Chapter 8

Summary and discussion

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Pulmonary diseases can be of various aetiologies including infectious, immunologic, malignant, environmental or an occupational origin. Traditionally, these diseases are evaluated by laboratory tests, lung function tests, imaging procedures, bronchoalveolar lavage (BAL) and tissue biopsies.
BAL is a safe, limited invasive, and generally well-tolerated method for obtaining cellular and a-cellular components of the lower respiratory tract[1,2]. In the diagnostic work-up of pulmonary diseases BAL can be used as an additional tool. Careful analysis of the BAL fluid (BALF) cell profile and presence of a-cellular components in BALF can, combined with clinical and radiological features, help to ascertain a diagnosis. Application of BALF in the diagnosis of pulmonary infections has already proven to be very useful[3], especially in case of ventilator-associated pneumonia (VAP)[4-6] and opportunistic infections such as Pneumocystis pneumonia (PCP)[7,8]. Figure 8.1 shows a flowchart of the BALF work-up as it is used in our laboratory used in immunocompetent intensive care patients on mechanical ventilation.

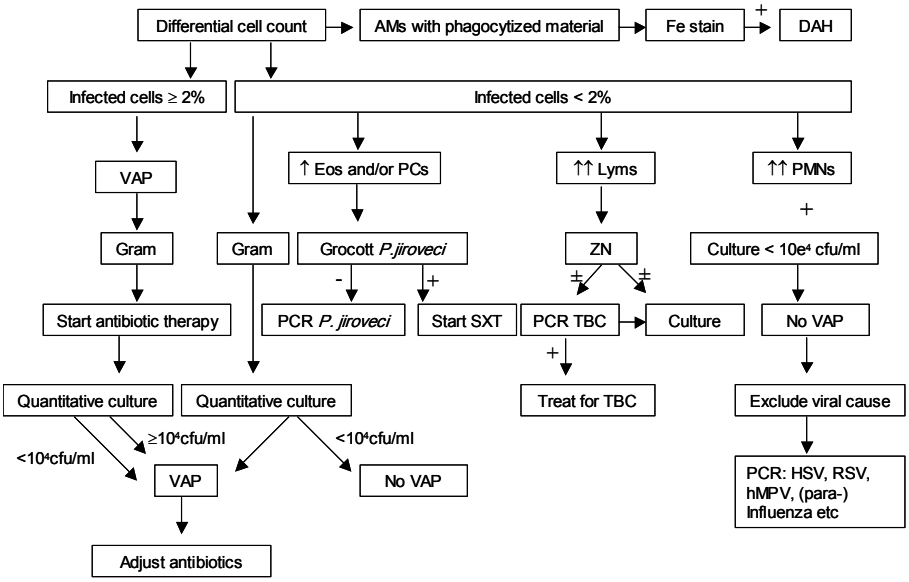

Figure 8.1 Flowchart showing the BALF work-up in immunocompetent intensive care patients on mechanical ventilation.
AMs: alveolar macrophages, cfu: colony-forming units, DAH: diffuse alveolar haemorrhage, Eos: eosinophil, Fe: iron, hMPV: human metapneumovirus, HSV: herpes simplex virus, Lyms: lymphocytes, PCs: plasma cells, PCR: polymerase chain reaction, PMNs: polymorphonuclear neutrophils, RSV: respiratory syncytial virus, SXT: trimethoprim + sulfamethoxazole, TBC: tuberculosis, VAP: ventilator-associated pneumonia, ZN: Ziehl-Neelsen (acid-fast stain).

Figure 8.2 shows a flowchart of the BALF work-up as it is used in our laboratory in immunocompromised intensive care patients on mechanical ventilation.

