EDITORIAL

Detection of Antigens in Urine

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The causes of pneumonia have traditionally been diagnosed with methods that involve culture of respiratory or blood samples. Sputum is the most common type of respiratory sample because sampling is simple. The culture, which should be preceded by Gram staining, requires at least 48 hours before the results are available, and problems of sensitivity and specificity have been reported. As a result, this approach performs poorly. Blood cultures are also subject to the problem of delays in returning the results and low sensitivity. The development of simple techniques for measuring antigens in urine in recent years represents an important step towards overcoming these problems.

This editorial aims to provide an update of the techniques which measure urinary components of antigens corresponding to bacteria such as *Streptococcus pneumoniae* and *Legionella pneumophila* to provide fast and reliable diagnosis of the resulting community-acquired pneumonia (CAP).

Streptococcus pneumoniae Antigens in Urine

Urine analysis is of interest because microbial antigens are more concentrated in urine than in other body fluids and because there is no interference from other antibodies. Pneumococcal antigens (normally polysaccharide capsular antigens) were first detected in urine in 1917.[1] Since then, various techniques have been used for their analysis (counterimmunoelectrophoresis, latex agglutination, coagglutination, enzyme immuno-assays), although the results had been disappointing and hope of a reliable urine test had been almost abandoned. Recently, Binax NOW, an immunochromatographic membrane assay, was approved by the United States Food and Drug Administration for rapid diagnosis (in 15 minutes) of pneumonia caused by *S pneumoniae*. The assay detects polysaccharide C, which is common to all serotypes and to pathogens such as *Streptococcus mitis* and *Streptococcus oralis*.

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The method is perfectly well explained in the instructions of Binax NOW, so we will not describe it here. Nevertheless, we do think it appropriate to dwell on some points of confusion. The result is positive when the control and sample lines are colored. A weak positive result should be considered negative to increase the specificity of the assay[2] and it should be remembered that a result can still be positive at least 1 month later.[3] Concentration of the urine is not considered necessary because, even though the sensitivity might be improved, the result would be delayed (1-3 hours) and the cost would increase.[1] Administration of antibiotics does not lead to negative results, as investigation of the test 7 days after initiation of antibiotic therapy has shown.[4]

The sensitivity in patients is between 50% and 80% for those without bacteriemia and between 75% and 85% when bacteriemia is present, whereas the specificity is greater than 95%.[1-6] The variation in sensitivity in patients without bacteriemia can be explained by the variable reliability of the methods used to compare the results. Colonization by *S pneumoniae*, *S oralis*, or *S mitis* is unlikely to result in false positives. No positives for *S pneumoniae* were found in control patients with chronic bronchitis, who are more likely to be carriers than the general population,[6] and in the case of *S oralis* and *S mitis,* neither is responsible for CAP and these bacteria are rarely present in sufficient concentrations to test positive for antigenes. Binax NOW, however, cannot be used for diagnosis of pneumonia in children because they are often carriers of the bacteria, particularly those with a history of bronchopulmonary disease, and because an increasing number are vaccinated.[7] In adults, the assay is not recommended during the 5 days after pneumococcal vaccination given the possibility of erroneous results.

Rationale for Using the Technique

Knowledge of the cause of a disease can certainly help provide targeted treatment, which, in Spain at least, implies using antimicrobial agents with a narrower spectrum of action or avoiding combinations. Likewise, such knowledge might prove decisive when is not clear whether to admit the patient to hospital.

According to a recent study of military trainees with nonsevere pneumonia, the results from Binax NOW assays could indicate treatment with amoxicillin without admission to hospital.[8] Evidence suggests that this approach is less effective in mild-moderate disease, but the examination nevertheless has to be carried out.[5] CAP associated with bacteremic pneumococcal infection is one of the forms associated with greatest morbidity and mortality. Diagnosis based on blood cultures cannot be made until at least 48 hours later. The sensitivity of the immunochromatographic method in such situations is very high[4]; therefore treatment can be selected with greater certainty. To date, no explanation has been forthcoming for the false negatives in patients with bacteriemia.

Legionella pneumophila Antigens in Urine

Infection by *Legionella* species is normally diagnosed with methods such as direct immunofluorescence and culture of respiratory secretions, which are not particularly sensitive, or with impractical methods (indirect immunofluorescence, which provides a late diagnosis). The presence of antigens in urine was determined by laborious radioimmunoassay techniques (1987-1988) until the use of enzyme immunoassay techniques became widespread[9] (Binax, Biotest, Bartels). Now, as with the detection of pneumococci, a membrane immunochromatographic assay is used (Binax NOW for the *Legionella* antigen). The performance of both enzyme immunoassay techniques and the membrane immunochromatographic assay is similar, although the latter has 2 clear advantages—a dedicated laboratory is not required and it is quicker (15 minutes vs 90 minutes). With enzyme immunoassays, positives have been detected for serogroups of *L pneumophila* other than serogroup 1, but the sensitivity and specificity have not been determined. Nevertheless, this does not seem to be an additional advantage given that most cases (at least 90%) belong to serogroup 1. Therefore, the rest of the article will focus on the immunochromatographic technique.

The test is positive if the control and sample lines are colored. If the color is weak after 15 minutes the result should be rechecked after a further 45 minutes.[10] If the color remains unchanged, the result is negative (although this is not indicated in the manufacturer's instructions). Positive results may still be obtained after more than 60 days in immunocompromised subjects in whom the fever takes longer to remit.[11] Anti-infective treatment does not interfere with the findings of the analysis. One study suggests that concentrating the urine increases sensitivity,[12] whereas other investigators consider that this makes the technique unnecessarily slow and costly.[10] If a suitable laboratory is available throughout the day, it may be preferable to concentrate the urine (something not indicated by the supplier). If not, untreated urine should be used. If the result is negative, and there is strong suspicion of CAP caused by *L pneumophila*, the assay should be repeated with concentrated urine. Sensitivity ranges from 56% (unconcentrated urine) to 97% (concentrated urine), with a specificity of 97%.[12] The issue remains in doubt because Helbig et al[10] reported that the specificity was similar for concentrated and unconcentrated urine. A valid alternative seems to be ultracentrifugation,[13] which requires 15 minutes and performs comparably.

Indications for Detection of *Streptococcus pneumoniae* and *Legionella pneumophila* Antigens in Urine

Accumulated worldwide experience suggests that determination of antigens in urine can be considered a step forward in the early detection of disease caused by both *S pneumoniae* and *L pneumophila* thanks to the high reliability of the technique. In fact, in it is widely mentioned in recent guidelines on CAP.[14,15]

In our opinion, at the very least, it is advisable to measure pneumococcal antigens in the urine of patients with CAP who do not require admission to hospital. Such an approach would allow diagnosis of a substantial proportion of pneumonias and specific treatments could be prescribed. When an outbreak of legionellosis occurs (regardless of where the patient is treated), the corresponding antigen assay should be requested. When a patient is admitted to hospital, we recommend initially requesting an assay for pneumococcal antigens. If this proves negative, and if epidemiological factors suggest legionellosis or if clinical suspicion of the disease is strong, an assay for the *L pneumophila* should be requested. Assays for both bacterial antigens should be done simultaneously if the patient is suffering from severe CAP.

To conclude, we think the cost-benefit ratio should be favorable although studies have yet to confirm this.

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