SUPPLEMENT ARTICLE

Novel Approaches to the Identification of *Streptococcus pneumoniae* as the Cause of Community-Acquired Pneumonia

Keith P. Klugman,[1,2] Shabir A. Madhi,[2,3] and Werner C. Albrich[3]

[1]Department of Global Health, Rollins School of Public Health, and Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, Georgia; [2]Medical Research Council/Wits Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa; and [3]Institute for Infectious Diseases, University Hospital Bern, Bern, Switzerland

Current diagnostic tests lack sensitivity for the identification of the bacterial etiology of pneumonia. Attempts during the past 2 decades to improve sensitivity of detection of bacterial constituents in blood by use of antibody-antigen complexes and polymerase chain reaction have been disappointing. Recent data using pneumococcal conjugate vaccines as probes suggest that increased levels of both C-reactive protein and procalcitonin may be useful adjuncts to chest radiographs in the selection of patients with presumed bacterial pneumonia for inclusion in clinical trials. Among pneumococcal diagnostics currently under investigation, quantitative real-time polymerase chain reaction of respiratory secretions, as well as urinary antigen detection and pneumococcal surface adhesin A serological analysis for adults, are candidates for use in future clinical trials of antibiotics.

Acute respiratory infections are the leading infectious cause of death globally among both infants and adults [1]. Although these deaths are, in large part, attributable to community-acquired pneumonia (CAP) caused by *Streptococcus pneumoniae* [2], our ability to identify pneumococci as the specific etiological agent of pneumonia is very limited.

Although culture of lung puncture aspirate may increase the yield of pneumococci, relative to blood culture alone, its sensitivity for diagnosing pneumococcal pneumonia is <20% [2, 3]. Additionally, other invasive techniques, such as bronchoalveolar lavage and protected specimen brush sampling, are not practical to use to define the role of pneumococci in patients with CAP who are routinely enrolled in clinical trials.

This article reviews advances in the specific diagnosis of pneumococcal infections in CAP. It starts with an overview of the insights gained from trials of conjugate pneumococcal vaccine on the role of chest radiographs and acute inflammatory responses in helping to define pneumococcal CAP and then looks at molecular and serological advances in the diagnosis of pneumococcal CAP for both children and adults.

PNEUMOCOCCAL CAP EXTENDS BEYOND CONSOLIDATION SEEN ON CHEST RADIOGRAPHS

Evidence of alveolar consolidation seen on chest radiograph is usually used to define the subset of patients with acute respiratory infections that have progressed to CAP. Although it is recognized that chest radiographs may not manifest alveolar consolidation early in the course of disease, particularly in pediatric studies, it has been difficult, in the absence of a sensitive diagnostic assay, to define a role for pneumococci in patients with pneumonia who do not have classic changes seen on chest radiographs [4].

Although pneumococcal conjugate vaccine trials involving children have demonstrated a decrease in the incidence of World Health Organization–defined consolidation seen on chest radiograph (chest radiograph

Reprints or correspondence: Dr. Keith P. Klugman, Dept. of Global Health, Rollins School of Public Health, Emory University, 1518 Clifton Rd. NE, Rm. 720, Atlanta, GA 30322 (keith.klugman@emory.edu).

Clinical Infectious Diseases 2008; 47:S202–6

1058-4838/2008/4711S3-0017$15.00
DOI: 10.1086/591405

end point consolidation) among 25%–37% of children receiving ⩾3 doses of 7- or 9-valent conjugate vaccine [2, 5, 6], the vaccine has also been used as a probe to investigate the role of C-reactive protein (CRP) and procalcitonin in defining a pneumococcal etiology. This is done by comparing the incidence of study end points between vaccine recipients and control individuals, after blinded determination of CRP and procalcitonin levels in archived specimens taken during the randomized clinical trials [7, 8]. In children with CAP and chest radiograph end point consolidation, an elevated CRP level identified an enriched population of patients with pneumonia among whom the fraction of pneumococcal-vaccine preventable pneumonia increased (in the intent-to-treat analysis) from 21% to 38% at a CRP level of >120 mg/L, and the fraction of pneumococcal-vaccine preventable pneumonia increased from 21% to 46% as the procalcitonin level increased to 5 ng/mL [7]. These levels of acute-phase reactants are too high to be clinically sensitive but demonstrate increasing specificity of the pneumococcal fraction of chest radiograph end point pneumonia as levels of acute-phase reactant increase. Among patients with the combination of high levels of CRP and high levels of procalcitonin, 64% of chest radiograph end point–confirmed CAP cases were preventable by the conjugate vaccine [7].