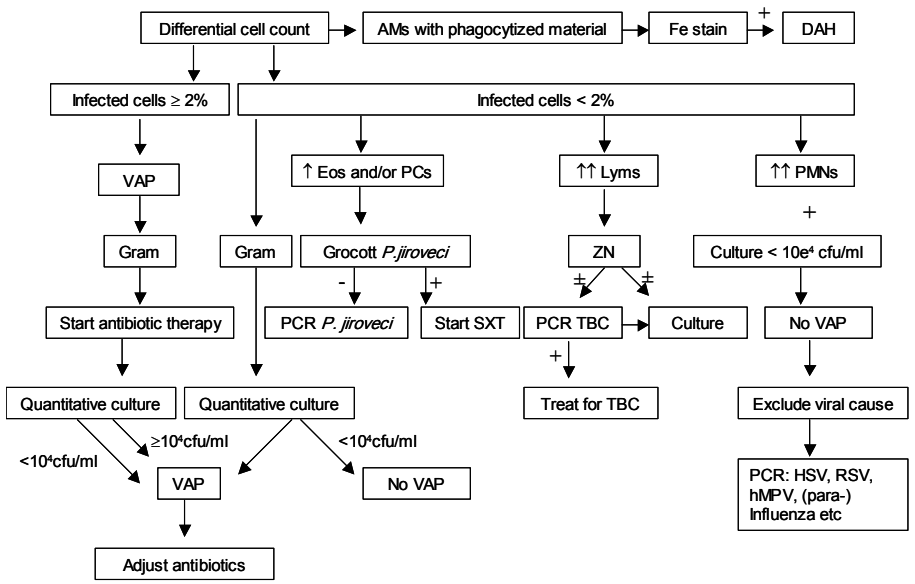

Figure 8.2 Flowchart showing the BALF work-up in immunocompromised intensive care patients on mechanical ventilation.
Cfu: colony-forming units, CMV: cytomegalovirus, hMPV: human metapneumovirus, HSV: herpes simplex virus, RSV: respiratory syncytial virus, SXT: trimethoprim + sulfamethoxazole, TBC: tuberculosis, VAP: ventilator-associated pneumonia, VOR: voriconazole, ZN: Ziehl-Neelsen (acid-fast stain).

BALF versus induced sputum

BALF as well as (induced) sputum samples have been used in the diagnostic work-up of pulmonary diseases. However, especially in infectious pulmonary diseases there is a difference in sensitivity between both types of samples. For example: the sensitivity of induced sputum for the microscopic diagnosis PCP varies between 50 and 90%, depending on the experience with recovery and the laboratory work-up of the sample[9,10]. With the introduction of a PCR for the detection of *P. jiroveci* however, the sensitivity of induced sputum for the diagnosis PCP has greatly improved[11]. Unfortunately, this PCR is not yet available in every hospital. Another example is the quantitative culture of BALF for the diagnosis VAP, this has recently led to a lot of discussion whether or not BALF is still the material of choice for this diagnosis[12,13]. Even though, the advantage of using BALF over (induced) sputum is the fact that quality control

is available for BALF (recovered volume, total cell count, presence of epithelial cells). This ensures that the sample used for further investigation originates from the alveolar space. Another advantage of BALF over (induced) sputum is the availability of a differential cell count. This makes it possible to explore non-infectious causes in the absence of an infectious cause[14]

Aim of the study

The aim of the studies presented in this thesis was attempting to improve the sensitivity of BAL as a diagnostic tool in pulmonary infectious diseases and to shorten the time to diagnosis.
BALF data obtained from patients during an eight year period from 1998 until 2005 were studied. In this thesis the use of BAL as a diagnostic tool for infectious diseases in patients suspected of a pulmonary infection is described.
In chapter 1, the general introduction, the BAL procedure, laboratory work-up of BALF and the value of BAL in infectious and non-infectious diseases mimicking VAP are discussed. Furthermore, the use of nucleic acid amplification techniques in pulmonary infectious diseases in general, is briefly addressed.

