The value of pneumococcal antigen detection in community-acquired pneumonia
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Despite advances in medicine and improvement in living conditions pneumonia is still a common disease in the community. It has been estimated that 6 million individuals annually develop pneumonia. In the United Kingdom and United States it is still the fifth to sixth leading cause of death, and the most common cause of infectious disease causing death. A higher incidence of community-acquired pneumonia is found in children aged under 2 years, in persons 65 years of age and older, in individuals with an impaired host defense and in patients with chronic illnesses. In the Western World, only 10% to 25% of patients with community-acquired pneumonia will be admitted to a hospital. The majority of the pneumonias will be treated at home by a general practitioner.

*S. pneumoniae* remains the most common cause of community-acquired pneumonia. In prospective studies pneumococci were bacteriologically identified in approximately one third of the hospitalized patients. Other pathogens such as *M. pneumoniae*, *Chlamydia* spp., *Legionella pneumophila* and *Coxiella burnetii* are the cause in 10% to 20% of the cases, viruses in 10%, and non-pneumococcal bacteria including Gram-negatives in only 5%. The aetiologic diagnosis of community-acquired pneumonia is not established in approximately 30% to 40% of the patients.

Accurate diagnosis of community-acquired pneumonia is not only complicated by antibiotic treatment prior to hospitalization, the absence of sputum, and difficulties in serological testing, but is also dependent on the ability to recognize new pathogens and by unusual microorganisms infecting normal and immunocompromised patients. Clinical symptoms and signs, laboratory tests, and chest radiographs may indicate a specific type of pneumonia, but there is a great overlap in features between the different causative microorganisms. Typical pneumococcal pneumonia is characterized by leucocytosis with a shift to the left, a lobar infiltrate and a rapid decline of fever to a normal body temperature level after initiation of adequate antibiotic treatment.

The extensive use of antibiotics for respiratory infections leads to an inability to detect the causative agent(s) and the possibility of inaccurate interpretation of culture results. Antimicrobial drugs with a broad-spectrum or a new mode of action may invite empiric administration without making an accurate diagnosis. In a later phase of the illness, when the pneumonia doesn’t get better or even deteriorates despite antibiotic therapy, diagnostic and therapeutic problems occur. In these cases, invasive techniques are justified in order to identify the causative agent(s) of pneumonia (Chapter 1).

Pneumococci are generally susceptible to penicillin in many European countries including the Netherlands. Therefore, it is not surprising that after initiation of antibiotic therapy clinical specimens from patients with community-acquired pneumonia do not yield pneumococci on culture. The specific diagnosis can therefore often not be assessed.

One approach to increasing the number of diagnoses of pneumococcal infections is to detect pneumococcal antigens in body fluids. Immunological tests for pneumococcal antigen detection do not depend on viable pneumococci, and may also yield results in deficiently processed- or collected specimens.

Due to continuous proliferation of pneumococci in the presence or absence of antibiotics capsular polysaccharides and C-polysaccharides (pneumococcal-specific cell wall component) are released into the lung tissue. The means by which antigen is cleared is only partially known, but is probably due to mucociliary transport, coughing, phagocytosis, and resorption into serum either as pure antigen or as circulating immune com-
plexes subsequently excreted as intact or partially degraded antigen. Different methods have been developed to detect pneumococcal antigen in specimens including sputum, serum, and urine.

For the current study latex agglutination (LA, Wellcogen S pneumoniae kit) was chosen because of its simplicity and speed in providing a result (within 30 minutes); important factors in clinical practice.

In Chapter 2 results of the value of antigen detection in clinical specimens (sputum, blood, urine and pleural fluid) in patients with community-acquired pneumonia are given. Sputum specimens were considered suitable for the study only if cytological examination by Gram-stain smear yielded a leucocyte/squamous epithelial cell ratio of ≥10 (magnification x 100). The specimens which fulfilled this criterium were defined as representative of the lower respiratory tract. We chose this ratio for logistic reasons; generally a ratio > 5 or criteria of > 25 leucocytes and < 10 squamous epithelial cells are used.8,9

Pneumococcal antigen, determined in definite (positive blood culture) and probable pneumococcal pneumonia, was detected by LA in first day (representative) sputum specimens with a sensitivity of 94%. In 23 (85%) of 27 patients antigen was detected in the first available representative sputum.

In sputum from patients with a pneumonia of other known aetiology and lung infarction a specificity of 87% was determined. The value of antigen detection was especially emphasized in patients who would otherwise have been diagnosed as pneumonia of unknown aetiology; in 12 (48%) of these 25 patients antigen could be detected.

