ERJ Express. Published on February 8, 2013 as doi: 10.1183/09031936.00137412

Diagnostic accuracy of a serotype specific antigen test in community-acquired pneumonia

Detecting pneumococcal antigens

Susanne M. Huijts M.D., Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

Michael W. Pride PhD, Pfizer Vaccines Research, Pearl River, New York, USA.

Josephine M.I. Vos M.D., Dept. of Haematology, Academic Medical Centre, Amsterdam, The Netherlands.

Kathrin U. Jansen PhD, Pfizer Vaccines Research, Pearl River, New York, USA.

Chris Webber M.D., Pfizer Vaccine Research, Maidenhead, Berkshire, UK.

William Gruber M.D., Pfizer Vaccine Clinical Research, New York, USA.

Wim G. Boersma M.D., PhD, Department of Pulmonary Diseases, Medical Centre Alkmaar, Alkmaar, The Netherlands.

Dominic Snijders M.D., Department of Pulmonary Diseases, Medical Centre Alkmaar, Alkmaar, The Netherlands.

Jan AJW. Kluytmans M.D., PhD, Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda, The Netherlands.

Ivo van der Lee M.D., Department of Pulmonology, Spaarne Hospital, Hoofddorp, The Netherlands.

Bart AF. Kuipers M.D., Pulmonary Diseases, Isala Klinieken, Zwolle, The Netherlands.

Arie van der Ende PhD, Academic Medical Centre, Department of Medical Microbiology, Amsterdam, the Netherlands. Also: Netherlands Reference Laboratory for Bacterial Meningitis, Academic Medical Centre, Amsterdam, the Netherlands.

Marc JM Bonten M.D., PhD, Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands. Also: Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

Copyright 2013 by the European Respiratory Society.

Corresponding author contact details:

UMC Utrecht / Julius Center for Health Sciences and Primary Care

Stratenum 6.131, Room 6.125

P.O.Box 85500, 3508 GA Utrecht, The Netherlands

Email: s.m.huijts@umcutrecht.nl

Funding:

This study was sponsored by Wyeth Pharmaceuticals Inc which was acquired by Pfizer in October 2009.

ABSTRACT

Aim: To evaluate the diagnostic accuracy and clinical utility of a serotype specific urinary antigen detection (UAD) multiplex assay for identification of 13 pneumococcal serotypes (1,3,4,5,6A,6B,7F,9V,14,18C,19A,19F,23F) in urine of patients with community acquired pneumonia (CAP).

Methods: Adult patients with clinical suspicion of CAP were included. In addition to standard diagnostic procedures, a urine sample was collected to perform the UAD test. Demographic, clinical, radiological and microbiological data were collected.

Results: Among 1,095 CAP patients *S. pneumoniae* was identified as causative pathogen in 257 (23%), when using conventional diagnostic methods and in 357 (32.6%) when UAD was added. Of the 49 bacteraemic episodes caused by 1 of the 13 serotypes covered by the UAD, 48 were detected by the UAD indicating a sensitivity of 98%. Of the 77 CAP episodes with a 'non-UAD' causative pathogen none had a positive UAD result, indicating a specificity of 100%.

Conclusion: Addition of the UAD test to conventional diagnostic methods increased the prevalence of *S. pneumoniae* CAP by 39%. Using bacteraemic episodes as reference sensitivity and specificity of the UAD was 98% and 100%, respectively.

KEYWORDS

Pneumococcal, sensitivity, specificity, *S. pneumonia*, urine.

INTRODUCTION

Community acquired pneumonia (CAP) is a major cause of morbidity and mortality worldwide. *Streptococcus pneumoniae* is the most common causative organism of CAP in all age groups[1, 2]. There are more than ninety different serotypes of *S. pneumoniae*, based on capsular polysaccharides, with marked serotype-specific differences in disease prevalence[3] and clinical outcome of invasive pneumococcal disease [4].

The diagnosis of pneumococcal CAP is usually based on clinical and radiographic evidence of pneumonia combined with microbiological results of blood and sputum samples and detection of pneumococcal antigens in urine. Yet, many episodes of pneumococcal CAP will remain undiagnosed. Bacteraemia with *S. pneumoniae* is demonstrated in 3.6 to 9.6% of the adult cases with CAP only[5] and sensitivity decreases due to prior use of antibiotics[6, 7]. Furthermore, only 36 to 64% of CAP patients produce sputum[8, 9], and samples are frequently contaminated with flora of the upper respiratory tract. Finally, detection of pneumococcal antigens in urine -usually with the immunogromatographic assay (ICA) of BinaxNOW®- may have a high specificity, yet reported sensitivities have varied from 76.9 to 100% in the subgroup of patients with pneumococci isolated from sterile sites (such as blood and pleural fluid) and from 43.7 to 69.2% in patients with pneumococci isolated in sputum only [10-12]. Serotyping is only possible for pneumococci isolated from sterile sites and sputum. As a result, serotype distributions and changes herein (for instance because of vaccination strategies) can only be measured in a small subset of CAP cases.

