(37) Lacherade JC, De JB, Guezennec P, Debbat K, Hayon J, Monsel A et al. Intermittent Subglottic Secretion Drainage and Ventilator-associated Pneumonia: A Multicenter Trial. Am J Respir Crit Care Med 2010 June 3.

(38) Laggner AN, Lenz K, Base W, Druml W, Schneeweiss B, Grimm G. Prevention of upper gastrointestinal bleeding in long-term ventilated patients. Sucralfate versus ranitidine. Am J Med 1989 June 9;86(6A):81-4.

(39) Lorente L, Lecuona M, Malaga J, Revert C, Mora ML, Sierra A. Bacterial filters in respiratory circuits: An unnecessary cost? Crit Care Med 2003;31(8):2126-30.

(40) Lorente L, Lecuona M, Martin MM, Garcia C, Mora ML, Sierra A. Ventilator-associated pneumonia using a closed versus an open tracheal suction system. Crit Care Med 2005 January;33(1):115-9.

(41) Lorente L, Lecuona M, Jimenez A, Mora ML, Sierra A. Ventilator-associated pneumonia using a heated humidifier or a heat and moisture exchanger: a randomized controlled trial. Crit Care 2006;10(4):R116.

(42) Lorente L, Lecuona M, Jimenez A, Mora ML, Sierra A. Tracheal suction by closed system without daily change versus open system. Intensive Care Med 2006 April;32(4):538-44.

(43) Lorente L, Lecuona M, Jimenez A, Mora ML, Sierra A. Influence of an endotracheal tube with polyurethane cuff and subglottic secretion drainage on pneumonia. Am J Respir Crit Care Med 2007 December 1;176(11):1079-83.

(44) Maier RV, Mitchell D, Gentilello L. Optimal therapy for stress gastritis. Ann Surg 1994 September;220(3):353-60.

(45) Memish ZA, Oni GA, Djazmati W, Cunningham G, Mah MW. A randomized clinical trial to compare the effects of a heat and moisture exchanger with a heated humidifying system on the occurrence rate of ventilator-associated pneumonia. Am J Infect Control 2001 October;29(5):301-5.

(46) Morrow LE, Kollef MH, Casale TB. Probiotic Prophylaxis of Ventilator-associated Pneumonia: A Blinded, Randomized, Controlled Trial. Am J Respir Crit Care Med 2010 June 3.

(47) Nardi G, Di Silvestre AD, De Monte A, Massarutti D, Proietti A, Grazia Troncon M et al. Reduction in gram-positive pneumonia and antibiotic consumption following the use of a SDD protocol including nasal and oral mupirocin. Eur J Emerg Med 2001;8(3):203-14.

(48) Ntoumenopoulos G, Presneill JJ, McElholum M, Cade JF. Chest physiotherapy for the prevention of ventilator-associated pneumonia. Intensive Care Med 2002 July;28(7):850-6.

(49) O'Keefe GE, Gentilello LM, Maier RV. Incidence of infectious complications associated with the use of histamine2-receptor antagonists in critically ill trauma patients. Ann Surg 1998 January;227(1):120-5.

(50) Palomar M, Alvarez LF, Jorda R, Bermejo B. Prevention of nosocomial infection in mechanically ventilated patients: Selective digestive decontamination versus sucralfate. Clinical Intensive Care 1997;8:228-35.

(51) Pickworth KK, Falcone RE, Hoogeboom JE, Santanello SA. Occurrence of nosocomial pneumonia in mechanically ventilated trauma patients: a comparison of sucralfate and ranitidine. Crit Care Med 1993 December;21(12):1856-62.

(52) Pneumatikos I, Koulouras V, Nathanail C, Goe D, Nakos G. Selective decontamination of subglottic area in mechanically ventilated patients with multiple trauma. Intensive Care Med 2002 April;28(4):432-7.

(53) Quinio B, Albanese J, BuesCharbit M, Viviand X, Martin C. Selective decontamination of the digestive tract in multiple trauma patients - A prospective double-blind, randomized, placebo-controlled study. Chest 1996;109(3):765-72.

(54) Ryan P, Dawson J, Teres D, Celoria G, Navab F. Nosocomial pneumonia during stress ulcer prophylaxis with cimetidine and sucralfate. Arch Surg 1993 December;128(12):1353-7.

(55) Seguin P, Tanguy M, Laviolle B, Tirel O, Mallédant Y. Effect of oropharyngeal decontamination by povidone-iodine on ventilator-associated pneumonia in patients with head trauma. Crit Care Med 2006;34:1514-9.

(56) Stoutenbeek CP, van Saene HKF, Little RA, Whitehead A. The effect of selective decontamination of the digestive tract on mortality in multiple trauma patients: A multicenter randomized controlled trial. Intensive Care Med 2007;33(2):261-70.

(57) Thomason MH, Payseur ES, Hakenewerth AM, Norton HJ, Mehta B, Reeves TR et al. Nosocomial pneumonia in ventilated trauma patients during stress ulcer prophylaxis with sucralfate, antacid, and ranitidine. The Journal of trauma 1996;41:503-8.

(58) Valencia M, Ferrer M, Farre R, Navajas D, Badia JR, Nicolas JM et al. Automatic control of tracheal tube cuff pressure in ventilated patients in semirecumbent position: a randomized trial. Crit Care Med 2007 June;35(6):1543-9.

(59) Valles J, Artigas A, Rello J, Bonsoms N, Fontanals D, Blanch L et al. Continuous Aspiration of Subglottic Secretions in Preventing Ventilator-Associated Pneumonia. Ann Intern Med 1995;122(3):179-86.

(60) Van Nieuwenhoven CA, Vandenbroucke-Grauls C, Van Tiel FH, Joore HC, van Schijndel RJ, van dT, I et al. Feasibility and effects of the semirecumbent position to prevent ventilator-associated pneumonia: a randomized study. Crit Care Med 2006 February;34(2):396-402.

(61) Verwaest C, Verhaegen J, Ferdinande P, Schetz M, Van den BG, Verbist L et al. Randomized, controlled trial of selective digestive decontamination in 600 mechanically ventilated patients in a multidisciplinary intensive care unit. Crit Care Med 1997 January;25(1):63-71.

(62) Wiener J, Itokazu G, Nathan C, Kabins SA, Weinstein RA. A randomized, double-blind, placebo-controlled trial of selective digestive decontamination in a medical-surgical intensive care unit. Clin Infect Dis 1995 April;20(4):861-7.

(63) Bonten MJ. Prevention of ventilator-associated pneumonia: bugs or drugs? Am J Respir Crit Care Med 2010 October 15;182(8):993-4.

(64) Klompas M. Ventilator-associated pneumonia: is zero possible? Clin Infect Dis 2010 November 15;51(10):1123-6.

CHAPTER 4

The attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomized prevention studies

W.G. Melsen; M.M Rovers; R.H.H. Groenwold; D.C.J.J. Bergmans; C.Camus; T.T.Bauer; E.W.Hanisch; B.Klarin; M.Koeman; W.A.Krueger; J.C. Lacherade; L.Lorente; Z.A.Memish; L.E. Morrow; G. Nardi; C.A. van Nieuwenhoven; G.E. O'Keefe; G. Nakos; F.A. Scannapieco; P.Seguin; T. Staudinger; A. Topeli; M.I.Ferrer and M.J.M. Bonten

Submitted

SUMMARY

Background

Estimating attributable mortality of Ventilator-Associated Pneumonia (VAP) has been hampered by confounding factors, small sample sizes and the impossibility to perform relevant subgroup analyses.

Objective

Theoretically, the question of attributable mortality could be answered by randomizing patients for VAP or not – which of course is ethically not possible. We therefore indirectly approached this question by extracting individual patient data of well published randomized VAP prevention trials.

Design

Relevant studies were identified through a systematic review and authors of eligible studies were invited to submit original patient data. Individual patient data were analyzed in a one-stage meta-analytical approach (in which attributable mortality was defined as the ratio between the relative risk reductions of mortality and VAP) and in competing risk analyses (for patients with information on length of stay in the ICU and time until VAP occurrence). Predefined subgroups included surgical, trauma and medical patients and patients with different categories of severity of illness scores.

Results

Individual patient data were available of 6,284 patients from 24 trials. The overall attributable mortality was 13%, with higher mortality rates among surgical patients and patients with mid-range severity scores at admission (i.e. APACHE 20-29 and SAPS2 35-58). Attributable mortality was close to zero in trauma, medical patients and patients with low (i.e. APACHE scores <20 or SAPS2 score<35) or high (APACHE> 30 or SAPS2 score>58) severity of illness scores. Competing risk analyses could be performed for 5162 patients from 19 studies and the overall daily hazard for ICU mortality after VAP was 1.13 (95% CI 0.98 to 1.31). The overall daily risk of discharge after VAP was 0.74 (95% CI 0.68 to 0.80), leading to an overall cumulative risk for dying in the ICU of 2.20 (95% CI 1.91 to 2.54). Highest cumulative risks for dying from VAP were found for surgical patients (2.97 (95%CI 2.24 to 3.94)) and patients with mid-range severity scores at admission (i.e. APACHE 20-29 and SAPS2 35-58 with cumulative risks of 2.49 (95%CI 1.81 to 3.44) and 2.72 (95%CI 1.95 to 3.78), respectively).

Conclusion

The overall attributable mortality of VAP is 13%, with higher rates for surgical patients and patients with a mid-range severity score at admission. Attributable mortality is mainly caused by prolonged exposure to the risk of dying due to increased length of ICU stay.

INTRODUCTION

In a one-day (May 8,2007) point prevalence survey of 13,796 adult patients in 1,265 Intensive Care Units (ICU) in 75 countries, 51% of all patients were infected of which 64% had an infection of the respiratory tract (1). Many of these episodes could have been categorized as Ventilator-Associated Pneumonia (VAP), which is one of the most common nosocomial infections with major consequences for patient outcome. Yet, to what extent VAP increases the likelihood of death in ICU is unknown.

Different methods have been used to determine attributable mortality of VAP, yielding estimates ranging from 0 to 60%. Most studies were observational, using cohorts of affected and non-affected patients to determine relative risks or odds ratios in univariate and multivariate analyses. Such studies inevitably suffer from the lack of adjustment for confounding and a meta-analysis of all published observational cohort studies did not allow a reliable estimate of attributable mortality of VAP due to extensive heterogeneity (2). Quantifying the effects of VAP on patient outcome is also hampered because of the time-dependent nature of VAP, which may include time-dependent bias, and the fact that ICU mortality and discharge act as competing endpoints. To overcome these problems innovative techniques, like multistate and competing risks models, have been applied recently to estimate attributable mortality of VAP (3;4). Although these methods carefully address time effects, adjustment for confounding is still not possible due to the observational nature of the data. Randomization is the only procedure to exclude the effects of confounding, and, therefore, studies in which patients have been randomized to a preventive measure would allow a non-confounded estimate of attributable mortality by analyzing the preventive effects on VAP and death, respectively. Based on a meta-analysis of aggregated data from 53 randomized prevention studies compromising 58 comparisons, we recently estimated the attributable mortality of VAP to be 9%(5). Yet, this approach was limited by lack of individual patient data, which precluded subgroup analyses as well as applying any of the newer statistical methods that adjust for competing endpoints. We, therefore, performed an individual patient data meta-analysis of VAP prevention studies, which offered the unique possibility to quantify attributable mortality of VAP in predefined subgroups, while avoiding effects of confounding and adjusting for competing endpoints.

METHODS

Search strategy and selection criteria

We searched for randomized trials evaluating VAP prevention measures in PubMed, Embase, the Cochrane library and Web of Science using the following terms and synonyms "ventilator-associated pneumonia" and "randomization". Eligible trials had to be published between January 1998 and July 2010. Inclusion criteria were: only patients who were mechanically ventilated should have been included, and both VAP and mortality rates during ICU stay had to be reported. Studies evaluating specific patient populations (supplement figure 1) or evaluating the following interventions were excluded; tracheostomy (as these studies only

include patients with prolonged stay in ICU), circuit changes of mechanical ventilation (as some of these studies are performed as cost saving studies, thus to extend the time of use of a system without increasing the incidence of VAP). Studies that were only published as abstracts, conference summaries or written in non-english language were excluded because thorough quality assessment was not possible.

Procedure

The original investigators of all selected trials were contacted to provide the raw data of their trials. The obtained data were thoroughly checked for consistency, plausibility and integrity of follow up. Discrepancies were queried with the responsible trial investigator and when the data were not complete or if discrepancies could not be resolved, the database was excluded from further analysis.

Studies were categorized according to the diagnostic methods used for VAP. Category I studies included those in which the following criteria were used for VAP: Roentgenographic criterion and at least two other criteria (i.e. fever, leukocytosis, purulent sputum, isolation of pathogenic bacteria from sputum or blood, or decreased alveolar-arterial oxygenation difference), and significant growth from samples obtained from lungs by bronchoscopic techniques (protected specimen brush, bronchoalveolar lavage (BAL), blind or not blinded) or by quantitative cultures of endotracheal aspirates. Category II studies had used the following criteria for VAP: Roentgenographic criterion and at least three other criteria (i.e. fever, leukocytosis, purulent sputum, isolation of pathogenic bacteria from sputum or blood, or decreased alveolar-arterial oxygenation difference), or CPIS score >6 with quantitative culture.

Statistical analyses

Attributable mortality of VAP was defined as the ratio of the relative risk reductions (RRRs) of mortality and VAP. This implies that if, for instance, the relative attributable mortality due to VAP would be 100%, a 50% RRR of VAP incidence due to a randomly applied intervention should lead to a 50% RRR of ICU mortality. The individual data of the different trials were pooled and the relative risks reduction of VAP and mortality and their corresponding 95% confidence interval were calculated using a random effects model, with RRR=1-relative risk (RR). A random effects model was used to account for cluster and between study effects. The 95% confidence interval of the attributable mortality was estimated by bootstrapping (n=1000). Subgroup analyses were performed to examine the effect of trauma, surgical or medical diagnosis as well as the severity of illness on the association of VAP and mortality. Severity of illness is expressed in APACHE II scores or SAPS2 scores measured at admission. To make three categories for APACHE II scores and SAPS 2 scores, we applied previously used cut-off points (3;6). In the subgroup analyses the distribution of different covariates (i.e. age, gender, severity of illness, admission diagnosis (trauma, surgical, medical)) among the intervention and control groups was examined to identify possible confounders. In case of significant differences adjusted RRRs were provided.

Furthermore, direct effects of VAP on outcome were examined in a competing risk analysis, which follows separate Cox models, estimating cause-specific hazard ratios for each possible event (i.e. ICU discharge or ICU death). VAP was treated in these models as a time-dependent variable. To directly judge the effect of VAP on death, taken the competing event (i.e. discharge) into account, the subdistribution hazard was calculated. Cluster effects were included in the different models to account for possible hospital and between study confounding effects. Data of patients in control and intervention groups were combined, as we considered that each of these interventions influenced mortality through VAP prevention only. All statistical tests were done with SPSS version 17.0 or R 2.8 software.

RESULTS

The systematic search identified 45 VAP prevention trials that were eligible for inclusion and all corresponding authors were contacted. Individual patient data were provided from 26 studies (supplement figure 2). After screening of the individual patient data, 24 studies remained for further analyses yielding 6,284 patients, of whom 3384 been randomized to a preventive measure (Table 1) (6-29). Overall, 1061 patients had developed VAP and 1683 had died in ICU. Seventeen (71%) trials were rated to category I regarding the diagnostic criteria for VAP (Table 1 of the supplement for the diagnostic criteria per study).

Table 1. Characteristics of included studies

Study	Year	Prevention method	Total*	VAP*	Mortality*	Subgroupanalysis
Bergmans	2004	Oral decontamination	87/139	13/42	25/53	Trauma/Medical/Surgical/APACHE II
Camus	2005	Oral decontamination	389/126	24/19	103/41	Trauma/Medical/Surgical/SAPS2
Cook	1998	Stress ulcer prophylaxis	604/596	98/114	138/140	Trauma/Medical/Surgical
Drakulovic	1999	Body positioning	39/47	2/11	7/13	Trauma/Medical/Surgical/APACHE II
Hanisch	1998	Stress Ulcer Prophylaxis	101/57	20/12	19/12	Trauma/Surgical/APACHE II
Klarin	2008	Probiotics	23/21	1/3	5/4	Trauma/Medical/Surgical/APACHE II
Koeman	2006	Chlorhexidine	255/130	29/23	95/38	Trauma/Medical/Surgical/APACHE II
Krueger	2002	Oral decontamination with ciprofloxacin iv	265/262	6/29	52/75	Trauma/Medical/Surgical/APACHE II
Lacherade	2005	Humidification system	185/184	47/53	60/63	Medical/Surgical/SAPS2
Lacherade	2010	Subglottic drainage	169/164	25/42	71/65	Trauma/Medical/Surgical/SAPS2
Lorente	2005	Suctioning system	210/233	43/42	52/50	Trauma/Medical/Surgical/APACHE II
Lorente	2006	Humdification system	53/51	21/8	13/12	Trauma/APACHE II
Lorente	2007	Polyurethane cuff and subglottic drainage	140/140	11/31	26/32	Trauma/Medical/Surgical/APACHE II
Memish	2001	Humidification system	123/120	14/19	40/30	Trauma/Medical/Surgical
Morrow	2010	Probiotic Prophylaxis	73/73	14/28	15/12	Trauma/APACHE II
Nardi	2001	Selective digestive decontamination	119/104	9/20	25/26	Trauma/Medical/Surgical
v. Nieuwenhoven	2006	Body positioning	112/109	13/8	33/33	Trauma/Medical/Surgical/APACHE II
O'Keefe	1998	Stress Ulcer Prophylaxis	47/49	10/14	6/11	Trauma
Pneumatikos	2002	Decontamination subglottic area	31/30	5/16	5/7	Trauma/APACHE II
Scannapieco	2009	Chlorhexidine	103/53	14/12	18/8	Trauma/Medical/Surgical/APACHE II
Seguin	2006	Oral decontamination	67/31	15/13	16/6	Trauma/SAPS2
Staudinger	2010	Continuous lateral rotation therapy	75/75	8/17	22/18	Medical/APACHE II/SAPS2
Topeli	2004	Suctioning system	41/37	13/9	27/25	APACHE II
Valencia	2007	Automatic control cuff pressure	73/69	11/10	20/16	Trauma/Medical/Surgical/APACHE II

*total number of patients in respectively prevention/control group.

Attributable mortality

Pooling the results of all studies yielded a statistically significant relative risk reduction of VAP in the total population (0.30 (95% CI 0.21 to 0.38)), as well as in all three subgroups of trauma, medical and surgical patients, and in all subgroups based on APACHE II scores (Table 2).
Relative risk reductions of mortality, though, were considerably lower than those of VAP, and in none of these analyses statistical significance was reached. Pooling the data of all studies resulted in a relative risk reduction of mortality of 0.04 (95%CI -0.06 to 0.12) and highest relative mortality reductions were observed in surgical patients 0.18 (95%CI -0.01 to 0.33) and patients with mid-range severity of illness scores (i.e. APACHE II 20-29 and SAPS2 35-58). The overall estimate of attributable mortality due to VAP was 13%, with considerable higher estimates for surgical patients (69%) and patients with mid-range severity scores (36% for APACHE II 20-29, 47% for SAPS2 35-58). There was no evidence for attributable mortality due to VAP among trauma and medical patients and patients with a low (i.e. APACHE scores <20 or SAPS2 score<35) or high (APACHE> 30 or SAPS2 score>58) severity of illness scores.

Table 2. Results primary analysis: Random effects model

Group	N	RRR VAP	RRR mort	AM	Bootstrap 95%CI
All Studies	6284	0.30 [0.21 to 0.38]	0.04 [-0.06 to 0.12]	13%	-0.14 to 0.38
Trauma	1159	0.40 [0.25 to 0.52]	-0.08 [-0.45 to 0.19]	0%	-1.06 to 0.45
Medical	3314	0.32 [0.17 to 0.43]	-0.01 [-0.14 to 0.11]	0%	-0.41 to 0.29
Surgical	1560	0.26 [0.04 to 0.43]	0.18 [-0.01 to 0.33]	69%	0.08 to 3.60
APACHE <20					
Unadjusted	1588	0.31 [0.10 to 0.47]	0.00 [-0.26 to 0.20]	0%	-0.94 to 0.72
Adjusted*	1521	0.34 [0.14 to 0.49]	-0.03 [-0.31 to 0.18]	0%	-0.97 to 0.77
APACHE 20-29	1176	0.28 [0.05 to 0.45]	0.10 [-0.12 to 0.27]	36%	-0.29 to 1.51
APACHE 30+	359	0.47 [0.08 to 0.70]	-0.03 [-0.39 to 0.23]	0%	-0.95 to 0.37
SAPS2 <35	364	0.45 [0.08 to 0.67]	-0.23 [-1.18 to 0.30]	0%	-4.48 to 0.82
SAPS2 35-58	723	0.38 [0.11 to 0.56]	0.18 [-0.07 to 0.38]	47%	-0.13 to 1.08
SAPS2 58+	377	0.35 [-0.05 to 0.60]	-0.12 [-0.50 to 0.16]	0%	-2.27 to 0.60

*adjusted for trauma
RRR=relative risk reduction
N=Total patients
AM= attributable mortality
95%CI= 95% confidence interval attributable mortality as estimated using bootstrap analyses

Competing risks analyses

In the competing risk analyses only patients with information on length of stay in the ICU, duration of mechanical ventilation until the occurrence of VAP and ICU mortality could be included. Therefore, of five studies all patients were excluded (11;16;19;22;24), as were 26 patients due to missing data from other studies. Eventually 5,162 patients were available for these analyses (see table 3 for the baseline characteristics of these patients).

Table 3. Baseline characteristics of patients included in the competing risk analysis

Group	Patients (n)	VAP n(%)	Onset VAP	Mortality VAP n(%)	Mortality others n(%)	LOS VAP	LOS others
All patients	5162	848 (16.4)	7.0 (7.0)	257 (30.3)	1176 (27.3)	21.0 (20.0)	9.0 (11.0)
Control	2376	488 (20.5)	7.0 (6.0)	149 (30.5)	527 (27.9)	20.0 (19.8)	9.0 (11.0)
Prevention	2786	360 (12.9)	7.0 (7.0)	108 (30.0)	649 (26.8)	22.5 (21.0)	9.0 (12.0)
Trauma	736	198 (26.9)	6.0 (6.0)	26 (13.1)	91 (16.9)	21.0 (16.0)	10.0 (10.0)
Surgery	1312	196 (14.9)	7.0 (6.0)	65 (33.2)	259 (23.2)	22.0 (22.0)	9.0 (11.0)
Medical	2876	395 (13.7)	7.0 (7.0)	138 (34.9)	765 (30.8)	21.0 (20.0)	9.0 (12.0)
APACHEII <20	1067	145 (13.6)	6.0 (5.5)	37 (25.5)	153 (16.6)	22.0 (19.0)	8.0 (10.0)
APACHEII 20-29	941	165 (17.5)	8.0 (8.5)	56 (33.9)	234 (30.2)	23.0 (26.0)	9.0 (13.0)
APACHEII 30+	338	51 (15.1)	6.0 (5.0)	24 (47.1)	149 (51.9)	17.0 (17.0)	8.0 (11.0)
SAPS2 <35	364	61 (16.8)	6.0 (5.0)	12 (19.7)	47 (15.5)	23.0 (20.0)	13.0 (15.0)
SAPS2 35-58	721	128 (17.8)	8.0 (7.0)	52 (40.6)	167 (28.2)	23.0 (23.8)	12.0 (14.0)
SAPS2 58+	377	73 (19.4)	7.0 (4.5)	28 (38.4)	159 (52.3)	23.0 (18.5)	11.0 (13.0)

VAP=ventilator associated pneumonia
N= number of patients
LOS=length of stay on the ICU (days).
Continuous variables are in median (IQR).

As compared to patients not developing VAP the cause specific hazard ratio (CSHR) of dying in ICU was 1.13 (95% CI 0.98 to 1.31), and after development of VAP, patients had a lower risk per day for ICU-discharge, as represented by the CSHR of discharge of 0.74 (95% CI 0.68 to 0.80) (Table 4). As a consequence these patients were exposed longer to a daily risk of dying in ICU. When combining the direct effects of VAP on the hazard of ICU mortality with the indirect effects imposed by a decreased risk of ICU discharge, the combined hazard for mortality (i.e. subdistribution hazard (SHR)) for patients with VAP was 2.20 (95% CI 1.91-2.54). These findings imply that the increased risk of dying in the ICU after VAP is merely the result of prolonged stay in the ICU than the direct influence of VAP on mortality. Results were comparable for patients randomized to preventive measures or to control strategies.

In subgroup analyses, surgical patients and patients with SAPS 2 score of 35-58 had a higher mortality risk per day after VAP (CSHRs of 1.37 and 1.49, respectively) as well as a lower risk of ICU discharge after VAP (CSHR of 0.69 and 0.62 respectively). This resulted in higher combined hazards for mortality of 2.97 (95%CI 2.24 to 3.94) and 2.72 (95%CI 1.95 to 3.78), for surgical patients and patients with SAPS 2 score of 35-58 respectively.

One of the lowest subdistribution hazard was obtained for trauma patients (1.48 (95% CI 0.93-2.36), with no evidence that VAP increased the daily risk of death (CSHR 0.73 (95% CI 0.43-1.23), although it appeared to reduce the likelihood of discharge (CSHR 0.65 (95% CI 0.54-0.78). Furthermore, the overall effects of VAP on mortality were lower in the extremes of the SAPS 2 scores (<35 and >58). This trend was less obvious for the three categories of

the APACHE scores: The direct effect of VAP on death was one of the lowest for patients with APACHE <20, but the effect on length of stay was highest in this category. Almost the opposite was observed for patients with APACHE >30.