In patients with pneumococcal pneumonia as well as in those with pneumonia of unknown aetiology antigen could also be detected for one week or even longer in sputum specimens during follow-up under adequate antibiotic therapy (Chapter 3).

However, after two days of antibiotic treatment hardly any pneumococci were detected by Gram-stain smear and culture. In addition, antigen detection in representative sputa was compared to non-representative sputa on admission and during follow-up. The same trend of antigen persistence was observed for both types of specimen in the pneumococcal pneumonia patients and in the group of pneumonia of unknown aetiology. The high specificity of the test was confirmed during follow-up in both representative and non-representative sputum specimens collected in the pneumonia of other known aetiology group. It seems that sputa not suitable for conventional microbiological examination can reliably be used for pneumococcal antigen detection.

In addition, it has to be emphasized that detectable antigen in lower respiratory tract secretion is not specific for the presence of pneumococcal pneumonia. Patients with chronic bronchitis but without pneumonia may have detectable antigen in sputum, especially if the lower respiratory tract harbours pneumococci.10–12 When these patients present with pneumonia, detectable antigen in sputum cannot discriminate between colonisation and acute infection. As stated by Schmid and coworkers detectable antigen may differentiate pneumococcal pneumonia from non-pneumococcal pneumonia, rather than from chronic bronchitis or other pneumococcal carriers.

The value of sputum examination for establishing the aetiological diagnosis of bacterial pneumonia has been widely discussed. Colonisation of the upper respiratory tract by potential pathogens, such as negative bacteria and anaerobes, leads to difficulties in the interpretation of laboratory results in order to reduce these objections.14,15 Theoretically, sputum antigen detection is another method to distinguish pneumococcal pneumonia from non-pneumococcal pneumonia.

However, in Chapter 4 it could be observed that the effect on the detection of pneumococcal antigen detection was found between the frequency of "only unwashed" and "only washed" urines, and not between the frequency of "only unwashed" and "only washed" sputa. These effects were due to the chemical properties of pneumococcal pneumonia and its pneumonia antigen content in oropharyngeal secretions. The tests used were not able to detect any difference in the chemical properties of pneumococcal pneumonia antigens from the pulmonary ones.16–18

The results of antigen detection in urine were also compared to the results of antigen detection in sputum from patients with pneumococcal pneumonia and from non-pneumococcal pneumonia. In Chapters 2 and 3, antigen was detected in urine of 23 (85%) of 27 patients with pneumococcal pneumonia. In the group of non-pneumococcal pneumonia patients, antigen could be detected in urine of 2 (8%) of 24 patients.

Concentration of urine specimens also seemed to increase the detection rate of pneumococcal pneumonia. In Chapters 2 and 3, antigen was detected in urine of 23 (85%) of 27 patients with pneumococcal pneumonia. In the group of non-pneumococcal pneumonia patients, antigen could be detected in urine of 2 (8%) of 24 patients.

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potential pathogens, such as *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, Gram-negative bacteria and anaerobes may contaminate sputum, resulting in less reliable interpretation of laboratory results. Therefore the sputum wash technique was introduced in order to reduce these oropharyngeal contaminants and to facilitate sputum examination. Theoretically, sputum washing may also reduce the amount of pneumococcal antigen present.

However, in Chapter 4 it is demonstrated that washing of sputum has no deleterious effect on the detection of pneumococcal antigen. No significant difference in the rate of detection was found between washed and unwashed specimens. Remarkable was the low frequency of “only unwashed antigen positive specimens”, which indicates that the antigen content in oropharyngeal secretions originating from pneumococci and/or cross-reacting microorganisms is only a minor disturbing factor. In none of the in parallel investigated specimens could antigen be detected in the washed portion, but not in the unwashed portion.

The results of antigen detection in serum as described in Chapter 2 were very disappointing. In only 3 of 14 patients with pneumococcal bacteraemia antigen was detected by LA; non-bacteraemic patients yielded no positive results. This confirms the poor results found in other studies. Serum yielded detectable antigen for several days in only one patient with bacteraemic pneumococcal pneumonia. A reason for this may be that at least 10⁶/ml pneumococci are required for antigen to be detectable, and in bacteraemic cases the concentration of pneumococci in blood is typically far below this detection level.

During pneumonia pneumococcal polysaccharides are excreted in urine in large quantities. Therefore urine would seem to be an ideal aid in diagnosing pneumococcal pneumonia. In Chapters 2 and 5 the results of pneumococcal antigen detection in urine specimens from different patient groups are reported. Pneumococcal polysaccharide in urine has been detected by LA and CIE, and by CoA and ELISA. As in the study by Ajello et al., we found LA to be inefficient. In only one patient with pneumococcal pneumonia could antigen be detected in urine, but this could also have been a false positive reaction due to the presence of a cross-reacting *C. albicans* which was present in the urine of this patient.