With the vaccine probe approach, the use of a CRP value >40 mg/L has been shown to identify a considerable burden of pneumococcal pneumonia in children with nonspecific changes seen on chest radiograph [8]. These data suggest that, at least in studies involving children, an increased CRP level in situations where any changes are seen on chest radiograph may be an enriched clinical trial population.

NONQUANTITATIVE PCR ASSAYS FOR *S. PNEUMONIAE*

The first generation of PCR assays of blood samples from adults with CAP had sensitivities less than or equal to those of blood cultures [9]. Throat swab specimens were probed by PCR of the *ply* gene (which encodes pneumolysin) in patients with pneumonia. Of 417 patients, 229 (55%) had positive results, but 73 (58%) of 126 control individuals had positive results [10]. The lack of specificity is now believed to be caused by the presence of the *ply* gene in some species of streptococcal mouth flora. In children, the use of pneumolysin-based nonquantitative PCR on blood samples is contradictory. Dagan et al. [11] showed a lack of sensitivity and specificity, whereas a study by Michelow et al. [12] suggested 100% sensitivity and 95% specificity, compared with blood culture. The data overall suggest a lack of specificity for the *ply* gene. A recent report shows improved specificity with use of a probe for the *lytA* gene [13].

QUANTITATIVE REAL-TIME PCR

Recent studies involving adults have investigated quantitative differences in pneumococcal colonization of the nasopharynx and sputum by using quantitative real-time PCR. Using the *ply* gene as a reporter and a cutoff of $>3.7 \times 10^4$ genomic equivalents per mL of good-quality sputum, sensitivity was 90% and specificity was 80% [14]. Using the same target at $>10^5$ copies of *ply* gene per mL of sputum was more sensitive than was culture, especially for patients receiving antibiotics [15, 16]. A more specific target, the Spn9802 gene fragment, at $>10^4$ copies/mL in nasopharyngeal aspirate specimens is reported to correlate with clinical disease [17]. The Spn9802 gene fragment compared favorably with quantitative *lytA*-based real-time PCR in distinguishing patients with pneumonia from control individuals [17]. There is some evidence that disease presentation and severity may correlate with real-time PCR levels of pneumococci in clinical specimens. In patients with pneumococcal meningitis, the concentration of pneumococcal DNA in blood and CSF specimens correlated with mortality [18]. Finally, in the diagnosis of pneumococcal empyema in children, real-time PCR is more sensitive than is culture [19, 20].

Real-time quantititave PCR of respiratory specimens with specific primers for genes such as *lytA* is thus a promising method for the identification of patients who should be enrolled in antibiotic trials of CAP. This approach appears to be more useful than is culture for identifying pneumococcal empyema.

DIFFERENTIAL PATTERNS OF GENE EXPRESSION

The patterns of gene expression in pneumococci vary between colonizing and invasive bacteria. In mice, opaque phenotypes of *S. pneumoniae* correlate with invasive disease, whereas transparent phenotypes correlate with colonization [21]. The phenotype is a function of capsular expression [22] and the expression of other virulence genes. Virulence genes other than capsular genes showed greater differential expression than did capsular genes, in lung and blood specimens of mice but not in nasal secretions [23]. These observations, however, were complicated by strain-specific differences in expression that were also found. Thus, differential gene expression may be a diagnostic modality of the future—a major hurdle that first must be overcome is the need to improve the sensitivity of in situ gene expression [23]. There are also large differences in host immune protein gene expression between different niches associated with pneumococcal infection in mice [23]. Thus, host protein expression may identify specific etiological agents, such as pneumococci. In mice, pneumococcal infection may be differentiated from influenza by patterns of host gene expression [24, 25], and the colonization of human pharyngeal epithelial cells with pneumococci can similarly be detected by

changes in the cells' gene expression [26]. The possibility therefore exists that, in the future, patterns of pneumococcal gene expression detected in the blood may identify invasive strains.

