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Investigation of Positive *Streptococcus pneumoniae* Urinary Antigen Test Results in a Korean University Hospital

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Background: The *Streptococcus pneumoniae* urinary antigen test (SPUAT) (Binax Now, USA) was developed for detecting polysaccharide C in urine samples for rapid diagnosis of pneumococcal pneumonia, the most common cause of community-acquired pneumonia (CAP). To validate positive results of these tests, we retrospectively investigated all positive results obtained from the emergency room of a Korean university hospital among patients with suspected CAP.
Methods: One hundred twenty-three positive SPUAT results were abstracted and analyzed from the authors' laboratory information system among the SPUAT results performed from 1,143 pneumonic patients admitted from the emergency room of a university hospital between 2007 and 2008. Medical records, including conventional microbiologic analysis results, were reviewed in detail for all positive test results.
Results: Among 123 patients with the positive SPUAT results, 24 patients were excluded due to hospitalization history during the preceding month. Nine of 99 patients (9.1%) with suspected CAP had confirmed pneumococcal pneumonia upon conventional sputum or blood culture. Thirty-five positive results (35.4%) showed other microorganisms upon conventional methods, which might be due to possible cross-reactivity. Among those, 23 positive results were considered bacterial pneumonic agents, and 12 positive results were regarded as urinary tract infection strains or contaminating agents. Fifty-five positive SPUAT results (55.6%) showed negative conventional microbiologic growth, and some positive SPUAT results might be caused by true pneumococcal infection although without cultural evidence.
Conclusion: Our retrospective study demonstrated that a positive SPUAT result typically does not agree well with conventional culture methods, suggesting that the value of a positive SPUAT result in etiology determination may be limited under practical conditions in a university hospital. **(Korean J Clin Microbiol 2010;13:14-18)**

Key Words: *Streptococcus pneumoniae*, Bacterial antigens, Urinary antigen test, Cross-reactivity

INTRODUCTION

Streptococcus pneumoniae is the most common cause of community-acquired pneumonia (CAP) worldwide[1]. The diagnosis of pneumococcal infection traditionally requires recovery of the microorganism from an uncontaminated specimen[2-4]. However, blood cultures are positive in only about one fourth of cases, and prior antibiotic therapy significantly reduces the likelihood of obtaining a positive blood culture. Cultures of expectorated sputum only provide a probable diagnosis because pneumococcal organisms are often carried in the oropharynx. In order to increase the number of etiologic diagnoses, a *Streptococcus pneumoniae* urinary antigen test (SPUAT) (Binax Now, Portland, ME, USA) was developed for detecting polysaccharide C in urine samples via a new immunochromatographic method[5-8]. The introduction of SPUAT in clinical practice has increased the incidence of this etiological diagnosis[9]. The test has proven to be rapid, sensitive, and specific in diagnosing pneumococcal pneumonia in adults[7-9]. However, due to persistent excretion of urinary antigen[10] or higher cross-reactivity with another pathogen[11], questions remain concerning the clinical usefulness of SPUAT tests. The clinical utility of a diagnostic test is determined not only by laboratory factors such as sensitivity, specificity, and ease of use, but also by such factors as the epidemiology of the target pathogen and the patterns of test usage. It is to be expected, therefore, that some diagnostic tests have excellent operating characteristics, yet provide no useful clinical information in actual practice. The aim of this study was to investigate the clinical implications of positive SPUAT results in patients with suspected CAP admitted from the emergency room of a university hospital.

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MATERIALS AND METHODS

1. Materials

We enrolled the adult patients who were admitted from the emergency room for suspected CAP and who underwent a SPUAT between January 2007 and December 2008. Among enrolled patients, the positive SPUAT results were abstracted and analyzed from the authors' laboratory information system. Patients were excluded from this study if their medical records were not available for review. The clinical criteria for CAP were acute illness, radiological signs of pulmonary consolidation, at least two of five signs and symptoms (fever of $>37.8^{o}$C, dyspnea, cough, pleuritic chest pain, and abnormal lung auscultation), and lack of hospitalization during the preceding month (except for transfer due to same event).