Concentration of urine specimens, maximally 300-fold, did not lead to more positive results. No difference in antigen results was demonstrated in a subgroup of urine specimens tested with another LA test (Slidex Pneumo-kit). Reasons for insensitivity of the LA tests in urine specimens remain unclear. It has been suggested that a change in physico-chemical properties of pneumococcal polysaccharides, the unfavourable microenvironment (pH, salt and protein concentrations) of urine, and limited antigen concentration are possible explanations.

Parapneumonic effusion is associated with acute bacterial pneumonia in approximately 40 to 60 percent of the patients. Pleural fluid is a result of the increased permeability of the visceral pleura due to inflammation. During the course of pneumococcal pneumonia there is a constant replenishment of antigens from the pulmonary infiltrate to the accumulated pleural fluid. Therefore, pleural
fluid, which can be obtained under sterile conditions, seems to be an ideal biological fluid for bacteriological examination as well as for antigen detection. Pleural fluid from our patients was examined only if empyema was suspected or the aetiologic diagnosis was not established (Chapter 6). Pleural fluid from 33 (24%) of the 135 included pneumonia patients was examined. LA detected antigen with a sensitivity of 89% in the pneumococcal pneumonia group. Pneumococci were microbiologically identified in pleural fluid in only 2 of 9 of these patients.

Pleural fluid from patients with pneumonia of other known aetiology was also investigated, yielding a specificity of 92%. In addition, antigen was present in pleural fluid from 7 of 12 patients with pneumonia of unknown aetiology. In these patients a pneumococcal aetiology could be definitely assumed. Antigen persistence for more than one week was found in patients who had an empyema. Similar results were reported in other studies using the CIE technique; in both children and adults antigen was detected with a high sensitivity. However, CIE is a less simple and more time-consuming test to perform than LA, and detects pneumococcal types 7 and 14 less effectively.27

In order to investigate whether the quantity of bacterial antigens in clinical specimens may be a determining factor for antigen detection, an in vitro experiment was set up to assess the exact number of pneumococci that is required for a positive antigen result by LA (Chapter 7). Suspensions of pneumococci originating from different clinical specimens were diluted serially. One part was investigated for the presence of pneumococcal antigen, and the other was used for quantitative cultures. It was observed that pneumococcal antigen was detected by LA only when the concentration of $10^6$ to $10^7$ microorganisms per ml was exceeded. No difference in threshold concentration was demonstrated in the differently tested capsular types. This was confirmed in another experiment using growth curves (Chapter 7). The same concentration of pneumococci was required for antigen to be detectable by LA. In addition it was demonstrated that once antigen could be detected, it remained detectable for the entire 72 h of the experiment, even when the concentration of viable pneumococci fell below the threshold concentration of $10^6$ to $10^7$ microorganisms per ml and even in the total absence of viable pneumococci. This observation of antigen persistence in the absence of viable pneumococci is in agreement with the results found in sputum and pleural fluid.

The threshold concentration of pneumococci required for a positive LA is comparable to that found by other methods, although ELISA seems to be slightly more sensitive than the other tests.19,31

It is known that the number of pneumococci present in body fluids bears a good relation to the severity of the pneumonia and the prognosis of the patient.32,33 In sputum from patients with pneumococcal pneumonia pneumococci are often cultured in a number exceeding $10^6$ per ml.34 Pneumococci are probably present in lower concentrations in the upper respiratory tract of pneumococcal carriers and in sputum of patients with chronic bronchitis.35,36 It is worth noting that the number of pneumococci that are required for clinical symptoms of infection to appear, is probably equal to the number required for a positive LA reaction.

The influence of penicillin on viable pneumococci during growth is enormous (Chapter 8). Only if the threshold concentration of approximately $10^6$ to $10^7$ pneumococci per ml had been reached, could all bacteria with bacteria at 0 hours or 8 hours curves. Only those pneumococci that remained detectable for the 72 h experiment, penicillin added to the threshold level had no influence.

Although the in vitro situation of concentrations of more than $10^8$ bacteria is not realistic, the threshold concentrations of $10^6$ to $10^7$ was used in this test result.4 In addition, in vivo concentrations than in vitro are not realistic. The number of bacteria present in the lungs, factors such as mucociliary clearance and antibiotic therapy. When in sputum material is used for sputum antigen detection, it is important that the material is used for sputum antigen detection, and not as in pneumococcal carriers can be detected in any of these specimens.