PNEUMOCOCCAL SEROLOGICAL ANALYSIS

Serological analysis is not useful for immediate treatment decisions, but the persistence of an antibody response despite preceding antibiotic exposure makes serological analysis appealing for the etiologic diagnosis of pneumococcal disease in epidemiologic studies. Pneumococcal surface adhesin A (PsaA) is present in the cell walls of all pneumococcal serotypes and some oral streptococci [27]. Anti-PsaA ELISA is a possible method of establishing a diagnosis of pneumococcal pneumonia. A serotype 4 outbreak in a nursing home led to hospitalization of 18 patients during a 2-week period. Blood culture results were positive for 3 patients; 6 other patients showed evidence of seroconversion to PsaA [28]. In patients with bacteremic pneumococcal pneumonia, a 2-fold increase in ELISA units of anti-PsaA from paired convalescent- and acute-phase serum samples had 85% sensitivity and 83% specificity [29]. The same assay achieved a sensitivity of 89% and a specificity of 98% among Kenyan adults with a 1.3-fold cutoff [30]. Unfortunately, among children with invasive pneumococcal disease, sensitivity was poor, and interpretation required higher cutoff values [31]. Comparison with convalescent-phase serum samples is necessary because single measurements of PsaA concentrations in acute-phase serum specimens lack sensitivity [30, 32]. This is a major limitation of the assay, because clinicians are unlikely to obtain convalescent-phase serum samples. Patients enrolled in clinical trials, however, are routinely seen at the end of treatment and/or at 21–30 days after initiation of therapy. PsaA seroconversion is currently an underutilized opportunity to make pneumococcal diagnoses in clinical trials. A major limitation of the assay for this purpose is that there is no US Food and Drug Administration (FDA)–licensed or commercially available assay.

Pneumococcal antigen-antibody immune complexes have been used in experimental assays to indicate pneumococcal infection. These assays are not standardized, and their utility in clinical studies remains to be established [33, 34]. Assays of pneumococcal antibody using ELISA and including absorption steps with C-polysaccharide and whole 22F pneumococci have been standardized by an international effort [35]. The possibility of measuring antibody-specific assays has become practical with use of simultaneous multiple-assay platforms, such as Luminex (Luminex Corporation), but, again, their use remains to be clinically established in the trial setting. In conclusion, the development of an FDA-licensed serological assay would be a useful adjunct to the diagnosis of pneumococcal pneumonia in clinical trials.

PNEUMOCOCCAL URINARY ANTIGEN TESTING

Serotype-specific latex-agglutination urinary assays for pneumococcal serotypes are limited because there are 91 identified pneumococcal serotypes [36, 37]. An ELISA for urinary pneumolysin detection in children has been recently developed. Compared with the immunochromatographic membrane assay (ICT) Binax Now *S. pneumoniae* (Binax), the urinary pneumolysin ELISA achieved lower sensitivity for pneumococcal infection in children with and without nasopharyngeal carriage, but markedly improved specificity [38]. The low sensitivity of 56.6% in adults likely does not suggest much added benefit in this age group [38]. The detection of pneumococcal C-polysaccharide with the ICT Binax NOW *S. pneumoniae* assay allows a bedside diagnosis of pneumococcal disease within 15 min after receipt of a urine sample [39]. The assay is reported to have a sensitivity of 74%, specificity of 94%, positive predictive value of 79%, and negative predictive value of 92% for adults not infected with HIV who have microbiologically confirmed pneumococcal disease [40]. The ICT may also be useful for special populations, such as HIV-infected patients [40] and critically ill patients [41]. Specificity issues have to be viewed in light of the limitations of traditional microbiological testing. In a latent-class analysis, the ICT performed favorably, with better sensitivity than that of blood and sputum culture and higher specificity than that of *lytA* or *ply* (nonquantitative) PCR of respiratory samples at comparable sensitivity [42]. The lower sensitivity of the ICT after antibiotic use that was found in some studies [37, 43] was not confirmed in others [12, 44]. Discordant results, with negative results of culture for pneumococci and positive results of ICT, were highly associated with previous use of antibiotics [45]. The measured fraction of patients with pneumonia of unknown etiology might be reduced by 23% [40], and the measured fraction of pneumococcal etiology could be increased up to 2-fold by use of this assay. The limitations of this assay include unacceptably low specificity in young children, because of correlation with pneumococcal carriage [39, 46], although not in adults [47], and possible cross-reactions with some α-hemolytic oral streptococci (*Streptococcus mitis* and *Streptococcus oralis*).