Microscopy of BALF

In chapter 2 we evaluated the use of polylysine coated slides for the diagnostic work-up of clinical BALF samples. Since polylysine coating of microscopy slides has been indicated to provide superior cell adhesion, we compared polylysine coated (PLC) slides to conventional slides in cyto-centrifuged BALF samples. A total of 20 BALF samples with representative numbers of alveolar macrophages (Ams), lymphocytes (Lyms) and polymorphonuclear neutrophils (PMNs) were cytocentrentrifuged on both conventional glass slides and polylysine coated slides. Using a May-Grünwald Giemsa stain they were evaluated for total cell recovery, differential cell count and cytomorphology. Clinical significance of differences in differential cell counts was assessed using a validated computer program predicting the most probable diagnosis. Interestingly, cell recovery was lower on PLC slides compared to the conventional, uncoated, slides. The PLC slides showed significantly lower recovery of Lyms as compared to uncoated slides (25.89%±28.26 versus 28.34±29.96, respectively). The reliability of counts of Ams, Lyms and PMNs was excellent for both type of slides. No discrepancies in diagnoses were found between both type of slides. We therefore concluded the PLC slides not to be superior to conventional slides. Besides polylysine, a few other coatings have

been evaluated in various clinical samples[15,16], often compared to the polylysine coated slides. In these studies polylysine coated slides demonstrated to be superior to albumin- and gelatine-coated slides. Since conventional uncoated glass slides are less expensive compared to coated slides and our study shows them to be non-inferior to the polylysine slides we opt the use of the uncoated slides in the diagnostic work-up of BALF in clinical laboratory practice.

Reactive type II pneumocytes

The aim of the study presented in chapter 3 was to evaluate the prevalence, the associated clinical conditions and the accompanying cytological findings of reactive type II pneumocytes (RPII) in a series of consecutive clinical BALF samples routinely submitted for analysis. All consecutive BALF samples obtained from patients in the period between 2000 and 2004 were included. The overall prevalence of RPII cells in our study population was 21.7%. No differences were found between BALF samples with and without RPII cells, with respect to the differential cell count. However, in BALF samples with RPII, foamy Ams, activated Lyms and plasma cells were seen more frequently. RPII were observed in the samples of patients diagnosed with acute respiratory distress syndrome, diffuse alveolar damage, acute eosinophilic pneumonia, extrinsic allergic alveolitis or hypersensitivity pneumonitis, drug-induced pulmonary disorders, PCP and VAP. No RPII cells were found in BALF from patients diagnosed with pulmonary tuberculosis or sarcoidosis. Even though RPII cells were found not to be pathognomonic for a specific disease, they are associated with severe pulmonary damage. Combined with the differential cell count and specific morphological findings such as foamy alveolar macrophages, activated Lyms and plasma cells, the presence of RPII may be of additional diagnostic value in the assessment of interstitial lung diseases and pulmonary infections.

Influence of antibiotic therapy on prediction of VAP

In intensive care patients on mechanical ventilation, VAP has a high prevalence[17,18] and is associated with a high mortality[17]. BALF analysis including differential cell count and enumeration of infected cells (IC) have been used in the diagnosis of VAP[4,6]. However, the influence of antibiotics on the differential cell count, including IC, remained unknown. Studies dealing with the subject showed conflicting results[19-21]. Therefore, in chapter 4, the influence

of antibiotics on the prediction of VAP was evaluated. The prospective study was conducted during a 61-month period (January 1999 until February 2004). All consecutive BALF samples of intensive care patients clinically suspected of VAP were included. Previous studies showed quantitative culture results to be influenced by antibiotic started up to 72 hours previous to the time of lavage. The main finding of this study was that, antibiotic therapy in 72 hours prior to performance of BAL, did not influence the reliability of the IC count in BALF in the prediction of VAP. The cytological parameter that most accurately predicted VAP was the percentage IC, using a cut-off value of 2%. Combining the percentage IC with cytological parameters did not result in a better prediction of VAP.