In preparation of a large randomized placebo-controlled double-blind trial[13] we evaluated the diagnostic properties and clinical utility of a novel serotype-specific urinary antigen detection (UAD) multiplex assay [14] for identification of 13 serotype-specific polysaccharides of *S. pneumoniae* (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) in human urine samples in patients hospitalized with CAP. The 13 polysaccharide

antigens correspond to the serotypes used in the 13-valent pneumococcal conjugate vaccine (PCV13).

MATERIAL AND METHODS

Patients

We conducted a prospective, observational, cohort study in 23 Dutch hospitals (4 academic, 15 teaching and 4 non-teaching hospitals), between January 2008 and April 2009. Not all hospitals started at the same time (last one started in July 2008) and 17 hospitals discontinued patient enrolment in October 2008 because of a competing pneumonia trial.

Adult patients, 18 years or older, with a clinical suspicion of CAP or lower respiratory tract infection (LRTI) presenting at the Emergency Room (ER) of the participating hospitals were eligible. A clinical suspicion of CAP or LRTI was defined as the presence of at least two of the following criteria: fever or hypothermia, cough or change in chronic coughing pattern, dyspnoea or tachypnoea or hypoxia, findings with percussion or auscultation consistent with pneumonia, leucocytosis or leucopenia or left shift or an infiltrate on the chest X-ray. In each hospital dedicated research nurses checked admission diagnoses of patients admitted through the ER two to three times weekly. Eligible patients not included at the ER, could be included until 48 hours after admission. The study was approved by all local Research Ethics Committees and written informed consent was obtained from all participants.

Diagnostic approach

In addition to history taking, physical examination, laboratory blood analysis and chest X-ray, diagnostic procedures included microbiological cultures of blood, sputum and pleural fluid (if

present), and collection of a urine sample immediately after presentation, but at least within 48 hours after admission.

Urine samples were processed locally in a standardized manner (treated with 0.5M PIPES buffer to a final concentration of 25mM) and subsequently frozen at -70^{o}C until processing in the reference laboratory of Pfizer Vaccine Research, Pearl River, New York. As ICA was not part of standard care in most hospitals, local processing of ICA was not part of study protocol. In the reference laboratory both the UAD test and the commercially available urinary pneumococcal antigen ICA (BinaxNOW® *S. pneumoniae* urine antigen test, Alere International, Ireland) were performed batch-wise according to manufacturers' instructions. The ICA results were interpreted by two analysts, blinded for any clinical information. A third analyst interpreted the results when the first two analysts did not agree. The UAD test is a Luminex technology based multiplex urinary antigen detection assay, that can detect simultaneously 13 different serotypes of *S. pneumoniae* (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) by capturing serotype-specific polysaccharides secreted in human urine[14]. The in vitro validation of the test (based on a subset of the current study population) and specificity determination in non-infected individuals has been reported elsewhere [14].

Microbiologic testing (blood and sputum cultures and BinaxNOW® urinary Legionella antigen test, if clinical applicable) was performed in local laboratories according to local and manufacturers' protocols. *S. pneumoniae* blood isolates were serotyped at the Netherlands Reference Laboratory for Bacterial Meningitis, by co-agglutination and subtyping with the capsular swelling method (Quellung reaction) using antisera (Statens Serum Institute, Copenhagen, Denmark) according to the manufacturer's protocol[15, 16]. Cogulase-negative staphylococci in blood cultures were considered as 'contaminants' and not as causative pathogen.

Data-collection

Demographics, prior antibiotic use, signs and symptoms of infection, radiological and microbiological test results and findings of physical examination were documented in a standardized case record form by trained research nurses and/or physicians in every hospital. Data were collected during or shortly after admission. Clinical severity of CAP was categorized upon the Pneumonia Severity Index score (PSI) [17].