The persistence of positive test results after recovery from pneumococcal disease is reported for up to 90% of patients 7 days after diagnosis [48] and for 40%–48% after 4–6 weeks [40, 49]. The persistence of positive assay findings is a particular problem (occuring for 70% at 1 month) if urine specimens are concentrated 25-fold with use of ultrafiltration [45]. The added time, effort, and cost, together with some loss in specificity from the use of concentrated urine, likely outweigh the sensitivity gained, but the increased sensitivity achieved by urine concentration might be useful for epidemiological studies.

Severe disease has been associated with higher detection rates with use of the ICT [50] and higher semiquantitative titers

[51], which might be an indication either of the severity of pneumococcal disease or simply of a higher pneumococcal burden in severe disease. Bacteremic infections tend to have higher rates of positivity [50, 52]. Promising results have been reported for testing of pleural fluid for the diagnosis of empyema [44, 53]. Finally, ICT has been used for management decisions. Targeted amoxicillin use among Russian military recruits with nonsevere pneumonia who had positive assay results achieved similar clinical success rates as did clarithromycin use among those with negative results [54]. Similarly, in a Swedish study, a positive result of ICT was associated with higher success rates of initial β-lactam monotherapy, compared with a negative result of ICT [55].

CONCLUSIONS

In clinical trials for CAP, *S. pneumoniae* is the most common and virulent bacterial pathogen against which empirical treatment is directed. We suggest that the following tests for *S. pneumoniae* be included in future clinical trials of both mild pneumonia in outpatients and severe pneumonia in hospitalized patients.

1. Culture of blood and of good-quality sputum
2. *S. pneumoniae* urinary antigen detection
3. Quantitative assays of CRP and procalcitonin
4. Serological analysis of acute- and convalescent-phase serum samples, pending an FDA-licensed assay for PsaA.

It is possible that, in the near future, specific primers for real-time PCR will allow the application of this method to respiratory secretions, and perhaps to blood and other bodily fluids, for quantitative differentiation of pneumococcal pneumonia from nasopharyngeal carriage.

Acknowledgments

Financial support. National Institutes of Health (grant 3A1050409-08).

Supplement sponsorship. This article was published as part of a supplement entitled "Workshop on Issues in the Design and Conduct of Clinical Trials of Antibacterial Drugs for the Treatment of Community-Acquired Pneumonia," sponsored by the US Food and Drug Administration and the Infectious Diseases Society of America.

Potential conflicts of interest. K.P.K. and S.A.M. acknowledge receipt of research funding and consultancy funding from Wyeth and GlaxoSmithKline Biologicals. W.C.A.: no conflicts.