Table 1. Demographic characteristics of community acquired pneumoniae patients with positive results of a *Streptococcus pneumoniae* urinary antigen testing (N=99)

Variables	N (%)
Gender	
Male	71 (71.7)
Female	28 (28.3)
Age (mean ± standard deviation)	65.8 ± 12.5
Final diagnosis considered	
Streptococcal pneumonia	9 (9.1)
Other bacterial pneumonia	23 (23.2)
Unknown etiology	67 (67.7)
Underlying disease	
Chronic obstructive pulmonary disease	44 (44.4)
Neoplasm	29 (29.3)
Lung cancer	9 (9.1)
Gastrointestinal malignancy	9 (9.1)
Hematologic malignancy	8 (8.1)
Head and neck tumor	2 (2.0)
Breast cancer	1 (1.0)
Tuberculosis	21 (21.3)
Diabetes	17 (17.2)
Renal failure	9 (9.1)
Mental disease	6 (6.1)
Heart failure	4 (4.0)
Rheumatoid disease	3 (3.0)
The number of possessing underlying disease	
None	10 (10.1)
≥1	89 (89.9)
≥2	41 (41.4)
X-Ray	
Unilobar	41 (41.4)
Bilateral	46 (46.5)
Parapneumonic effusion	12 (12.1)
Antibiotic treatment at emergency room arrival	
Prior antibiotic therapy	43 (43.4)
No prior antibiotic therapy	45 (45.5)
Unavailable data	11 (11.1)

2. Data collection

Among the 1,143 patients performed the SPUAT test, the medical records of the 123 patients with the positive SPUAT results were reviewed carefully. At chart review, the following data were recorded: age, sex, medical record number, sample type, sample collection method, clinical history, prior antibiotic therapy history, admission history during the preceding month, antibiotic therapy, culture results from any source, SPUAT results from urine, gram stain results from any source, clinical impression, and radiological findings.

3. *S. pneumoniae* urinary antigen test

Non-concentrated urine was used for SPUAT according to the instructions of the manufacturer. The result was read visually after 15 minutes and was interpreted on the basis of the presence or absence of a detectable pink to purple lane.

RESULTS

Among the 1,143 patients with available SPUAT results, 123 (10.8%) showed positive SPUAT results. Twenty-four of these 123 patients were excluded because they had hospitalization during the preceding month and were diagnosed as having hospital-acquired pneumonia. Eighty-nine of the 99 patients who met the criteria for CAP (89.9%) had more than one underlying disease, and 43 patients (43.4%) had prior antibiotic treatment history. The demographic characteristics of these patients are shown in Table 1. Only nine (9.1%) of the pneumonic patients had pneumococcal pneumonia with positive sputum culture results

Table 2. Possible cross-reacting microorganisms in this study (N=35)

Positive agent	Total (N=35)	Sputum (N=25)	Blood culture (N=10)
Pneumonic etiologic agents considered (N=23)			
Klebsiella pneumoniae	7	6	1
Staphylococcus aureus	6	5	1
Pseudomonas aeruginosa	5	5	
Acinetobacter baumannii	2	2	
Stenotrophomonas maltophilia	1	1	
Streptococcus agalactiae (Group B streptococcus)	1	1	
Klebsiella ornithinolytica	1	1	
Non-pneumonic agents considered (N=12)			
Coagulase negative staphylococcus	5		5
Alpha-hemolytic streptococcus	2	2	
Yeast	2	2	
Escherichia coli (Urinary tract infection)	1		1
Proteus vulgaris (Urinary tract infection)	1		1
Enterococcus fecalis	1		1

(N=7) and positive blood culture results (N=2). Four (44.4%) of these nine patients had prior antibiotic treatment history.

Thirty-five (35.4%) patients had cultural evidence with positive sputum culture results (N=25) and positive blood culture results (N=10) (Table 2). These microorganisms were regarded as the possible causative agents through SPUAT cross-reactivity. Twenty-three patients (23.2%) with proven microorganisms such as *Klebsiella pneumoniae* (N=7), *Staphylococcus aureus* (N=6), *Pseudomonas aeruginosa* (N=5), *Acinetobacter baumannii* (N=2), *Stenotrophomonas maltophilia* (N=1), *Streptococcus agalactiae* (N=1), and *Klebsiella ornithinolytica* (N=1) were diagnosed as having bacterial pneumonia. Another 12 patients (12.1%) showed microbial growth such as coagulase-negative *Staphylococcus*, alpha-hemolytic *Streptococcus*, yeast, *Escherichia coli*, *Proteus vulgaris*, and *Enterococcus fecalis*, however, we considered these microorganisms to be the etiologic agent of urinary tract infection or contamination. Therefore, no etiology was determined in more than half (N=67, 67.7%) of the pneumonic patients including 12 (12.1%) positive SPUAT results regarded as urinary tract infection or contamination and 55 (55.6%) positive results with negative conventional microbiologic growth in our study group.

DISCUSSION

Compared with conventional culture methods used as the gold standard, the presenting findings showed that positive SPUAT results had a low positive agreement results, a high false-positive rate, with high cross-reactivity with other bacterial strains. Although these tests for diagnosing pneumococcal pneumonia have traditionally compared with conventional culture methods, the gold standard is of limited sensitivity. Given the absence of a gold standard with good sensitivity, the precise significance and performance of these tests cannot be assessed.

