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Rapid detection of pneumococcal antigen in serum samples for diagnosing pneumococcal pneumonia

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Summary *Objectives*: The aim of the study is to assess the usefulness of C polysaccharide and polysaccharide capsular antigen detection by immunochromatography (ICT) and enzyme immunoassay (EIA), respectively, in serum samples for diagnosing pneumococcal pneumonia.
Methods: Adult patients included in the study were classified in the following groups: In group 1 we studied 101 serum samples from patients with pneumonia due to *Streptococcus pneumoniae*. In 53 cases the pneumonia was bacteremic. The second group contained 113 serum samples from patients with no pneumococcal pneumonia. Group 3 was made up of 40 serum samples from healthy subjects with no clinical or radiological signs of pneumonia.
Results: Using ICT, antigen was detected in 50% of patients with pneumococcal pneumonia, in 64.3 and 40.9% of patients with bacteremic and non-bacteremic pneumococcal pneumonia, respectively. Using EIA, antigens were detected in 35.8% of patients with pneumococcal pneumonia, in 45 and 22.2% of patients with bacteremic and non-bacteremic pneumococcal pneumonia, respectively.
Conclusions: In conclusion, the sensitivity of the tests is low. However, in special situations, where obtaining large volume of urine is difficult, they could be a complementary method in the rapid diagnosis of pneumococcal pneumonia.

Introduction

A definitive diagnosis of pneumococcal pneumonia is difficult.[1] Immunological tests for detecting pneumococcal antigens provide a rapid diagnosis, and are

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also particularly useful for patients who have been treated with antibiotics. Several studies have shown the potential usefulness of detecting antigens by latex agglutination,[2] counterimmunoelectrophoresis (CIE)[3,4] and enzyme immunoassay (EIA).[5,6] An immunochromatographic test (ICT) (Binax Now *Streptococcus pneumoniae* Antigen Test, Portland, Maine, U.S.A.) has been developed to detect C polysaccharide (PnC) antigen in urine samples. The test has proven to be rapid, sensitive and specific in pneumococcal pneumonia in adults.[7] There are several situations in which detecting urinary antigen is difficult. Obtaining large volumes of urine is not possible for some patients, especially for those who have oliguria secondary to sepsis and acute or chronic kidney failure, as well as, patients with altered mental status. Therefore, antigen detection in samples other than urine might be useful for obtaining a rapid and accurate diagnosis. The aim of the study is to assess the usefulness of PnC and PCA detection by ICT and EIA, respectively, in serum samples for diagnosing pneumococcal pneumonia.

Patients and methods

Bacterial strains

In order to establish the reactivity of pneumococcal organisms and specificity of the assays, we used the following clinical isolates: *S. pneumoniae* serotypes 1 (2 strains), 3, 5, 6B, 7, 8, 9N, 9V, 10, 11, 12, 13, 14, 18C, 19, 20 and 23F, *Streptococcus pyogenes Streptococcus constellatus*, *Streptococcus mitis*, *Streptococcus bovis* II, *Streptococcus salivarius*, three strains of *Streptococcus* viridans group, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Haemophilus influenzae* biotype I (2 strains) and IV, *Moraxella catarrhalis*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, *Escherichia coli* and *Bacteroides fragilis*.

Bacterial strains were harvested from solid cultures with a cotton swab, and suspensions containing 10^6 organisms per ml were prepared in physiological saline. Suspensions were done in PBS, boiled for 5 min and centrifuged at 3000 g for 15 min before being tested for antigen detection by EIA and ICT.

Groups of patients

Adult patients included in the study were classified in the following groups: In group 1 we studied 101 serum samples from patients with pneumonia due to *S. pneumoniae*. In 53 cases the pneumonia was bacteremic, where *S. pneumoniae* was isolated by the blood culture BactAlert system (BioMérieux SA, Marcy-L'Etoile, France) and identification and serotyping was based on the usual criteria.[8] In the remaining 48 cases pneumonia was non-bacteremic, and diagnosis was based on PCA detection in urine using CIE[3] or PnC detection using ICT. The second group contained 113 serum samples from patients with no pneumococcal pneumonia (28 *Mycoplasma pneumoniae*, 46 *Legionella pneumophila*, 14 *Chlamydophila pneumoniae*, four *Coxiella burnetii*, 14 *H. influenzae*, four *P. aeruginosa*, one *M. catarrhalis*). Group 3 was made up of 40 serum samples from healthy subjects with no clinical or radiological signs of pneumonia.

All serum specimens were collected and frozen at −20 °C until use and thawed immediately before being tested. EIA and ICT assays were performed in different times so it was not always possible to perform the antigen detection by the two techniques in all the samples. Serum samples were diluted 3:1 in EDTA 4% (w/v) solution. In order to minimize possible non-specific reactions and for circulating immune complexes dissociations, all serum samples were boiled for 5 min and centrifuged at 3000 g for 15 min.[9]

EIA method

As specific antibodies, we used the purified immunoglobulin G (IgG) fraction from pools P (types 1 and 12; group 7 and 19), Q (types 6, 8 and 23), R (types 3 and 4; groups 9 and 12), S (types 5 and 8, group 10, 15 and 17), and T (types 2 and 20, groups 11, 22 and 33) (Statens Serum Institut, Copenhagen, Denmark), which contain specific antibodies against the 23 most prevalent pneumococcal polysaccharide types (*23-pool*) in sepsis and bacteremia cases.[10] The IgG fraction was obtained by passing the *23-pool* through an exchange protein G chromatography column (ImmunoPure® (G) IgG Purification kit. Pierce Chemical Company. U.S.A.). Byotinylated antibodies were prepared, from a fraction of purified *23-pool*, according to the previous described methods.[11]

All parameters were determined by checkerboarding. Microtiter plates (Maxisorp. Nunc. Roskilde, Denmark) were coated with 100 μl of diluted *23-pool* fraction (50 μg/ml) in 0.05 mM carbonate buffer (pH 9.6). The plates were incubated overnight at 4 °C in a moist chamber and washed four times with PBS containing 0.05% Tween-20 and blocked with 200 μl of 0.5% BSA.