Identifying the pneumococcal antigen in the cephalorachidian fluid using the immunochromatographic method

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Abstract. We determined the accuracy of the NOW S. pneumoniae Urinary Antigen Test (Binax, Portland, ME, USA) when it comes to detecting the pneumococcal antigen in the cephalorachidian fluid. 30 patients under suspicion of acute meningitis were tested for the presence of pneumococcal antigen in the cephalorachidian fluid using both the latex and the NOW S. pneumoniae Urinary Antigen test. We also performed direct microscopic examination and insemination on solid and fluid culture media of the cephalorachidian fluid. Samples of cephalorachidian fluid taken from 5 patients (smear and in 2 cases in cultures) tested positive for pneumococcus. Both the NOW and the latex test were positive in all 5 cases (with a 100% sensitivity). In the rest of the cases the NOW test was negative (with a 100% specificity). By contrast, the latex test was positive in 2 cases of meningitis of other aetiology (with a 92% specificity). The NOW test allows a rapid and correct diagnosis for pneumococcal meningitis, determining the choice of appropriate treatment.

Key words: meningitis, S. pneumoniae, immunochromatographic method.

Introduction

Streptococcus pneumoniae represents the main aetiological agent when it comes to acute bacterial meningitis in adults. Due to the seriousness of the disease, expressed both by high mortality (20%) and by a high percentage of sequelae (30%) in survivors, the aetiological diagnosis must be correctly and rapidly established. Gram staining allows the identification of the pathogenic agent, with a sensitivity of 75%, lower in the case of patients that have been previously under treatment with antibiotics (50%). The test tube can identify the pathogenic agent with a sensitivity of 70-85% in patients who have not been under previous treatment with antibiotics. Once the antibiotic treatment begins, the cephalorachidian fluid destroys bacteria in 4 hours in the case of the pneumococcus (Koedel et al. 2002, Jit 2010, Edmond et al. 2010). The PCR technique presents the advantage of having a sensitivity and specificity close to 100%. The aim of this study was to assess the accuracy of this particular test, which was initially conceived to detect the pneumococcal urinary antigen, when it comes to identifying the agent in the cephalorachidian fluid (Samra et al. 2003).

Materials and methods

The study was carried out at the Clinic of Infectious Diseases from December 2004 to August 2006. The study included 30 patients under initial suspicion of acute meningitis (21 women and 9 men) aged 18 to 68. Out of these, 19 came from urban area and 11 from rural area. Samples of cephalorachidian fluid were taken from all patients. The samples were tested for polyglycides C in the structure of the cell wall of the S. pneumoniae employing the immunochromatographic method, using the NOW S. pneumoniae Urinary Antigen Test (Binax, Portland, ME, USA). The test consists in a book-shaped device containing a nitrocellulose membrane on which the anti- S. pneumoniae antibodies sampled from rabbit are adsorbed (the line indicating a positive test). A swab is dipped into the cephalorachidian sample and is then inserted into the device. A few drops of a solution are then dropped onto the swab after which the device is closed. The role of the solution is to facilitate contact between the antigen from the cephalorachidian fluid and
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When comparing the results obtained using both methods, it can be noticed a significant statistical difference (p=0.00015).

Acute bacterial meningitis is a disease with high morbidity and mortality. This is the reason why it is necessary to establish its aetiological diagnosis as accurately and rapidly as possible. As in most of the cases the patients are treated with antibiotics before they are admitted to hospital, the chances to identify the pathogenic agent involved based on the medium (cephalorachidian fluid or blood) are dramatically low. At the same time, identifying the bacteria using Gram stain of the smear also presents a relatively low sensitivity.

Under these circumstances, new bacterial antigen detection tests using cephalorachidian fluid are more than welcome. The NOW test is actually an immunochromatographic method used to identify polyglucides C in the structure of the pneumococcal cellular wall present in all types of *S. pneumoniae* involved in the pathology (Samra et al. 2003). Unlike this one, other antigen-identification methods such as latex-agglutination and counter-immune-electrophoresis reveal more pneumococcal antigens, but which are not present in all types of *S. pneumoniae* involved in the pathology, only in the most frequently met ones.

The NOW test can be used in several situations. A study (Faden et al. 2002) carried out on 138 children suffering or not from acute otitis media, demonstrated the presence of the pneumococcus at the nasopharyngeal level in 37% of the cases, the test having a 92.2% sensitivity and a 97.7% specificity. Other studies (Dominguez et al. 2001 cited in by Samra et al. 2003) demonstrated the usefulness of the test in detecting the pneumococcal antigen in the urine of patients suffering from pneumococcal pneumonia. The sensitivity of the test was of 78-82%, while its specificity was 97% (Samra et al. 2003, Faden et al. 2002).

Other studies which demonstrated the presence of the urinary pneumococcal agent in pa-

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Cephalorachidian fluid: Smear</th>
<th>Cephalorachidian fluid: culture medium</th>
<th>NOW Test</th>
<th>Latex Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>66</td>
<td>Encapsulated Gram-positive diplococci</td>
<td>Negative pneumococcus</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>66</td>
<td>Lanceolated diplococci</td>
<td>Positive pneumococcus</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>56</td>
<td>Encapsulated diplococci. Coccii in clusters</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>67</td>
<td>Rare-encapsulated diplococci in short chains</td>
<td>Negative pneumococcus</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>66</td>
<td>Encapsulated Gram-positive diplococci</td>
<td>Negative pneumococcus</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Patients suffering from pneumococcal pneumonia had a much more varied sensitivity (ranging between 0-80%, normally below 50%) (Murdoch et al. 2001, Stralin et al. 2004, Matta et al. 2010). By contrast, other studies (Powell et al. 2001 cited in Samra et al. 2003) outlined the method’s lack of utility when it comes to differentiating between patients suffering from pneumococcal pneumonia and those from the control group, as well as when it comes to differentiating between patients suffering from pneumococcal pneumonia and those infected with *S. pneumoniae* (Samra et al. 2003).

In pneumococcal meningitis the NOW test proved to be extremely useful. Its sensitivity in a study (Samra et al. 2003) carried out on a group of 514 patients (out of which 22 suffered from pneumococcal meningitis) was of 95.4% and its specificity of 100% when it came to identifying antigens in cerebrospinal fluid. Nevertheless, its sensitivity was low (57.1%) in determining urinary antigen, which leads to the conclusion that this is not a useful diagnosis method (Samra et al. 2003). In another study conducted on patients suffering from meningitis the NOW test performed on cerebrospinal fluid showed a 100% sensitivity and specificity (Marcos et al. 2001).

In the study carried out on the 30 patients admitted to the Clinic of Infectious Diseases of Cluj-Napoca the NOW test showed a 100% sensitivity and specificity. When compared with the latex-agglutination test, a significant statistical difference can be noticed between the two methods. Nevertheless, it must be mentioned that the results would have been much more conclusive if the study group had included more patients.

Conclusions

The NOW test presents a few definite advantages: it is fast, easy to carry out, relatively cheap, it requires only a small amount of cephalarachidian fluid, and it has a very high sensitivity and specificity.

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References