The high number of pneumococci, is probably present in concentrations that may be detected by cross-reactions with other antigens or antigen originating from the saliva. It has been suggested that the sensitivity of LA for detection of pneumococcal antigen is low (29%) isolated from the positive cultures in 8% of pharyngeal specimens from pneumococcal carriers by LA. Quantitative culture of pneumococcal carriers, yielded positive cultures below the threshold level (100 to 107) pneumococcal carriers and only (8%) as in pneumococcal carriers not detected by cross-reactions with other antigens. In pneumococcal carriers, it is often concluded that detection...
ml had been reached, could antigen be detected. Whether penicillin was added to the tubes with bacteria at 0 hours or 8 hours had no influence on antigen detection during the growth curves. Only those pneumococci which had had the opportunity of growing to the threshold concentration before penicillin was added, yielded detectable antigen, which remained detectable for the entire 72 hours of the experiment. In contrast to what one may expect, penicillin added to the pneumococci when they were at a concentration just below the threshold level had no influence on antigen detection.

Although the in vitro situation cannot be compared with that present in vivo, bacterial concentrations of more than 10⁶/ml occur in humans. It has been reported that higher threshold concentrations of bacteria are needed in vitro than in vivo for a positive antigen test result. In addition, in vivo antigen is probably synthesized or released in higher concentrations than in vitro.

The number of bacteria present in the lower respiratory tract is dependent on several factors such as mucociliary clearance, phagocytosis, immunologic reactions, and antibiotic therapy. When in spite of negative cultures antigen persists, it means that the threshold value for antigen detection is still valid, and sufficient antigen is available.

It has been suggested that pneumococcal colonisation of the oropharynx may interfere with antigen detection in lower respiratory tract secretions. However, the question whether antigen can be detected in specimens from individuals colonized with pneumococci has not been determined. To assess this potentially misleading factor in the diagnosis of pneumococcal pneumonia, upper respiratory tract secretions from patients with a potentially high carrier rate (COPD and asthma patients), were cultured and tested for the presence of pneumococcal antigen by LA (Chapter 9). Pneumococci were most frequently (29%) isolated from the oropharynx, whereas the nasopharynx and saliva yielded positive cultures in 8% and 16% of the patients respectively. In only 4 (9%) of 46 oropharyngeal specimens from which pneumococci were isolated, could antigen be detected by LA. Quantitative cultures of the oropharynx, performed in a sub-group of pneumococcal carriers, yielded pneumococcal concentrations of 10³ to 10⁶ per ml, which is far below the threshold level (10⁸ -10⁹ CFU/ml) required for antigen to be detectable. In non-pneumococcal carriers antigen was detected in the oropharynx in the same proportion (8%) as in pneumococcal carriers. These positive antigen results may be explained either by cross-reactions with other Streptococcus species from that area or by pneumococcal antigen originating from the lower respiratory tract.

The high number of antigen positive saliva specimens, in the absence of viable pneumococci, is probably due to cross-reactions of aerobic and anaerobic streptococci present in concentrations exceeding 10⁷ to 10⁸ bacteria per ml. Antigen present in saliva probably seldom influences antigen detection in sputum, because only tenacious material is used for sputum examination, and saliva is washed away by using the wash technique. This assumption is confirmed by the results noted in Chapter 4, in which, by comparing washed and unwashed sputum specimens, a low frequency of “only unwashed antigen positive specimens” was found.

The effect of pneumococcal carriership on antigen detection has been studied only in pneumococcal carriers with colds (acute coryzal infection). Antigen could not be detected in any of these individuals by CIE in respiratory tract secretions. The authors concluded that detection of antigen in respiratory tract secretions indicates infection
caused by \textit{S. pneumoniae}. It should be emphasized that this conclusion is not always applicable to patients with chronic bronchitis, since antigen can be detected in their sputa during stable periods without an exacerbation of the disease.\textsuperscript{10,11,13}