Definitions

CAP was defined as the presence of an infiltrate on the chest X-ray within 48 hours after admission based on the diagnosis of the local radiologist together with at least two of the following signs or symptoms: cough, sputum production, fever, auscultatory findings consistent with pneumonia, leucocytosis or leucopenia, C reactive protein more than three times the upper limit of normal, hypoxemia with pO_2< 60mmHg while patient is breathing room air or signs of dyspnoea/ tachypnoea .

The causative microorganism of CAP was considered ‘definite’ if it was cultured from blood or any other sterile body fluid or if the urinary antigen test was positive (either for pneumococcal or legionella antigen with ICA or with the UAD test). Bacteria considered as contaminants (e.g. coagulase-negative staphylococci) were not considered as causative pathogens. A microorganism was considered a ‘probable’ cause of CAP in the absence of a definite pathogen and if it was present as dominant flora in the sputum culture. Pneumococcal CAP was defined as CAP with *S. pneumoniae* as the ‘definite’ or ‘probable’ causative microorganism.

Data-analysis

Descriptive analyses were carried out by calculating frequencies, mean or median with SPSS statistical package (version 17.0, SPSS Inc, Chicago, IL, USA). To compare groups the

Pearson's Chi Square was used for dichotomous determinants and the Students T test or Mann Whitney U test was used for continuous data. A p-value < 0.05 was considered significant.

To calculate the sensitivity of the UAD test 'true positives' were defined as CAP with bacteraemia (blood culture positive) caused by one of the 13 serotypes of the UAD test and the number of positive UAD tests was compared to the number of 'true positives'. Episodes of probable pneumococcal CAP that were based on sputum cultures only were excluded from this analysis. 'True negatives' were defined as CAP cases with bacteraemia caused by another pathogen or by pneumococcal serotypes not covered by the UAD test or CAP cases with only a positive legionella urinary antigen test. Specificity was determined by the number of negative UAD tests compared to the number of 'true negatives'.

RESULTS

Study Population

In all 1,758 patients with a clinical suspicion of CAP or LRTI were included, of whom 552 (31%) had no infiltrate on chest X-ray within 48 hours after admission. Urine samples had not been obtained from 101 patients and from 10 patients informed consent was not obtained, yielding a total study population of 1,095 patients with CAP (Figure 1). The median age of these 1,095 patients was 69 years (IQR 57 – 79), the majority was male (62.7%) and 50 (4.6%) patients were treated as outpatients. Median duration of complaints before presentation was 4 days (IQR 2-7). PSI categorization yielded a distribution of 9.9%, 22.1%, 23.0%, 34.6% and 10.4% for categories I through V, respectively. Four patients (0,4%) reported receipt of pneumococcal vaccination. Information on pre-admission antibiotic use was available for 1077 patients and 325 had received antibiotics before admission.

Aetiology

Blood cultures were available in 922 patients (84.2%), sputum cultures in 570 patients (52.1%) and Legionella urinary antigen tests in 762 patients (69.6%). All 1,095 urine samples were tested with ICA and UAD in the reference laboratory. There were three CAP episodes with urine antigen tests positive for both pneumococci and Legionella. In all three episodes both ICA and UAD test were positive, and in one episode there was also pneumococcal bacteraemia. *S. pneumoniae* was considered the causative pathogen of CAP in all three episodes. One CAP episode was associated with *E. coli* bacteraemia, a positive ICA pneumococcal urinary antigen test and a negative UAD test. In this episode *S. pneumoniae* was considered as the causative pathogen of CAP.

Based on microbiological culture results and ICA an aetiological cause of CAP could be determined in 403 (36.8%) episodes (Table 1). When the UAD test results were added to these methods, aetiology could be determined in 493 (45.0%) episodes, and proportions of episodes of pneumococcal CAP were 23.5% (n=257) and 32.6% (n=357) without and, with inclusion of UAD test results. This represents a relative increase of the diagnostic yield for *S. pneumoniae* of 39% and an absolute increase of 9.1%.

Diagnostic accuracy

In all, 249 UAD tests (22.7%) and 211 ICA tests (19.3%) were positive in the study population. Of the 249 UAD positive samples, 122 samples were negative in the ICA.

There were 49 bacteraemic isolates that belonged to one of the 13 serotypes included in the UAD test, of which 48 were detected by UAD, yielding a sensitivity of 98% for bacteraemic pneumococcal CAP with serotypes included in the UAD assay (Table 2). The only negative urine sample yielded a positive signal that did not reach the predefined positivity cut-off limit for serotype 3, which was the serotype of the blood culture isolate. The ICA yielded positive results in 34 of these 49 episodes, yielding a sensitivity of 69.4% for the bacteraemic CAP

with one of the 13 pneumococcal serotypes included in the UAD assay. The ICA detected 49 of the total of 77 bacteraemic pneumococcal CAP cases (including the serotypes not included in the UAD), indicating an overall sensitivity of 63.6%.