Soluble factors in BALF in the prediction of VAP

Even though routine application of BALF analysis is a valuable tool in the identification of infectious and non-infectious pulmonary diseases, it is limited by the fact that it is expensive, time-consuming, and relies upon specialised technicians. In most hospitals facilities for BALF cytology are not available on a 24-hour base, therefore we were interested in a fast method, present in most hospitals, to predict VAP. The use of acute phase proteins seemed promising[22] and these tests are available in most hospitals on a 24 hour base. Chapter 5 describes the usefulness of C-reactive protein (CRP) and procalcitonin (PCT) in the prediction of VAP. Both CRP and PCT were determined in BALF and serum of patients with microbiologically proven VAP. Initially, the kits for high-sensitive CRP (CRPH) and the kit for high-sensitive PCT (ProCa-S) were validated for BALF. Both tests performed well when assessed for matrix-effect, linearity and reproducibility, and, were therefore applicable for use on BALF. However, there was a large overlap in PCT and CRP concentrations in BALF samples between patients with and without VAP and both PCT and CRP concentrations in BALF resulted in a small area under the curve in the receiver operating characteristic curve. Therefore, the major conclusion from this study was that both parameters, in BALF as well as in serum, were not useful as a predictor for VAP.

However, other soluble factors have proven to be useful in the diagnosis of pulmonary diseases. The need for a rapid method for confirming or excluding VAP remains, especially a method that would be available in every hospital at any time of day. Other soluble factors are promising, for instance the soluble triggering receptor expressed on myeloid cells (TREM-1). sTREM-1 is a member of the immunoglobulin superfamily which is a group of cell surface and soluble proteins involved in recognition, binding or adhesion of cells[23]. Expression of TREM-1 has been found on neutrophils and monocytes[24], but

also on alveolar macrophages[25]. The expression of this triggering receptor is up regulated by the presence of bacteria and fungi[26], thereby mediating the acute inflammatory response to these micro-organisms[26]. For the detection of sTREM-1, in blood and sputum, an enzyme-linked immuno sorbent assay (ELISA) is currently on the market for research purposes (Human TREM-1 Quantikine kit, R&D systems inc, Minneapolis, U.S.A.) taking approximately 4 hours to generate a result. Few studies have investigated the usefulness of s-TREM-1 in the diagnosis pneumonia[27,28]. A multiple logistic-regression analysis conducted by Gibot *et al.*[27] showed the presence of s-TREM-1 to be the strongest single predictor of pneumonia in a study with 148 patients, including 46 patients with VAP, undergoing mini-BAL[27]. They found a cut-off value of 5 pg/ml to be the best predictor for the presence of pneumonia. Unfortunately, they did not take into account the dilution 10-100 times when BALF is compared to ELF. Richeldi and colleagues investigated sTREM-1 as a potential marker in community-acquired pneumonia and tuberculosis[28]. Their study showed that sTREM-1 was recovered in BALF of patients with community-acquired pneumonia, but not in patients with tuberculosis. Neither Gibot *et al.*[27] nor Richeldi *et al.*[28] used the above described ELISA. Until now, only one study used this kit in a small series of patients suspected of VAP (n=28)[29]. They found sTREM-1 to be an excellent predictor of VAP at a cut-off value of 200 pg/ml. Unfortunately, Determann *et al.*[29] did not validate the kit for use on BALF neither did they correct for the dilution factor. Therefore, the Human TREM-1 Quantikine kit should be validated for the use on BALF. Additionally, it should be tested in a prospective study of ICU patients suspected of VAP. The aim of this study could be determining the predictive value of sTREM-1 in the diagnosis VAP and eventually defining a cut-off value to distinguish between VAP and non-VAP.

Molecular techniques in the work-up of BALF

In the past, the clinical standard for assessing many infectious agents was based on culture and serology. In recent years, detection of many micro-organisms by means of nucleic acid amplification methods has been introduced as superior methods when compared to conventional methods in terms of sensitivity and specificity. Nucleic acid amplification techniques, such as polymerase chain reaction (PCR) can also contribute in the diagnostic work-up of BALF. Chapter 6 and 7 described two studies dealing with implementation and interpretation of PCR techniques in BALF work-up.