Table 4. Results competing risks analysis

Group	CSHR mortality	95% CI	CSHR discharge	95% CI	SHR mortality	95% CI
All patients	1.13	0.98 to 1.31	0.74	0.68 to 0.80	2.20	1.91 to 2.54
Control	1.13	0.93 to 1.38	0.75	0.67 to 0.84	2.15	1.77 to 2.61
Prevention	1.12	0.90 to 1.39	0.72	0.64 to 0.81	2.24	1.81 to 2.77
Trauma	0.73	0.43 to 1.23	0.65	0.54 to 0.78	1.48	0.93 to 2.36
Medical	1.20	0.99 to 1.46	0.75	0.67 to 0.84	2.23	1.84 to 2.70
Surgical	1.37	1.03 to 1.83	0.69	0.58 to 0.82	2.97	2.24 to 3.94
APACHE <20	1.03	0.70 to 1.52	0.54	0.44 to 0.66	2.66	1.84 to 3.84
APACHE 20-29	1.31	0.94 to 1.83	0.73	0.60 to 0.88	2.49	1.81 to 3.44
APACHE 30+	1.22	0.79 to 1.89	1.04	0.71 to 1.52	1.72	1.09 to 2.71
SAPS2 <35	1.31	0.65 to 2.62	0.92	0.72 to 1.17	1.88	0.96 to 3.70
SAPS2 35-58	1.49	1.05 to 2.11	0.62	0.50 to 0.77	2.72	1.95 to 3.78
SAPS2 58+	0.81	0.53 to 1.22	0.76	0.55 to 1.03	1.16	0.77 to 1.76

CSHR= Cause-specific hazard ratio
SHR= Subdistribution hazard ratio
95%CI= 95% Confidence Interval

DISCUSSION

Based on a meta-analysis of 6,284 individual patient data from 24 VAP prevention trials we estimate that the attributable mortality of VAP is 13%. Yet, there are large differences between subgroups of patients, with attributable mortality rates of 69% and 36% among surgical patients and patients with an intermediate severity of illness score (i.e. APACHE 20-29), respectively. The attributable mortality was close to zero in trauma and medical patients and in patients with a low (i.e. APACHE scores <20 or SAPS2 score<35) or high (APACHE> 30 or SAPS2 score>58) severity of illness scores. These findings were confirmed by competing risk analyses. Our findings elucidate that attributable mortality mainly results from longer stay in the ICU. This prolonged stay increases the risk of dying, possible reasons are increased risk of ICU-related complications like other nosocomial infections and complications related to invasive procedures. For trauma patients and patients with low severity of illness scores this prolonged stay due to VAP does not increases mortality, which could be explained by the better clinical condition to cope with these complications. Severly ill patients (i.e. APACHE II>30, SAPS2>58) are the ones likely to have prolonged ICU stays already so the presence of VAP does

not contribute to additional ICU days with the attendent mortality.

Our estimate of attributable mortality is remarkably consistent with estimates from other studies. Nguile-Makao et al.(3) estimated attributable mortality in a cohort of 2,873 patients, with 434 of them developing VAP, using three statistical methods. Based on unadjusted logistic regression and a progressive disability model attributable mortality of VAP was estimated to be 8.1% (95% CI 3.1% to 13.1%). In conditional logistic regression on a matched population (matching on duration of mechanical ventilation) attributable mortality was estimated to be 10.4% (95% CI 5.6% to 24.5%). Schumacher et al(4) estimated attributable mortality of nosocomial pneumonia (not only VAP, because not all patients were mechanically ventilated) to be 10.6% using multistate models in a cohort of 1,876 patients with a duration of ICU-stay of at least 48 hours.

Our finding that surgical patients and patients with mid-range severity of illness had the highest attributable mortality due to VAP corroborates with findings from Nguile-Makao et al(3). Moreover, our finding of absence of attributable mortality among trauma patients corroborates with findings from Magret et al(30). In a prospective observational survey of 2,436 patients from 27 ICUs in nine European countries mortality was 73% lower among trauma patients with VAP as compared to non-trauma patients with VAP (adjusted odds ratio of 0.37 (95% CI 0.21-0.65).

We have used an innovative approach by performing a meta-analysis of studies evaluating different intervention methods. This implies that this study did not aim (and should not be used) to determine the preventive effects of individual measures. However, this approach offers a unique opportunity to estimate attributable mortality of VAP, as long as the preventive measures only influence mortality through reducing the risk of developing VAP. The main strengths of our analyses are the reliability of the data as they were prospectively obtained during randomised controlled trials, the size of the study population increasing the power to evaluate the effects of VAP in subgroups of patients and the lack of confounding due to randomisation in the calculation of attributable mortality. Moreover, adjustments for cluster effects to account for hospital effects and between study effects were included in all statistical analyses, and sensitivity analyses were performed to evaluate the influence of the diagnostic methods for VAP. This did not change conclusions (data not shown).

Some limitations should also be discussed. Not all investigators could provide individual patient data, and, therefore, data from 21 studies were not included. However, if we analyze the aggregated data of the 24 studies included in a classical meta-analysis approach, results are in agreement with those reported in our previous published meta-analysis, in which all VAP prevention studies were included (58 comparisons with 12,830 patients)(5). We, therefore, conclude that the included studies are reliable representatives of all VAP prevention studies. Another limitation is that we could not include adequacy of antimicrobial treatment of patients with VAP, which could be a confounder if there was a difference between the prevention and control patients.

In conclusion, based on the individual patient data of 24 VAP prevention trials the attributable mortality of VAP was estimated to be 13%. A higher attributable mortality rate was seen in surgical patients and patients with mid-range severity of illness on admission. These findings are of critical importance for the future design and analyses of intervention studies. It has been stated that, considering the difficulties in diagnosing VAP, prevention studies should focus on demonstrating favourable effects on more solid endpoints, such as ICU-survival (31;32). Consequently, prevention studies should include at least thousands of patients to be adequately powered for demonstrating beneficial effects on mortality. Furthermore, considering the large differences in attributable mortality between subgroups, investigators may consider to primarily focus on those subgroups of patients with the highest risk.

SUPPLEMENT.

Figure 1 Flowchart search

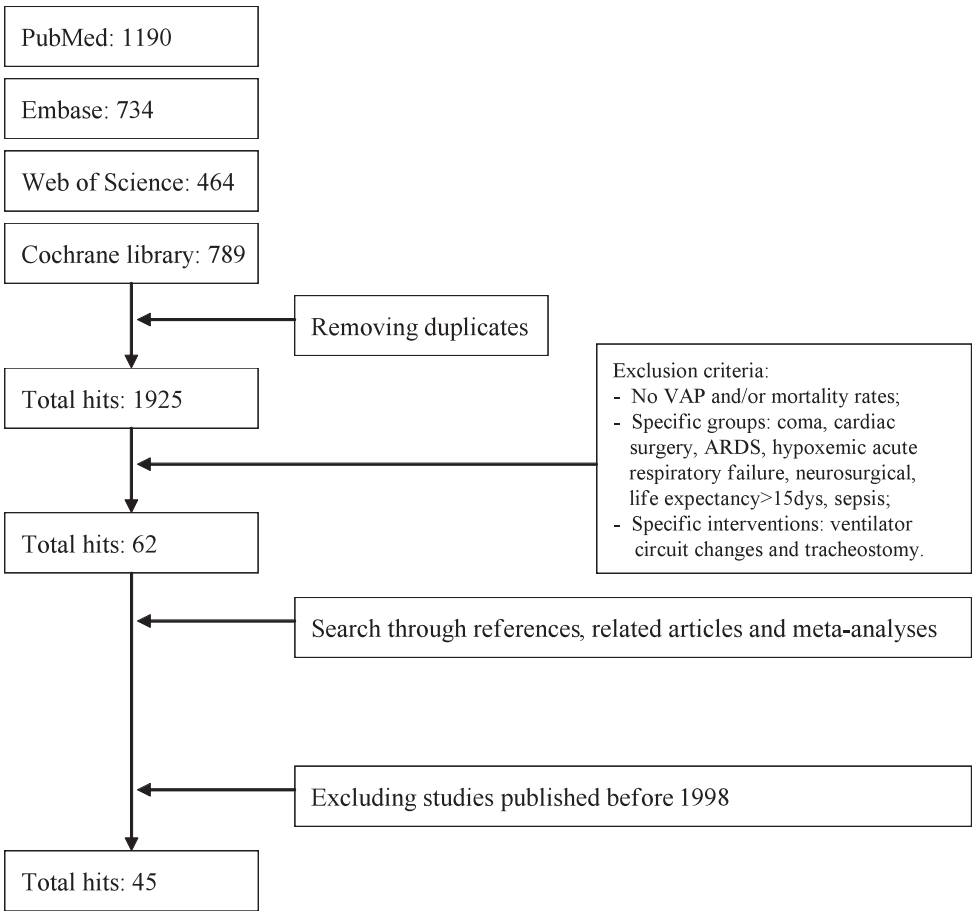


Figure 2 Selected studies

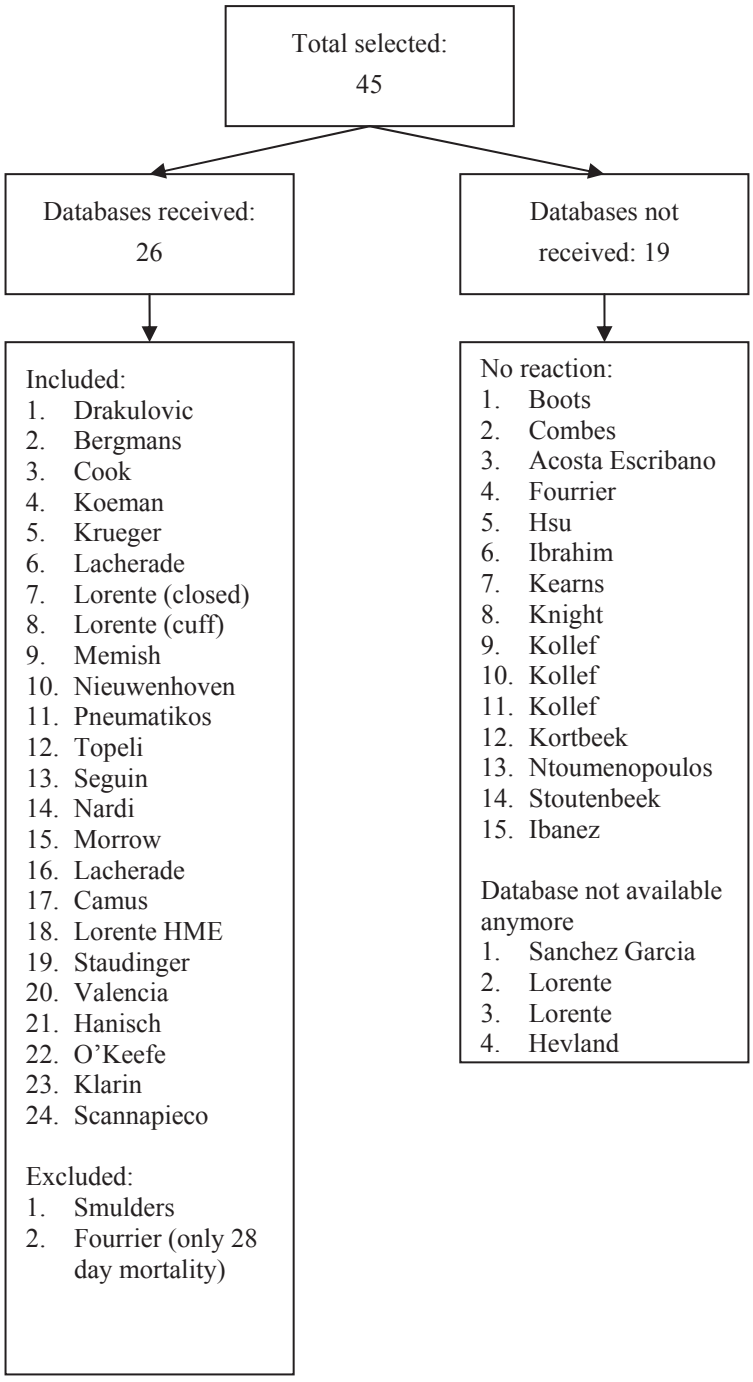


Table 1 Diagnostic Methods

Study	Clinical criteria	Microbiological cultures	Category*
Bergmans	New, persistent or progressive infiltrate on chest X ray and 3 or more of the following: fever or hypothermia, leukocytosis or leukopenia, >10 leukocytes per high power field in gram stain of tracheal aspirate and a positive culture from tracheal aspirate.	Quantitative cultures from BAL ($>10^4$ cfu/ml) or PSB ($>10^3$ cfu/ml) or a positive bloodculture unrelated to another source of infection or a positive culture from pleural fluid in the absence of previous pleural instrumentation.	1
Camus	CDC criteria	Quantitative culture of a bronchoscopic protected specimen at a concentration of 10^3 cfu/ml for brush or plugged catheter and $>10^4$ cfu/ml for bronchoalveolar lavage	1
Cook	New radiographic infiltrate that had persisted for at least 48 hrs, plus at least two of the following: fever or hypothermia, leukocytosis or leukopenia, purulent sputum or isolation of pathogenic bacteria from an endotracheal aspirate.	Quantitative cultures of bronchoalveolar lavage or protected brush catheter sampling. Two members of the pneumonia-adjudication committee examined all relevant clinical and diagnostic documents.	1
Drakulovic	New and persistent infiltrates on chest radiography and at least two of the following criteria: fever, leucopenia or leucocytosis, purulent tracheal secretions.	Positive culture of tracheobronchial aspirate ($>10^5$ cfu/ml), bronchoalveolar lavage ($>10^4$ cfu/ml) or protected specimen brush ($>10^3$ cfu/ml) in protected specimen brush cultures	1
Hanisch	Radiological signs of pneumonia and purulent tracheal secretion or positive microbiological findings in tracheal aspirate and fever and leukocytosis.	In all patients bronchoalveolair lavage was routinely performed but no quantitative cultures were done.	2
Klarin	New and persistent or progressive infiltrate on chest radiograph combined with at least three of the other four criteria: purulent tracheal aspirate; positive culture of tracheal aspirates; fever or hypothermia, leukocytosis or leukopenia.		2
Koeman	New persistent or progressive infiltrate on chest X ray in combination with at least three of four criteria: fever or hypothermia, leukocytosis and.or left shift or leukopenia, purulent aspect of tracheal aspirate	Semiquantitative culture from tracheal aspirates ($>10^5$ cfu/ml)	2
Krueger	Purulent tracheobronchial secretions, chest radiographic examination with indication of a new or progressive infiltrate, consolidation or cavitation or pleural effusion, increase in the inspiratory oxygen fraction of more than 0.15 necessary to maintain arterial oxygen tension at the same level and at least one of the following symptoms: fever, leukcocytosis, or more than 10% band forms of neutrophil granulocytes.	Microbiological cultures (bloodcultures,tracheobronchial secretions, protected specimen brush, BAL, pleural fluid or lung biopsy) were attempted but not prerequisted, but in all pneumonia cases a causative pathogen was identified (89% with cultures of tracheobronchial secretions).	1
Lacherade '05	New and persistent infiltrate on chest x ray and two of the following: fever or hypothermia, leukocytosis or leukopenia, and purulent tracheal secretions.	Quantitative culture of specimens obtained using a protected telescoping catheter or broncholalveolair lavage,	1

Table 1 (Continued)

Study	Clinical criteria	Microbiological cultures	Category*
Lacherade '10	New and persistent infiltrate on chest X-ray and at least two of the following criteria: fever or hypothermia, leucocytosis or leucopenia, and purulent tracheal secretions.	Quantitative culture of either a protected telescoping catheter sample (>10^3cfu/ml) or bronchoalveolar lavage fluid (>10^4 cfu/ml).	1
Lorente 2005	New onset of purulent bronchial sputum, fever or hypothermia, leukopenia or leukocytosis, chest radiograph showing new or progressive infiltrates.	Quantitative culture of respiratory secretions (tracheal aspirate (>10^6 cfu/ml), BAL (>10^4 cfu/ml), protected specimen brush (>10^3 cfu/ml)) or bloodculture coinciding with the culture of the respiratory secretion which is not statistically significant.	1
Lorente 2006	New onset of purulent bronchial sputum, fever or hypothermia, leukopenia or leukocytosis, chest radiograph showing new or progressive infiltrates	Quantitative culture of respiratory by tracheal aspirate (>10^6 cfu/ml)	1
Lorente 2007	New onset of purulent bronchial sputum, fever or hypothermia, leukopenia or leukocytosis, chest radiograph showing new or progressive infiltrates.	Quantitative culture of respiratory secretions by tracheal aspirate (>10^6 cfu/ml)	1
Memish	The CDC criteria and at least one of the following conditions: new onset of purulent sputum or change in sputum, organisms cultured from blood, isolation of an etiologic agent from a specimen that was obtained by transtracheal aspirate, isolation of virus from or detection of viral antigen in respiratory secretions, diagnostic single antibody titer or 4-fold increase in paired sera for pathogen, histopatologic evidence of pneumonia.		2
Morrow	New and persistent infiltrate on chest radiographs with two of three supporting findings: fever or hypothermia, leukocytosis or leukopenia and/or purulent sputum.	Quantitative cultures obtained by non-bronchoscopic bronchoalveolar lavage using a protected catheter (>10^4 cfu/ml).	1
Nardi	New and persistent pulmonary infiltrates, purulent tracheal secretion, fever, leukocytosis or leukopenia, and hypoxaemia (Pa)2/FiO2<250).	A bronchoscopic protected specimen brush together wit a distal BAL were performed to confirm the diagnosis.	1
Nieuwenhoven	New persistent or progressive radiographic infiltrate with at least two of the following: fever or hypothermia, leukocytosis or leukopenia, positive culture of tracheal aspirate.	Quantitative cultures of bronchoalveolar lavage yielded >10^4 cfu.ml or if blood cultures were positive for a micro-organism colonizing the respiratory tract.	1

Table 1 (Continued)

Study	Clinical criteria	Microbiological cultures	Category*
O'Keefe	Pneumonia was defined by the presence of leukocytosis, a new or changing infiltrate on chest X ray, fever or hypothermia, and positive sputum and gram stain for specific pathogen		2
Pneumatikos	New and persistent pulmonary infiltrates in addition to two of the following criteria: fever, leukocytosis or leukopenia and purulent tracheal secretions.	The diagnosis of VAP was confirmed by quantitative cultures.	1
Scannapieco	A CPIS score of 6 or more triggered the sampling of the lower airway by bqBAL.	Blind quantitative bronchoalveolar Lavage (bqBAL) using a miniBAL technique ($> 10^4$ cfu/ml).	2
Seguin	New pulmonary infiltrates detected by chest radiography plus two of the following: fever or hypothermia, purulent endotracheal aspirate and leukocytosis or leukopenia.	Quantitative culture of bronchoscopic or a non-bronchoscopic "blind"bronchoalveolar lavage ($>10^4$ cfu/ml)	1
Staudinger	New and persistent radiographic infiltrate on the chest radiograph plus newly developed purulent tracheal secretions plus increasing signs of inflammation (fever, leukocytosis or increasing levels of C-reactive protein)	Positive quantitative culture of BAL ($>10^4$ cfu/ml)	1
Topeli	New and persistent infiltration on the chest X ray and presence of any two out of three criteria were used; fever or hypothermia, leukocytosis or leukopenia, purulent tracheal secretions or at least 10 leukocytes per high power field in gram's stain of the endotracheal aspirates.	After the clinical diagnosis endotracheal suction cultures were performed.	2
Valencia	New or progressive pulmonary infiltrates together with at least two of the following: fever or hypothermia, leukocytosis or leukopenia and purulent tracheal secretions.	Positive quantitative culture of protected specimen brush ($>10^3$ cfu/ml) or BAL($>10^4$ cfu/ml) or tracheobronchial aspirates cultures ($>10^5$ cfu/ml), in pleural fluid or bloodcultures without alternative cause of bacteremia .	1

*Category 1: Roentgenographic criterion and at least two other criteria (ie fever, leukocytosis, purulent sputum, isolation of pathogenic bacteria from sputum or blood, or decreased alveolar-arterial oxygenation difference) , and significant growth from samples obtained from lungs by bronchoscopic techniques (protected specimen brush, bronchoalveolar lavage (BAL), blind or not blinded) or by quantitative cultures of endotracheal aspirates.

Category 2: Roentgenographic criterion and at least three other criteria (ie fever, leukocytosis, purulent sputum, isolation of pathogenic bacteria from sputum or blood, or decreased alveolar-arterial oxygenation difference), or CPIS score >6 with quantitative culture.

REFERENCES

(1) Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA 2009 December 2;302(21):2323-9.

(2) Melsen WG, Rovers MM, Bonten MJ. Ventilator-associated pneumonia and mortality: a systematic review of observational studies. Crit Care Med 2009 October;37(10):2709-18.

(3) Nguile-Makao M, Zahar JR, Francais A, Tabah A, Garrouste-Org, Allaouchiche B et al. Attributable mortality of ventilator-associated pneumonia: respective impact of main characteristics at ICU admission and VAP onset using conditional logistic regression and multi-state models. Intensive Care Med 2010 May;36(5):781-9.

(4) Schumacher M, Wangler M, Wolkewitz M, Beyersmann J. Attributable mortality due to nosocomial infections. A simple and useful application of multistate models. Methods Inf Med 2007;46(5):595-600.

(5) Melsen WG, Rovers MM, Koeman M, Bonten MJ. Estimating the attributable mortality of ventilator-associated pneumonia from randomized prevention studies. Crit Care Med 2011 December;39(12):2736-42.

(6) Krueger WA, Lenhart FP, Neeser G, Ruckdeschel G, Schreckhase H, Eissner HJ et al. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. Am J Respir Crit Care Med 2002 October 15;166(8):1029-37.

(7) Bergmans DC, Bonten MJ, Gaillard CA, Paling JC, van-der GS, van-Tiel FH et al. Prevention of ventilator-associated pneumonia by oral decontamination: a prospective, randomized, double-blind, placebo-controlled study. Am J Respir Crit Care Med 2001;164:382-8.

(8) Camus C, Bellissant E, Sebille V, Perrotin D, Garo B, Legras A et al. Prevention of acquired infections in intubated patients with the combination of two decontamination regimens. Crit Care Med 2005;33(2):307-14.

(9) Cook D, Guyatt G, Marshall J, Leasa D, Fuller H, Hall R et al. A comparison of sucralfate and ranitidine for the prevention of upper gastrointestinal bleeding in patients requiring mechanical ventilation. New Engl J Med 1998;338(12):791-7.

(10) Drakulovic MB, Torres A, Bauer TT, Nicolas JM, Nogue S, Ferrer M. Supine body position as a risk factor for nosocomial pneumonia in mechanically ventilated patients: a randomised trial. Lancet 1999 November 27;354(9193):1851-8.

(11) Hanisch EW, Encke A, Naujoks F, Windolf J. A randomized, double-blind trial for stress ulcer prophylaxis shows no evidence of increased pneumonia. Am J Surg 1998 November;176(5):453-7.

(12) Klarin B, Molin G, Jeppsson B, Larsson A. Use of the probiotic Lactobacillus plantarum 299 to reduce pathogenic bacteria in the oropharynx of intubated patients: a randomised controlled open pilot study. Crit Care 2008;12(6):R136.

(13) Koeman M, van d, V, Hak E, Joore HC, Kaasjager K, de Smet AG et al. Oral decontamination with chlorhexidine reduces the incidence of ventilator-associated pneumonia. Am J Respir Crit Care Med 2006 June 15;173(12):1348-55.

(14) Lacherade JC, Auburtin M, Cerf C, Van de LA, Soufir L, Rebufat Y et al. Impact of humidification systems on ventilator-associated pneumonia: a randomized multicenter trial. Am J Respir Crit Care Med 2005 November 15;172(10):1276-82.

(15) Lacherade JC, De JB, Guezennec P, Debbat K, Hayon J, Monsel A et al. Intermittent Subglottic Secretion Drainage and Ventilator-associated Pneumonia: A Multicenter Trial. Am J Respir Crit Care Med 2010 June 3.

(16) Lorente L, Lecuona M, Martin MM, Garcia C, Mora ML, Sierra A. Ventilator-associated pneumonia using a closed versus an open tracheal suction system. Crit Care Med 2005 January;33(1):115-9.

(17) Lorente L, Lecuona M, Jimenez A, Mora ML, Sierra A. Ventilator-associated pneumonia using a heated humidifier or a heat and moisture exchanger: a randomized controlled trial. Crit Care 2006;10(4):R116.

(18) Lorente L, Lecuona M, Jimenez A, Mora ML, Sierra A. Influence of an endotracheal tube with polyurethane cuff and subglottic secretion drainage on pneumonia. Am J Respir Crit Care Med 2007 December 1;176(11):1079-83.

(19) Memish ZA, Oni GA, Djazmati W, Cunningham G, Mah MW. A randomized clinical trial to compare the effects of a heat and moisture exchanger with a heated humidifying system on the occurrence rate of ventilator-associated pneumonia. Am J Infect Control 2001 October;29(5):301-5.

(20) Morrow LE, Kollef MH, Casale TB. Probiotic Prophylaxis of Ventilator-associated Pneumonia: A Blinded, Randomized, Controlled Trial. Am J Respir Crit Care Med 2010 June 3.

(21) Nardi G, Di Silvestre AD, De Monte A, Massarutti D, Proietti A, Grazia Troncon M et al. Reduction in gram-positive pneumonia and antibiotic consumption following the use of a SDD protocol including nasal and oral mupirocin. Eur J Emerg Med 2001;8(3):203-14.

(22) O'Keefe GE, Gentilello LM, Maier RV. Incidence of infectious complications associated with the use of histamine2-receptor antagonists in critically ill trauma patients. Ann Surg 1998 January;227(1):120-5.

(23) Pneumatikos I, Koulouras V, Nathanail C, Goe D, Nakos G. Selective decontamination of subglottic area in mechanically ventilated patients with multiple trauma. Intensive Care Med 2002 April;28(4):432-7.

(24) Scannapieco FA, Yu J, Raghavendran K, Vacanti A, Owens SI, Wood K et al. A randomized trial of chlorhexidine gluconate on oral bacterial pathogens in mechanically ventilated patients. Crit Care 2009;13(4):R117.