There were 76 CAP episodes with other causative pathogens than *S. pneumoniae* serotypes included in the UAD test: 23 bacteraemic episodes due to non-UAD test *S. pneumoniae* serotypes, 28 non-pneumococcal bacteraemic cases and 25 episodes with mono-infection caused by *L. pneumophila.* There was no positive UAD test in this cohort, indicating an UAD assay specificity of 100%. Of the 28 non-pneumococcal bacteraemic cases and the 25 episodes caused by *L. pneumophila*, there was one positive ICA test, indicating a specificity of 98%.

Serotype distribution

Among the 77 episodes of bacteraemic pneumococcal CAP, serotypes 1 and 14 were the most common serotypes (n=9), followed by 19A and 7F (n=7) and 22F (n=6). Five isolates could not be serotyped. Based on the UAD results, serotype distribution of the 13 serotypes included appeared to change (Figure 2). Serotype 1 remained the most frequent serotype (n=38) (Table 3), but the relative frequencies of serotypes 3 and 18C increased, whereas that of serotype 14 decreased (from 18.4% (95%CI 7.5 – 29.2%) to 8.4% (95%CI 4.7 – 11.3%) among bacteraemia isolates).

Based on the UAD results eleven CAP episodes (4.4% of pneumococcal CAP cases diagnosed by UAD, 1.0% of all samples tested) appeared to be caused by multiple serotypes, which was confirmed by retesting and inhibition experiments (data not shown[14]). Ten episodes were caused by two serotypes included in the UAD test and one episode by three. Two of these patients also had concomitant bacteraemia and in both cases serotype of bacteraemia isolates matched with one of the serotypes detected by the UAD assay.

DISCUSSION

Addition of a novel serotype-specific UAD test to commonly used standard diagnostic methods, such as microbiological cultures and ICA of urine for pneumococcal antigens, increased the diagnostic yield for *S. pneumoniae* CAP by 39%. The sensitivity of the UAD test was 98% for pneumococcal CAP episodes caused by one of the serotypes included in the UAD test, as compared to 69% for ICA. Specificity for detecting 13 serotype specific pneumococcal antigens in urine of adult patients with CAP was 100%, and similar to the ICA test. Moreover, four percent of pneumococcal CAP episodes diagnosed by the UAD test resulted from co-infection with multiple serotypes. Although not commercially available, this approach could enhance our diagnostic yield for pneumococcal infections and could provide more detailed information on the epidemiology of infection-associated pneumococcal serotypes.

Detection of pneumococcal antigen in urine was first described in 1917 [18], and became widely applied as of 1999 with the commercial availability of the ICA of BinaxNOW. Serotype-specific antigen detection in urine has been attempted before, with excellent specificity (98%-99%), but disappointing sensitivity (55%-84%), when compared to *S. pneumoniae* isolates detected in sterile sites [19-22]. The sensitivity of the ICA test in the present study was 63%.

With the addition of the UAD test to the conventional diagnostic methods the proportion of CAP episodes caused by *S. pneumoniae* increased from 23.3% to 32.6%. However, the UAD test only detects 13 serotypes and there were 71 pneumococcal CAP episodes diagnosed through a positive ICA result only. Extrapolation of the observed 63% sensitivity of the ICA test for bacteraemic CAP would imply that there were 113 instead of the observed 71 additional episodes of pneumococcal CAP caused by serotypes not covered by the UAD test. That would imply that the total proportion of CAP episodes caused by pneumococci would be 36.4% (399/1095). The UAD test as used in this study “serotyped” three times more episodes

of pneumococcal CAP (249 as compared to 77 for isolates from sterile sites and sputum), and, therefore, enhances our capacity to investigate the serotype-epidemiology of *S. pneumoniae*. Moreover, if the turn-around-time of this test could be reduced to that of a real-time test, demonstrating *S. pneumoniae* as a cause of CAP could assist in rapid de-escalation of unnecessarily broad empirical antibiotic treatment for CAP. As many serotypes will not be detected, a negative test result will not be useful for clinical decision making.