Detection of *P. jiroveci* in BALF

The implementation of a new method as a diagnostic tool always requires extensive standardisation and validation before it can be used in daily practice. One additional measurement used to ensure good quality and to identify possible flaws in the used test is a quality control panel distributed by a coordinating organisation. However, these quality control panels are not available for all micro-organisms, one example of such a micro-organism is *P. jiroveci*. Real-time PCR methods for the diagnosis of *P. jiroveci* in pulmonary samples have recently made the switch from research tool to diagnostic tool[30,31]. However, since no quality control panels are available, validation, implementation and comparison of intra-laboratory results is difficult. Therefore, chapter 6 described a study which was initiated to compare the performance of three independently developed real-time PCR assays for the detection of *P. jiroveci* in three different tertiary care centres in The Netherlands. A cooperation was formed between the university hospitals of Nijmegen, Leiden and Maastricht. In the period August 1999 until April 2004 a total of 124 BALF samples were included. They were obtained from either HIV-positive or HIV-negative patients with a known risk factor for PCP, such as a (hematological) malignancy, bone marrow or organ transplantation, Wegener's granulomatosis, and immunosuppressive or corticosteroid therapy (n=84). Additionally a number of BALF samples obtained from patients with no known risk factor for PCP, but with either VAP or newly diagnosed sarcoidosis, were included (n=40). From these 124 BALF samples 41 samples showed the presence of *P. jiroveci* by microscopy, the remaining 83 were microscopy negative. For 114 samples the PCR results from the three laboratories were conclusive. Forty out of 41 microscopy-positive samples were found positive in all three PCR assays. Out of the 83 microscopy-negative samples, 69 were also negative in all three PCR assays. From the remaining 14 microscopy-negative samples, five were positive in all three PCR assays. A high agreement between the three *P. jiroveci* real-time PCR assays was found, varying from 94.4% (Kappa value: 0.88) till 96.8% (Kappa value: 0.93) was found. Since *P. jiroveci* can be present in low quantities in the pulmonary tract without causing disease (carriership). It is mandatory to distinguish between carriership and disease. It was hypothesized that, in case of PCP, *P. jiroveci* would be present in patients in higher quantities compared to carriers. Unfortunately, there was an overlap between patients with microscopically confirmed PCP and patients without (microscopically confirmed) PCP with regard to quantities of *P. jiroveci* present. In this retrospective study no absolute cut-off value to discriminate between disease and carrier status for *P. jiroveci* could be established.

Improving the clinical relevance of a *P. jiroveci* PCR

Different strategies could be investigated to eventually reduce the grey area. One strategy could be combining PCR results with serum indicators. Some indicators that may be helpful are: serum lactate dehydrogenase (LDH) and β-D-glucan. LDH in BALF has shown to be of use in differentiating between infectious and non-infectious pulmonary disease[32]. Especially in patients with PCP, LDH has shown to be elevated[33-36]. In a study by Zaman *et al.*[35] they found a LDH serum concentration of 450 international units (IU) to predict the presence of PCP in their population. Quist *et al.*[33] investigated 42 patients with PCP, 71 with disseminated tuberculosis, 40 with pulmonary tuberculosis and 37 with bacterial pneumonia. They found peak LDH levels to be significantly higher in patients with PCP compared with the other groups. Unfortunately, there was an overlap in measured serum LDH between patients with PCP and the other three groups, limiting its specificity[33]. A recent, retrospective, study by Tasaka *et al.*[36] included 295 patients who underwent BAL for the specific diagnosis of PCP. In 57 patients PCP was confirmed based upon microscopic findings of sedimented BALF. They showed serum levels of LDH to be significantly higher in patients with PCP compared with other pulmonary infections. The second serum marker which could be of interest is β-D-glucan, which is part of the cell wall of most fungi. It has already been used for the diagnosis of invasive mycosis such as candidiasis and invasive aspergillosis (IA). Tasaka *et al.*[36] evaluated the use of serum β-D-glucan in the diagnosis PCP. They showed that when using their cut-off value of 31.1 pg/ml β-D-glucan in serum, led to a sensitivity of 92.3% with a specificity of 83.1%. In their population this led to a positive predictive value (PPV) of 0.61 and a negative predictive value (NPV) of 0.98. The relatively low NPV could be explained by other invasive fungi such as Candida species or Aspergillus species. A limitation of this study is the fact that they use a less sensitive method (microscopy of sedimentated BALF) as their gold standard. Especially the use of cytocentrifuged preparations can increase the sensitivity. They argue that microscopy has a PPV and NPV exceeding 90%. However, in our experience this is only true in the hands of experiences technicians. Tasaka *et al.*[36] state they did not use PCR to diagnose PCP because of the possible detection of carriers. However, the combination of PCR and β-D-glucan may be very interesting. We hypothesize that since there is no pulmonary tissue damage in carriership, there may not be any (or only a low concentration of) β-D-glucan detectable in the peripheral blood. Therefore, a study determining the concentration β-D-glucan in serum (and BALF) of patients with proven PCP, carriers, patients with a PCR-result within the grey zone and patients without suspected PCP should be investigated and correlated with the PCR result. This