References

1. World Health Organization (WHO). World health report. Geneva, Switzerland: WHO, **2005**.
2. Cutts FT, Zaman SM, Enwere G, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in the Gambia: randomised, double-blind, placebo-controlled trial. Lancet **2005**; 365:1139–46.
3. Obaro SK, Madhi SA. Bacterial pneumonia vaccines and childhood pneumonia: are we winning, refining, or redefining? Lancet Infect Dis **2006**; 6:150–61.
4. Cherian T, Mulholland EK, Carlin JB, et al. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. Bull World Health Organ **2005**; 83:353–9.
5. Hansen J, Black S, Shinefield H, et al. Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than 5 years of age for prevention of pneumonia: updated analysis using World Health Organization standardized interpretation of chest radiographs. Pediatr Infect Dis J **2006**; 25:779–81.
6. Klugman KP, Madhi SA, Huebner RE, et al. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. N Engl J Med **2003**; 349:1341–8.
7. Madhi SA, Heera JR, Kuwanda L, Klugman KP. Use of procalcitonin and C-reactive protein to evaluate vaccine efficacy against pneumonia. PLoS Med **2005**; 2:e38.
8. Madhi SA, Klugman KP. World Health Organisation definition of "radiologically-confirmed pneumonia" may under-estimate the true public health value of conjugate pneumococcal vaccines. Vaccine **2007**; 25: 2413–9.
9. Murdoch DR. Molecular genetic methods in the diagnosis of lower respiratory tract infections. APMIS **2004**; 112:713–27.
10. Murdoch DR, Anderson TP, Beynon KA, et al. Evaluation of a PCR assay for detection of *Streptococcus pneumoniae* in respiratory and nonrespiratory samples from adults with community-acquired pneumonia. J Clin Microbiol **2003**; 41:63–6.
11. Dagan R, Shriker O, Hazan I, et al. Prospective study to determine clinical relevance of detection of pneumococcal DNA in sera of children by PCR. J Clin Microbiol **1998**; 36:669–73.
12. Michelow I, Lozano J, Olsen K, et al. Diagnosis of *Streptococcus pneumoniae* lower respiratory infection in hospitalized children by culture, polymerase chain reaction, serological testing, and urinary antigen detection. Clin Infect Dis **2002**; 34:e1–11.
13. Carvalho MdGS, Tondella ML, McCaustland K, et al. Evaluation and improvement of real-time PCR assays targeting *lytA, ply,* and *psaA* genes for detection of pneumococcal DNA. J Clin Microbiol **2007**; 45: 2460–6.
14. Yang S, Lin S, Khalil A, et al. Quantitative PCR assay using sputum samples for rapid diagnosis of pneumococcal pneumonia in adult emergency department patients. J Clin Microbiol **2005**; 43:3221–6.
15. Johansson N, Kalin M, Giske CG, Hedlund J. Quantitative detection of *Streptococcus pneumoniae* from sputum samples with real-time quantitative polymerase chain reaction for etiologic diagnosis of community-acquired pneumonia. Diagn Microbiol Infect Dis **2008**; 60:255–61.
16. Kais M, Spindler C, Kalin M, Ortqvist A, Giske CG. Quantitative detection of *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella cattarhalis* in lower respiratory tract samples by real-time PCR. Diagn Microbiol Infect Dis **2006**; 55:169–78.
17. Abdeldaim GM, Stralin K, Olcen P, Blomberg J, Herrmann B. Toward a quantitative DNA-based definition of pneumococcal pneumonia: a comparison of *Streptococcus pneumoniae* target genes, with special reference to the Spn9802 fragment. Diagn Microbiol Infect Dis **2008**; 60: 143–50.
18. Carrol ED, Guiver M, Nkhoma S, et al. High pneumococcal DNA loads are associated with mortality in Malawian children with invasive pneumococcal disease. Pediatr Infect Dis J **2007**; 26:416–22.
19. Eastham KM, Freeman R, Kearns AM, et al. Clinical features, aetiology and outcome of empyema in children in the north east of England. Thorax **2004**; 59:522–5.
20. Lahti E, Mertsola J, Kontiokari T, Eerola E, Ruuskanen O, Jalava J. Pneumolysin polymerase chain reaction for diagnosis of pneumococcal pneumonia and empyema in children. Eur J Clin Microbiol Infect Dis **2006**; 25:783–9.
21. Weiser JN, Austrian R, Sreenivasan PK, Masure HR. Phase variation in pneumococcal opacity: relationship between colonial morphology and nasopharyngeal colonization. Infect Immun **1994**; 62:2582–9.
22. Kim JO, Weiser JN. Association of intrastrain phase variation in quantity of capsular polysaccharide and teichoic acid with the virulence of *Streptococcus pneumoniae.* J Infect Dis **1998**; 177:368–77.