Of the pneumococcal CAP cases detected by the UAD test, 4.4% appeared to be caused by multiple serotypes. Although the presence of carriage with multiple serotypes has been described[23-25], infections caused by multiple serotypes have been reported only sporadically[20, 21, 26, 27]. Recently five of 366 patients with pneumococcal CAP had pneumonia caused by two serotypes based on a multiplex immunoassay for 14 serotypes in urine samples[28]. Thus, apart from higher sensitivity, the UAD test also improves our capacity to detect co-infections with multiple serotypes.

Our study has some limitations. As in all clinical studies investigating diagnosis of non-invasive *S. pneumoniae* infections we suffered from the absence of a true 'gold standard'. This results in low numbers of 'true positives' (n=49) and 'true negatives' (n=76). However, at this point there is no other reference standard to better test accuracy of serotype specific diagnostic tools. Secondly, pneumococcal antigens can persist in urine samples after infection for periods as long as 7 days[19, 29] to 3 months[30]. Although we did not have detailed information about disease history in the weeks before the clinical episode of CAP, we could determine that only 8 patients (0.7%) had been hospitalized in the weeks before the CAP episode. Moreover, persistent antigen positivity is more likely when concentrated urine is used[11, 30]. In our study only unconcentrated urine was used, reducing the possibility of false positive tests. Therefore, we consider it unlikely that false-positive test results due to previous pneumococcal infections influenced our findings. And finally, the absence of a urine sample in 101 patients could have created a selection bias. Indeed, these 101 patients were

more frequently categorized as ‘severe CAP’ (PSI class V) (p=0,012, data not shown) and previous studies have suggested that the ICA is more sensitive in severely ill patients[21, 31], although this finding was not confirmed by others[10, 32]. If severity of CAP is associated with higher likelihood of antigen detection in urine the diagnostic yield of the UAD test might actually be higher than reported here.

Acknowledgements:

The authors would like to acknowledge the substantial contribution to this study made by Dr. Marcel Peeters (St. Elisabeth Hospital, Tilburg, The Netherlands), who sadly passed away in June 2011.

Furthermore we would like to thank all CAP-diagnostic investigators for their time and effort.

Word count: 2734

Reference list

1. Woodhead MA, Macfarlane JT, McCracken JS, Rose DH, Finch RG. Prospective study of the aetiology and outcome of pneumonia in the community. *Lancet*. 1987 Mar 21;1(8534):671-4.

2. Jokinen C, Heiskanen L, Juvonen H, Kallinen S, Kleemola M, Koskela M, Leinonen M, Ronnberg PR, Saikku P, Sten M, Tarkiainen A, Tukiainen H, Pyorala K, Makela PH. Microbial etiology of community-acquired pneumonia in the adult population of 4 municipalities in eastern Finland. *Clin Infect Dis*. 2001 Apr 15;32(8):1141-54.

3. Hausdorff WP, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. *Clin Infect Dis*. 2000 Jan;30(1):100-21.

4. Harboe ZB, Thomsen RW, Riis A, Valentiner-Branth P, Christensen JJ, Lambertsen L, Krogfelt KA, Konradsen HB, Benfield TL. Pneumococcal serotypes and mortality following invasive pneumococcal disease: a population-based cohort study. *PLoS Med*. 2009 May 26;6(5):e1000081.

5. Campbell SG, Marrie TJ, Anstey R, Dickinson G, Ackroyd-Stolarz S. The contribution of blood cultures to the clinical management of adult patients admitted to the hospital with community-acquired pneumonia: a prospective observational study. *Chest*. 2003 Apr;123(4):1142-50.

6. van de Garde EM, Endeman H, van Hemert RN, Voorn GP, Deneer VH, Leufkens HG, van den Bosch JM, Biesma DH. Prior outpatient antibiotic use as predictor for microbial aetiology of community-acquired pneumonia: hospital-based study. *Eur J Clin Pharmacol*. 2008 Apr;64(4):405-10.

7. Metersky ML, Ma A, Bratzler DW, Houck PM. Predicting bacteremia in patients with community-acquired pneumonia. *Am J Respir Crit Care Med*. 2004 Feb 1;169(3):342-7.

8. Roson B, Carratala J, Verdaguer R, Dorca J, Manresa F, Gudiol F. Prospective study of the usefulness of sputum Gram stain in the initial approach to community-acquired pneumonia requiring hospitalization. *Clin Infect Dis*. 2000 Oct;31(4):869-74.

9. Ewig S, Schlochtermeier M, Goke N, Niederman MS. Applying sputum as a diagnostic tool in pneumonia: limited yield, minimal impact on treatment decisions. *Chest*. 2002 May;121(5):1486-92.