(25) Seguin P, Tanguy M, Laviolle B, Tirel O, Mallédant Y. Effect of oropharyngeal decontamination by povidone-iodine on ventilator-associated pneumonia in patients with head trauma. Crit Care Med 2006;34:1514-9.

(26) Staudinger T, Bojic A, Holzinger U, Meyer B, Rohwer M, Mallner F et al. Continuous lateral rotation therapy to prevent ventilator-associated pneumonia. Crit Care Med 2010 February;38(2):486-90.

(27) Topeli A, Harmanci A, Cetinkaya Y, Akdeniz S, Unal S. Comparison of the effect of closed versus open endotracheal suction systems on the development of ventilator-associated pneumonia. J Hosp Infect 2004 September;58(1):14-9.

(28) Valles J, Artigas A, Rello J, Bonsoms N, Fontanals D, Blanch L et al. Continuous Aspiration of Subglottic Secretions in Preventing Ventilator-Associated Pneumonia. Ann Intern Med 1995;122(3):179-86.

(29) Van Nieuwenhoven CA, Vandenbroucke-Grauls C, Van Tiel FH, Joore HC, van Schijndel RJ, van dT, I et al. Feasibility and effects of the semirecumbent position to prevent ventilator-associated pneumonia: a randomized study. Crit Care Med 2006 February;34(2):396-402.

(30) Magret M, maya-Villar R, Garnacho J, Lisboa T, Diaz E, Dewaele J et al. Ventilator-associated pneumonia in trauma patients is associated with lower mortality: results from EU-VAP study. J Trauma 2010 October;69(4):849-54.

(31) Bonten MJ. Prevention of ventilator-associated pneumonia: bugs or drugs? Am J Respir Crit Care Med 2010 October 15;182(8):993-4.

(32) Klompas M. Ventilator-associated pneumonia: is zero possible? Clin Infect Dis 2010 November 15;51(10):1123-6.

CHAPTER 5

Microbial etiology and attributable mortality of ventilator-associated pneumonia: results from an individual patient data meta-analysis

W.G.Melsen; M.M.Rovers; D.C.J.J.Bergmans; C.Camus; T.T.Bauer; B.Klarin; W.A. Krueger; J.C. Lacherade; L.Lorente; G. Nardi; G. Nakos; P. Seguin; T. Staudinger; A. Topeli; M.I. Ferrer and M.J.M. Bonten

SUMMARY

Background

Attributable mortality of Ventilator-associated Pneumonia (VAP) in intensive care patients has been estimated to range from 10% to 13%, but only few studies estimated pathogen-specific attributable mortality.

Methods

We analysed the individual patient data of fifteen VAP prevention trials. For every causative pathogen, we compared outcomes between patients with and without this pathogen by use of time-dependent regression modelling. We adjusted results for the timing of VAP and the competing endpoint ICU-discharge. Cause-specific hazard ratios for ICU mortality and ICU discharge as well as the subdistribution hazard ratio (SHR) was estimated for all patients and in subgroups (surgical, medical and trauma). Pathogens were categorized as *Staphylococcus aureus*, non-fermenters (*Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and Acinetobacter species), Enterobacteriaceae, Other and Polymicrobial.

Results

Of the 3,231 patients, 532 (16%) experienced VAP during their ICU period, with the group non-fermenters being most prevalent (27%). The SHR for all-cause VAP was 2.42 (95% CI 2.04 to 2.87). Patients with VAP caused by pathogens categorised as non-fermenters and polymicrobial had the highest risk of mortality (SHR 3.59 (95% CI 2.71 to 4.74) and 2.77 (95% CI 2.00 to 3.84), respectively). Surgical patients had the highest attributable mortality of VAP (SHR 3.80 (95%CI 2.75 to 5.25), whereas VAP was not associated with higher mortality in trauma patients, irrespective of causative pathogens.

Conclusion

Polymicrobial VAP and VAP caused by non-fermenters are associated with the highest mortality rates. Attributable mortality is highest among surgical patients, independent of causative pathogens.

INTRODUCTION

Ventilator associated Pneumonia (VAP) is the most frequently occurring infection among ICU patients, yet the attributable effects of VAP on patient outcome are difficult to accurately quantify. Based on recent studies applying statistical techniques that account for confounding, timing of infection and competing risks caused by informative censoring (i.e. patients who are discharged from the ICU are by definition in a better or worse clinical condition than those that stay), attributable mortality of VAP was estimated to be 10-13% (1-3). Furthermore, attributable mortality varies considerably among subgroups, with higher mortality rates among surgical patients and patients with intermediate severity of illness scores on admission(1;2). To what extent attributable mortality depends on pathogens, is, however, largely unknown. Older studies suffer from methodological limitations (4;5) and more recent estimates (2;6) were based on a limited number of pathogens only (*Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*).

We previously quantified associations between VAP and mortality using meta-analyses of observational and randomized intervention studies, as well as individual patient data of 24 randomised VAP prevention trials (1). Here, we have extended the individual patient data meta-analysis by evaluating the available data on causative pathogens in order to determine excess mortality and length of stay in ICU of the most common causative pathogens of VAP.

METHODS

A systematic literature search was performed using PubMed, Embase, the Cochrane Library and Web of Science to identify all randomised VAP prevention trials. In each of these trials a certain intervention aimed to reduce the incidence of VAP was randomly allocated to an intervention and a control group. To be selected, trials had to include only patients who were mechanically ventilated and evaluated VAP and mortality rates during the total ICU stay of the patients (the search methods and its results are described in detail elsewhere(1)). Individual patient data were available from 24 randomized studies and all investigators were contacted and asked to provide data on causative pathogens of VAP. The obtained data were thoroughly checked for consistency, plausibility and integrity. Only the first event of VAP was taken into account.

Isolated micro-organisms were classified into five groups; 1) *Staphylococcus aureus* (*S. aureus*), 2) Non-fermenters (non-fermenting Gram-negative rods like *Pseudomonas aeruginosa* (*P. aeruginosa*), *Stenotrophomonas maltophilia* (*S. maltophilia*) and Acinetobacter species), 3) Enterobacteriaceae (e.g, *Escherichia coli* (*E. coli*), Citrobacter species, Enterobacter species, Klebsiella species, Morganella species, Proteus species, Serratia species), 4) Other (e.g Enterococcus species, Streptococcus spp, *Haemophilus influenzae* (*H. influenzae*), *Moraxella catarrhalis*) 5) Polymicrobial VAP, defined as isolation of two or more different pathogens in the same VAP episode.

Statistical analyses

The influence of VAP on mortality was evaluated using a competing risk analysis taking the time-dependent nature of VAP and the competing endpoint ICU-discharge into account. In this analysis the cause specific hazard ratios for ICU mortality and ICU discharge are estimated using a Cox proportional hazards model. The effect of the different pathogens was compared by incorporating VAP in the model as a categorical time-dependent variable, considering patients who did not acquired VAP (yet) as a reference. Once a patient experiences VAP, their VAP status remained yes, until one of the endpoints (ICU death or discharge) occurred. To directly judge the effect of VAP on death, taking the competing endpoint ICU discharge into account, the subdistribution hazard was calculated. Cluster effects were included in the different models to account for possible hospital and between study confounding effects. Data of patients in the control and intervention groups were combined, as we considered that each of these interventions influenced mortality through VAP prevention only and had no effect on the attributable mortality per pathogen. Outcome parameters were determined for all patients, as well as for subgroups (surgical, trauma and medical patients).

Tabel 1. Characteristics of included studies

Study	Year	Prevention method	Total*	VAP*	Mortality*
Bergmans	2004	Oral decontamination	87/139	13/42	25/53
Camus	2005	Oral decontamination	389/126	24/19	103/41
Drakulovic	1999	Body positioning	39/47	2/11	7/13
Klarin	2008	Probiotics	23/21	1/3	5/4
Krueger	2002	Oral decontamination with ciprofloxacin iv	265/262	6/29	52/75
Lacherade	2005	Humidification system	185/184	47/53	60/63
Lacherade	2010	Subglottic drainage	169/164	25/42	71/65
Lorente	2006	Humdification system	53/51	21/8	13/12
Lorente	2007	Polyurethane cuff and subglottic drainage	140/140	11/31	26/32
Nardi	2001	Selective digestive decontamination	119/104	9/20	25/26
Pneumatikos	2002	Decontamination subglottic area	31/30	5/16	5/7
Seguin	2006	Oral decontamination	67/31	15/13	16/6
Staudinger	2010	Continuous lateral rotation therapy	75/75	8/17	22/18
Topeli	2004	Suctioning system	41/37	13/9	27/25
Valencia	2007	Automatic control cuff pressure	73/69	11/10	20/16

*total number of patients in respectively prevention/control group.

RESULTS

Data were available from 15 VAP prevention trials with 3,236 patients, of whom five were excluded because of incomplete data (Table 1). Of the remaining 3,231 patients, 532 (16%) experienced VAP during ICU-stay (Table 2). In most studies quantitative cultures of respiratory tract secretions collected by invasive techniques were used to diagnose VAP and to identify causative pathogens (supplement Table 1).

Table 2. Baseline characteristics of patients included in the competing risk analysis

Group	Patients	Age	Gender(M)	APACHE	SAPS2	Surgery	Medical	Trauma	Mortality	LOS	MV	MVVAP
No VAP	2699	63(26)	1605(64.1)	19(9)	46.5(24)	734 (28.6)	1491 (58.3)	362 (15.2)	747(27.7)	10(12)	7(8)	-
All VAP	532	57(32)	346(68.8)	21(8)	46.5(23)	125 (26.0)	246 (51.4)	119 (29.0)	185 (34.8)	23(22)	17(18)	7(8)
Staph Aureus	89	51(35)	53 (63.9)	18(9)	38(21)	23 (28.4)	40 (50.0)	23 (38.3)	25 (28.1)	25(24)	20(18)	6(6)
MSSA	62	48(36)	36(64.3)	20(8)	38(17)	17(30.4)	24(42.9)	19(44.2)	17(27.4)	24(25)	19.5(19)	5(5)
MRSA	21	65(25)	14 (66.7)	18(10)	48(28)	5(26.3)	13(72.2)	2(18.2)	6(28.6)	24(23)	17(15)	8(7)
Non-Fermenters	139	62(28)	92 (68.7)	20(9)	52(22)	32 (25.6)	76 (60.8)	17 (15.9)	56 (40.3)	25(23)	20(22)	9.5(8)
Pseudomonas	108	62.5(28)	71(68.3)	20(9)	51(21)	28(28.9)	57(58.8)	12(14.5)	42(38.9)	25(26)	19.5(21)	9(10)
Enterobacteriaceae	94	64(30)	61 (66.3)	20(6)	56(31)	18(22.5)	45(56.3)	21 (29.2)	32 (34.0)	22.5(26)	17(20)	7(6)
Other	90	48.5 (36)	56 (69.1)	18 (9)	44.5(22)	18(21.2)	39 (45.9)	28 (38.4)	24 (26.7)	21(20)	17(17)	6(6)
Streptoccoci/H.Influenzae	44	45 (32)	32(74.4)	15 (8)	40.5 (19)	10 (24.4)	21 (51.2)	10 (29.4)	9 (20.5)	22 (21)	17 (21)	5(5)
Polymicrobial	101	58 (39)	73 (76.8)	23.5(7)	43(26)	30 (31.3)	39 (41.1)	27 (30.7)	39(38.6)	22(17)	17(16)	6(7)

VAP=ventilator associated pneumonia
LOS=length of stay.
MV= total duration of mechanical ventilation
MVVAP= duration of mechanical ventilation until VAP
Continuous variables are in median
Missings gender (223), Surgery (182), Medical (195), Trauma (445). APACHE (1605), SAPS (1769)
Unknown pathogens in 19 patients.
Non-Fermenters: non-fermenting Gram-negative rods like *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and Acinetobacter species

Microbial causes of VAP were documented in 513 patients with VAP (Table 2). Non-fermenters were most prevalent (27%), followed by polymicrobial episodes (19%), Enterobacteriaceae (18%) and *S. aureus* (17%). In the polymicrobial episodes either two (n=81), three (n=18), four (n=1) or five (n=1) categories of pathogens were involved (supplement Table 2). There were no major differences in VAP etiology between surgical, medical and trauma patients, although non-fermenters were less prevalent among trauma patients (Table 3).

Table 3. Pathogens per admission diagnosis

Category	Surgical	Medical	Trauma
Staph Aureus	23 (18.4)	40 (16.2)	23(19.3)
MSSA	17	24	19
MRSA	5	13	2
Non Fermenters	32 (25.6)	76 (30.9)	17(15.1)
Pseudomonas	28	57	12
Enterobacteriaceae	28 (22.4)	45 (18.3)	21(17.6)
Other	18 (14.4)	39 (15.9)	28 (23.5)
Streptococci / H.Influenzae	10	21	10
Polymicrobial	30 (24.0)	39 (15.9)	27 (22.7)
Unknown	4	7	3

Non-Fermenters: non-fermenting Gram-negative rods like *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and Acinetobacter species

Patients with VAP had a longer median length of stay in ICU (23 days) than patients without VAP (10 days) (Table 2). The median length of stay until VAP was 7 days, and ranged from 6 days for patients with *S. aureus* to 9.5 days for patients with VAP caused by non-fermenters. Of the 532 patients with VAP, 185 (34.8%) died in ICU, as compared to 747 (27.7%) of 2699 patients without VAP ($p<0.001$). Crude mortality rates varied for different pathogen groups and ranged from 40.3% for VAP caused by non-fermenters to 26.7% for VAP caused by "other" pathogens, respectively.

VAP was associated with a higher daily risk of mortality in ICU reflected by a cause specific hazard of 1.23 (95% CI 1.03to1.47) and with a lower daily risk of discharge (cause specific hazard ratio for ICU discharge of 0.65 (95% CI 0.59 to 0.72)). The combined effects resulted in a subdistribution hazard ratio (SHR) for mortality of 2.42 (95% CI 2.04 to 2.87) (Table 4). For the individual pathogen groups the SHR was above average for non-fermenters (3.59 (95% CI 2.71 to 4.74)) and polymicrobial episodes (2.77 (95% CI 2.00 to 3.84)). For both pathogen groups patients experienced, after VAP was diagnosed, an increased risk per day to die and a decreased rate to be discharged while still alive. Below average SHR were obtained for *S. aureus* (1.86 (95% CI 1.24 to 2.79)) and pathogens categorized as other (1.74 (95% CI 1.13 to 2.66)).

Subsequently, we assessed the effects of antibiotic resistance in *S. aureus* (methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA)), of *P. aeruginosa* (most important pathogen in the group non-fermenters) and of streptococci and *H. influenzae* (pathogens associated with early-onset VAP). The SHRs of VAP caused by MRSA and MSSA was 2.30 (95% CI 0.99 to 5.31) and 1.71 (95%CI 1.05 to 2.78), respectively. The mortality associated with *P. aeruginosa* was comparable to that of all other pathogens in the group of non-fermenters. VAP caused by streptococci or *H. influenzae* was not associated with attributable mortality (SHR 1.16 (95% CI 0.59 to 2.27).

Table 4. Results competing risks analysis

Group	CSHR mortality	95% CI	CSHR discharge	95% CI	SHR mortality	95% CI
All VAP patients	1.23	1.03 to 1.47	0.65	0.59 to 0.72	2.42	2.04 to 2.87
Staph Aureus	0.88	0.59 to 1.33	0.66	0.55 to 0.80	1.86	1.24 to 2.79
MSSA	0.83	0.51 to 1.34	0.65	0.52 to 0.81	1.71	1.05 to 2.78
MRSA	1.04	0.44 to 2.46	0.75	0.54 to 1.04	2.30	0.99 to 5.31
Non-Fermenters	1.46	1.09 to 1.96	0.62	0.51 to 0.75	3.59	2.71 to 4.74
Pseudomonas	1.47	1.06 to 2.03	0.63	0.51 to 0.78	3.49	2.53 to 4.80
Enterobacteriaceae	1.04	0.72 to 1.51	0.59	0.48 to 0.73	2.33	1.62 to 3.36
Other	0.96	0.62 to 1.48	0.79	0.65 to 0.96	1.74	1.13 to 2.66
Streptoccoci / Haemophilus Influenzae	0.62	0.31 to 1.23	0.72	0.56 to 0.94	1.16	0.59 to 2.27
Polymicrobial	1.47	1.07 to 2.02	0.69	0.56 to 0.85	2.77	2.00 to 3.84

CSHR= Cause-specific hazard ratio
SHR= Subdistribution hazard ratio
95%CI= 95% Confidence Interval
MSSA: methicillin-susceptible *Staphylococcus aureus*
MRSA: methicillin-resistant *Staphylococcus aureus*
Non-Fermenters: non-fermenting Gram-negative rods like *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and Acinetobacter species

Finally, the effects of VAP caused by different pathogens were determined in subgroups of surgical, medical and trauma patients (Table 5). Causative pathogens were grouped into three categories: all cause VAP, VAP caused by groups of pathogens associated with higher risk of mortality (VAPhigh; non-fermenters or polymicrobial VAP) and VAP caused by pathogens belonging to the categories *S. aureus*, *Enterobacteriaceae* or others (VAPlow). In surgical patients the daily risk of mortality increased and the daily risk of discharge decreased for all-cause VAP and for VAPhigh pathogens, yielding combined effects on mortality of 3.80 (95% CI 2.75 to 5.25) and 5.51 (95%CI 3.75 to 8.09), respectively. In surgical patients VAP has, as compared to medical and trauma patients, the largest effect on mortality, irrespective of the category of causative pathogens (as reflected by the SHRs of 3.80, 5.51 and 2.72 for all-cause VAP, VAPhigh

and VAPlow, respectively). In medical patients the higher mortality rate due to VAP results from a decreased daily risk of discharge after VAP was diagnosed in each category. In trauma patients, VAP decreases the daily risk of discharge, without increasing mortality in any of the categories.

Table 5. Results competing risks analyses per admission diagnosis

Subgroup	Patients, N	CSHR Mortality	95% CI	CSHR Discharge	95%CI	SHR mortality	95% CI
Surgical							
VAP	125	1.52	1.09 to 2.13	0.51	0.41 to 0.64	3.80	2.75 to 5.25
VAPhigh	59	2.22	1.50 to 3.29	0.48	0.34 to 0.68	5.51	3.75 to 8.09
VAPlow	62	1.05	0.65 to 1.68	0.55	0.42 to 0.71	2.72	1.69 to 4.37
Medical							
VAP	246	1.20	0.94 to 1.54	0.73	0.63 to 0.84	2.10	1.65 to 2.67
VAPhigh	108	1.32	0.97 to 1.82	0.73	0.59 to 0.90	2.55	1.85 to 3.52
VAPlow	131	1.04	0.74 to 1.48	0.72	0.60 to 0.87	1.82	1.30 to 2.53
Trauma							
VAP	119	0.83	0.46 to 1.51	0.57	0.45 to 0.72	1.69	0.99 to 2.88
VAPhigh	41	0.82	0.35 to 1.91	0.58	0.42 to 0.80	1.77	0.80 to 3.88
VAPlow	75	0.74	0.37 to 1.51	0.58	0.45 to 0.76	1.66	0.86 to 3.22

VAP= All cause VAP
VAPhigh= VAP caused by pathogens belonging to the categories non-fermenters or polymicrobial VAP
VAPlow=VAP caused by pathogens belonging to the categories *Staphylococcus aureus*, *Enterobacteriaceae* or others

DISCUSSION

In this individual patient data meta-analysis of 15 VAP prevention studies marked differences of attributable mortality between different pathogens was demonstrated. The highest risk of mortality was demonstrated among patients with VAP caused by non-fermenters (mainly *P. aeruginosa*) and with polymicrobial VAP, with subdistribution hazard ratios of 3.59 (95% CI 2.71 to 4.74) and 2.77 (95% CI 2.00 to 3.84), respectively. Surgical patients had, as compared to medical patients, higher excess mortality rates, irrespective of the causative pathogens. In trauma patients VAP was not associated with an increased risk of mortality.

There are more studies in which associations between mortality and VAP caused by different pathogens have been evaluated. However, the statistical methods applied had major limitations, sample sizes were small and results are, therefore, difficult to compare with our findings. There are only two studies in which similar statistical techniques were used to account for the timing and duration of exposure and the competing risks caused by informative censoring. Lambert et al(6) estimated the excess mortality of ICU-acquired pneumonia (of which approximately 90% were ventilator associated) caused by different pathogens (*Acinetobacter baumannii* (*A. baumanni*), *E.*

coli, *P. aeruginosa* and *S. aureus*) with and without antimicrobial resistance. Subdistribution hazards, with adjustments for timing of infection and baseline covariates, were calculated for each individual pathogen and combination of pathogens with or without antimicrobial resistance. The SHR for all cause VAP was 2.3 (95%CI 2.1 to 2.5) and 2.8 (95%CI 2.5 to 3.1), for antibiotic sensitive and resistant pathogens, respectively, which is comparable to our estimate of 2.42 (95% CI 2.04 to 2.87) (Table 4). The highest excess mortality of VAP was found for *P. aeruginosa* (ceftazidim resistant) with a SHR of 3.5 (95% CI 2.9 to 4.2), again comparable to the SHR found in this study (Table 4; 3.49 (95% CI 2.53 to 4.80). In that study SHRs of MSSA and MRSA were 1.7 (95% CI 1.4 to 1.9) and 2.1 (95% CI 1.8 to 2.5), respectively, again rather similar to our estimates of 1.71 (95% CI 1.05 to 2.78) and 2.30 (95%CI 0.99 to 5.31) for MSSA and MRSA, respectively. Effects of polymicrobial VAP or VAP caused by Enterobacteriaceae other than *E. coli*, were not evaluated.

Nguile-Makao et al estimated the attributable mortality of VAP in subgroups of pathogens (MSSA, MRSA, ureidopenicillin susceptible and resistant *P. aeruginosa*) using a multistate model (progressive disability model) also accounting for the timing of infection and competing risks (2). Higher mortality rates were found among VAP caused by susceptible *P. aeruginosa*. Also late-onset VAP (i.e. VAP developing after ≥ 8 days), often associated with pathogens comparable to those that were included our group of non-fermenters, was associated with a higher attributable mortality.

The effect of polymicrobial VAP on mortality has been poorly evaluated, although many studies reported the frequent occurrence of polymicrobial VAP(4;5;7-9). Combes et al evaluated the outcome of polymicrobial VAP as compared to monomicrobial VAP in 124 patients, using a chi-square test or the Kaplan-Meier method with log rank test(4). No statistically significant difference in mortality was demonstrated between the two groups.
However, this study is hampered by the statistical methods and had limited power to detect differences.

To our knowledge this is the first study that evaluates mortality of VAP caused by different pathogens in surgical, medical and trauma patients, using appropriate statistical methods. The impact of VAP on excess mortality was most prominent in surgical patients, which could not be explained by differences in causative pathogens, as excess mortality (as compared to medical and trauma patients) was present in all categories of pathogens (Table 5). The underlying mechanisms of increased mortality due to VAP in surgical patients remain to be elucidated.

Some potential limitations of our study should also be discussed. The first limitation is that we had, other than MRSA incidence rates, no data on antibiotic resistance patterns of pathogens. The study by Lambert et al, specifically assessed the role of antimicrobial resistance, which was associated with worse outcome in patients with VAP caused by MRSA and ceftazidim-resistant *P. aeruginosa* (ratios of SHRs resistant versus sensitive of 1.3 (95% CI 1.0 to 1.6) and 1.2 (95% CI 1.0 to 1.5), respectively). No effects of resistance could be demonstrated for VAP caused by *A. baumannii* or *E. coli* (6).

Second, the adequacy of treatment was not evaluated. Especially VAP caused by non-fermenters and polymicrobial VAP might be associated with inadequate treatment. However,

since it was the objective of this manuscript to asses the real-life effect of an episode of VAP with a certain pathogen on mortality, accounting for adequacy of treatment was not necessary.

In conclusion, in this study VAP caused by *P. aeruginosa*, *S. maltophilia* and Acinetobacter species or caused by multiple species was associated with increased mortality, whereas attributable mortality appeared absent in VAP caused by streptococci or *H. influenzae*. Furthermore, surgical patients had the highest mortality risk due to VAP and in trauma patients VAP was not associated with attributable mortality, in both populations irrespective of causative pathogens.

SUPPLEMENT

Table 1. Diagnostic Methods

Study	Clinical criteria	Microbiological cultures
Bergmans	New, persistent or progressive infiltrate on chest X ray and 3 or more of the following: fever or hypothermia, leukocytosis or leukopenia, >10 leukocytes per high power field in gram stain of tracheal aspirate and a positive culture from tracheal aspirate.	Quantitative cultures from BAL ($>10^4$ cfu/ml) or PSB ($>10^3$ cfu/ml) or a positive bloodculture unrelated to another source of infection or a positive culture from pleural fluid in the absence of previous pleural instrumentation.
Camus	CDC criteria	Quantitative culture of a bronchoscopic protected specimen at a concentration of 10^3 cfu/ml for brush or plugged catheter and $>10^4$ cfu/ml for bronchoalveolar lavage
Drakulovic	New and persistent infiltrates on chest radiography and at least two of the following criteria: fever, leucopenia or leucocytosis, purulent tracheal secretions.	Positive culture of tracheobronchial aspirate ($>10^5$ cfu/ml), bronchoalveolar lavage ($>10^4$ cfu/ml) or protected specimen brush ($>10^3$ cfu/ml) in protected specimen brush cultures
Klarin	New and persistent or progressive infiltrate on chest radiograph combined with at least three of the other four criteria: purulent tracheal aspirate; positive culture of tracheal aspirates; fever or hypothermia, leukocytosis or leukopenia.	Positive culture of tracheal aspirate
Krueger	Purulent tracheobronchial secretions, chest radiographic examination with indication of a new or progressive infiltrate, consolidation or cavitation or pleural effusion, increase in the inspiratory oxygen fraction of more than 0.15 necessary to maintain arterial oxygen tension at the same level and at least one of the following symptoms: fever, leukcocytosis, or more than 10% band forms of neutrophil granulocytes.	Microbiological cultures (bloodcultures,tracheobronchial secretions, protected specimen brush, BAL, pleural fluid or lung biopsy) were attempted but not prerequisted, but in all pneumonia cases a causative pathogen was identified (89% with cultures of tracheobronchial secretions).
Lacherade '05	New and persistent infiltrate on chest x ray and two of the following: fever or hypothermia, leukocytosis or leukopenia, and purulent tracheal secretions.	Quantitative culture of specimens obtained using a protected telescoping catheter or broncholalveolair lavage,
Lacherade '10	New and persistent infiltrate on chest X-ray and at least two of the following criteria: fever or hypothermia, leucocytosis or leucopenia, and purulent tracheal secretions.	Quantitative culture of either a protected telescoping catheter sample ($>10^3$cfu/ml) or bronchoalveolar lavage fluid ($>10^4$ cfu/ml).