23. Mahdi LK, Ogunniyi AD, Lemessurier KS, Paton JC. Pneumococcal virulence gene expression and host cytokine profiles during pathogenesis of invasive disease. Infect Immun **2008**; 76:646–57.
24. Rosseau S, Hocke A, Mollenkopf H, et al. Comparative transcriptional profiling of the lung reveals shared and distinct features of *Streptococcus pneumoniae* and influenza A virus infection. Immunology **2007**; 120: 380–91.
25. Zhang H, Su YA, Hu P, et al. Signature patterns revealed by microarray analyses of mice infected with influenza virus A and *Streptococcus pneumoniae.* Microbes Infect **2006**; 8:2172–85.
26. Bootsma HJ, Egmont-Petersen M, Hermans PW. Analysis of the in vitro transcriptional response of human pharyngeal epithelial cells to adherent *Streptococcus pneumoniae:* evidence for a distinct response to encapsulated strains. Infect Immun **2007**; 75:5489–99.
27. Jado I, Fenoll A, Casal J, Perez A. Identification of the *psaA* gene, coding for pneumococcal surface adhesin A, in viridans group streptococci other than *Streptococcus pneumoniae.* Clin Diagn Lab Immunol **2001**; 8:895–8.
28. Gleich S, Morad Y, Echague R, et al. *Streptococcus pneumoniae* serotype 4 outbreak in a home for the aged: report and review of recent outbreaks. Infect Control Hosp Epidemiol **2000**; 21:711–7.
29. Tharpe JA, Russell H, Leinonen M, et al. Comparison of a pneumococcal common protein (PsaA) antibody ELISA and a PsaA immune complex ELISA for detection of pneumococcal serum antibody. Pathobiology **1998**; 66:77–83.
30. Scott JA, Obiero J, Hall AJ, Marsh K. Validation of immunoglobulin G enzyme-linked immunosorbent assay for antibodies to pneumococcal surface adhesin A in the diagnosis of pneumococcal pneumonia among adults in Kenya. J Infect Dis **2002**; 186:220–6.
31. Scott JA, Mlacha Z, Nyiro J, et al. Diagnosis of invasive pneumococcal disease among children in Kenya with enzyme-linked immunosorbent assay for immunoglobulin G antibodies to pneumococcal surface adhesin A. Clin Diagn Lab Immunol **2005**; 12:1195–201.
32. Baril L, Briles DE, Crozier P, et al. Characterization of antibodies to PspA and PsaA in adults over 50 years of age with invasive pneumococcal disease. Vaccine **2004**; 23:789–93.
33. Lankinen KS, Ruutu P, Nohynek H, Lucero M, Paton JC, Leinonen M. Pneumococcal pneumonia diagnosis by demonstration of pneumolysin antibodies in precipitated immune complexes: a study in 350 Philippine children with acute lower respiratory infection. Scand J Infect Dis **1999**; 31:155–61.
34. Musher DM, Mediwala R, Phan HM, Chen G, Baughn RE. Nonspecificity of assaying for IgG antibody to pneumolysin in circulating immune complexes as a means to diagnose pneumococcal pneumonia. Clin Infect Dis **2001**; 32:534–8.
35. Wernette CM, Frasch CE, Madore D, et al. Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. Clin Diagn Lab Immunol **2003**; 10:514–9.
36. Scott JA, Hannington A, Marsh K, Hall AJ. Diagnosis of pneumococcal pneumonia in epidemiological studies: evaluation in Kenyan adults of a serotype-specific urine latex agglutination assay. Clin Infect Dis **1999**; 28:764–9.
37. Stralin K, Kaltoft MS, Konradsen HB, Olcen P, Holmberg H. Comparison of two urinary antigen tests for establishment of pneumococcal etiology of adult community-acquired pneumonia. J Clin Microbiol **2004**; 42:3620–5.
38. del Mar Garcia-Suarez M, Cima-Cabal MD, Villaverde R, et al. Performance of a pneumolysin enzyme-linked immunosorbent assay for diagnosis of pneumococcal infections. J Clin Microbiol **2007**; 45: 3549–54.