10. Gutierrez F, Masia M, Rodriguez JC, Ayelo A, Soldan B, Cebrian L, Mirete C, Royo G, Hidalgo AM. Evaluation of the immunochromatographic Binax NOW assay for detection of Streptococcus pneumoniae urinary antigen in a prospective study of community-acquired pneumonia in Spain. *Clin Infect Dis*. 2003 Feb 1;36(3):286-92.

11. Marcos MA, Jimenez de Anta MT, de la Bellacasa JP, Gonzalez J, Martinez E, Garcia E, Mensa J, de Roux A, Torres A. Rapid urinary antigen test for diagnosis of pneumococcal community-acquired pneumonia in adults. *European Respiratory Journal*. 2003;21(2):209.

12. Dominguez J, Gali N, Blanco S, Pedroso P, Prat C, Matas L, Ausina V. Detection of Streptococcus pneumoniae antigen by a rapid immunochromatographic assay in urine samples. *Chest*. 2001;119(1):243.

13. Hak E, Grobbee DE, Sanders EA, Verheij TJ, Bolkenbaas M, Huijts SM, Gruber WC, Tansey S, McDonough A, Thoma B, Patterson S, van Alphen AJ, Bonten MJ. Rationale and design of CAPITA: a RCT of 13-valent conjugated pneumococcal vaccine efficacy among older adults. *Neth J Med*. 2008 Oct;66(9):378-83.

14. Pride MW, Huijts SM, Wu K, Souza V, Passador S, Tinder C, Song E, Elfassy A, McNeil L, Menton R, French R, Callahan J, Webber C, Gruber WC, Bonten MJ, Jansen KU. Validation of an Immunodiagnostic Assay for Detection of 13 Streptococcus pneumoniae Serotype-Specific Polysaccharides in Human Urine. *Clin Vaccine Immunol*. 2012 Aug;19(8):1131-41.

15. Austrian R. The quellung reaction, a neglected microbiologic technique. *Mt Sinai J Med*. 1976 Nov-Dec;43(6):699-709.

16. Lund E HJ. Laboratory diagnosis, serology and epidemiology of Streptococcus pneumoniae. *Methods in microbiology*. 1978;12:244-55.

17. Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE, Coley CM, Marrie TJ, Kapoor WN. A prediction rule to identify low-risk patients with community-acquired pneumonia. *New England Journal of Medicine*. 1997;336(4):243.

18. Dochez AR, Avery OT. The Elaboration Of Specific Soluble Substance By Pneumococcus During Growth. *J Exp Med*. 1917 Oct 1;26(4):477-93.

19. Leeming JP, Cartwright K, Morris R, Martin SA, Smith MD. Diagnosis of invasive pneumococcal infection by serotype-specific urinary antigen detection. *J Clin Microbiol*. 2005 Oct;43(10):4972-6.

20. 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 Apr;28(4):764-9.

21. 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 Aug;42(8):3620-5.

22. Sheppard CL, Harrison TG, Smith MD, George RC. Development of a sensitive, multiplexed immunoassay using xMAP beads for detection of serotype-specific streptococcus pneumoniae antigen in urine samples. *J Med Microbiol*. 2011 Jan;60(Pt 1):49-55.

23. Carvalho MDG, Pimenta FC, Jackson D, Roundtree A, Ahmad Y, Millar EV, O'Brien KL, Whitney CG, Cohen AL, Beall BW. Revisiting pneumococcal carriage by use of broth enrichment and PCR techniques for enhanced detection of carriage and serotypes. *Journal of Clinical Microbiology*. 2010;48(5):1611.

24. Huebner RE, Dagan R, Porath N, Wasas AD, Klugman KP. Lack of utility of serotyping multiple colonies for detection of simultaneous nasopharyngeal carriage of different pneumococcal serotypes. *Pediatr Infect Dis J*. 2000 Oct;19(10):1017-20.

25. Kaltoft MS, Skov Sorensen UB, Slotved HC, Konradsen HB. An easy method for detection of nasopharyngeal carriage of multiple Streptococcus pneumoniae serotypes. *J Microbiol Methods*. 2008 Dec;75(3):540-4.

26. Brandileone MCDC, Vieira VSD, Casagrande ST, Zanella RC, Guerra MLLS, Bokermann S, De Moraes JC, Baldacci ER, Chamone CB, Oliveira MAA, De Matos DGC, Arruda TMC, Da Costa Coelho MF, D'Avila SM, Dos Santos AR, Di Fabio JL. Prevalence of serotypes and antimicrobial resistance of Streptococcus pneurnoniae strains isolated from Brazilian children with invasive infections. *Microbial Drug Resistance*. 1997;3(2):141.