may help to limit the grey zone of the PCR and confirm the usefulness of β-D-glucan in the diagnosis PCP.

Detection of HSV-1 in BALF

Finally, in **chapter 7,** we tried to unravel the clinical relevance of a high herpes simplex virus load in BALF. The two subtypes of human herpes simplex virus, HSV-1 and HSV-2, are highly prevalent and ubiquitously distributed. HSV-1 has been described as an (unusual) cause of pneumonia in immune compromised patients[37,38]. However, the clinical relevance of detecting HSV-1 and -2 in BALF is unclear.

We aimed to evaluate the relationship between the HSV-1 and -2 loads in BALF and clinical outcome. Our data showed HSV-1 to be present in approximately one third of all BALF samples acquired from ICU patients. Furthermore, it indicates that a HSV-1 load in BALF of $>10^5$ ge/ml is an independent predictor for a poor outcome in critically ill patients, with an increase in mortality rate of 21%. Even though, it remains to be determined whether HSV-1 is causally linked with a low survival or whether it is a marker of a severely disturbed immune system. Our study was limited by the fact that it was conducted retrospectively, therefore, the influence of aciclovir on the mortality and morbidity could not be evaluated. A prospective intervention study using the cut-off value of 10^5 ge/ml BALF may generate the data needed. If HSV-1 is the cause of an increased mortality of ICU patients,with a HSV-1 load exceeding 10^5, treatment with aciclovir should at least decrease the mortality rate in these patients Of course HSV is not the only virus that could cause pneumonia. Other viruses that cause pneumonia[3,39] include influenza virus A, B, para-influenza virus 1,2,3,4, cytomegalovirus, varicella zoster virus, coronavirus, human respiratory syncytial virus (RSV) and rhinovirus. In recent years, attention has been drawn towards human metapneumovirus (hMPV) as a cause of pneumonia. HMPV is an paramyxovirus identified in 2001 in the Netherlands[40]. The study by van den Hoogen *et al.*[40] included nasopharyngeal aspirate samples collected, over a period of 20 years, from 28 young children (<5 years) with symptoms of respiratory tract infections similar to those caused by RSV. Epidemiologically, the children were unrelated[40]. The infection seems to have a seasonal cycle with most infections occurring in the winter months (resembling infection caused by RSV[41]). Most studies use reverse transcriptase PCR (RT-PCR) for the detection of hMPV RNA in respiratory samples[42-45]. It is possible to culture the virus, it grows slowly on tertiary monkey kidney cells[40,43] and shows poor growth in Vero cells and A549 cells with a cytopathogenic effect strongly resembling that initiated by RSV[40]. Since its discovery, data have been published about its role in pneumonia in young children[39,40,44,46], the

elderly[42] and immunocompromised patients[42,45]. The virus has been described in a variety of patients. Accordingly, it may also play a role in intubated patients at the ICU. Until now, only one study has been published including adult intensive care patients in the study population[43]. The study by Gray *et al.*[43] included 1500 respiratory samples (75% collected by nasal wash, only 10% BALF) from 1294 patients. By using RT-PCR they found 34 patients to be hMPV positive. These 34 patients included 9 adult patients with various underlying disorders such as malignancy (n=2), obstructive bronchitis (n=2), bacterial pneumonia (n=1), influenza (n=1) and respiratory failure of unknown origin (n=3). A number of patients admitted to our ICU develop additional respiratory problems during ventilation. Since only approximately 25-33% can be explained by bacterial infection, we hypothesize that some may be associated with a viral infection Therefore, a respiratory multiplex PCR (including hMPV) may discover additional viral causes for the respiratory deterioration of the patients admitted to our ICU.