Table 1 (Continued

Study	Clinical criteria	Microbiological cultures
Lorente 2006	New onset of purulent bronchial sputum, fever or hypothermia, leukopenia or leukocytosis, chest radiograph showing new or progressive infiltrates	Quantitative culture of respiratory by tracheal aspirate ($>10^6$ cfu/ml)
Lorente 2007	New onset of purulent bronchial sputum, fever or hypothermia, leukopenia or leukocytosis, chest radiograph showing new or progressive infiltrates.	Quantitative culture of respiratory secretions by tracheal aspirate ($>10^6$ cfu/ml)
Nardi	New and persistent pulmonary infiltrates, purulent tracheal secretion, fever, leukocytosis or leukopenia, and hypoxaemia (Pa)2/FiO2<250).	A bronchoscopic protected specimen brush together with a distal BAL were performed to confirm the diagnosis.
Pneumatikos	New and persistent pulmonary infiltrates in addition to two of the following criteria: fever, leukocytosis or leukopenia and purulent tracheal secretions.	The diagnosis of VAP was confirmed by quantitative cultures.
Seguin	New pulmonary infiltrates detected by chest radiography plus two of the following: fever or hypothermia, purulent endotracheal aspirate and leukocytosis or leukopenia.	Quantitative culture of bronchoscopic or a non-bronchoscopic "blind"bronchoalveolar lavage ($>10^4$ cfu/ml)
Staudinger	New and persistent radiographic infiltrate on the chest radiograph plus newly developed purulent tracheal secretions plus increasing signs of inflammation (fever, leukocytosis or increasing levels of C-reactive protein)	Positive quantitative culture of BAL ($>10^4$ cfu/ml)
Topeli	New and persistent infiltration on the chest X ray and presence of any two out of three criteria were used; fever or hypothermia, leukocytosis or leukopenia, purulent tracheal secretions or at least 10 leukocytes per high power field in gram's stain of the endotracheal aspirates.	After the clinical diagnosis endotracheal suction cultures were performed.
Valencia	New or progressive pulmonary infiltrates together with at least two of the following: fever or hypothermia, leukocytosis or leukopenia and purulent tracheal secretions.	Positive quantitative culture of protected specimen brush ($>10^3$ cfu/ml) or BAL($>10^4$ cfu/ml) or tracheobronchial aspirates cultures ($>10^5$ cfu/ml), in pleural fluid or bloodcultures without alternative cause of bacteremia .

Table 2. Polymicrobial VAP: combination of pathogens and frequency.

Pathogen 1	Pathogen 2	Pathogen 3	Pathogen 4	Pathogen 5	Patients,n
Staphylococcus aureus	Others	-			17
Staphylococcus aureus	Others	Others			2
Staphylococcus aureus	Enterobacteriaceae	-			11
Staphylococcus aureus	Enterobacteriaceae	Enterobacteriaceae			1
Staphylococcus aureus	Enterobacteriaceae	Enterobacteriaceae	Other	Other	1
Staphylococcus aureus	Enterobacteriaceae	Other			2
Staphylococcus aureus	Enterobacteriaceae	Other	Other		1
Staphylococcus aureus	Non Fermenters				10
Staphylococcus aureus	Non Fermenters	Enterobacteriaceae			3
Staphylococcus aureus	Non Fermenters	Other			2
Enterobacteriaceae	Enterobacteriaceae				2
Enterobacteriaceae	Enterobacteriaceae	Other			1
Enterobacteriaceae	Other				8
Enterobacteriaceae	Other	Other			1
Non Fermenters	Enterobacteriaceae				11
Non Fermenters	Enterobacteriaceae	Enterobacteriaceae			2
Non Fermenters	Enterobacteriaceae	Other			3
Non Fermenters	Other				8
Non Fermenters	Non Fermenters				1
Other	Other				10
Other	Other	Other			1
Staphylococcus aureus	Unknown				1
Non Fermenters	Unknown				1
Other	Unknown				1

Non-Fermenters: non-fermenting Gram-negative rods like *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and Acinetobacter species

REFERENCES

(1) Melsen W.G., Rovers M.M., Groenwold R.H.H., Bergmans D.C.J.J, Camus C., Bauer T.T. et al. The attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomized prevention studies. 2011.
Unpublished Work

(2) Nguile-Makao M, Zahar JR, Francais A, Tabah A, Garrouste-Org, Allaouchiche B et al. Attributable mortality of ventilator-associated pneumonia: respective impact of main characteristics at ICU admission and VAP onset using conditional logistic regression and multi-state models. Intensive Care Med 2010 May;36(5):781-9.

(3) Schumacher M, Wangler M, Wolkewitz M, Beyersmann J. Attributable mortality due to nosocomial infections. A simple and useful application of multistate models. Methods Inf Med 2007;46(5):595-600.

(4) Combes A, Figliolini C, Trouillet JL, Kassis N, Wolff M, Gibert C et al. Incidence and outcome of polymicrobial ventilator-associated pneumonia. Chest 2002 May;121(5):1618-23.

(5) Vidaur L, Planas K, Sierra R, Dimopoulos G, Ramirez A, Lisboa T et al. Ventilator-associated pneumonia: impact of organisms on clinical resolution and medical resources utilization. Chest 2008 March;133(3):625-32.

(6) Lambert ML, Suetens C, Savey A, Palomar M, Hiesmayr M, Morales I et al. Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. Lancet Infect Dis 2011 January;11(1):30-8.

(7) Verhamme KM, De CW, De RL, De BH, Nollet G, Verbeke J et al. Pathogens in early-onset and late-onset intensive care unit-acquired pneumonia. Infect Control Hosp Epidemiol 2007 April;28(4):389-97.

(8) Trouillet JL, Chastre J, Vuagnat A, Joly-Guillou ML, Combaux D, Dombret MC et al. Ventilator-associated pneumonia caused by potentially drug-resistant bacteria. Am J Respir Crit Care Med 1998 February;157(2):531-9.

(9) Ahl J, Tham J, Walder M, Melander E, Odenholt I. Bacterial aetiology in ventilator-associated pneumonia at a Swedish university hospital. Scand J Infect Dis 2010 July;42(6-7):469-74.

CHAPTER 6

The effects of clinical and statistical heterogeneity on the predictive values of results from meta-analyses

W.G.Melsen; M.C.J. Bootsma; M.M. Rovers and M.J.M. Bonten

SUMMARY

Objective

To investigate, using a simulation model, the accuracy of the I^2 statistic in the assessment and quantification of heterogeneity, and how heterogeneity across studies relates to the predictive value of meta-analyses.

Methods

Meta-analyses of randomized controlled trials evaluating a certain intervention were simulated with varying amounts of heterogeneity. The amount of heterogeneity was calculated using the I^2-statistic. The results of the meta-analyses were compared with a study with an infinite amount of patients regarded as a reference for the true effect of the intervention. The results of meta-analyses were considered correct, when the outcome of this large study felt within the 95% confidence area of the preceding meta-analysis. This is repeated 10^7 times to determine associations between the likelihood of predicting the correct estimate of effect, I^2 and the amount of heterogeneity. The findings of the simulations are illustrated by presenting common scenarios including several examples of meta-analyses evaluating different interventions published in the field of critical care medicine.

Conclusion

The crucial importance of study selection (or in other words of minimizing clinical and methodological heterogeneity) for the accuracy of pooled estimates derived from meta-analyses was demonstrated. Quantifying statistical heterogeneity, through I^2-statistics, can be helpful in some scenarios (when the amount of heterogeneity is unknown and I^2 is high), but is of no help in other scenarios (at the extremes of heterogeneity levels and when the amount of heterogeneity is unknown and I^2 is low). Our findings justify a critical appraisal of meta-analyses before accepting their results and underscore the huge responsibility of meta-analysts (and peer reviewers and editors) in adequately performing, interpreting and reporting of meta-analyses.

INTRODUCTION

Meta-analyses have become one of the most widely used methods to quantify effects of medical interventions. In fact, in grading the evidence base of medical practice, a properly designed meta-analysis is considered equally relevant as a large randomized-controlled trial, as one of both is needed to reach so-called level I evidence(1). As such, meta-analyses are generally the starting point, and frequently most prominent component, of guidelines for clinical management. Furthermore, clinicians increasingly use meta-analyses to remain up-to-date, and funding agencies frequently require such an analysis to justify further research. The number of published systematic reviews and meta-analyses has increased substantially in the last decade, also in the field of critical care medicine. A PubMed search in literature using the search terms 'meta-analysis' or 'meta-analyses' and 'critical care' or 'intensive care' yielded totals of 103 publications between 1995 and 2000, 272 between 2000 and 2005, and 613 between 2005 and 2010.

Ideally, a meta-analysis combines the results of several studies, highly comparable in design, intervention and patient population. The individual studies had similar trends in outcome, but lacked sufficient statistical power for a definite conclusion. Yet, in real life, meta-analyses frequently contain multiple, relatively small studies, that differ in many aspects (such as in dosing schedules, duration of follow-up, types of participants, and modes of treatment and diagnosis).

Naturally, studies brought together in a meta-analysis will differ, which is also called "heterogeneity". Generally a distinction is made in clinical heterogeneity (differences in e.g. patients populations, treatment protocol), methodological heterogeneity (differences in study design, risk of bias) and statistical heterogeneity (differences in the outcome of the individual studies, which may be the result of clinical or methodological heterogeneity). A more extensive description of the different types of heterogeneity is available in the supplementary material.

Tests for heterogeneity, like Cochran's Q-statistic and the I^2-statistic, are commonly used in meta-analysis to determine whether there are genuine differences underlying the results of the studies, or whether the variation in findings is compatible with chance alone. The most commonly used test is the I^2 statistic, which expresses the level of heterogeneity as a percentage and can be compared across meta-analyses with different sizes and outcomes. Generally heterogeneity is regarded as being low, moderate and high with upper limits of 25%, 50% and 75% for I^2, respectively (2).

The appraisal of the similarity of studies with regard to clinical and methodological heterogeneity and the ultimate decision whether to include (or exclude) a certain study in a meta-analysis is the sole responsibility and therefore totally dependent on the meta-analysts. As there are no criteria to quantify clinical and methodological heterogeneity this appraisal is subjective. Although the quantification of statistical heterogeneity seems to be more objective (for instance by calculating the I^2 value) the predictive value of this test for the accuracy of the estimate derived from the meta-analysis is unknown. Furthermore, there is no uniform approach to deal with heterogeneity. Multiple strategies have been proposed (3) and there are many examples of meta-analyses being performed in the presence of substantial

heterogeneity. In this study we investigated, using a simulation model, the accuracy of the I^2 statistic in the assessment and quantification of heterogeneity, and how heterogeneity across studies relates to the predictive value of meta-analyses. We illustrate and clarify our findings by presenting common scenarios including several examples of meta-analyses evaluating different interventions published in the field of critical care medicine.

METHODS

Meta-analyses of randomized controlled trials evaluating a certain intervention were simulated with varying amounts of heterogeneity, making the following assumptions. We assume an intervention with 25% efficacy (for instance mortality reduction), and the outcome occurs in 15% of the population when the intervention is performed (and thus in 20% without intervention). Because of heterogeneity due to chance the 95% margin of uncertainty around this 15% is assumed to be 14% (95% confidence interval ranging from 8% to 22%). However, when there is systematic (statistical) heterogeneity, this 95% margin can increase to widths of 20% (ranges from 5% to 25%), although the average reduction remains 15%. The amount of systematic heterogeneity is expressed by σ, which is 0 in the absence of systematic heterogeneity. Monte-Carlo simulations are used to perform multiple meta-analyses, each including ten studies with two groups of 100 patients. For each simulated study, the expected mortality (m) in the control group of the study is 0.20 and the expected mortality in the intervention arm of the study is drawn from a beta distribution with a mean of 0.15 and with variance of σ. Given this expected mortality, the outcome (mortality) of the 100 patients in each study arm is randomly determined by sampling 100 times from a Bernoulli distribution with parameter m, and relative risks are calculated from outcomes of patients in control and intervention groups. This is performed ten times, followed by a meta-analysis of these ten (simulated) studies with calculation of a pooled estimate with 95% confidence interval and I^2 using a fixed effects model (Mantel-Haenszel method) and a random effects model (Mantel-Haenszel method). Then, another study is picked from the same distribution, but this eleventh study has an infinite number of patients, and, therefore can be considered as a reference for the true effect of the intervention. Finally, we determine whether the outcome of this eleventh study falls within the 95% confidence area of the preceding meta-analysis. If so, the meta-analysis would have predicted a correct estimate of the intervention. This is repeated 10^7 times to determine associations between the likelihood of predicting the correct estimate of effect, I^2 and the amount of heterogeneity (σ).

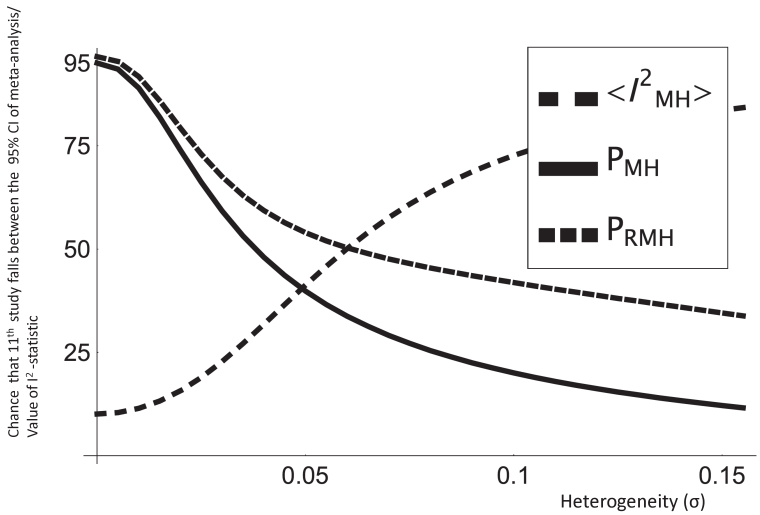


Figure 1. Results Monte Carlo Simulation Study
The larger the I^2-statistic, the lower the likelihood that the result of the 11th trial will fall within the 95% CI of the meta-analysis.
I^2MH= I^2-statistic
MH=calculations based on fixed effects model using the Mantel-Haenszel method
RMH= calculations based on random effects model using the Mantel-Haenszel method

RESULTS

In a series of figures (Figures 1-3) we have depicted the associations between the accuracy of estimates derived from meta-analyses (on the vertical axes as the likelihood that the estimate is correct, i.e., the chance that the result of 11th study falls within the 95% confidence interval of the meta-analysis estimate) and increasing amounts of heterogeneity (σ) on the x-axes. As expected I^2 increases and the likelihood to draw correct inferences from a meta-analysis decreases with increasing heterogeneity (Figure 1). With a random effects model chances of correct estimates are higher, especially when heterogeneity increases (Figure 1).
Surprisingly, though, in case of low levels of heterogeneity (σ close to 0) the I^2 value appears not predictive for the accuracy of the meta-analysis result. With low amounts of heterogeneity (σ close to 0%) even meta-analyses with I^2 >75% yield highly accurate results (Figure 2). Vice versa, with high levels of heterogeneity even meta-analyses with low I^2 values are associated with low predictive values of results (Figure 2).

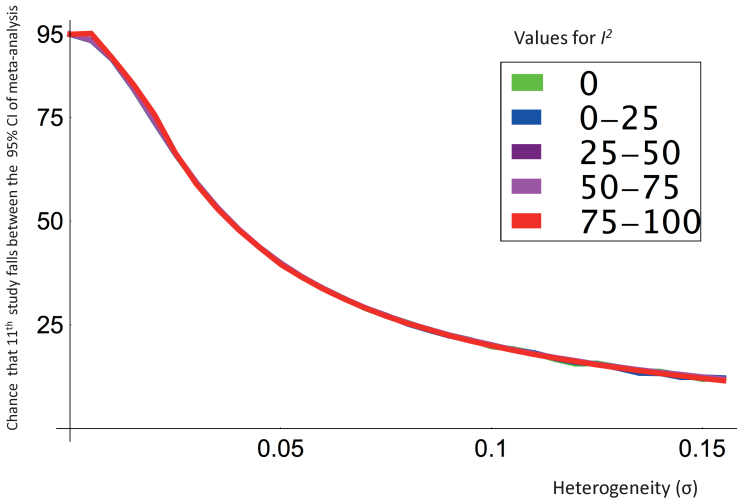


Figure 2. Results Monte Carlo Simulation Study: Fixed effects analysis
Chance that, for a certain value of heterogeneity (x-axis) and a certain calculated I^2 statistic, the 11th study falls between the 95% confidence interval of the meta-analysis.

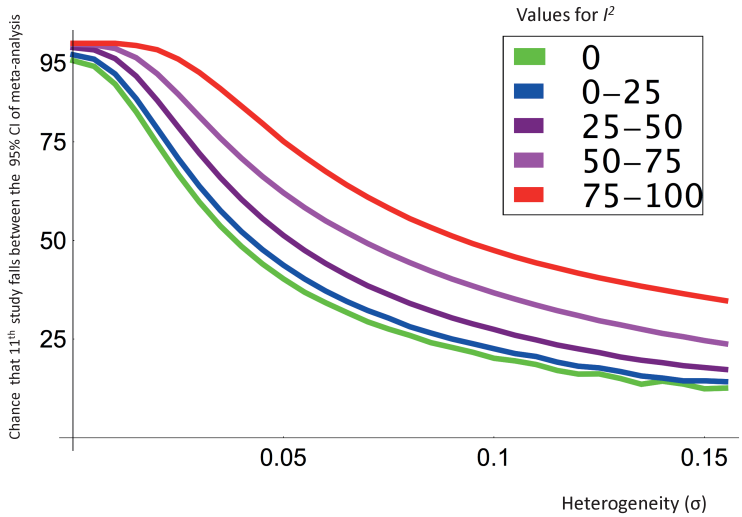


Figure 3. Results Monte Carlo Simulation Study: Random effects analysis
Chance that, for a certain value of heterogeneity (x-axis) and a certain calculated I^2 statistic, the 11th study falls between the 95% confidence interval of the meta-analysis. Calculations based on a random effects meta-analysis.

With a random effects model the width of the 95% confidence intervals increases with increasing I^2 statistic, which increases the likelihood of that the 11th study result falls within the confidence interval limits (Figure 3). Yet, the likelihood to obtain low I^2 values also depends on the amount of heterogeneity (Figure 4). In the absence of any heterogeneity (a pure theoretical

option) the chance of finding a high I^2 statistic (>50%) is very low, but this rapidly increases with increasing levels of heterogeneity. But even with high heterogeneity (σ large) low I^2 values can be derived. All simulations were repeated using an Inverse variance method (instead of the Mantel-Haenszel method) leading to identical results

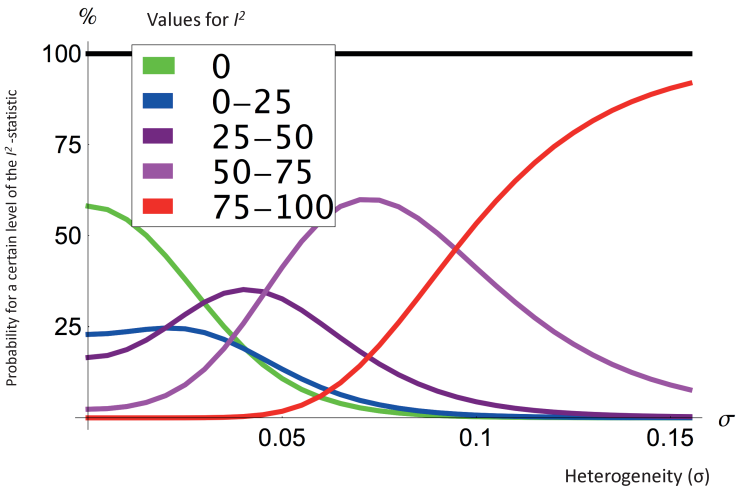


Figure 4: Results Monte Carlo Simulation Study: Chance to observe a certain level of the I^2 statistic

How do these results relate to the daily practice of performing meta-analyses? We propose six different scenarios, depending on the amount of expected methodological and clinical heterogeneity and the results of I^2-statistics, that may occur in the preparation of a meta-analysis (Figure 5).

Scenario 1 and 2: These scenarios relate to situations in which, due to differences between studies, large amounts of heterogeneity can be expected. Calculated I^2 values can be low (scenario 1) or high (scenario 2). As shown in Figure 4, the I^2 value can be low (scenario 1), but still be associated with a low predictive value of the accuracy of the meta-analysis result (Figure 2). In this scenario the low I^2 value may create a false-positive signal of low heterogeneity. A high I^2 value (scenario 2) is intuitively correct, considering the obvious amount of clinical and methodological heterogeneity, and only confirms what was already expected. Also in this scenario the accuracy of the meta-analysis result will be low.

Scenario 3 and 4: These scenarios relate to the (rare) situation where, based on the similarity of the included studies, a low amount of heterogeneity is expected. The chance of obtaining a high I^2 value (scenario 4) is low (Figure 4), but even if so the accuracy of the meta-analysis result will be equally high as with low I^2 values (scenario 3) (Figure 2). Therefore, with extremely low levels of heterogeneity I^2 values are not informative for the accuracy of meta-analyses.

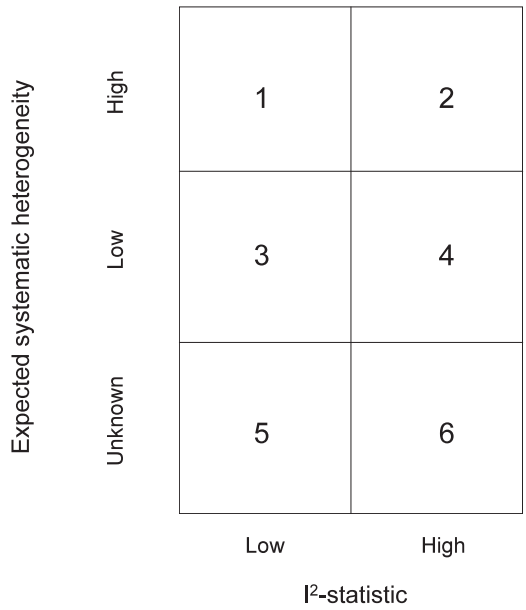


Figure 5. Clinical Scenarios

Scenario 5 and 6: These scenarios relate to (probably frequent) situations in which differences between studies exist, but where the impact of these differences on the pooled estimates are unknown. Especially in such situations a reliable statistical method to quantify the amount of heterogeneity is needed. In our simulation studies high I^2 values (>75%) (scenario 6) are predictive for the presence of heterogeneity (Figure 4) and low predictive values of estimates derived (Figure 2). Yet, low I^2 values (scenario 5) correspond to a wide range of systematic heterogeneity levels, and, thus, to a high level of uncertainty about the predictive value of meta-analysis results. Even when $I^2=0$ systematic heterogeneity can exist, which will reduce the reliability of the pooled estimate.

We illustrate only the first two scenarios, as scenarios 3 and 4 are scarce and scenarios 5 and 6 unknown, with two clinical examples of meta-analyses published in the field of critical care medicine.

Clinical example I

The effectiveness of Selective Decontamination of the Digestive Tract (SDD) has been evaluated in several meta-analyses (Table 1) (4-10). These meta-analyses included different studies, partly because not all studies were available at the time of preparation or because different selection criteria were applied. There were also differences in the aggregate data used per study; some meta-analyses preferably used intention to treat data, while others preferably used the data of patients with a length of stay of at least 48 hrs. Also some authors used the hospital mortality

data when available, while others only used ICU mortality. Despite these differences the pooled estimate of efficacy of SDD in reducing mortality remained more or less stable with odds ratios around 0,80, being statistically significant in the most recent analyses. Silvestri et al(9) summarized the characteristics of the 30 studies included in their meta-analysis. Mortality in control patients ranged from 3% to 58% (average is 25% with standard deviation (SD) of 15%), the methodological study quality ranged (on a scale of 16) from 6 to 14 (average is 9 $\pm$5), eleven different patient populations were studied, eleven different intravenous medications were tested (including no prophylaxis), eleven of 30 studies used intravenous prophylaxis in control patients, two studies evaluated oropharyngeal decontamination only and three evaluated intestinal decontamination only. Because of these differences, we firmly believe that there is considerable heterogeneity between studies. Yet even with all these differences the calculated I^2 in this meta-analysis is 0%, and similarly low as for the other meta-analyses. More recently, the effects of SDD on patient outcome were determined in a multicenter trial including more patients (n=5939) than in all studies included in the most recent meta-analysis (11). In this multicenter study SDD was, as compared to standard care, associated with 13% reduction in day-28 mortality, which corresponded to an adjusted odds ratio of 0.83. This result was remarkably similar to the results obtained in previous meta-analyses. Thus, despite obvious methodological and clinical differences between individual studies, the meta-analyses seemed to have accurately predicted the effects of SDD.