27. Cockrell. Multiple Pneumococcus Infections in Pneumonia. *The Journal of Infectious Diseases*. 1941;68(1):33-6.

28. Bewick T, Sheppard C, Greenwood S, Slack M, Trotter C, George R, Lim WS. Serotype prevalence in adults hospitalised with pneumococcal non-invasive community-acquired pneumonia. *Thorax*. 2012 Feb 28.

29. Smith MD, Derrington P, Evans R, Creek M, Morris R, Dance DA, Cartwright K. 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 Jul;41(7):2810-3.

30. Andreo F, Prat C, Ruiz-Manzano J, Lores L, Blanco S, Cuesta MA, Gimenez M, Dominguez J. Persistence of Streptococcus pneumoniae urinary antigen excretion after pneumococcal pneumonia. *Eur J Clin Microbiol Infect Dis*. 2009 Feb;28(2):197-201.

31. Hohenthal U, Vainionpaa R, Meurman O, Vahtera A, Katiskalahti T, Nikoskelainen J, Kotilainen P. Aetiological diagnosis of community acquired pneumonia: utility of rapid

microbiological methods with respect to disease severity. *Scand J Infect Dis*. 2008;40(2):131-8.

32. van der Eerden MM, Vlaspolder F, de Graaff CS, Groot T, Jansen HM, Boersma WG. Value of intensive diagnostic microbiological investigation in low- and high-risk patients with community-acquired pneumonia. *Eur J Clin Microbiol Infect Dis*. 2005 Apr;24(4):241-9.

Tables

Table 1 – *Aetiology of 1,095 patients with confirmed CAP*.

	Conventional diagnostics*			**Conventional diagnostics + UAD****		
Pathogen	**Definite ^ pathogen**	**Probable ^^ pathogen**	**Total (n=1,095)**	**Definite^ pathogen**	**Probable ^^ pathogen**	**Total (n=1,095)**
S. pneumoniae	240	17	257 (23.5%)	347	10	357 (32.6%)
H. influenzae	1	37	38 (3.5%)	1	34	35 (3.2%)
L. pneumophila	25	-	25 (2.3%)	25	-	25 (2.3%)
P. aeruginosa	4	11	15 (1.4%)	4	7	11 (1.0%)
M. catarrhalis	-	8	8 (0.7%)	-	7	7 (0.6%)
Klebsiella spp.	3	9	12 (1.1%)	3	8	11 (1.0%)
E. coli	5	6	11 (1.0%)	5	6	11 (1.0%)
S. aureus	3	4	7 (0.6%)	3	4	7 (0.6%)
Other	11	19	30 (2.7%)	11	18	29 (2.6%)
Unknown			692 (63.2%)			602 (55.0%)

**Conventional diagnostics*: Causative pathogen based on the results of blood or other sterile fluid culture, ICA urinary antigen test only and sputum culture. ** *Conventional diagnostics + UAD:* Causative pathogen based on results of blood or other sterile fluid culture, ICA urinary antigen test and UAD test results and sputum culture. ^ Definite pathogen: pathogen cultured from blood or any other sterile body fluid or if the urinary antigen test was positive. ^^ Probable pathogen: pathogen identified in the absence of a definite pathogen and if it was present as dominant flora in the sputum culture.

Table 2: *Results of UAD assay and ICA in patients with bacteraemic and non-bacteraemic pneumococcal CAP*.

			UAD Negative	Positive	Total
BC negative for *S. pneumoniae*	**ICA**	Negative	749	107	856
		Positive	71	91	162
	Total		820	198	1018
BC positive for *S. pneumoniae*, UAD serotype	**ICA**	Negative	1	14	15
		Positive	0	34	34
	Total		1	48	49
BC positive for *S. pneumoniae*, non-UAD serotype	**ICA**	Negative	12	0	12
		Positive	11	0	11
	Total		23	0	23
BC positive for *S. pneumoniae*, non-typeable serotype	**ICA**	Negative	0	1	1
		Positive	2	2	4
	Total		2	3	5
Total	**ICA**	Negative	762	122	884
		Positive	84	127	211
	Total		846	249	1095

Abbreviations: *S. pneumoniae*= Streptococcus pneumoniae; BC= blood culture; UAD= urinary antigen detection assay; ICA= immunogromatographic assay.

