In search of new diagnostic modalities and techniques in ventilator-associated pneumonia

Chapter 8

General discussion and summary

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The present thesis describes the search for new diagnostic modalities and techniques in ventilator-associated pneumonia (VAP), a common nosocomial infection in intensive care patients. All studies were conducted in a university hospital intensive care unit in the Netherlands. The diagnostic standard for VAP following clinical suspicion has been bronchoalveolar lavage (BAL) and subsequently microbiological culture with quantitative analysis for more than a decade. Hence a substantial database of BAL fluid analyses was available. Combined with clinical data this allowed further investigation of the diagnostic process of VAP.

Alternative diagnoses in the putative ventilator-associated pneumonia patient not meeting lavage-based diagnostic criteria

It has been known that on average in only a quarter of all patients clinically suspected of having VAP, bacterial pneumonia can be confirmed by BAL fluid analysis.[1] The clinical picture of VAP can be mimicked by various other infectious and non-infectious diseases.[2] Establishing a correct alternative diagnosis rapidly may be vital for initiating adequate treatment and benefiting patient outcome. Aim of the study was to determine the frequency of alternative diagnoses in putative VAP patients with negative lavage results. For this purpose data of BAL fluid analyses and clinical data of patients were retrospectively analysed. Results delivered numerous different alternative diagnoses of a wide clinical spectrum. The most frequently found alternative diagnoses and recommended diagnostic tests in the further workup are summarized in Table 8.1. A generally applicable diagnostic flowchart could not be deduced from the analysis. Diagnostic work-up has therefore to be individualized and guided by repeated clinical assessment. A frequent finding in our study was the presence of *Herpes simplex* virus DNA in BAL fluid. However, the clinical significance has been debated whether *Herpes simplex* virus has a genuine pathogenic role or is only a bystander.[3] It was previously demonstrated that *Herpes simplex* DNA can be detected in the lower airways in about 30% of critically ill ventilated patients correlated to the severity of illness. More severe illness may lead to immune suppression, viral reactivation and shedding of microorganisms from the throat. No relationship between HSV-1 viral load and pulmonary injury or additional mortality could be established. This suggests a lack of pulmonary pathogenicity.[4] Other studies contradict these data claiming some of HSV-1 positive critically ill patients may have evidence of viral pneumonia.[5] If HSV-1 has a genuine pathogenic role, then treatment with acyclovir should result in better outcome. A recent study could establish a better mortality outcome when patients with HSV-1 positivity in BAL fluid were treated with acyclovir.[6] Although the exact significance of HSV pneumonia remains to be established, treatment should be

considered if no other likely causes of respiratory failure in severely ill patients can be identified.

Table 8.1 Frequent alternative diagnoses in patients with negative bacterial growth BAL result and recommended diagnostic tests

Alternative diagnoses	recommended diagnostic tests
Non-bacterial infectious pneumonia	PCR for presence of viruses, *Pneumocystis jirovecii*, fungi in BAL fluid; Galactomannan
Non-infectious pneumonitis	computed tomography; lung biopsy
Cardiovascular (heart failure, pulmonary embolism, endocarditis)	echocardiography; computed tomography
Different septic focus (abdominal, urogenital, cerebral)	repeated blood culture, computed tomography

Candida pneumonia in critically ill ventilated patients

Whereas the role of bacteria in the etiology of VAP has been established and diagnostic cut-off values have been defined, less is known about the role of fungi in nosocomial pneumonia. *Candida species* are the most common isolated yeasts in critically ill patients but the clinical significance and pathogenic potency are still argued.[7-9] *Candida spp.* isolation in the respiratory tract was associated with prolonged stay and increased risk of VAP.[10] The presence of *Candida spp.* in respiratory secretions of patients with VAP could be associated with longer mechanical ventilation, prolonged stay and worse outcome.[7,11] Respiratory tract colonization with *Candida spp*. can originate from the oropharyngeal cavity and upper respiratory tract in severely ill patients with diminished immune response.[12] But many believe *Candida* does not directly contribute to respiratory disease and is merely a marker for severity of illness.[4] A recent analysis investigated immune competent patients with nosocomial intensive-care unit acquired pneumonia with or without *Candida spp.* isolation in the respiratory tract. Although patients with *Candida spp.* had higher severity scores and organ dysfunction on admission and at onset of pneumonia, there were no differences in the systemic inflammatory response, length of stay and mortality. Antifungal therapy did not significantly influence outcome.[11] *Candida* pneumonia as a clinical entity is questioned altogether.[13] Specific cut-off values for fungi in BAL fluid are lacking. Only histopathology could establish the definitive diagnosis. Less invasive diagnostic strategies lack specificity and have been insufficiently validated. In our study we linked microbiological data from the BAL database to clinical information and available autopsy data. Critically ill patients with respiratory failure with no other microbiological or clinical explanation than exclusive presence of *Candida* species in bronchoalveolar lavage fluid were described. Reviewing 701 included BAL specimens and subsequently linking it to the clinical cases, five patients (0.7%) could be identified with possible *Candida* pneumonia. It definitely is a rare entity but clinical evidence suggests that the

condition can occur under certain circumstances. [Table 8.2] In cases of serious respiratory failure, radiographic and laboratory evidence for pneumonia and no other growth than *Candida* in the BAL fluid C*andida* pneumonia should be considered and subsequently treated.