Table 1. Meta-analyses published evaluating selective digestive decontamination

Author	Year	No. of studies	Odds ratio	I2
Vandenbroucke-Grauls	1991	7	0.70 (0.45-1.09)	0%
SDD CTG	1993	15	0.80 (0.67-0.97)	0%
Heyland	1994	24	0.83 (0.71-0.98)	0%
Kollef	1994	16	0.88 (0.72-1.08)	0%
Hurley	1995	26	0.86 (0.74-0.99)	5%
D'Amico	1998	17	0.80 (0.69-0.93)	10%
Silvestri	2007	30	0.80 (0.69-0.94)	0%

Pooled odds ratio's as provided in the meta-analysis or calculated using the information in the manuscript of the meta-analyses. I^2 when unavailable was calculated using the chi squared statistic and degrees of freedom. The meta-analysis of SDD CTG was updated, here only the results of the first publication is provided.

Clinical example II

In another meta-analysis the effects of weaning protocols on duration of mechanical ventilation in critically ill adult patients was determined (12). Eleven studies, both randomized and quasi randomized controlled trials, were selected, evaluating 1,971 patients admitted to 7 different types of intensive care units. Only two studies used the same weaning protocol and also the "usual care" in the control group compromised a wide variety of practices. The authors

used fixed effects models for meta-analysis, and a random effects model in case of statistical heterogeneity (defined as I^2 statistic >50% and/or chi-square statistic p<0.05). The primary outcome was the duration of mechanical ventilation with and without weaning protocol, which was estimated (with random effects model) as mean log -0.29 (95% CI -0.5 to -0.09). However a substantial amount of heterogeneity was quantified with the I^2 statistic (I^2=76%). Subgroup analyses to assess the impact of type of ICU were small (two to four studies) and did not reduce heterogeneity as indicated with the statistical test. Several secondary outcomes were tested, no heterogeneity was indicated (I^2 was estimated as low) in the analyses concerning hospital mortality (pooled estimate 1.10 (95% CI 0.86 to 1.41, I^2=0%, p=0.46) and length of stay in de ICU (pooled estimate -0.11 (95% CI -0.21 to -0.02 I^2=0%, p=0.45), and marked heterogeneity was indicated in the analyses of ICU mortality (0.98 (95% CI 0.48 to 2.02) I^2=57, p=0.07) and duration of weaning (-1.52 (95% CI -2.66 to -0.37) I^2=97%, p<0.001). The authors concluded that "compared with usual care, use of weaning protocols can reduce the duration of mechanical ventilation by 25%, weaning duration by 78% and length of stay in the ICU by 10%. As there was significant heterogeneity in included trials and most were conducted in the US, these findings might not be generalisable." Indeed, heterogeneity was expected to be high in this meta-analyses (due to differences in intervention, control groups, patient population), which was confirmed by a high I^2 value in many analyses, thereby resembling scenario 2. The estimates obtained should, therefore, be interpreted with extreme caution.

DISCUSSION

In this study we have demonstrated the crucial importance of study selection (or in other words of minimizing clinical and methodological heterogeneity) for the accuracy of pooled estimates derived from meta-analyses. Quantifying statistical heterogeneity, through I^2-statistics, can be helpful in some scenarios (when the amount of heterogeneity is unknown and I^2 is high), but is of no help in other scenarios (at the extremes of heterogeneity levels and when the amount of heterogeneity is unknown and I^2 is low). Our findings justify a critical appraisal of meta-analyses before accepting their results and underscore the huge responsibility of meta-analysts (and peer reviewers and editors) in adequately performing, interpreting and reporting of meta-analyses.

The reliability of I^2 statistics in quantifying levels of heterogeneity has been questioned before, albeit without determination of its association with estimate accuracy. Huedo-Medina et al (13) demonstrated that the I^2 statistic suffers from low statistical power, potentially revealing misleading results, when the number of studies is small. Ioaniddis et al (14) emphasized that like any metric I^2 has some uncertainty that can be expressed in 95% confidence intervals. In Cochrane meta-analyses with I^2 values ≤ 25%, 83% of these values had upper 95% confidence intervals that crossed into the range of large heterogeneity (≥50%). Even when I^2 was 0%, 81% had confidence intervals exceeding 50%. Yet, these intervals are still rarely provided.

As meta-analyses have become so important in evidence based medicine, their results

should be reliable and accurate. Our findings demonstrate that heterogeneity importantly influences both aspects. As of yet, there are no reliable methods to quantify the amount of clinical and methodological heterogeneity and careful selection of appropriate studies is the only tool to derive correct inferences from meta-analyses. Unfortunately, this selection will always be, at least to some extent, subjective. Our findings demonstrate that determination of I^2 is of little value at the extremes of heterogeneity, and it would be helpful to derive criteria for categorizing meta-analyses into either low or high levels of clinical and methodological heterogeneity. The consequence would be that meta-analyses with high levels of clinical and methodological heterogeneity should not provide a pooled estimate (as the level of accuracy will always be low, regardless of I^2) and that meta-analyses with low levels of heterogeneity should not provide an estimate of I^2. In real life, though, levels of heterogeneity of most meta-analyses will be unknown. In such scenarios I^2 determination may help to identify estimates with low predictive values (high I^2), for which we recommend not to provide pooled estimates. With low I^2 values and unknown levels of clinical and methodological heterogeneity predictive values of pooled estimates may range extensively, and findings should be interpreted with caution. Objective methods to quantify the levels of clinical and methodological heterogeneity are urgently needed to allow reliable determination of the accuracy of meta-analyses. Until that time we propose that investigators describe the pre-test likelihood of clinical and methodological heterogeneity and carefully discuss the potential effects on study results.

SUPPLEMENTARY MATERIAL: HETEROGENEITY

Heterogeneity across studies includes all differences between individual studies related to, amongst others, study design, populations included, treatment strategies and outcomes. For simplicity we distinguish two types of heterogeneity: "due to chance" and "systematic".

Even when using the strictest selection criteria for study inclusion, it is impossible to avoid some kind of heterogeneity between studies performed under different conditions. In fact, even in the hypothetical situation that a single study would have been executed multiple times under exactly the same conditions, the outcome would, due to chance events, not be exactly the same for each evaluation. In addition to this, unavoidable, heterogeneity due to chance, there is a possibility of heterogeneity due to systematic differences between the studies, such as differences in study design, patient populations, diagnostic methods, application of interventions, or definitions of outcome. Some level of heterogeneity can be avoided by using strict criteria of study selection, based on design (i.e., only double-blind randomized trials instead of any randomized trial), populations (only mechanically ventilated trauma patients instead of all types of mechanically ventilated patients) and outcomes (i.e., only day 28 mortality instead of mortality measured at different time points). Therefore, although heterogeneity can be avoided to some extent, it can never be prevented completely. Yet, the predictive value of meta-analyses is unknown in case of systematic heterogeneity.

Several methods have been proposed for quantification of heterogeneity in meta-analysis (3). Such a test examines the null hypothesis that all studies have evaluated the same effect. Cochran's Q reflects the sum of the squared deviations of study's estimate from the overall pooled estimate, weighing each study's contribution in the same way. P values are obtained by comparing the statistic with a χ^2 distribution with k-1 degrees of freedom (where k is the number of studies). Yet, this test is poor in detecting true heterogeneity, especially when dealing with small numbers of studies.

More recently, the quantity I^2 has been proposed as a better measure to quantify heterogeneity (2). I^2 reflects the percentage of total variation across studies that is due to heterogeneity rather than chance, and is calculated using Cochran's Q as 100% x (Q-df)/Q. Negative values for I^2 are considered as 0%, which indicates no observed heterogeneity. Heterogeneity can be quantified as low, moderate and high with upper limits of 25%, 50% and 75% for I^2, respectively. Calculation of I^2 has now become the standard way of reporting heterogeneity in all Cochrane reviews (2;3). Interestingly, I^2 is almost always reported as a single value without 95% confidence area, although these areas can be wide, already demonstrating the inherent uncertainty of this value(14). It is neither possible to quantify the exact level of heterogeneity across studies nor to distinguish the contribution of chance and systematic heterogeneity.

Adequately dealing with heterogeneity is often difficult, although some guidelines are provided (3), many different approaches are undertaken. Particularly concerning the assessment of when heterogeneity is to much for a meaningful meta-analysis as well as the choice of a particular model to calculate the pooled estimate. Concerning the latter two models are used (3): 1) the fixed effect model which assumes that all the included studies are estimating the true effect and that variation in findings among the studies is therefore due to chance only. 2) A

random effects model which assumes that the effects estimated in the different studies follow a distribution. The between trial variance is added to the within variance, resulting in a wider confidence interval of the random effects pooled estimate as compared to the fixed effects pooled estimate.

REFERENCES

(1) Harbour R, Miller J. A new system for grading recommendations in evidence based guidelines. BMJ 2001 August 11;323(7308):334-6.

(2) Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003 September 6;327(7414):557-60.

(3) Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org. 2011.

(4) Meta-analysis of randomised controlled trials of selective decontamination of the digestive tract. Selective Decontamination of the Digestive Tract Trialists' Collaborative Group. BMJ 1993 August 28;307(6903):525-32.

(5) D'Amico R, Pifferi S, Leonetti C, Torri V, Tinazzi A, Liberati A. Effectiveness of antibiotic prophylaxis in critically ill adult patients: systematic review of randomised controlled trials. BMJ 1998 April 25;316(7140):1275-85.

(6) Heyland DK, Cook DJ, Jaeschke R, Griffith L, Lee HN, Guyatt GH. Selective decontamination of the digestive tract. An overview. Chest 1994 April;105(4):1221-9.

(7) Hurley JC. Prophylaxis with enteral antibiotics in ventilated patients: selective decontamination or selective cross-infection? Antimicrob Agents Chemother 1995 April;39(4):941-7.

(8) Kollef MH. The role of selective digestive tract decontamination on mortality and respiratory tract infections. A meta-analysis. Chest 1994 April;105(4):1101-8.

(9) Silvestri L, van Saene HK, Milanese M, Gregori D, Gullo A. Selective decontamination of the digestive tract reduces bacterial bloodstream infection and mortality in critically ill patients. Systematic review of randomized, controlled trials. J Hosp Infect 2007 March;65(3):187-203.

(10) Vandenbroucke-Grauls CM, Vandenbroucke JP. Effect of selective decontamination of the digestive tract on respiratory tract infections and mortality in the intensive care unit. Lancet 1991 October 5;338(8771):859-62.

(11) de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS et al. Decontamination of the digestive tract and oropharynx in ICU patients. N Engl J Med 2009 January 1;360(1):20-31.

(12) Blackwood B, Alderdice F, Burns K, Cardwell C, Lavery G, O'Halloran P. Use of weaning protocols for reducing duration of mechanical ventilation in critically ill adult patients: Cochrane systematic review and meta-analysis. BMJ 2011;342:c7237.

(13) Huedo-Medina TB, Sanchez-Meca J, Marin-Martinez F, Botella J. Assessing heterogeneity in meta-analysis: Q statistic or I2 index? Psychol Methods 2006 June;11(2):193-206.

(14) Ioannidis JP, Patsopoulos NA, Evangelou E. Uncertainty in heterogeneity estimates in meta-analyses. BMJ 2007 November 3;335(7626):914-6.

CHAPTER 7

Selective Decontamination of The Digestive Tract and Oropharynx in Surgical versus Non-surgical Intensive Care Patients enrolled in a Cluster-randomised Clinical Trial

W.G. Melsen; A.M.G.A. de Smet; J.A.J.W. Kluytmans and M.J.M. Bonten
on behalf of the Dutch SOD-SDD Trialists' Group

SUMMARY

Background

Selective Digestive Decontamination (SDD) and Selective Oropharyngeal Decontamination (SOD) are effective in improving survival in patients under intensive care. In this study possible differential effects in surgical and non-surgical patients are investigated

Methods

A post-hoc subgroup analysis of data from a cluster randomized multicenter trial comparing three groups (SDD, SOD or standard care) to quantify effects among surgical and non-surgical patients.. The primary study outcome was mortality at day 28. Duration of mechanical ventilation, duration of ICU- and, hospital length of stay and bacteremia rates were secondary outcomes.

Results

The subgroup analyses compromised a total of 2762 surgical patients and 3165 non-surgical patients. Compared to standard care, adjusted odds ratios (ORs) for mortality were comparable in SDD-treated surgical and non-surgical patients (OR 0.86, 95% Confidence Interval (CI) 0.69 to 1.09) and OR 0.85 (95% CI 0.70 to 1.03), respectively, but durations of mechanical ventilation, ICU-stay and hospital stay were significantly reduced in surgical patients only. SOD did not reduce mortality (adjusted OR 0.97 (95% CI 0.77 to 1.22) in surgical patients, but reduced mortality in non-surgical patients (adjusted OR 0.77 (95% CI 0.63 to 0.94)) by 16.6%, for an absolute mortality reduction of 5.5% with number needed to treat of 18. In patients receiving SOD, incidences of ICU-acquired bacteremia were comparable for surgical and non-surgical patients.

Conclusion

Subgroup analysis found equal effects of SDD in reducing mortality in surgical and non-surgical ICU patients, whereas SOD was only effective in non-surgical patients. The hypothesis-generating findings mandate investigation into mechanisms between different ICU populations.

INTRODUCTION

Nosocomial infections frequently occur in critically ill patients which cause added morbidity and even mortality in the intensive care unit (ICU). Selective decontamination of the digestive tract (SDD) is a frequently studied method aimed to prevent infections acquired in the ICU (1;2). SDD consists of an oral paste containing non-absorbable antibiotics (e.g. polymyxin E, tobramycin and amphotericin B) which is applied in the oral cavity, application of a suspension with the same antibiotics in the gastrointestinal tract and a short course of systemic antibiotics. Selective Oropharyngeal Decontamination (SOD), in which the same topical antibiotics are applied in the oropharynx only, is considered as an alternative, especially for preventing ventilator-associated pneumonia (VAP).

Multiple trials have evaluated the effects of SDD and SOD, with beneficial effects on infection rates being demonstrated in many studies (1;2), while improved survival rates are documented in three studies for SDD (3-5) and in one for SOD(3). In a recent study both SDD and SOD were associated with a significant relative reduction of day 28 mortality, of 13% and 11% respectively, as compared to standard care in a mixed ICU population of 5939 patients (3).

The question remains if this overall effect is different in certain subgroups of patients. Results from a meta-analysis (6) suggest that surgical ICU patients might benefit more from SDD than medical ICU patients. It is also unknown whether surgical and non-surgical patients benefit differently from SDD or SOD. The present post hoc analysis of a recent multicenter trial (3) was conducted to determine the effects of SDD and SOD in surgical and non-surgical ICU patients.

METHODS

Study Design

This study uses the data of a large open-label clustered group-randomized controlled cross-over study of the effect of SDD and SOD on mortality at day 28 in 13 ICUs in the Netherlands. Details of this study are described elsewhere (3). In short, 5939 patients with an expected duration of intubation >48 hours and/or an expected ICU stay >72 hours were enrolled. In each of the 13 participating ICUs, the three regimens (SDD, SOD and standard care) were applied during 6 months in random order. SOD-treated patients received oropharyngeal application (every 6 h) of a paste containing polymyxin E, tobramycin and amphotericin B each in a 2% concentration. In SDD-treated patients, administration (every 6 h) of a 10 ml suspension containing 100 mg polymyxin E, 80 mg tobramycin and 500 mg amphotericin B via the nasogastric tube was added and cefotaxime (1000mg, every 6 h) was administered intravenously during the first four days of the study. Topical antibiotics were applied until ICU-discharge. The surgical or non-surgical status of a patient was determined by the attending ICU physician at admission. Patients were defined as surgical patients when they were admitted post-operatively and/or due to surgical conditions. Patients of whom the admitting specialism was surgery, cardiothoracic surgery or neurosurgery but that were admitted for non-surgical conditions without prior surgery, were regarded as non surgical patients.

Outcomes

The primary study outcome was mortality at day 28. Duration of mechanical ventilation, duration of ICU- and hospital length of stay and bacteremia rates were secondary outcomes.

Statistical analysis

The statistical analysis was performed using SPSS (SPSS, Inc., Chicago, IL.). Our analysis focused solely on the possible interaction between surgical status and SDD or SOD, no other subgroup analyses were conducted.

The existence of an interaction between surgical status (i.e. surgical patient yes/no) and SDD or SOD on the outcome mortality at day 28 was formally evaluated using logistic regression analyses incorporating terms for SDD or SOD, surgical status and the interaction between SDD or SOD and surgical status. Because of baseline differences between patients receiving SDD or SOD and standard care (3), we adjusted for all available covariates (APACHE >20, Age >65, mechanical ventilation and gender). A Cox proportional-hazards analysis, with all observations censored at day 28, was conducted to evaluate the interaction between surgical status and SDD or SOD regarding time to cessation of ventilation, ICU discharge and hospital discharge. Patients who died were considered to have infinite times to cessation of ventilation and discharge, since deaths will lead to informative censoring and act in the opposite direction to any positive effect of the interventions on these outcomes. To quantify the effect of SDD and SOD among surgical and non-surgical patients regarding the different endpoints, separate analyses for all endpoints were performed. ICU clustering effects were not taken into account since cluster effects were not found in earlier analyses and would therefore not change the results.(3) To calculate the number needed tot treat (NNT) we used the following formula NNT = (1/((OR-1) x UER)) + (OR/((OR-1) x (1- UER))), where OR>1 is the adjusted OR, and UER is the unexposed event rate. If OR<1,the corresponding number needed to treat= -NNT(7).

Table 1. Baseline characteristics of the surgical and non-surgical patients

	Surgical			Non-Surgical		
Variable	Standard N=973	SDD N=923	SOD N=866	Standard N=1016	SDD N=1111	SOD N=1038
Age	62.6±16.0	64.0±15.3*	63.4±16.0	60.2±16.3	61.1±16.2	59.8±16.4
Male sex (%)	617(63.4)	588(63.7)	560(64.7)	603(59.4)	655(59.0)	653(62.9)
Mean APACHE II score	17.1±7.3	17.7±6.9*	17.6±7.6	20.1±8.1	21.2±8.2*	21.1±8.4*
Mechanical ventilation	899(92.4)	885(95.9)*	835(96.4)*	854(84.1)	1005(90.5)*	958(92.3)*
Previous or pre-existent disorders						
Cardiovascular	526(54.1)	550(59.6)*	474(54.7)	450(44.3)	481(43.3)	425(40.9)
Pulmonary	221(22.7)	214(23.2)	149(17.2)*	268(26.4)	316(28.4)	299(28.8)
Diabetes Mellitus	142(14.6)	129(14.0)	138(15.9)	160(15.7)	152(13.7)	136(13.1)

	Surgical			Non-Surgical		
Variable	Standard N=973	SDD N=923	SOD N=866	Standard N=1016	SDD N=1111	SOD N=1038
Acute renal failure	29 (3.0)	28 (3.0)	25 (2.9)	50(4.9)	44 (4.0)	46 (4.4)
Chronic renal failure	52 (5.3)	73 (7.9)*	66 (7.6)*	67(6.6)	82(7.4)	69(6.6)
Malignancy solid organ	116(11.9)	139(15.1)	116(13.4)	80 (7.9)	81 (7.3)	77 (7.4)
Metastasized malignancy	35 (3.6)	33 (3.6)	25 (2.9)	29 (2.9)	38(3.4)	31 (3.0)
Haematological malignancy	10 (1.0)	9 (1.0)	6 (0.7)	38(3.7)	47 (4.2)	45(4.3)
Immunodepression/ AIDS	15 (1.5)	8 (0.9)	11 (1.3)	32 (3.1)	52 (4.7)	36 (3.5)
Alcohol and/or drug abuse	42 (4.3)	35 (3.8)	33 (3.8)	69 (6.8)	77 (6.9)	87 (8.4)
Specialism						
Surgery	489(50.3)	470(50.9)	440(50.8)	120(11.8)	135(12.2)	111(10.7)
Cardiothoracic surgery	293(30.1)	319(34.6)	255(29.4)	28(2.8)	34(3.1)	29(2.8)
Neurosurgery	92 (9.5)	59 (6.4)	80 (9.2)	53(5.2)	46(4.1)	60(5.8)
Neurology	13(1.3)	5 (0.5)	10(1.2)	115(11.3)	119(10.7)	134(12.9)
Medical	21 (2.2)	10 (1.1)	26 (3.0)	372(36.6)	372(33.5)	345(33.2)
Cardiology	10 (1.0)	8 (0.9)	8 (0.9)	119(11.7)	151(13.6)	139(13.4)
Pulmonology	5 (0.5)	6 (0.7)	5 (0.6)	122(12.0)	146(13.1)	133(12.8)
Other	50 (5.1)	46 (5.0)	41 (4.7)	87(8.6)	107(9.6)	85(8.2)
Patient admitted to ICU from						
Emergency room	140 (14.4)	130 (14.1)	134 (15.9)	325 (32.0)	379 (34.1)	341 (32.9)
Other ICU in the Netherlands	44 (4.5)	44 (4.7)	36 (4.2)	72 (7.1)	91 (8.2)	85 (8.2)
Nursing ward	519 (53.3)	521 (56.1)	482 (55.7)	424 (41.7)	440 (39.6)	433 (41.7)
Other	270 (27.7)	228 (24.7)	214 (24.7)	195 (19.2)	201 (18.1)	179 (17.2)

*P<0.05 as compared to standard care (calculated using the Mann Whitney U test (continuous variables) or chi square test (dichotomous variables))

RESULTS

In total 5.939 patients were included in the trial; data about the surgical status of 12 patients were missing and these were excluded from our subgroup analysis. There were 2762 surgical patients; 973 received standard care, 866 received SOD and 923 SDD. Of the 3165 non-surgical patients; 1016 received standard care, 1038 received SOD and 1111 received SDD. All 616

patients (19.5%), of which the admitting specialism was surgery, cardiothoracic surgery or neurosurgery (table 1), were admitted for non-surgical conditions without prior surgery and thus were regarded as non-surgical patients. Overall, patients in the SOD and SDD treatment groups were slightly older, had higher APACHE II scores and were more frequently ventilated compared to patients treated in the control period (table 1). There were no significant differences in baseline characteristics between patients receiving SDD and SOD.

PRIMARY OUTCOME

Surgical patients

The crude mortality rates at day 28 were 21.6%, 20.8% and 22.6% for the surgical patients in the standard care, SDD and SOD group, respectively. After adjustment for baseline differences in age, APACHE II scores, proportion being ventilated and gender, ORs were 0.86 (95% Confidence Interval (CI) 0.69 to 1.09) for SDD and 0.97 (95% CI 0.77 to 1.22) for SOD (table 2). There was no significant interaction between surgical status and SDD or SOD regarding mortality. The adjusted OR for day 28 mortality for SDD versus SOD for surgical patients was 0.88 (95% CI 0.70 to 1.11).

Non-surgical patients

Among the non-surgical patients crude mortality rates at day 28 were 33.2%, 31.7% and 30.0% for the standard care, SDD and SOD groups, respectively, with adjusted ORs of 0.85 (95% CI 0.70 to 1.03) for SDD and 0.77 (95% CI 0.63 to 0.94) for SOD (table 2). Of note, the adjusted ORs for SDD were almost similar among surgical and non-surgical patients (0.86 and 0.85, respectively), but differed extensively between surgical and non-surgical patients receiving SOD (0.97 and 0.77, respectively). The OR of 0.77 for mortality in non-surgical patients receiving SOD (as compared to patients receiving standard care with a mortality rate of 33.0%) equals a relative mortality reduction of 16.6%, an absolute mortality reduction of 5.5% with a number needed to treat of 18. The adjusted OR for day 28 mortality for SDD versus SOD for non-surgical patients was 1.09 (95% CI 0.90 to 1.32).

Secondary outcomes

The duration of mechanical ventilation, ICU and hospital length of stay were, after adjustment for covariates, significantly reduced in surgical patients receiving SDD with Hazard Ratio's of 1.15 (95% CI 1.03 to 1.28), 1.15 (95% CI 1.03 to 1.28) and 1.17 (95% CI 1.01 to 1.35) (table 2). SOD had no apparent effects on any of the secondary endpoints in surgical patients. In non-surgical patients, though, SOD was associated with a significant reduction in hospital stay (HR 1.18 (95% CI 1.02 to 1.37). In the non-surgical subpopulation SDD was not associated with significant reductions on any secondary outcome.

Table 2. Primary and Secondary Endpoints

	Standard Care	SDD	SOD
Surgical patients			
N	973	923	866
Mortality at day 28			
N (%)	209 (21.6)	191 (20.8)	194 (22.6)
Unadjusted Odds Ratio	1	0.96 (0.77 to 1.19)	1.06 (0.85 to 1.32),
Adjusted Odds Ratio[*]	1	0.86 (0.69 to 1.09)	0.97 (0.77 to 1.22),
Cessation of mechanical ventilation			
Median (IQR) [†]	7 (13)	6 (9)	8 (11)
Unadjusted Hazard Ratio	1	1.13 (1.02 to 1.27)	1.01 (0.90 to 1.13)
Adjusted Hazard Ratio[#]	1	1.15 (1.03 to 1.28)	1.00 (0.90 to 1.12)
Duration of ICU stay			
Median (IQR) [†]	9 (13)	9 (9)	9 (12)
Unadjusted Hazard Ratio	1	1.10 (0.99 to 1.23)	0.99 (0.89 to 1.11)
Adjusted Hazard Ratio[#]	1	1.15 (1.03 to 1.28)	1.04 (0.93 to 1.17)
Duration of hospital stay			
Median (IQR) [†]	29 (33)	26 (29)	29.5 (30)
Unadjusted Hazard Ratio	1	1.12 (0.97 to 1.29)	1.01 (0.88 to 1.18)
Adjusted Hazard Ratio[#]	1	1.17 (1.01 to 1.35)	1.08 (0.93 to 1.25)
Non-surgical patients			
N	1016	1111	1038
Mortality at day 28			
N (%)	335 (33.2)	349 (31.7)	308 (30.0)
Unadjusted Odds Ratio	1	0.94 (0.78 to 1.12)	0.86 (0.72 to 1.04)
Adjusted Odds Ratio[*]	1	0.85 (0.70 to 1.03)	0.77 (0.63 to 0.94)
Cessation of mechanical ventilation			
Median (IQR) [†]	8 (14)	8 (14)	8 (10)
Unadjusted Hazard Ratio	1	1.03 (0.91 to 1.16)	1.07 (0.95 to 1.21)
Adjusted Hazard Ratio[#]	1	1.04 (0.92 to 1.17)	1.07 (0.95 to 1.21)
Duration of ICU stay			
Median (IQR) [†]	10 (12)	10 (14)	10 (11)
Unadjusted Hazard Ratio	1	0.97 (0.87 to 1.09)	1.03 (0.92 to 1.16)
Adjusted Hazard Ratio[#]	1	1.04 (0.93 to 1.16)	1.11 (0.99 to 1.25)
Duration of hospital stay			
Median (IQR) [†]	29 (32)	29 (30)	27 (31)
Unadjusted Hazard Ratio	1	1.01 (0.87 to 1.17)	1.11 (0.96 to 1.28)
Adjusted Hazard Ratio[#]	1	1.08 (0.93 to 1.25)	1.18 (1.02 to 1.37)

Surgical patients: The 28 day mortality outcomes exclude 16 patients (i.e. 4 standard care, 6 SOD and 6 SDD patients) for whom the data were unavailable. Data on the duration of mechanical ventilation was unavailable for 1 patient in the standard care group.