Table 3 – *Serotype distribution based on UAD results*

Serotype	**Frequencies, only single serotypes**	**Frequencies, incl. multiple serotypes**
1	38	40
3	35	38
7F	32	35
19A	24	27
14	22	22
23F	21	25
18C	18	21
9V	16	18
4	14	14
6A	10	12
5	3	4
19F	3	3
6B	2	2
Total	238	261

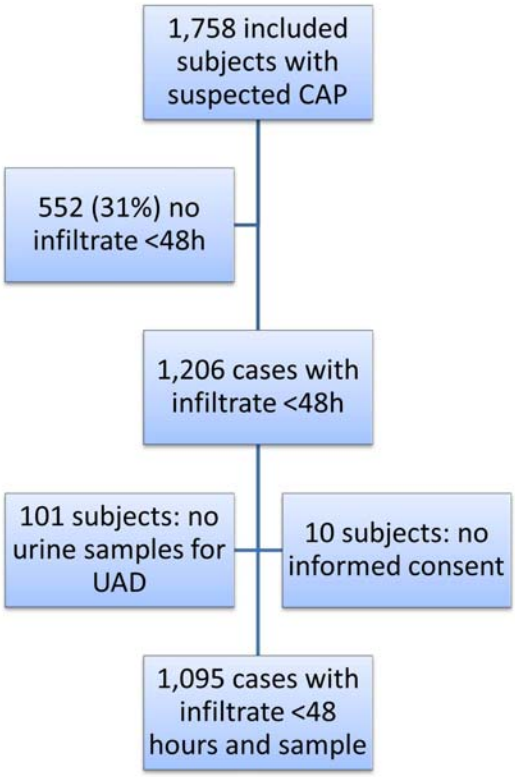

Figure 1 – *Flowchart study population suspected with CAP.*

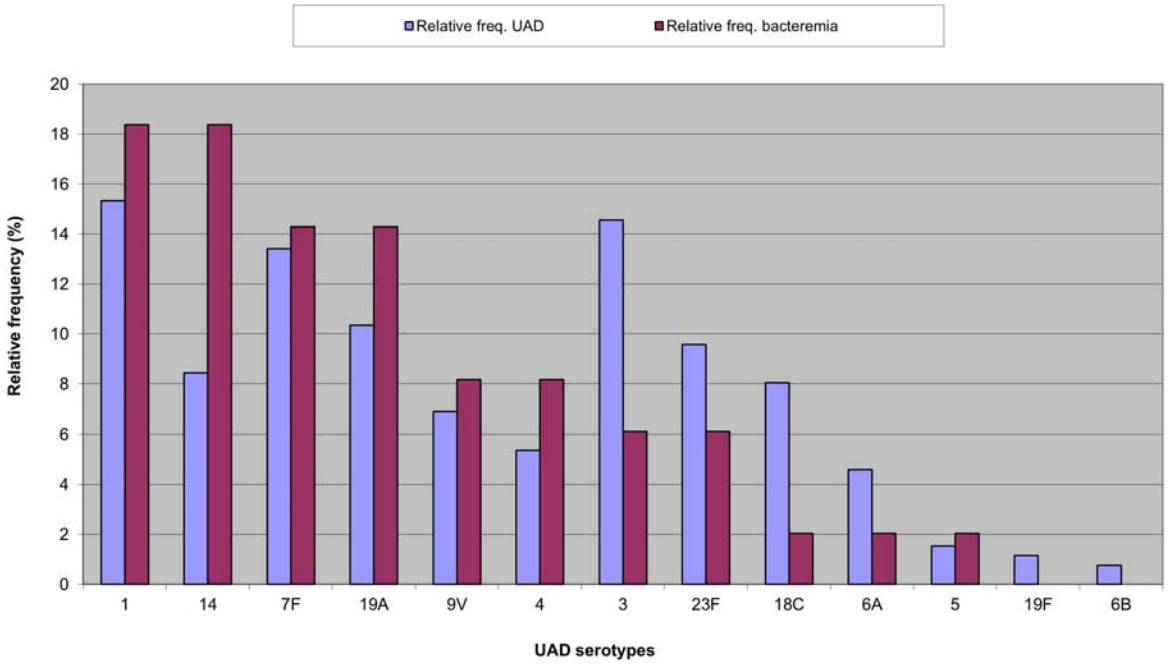

Figure 2 – *Relative serotype distribution based on UAD data (blue bars, n=261, including multiple serotypes) and on bacteraemia data (purple bars, n=49). For absolute numbers of UAD data see table 2.*