Table 8.2 Risk factors under which *Candida* pneumonia might occur in critically ill patients

Risk factor for *Candida* pneumonia	
Increased *Candida* load	diverticulum of the oesophagus, diabetes mellitus, nicotine and alcohol abuse, aspiration of gastric fluids
Immunosuppression	cancer, immunosuppressive drugs, malnutrition
Broad spectrum antibiotic treatment	

Acanthamoeba polyphaga mimivirus as a respiratory pathogen in critically ill ventilated patients

An investigation of a pneumonia outbreak with no clear causative agent led to the discovery of *Acanthamoeba polyphaga Mimivirus* (APMV) named so due to its mimicry of a bacterium by its size and appearance in Gram staining. Viruses belonging to the Mimiviridae family potentially cause community-acquired and healthcare-associated pneumonia.[14] The clinical significance of APMV in the etiology of VAP has been suggested in earlier studies. APMV is known to induce histological evidence of pneumonia with the formation of diffuse alveolar damage in mice. Since the virus is able to induce pneumonia in mice, it was hypothesized that it would also be able to induce pneumonia in humans. Evidence of APMV as a respiratory pathogen in humans has mainly been based on serologic studies. It was shown that a fifth of intensive care patients with a suspicion of VAP are seropositive for APMV and this was associated with an increased duration of mechanical ventilation and ICU stay.[15,16] However, the mere presence of antibodies only showed that the individual had been into contact with the virus, but did not always imply disease. Therefore we looked for the presence of the virus in patients with a clinical suspicion of VAP. We were unable to detect APMV DNA in BAL fluid and therefore concluded that APMV does not cause VAP.

Clinical course and complications following diagnostic bronchoalveolar lavage

Bronchoscopic BAL from the presumed site of infection with cytological analysis and quantitative microbiological culture of the lavage fluid has been established as a diagnostic standard for VAP.[17-19] However, BAL is an invasive diagnostic technique

carried out in vulnerable ventilated patients. Studies concerning the safety of bronchoscopic diagnostic techniques in critical care patients were small in size, differed in patient population, setting, applied diagnostic techniques and length of observation.[20-27] The present study was the largest analysis of the clinical course and complications in patients following bronchoscopic BAL in the diagnosis of VAP. Frequently occurring haemodynamic and respiratory instability could be attributable to diagnostic BAL but no cases of severe cardiac rhythm disturbances, bleeding, pneumothorax or procedure related death were observed. [Table 8.3] The study did not allow to define clear-cut clinical criteria for withholding BAL because the limited number of patients with severe acidosis (pH<7.25), hypercapnia (PaCO2 ≥7.5kPa/56 mmHg) and hypoxia (PaO2 ≤8kPa/60mmHg or PaO2/FiO2 ratio ≤13kPa/100 mmHg).

Table 8.3 Summary of the clinical course and complications occurring within 24 hours following diagnostic FFB and BAL

Hypo-oxygenation (SaO_2 ≤ 88%) during BAL and/or bronchospasm in 9% of patients
Decrease in the average PaO_2/FiO_2 ratio which fully recovered to baseline after 24 h
Respiratory instability in 29% of patients one hour after BAL
Haemodynamic instability in 22% of patients within 24h
Haemodynamic instability correlated with a cardiovascular diagnosis at admission and the presence of cardiovascular co-morbidity
Newly positive blood cultures following BAL in 7% of patients
No patients with hypertension or cardiac rhythm disturbances
No cases of clinically significant bleeding requiring interruption of the procedure or treatment
No case of pneumothorax
No case of procedure related death

Diagnosis of ventilator-associated pneumonia from exhaled breath analysis

Bronchoscopic BAL is invasive and has its limitations in patients with severe pulmonary disease, high respiratory support settings and coagulation abnormalities. Analysis of BAL is laborious, costly and time-consuming before definitive results are available and the diagnosis of VAP can be confirmed or rejected. Therefore, it has been of interest to find new methods that allow a fast, reliable, non-invasive diagnosis of VAP. Exhaled breath analysis is an emerging and promising technique that may be able to meet some of these criteria. It is based on the analysis of volatile organic compounds (VOCs) present in exhaled breath. VOCs originate from both exogenous and endogenous sources. VOCs are produced by biological processes including oxidative stress and inflammation in the human body [28,29] as well as by invading microorganisms [30]. Upon their production, VOCs are excreted into the blood after which they diffuse into the

