Chapter 9

General Discussion and Summary
INTRODUCTION

The introduction of molecular methods has led to an exponential increase in the detection of respiratory pathogens. This is especially true for viruses. Although it appears beneficial that molecular methods are available to test with high sensitivity for a broader range of viruses, it is not done routinely in many laboratories. Moreover, the detection of microorganisms in the respiratory tract does not necessarily mean that they are the cause of the pulmonary symptoms.

In this thesis we assessed the added value of enhanced laboratory testing in a routine medical microbiological setting, and assessed the added value of a broad range of diagnostics. We will discuss the implications of our findings, and set out how these could strengthen a targeted test algorithm.

AETIOLOGY

At the beginning of the 19th century, Sir William Osler wrote that “the most widespread and fatal of all acute diseases, pneumonia, is now Captain of the Men of Death” [1]. Nowadays, respiratory tract infections, including community-acquired pneumonia (CAP) are still common and potentially fatal but they are not among the leading causes of death anymore. In high-income countries, CAP still is the most important cause of death from infectious diseases [2]. Several studies show that Streptococcus pneumoniae is the predominant aetiologic agent of CAP, followed by other bacteria such as Haemophilus influenzae, and Mycoplasma pneumoniae [3, 4]. In general respiratory viruses are poorly detected by conventional techniques, but the recent developments in molecular diagnostics have resulted in increased detection of respiratory viruses in patients with CAP [5, 6].

In chapter 2 we have determined the aetiology of CAP in patients admitted to the hospital using extensive molecular and serological testing for viral and bacterial pathogens. Viral pathogens play an important role in the aetiology of CAP. Influenza A virus was the third common single organism found after Streptococcus pneumoniae and Coxiella burnetti. The high rank of the latter organism was probably due to a concurrent outbreak of Q-fever in the area of the study [7]. To improve the insight in occurrence, fluctuations and seasonality of pathogens associated with CAP, testing for respiratory pathogens is needed.

In chapter 3 we demonstrated that improvement of the sample collection process increases the yield to 80%. In the literature, up to 50% of the patients no bacterial and viral pathogen is found in patients with CAP, possible explanations are use of antibiotics before collecting samples, sample type tested, and the diagnostic panel used for patient evaluation [4, 8-13]. Unfortunately the majority of patients with CAP undergo limited diagnostic tests to demonstrate an aetiologic agent, other than urine antigen test and, only if available, a bacterial sputum culture. An easily obtained throat swab is sufficient to detected most viruses, with the exception of human rhinovirus (HRV) and respiratory syncytial virus (RSV). For detecting bacterial pathogens, Legionella pneumophila,
Mycoplasma pneumoniae, Coxiella burnetii, Chlamyaphila psittaci, Chlamyaphila pneumoniae, a sputum sample is still preferred.

DIAGNOSTIC NEEDS FROM THE PERSPECTIVE OF A TREATING PHYSICIAN..

In chapter 4 we compared clinical and laboratory parameters of patients with CAP caused by a pure bacterial, pure viral, mixed viral and bacterial aetiology or cases in which no aetiology was found. We tested if we could differentiate on admission between the groups. Unfortunately, a substantial overlap and variability in clinical signs and symptoms of patients with a viral and/or bacterial pneumonia were found. Only age, coughing, pneumonia severity index (PSI) and immunodeficiency could be used to predict a possible aetiology of CAP. Of relevance, however, is that we found that mixed infections were associated with an increased disease severity and a longer hospital stay. It remains to be seen if early detection of such mixed infections would reduce this factors, which then would render support for an enhanced diagnostic workup.

We found that almost half (44.5%) of the patients were positive for one or more viral pathogens. Increased detection of viruses, often detected in the same sample, has made diagnosis challenging. Viral-viral interaction is poorly understood and whether dual viral infection leads to more severe disease is still a matter of debate. Viruses might interact indirectly by altering the host environment and by immunological interactions or directly through exchanging viral genes, resulting in complementation or inhibition. More research is required to elucidate the mechanisms associated with and the clinical significance of multiple viral infections.

Not only viruses are increasingly detected with qPCR but also bacterial pathogens such as S. pneumoniae can be detected in a more sensitive way. As a diagnostic tool for pneumococcal CAP mixed results are reported because distinguishing colonization from infection using S. pneumoniae PCR is difficult even by quantifying the load. Similarly, culturing Streptococci from sputum samples is not conclusive evidence for their aetiological role. To implement the S. pneumoniae qPCR in a routine medical microbiological setting more knowledge need to be gathered to assure appropriate use of a S. pneumoniae qPCR on sputum samples.

Increasing pressures to reduce healthcare costs warrant limiting the use of (costly) diagnostic tests, particularly for virus testing which often does not inform patient treatment. In theory, rapid detection of respiratory viruses might also result in clinical and economic benefits. First, the cost-effectiveness of qPCR could be improved by selecting a targeted number of pathogens based on epidemiology and clinical severity of the pathogen. Alternatively, establishing selection criteria for the patients to be examined could also decrease costs. For example, patients with low C-reactive protein levels or with coughing were most likely to have viral pathogens detected.

Second, unnecessary antibiotic use contributes to the emergence and dissemination of antimicrobial-resistant pathogens. Unnecessary use of antibiotics will lead to extra costs due to management of adverse effects or complications e.g. Clostridium difficile infection.
Choosing the correct therapy should be based on a thorough knowledge of the local etiology. In the Netherlands before 2011 the initial treatment of CAP was macrolide monotherapy for outpatient treatment. As a result of an increasing rate of resistance of *S. pneumoniae* for this antibiotic the initial treatment has been switched to amoxicillin, a treatment not suitable for *C. burnetii* [27]. This would not have been a favourable change during the 2007-2010 Q-fever outbreak. Therefore rapid molecular diagnostic tests need to be used to streamline or cease antibiotics early in the course of the disease.