BAL in immunosuppressed patients

The same may be applicable to patients admitted to the hematology-oncology ward with signs of pulmonary infection. Often analysis of BALF samples obtained from these patients do not result in the identification of a causative organism. Due to their underlying disease and subsequent therapy (chemotherapy) these patients often have no (functional) granulocytes. Therefore, they are vulnerable for all kinds of (pulmonary) infections. Besides bacterial, viral, and fungal infections in parasitic causes should be considered[3]. The advantage of a sensitive and fast method for detection of these organisms are mainly clear when there is therapy available directed against the suspected micro-organism. For instance *Aspergillus fumigatus*, this fungus is notorious for causing life-threatening pulmonary infections in immunocompromised patients[47] and seldom in immunocompetent patients[48]. Invasive Aspergillosis (IA) is associated with a high mortality varying from 30-80%[49,50] depending on the time of diagnosis and the initiation of therapy. The diagnosis IA is difficult since clinical symptoms and radiological features are often non-specific. Cultures of BALF or biopsies are very specific, however the sensitivity is low (30-50%)[51]. In recent years, the detection of galactomannan, a part of the cell wall of *A. fumigatus*, in BALF and serum has contributed to an earlier diagnosis of IA[52]. PCR methods for the detection of *A. fumigatus* in respiratory samples have been described in literature[53,54]. However, studies evaluating the value of an *A. fumigatus* specific PCR compared to the detection of galactomannan, in the diagnosis IA has led to conflicting results. Becker *et al.*[55] using an animal model to study IA found the quantitative galactomannan detection to be

superior over the PCR in both serum as BALF. A study by Costa *et al.*[56] comparing galactomannan detection with PCR in serum from patients suspected of IA, showed comparable results. A study conducted by Kami *et al.*[57] showed a higher sensitivity and specificity for the PCR compared to the detection of galactomannan in serum from patients suspected of IA. All three described studies used the same ELISA for the detection of galactomannan in their samples; however the molecular methods they used differed significantly. One use a conventional PCR[55], whilst the others used a real-time PCR[56,57]. The isolation of DNA, the target and the platform used to run the PCR differed in all three studies. This could, in part explain the differences found. Combining a real-time PCR assay with the ELISA may increase the diagnostic yield in the diagnosis IA.

As stated earlier, even parasitic pulmonary infection should be considered in immunocompromised patients suspected of pulmonary infection. One of the possibilities is *Toxoplasma gondii*. *T. gondii* is an obligate intracellular parasite with a ubiquitous distribution. After the acute, in immunocompetent hosts often asymptomatic phase of infection, bradyzoites remain within the human body. In immunocompromised hosts it is known for its tendency to reactivate and to cause disseminated disease[58,59]. Pulmonary infection with *T. gondii* is associated with a mortality exceeding 50%[60]. The diagnosis pulmonary infection by *T. gondii* can be ascertained in a way comparable to *P. jiroveci*, by microscopic examination of BALF[61]. However, patients at risk for *T. gondii* pulmonary infections are identical to patients at risk for PCP, therefore they may already be receiving PCP prophylaxis (co-trimoxazole). Co-trimoxazole also has an effect on *T. gondii*, which may influence the load of *T. gondii* present in BALF, just as it does influence the load of *P. jiroveci* in BALF. This may be enough to make it undetectable in microscopic preparations, but not enough to fully protect against disseminated *T. gondii* infection[62]. To increase the detection rate of *T. gondii* in BALF, PCR may be useful[63]. Petersen *et al.*[62] examined 332 BALF samples of 290 HIV positive patients by means of real-time PCR. They found *T. gondii* DNA in 7 patients, resulting in a prevalence of 2%. Since pulmonary toxoplasmosis has not only been described in patients with HIV, but also in otherwise immunocompromised patients[64], the addition of a PCR for *T. gondii* to the diagnostic work-up of BALF could be beneficial. To evaluate this, BALF samples from immunocompetent and immuno-compromised patients (both HIV-positive and -negative) should be evaluated for the presence of *T. gondii* DNA by means of real-time PCR. This could eventually lead to an increase of diagnosed *T. gondii* pulmonary infections and subsequent treatment with high dose co-trimoxazole.