lungs where they are exhaled. Two different techniques of exhaled breath analysis in VAP were studied. Gas chromatography coupled with mass spectrometry (GC-MS) can directly detect VOCs. It separates molecules according to their volatility and interaction with the stationary phase of an absorbing column. During mass-spectrometry components are fragmented into charged particles, carried through an electromagnetic field and quantitatively detected.[31,32] Electronic nose (e-nose) technology with hybrid metal oxide semiconductor sensors cannot directly detect different VOCs. E-noses are artificial sensor systems with an algorithm for pattern recognition. Changes in temperature-time-conductivity curves caused by presumed differences in the VOC composition of exhaled breath in patients with and without VAP were analysed. The results were compiled in Table 8.4 and compared with demands for an ideal diagnostic technique for VAP and the current diagnostic standard, the bronchoscopic BAL analysis. Exhaled breath analysis with GC-MS could not identify VOCs that exclusively appear in patients with VAP. But it could be demonstrated that it is possible to distinguish ICU patients with VAP from patients without VAP based on a profile of only 12 VOCs. However, the diagnostic sensitivity and specificity are insufficient for current clinical application. In the present study, the applied e-nose sensors lacked the clinically required diagnostic sensitivity and specificity in the direct exhaled breath detection of patients with VAP. Both studies had a relatively small number of subjects to test for specific strains of bacteria. As VAP is generally caused by an array of bacteria, there were only a few patients per bacterial strain available at most, hindering the use of multivariate statistics to identify strain-specific VOCs *in vivo*.

The selected VOCs to discriminate between VAP(+) and VAP(-) critically ill patients were associated with inflammation and activation of the immune system (Acrolein) or with activation of the metabolism (Ethanol, Aceton) in general. As two groups of comparably ill ICU patients were investigated, it might be difficult to discriminate with parameters that react to activation of inflammatory cascades in the body between subjects with and without additional presence of VAP. The majority of VOCs were (branched) alkanes (heptane, 2-methylbutane, dodecane, tetradecane and tetradecanal). Alkanes are present in the environment and are inhaled on a daily basis. After ingestion, the compounds are broken down in the liver by cytochrome P450 enzymes (CYP). The activity of these enzymes decreases with aging, but also with disease, implicating reduced CYP activity in severely ill patients.[33] Differences in these VOCs could be caused by variations in age and severity of illness, inflammation and metabolic state. Ethylbenzene is a benzene derivative and an indoor pollutant.[34] Tetrahydrofuran and carane are also environmental pollutants without known endogenous source. Benzene and its derivatives are also broken down in the liver by CYP enzymes, which may have an altered activity in critically ill patients, resulting in different exhaled abundances of ethylbenzene. Thus, different concentrations in exhaled breath could likely reflect differences in exogenous source or liver metabolism.

Table 8.4 Comparison between an ideal diagnostic technique for VAP and bronchoalveolar lavage (BAL), electronic nose (e-nose) and gas chromatography-mass spectrometry (GC-MS)

Ideal technique	BAL	e-nose	GC-MS
Non–invasive sampling	invasive technique, requires bronchoscopy and lavage with frequent respiratory and haemodynamic deterioration	non-invasive exhaled breath collection	non-invasive exhaled breath collection
Feasible in all patients	contraindicated in patients with coagulation abnormalities	no restrictions	no restrictions
Point-of-care results	no	potentially possible	no
Short duration until results available	days	<1h	hours
Not labour-intensive	laborious, requires bronchoscopy, lavage, cellular analysis and microbiological culturing	non-laborious, could become automated	laborious, requires exhaled breath sampling, carbon element fixation, lab analysis
Inexpensive	no; requires manpower during sampling and analysis, expensive equipment, single use material necessary	no; limited durability of current sensors	no; requires manpower during analysis, expensive equipment
Strain specific diagnosis	yes	not yet available	not yet available
Antibiogram available	yes	no	no
High sensitivity / specificity	42-93 / 45-100%[1]	76 / 56%[2]	76 / 73%[2]

[1] compared to histopathology; [2] compared to BAL

In general, exhaled breath data contain several sources of variance, which include information of interest with regard to the examined disease, but also irrelevant variance associated with biological variation and noise.[35,36] Ideally, in diagnostic exhaled breath analysis all sources of exogenous compounds should be eliminated and VOCs derived from the environment should be excluded from the data analysis and evaluation of biomarkers.[33] Selected VOCs should have at least a 15% higher concentration in exhaled breath than in ambient air, the use of plastic for breath sampling should be reduced and exogenous compounds derived from room air, consumer products, food, analytical devices as well as compounds associated with cigarette smoking should all be excluded.[37] Furthermore, exhaled breath collection and data processing methodologies could be more standardised.[35] These measures could reduce inconsistency in results derived from exhaled breath analysis in the detection of respiratory diseases and thus facilitate the development of true volatile biomarker profiles. The availability of biomarker VOCs could enable the development of more specific electronic nose sensors to transfer exhaled breath analysis of respiratory disease towards the bedside. Further developments could focus on integrated devices for an early prediction of pneumonia development in ventilated patients. Ideally, it should be also possible to follow the response to therapeutic interventions to give guidance in antibiotic stewardship.

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