Non-surgical patients: The 28 day mortality outcomes exclude 28 patients for whom the data were unavailable (6 standard care, 12 SOD, 10 SDD). Data on duration of the hospital stay and duration of mechanical ventilation were unavailable for three patients (two in the SOD and one in the SDD group) and for seven patients (two in the standard care group and five in the SOD group)

[*]adjusted for APACHE II>20, Age >65, Mechanical Ventilation, Gender

[†]Median (interquartile range) in days for survivors at day 28

[#]Hazard ratios from Cox regression model with censoring at day 28 (hazard ratios larger than one indicate a tendency for shorter durations of ventilation, ICU/ hospital stay). Models for adjusted outcomes included the same covariates as in the logistic regression. Infinite durations were used for patients who died.

ICU-acquired bacteremia

Crude incidences of ICU-acquired bacteremias were lower for patients receiving SDD or SOD as compared to standard care (table 3), with the largest differences for Enterobacteriaceae. However, among patients receiving either SDD or SOD there were no significant differences in incidences of ICU-acquired bacteremias between surgical and non-surgical patients.

Table 3.Incidences of ICU acquired bacteremia

	Surgical			Non-Surgical		
microorganism	Standard Care N= 973	SDD N= 923	SOD N= 866	Standard Care N= 1016	SDD N= 1111	SOD N= 1038
	No (%)					
Staph. Aureus	11 (1.1)	3 (0.3)*	5 (0.6)	11 (1.0)	6 (0.5)	4 (0.4)
Strept. Pneumoniae	1 (0.1)	0 (0.0)	1 (0.1)	2 (0.2)	1 (0.1)	0 (0.0)
GNF-GNR	17 (1.7)	10 (1.1)	6 (0.7)*	19 (1.9)	6 (0.5)*	11 (1.1)
Enterobacteriaceae	51 (5.2)	10 (1.1)*†	25 (2.9)*	36 (3.5)	8 (0.7)*†	34 (3.3)
Enterococcus spp	24 (2.5)	24 (2.6)	23 (2.7)	31 (3.1)	24 (2.2)	26 (2.5)
Pts with at least one episode of bacteremia	86 (8.8)	39 (4.2)*	50 (5.8)*	84 (8.3)	41 (3.7)*†	60 (5.8)*

*Significant reductions ($p<0.05$) SOD and SDD vs standard care.
†Significant differences ($p<0.05$) between SOD and SDD within the same population.
GNF-GNR: Glucose Non-Fermenting Gram-negative Rods; *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and Acinetobacter species.

DISCUSSION

In a ICU population from a cluster randomized cross-over study with 5939 patients with low levels of antibiotic resistance the use of SOD and SDD were almost equally effective in reducing 28 day mortality(3).The current subgroup analysis demonstrates that SDD had similar effects in surgical and non-surgical patients, whereas non-surgical patients had a markedly higher benefit from SOD than surgical patients.. Of note, this study is performed in ICUs with low levels of antibiotic resistance.

These findings suggest that surgical patients might benefit from the addition of the enteric and/or systemic component to the SDD regimen. A higher efficacy of SDD, compared to SOD, among surgical patients has been suggested before upon the results of a meta-analysis (6). In that analysis results of studies evaluating SDD or SOD in populations with at least 75% surgical or trauma patients were pooled and compared to the pooled results of studies with lower proportions of surgical patients. Mortality was significantly lower in the eleven studies evaluating SDD or SOD in a predominant surgical population (pooled OR 0.70; 95% CI: 0.52 to 0.93), compared to ten trials with predominantly medical patients. Within these studies,

survival benefit was largest in the studies using both topical and systemic antibiotic prophylaxis (pooled OR 0.60 (95% CI: 0.41 to 0.88)), as compared to those using topical prophylaxis alone (pooled OR 0.86 (95% CI: 0.51 to 1.45)). Our analysis represents the first head-to-head comparison of SDD and SOD in surgical and non-surgical patients. Strengths of our study are that the definition of subgroups is more specific than in the previous meta-analysis, that treatments were uniform in the different study groups (as compared to multiple different protocols in the meta-analysis) and that it was possible to adjust for confounders.

A concern would be increase in antibiotic resistance with the use of SDD and SOD, but the occurrence of antibiotic resistance in treated patients was not found in the current study (8).

Nevertheless some limitations should be addressed. As a consequence of the study design and absence of concealment of randomisation (in detail described in the original publication(3)), baseline differences were present in the original study and thus also in this subgroup analysis for which we had to adjust in the analyses. Also the analyses do not provide an explanation for a different efficacy of SDD and SOD in surgical patients. As such, the current study should be regarded as hypothesis-generating rather than hypothesis testing, due to the secondary design based on primary data from a randomised trial.

Prior to the analysis, we hypothesized that the addition of systemic prophylaxis with cefotaxime and enteric decontamination, to oral decontamination alone, would reduce the incidence of Gram-negative infections, from which surgical patients might benefit more than non-surgical patients. Indeed, incidences of Gram-negative bacteremias were lower among patients receiving SDD compared to those that received SOD or standard care. However, a similar reduction in surgical and non-surgical patients was observed, indicating that this mechanism of action is unlikely to explain the observed difference between both patient groups. Since the effects of SDD on day 28 mortality are also similar in both subgroups, it appears unlikely that Gram negative bacteremia differently affects outcome in these two populations. Furthermore, there were no differences in day 28 mortality between SDD and SOD patients that had developed Gram-negative bacteremia (OR 0.88 (95% CI 0.36 to 2.16)). Unfortunately data of infections other than bacteremia were not available.

Subgroup analyses are generally not considered as providing definite evidence for several reasons, including spurious associations that may arise because of data dredging, multiple testing and chance findings. However, we believe these issues do not play a role of major importance in the current analyses. First, this subgroup analysis was performed because of a hypothesis that was already known and described before(6). A single subgroup (surgical status yes/no) was tested, so no data dredging was performed to identify smaller subgroups of possible patient populations with increased benefit. Second, this subgroup analysis was not performed because of absence of beneficial effects in the trial (the situation in which subgroup analyses are most commonly conducted).

Further studies of effect in ICU populations with higher rates of resistance are needed for generalisability of results. Also, research to elucidate the underlying mechanisms of perceived differences in effectiveness is warranted.

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REFERENCES

(1) D'Amico R, Pifferi S, Leonetti C, Torri V, Tinazzi A, Liberati A. Effectiveness of antibiotic prophylaxis in critically ill adult patients: systematic review of randomised controlled trials. BMJ 1998 April 25;316(7140):1275-85.

(2) Liberati A, D'Amico R, Pifferi S, Torri V, Brazzi L, Parmelli E. Antibiotic prophylaxis to reduce respiratory tract infections and mortality in adults receiving intensive care. Cochrane Database Syst Rev 2009;(4):CD000022.

(3) de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS et al. Decontamination of the digestive tract and oropharynx in ICU patients. N Engl J Med 2009 January 1;360(1):20-31.

(4) de JE, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. Lancet 2003 September 27;362(9389):1011-6.

(5) Krueger WA, Lenhart FP, Neeser G, Ruckdeschel G, Schreckhase H, Eissner HJ et al. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. Am J Respir Crit Care Med 2002 October 15;166(8):1029-37.

(6) Nathens AB, Marshall JC. Selective decontamination of the digestive tract in surgical patients: a systematic review of the evidence. Arch Surg 1999 February;134(2):170-6.

(7) Bender R, Blettner M. Calculating the "number needed to be exposed" with adjustment for confounding variables in epidemiological studies. J Clin Epidemiol 2002 May;55(5):525-30.

(8) de Smet AM, Kluytmans JA, Blok HE, Mascini EM, Benus RF, Bernards AT et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. Lancet Infect Dis 2011 March 18.

CHAPTER 8

General discussion

This thesis focuses on quantification of attributable mortality of Ventilator-associated Pneumonia (VAP). Determination of attributable mortality of nosocomial infections is challenging, as no "golden standard" methodology exists and the used methods had major limitations. Yet, over the last four years novel insights in the methodology of determining effects of nosocomial infections on mortality have emerged. These novel insights were used, where possible, in the studies presented in this thesis. Nevertheless various statistical methods have been used to determine the effects of nosocomial infections. We will provide an overview of these methods and subsequently discuss their strengths and limitations. Furthermore, we will provide some recommendations for future studies.

8.1 OVERVIEW OF CURRENTLY APPLIED STATISTICAL METHODS.

We performed a literature search to assess the currently used statistical methods in determining associations between a nosocomial infection and mortality. PubMed was searched between January 2008 and 2011 using the following search terms: (((*nosocomial* OR *hospital-acquired* OR *ICU-acquired*) AND (*infection* OR *pneumonia* OR *urinary tract infection*)) OR (*bloodstream infection* OR *blood stream infection* OR *bacteremia* OR *bacteraemia*) OR (*VAP* OR *ventilator-associated pneumonia*)) AND (*mortality* OR *survival* OR *outcome* OR *death*). Only studies, published in English, comparing the mortality between adult patients with and without a nosocomial infection were selected.

In total 3,066 publications were retrieved of which 43 studies (1-43) (see supplement Table I for a complete overview of the characteristics of these studies) met the inclusion criteria and could be included (Table 1). In total 49 statistical approaches were used (four studies used two and one study used three different statistical methods), and 14 different strategies were applied, with multivariate logistic regression analysis as the most common strategy. Eleven studies used survival analyses to determine associations between nosocomial infections and mortality.

Table 1. Statistical methods used in 43 studies to determine associations between nosocomial infections and mortality

Statistical method	Number of studies
Chi-square test (1)	8
Fisher's exact test (2)	5
Relative risk (3)	3
Comparison of mortality rates with calculated 95% Confidence Interval (4)	2
Standardized mortality ratio's (5)	3
Population attributable fraction (6)	1
Attributable mortality defined as difference in mortality rate per 100 matched pairs.	1
Logistic regression analysis (7)	4
Multivariate logistic regression analysis (8)	11

Table 1 (Continued)

Statistical method	Number of studies
Kaplan-Meier Survival Curves with log rank test (9)	3
Multivariate Cox regression analysis	1
Multivariate cox regression analysis with time dependent variable (10)	2
Multistate model (including competing risk model) (11)	4
Competing risk analysis with cox regression analysis accounting for time dependency and adjustments for potential confounders. (12)	1

Table 2. Overview of methods for addressing confounding, time-dependent bias and competing risks in 43 studies on quantifying associations between nosocomial infections and mortality

	Number
Confounding*	
Matching criteria	16
Adjustments in the analysis	14
Standardized mortality ratio's (standardized for expected mortality)	3
No correction or adjustments	15
Time-dependent bias	
Including nosocomial infection as a time dependent covariate	7
Survival analysis without correction for time dependent bias	4
No survival analysis	32
Competing risks	
Accounting for competing risk in survival analysis	5
Fixed endpoint in survival analysis (e.g. 30 day mortality)	4
Survival analysis without accounting for competing risk	2
No survival analysis	32

*Some studies used more strategies to control for confounding.

8.2 POTENTIAL LIMITATIONS AND STRENGTHS OF THE CURRENTLY APPLIED STATISTICAL METHODS

Confounding

When estimating mortality due to nosocomial infections confounding is a major issue since most studies are observational. There is confounding when covariates are associated both with the development of nosocomial infections as well as with mortality and when these covariates are unequally distributed. For example, severely ill patients have a higher chance of developing nosocomial infections and a higher chance of dying. When patients with a nosocomial infection are more severely ill than control patients without infection, the effects of nosocomial infections on mortality will be overestimated. Ignoring confounding may, therefore, lead to biased estimates of the association of interest. Confounding can be subdivided in observed

and unobserved confounding, and we can only adjust for observed confounding. The most common confounders that are usually addressed are age, gender, severity of illness and diagnosis on admission(44).

Only 65% of the 43 studies investigating associations between nosocomial infections and mortality, published in the past 3 years, attempted to adjust for confounding by applying matching criteria, performing multivariate analyses or using standardized mortality ratios (Table 2). Moreover, large differences between studies in type and quantity of matching criteria and/or possible confounders exist.

Several methods have been proposed to limit the effect of confounding. First, stratification can be used, i.e. estimating the relation of a nosocomial infection among different strata. This method is, however, rarely used (none of the studies in the overview used this method) since the power per stratum is often too low.

Second, to increase the comparability of patients with and without a nosocomial infection, matching criteria, like e.g. age, gender, admission diagnosis, APACHE II or SAPS 2 scores or time in the hospital, can be applied. The more criteria used the more difficult it will be to find a possible match, and increasingly larger populations of possible control patients are necessary.

Third, multivariate logistic regression or Cox regression analyses can be performed (45). The drawback of these analyses is that instable estimates may occur due to overfitting if more than one potential confounder per 10 cases (meeting the endpoint of interest) is included (46;47). Again, this would necessitate larger populations of unaffected "control" patients. Moreover, one can only control for observed confounding, and not for residual (i.e. unobserved) confounding.

Some of the studies used a standardized mortality ratio, which is the ratio of observed deaths to expected deaths (48). The expected chance of mortality is calculated at admission with a severity of illness score (e.g APACHE II OR SAPS2 score). This method can be seen as an indirect mean of adjusting a rate, but one can only control for the expected chance of mortality and no adjustment is made for other possible confounders.

Adjusting simultaneously for more confounders is possible by applying a propensity score (49;50). The construction of this score is based on associations between included confounders and exposure (i.e. nosocomial infections). Groups of subjects with similar propensity scores can be expected to have similar distribution of all potential confounders. With this method, selection of control patients is based on their propensity score. Also with this method one can only adjust for observed confounding and not for unobserved confounding.

Logistic regression analysis versus survival analysis

In a logistic regression model the survival status at the end of ICU or hospital stay is used to model the probability of dying during these periods. The main disadvantage of this approach is the assumption that observation periods are similar for all patients studied, which hardly ever occurs in ICU populations. Violation of this assumption can bias the results, as the time in ICU or hospital is in itself a risk factor for mortality (51;52). Moreover, a nosocomial infection is treated as present or absent and the timing of this infection during ICU or hospital stay is ignored.

To adequately model time to event, survival analyses (e.g. Kaplan Meier method or Cox proportional hazard model) can be used to evaluate the association between nosocomial infections and mortality. With the Kaplan-Meier method two survival curves are constructed (the survival curves of patients with and without infection), which can be statistically compared using the log-rank test. The main limitation of this method is that adjustments for confounding is not possible. With a Cox proportional hazard model the effect of multiple covariates on survival can be assessed allowing adjustment for possible confounders.

During the last 3 years more studies used logistic regression analyses instead of survival analyses to evaluate the relation nosocomial infection and mortality (15 versus 11 studies, respectively. Table 1), thereby ignoring the effects of time and increasing the likelihood of obtaining biased results.

Competing risks in survival analysis

The main argument against using survival methods to analyse ICU mortality or hospital mortality pertains to censoring. Importantly, the Kaplan-Meier method and Cox model assume that censoring is non-informative (i.e., that the survival time of an individual patient is independent of censoring). That is, patients discharged alive from the ICU or hospital must be representative of all other individuals who have survived to this time of discharge but who are still in the ICU or hospital. Censoring is informative when the survival time of an individual is censored as a result of improved or deteriorated clinical condition, which mostly applies to patients discharged from ICU: they either do not need longer treatment in ICU, usually because their clinical condition has improved or deteriorated to such an extent that further treatment in ICU is not considered appropriate. As such, censoring is informative as it carries information about the survival time. A competing risk is present when informative censoring implies that discharge from the ICU affects the probability of experiencing an event of interest (death before discharge). In this setting standard survival curves are not valid (51). When censoring would occur randomly competing risks would not be a problem, e.g. when mortality would be assessed at some, predefined, fixed time point (e.g., day 28 mortality).

There are several methods to deal with the problem of competing risks in survival analyses. In hospital epidemiology the use of competing risks analyses in the evaluation of risk factors for ICU or hospital mortality, like infections, is increasing due to some recent publications (24;41;53). Of the selected studies, 2 of the 11 studies that performed survival analyses ignored the problem of competing risks. Four studies avoided the problem of competing risks by assessing the endpoint at a predefined fixed time point.

The most commonly used method for addressing competing risks is to calculate cause specific hazards for each possible event, e.g. the cause specific hazard for discharge (ICU or hospital) and death (ICU or hospital), which can be done by using separate Cox models for each event. A limitation of only providing the cause specific hazards of mortality and discharge is that no inferences can be made on the direct effect of the covariate (i.e. nosocomial infections) on the event of interest. For example, the cause specific hazard for mortality may not be increased for patients with a nosocomial infection, while the cause specific hazard for

discharge may be significantly reduced, i.e. patients have a longer stay on the ICU or hospital. As we know that patients do not benefit from prolonged stay on the ICU or the hospital, the overall effect may still be that there is an increased mortality rate among patients in the ICU.

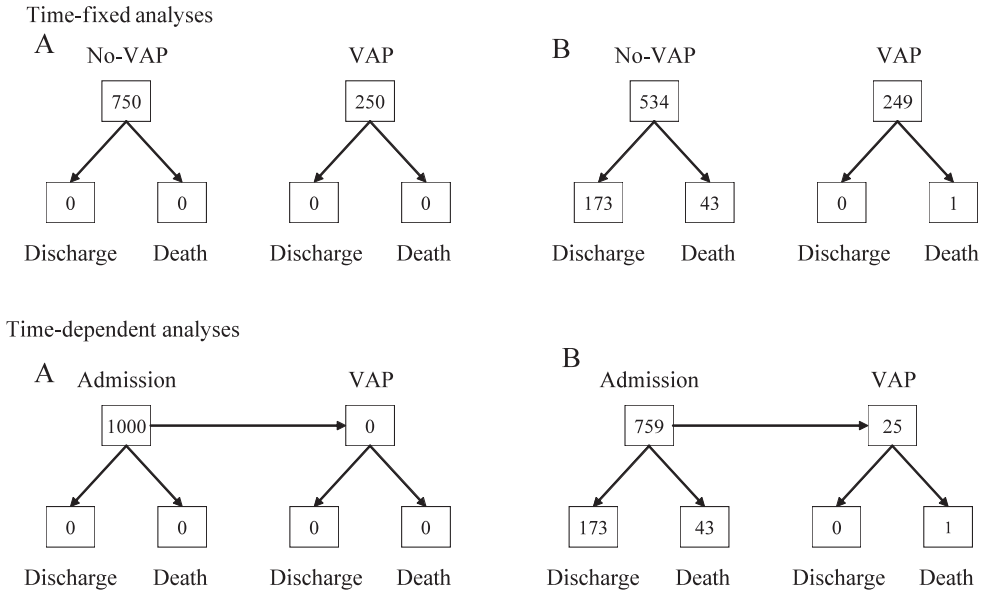
To directly assess the influence of a covariate on the event of interest, Fine and Gray (54) introduced a proportional hazards model for the subdistribution, which provides a hazard ratio for the event of interest (ICU mortality) taken the competing event (ICU discharge) into account. The cumulative incidence function in a competing risk analysis tends to the raw proportion of deaths and is, therefore, also called a "subdistribution function"(51). The estimate of the subdistribution hazard directly reflects the overall effect of a nosocomial infection on mortality.

Follow up of patients is frequently complete in studies in ICUs or hospital settings, which enables the use of standard software packages to perform Cox regression analyses to calculate subdistribution hazards. Only the exposure time of patients who were discharged alive should be recoded at the largest observed time of death (54). If censoring is present, the R package *cmprsk* (55) can be used, and in case of time-dependent variables, the R package *kmi* (56) can be used.

Time dependent bias in survival analyses

The occurrence of a nosocomial infection is a time-dependent event, since it may occur after a patient has been admitted to the hospital. Time-dependent bias occurs when a future exposure status is treated as if it was known at baseline. For nosocomial infections a time-fixed approach means that patients are retrospectively divided into infected and non-infected patients. This approach has been used in many studies, leading to inevitable bias in the estimates of risk factors and a misjudgement of the true effects of nosocomial infections on patient outcome. The bias in these estimates implies that the estimator, for example a hazard ratio, will be less than the unbiased (and true) estimator. In the case of nosocomial infections harmful effects will, therefore, be underestimated (57). Whether adjustment of time-dependent bias qualitatively changes conclusions depends on the value of the biased estimate. If, for example, the hazard ratio in the biased approach significantly exceeds 1, the study's conclusion will not qualitatively change when controlling for time-dependent bias. If, however, the hazard ratio is not statistically significant, controlling for time-dependent bias could result in a statistically significant point estimate. If a hazard ratio is less than 1, controlling for the bias could change a significantly preventive effect into an effect that is not significant or even into a harmful effect (58;59). In figure 1 the effect of time-dependent bias is illustrated using a hypothetical cohort of 1000 ICU patients admitted to ICU.

Figure 1. Time-dependent bias illustrated



On the top row are the models depicted as occurring in the time-fixed analyses (biased analyses) and at the bottom row the models as occurring in the time-dependent analyses (correct analyses). On the left side (A) the situation on admission; in the time-dependent analyses all individuals are in the initial state on admission, in the time-fixed analyses individuals who acquire VAP during their stay in the ICU are already in the VAP state. On the right (B) the situation at the start of day 4 when the first patient with VAP dies. In the time-fixed analysis the death hazard of VAP will be too low, as patients with VAP are analyzed as if nosocomial infection had been present on admission, which increases the denominator (249 instead of 25) in the hazard of VAP. As a result, the hazard is decreased, and the death hazard of patients without VAP will be too high (patients with VAP drop out of the denominator of the death hazard without infection for the time period in which they have not yet acquired infection). As a consequence, the denominator is decreased (534 instead of 759) and the hazard is increased. As the death hazard of patients with VAP is too low and the death hazard of patients without VAP is too high, the hazard ratio will be too low.

Time-dependent bias can easily be avoided by adding a covariate as a categorical time-dependent variable in a survival analyses. Once a patient experiences an episode of a nosocomial infection, their infection status changes to "yes", and remains as such until one of the endpoints (i.e. death or discharge) occurs. This implies that a patient that experiences such an infection will be represented twice in the database; one row for the period before the infection and one for the period after infection. As the statistical model of survival analyses is based on a counting process, reliable estimates will be obtained (60). During the last three years 65% of the studies performing survival analyses used this method to avoid time-dependent bias (Table 2).

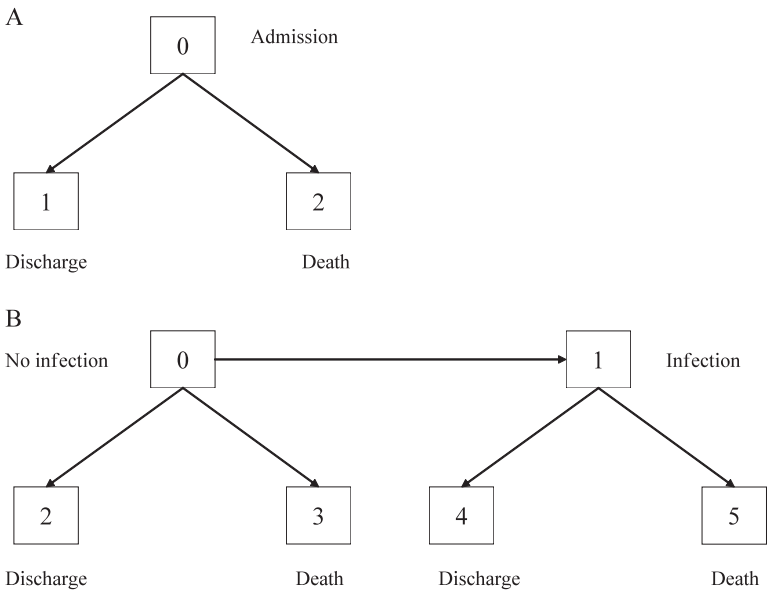
Multistate models

The class of multistate models forms an extension to that of competing risks models, where there is only an initial state and several exclusive absorbing states (figure 2a). Typically in hospital epidemiology patients will also go to intermediate events (e.g. nosocomial infections, invasive procedures like mechanical ventilation, surgery etc) which are neither initial states nor final states.

Multistate models describe events over the course of time as transitions between multiple states. In multistate models both time-dependent covariates and competing risks can be considered. The progressive disability model (see figure 2b) (61) is particularly applicable to nosocomial infections.

Estimation of the transition intensities can be done in most statistical packages by fitting a time-dependent Cox model. Estimation of the cumulative effects is more complicated. Schumacher et al introduced the R-package *changeLOS* where the transition probabilities are estimated with the Aalen-Johanson estimator. From these, attributable mortality and the population attributable fraction are derived (61).

Figure 2. Multistate model



A. competing risks model with initial state (0) and two absorbing states (1 and 2)
B. progressive disability model with an initial state (0), an intermediate state (1), and four absorbing states (2 t/m 5).

