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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%, and the disadvantage of being an expensive and less rapid method.

The latex-agglutination allows for the identification of the pathogenic agent especially in patients under previous treatment with antibiotics, the sensitivity of the method being of 70% (Koedel et al. 2002, Park et al. 2010). The immunochromatographic method allows a rapid identification of bacterial antigens in the cephalorachidian fluid. There are also tests such as NOW *S. pneumoniae* Urinary Antigen Test (Binax, Portland, ME, USA) with 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 polyglucides C in the structure of the cellular 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). On the same membrane there is also IgG type immunoglobulin directed against the antibodies sampled from rabbit (indicating the control line). 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

the antibodies at the level of the nitrocellulose membrane. The antigen present in the sample binds to the anti-*S.pneumoniae* antibodies forming the line that indicates a positive test. The IgG type antibodies interact with the excess of antibodies sampled from rabbit, forming the control line. The result of the test can be seen 15 minutes later. The test is considered positive if both purple lines appear: both the control and the test one. The test is considered negative if only the control line can be seen. The cephalorachidian fluid samples were inseminated on solid and fluid culture media, using the BacT/Alert (Organon Tecnika) system. We used the Fisher exact test for statistical processing of data.

Results and discussion

Data gathering was conducted on a representative sample. Among the 30 patients, 5 were diagnosed with acute pneumococcal meningitis. Table 1 presents the characteristics of these patients, as well as the laboratory results.

19 patients displayed other-aetiology meningitis: 5 of probably viral aetiology, 5 of bacterial aetiology, other than pneumococcal (in one case we isolated *S. pyogenes* non-A group), 1 neoplasic meningitis and 8 of probably bacillary aetiology. The NOW test was negative in all these cases. By contrast, the latex test for pneumococcus was positive in 2 cases: in the case of a bacterial meningitis (without confirmation through smear or culture) and in a case of bacillary meningitis.

The remaining 6 patients from the studied group were not suffering from meningitis. Both the NOW and the latex-agglutination tests were negative for pneumococcus in all these 6 cases.

Analyzing the data gathered from the study-group, one can notice a 100% sensitivity and a 100% specificity in the case of the NOW test, which has a positive predictive value of 1 and a negative predictive value of 1. The sensitivity of the latex test amounted to 100%, with a specificity of 0.92, a positive predictive value of 0.71, and a negative predictive value of 1.

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 1. The characteristics and laboratory results of patients suffering from pneumococcal meningitis (F- female, M-male).

No.	Sex	Age	Cephalorachidian fluid: Smear	Cephalorachidian fluid: culture medium	NOW Test	Latex Test
1	F	66	Encapsulated Gram-positive diplococci	Negative	Positive	Positive
2	M	66	Lanceolated diplococci	pneumococcus	Positive	Positive
3	F	56	Encapsulated diplococci. Cocci in clusters	Negative	Positive	Positive
4	F	67	Rare encapsulated diplococci in short chains	Negative	Positive	Positive
5	M	66	Encapsulated Gram- positive diplococci	pneumococcus	Positive	Positive

tients 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 incephalorachidian 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 cephalorachidian fluid showed a 100% sensitivity and sensibility (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 cephalorachidian fluid, and it has a very high sensitivity and sensibility.

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