In summary

- polylysine coated slides are not superior over conventional uncoated glass slides in the work-up of clinical bronchoalveolar lavage fluid samples
- Normally, reactive pneumocytes type II are not present in bronchoalveolar lavage fluid
- The presence of reactive pneumocytes type II is associated with conditions of acute lung injury such as alveolar damage as extrinsic allergic alveolitis and drug-induces pulmonary disorders, pneumocystis pneumonia and ventilator-associated pneumonia
- Antibiotic therapy in the 72 hours preceding the bronchoalveolar lavage does not influence the predictive value of cytological bronchoalveolar lavage fluid parameters in the microscopic diagnosis ventilator-associated pneumonia.
- Amongst the cells present in bronchoalveolar lavage fluid, the percentage of infected cells is the most important parameter for distinguishing ventilator-associated pneumonia from a non-ventilator-associated pneumonia condition.
- Using a standardised method, the best cut-off value for the percentage infected cells was set at 2%. Combining the percentage infected cells with any other cytological parameter did not reveal better predictive values.
- The three different in-house real-time polymerase chain reaction assays for *P. jiroveci* developed in the university hospitals of Leiden, Nijmegen and Maastricht show an excellent agreement in performance.
- *P. jiroveci* quantity determined by real-time polymerase chain reaction and microscopic quantification are comparable.
- At this moment an absolute cut-off value discriminating disease and carrier status for *P. jiroveci* can not be established due to the presence of a grey zone.
- In 32% of all bronchoalveolar lavage fluid samples acquired from intensive care patients, a herpes simplex type 1 load can be detected
- A herpes simplex type 1 load in bronchoalveolar lavage fluid of $>10^5$ ge/ml is an independent predictor for a poor outcome in critically ill patients, with an increase in mortality rate of 21%.

Directions for future research:

This thesis underlines the value of BALF in the diagnostic work-up of infectious pulmonary diseases. Especially the incorporation of molecular methods will increase the diagnostic yield and sensitivity of BAL in infectious diseases. However, future studies are needed to:

1. Validate the usefulness of the Human TREM-1 Quantikine kit on bronchoalveolar lavage fluid
2. Evaluation of the Human TREM-1 Quantikine kit in the prediction of ventilator-associated pneumonia
3. Refine the value of new techniques such as the limitation of the grey zone in the *P. jiroveci* PCR because this will result in an increase of clinical applicability. Two possible strategies could be:
    a. The detection of LDH in both bronchoalveolar lavage fluid and serum.
    b. The detection of β-D-glucan in both bronchoalveolar lavage fluid and serum
4. Evaluate the herpes simplex type 1 polymerase chain reaction cut-off value of 10^5 ge/ml in a prospective intervention study.
5. Evaluate the clinical value of a respiratory multiplex (including human metapneumovirus) for patients with respiratory deterioration whilst on mechanical ventilation at the ICU.
6. Evaluate the clinical value of a respiratory multiplex polymerase chain reaction (including human metapneumovirus) for patients admitted to the hematology-oncology ward with signs of pulmonary infection.
7. Evaluate the combination of an *Aspergillus fumigatus* specific real-time polymerase chain reaction assay and the enzyme-linked immuno sorbent assay for the detection of circulating galactomannan in both serum and blood of patients suspected of invasive Aspergillosis.
8. Evaluate the clinical value of a *T. gondii* real-time polymerase chain reaction on bronchoalveolar lavage fluid samples of immunocompromised patients suspected of a pulmonary infection.

In conclusion, BALF work-up is of great value in the diagnosis of pulmonary infections. Especially the incorporation of molecular techniques for viral pathogens, fungi and parasites will increase the number of confirmed infections which in turn will lead to an improvement of patient care because of a targeted therapy.

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