39. Dominguez J, Blanco S, Rodrigo C, et al. Usefulness of urinary antigen detection by an immunochromatographic test for diagnosis of pneumococcal pneumonia in children. J Clin Microbiol **2003**; 41:2161–3.
40. Boulware DR, Daley CL, Merrifield C, Hopewell PC, Janoff EN. Rapid diagnosis of pneumococcal pneumonia among HIV-infected adults with urine antigen detection. J Infect **2007**; 55:300–9.
41. Lasocki S, Scanvic A, Le Turdu F, et al. Evaluation of the Binax NOW *Streptococcus pneumoniae* urinary antigen assay in intensive care patients hospitalized for pneumonia. Intensive Care Med **2006**; 32: 1766–72.
42. Butler JC, Bosshardt SC, Phelan M, et al. Classical and latent class analysis evaluation of sputum polymerase chain reaction and urine antigen testing for diagnosis of pneumococcal pneumonia in adults. J Infect Dis **2003**; 187:1416–23.
43. Ishida T, Hashimoto T, Arita M, Tojo Y, Tachibana H, Jinnai M. A 3-year prospective study of a urinary antigen-detection test for *Streptococcus pneumoniae* in community-acquired pneumonia: utility and clinical impact on the reported etiology. J Infect Chemother **2004**; 10: 359–63.
44. Porcel JM, Ruiz-Gonzalez A, Falguera M, et al. Contribution of a pleural antigen assay (Binax NOW) to the diagnosis of pneumococcal pneumonia. Chest **2007**; 131:1442–7.
45. Marcos MA, Jimenez de Anta MT, de la Bellacasa JP, et al. Rapid urinary antigen test for diagnosis of pneumococcal community-acquired pneumonia in adults. Eur Respir J **2003**; 21:209–14.
46. Dowell S, Garman R, Liu G, Levine O, Yang Y. Evaluation of Binax NOW, an assay for the detection of pneumococcal antigen in urine samples, performed among pediatric patients. Clin Infect Dis **2001**; 32:824–5.
47. Dominguez J, Gali N, Blanco S, et al. Detection of *Streptococcus pneumoniae* antigen by a rapid immunochromatographic assay in urine samples. Chest **2001**; 119:243–9.
48. Smith MD, Derrington P, Evans R, et al. Rapid diagnosis of bacteremic pneumococcal infections in adults by using the Binax NOW *Streptococcus pneumoniae* urinary antigen test: a prospective, controlled clinical evaluation. J Clin Microbiol **2003**; 41:2810–3.
49. Murdoch DR, Laing RT, Mills GD, et al. Evaluation of a rapid immunochromatographic test for detection of *Streptococcus pneumoniae* antigen in urine samples from adults with community-acquired pneumonia. J Clin Microbiol **2001**; 39:3495–8.
50. Roson B, Fernandez-Sabe N, Carratala J, et al. Contribution of a urinary antigen assay (Binax NOW) to the early diagnosis of pneumococcal pneumonia. Clin Infect Dis **2004**; 38:222–6.
51. Tateda K, Kusano E, Matsumoto T, et al. Semi-quantitative analysis of *Streptococcus pneumoniae* urinary antigen: kinetics of antigen titers and severity of diseases. Scand J Infect Dis **2006**; 38:166–71.
52. Diederen BM, Peeters MF. Rapid diagnosis of pneumococcal pneumonia in adults using the Binax NOW *Streptococcus pneumoniae* urinary antigen test. Int J Infect Dis **2007**; 11:284–5.
53. Andreo F, Dominguez J, Ruiz-Manzano J, et al. Usefulness of pneumococcal antigen detection in pleural fluid samples by immunochromatographic assay for diagnosis of pneumococcal pneumonia. Clin Microbiol Infect **2006**; 12:682–4.
54. Guchev IA, Yu VL, Sinopalnikov A, Klochkov OI, Kozlov RS, Stratchounski LS. Management of nonsevere pneumonia in military trainees with the urinary antigen test for *Streptococcus pneumoniae:* an innovative approach to targeted therapy. Clin Infect Dis **2005**; 40:1608–16 (erratum: Clin Infect Dis 2005; 41:580–1).
55. Stralin K, Holmberg H. Usefulness of the *Streptococcus pneumoniae* urinary antigen test in the treatment of community-acquired pneumonia. Clin Infect Dis **2005**; 41:1209–10.