Available online at www.sciencedirect.com
ScienceDirect
Pathologie Biologie 54 (2006) 498–501
PATHOLOGIE BIOLOGIE
http://france.elsevier.com/direct/PATBIO/

Streptococcus pneumoniae thoracic empyema in children: rapid diagnosis by using the Binax NOW immunochromatographic membrane test in pleural fluids

Pleuropneumopathie à *Streptococcus pneumoniae* chez l'enfant : diagnostic rapide avec le test immunochromatographique Binax NOW sur les liquides pleuraux

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Available online 05 October 2006

Abstract

Aim. – To evaluate an immunochromatographic membrane test for *Streptococcus pneumoniae* antigen (Binax NOW, Inverness medical France) applied to pleural fluid samples.

Methods. – Binax NOW was applied to the pleural fluids of 69 children with thoracic empyema, in comparison with conventional culture and molecular techniques.

Results. – Binax NOW was positive on all 15 pleural fluid samples that yielded *S. pneumoniae* in culture, on two samples that yielded *S. oralis* and *S. salivarius* in culture and on 34 culture-negative samples. Fifteen of these 34 culture-negative samples were retrospectively tested by PCR methods, and 14 were shown to contain *S. pneumoniae* DNA. Thus, *S. pneumoniae* was identified by culture in 22% of samples and by Binax NOW in 69% of samples.

Conclusion. – Binax NOW may thus be useful for rapid diagnosis of *S. pneumoniae* thoracic empyema.

Résumé

But de l'étude. – Évaluer, sur les liquides pleuraux, le test immunochromatogaphique Binax NOW (Inverness Medical France) pour la recherche d'antigène de *Streptococcus pneumoniae*.

Méthodes. – Binax NOW a été testé sur les liquides pleuraux de 69 enfants hospitalisés pour pleuropneumopathie. Les résultats ont été comparés aux résultats de la culture conventionnelle et des techniques moléculaires.

Résultats. – Le test Binax NOW s'est avéré positif pour les 15 liquides pleuraux ayant donné une culture positive à *S. pneumoniae*, deux liquides pleuraux ayant une culture positive à *S. oralis* et *S. salivarius*, et 34 liquides pleuraux ayant une culture négative. Des techniques de PCR (universelle et/ou spécifique *S. pneumoniae*) effectuées sur 15 de ces 34 liquides pleuraux montrent que 14 contenaient de l'ADN de *S. pneumoniae*. Donc S. *pneumoniae* a été diagnostiqué par culture dans 22 % des prélèvements et par le test Binax NOW dans 69 % des prélèvements.

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doi:10.1016/j.patbio.2006.07.031

Conclusion. – Binax NOW, testé sur les liquides pleuraux, est un outil intéressant pour le diagnostic rapide des pleuropneumopathies à *S. pneumoniae*.

Keywords: Thoracic empyema; Children; Immunochromatographic membrane Binax NOW test; Pleural fluid; *Streptococcus pneumoniae*; Rapid diagnosis

Mots clés : Pleuropneumopathie ; Enfant ; Test immunochromatographique Binax NOW ; Liquide pleural ; *Streptococcus pneumoniae* ; Diagnostic rapide

1. Introduction

The frequency of thoracic empyema and parapneumonic effusion in children with pneumonia has increased markedly over the last decade, for unknown reasons [1–4]. Routine bacterial culture is negative in many cases, often because antibiotics have been administered before sampling. Another problem is that *Streptococcus pneumoniae*, the main causative pathogen in childhood empyema, produces autolysin, a cell-wall enzyme that causes its own lysis.

A recent rapid immunochromatographic membrane assay (Binax NOW *Streptococcus pneumoniae* test, from Inverness Medical France) detects the C polysaccharide cell wall antigen common to all pneumococcal serotypes. The results are available in 15 min. The test has proven useful for rapid diagnosis of *S. pneumoniae* pneumonia, based on urine samples [5–8] and of *S. pneumoniae* meningitis, based on cerebrospinal fluid samples [9,10].

The objective of this prospective study was to evaluate the Binax NOW test on pleural fluid, by comparison with conventional culture and molecular techniques, in children hospitalized for thoracic empyema.

2. Methods

2.1. Patients

This prospective study focused on pleural fluid samples collected from 69 consecutive children admitted to Debrousse Pediatric Hospital (Lyon, France) for thoracic empyema between January 2003 and April 2005. Median age was 4.1 years (0.7 months to 16.5 years), and 41 of the children were boys. All the children had clinical and radiological evidence of thoracic empyema requiring a pleural fluid puncture.

2.2. Microbiological and molecular methods

2.2.1. Binax NOW rapid immunochromatographic *Streptococcus pneumoniae* antigen test

The Binax NOW test was applied to pleural fluid samples in the same way as recommended by the manufacturer for urine and CSF samples. Binax NOW uses a rabbit anti-*S. pneumoniae* antibody conjugated to visualizing particles to bind soluble pneumococcal antigen (C polysaccharide) present in the sample. The resulting complexes are immobilized by a band of rabbit anti-*S. pneumoniae* antibodies adsorbed to a nitrocellulose membrane (sample line). A second band of goat anti-rabbit immunoglobulin G (control line) captures excess visualizing complexes. A swab is dipped into the sample and inserted into the test device; a buffer solution is added and the device is closed. The result is read by visual inspection after 15 minutes. A pink to purple color on both the sample and control lines indicates a positive antigen test. Color on the control line alone indicates a negative test. Absence of color on the control line indicates an invalid test. Each set of tests includes positive and negative control swabs.

2.2.2. Conventional culture

Pleural fluid samples were also inoculated on horse blood agar incubated in aerobic conditions, chocolate agar incubated in CO_2-enriched air, Columbia agar incubated in an anaerobic atmosphere at 36 °C for 48 h, and Schaedler broth incubated for 10 days. All isolates were identified by Gram staining and colony morphology, hemolysis, oxidase and catalase enzymatic tests, optochin susceptibility, and biochemical profiles (Rapid ID STREP or ID 32 STAPH, (bioMérieux), depending on presumed identity). The Lancefield group of beta-hemolytic streptococci was determined by latex agglutination with a commercial grouping kit (SERVITEX Streptocoque; Servibio). Antibiotic susceptibility was determined with a dilution micromethod using the ATB expression system (bioMérieux).

2.2.3. Molecular techniques

When sufficient pleural fluid was available, DNA extraction, 16S rDNA amplification and sequencing were performed as described elsewhere [11,12]. In addition, 16S rDNA PCR-negative and Binax NOW-positive samples were submitted to real-time *S. pneumoniae* PCR amplification (LightCycler system, Roche Diagnostics, France) of the autolysin gene (*lytA*) with the specific primers pno1 (5′ GCATTCAACCGTACA GAATGAAGCG 3′) and pno2 (5′ CCTGCTTCATCTGCTA GAATTGCG 3′) [4].

3. Results

Between January 2003 and April 2005, a total of 69 pleural fluid samples from children admitted to our institution for thoracic empyema or pleural effusion necessitating thoracocentesis and/or decortication were tested with the Binax NOW test by comparison with conventional culture.

Conventional culture was positive on 20 (29%) of the 69 pleural fluid samples. *S. pneumoniae* was isolated from 15 samples (22%) Table 1. The other isolates were beta-hemolytic group A streptococci ($N = 3$), *Streptococcus oralis* ($N = 1$) and *S. salivarius* ($N = 1$). Among the 15 *S. pneumoniae*, 13 (87%) were penicillin-sensitive and 10 (66%) were of capsular serotype 1.