8.3 FUTURE CHALLENGES

To improve the quality and comparability of the studies investigating associations between nosocomial infections and patient outcomes a more uniform approach in data analysis is needed. The use of survival analysis should be encouraged, incorporating the time-dependent nature of nosocomial infections, as well as accounting for competing risks. The combination of these two (time-dependency and competing risks) complicates the use of standard available software packages, making these approaches less attractive for clinical researchers (non-statistians). However, guidelines for performing these analyses have been improved. Competing risks analyses in standard software programs can incorporate time dependent variables. To use time-dependent variables in this setting the data-format needs to be re-arranged in a non-standard format. Wolkewitz et al (41) provide as a supplement with their article a practical example of the data-format and the SAS and R codes for performing the calculations of cause-specific hazards ratio in a competing risks analysis with time-dependent covariates. A big advantage of the competing risks methodology is that it also allows for a better understanding of why a nosocomial infection increases mortality. In our study estimating the association of VAP and mortality it was shown that, as compared to patients not developing VAP, the cause specific hazard ratio (CSHR) of dying in the ICU was 1.13 (95% CI 0.98 to 1.31). Yet, after the development of VAP patients had a lower risk per day for ICU discharge, as represented by the CSHR of discharge of 0.74 (95% CI 0.68 to 0.80) (62). The attributable mortality of VAP is, therefore, mainly caused by prolonged exposure to the risk of dying due to increased length of stay on the ICU. One should, however, also realise that the estimates of the subdistribution hazard may not apply to other populations when the risk of the competing event is totally different to that of the research population.

The role of multistate models in estimating the mortality of nosocomial infections is very promising. However their use is, even more than the competing risk methodology, limited to experienced statisticians. Some progress to make it more available for a clinician has been made by Wangler et al with the R package changeLOS (63) in which the progressive disability model (figure 2b) is incorporated.

The recent new insights in the attributable mortality of VAP, which was estimated lower than formerly expected, indicates that the population studied should be large enough to have enough power to demonstrate any kind of association between infection and mortality. To accomplish such large populations, multicenter and maybe even international collaborations will be necessary. Fortunately, such collaborations are currently existing (24;30;61).

Despite the progress that has been made with these new statistical methods there are still many challenges to overcome. One of the most challenging aspects is adequately dealing with non-observed confounding, i.e., residual confounding. Almost all studies evaluating the impact of nosocomial infections on patient outcome are observational, and the risk of confounding will, therefore, always exist. As discussed before in this thesis, the best – theoretical - way to eliminate the problem of confounding would be to perform a randomized controlled trial intentionally inducing a nosocomial infection to patients randomized to the intervention group. However as this will never be considered ethical other methods should be sought. In

this thesis we determined attributable mortality of VAP by calculating a ratio of the relative risk reduction of mortality and VAP using the data of randomized VAP prevention trials. As the intervention was randomly allocated confounding, and especially residual confounding, was limited.

In the future researchers should specifically address the problem of confounding in the design of their study. The more general methods to deal with confounding, like stratification, matching, adjustments in the analyses, and propensity scores require certain conditions. First of all one must upfront determine the potential confounders, which should be based on previous knowledge or biological mechanisms. Then, these potential confounders should be accurately measured, preferably prospectively and one should strive for completeness. Also a considerable amount of patients should be included. This to maintain sufficient power in case of stratification, an increased chance of perfect matching in case of matching and enough cases in case of multivariate regression analyses. Of note, one should not correct for variables that result from the infection itself, like length of stay in the ICU or hospital, which was frequently done in the past. As confounding is a problem of all observational studies in every field of research many new strategies are being developed which should also be evaluated in the field of hospital-acquired infectious diseases.

A second challenge is to adequately model the influence of VAP. Survival methods incorporating VAP as a time dependent dichotomized variable assumes that the influence of VAP on patient outcome will remain stable during ICU stay. This assumption may hold when a patient experiences VAP on day 6 and dies on day 10. But what if a patient experiences VAP on day 6 and dies on day 30. In the current analyses it is assumed that mortality at day 30 still results from VAP at day 6, neglecting all possible circumstances that could have contributed to death. Yet, VAP probably increased the length of ICU-stay which in itself increased the risks of other complications and eventually death. Multistate models, that enable various intermediate events, could offer more flexibility in the analyses and better capture the (chronological) complexity of factors influencing mortality. A disadvantage of this method is that it will be more difficult to understand for general clinicians and difficult to compare between studies. Also comprehensive databases, with daily information per possible (intermediate) event, are needed to perform such analyses.

8.4 CONCLUDING REMARKS

In the past accurateness of estimates of associations between nosocomial infections and mortality have been limited by the statistical methods used. Recent studies have demonstrated the advantages of survival analyses taking into account the time dependent nature of nosocomial infections and competing risks. These techniques should now be state-of-the-art, to derive more reliable and better comparable findings. From here, further methodological improvements are needed, especially concerning methods to limit the influence of confounding.

SUPPLEMENT

Table 1: Overview of studies

Author	Population	Infection	Statistical method
Adiguzel, 2010	Respiratory intensive care unit patients	Nosocomial candida infections	Chi-square test
Aly, 2008	Medical, surgical ICU	Nosocomial infections	Chi-square test
Berger, 2010	Hospital patients	Nosocomial bloodstream infections caused by St. Aureus or E. Coli	Fisher's exact test (matching)
Borer, 2009	Hospital patients	Bloodstream infection with carbapenem resistant K. pneumoniae	Risk ratio (matching)
Bou, 2009	ICU patients	Pseudomonas aeruginosa (outbreak) infections	Risk ratio
Brusselaers, 2010	Severe burn injury patients	Bloodstream infections	Multivariate logistic regression analysis (matching)
Burgmann, 2010	ICU patients	Nosocomial infections	Standardized mortality ratio
Cook, 2010	Trauma center	Ventilator-associated Pneumonia	Fisher's exact test
De Oliveira, 201	Intensive care patients	Nosocomial infection	Fisher's exact test
De Santo, 2008	Cardiac surgery ICU patients	Nosocomial infections	Multivariate logistic regression analysis
Frontera, 2008	Patients with subarachnoid hemorrhage	Nosocomial infections	Multivariate logistic regression analysis
Furtado, 2009	ICU patients	Imipenem-resistant Pseudomona aeruginosa infection	Logistic regression analysis (matching)
Geffers, 2008	Surgical patients	Nosocomial infections	Attributable mortality defined as difference in mortality rate per 100 matched pairs (matching)
Gikas, 2010	ICU patients	Nosocomial infections	Comparison of mortality rates with calculated 95% confidence intervals.
Han, 2010	Medical ICU patients	Candidemia	Kaplan-Meier survival curves with log-rank test. Logistic regression analysis. (matching)
Hortal, 2009	Cardiac surgery patients	Ventilator-associated pneumonia	Logistic regression analysis

Table 1 (Continued)

Author	Population	Infection	Statistical method
Jang, 2009	Intensive care patients	Nosocomial Acinetobacter bloodstream infections	Kaplan-Meier survival curves with log rank test (matching)
Januel, 2010	Intensive care unit patients	Nosocomial infections	Population attributable fraction (matching)
Josephson, 2010	Neurologic intensive care unit	Ventilator-associated Pneumonia	Multivariate logistic regression analysis
Karaoglan, 2010	Medical, Surgical ICU patients	Ventilator-associated Pneumonia	Chi-square test (matching)
Kasuya, 2010	Critically ill stroke patients	Ventilator-associated Pneumonia	Chi-square test
Kothari, 2009	Cardiac surgery patients	Nosocomial bloodstream infection	Fisher's exact test (matching)
Kourkoumpetis, 2010	Surgery patients	Nosocomial candida infection	Chi-square test
Lambert, 2011	ICU patients	Nosocomial bloodstream infections and pneumonia	Competing risk analysis with cox regression analysis accounting for time dependency and adjustment for potential confounders.
Madani, 2009	Intensive care patients	Nosocomial infections	Relative risk
Magnason, 2008	Intensive care patients	Nosocomial infections	Multivariate cox regression analysis with time-dependent variable
Malacarne, 2010	Intensive care patients	Nosocomial infections	Comparison of mortality rates with calculated 95% confidence intervals
Markogiannakis 2009	Surgical ICU patients	Nosocomial infections	Multivariate logistic regression
Michalapoulos 2010	Intensive care patients	Nosocomial bloodstream infection	Fisher's exact test (matching)
Nguile-Makao 2010	Intenive care patients	Ventilator associated pneumonia	multistate model/ conditional logistic regression/multivariate logistic regression analysis
Oake 2010	Hospital patients	Nosocomial Clostridium difficile infections	Cox multivariable regression model. Stratified analysis of baseline mortality risk
Olsen 2008	Heart surgery patients	Nosocomial bloodstream infection	Multivariate cox regression analysis with time dependent variable

Table 1 (Continued)

Author	Population	Infection	Statistical method
Ressner 2008	Burn patients	Nosocomial bloodstream infection	Multivariate logistic regression analysis
Rodrigues 2009	Intensive care patients	Ventilator associated pneumonia	Multivariate logistic regression analysis
Rosenthal 2011	Intensive care patients	Catheter associated urinary tract infection	Multistate model
Rudiger 2010	Critically ill acute heart failure patients	Nosocomial infections	Kaplan-Meier survival curves with log rank test
Shupp 2010	Burn patients	Nosocomial bloodstream infection	Multivariate logistic regression (matching)
Tay 2010	Intensive care patients	Nosocomial urinary tract infection	Multivariate logistic regression
Thompson 2008	Intensive care patients	Nosocomial bloodstream infection	Chi-square test (matching)
Thompson 2008	Intensive care patients	Nosocomial bloodstream infection (MRSA)	Chi-square test and standardised mortality rate (matching)
Wolkewitz 2008	Intensive care patients	Nosocomial pneumonia	Multistate model/ Competing risk analysis with time dependent variable
Wolkewitz 2010	Hospital patients	Nosocomial bloodstream infection	Multistate model
Wu 2008	Patients with acute pancreatitis	Nosocomial infection	Chi-square test/pairwise testing (matching)

REFERENCES

(1) Adiguzel N, Karakurt Z, Gungor G, Yazicioglu MO, Acarturk E, Sogukpinar O et al. Mortality rates and risk factors associated with nosocomial Candida infection in a respiratory intensive care unit. Tuberk Toraks 2010 January;58(1):35-43.

(2) Aly NY, Al-Mousa HH, Al Asar el SM. Nosocomial infections in a medical-surgical intensive care unit. Med Princ Pract 2008;17(5):373-7.

(3) Berger J, ab-Elschahawi M, Blacky A, Pernicka E, Spertini V, Assadian O et al. A matched prospective cohort study on Staphylococcus aureus and Escherichia coli bloodstream infections: extended perspectives beyond resistance. Am J Infect Control 2010 December;38(10):839-45.

(4) Borer A, Saidel-Odes L, Riesenberg K, Eskira S, Peled N, Nativ R et al. Attributable mortality rate for carbapenem-resistant Klebsiella pneumoniae bacteremia. Infect Control Hosp Epidemiol 2009 October;30(10):972-6.

(5) Bou R, Lorente L, Aguilar A, Perpinan J, Ramos P, Peris M et al. Hospital economic impact of an outbreak of Pseudomonas aeruginosa infections. J Hosp Infect 2009 February;71(2):138-42.

(6) Brusselaers N, Monstrey S, Snoeij T, Vandijck D, Lizy C, Hoste E et al. Morbidity and mortality of bloodstream infections in patients with severe burn injury. Am J Crit Care 2010 November;19(6):e81-e87.

(7) Burgmann H, Hiesmayr JM, Savey A, Bauer P, Metnitz B, Metnitz PG. Impact of nosocomial infections on clinical outcome and resource consumption in critically ill patients. Intensive Care Med 2010 September;36(9):1597-601.

(8) Cook A, Norwood S, Berne J. Ventilator-associated pneumonia is more common and of less consequence in trauma patients compared with other critically ill patients. J Trauma 2010 November;69(5):1083-91.

(9) de Oliveira AC, Kovner CT, da Silva RS. Nosocomial infection in an intensive care unit in a Brazilian university hospital. Rev Lat Am Enfermagem 2010 March;18(2):233-9.

(10) De Santo LS, Bancone C, Santarpino G, Romano G, De FM, Scardone M et al. Microbiologically documented nosocomial infections after cardiac surgery: an 18-month prospective tertiary care centre report. Eur J Cardiothorac Surg 2008 April;33(4):666-72.

(11) Frontera JA, Fernandez A, Schmidt JM, Claassen J, Wartenberg KE, Badjatia N et al. Impact of nosocomial infectious complications after subarachnoid hemorrhage. Neurosurgery 2008 January;62(1):80-7.

(12) Furtado GH, Bergamasco MD, Menezes FG, Marques D, Silva A, Perdiz LB et al. Imipenem-resistant Pseudomonas aeruginosa infection at a medical-surgical intensive care unit: risk factors and mortality. J Crit Care 2009 December;24(4):625-14.

(13) Geffers C, Sohr D, Gastmeier P. Mortality attributable to hospital-acquired infections among surgical patients. Infect Control Hosp Epidemiol 2008 December;29(12):1167-70.

(14) Gikas A, Roumbelaki M, Bagatzouni-Pieridou D, Alexandrou M, Zinieri V, Dimitriadis I et al. Device-associated infections in the intensive care units of Cyprus: results of the first national incidence study. Infection 2010 June;38(3):165-71.

(15) Han SS, Yim JJ, Yoo CG, Kim YW, Han SK, Shim YS et al. Clinical characteristics and risk factors for nosocomial candidemia in medical intensive care units: experience in a single hospital in Korea for 6.6 years. J Korean Med Sci 2010 May;25(5):671-6.

(16) Hortal J, Munoz P, Cuerpo G, Litvan H, Rosseel PM, Bouza E. Ventilator-associated pneumonia in patients undergoing major heart surgery: an incidence study in Europe. Crit Care 2009;13(3):R80.

(17) Jang TN, Lee SH, Huang CH, Lee CL, Chen WY. Risk factors and impact of nosocomial Acinetobacter baumannii bloodstream infections in the adult intensive care unit: a case-control study. J Hosp Infect 2009 October;73(2):143-50.

(18) Januel JM, Harbarth S, Allard R, Voirin N, Lepape A, Allaouchiche B et al. Estimating attributable mortality due to nosocomial infections acquired in intensive care units. Infect Control Hosp Epidemiol 2010 April;31(4):388-94.

(19) Josephson SA, Moheet AM, Gropper MA, Nichols AD, Smith WS. Ventilator-associated pneumonia in a neurologic intensive care unit does not lead to increased mortality. Neurocrit Care 2010 April;12(2):155-8.

(20) Karaoglan H, Yalcin AN, Cengiz M, Ramazanoglu A, Ogunc D, Hakan R et al. Cost analysis of ventilator-associated pneumonia in Turkish medical-surgical intensive care units. Infez Med 2010 December 1;18(4):248-55.

(21) Kasuya Y, Hargett JL, Lenhardt R, Heine MF, Doufas AG, Remmel KS et al. Ventilator-associated pneumonia in critically ill stroke patients: Frequency, risk factors, and outcomes. J Crit Care 2010 November 22.

(22) Kothari A, Sagar V, Ahluwalia V, Pillai BS, Madan M. Costs associated with hospital-acquired bacteraemia in an Indian hospital: a case-control study. J Hosp Infect 2009 February;71(2):143-8.

(23) Kourkoumpetis T, Manolakaki D, Velmahos G, Chang Y, Alam HB, De Moya MM et al. Candida infection and colonization among non-trauma emergency surgery patients. Virulence 2010 September;1(5):359-66.

(24) Lambert ML, Suetens C, Savey A, Palomar M, Hiesmayr M, Morales I et al. Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. Lancet Infect Dis 2011 January;11(1):30-8.

(25) Madani N, Rosenthal VD, Dendane T, Abidi K, Zeggwagh AA, Abouqal R. Health-care associated infections rates, length of stay, and bacterial resistance in an intensive care unit of Morocco: findings of the International Nosocomial Infection Control Consortium (INICC). Int Arch Med 2009;2(1):29.

(26) Magnason S, Kristinsson KG, Stefansson T, Erlendsdottir H, Jonsdottir K, Kristjansson M et al. Risk factors and outcome in ICU-acquired infections. Acta Anaesthesiol Scand 2008 October;52(9):1238-45.

(27) Malacarne P, Boccalatte D, Acquarolo A, Agostini F, Anghileri A, Giardino M et al. Epidemiology of nosocomial infection in 125 Italian intensive care units. Minerva Anestesiol 2010 January;76(1):13-23.

(28) Markogiannakis H, Pachylaki N, Samara E, Kalderi M, Minettou M, Toutouza M et al. Infections in a surgical intensive care unit of a university hospital in Greece. Int J Infect Dis 2009 March;13(2):145-53.

(29) Michalopoulos A, Falagas ME, Karatza DC, Alexandropoulou P, Papadakis E, Gregorakos L et al. Epidemiologic, clinical characteristics, and risk factors for adverse outcome in multiresistant gram-negative primary bacteremia of critically ill patients. Am J Infect Control 2010 October 28.

(30) Nguile-Makao M, Zahar JR, Francais A, Tabah A, Garrouste-Org, Allaouchiche B et al. Attributable mortality of ventilator-associated pneumonia: respective impact of main characteristics at ICU admission and VAP onset using conditional logistic regression and multi-state models. Intensive Care Med 2010 May;36(5):781-9.

(31) Oake N, Taljaard M, van WC, Wilson K, Roth V, Forster AJ. The effect of hospital-acquired Clostridium difficile infection on in-hospital mortality. Arch Intern Med 2010 November 8;170(20):1804-10.

(32) Olsen MA, Krauss M, Agniel D, Schootman M, Gentry CN, Yan Y et al. Mortality associated with bloodstream infection after coronary artery bypass surgery. Clin Infect Dis 2008 May 15;46(10):1537-46.

(33) Ressner RA, Murray CK, Griffith ME, Rasnake MS, Hospenthal DR, Wolf SE. Outcomes of bacteremia in burn patients involved in combat operations overseas. J Am Coll Surg 2008 March;206(3):439-44.

(34) Rodrigues PM, Carmo NE, Santos LR, Knibel MF. Ventilator-associated pneumonia: epidemiology and impact on the clinical evolution of ICU patients. J Bras Pneumol 2009 November;35(11):1084-91.

(35) Rosenthal VD, Dwivedy A, Rodriguez Calderon ME, Esen S, Hernandez HT, Abouqal R et al. Time-dependent analysis of length of stay and mortality due to urinary tract infections in ten developing countries: INICC findings. J Infect 2011 February;62(2):136-41.

(36) Rudiger A, Streit M, Businger F, Schmid ER, Follath F, Maggiorini M. The impact of infections on critically ill acute heart failure patients: an observational study. Swiss Med Wkly 2010;140:w13125.

(37) Shupp JW, Pavlovich AR, Jeng JC, Pezzullo JC, Oetgen WJ, Jaskille AD et al. Epidemiology of bloodstream infections in burn-injured patients: a review of the national burn repository. J Burn Care Res 2010 July;31(4):521-8.

(38) Tay MK, Lee JY, Wee IY, Oh HM. Evaluation of intensive care unit-acquired urinary tract infections in Singapore. Ann Acad Med Singapore 2010 June;39(6):460-5.

(39) Thompson DS, Workman R, Strutt M. Contribution of acquired meticillin-resistant Staphylococcus aureus bacteraemia to overall mortality in a general intensive care unit. J Hosp Infect 2008 November;70(3):223-7.

(40) Thompson DS. Estimates of the rate of acquisition of bacteraemia and associated excess mortality in a general intensive care unit: a 10 year study. J Hosp Infect 2008 May;69(1):56-61.

(41) Wolkewitz M, Vonberg RP, Grundmann H, Beyersmann J, Gastmeier P, Barwolff S et al. Risk factors for the development of nosocomial pneumonia and mortality on intensive care units: application of competing risks models. Crit Care 2008;12(2):R44.

(42) Wolkewitz M, Frank U, Philips G, Schumacher M, Davey P. Mortality associated with in-hospital bacteraemia caused by Staphylococcus aureus: a multistate analysis with follow-up beyond hospital discharge. J Antimicrob Chemother 2010 November 23.

(43) Wu BU, Johannes RS, Kurtz S, Banks PA. The impact of hospital-acquired infection on outcome in acute pancreatitis. Gastroenterology 2008 September;135(3):816-20.

(44) Melsen WG, Rovers MM, Bonten MJ. Ventilator-associated pneumonia and mortality: a systematic review of observational studies. Crit Care Med 2009 October;37(10):2709-18.

(45) Klungel OH, Martens EP, Psaty BM, Grobbee DE, Sullivan SD, Stricker BH et al. Methods to assess intended effects of drug treatment in observational studies are reviewed. J Clin Epidemiol 2004 December;57(12):1223-31.

(46) Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol 1996 December;49(12):1373-9.

(47) Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. Am J Epidemiol 2007 March 15;165(6):710-8.

(48) Higgins TL, Kramer AA, Nathanson BH, Copes W, Stark M, Teres D. Prospective validation of the intensive care unit admission Mortality Probability Model (MPM0-III). Crit Care Med 2009 May;37(5):1619-23.

(49) Rubin DB, Thomas N. Matching using estimated propensity scores: relating theory to practice. Biometrics 1996 March;52(1):249-64.

(50) Rubin DB. Estimating causal effects from large data sets using propensity scores. Ann Intern Med 1997 October 15;127(8 Pt 2):757-63.

(51) Resche-Rigon M, Azoulay E, Chevret S. Evaluating mortality in intensive care units: contribution of competing risks analyses. Crit Care 2006 February;10(1):R5.

(52) Wolkewitz M, Beyersmann J, Gastmeier P, Schumacher M. Modeling the effect of time-dependent exposure on intensive care unit mortality. Intensive Care Med 2009 May;35(5):826-32.

(53) De AG, Allignol A, Murthy A, Wolkewitz M, Beyersmann J, Safran E et al. Multistate modelling to estimate the excess length of stay associated with meticillin-resistant Staphylococcus aureus colonisation and infection in surgical patients. J Hosp Infect 2011 June;78(2):86-91.

(54) Fine J.P., Gray R.J. A proportional hazards model for the subdistribution of a competing risk. Journal of the American Statistical Association 1999;94(446):496-509.

(55) Gray B. cmprsk package. http://cran.r-project.org/web/packages/cmprsk/index.html. 2011.

(56) Allignol A.. kmi package. http://cran.r-project.org/web/packages/kmi/kmi.pdf. 4-5-2011.

(57) Beyersmann J, Wolkewitz M, Schumacher M. The impact of time-dependent bias in proportional hazards modelling. Stat Med 2008 December 30;27(30):6439-54.

(58) Beyersmann J, Gastmeier P, Wolkewitz M, Schumacher M. An easy mathematical proof showed that time-dependent bias inevitably leads to biased effect estimation. J Clin Epidemiol 2008 December;61(12):1216-21.

(59) van WC, Davis D, Forster AJ, Wells GA. Time-dependent bias was common in survival analyses published in leading clinical journals. J Clin Epidemiol 2004 July;57(7):672-82.

(60) Therneau T.M., Grambsch P.M. Modeling Survival Data: Extending the Cox Model. 2000.

(61) Schumacher M, Wangler M, Wolkewitz M, Beyersmann J. Attributable mortality due to nosocomial infections. A simple and useful application of multistate models. Methods Inf Med 2007;46(5):595-600.

(62) Melsen W.G., Rovers M.M., Groenwold R.H.H., Bergmans D.C.J.J, Camus C., Bauer T.T. et al. The attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomized prevention studies. 2011. Unpublished Work

(63) Wangler M., Beyersmann J. ChangeLOS package. http://cran.r-project.org/web/packages/changeLOS/index.html.

SUMMARY

SAMENVATTING

DANKWOORD

CURRICULUM VITAE

Attributable mortality of Ventilator-associated Pneumonia

Ventilator-associated Pneumonia (VAP) is one of the most common nosocomial infections among patients admitted to the intensive care unit (ICU). Generally it is believed that VAP increases both morbidity and mortality of ICU patients. The latter notion is primarily based on the results of observational studies using a (matched) cohort design.

As no complete overview of all studies evaluating the relation between VAP and mortality existed, an systematic approach to quantitatively combine the results of all available studies was executed. The results are described in **Chapter 2**. A systematic search was performed using PubMed, Web of Science and Embase from their inception to February 2007 to identify all eligible studies. A total of 52 studies with a total of 17.347 patients were selected.

In these studies a considerable variation in mortality rates of patients with VAP was found: 14 to 78%. The pooled relative risk of the association VAP and mortality was 1.27 (95% confidence interval (CI) 1.15 to 1.39), but with an I^2 statistic of 69% indicating considerable heterogeneity. This high level of heterogeneity could not be reduced by pooling studies with similar methodology, clinical characteristics and quality. Heterogeneity was limited when pooling studies investigating only trauma patients (9 studies) or acute respiratory distress syndrome (ARDS, 4 studies) with estimated relative risks of 1.09 (95% CI 0.87 to 1.37) and 0.86 (95% CI 0.72 to 1.04) respectively. Nevertheless despite extensive heterogeneity, estimates of the studies that were not restricted to trauma patients or ARDS patients suggest that VAP may be associated with an increased mortality as most of these studies (31 of 38) had a relative risk above 1. The exact amount of attributable mortality of VAP could however not be quantified based on the studies. A striking observation was that only a minority of these studies performed multivariate analyses to control for possible confounders, which may also play a role in the observed associations between VAP and mortality.

The study described in **chapter 2** clearly demonstrates that the evidence regarding the attributable mortality of VAP is limited by small studies, the influence of confounding and marked differences in estimations of attributable mortality. Confounding can best be precluded by conducting a randomized controlled trial with "intentionally" induced VAP in one group, which of course is highly unethical. Based on this we aimed to determine the attributable mortality of VAP using the results from randomized controlled trials on different VAP prevention measures. We reasoned that all patients run a certain risk of developing VAP and that this is-at random-prevented. If the attributable mortality due to VAP would be 100%, a 50% relative risk reduction (RRR) of VAP incidence due to a randomly applied intervention should lead to a 50% RRR of ICU mortality. The ratio of the RRR of mortality and RRR of VAP will, therefore, provide an estimate of the attributable mortality of VAP.
In **chapter 3** a total of 58 randomized VAP prevention studies published until July 2010 were selected with a systematic search strategy using PubMed, Embase, Cochrane Library and Embase. The results of all these studies were pooled to calculate the RRRs of VAP and mortality.