In chapter 5 we evaluate the clinical and epidemiological features of detecting respiratory viruses and *Mycoplasma pneumoniae* in children, in relation to clinical decision making. We retrospectively determined the influence of a positive viral test result on the use of antibiotics. In general, in our study antibiotic use was low, and the majority of children with the clinical diagnosis viral respiratory tract infection did not receive any antibiotics. However, of the patients started on antibiotic treatment, therapy was discontinued only in 40% of cases if a positive viral test result was known. In order to change clinical management based on viral molecular diagnostics, studies with clear algorithms and more rigorous patient management based on molecular diagnostic tests are needed to evaluate the impact on development of antimicrobial resistance and the costs and effects on patient management.

........DIAGNOSTIC NEEDS FROM THE PERSPECTIVE OF EMERGING DISEASE PREPAREDNESS

Timely detection is a critical component in the course of a possible outbreak. Although clinicians examine patients with respiratory symptoms and illness every day, still they are the first to recognize clusters of unusual syndroms. Distinguishing between normal respiratory pathogens and potential new pandemic pathogens is challenging, but emerging infectious diseases represent an ongoing threat.

In chapter 6 we explored the possibilities of spatial analysis to detect clusters of patients with CAP with known and unknown aetiology in an area with a high density of farm animals. CAP caused by *Coxiella burnetii* was associated with living near sheep farms or in regions with high numbers of goats. CAP with unknown aetiology was not associated with the presence of animal farms. Disease surveillance, based on spatial analysis and linked to diagnostic sampling, could be a simple and powerful tool to detect unusual patterns of patients with respiratory illness.

Timely detection is of essence in understanding and controlling an outbreak. Laboratories play an important role in emerging disease outbreaks, by provision of diagnostics, but also in helping to provide answers to critical data needs during early stages of an outbreak. Early case reports of the 2009 influenza pandemic suggested serious morbidity and mortality but after some time it became clear that most influenza cases were self-limited [28-30]. During the start of the outbreak only the serious cases had been tested for the causative organism. Therefore, it is needed to combine clinical and epidemiological data with detailed sampling information, laboratory analyses...
and results in order to quickly understand the course of an outbreak. To ensure optimal infection control also ruling out common pathogens in the beginning of an outbreak when targeted diagnostics are not yet available is important. The clinical presentation of the 2002-2003 outbreak with severe acute respiratory syndrome (SARS) resembled atypical pneumonia caused by much more common pathogens, like influenza viruses.

Gaining information about the severity of an outbreak in patients, in time and location is essential to guide control activities. This knowledge will help in the development of specific treatments aimed at these newly emerged pathogens, as well as in the development of preventive measures through the use of vaccines. In chapter 7 we measure the humoral immunity before and after vaccination with the 2009 influenza A virus (H1N1) monovalent MF59-adjuvanted vaccine and examined the duration of the immune response. A single dose of the H1N1 2009 vaccine produced an antibody response in almost 80% of the healthcare workers, whereas an additional dose resulted in significantly increased titers only in persons over 50. More factors are of influence on the immune response such as age and a history of seasonal influenza vaccination. Yearly seasonal vaccination is recommended for certain groups, including for healthcare workers.

Seasonal influenza vaccination is considered to be a keystone intervention in reducing the risk of nosocomial transmission. Together with hand hygiene, isolation of infected patients, and leave of absence for healthcare workers with influenza-like illness they are part of influenza infection control measurements. Yet yearly seasonal influenza vaccination remains a matter of debate.

In chapter 8 we compared the profile of antibody responses elicited by natural infection, and vaccination for influenza A(H1N1)pdm09 in adults with and without a history of seasonal influenza vaccination using the protein microarray. We demonstrated that subjects with a history of seasonal vaccination generally exhibited higher baseline titers for the various HA antigens than subjects without such history. The immune response against influenza viruses is shaped through prior infections clinical or subclinical and through vaccination. In literature conflicting results on the influence of the immune system are found. Some studies concluded that vaccination against seasonal influenza interferes with the development of cross-reactive immunity against influenza A viruses of other subtypes whereas others demonstrated heterosubtypic neutralizing antibodies after vaccination.

More research is needed to determine whether seasonal vaccination is of influence, positive or negative, on protective immunity. In addition, the results of our studies highlight the importance of thorough research in the development of vaccines that are able to provide protection against influenza A viruses of all subtypes, so called universal vaccines.

**CONCLUSION**

Since the turn of the century there has been an explosive growth of diagnostic tools in clinical microbiology. The challenge is to determine the optimal diagnostic strategy for several groups of patients. For respiratory complaints a diagnosis can often be made using a throat swab only.
Sputum samples are still needed to optimize the yield for bacterial respiratory pathogens. The use of extensive molecular detection methods in this thesis has shown that viral pathogens are often found in patients with CAP. Their role should not be underestimated as patients with mixed infections had an increased disease severity and a longer hospital stay. Also molecular diagnostics need to be used to adjust antibiotic therapy and thereby decreasing unnecessary use of antibiotics. Furthermore of importance is early awareness of unusual clusters of respiratory illness or respiratory pathogens to quickly detect emerging diseases. Spatial analysis linked with detection of respiratory pathogens could be used as an early warning system. And finally, influenza vaccination is a strong countermeasure against influenza virus infection but seasonal vaccination is of significant influence on the antibody response, and further research is needed to understand the effect on protective immunity.