Pneumococcal pneumonia develops when pneumococci colonizing the upper respiratory tract are aspirated into the terminal airways of the lung(s).\textsuperscript{12} In these patients an increased pneumococcal carrier state of the upper respiratory tract has been observed.\textsuperscript{43,44} Therefore, some studies have suggested that the isolation of pneumococci from the nasopharynx and oropharynx may be of supplemental value in diagnosing pneumococcal pneumonia.\textsuperscript{45,46} As already shown, during antibiotic treatment cultures for pneumococci become negative more rapidly than tests for antigen detection. Therefore, detectable antigen in the oropharynx may also provide an assignment for aetiology, especially in patients who cannot expectorate sputum. In order to confirm this hypothesis, we examined oropharyngeal secretions from different patient categories and from healthy individuals (Chapter 10). It was found that the rate of antigen positive specimens gradually increases with the severity of pneumococcal carriage or infection (healthy individuals, COPD or asthma, LRTI, pneumonia). Antigen was detected in the oropharynx in 8 (38\%) of 21 patients with pneumococcal pneumonia, whereas patients with LRTI and carriers yielded positive results in 20\% and 9\% respectively. No false positive reactions were observed in healthy individuals, suggesting that cross-reactions with other bacteria (e.g. \textit{c}-haemolytic streptococci) are irrelevant for pneumococcal antigen detection in this area. In contrast to others\textsuperscript{43-46} a relatively low pneumococcal isolation rate (14\%) was established in the upper respiratory tract of patients with pneumococcal pneumonia. This might be explained by the different method that was used.

In our opinion detectable antigen in oropharyngeal secretions originates from the lower respiratory tract. During coughing aerosolized droplets of lower respiratory tract secretions are deposited on the oropharynx. Most of the respiratory tract secretions are transported to the oropharynx by mucociliary action. Impaired laryngeal-pharyngeal function, especially in critically ill patients, may lead to stasis of these secretions. The assumption that oropharyngeal antigen originates from the lower respiratory tract was provided by the observation that antigen was detected in the oropharynx of patients with pneumonia of unknown aetiology. Another argument for this assumption was provided in patients with pneumococcal pneumonia; antigen detection yielded concordant results in sputum and oropharyngeal specimens collected on the same day.

Although the sensitivity (approximately 40\%) of LA in detecting antigen in oropharyngeal secretions from patients with pneumococcal pneumonia is much lower than that found in sputum (95\%), the simplicity of obtaining a specimen promotes this method for supplemental application, especially when sputum is not being produced.

The case reports in Chapter 11 illustrate the application and usefulness of antigen detection in clinical specimens from patients with community-acquired pneumonia.

\textbf{Conclusions}

1. Pneumococcal capsulation (LA), is of diagnostic importance in sputum (preferably residual) and is of important value in pneumococcal pneumonia.Limited utility for diagnosis of pneumococcal pneumonia.

2. Pneumococcal antigen detection and culture become positive more rapidly than tests for antigen detection.

3. The sputum wash technique is preferred in pneumococcal antigen detection as in unwashed samples.

4. Approximately 10$^8$ pneumococci is necessary for detection of antigen in sputum and culture becomes positive.

5. Pneumococcal carrier state and culture becomes positive more rapidly than tests for antigen detection.

6. Detectable antigen for pneumococcal pneumonia is a useful simple method for diagnosis.
Conclusions

1. Pneumococcal capsular antigen detection in clinical specimens, using latex agglutination (LA), is of diagnostic value in patients with community-acquired pneumonia. In sputum (preferably representative specimens) and in pleural fluid antigen determination is of important value in addition to Gram staining and culture for the diagnosis of pneumococcal pneumonia. Antigen detection in urine and serum appears to have limited utility for diagnosing pneumococcal pneumonia.

2. Pneumococcal antigen remains detectable in sputum or pleural fluid even after Gram stain and culture become negative. Therefore, the number of patients diagnosed as pneumococcal pneumonia, who would otherwise be classified as unknown aetiology, will be increased. Some restrictions should be kept in mind for patients with chronic bronchitis, since detectable antigen in sputum could also be found in the absence of pneumonia.

3. The sputum wash technique, which has been developed to reduce contaminating oropharyngeal flora while potential pathogens are not affected, has only a minor influence on pneumococcal antigen detection. Antigen can be detected as reliably in washed sputum as in unwashed sputum.

4. Approximately 10⁶ pneumococci per ml are required in vitro for a positive LA. Once this threshold concentration was exceeded, antigen remained detectable in the presence of absence of penicillin for the entire 72 hours of the in vivo experiments. When penicillin was added at a concentration below the threshold level antigen could not be detected in the later phases of the experiment. Pneumococci isolated in concentrations of more than 10⁶/ml are generally associated with clinical manifestations of infection.

5. Pneumococcal carriership in the upper respiratory tract seldom yields a positive antigen result, because the number of pneumococci present are below the threshold level. The amount of pneumococcal antigen present in the upper respiratory tract probably does not influence antigen detection in sputum specimens.

6. Detectable antigen found in oropharyngeal secretions probably originates from the lower respiratory tract and is suggestive of pneumococcal infection. In patients with community-acquired pneumonia, oropharyngeal sampling may be an additional and simple method for diagnosing pneumococcal aetiology.