The attributable mortality was subsequently calculated as the ratio of RRR of mortality and RRR of VAP and estimated as 9%. In subgroup analyses, evaluating de influence of study quality, diagnostic methods and effectiveness of VAP prevention, attributable mortality varied between 3% and 17%. In **chapter 3** only the data of the studies, as published in the original publication, were used to perform the analyses, limiting the ability to perform subgroup analyses and the ability to use more sophisticated statistical methods.

In **chapter 4** the attributable mortality is estimated by means of a meta-analysis with original patient data (so-called individual patient data meta-analysis (IPD-meta-analysis)). In this study all authors of VAP prevention studies published between 1998 and 2010 were invited to collaborate by sending the original database of their studies. This resulted in an IPD-meta-analysis with in total 24 studies from 13 countries and the original patient data of 6284 patients. De association between VAP and mortality was analyzed using two different methods.

First, the attributable mortality of VAP was estimated, similarly as described in **chapter 3**, i.e. as the ratio of the RRR of mortality and VAP.
Second, a more sophisticated method was used: a competing risk survival analysis. The use of a competing risk approach enabled us to model VAP as a time-dependent exposure and evaluate its effects on all competing endpoints, being ICU mortality and discharge from the ICU alive, as these endpoints prevent each other to occur first. The latter is important as ICU discharge is informative regarding the mortality pattern of the study population (patients are usually discharged from the ICU when their clinical condition is significantly improved or deteriorated), and should be taken into account when analyzing ICU mortality. By modeling VAP as a time-dependent determinant, specifically the effect on the prognosis of ICU patients after VAP could be evaluated.

In both methods predefined subgroup analyses included:
1. Surgical patients;
2. Trauma patients;
3. Medical patients; and
4. Patients with different categories of severity of illness scores ad admission (low (APACHE II <20 and SAPS2<35), midrange (APACHE II 20-29 and SAPS2 35-58) and high (APACHE II >30 and SAPS2 >58)).

The attributable mortality as calculated with the first method was estimated as 13%, with higher mortality rates among surgical patients and patients with mid-range severity scores at admission. Attributable mortality was close to zero in trauma patients, medical patients and patients with low or high severity of illness scores at admission.
Competing risk analyses were performed using the data of 5162 patients. The overall daily hazard for ICU mortality after VAP was 1.13 (95% CI 0.98 to 1.31) and the overall daily risk of discharge after VAP was 0.74 (95% CI 0.68 to 0.80), leading to an overall cumulative risk of

dying in the ICU of 2.20 (95% CI 1.91 to 2.54). As with the first method, highest cumulative risk for dying were found for surgical patients (2.97 (95% CI 2.24 to 3.94) and patients with midrange severity of illness score at admission (2.49 (95% CI 1.81 to 3.44) and 2.72 (95% CI 1.95 to 3.78)). Based on the competing risks analysis it was concluded that attributable mortality of VAP is mainly caused by a prolonged exposure to the risk of dying due to increased length of ICU stay.

The influence of the different pathogens on the attributable mortality of VAP is investigated in **chapter 5**. The individual patient data of fifteen VAP prevention trials were analyzed.
Pathogens were categorized as:

1. *Staphyloccus aureus;*,
2. Non fermenters (*Pseudomonas aeruginosa*, *Stenothrophomonas maltophilia* and Acinetobacter species);
3. Enterobacteriaceae,
4. Polymicrobial; and
5. Other

Competing risk analyses revealed that VAP caused by non fermenters and Polymicrobial VAP are associated with the highest mortality. Surgical patients had the highest attributable mortality of VAP, whereas VAP was not associated with higher mortality in trauma patients, both irrespectively of causative pathogens.

Heterogeneity

In this thesis meta-analytic techniques were included in the evaluation of attributable mortality of VAP. Important in performing meta-analyses is the judgment of heterogeneity, which is currently based on calculation of the I^2-statistic. In **chapter 2** high levels of heterogeneity were important for assessing the reliability of the pooled relative risk. Currently, there is no consensus on how to deal with heterogeneity, and many systematic reviews calculate pooled estimates even when heterogeneity is high.

In **chapter 6** a simulation model was used to investigate the accuracy of the I^2 statistic in the assessment and quantification of heterogeneity. Also, the predictive value in relation to heterogeneity across studies was evaluated. The crucial importance of study selection (in other words minimizing clinical and methodological heterogeneity) for the accuracy of pooled estimates derived from meta-analyses was demonstrated. Quantifying statistical heterogeneity, through I^2 statistic, is only helpful when the amount of heterogeneity is unknown and the I^2-statistic is high, in all other situations the I^2-statistic does not contribute to the assessment of heterogeneity.

As meta-analyses have become so important in evidence based medicine, their results should be reliable and accurate. It was demonstrated that heterogeneity importantly influences both aspects. As there are no reliable methods to quantify the amount of heterogeneity yet, careful selection of appropriate studies is the only available tool. Unfortunately this selection will, at least to some extent, be subjective. Furthermore, pooled estimates should not be provided in case of high levels of heterogeneity. A critical appraisal of meta-analyses before accepting their results is warranted.

Subgroup effects in prevention studies

The intensive care population is a very diverse patient population consisting of patients with differences in admitting specialism (e.g. surgical, medical, trauma), differences in underlying severity of illness, differences in reasons for admission, and so on. One should take these differences into account when designing and analyzing studies performed in ICU. The effect of subgroups are not only important when evaluating the role of VAP or other nosocomial infections, but also when evaluating the effectiveness of measures in prevention studies.

In **chapter 7** potential differences in effectiveness of selective digestive decontamination (SDD) and selective oropharyngeal decontamination (SOD) in surgical and non-surgical ICU patients was assessed. Both measures are aimed to reduce the incidence of nosocomial ICU infections and thus to improve the survival of patients. The difference between SOD and SDD is that SDD has the addition of systemic prophylaxis and enteric decontamination. A large cluster randomized multicenter trial showed almost equal effectivity of SOD and SDD in reducing 28 day mortality (odds ratio (OR) 0.86 (95%CI 0.74 to 0.99) en 0.83 (95% CI 0.72 to 0.97), respectively). The post-hoc subgroup analysis of data from this trial reported in **chapter 7** found equal effects of SDD in reducing mortality in surgical and non-surgical ICU patients ((OR) 0.86 (95%CI 0.69 to 1.09) en 0.85 (95%CI 0.70 to 1.03)), whereas SOD was only effective in non-surgical patients (OR 0.77 (95%CI 0.63 to 0.94)).

Conclusion and recommendation

As demonstrated in this thesis, the attributable mortality of VAP can be investigated in several ways:

1. The use of observational studies of patients at risk for VAP, of whom some will develop VAP (and some will not) and subsequent comparison of crude mortality rates of those with and without VAP. This is obviously flawed by the many variables - aside from developing VAP or not - that differ between both patient groups that also influence mortality.
2. The use of the same observational studies, but now with additional adjustments for differences that are not equally distributed between those developing and not developing VAP. Yet, in many studies these methods overestimate the attributable mortality of VAP because of – inevitable – incomplete adjustment for differences between groups – both unmeasured variables at baseline, as well as changes in clinical condition during ICU stay.

3. One could - theoretically but not ethically - randomize patients to receive VAP or not and count deaths in each group.
4. Using the data of randomized controlled trials on VAP prevention. This provides the benefit of randomization, which excludes the effects of measured and unmeasured differences between groups.
5. Using novel statistical methods, such as competing risk survival analyses including VAP as a time dependent exposure.

From the studies described in this thesis it can be concluded that the estimated attributable mortality of VAP is around 10% (9% in chapter 3 and 13% in chapter 4), which is much lower than estimates provided in previous studies. Subgroup analyses revealed an absence of attributable mortality in trauma patients, medical patients and patients with a low or high severity of illness at admission. Highest attributable mortality rates are found in surgical patients and patients with an intermediate score of severity of illness at admission.

The lower estimates of attributable mortality and the differences of mortality in subgroups of patients are of critical importance for the design and interpretation of VAP prevention trials. Many studies were hugely underpowered to demonstrate improvements in patient outcome. The new knowledge on attributable mortality underpins the need of large studies (>1000 patients per study group) to demonstrate whether VAP prevention improves patient outcome. Furthermore, it is rational to focus on the subgroup of patients with the highest mortality rates, considering the large differences in mortality between subgroups.

Further methodological improvements are needed in determining the attributable mortality of VAP, especially concerning methods to limit the influence of confounding. The use of multistate models might improve the determination of the attributable mortality of VAP. As these methods enable various intermediate events, and thus could offer more flexibility in the analyses and better capture the (chronological) complexity of factors influencing mortality.

SUMMARY

SAMENVATTING

DANKWOORD

CURRICULUM VITAE

Attributieve mortaliteit van beademings-geassocieerde longontsteking

Beademings-geassocieerde longontsteking of Ventilator-geassocieerde pneumonie (VAP) is een van de meest voorkomende infecties bij patiënten die opgenomen zijn op de intensive care (IC). Algemeen wordt aangenomen dat VAP de morbiditeit en de mortaliteit van IC patiënten verhoogt. Deze aanname is met name gebaseerd op de resultaten van observationele studies.

In **hoofdstuk 2** van dit proefschrift geven wij een overzicht van alle observationele studies die tot op heden zijn verricht om de relatie tussen VAP en sterfte te onderzoeken. Hiertoe hebben we een systematische literatuurstudie uitgevoerd (PubMed, Web of Science en Embase). In totaal hebben we 52 observationele studies geselecteerd met in totaal 17.347 patiënten. De resultaten van al deze studies zijn gepoold, wat resulteerde in een relatief risico (RR) van 1,27 (95% betrouwbaarheidsinterval (BI) 1,15 tot 1,39).

De studies vertoonden een grote verscheidenheid (heterogeniteit) in resultaten (I^2 van 69%). Dit betekent dat het relatief risico niet betrouwbaar is. De verschillen in resultaten konden niet worden verklaard door verschillen in opzet van de studies, kwaliteit van de studies en verschillen in diagnostische VAP criteria die de diverse studies hanteerden. Studies die alleen trauma patiënten (9 studies) of alleen patiënten met "acute respiratory distress" syndroom (ARDS, 4 studies) includeerden lieten andere resultaten zien, met relatieve risico's van respectievelijk 1,09 (95% BI 0,87 tot 1,37) en 0,86 (95% BI 0,72 tot 1,04). Op grond van al deze studies lijkt er geen bewijs te zijn dat VAP de sterfte verhoogt bij traumapatiënten of patiënten met ARDS. Echter, in andere groepen patiënten lijkt VAP wel tot meer sterfte te leiden (de meeste studies hebben een relatief risico groter dan 1). Opvallend is het gegeven dat slechts een minderheid van de studies een multivariate analyse verrichtte om te corrigeren voor eventuele confounding.

De studie beschreven in **hoofdstuk 2** laat duidelijk zien dat de uitkomsten van de studies die de mortaliteit van VAP onderzoeken tegenstrijdig zijn: de attributieve mortaliteit van VAP varieert en sommige studies laten helemaal geen verhoogde mortaliteit zien. De meeste studies includeren een klein aantal patiënten en vanwege het observationele design kan confounding niet worden uitgesloten.

Een gerandomiseerde studie (waarin VAP bewust geïnduceerd wordt) is het beste design om confounding uit te sluiten maar uiteraard is dit niet-uitvoerbaar en bovendien onethisch. De studie beschreven in **hoofdstuk 3** is echter wel op het principe van een gerandomiseerde studie gebaseerd. In dit hoofdstuk hebben we namelijk de mortaliteit van VAP berekend, gebruik makend van de resultaten van gerandomiseerde VAP preventie studies. De hypothese hierbij is dat alle patiënten een bepaald risico lopen op het ontwikkelen van VAP en dat dit, willekeurig ("at random") wordt voorkomen door een preventiemaatregel. Dat wil zeggen dat indien de attributieve mortaliteit van VAP 100% is, een relatieve risico reductie (RRR) van VAP van 50% leidt tot een 50% RRR in mortaliteit. De ratio van de RRR van mortaliteit en de RRR van VAP is daarom een weergave van de attributieve mortaliteit.

In totaal hebben we 58 gerandomiseerde preventie studies geselecteerd die tot juli 2010 zijn gepubliceerd. We hebben deze selectie uitgevoerd door middel van een systematische zoekstrategie in verscheidene medische databases (PubMed, Embase, Cochrane Library en Embase). De resultaten van al deze studies zijn gepoold om de RRR's van VAP en mortaliteit te berekenen. De attributieve mortaliteit, die is berekend als de ratio van de RRR van de mortaliteit en de RRR van VAP, was 9%. In subgroepanalyses, waarin we de invloed van de kwaliteit van de studies, diagnostische methoden en effectiviteit van VAP preventie hebben bekeken, varieerde de mortaliteit tussen de 3% en 17%. Omdat we in deze studie alleen de data hebben gebruikt die beschikbaar waren in de originele publicaties van de verschillende studies, was het niet mogelijk om relevante klinische subgroepanalyses te verrichten en gebruik te maken van nieuwere statistische technieken.

In **hoofdstuk 4** hebben we de attributieve mortaliteit van VAP berekend via een meta-analyse met originele patiënten gegevens (ook wel een individuele patiënten data (IPD) meta-analyse genoemd). Voor deze studie hebben we alle auteurs, die tussen 1998 en 2010 een VAP preventie studie publiceerden gevraagd, om hun originele database met alle patiëntgegevens ter beschikking te stellen. Dit heeft geresulteerd in een database van 24 internationale studies met de originele gegevens van in totaal 6.284 patiënten. De relatie VAP en sterfte hebben we middels twee verschillende methoden bestudeerd.

Allereerst hebben we de attributieve mortaliteit van VAP, namelijk de ratio van de RRR van mortaliteit en VAP, berekend (zoals in hoofdstuk 3).
Daarnaast hebben we een "competing risks survival" analyse uitgevoerd, waarin VAP als een tijdsafhankelijke variabele is meegenomen. Het gebruik van een "competing risks survival" analyse met VAP als tijdsafhankelijke determinant maakte het mogelijk om het effect van VAP op de concurrerende eindpunten te onderzoeken. Wanneer IC-sterfte het primaire eindpunt van de studie is, dient ontslag van de IC altijd te worden meegenomen als concurrerend eindpunt. Ontslag van de IC is namelijk informatief: de toestand van een patiënt is dusdanig veranderd (verbeterd of verslechterd) dat verdere behandeling op de IC niet noodzakelijk is. Bovendien sluit ontslag van de IC overlijden op de IC uit.

Binnen beide methoden hebben we de volgende subgroepen bestudeerd:
1. chirurgische patiënten;
2. traumapatiënten;
3. interne geneeskunde patiënten; en
4. patiënten met bij opname verschillen in ernst van ziekte (uitgedrukt in een APACHE II en SAPS 2 scoresysteem, die elk worden onderverdeeld in drie categorieën: gering (APACHE II <20 en SAPS2 <35), gemiddeld (APACHE II 20-29 en SAPS2 35-58) en ernstig (APACHE II >30 en SAPS2 >58)).

De volgens de eerste methode berekende attributieve mortaliteit was 13%. De mortaliteit was hoger bij chirurgische patiënten en bij patiënten met een gemiddelde ernst van ziekte. De attributieve mortaliteit bij traumapatiënten, interne geneeskunde patiënten en patiënten met een geringe ernst van ziekte was nihil.

Bij 5.162 patiënten hebben we een "competing risks survival" analyse uitgevoerd. De hazard om te overlijden ten gevolge van VAP was 1,13 per dag (95% BI 0,98 tot 1,31). De hazard voor ontslag van de IC na VAP was 0,74 per dag (95% BI 0,68 tot 0,80). Het cumulatieve risico voor sterfte op de IC, dat wordt bepaald door zowel de hazard per dag om te overlijden als de hazard per dag voor ontslag, is 2,20 (95% BI 1,91 tot 2,54).

Uit de resultaten van de "competing risk survival" analyse blijkt dat VAP niet direct de mortaliteit verhoogt, aangezien de hazard per dag om te overlijden niet significant is. Door de langere ligduur op de IC als gevolg van de VAP (hazard per dag voor ontslag is 0,74 (95% BI 0,68 tot 0,80)) is er echter een langere blootstelling aan het risico op sterfte, wat cumulatief leidt tot een verhoogde mortaliteit. In de "competing risks survival" analyse vonden we een verhoogde mortaliteit bij chirurgische patiënten en bij patiënten met een gemiddelde ernst van ziekte.

In **hoofdstuk 5** beschrijven we het effect van verschillende pathogenen op de attributieve mortaliteit van VAP. Hiervoor hebben we wederom originele patiëntengegevens gebruikt. Voor dit onderzoek hebben we 15 VAP preventie trials geselecteerd met in totaal 3.231 patiënten, waarvan 532 patiënten (16%) VAP hebben gekregen tijdens hun verblijf op de IC.

De pathogenen werden onderverdeeld in de volgende groepen:
1. *Staphyloccus aureus*;
2. niet-fermenterende Gram-negatieve staven (voornamelijk *Pseudomonas aeruginosa*);
3. Enterobacteriaceae;
4. polymicrobieel; en
5. overig.

De resultaten laten zien dat door meerdere pathogenen veroorzaakte VAP en door niet-fermenterende Gram-negatieve staven veroorzaakte VAP geassocieerd zijn met de hoogste mortaliteit. Chirurgische patiënten bleken de hoogste attributieve mortaliteit te hebben. Bij traumapatiënten was er geen sprake van een verhoogde mortaliteit onder patiënten met VAP. Beide bevindingen waren onafhankelijk van het type pathogeen.

Heterogeniteit

In dit proefschrift hebben we onder meer gebruik gemaakt van meta-analyses. Belangrijk bij het verrichten van een meta-analyse is het bepalen van de mate van heterogeniteit (de verschillen tussen de studies). Een statistische test die hier vaak voor wordt gebruikt, is de "I^2 statistic". In **hoofdstuk 2** gaven we zelf reeds aan te twijfelen aan de betrouwbaarheid van de uitkomst van de meta-analyse, vanwege de grote mate van heterogeniteit tussen de studies.

Er is geen duidelijke consensus over de vraag hoe om te gaan met heterogeniteit. In veel reviews worden gepoolde effectmaten berekend, zelfs wanneer er een aanzienlijke mate van heterogeniteit aanwezig is.

In **hoofdstuk 6** hebben we een simulatie model beschreven waarmee we de nauwkeurigheid van de I^2 statistic in het beoordelen van de mate van heterogeniteit bestuderen. Tevens hebben we gekeken naar de invloed van heterogeniteit op het voorspellend vermogen van een meta-analyse. De betrouwbaarheid van een meta-analyse blijkt sterk afhankelijk van de selectie van studies (met andere woorden: het beperken van heterogeniteit).
Het kwantificeren van heterogeniteit met behulp van de I^2 is alleen nuttig wanneer onduidelijk is of er sprake is van heterogeniteit en de I^2 hoog is. In alle andere situaties heeft het bepalen van de I^2 geen toegevoegde waarde. Gelet op de belangrijke rol die meta-analyses tegenwoordig vervullen in de evidence based medicine – en daarmee bij het tot stand komen van allerlei richtlijnen en aanbevelingen – is de betrouwbaarheid van de resultaten van een meta-analyse van groot belang.

Tot op heden zijn er geen betrouwbare methoden om de mate van heterogeniteit te bepalen. Als gevolg hiervan is een strenge selectie van te includeren studies in een meta-analyse noodzakelijk. Een nadeel van deze selectie is dat deze – in elk geval voor een deel – subjectief is en blijft. Wanneer sprake is van heterogeniteit raden wij af om een gepoolde effectmaat te berekenen. Een kritische beoordeling van meta-analyses alvorens de resultaten te accepteren is ons inziens noodzakelijk.

Subgroepeffecten bij preventie studies

Een intensive care populatie is een zeer gevarieerde patiënten populatie, die verschilt in de ernst van ziekte, specialisme (bijvoorbeeld trauma, chirurgisch, interne geneeskunde), reden van opname enzovoorts. Bij onderzoek verricht onder IC-patiënten moet met deze verschillen rekening worden gehouden.

In **hoofdstuk 7** onderzochten wij verschillen in de effectiviteit van SDD (selective digestive decontamination) en SOD (selective oropharyngeal decontamination) tussen chirurgische en niet chirurgische patiënten. Zowel SDD als SOD zijn er op gericht om infecties bij IC-patiënten te voorkomen en dus hun toestand te verbeteren. Het verschil tussen SOD en SDD is dat bij SDD decontaminatie van de darm en routinematig profylactisch gebruik van parenterale antibiotica tijdens de eerste vier dagen wordt toegevoegd. Een grote cluster-gerandomiseerde trial vond bijna geen verschil in de effectiviteit van SOD en SDD (odds ratio (OR) 0,86 (95%BI 0,74 tot 0,99) en 0,83 (95% BI 0,72 tot 0,97)).

In de subgroep analyse, beschreven in **hoofdstuk 7**, hebben wij wel verschillen in de effecten van SDD en SOD voor chirurgische en niet-chirurgische patiënten gevonden. SDD is net zo effectief voor chirurgische als voor niet-chirurgische patiënten in het verminderen van de

mortaliteit na 28 dagen ((OR) 0,86 (95%BI 0,69 tot 1,09) en 0.85 (95%BI 0,70 tot 1,03)). SOD blijkt met name effectief voor niet-chirurgische patiënten (OR 0.77 (95%BI 0.63 tot 0.94)). SOD wordt niet geassocieerd met een reductie in de mortaliteit na 28 dagen bij chirurgische patiënten.

Conclusies en aanbevelingen

In dit proefschrift laten wij zien dat de attributieve mortaliteit van VAP op verschillende manieren kan worden onderzocht, te weten middels:

1. observationele studies met patiënten at risk voor VAP waarbij de sterftecijfers van patiënten met VAP en patiënten zonder VAP worden vergeleken. Deze methode is echter onbetrouwbaar omdat naast VAP ook andere variabelen die van invloed kunnen zijn op de mortaliteit, kunnen verschillen;
2. dezelfde observationele studies, waarbij wel wordt gecorrigeerd voor verschillen tussen patiënten met VAP en patiënten zonder VAP. Ook deze methode kent beperkingen omdat alleen gecorrigeerd kan worden voor bepaalde baseline karakteristieken (zoals APACHE, geslacht, leeftijd, reden van opname). Deze methode overschat de attributieve mortaliteit van VAP aangezien niet gecorrigeerd kan worden voor variabelen die niet zijn gemeten en variabelen die wijzigen gedurende het verblijf op de IC;
3. een gerandomiseerde studie, maar dit is niet-uitvoerbaar en bovendien onethisch;
4. data van gerandomiseerde VAP preventie trials waarmee, door de randomisatie, zowel gemeten als niet-gemeten verschillen in variabelen worden voorkomen;
5. het gebruik van state of the art statistische methoden, zoals de competing risks survival analyses, waarbij VAP als tijdafhankelijke variabele wordt gemodelleerd.

De geschatte attributieve mortaliteit van VAP ligt rond de 10% (9% in **hoofdstuk 3** en 13% in **hoofdstuk 4**). Deze schatting is veel lager dan de schattingen in eerdere studies. Bij subgroepanalyses vonden we geen attributieve mortaliteit van VAP bij trauma patiënten, interne geneeskunde patiënten en patiënten met een geringe of ernstige ziekte bij opname. Chirurgische patiënten en patiënten met een gemiddelde ziekte-ernst bij opname bleken de hoogste attributieve mortaliteit te hebben.
Deze bevindingen, de lagere attributieve mortaliteit en het effect van subgroepen, zijn essentieel bij het uitvoeren van VAP preventie trials en bij het interpreteren van de resultaten daarvan. Veel studies waren te klein om een verschil in mortaliteit tussen de groepen aan te tonen. Grote studies, met de inclusie van meer dan 1.000 patiënten per studiearm, zijn noodzakelijk om verschillen aan te kunnen tonen. Bovendien is het verstandig om zich te concentreren op de patiëntengroepen met de hoogste mortaliteit.

Verdere verbeteringen in de methodologie die wordt toegepast om de attributieve mortaliteit van VAP te berekenen is noodzakelijk, met name met betrekking tot het verminderen van de invloed van confounding. Het gebruik van nieuwere statische technieken (zoals bijvoorbeeld een "multistate model") waarbij er meer flexibiliteit is voor het modeleren van (tijdens een IC opname veranderende) factoren die de sterfte beïnvloeden, zal moeten worden aangemoedigd.

SUMMARY

SAMENVATTING

DANKWOORD

CURRICULUM VITAE

Iedereen die heeft bijgedragen aan de totstandkoming van dit proefschrift wil ik daarvoor heel hartelijk bedanken. Een aantal mensen wil ik in het bijzonder noemen.

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Marianne

SUMMARY

SAMENVATTING

DANKWOORD

CURRICULUM VITAE

Wilhelmina Gerrianne (Marianne) Melsen was born on March 8th, 1981, in Zoetermeer, the Netherlands. After graduating grammar school at the Driestar College in Gouda in 1999, she started her medical training at the University of Antwerp, Belgium and received her first Candidature degree with distinction. She continued her study medicine in 2000 at the University of Utrecht, the Netherlands. In 2004 she joined the research group of Prof. dr. M.J.M. Bonten and in 2006 she started investigating the attributable mortality of Ventilator-Associated Pneumonia. After receiving her medical degree cum laude in September 2006 she continued her work, as described in this thesis, at the Julius Center for Health Sciences and Primary Care, University Medical Centre Utrecht (UMC Utrecht). In 2008 she received her Master of Science degree in Epidemiology at the University of Utrecht. In 2009 she started her specialist training in Internal Medicine at the St. Antonius Hospital, Nieuwegein, the Netherlands, under the supervision of dr. A.B.M. Geers and dr. M.M.E. Schneider (UMC Utrecht).