Possible false-positive or cross-reacting microorganisms in this study included gram-positive and gram-negative bacterial strains (Table 2). As alpha-hemolytic *Streptococci* contain cell wall components similar to the pneumococcal C polysaccharide, they have been shown to yield false-positive SPUAT results[12]. Charkaluk et al[13] reported that *Staphylococcus* and *Streptococcus* species showed cross-reactivity with SPUAT. In the current study, pneumococcal capsular polysaccharides also cross-reacted with gram-negative strains such as *E. coli*, *Klebsiella* species, *P. aeruginosa*, *A. baumannii*, *S. maltophilia*, and *E. fecalis*. Previously, Stalin et al[12] also demonstrated that an in-house serotype-specific latex agglutination (LA) test developed by the Streptococcus Unit at Statens Serum Institut (Copenhagen, Denmark) yielded false-positive LA results with strains of *E. coli*, *Klebsiella* species, and *Neisseria meningitidis*. SPUAT can detect the common C polysaccharide antigen seen in these 90 serotypes, and any component of gram-negative bacteria might react with SPUAT. However, in such cases, we could not exclude true *S. pneumoniae* infection or nasopharyngeal colonization, which could also explain the positive SPUAT results.

No etiology was determined in more than half the pneumonic patients in this study. Unknown etiology with a positive SPUAT is an obvious clinical problem, and a positive SPUAT is not helpful if bacteriologic confirmation of pneumococcal pneumonia is questionable. In some studies, it has been hypothesized that most of these patients have undetected pneumococcal pneumonia and that an alternative test, like a urinary antigen assay, can improve diagnosis[9,14,15]. Therefore, true pneumococcal infection without cultural evidence of infection should be considered.

Other possible explanations of false-positive results include insufficient specimen for culture, prior antibiotic administration, persistent urinary antigen excretion after prior pneumococcal pneumonia, non-specific cross-reactivity, nasopharyngeal colonization with *S. pneumoniae*, systemic absorption of *S. pneumoniae* antigen, contamination of urine by skin flora, and no detectable serological or virological cultures. Although Marcos et al. [16] showed that pneumococcal carriage in adults was not associated with SPUAT positivity in eight patients, Stalin et al[12] showed that one of five carriers had weakly positive results. Thus, pneumococcal carriage may cause false-positive results in adults. As the rate of nasopharyngeal colonization in adults is lower than that in children, nasopharyngeal colonization is less important in the former. Persistence of both capsular antigens and C polysaccharide in the urine has been demonstrated after pneumococcal pneumonia[16,17]. Thus, positivity due to previous pneumococcal infection should always be considered in urine antigen-positive patients. Recent vaccination with pneumococcal polysaccharide vaccine might also explain the presence of pneumococcal antigen in the urine. Urine is a convenient sample in which to detect capsular antigen; however, contaminating flora in samples obtained in a non-sterile manner may cause false-positive SPUAT results.

Although certain risk factors, clinical features, and laboratory abnormalities may suggest a diagnosis of pneumococcal pneumonia, differentiation from common bacterial pneumonias is usually difficult in clinical practice. Before emergency room arrival, 43.4% of patients are given antibiotics. Almost 90% of patients in this study had underlying diseases such as chronic obstructive pulmonary disease, neoplasia, or tuberculosis. The treatment strategy would not have changed according to the SPUAT results under many actual scenarios. Many previous studies have demonstrated that SPUAT has high specificity and high negative predictive values in adults with CAP[14,18-22]. These findings indicate that a negative result may be more useful than a positive one is in clinical practice.

This SPUAT test was recently recommended for diagnostic use by the Infectious Diseases Society of America[4]. However, it is not clear how SPUAT should be used and interpreted. Dominguez et al. suggested that the specificity of the test could be enhanced if result lines weaker than the control line were considered negative[7]. Stalin et al suggested using unconcentrated urine and dividing SPUAT-positive results into strong and weak positivity[12]. While weak SPUAT positivity should be interpreted with caution, strong positivity should be considered indicative of pneumococcal etiology in adult CAP. When weak SPUAT positivity was interpreted as positive, SPUAT showed low specificity

and a low positive predictive value. Because of the low specificity, weakly SPUAT-positive results appear to be unreliable for diagnostic use. The low positive predictive values of SPUAT discourage their use in order to rule out a pneumococcal etiology in CAP. Therefore, it might be wise to consider weak SPUAT positivity as negative in patients with underlying disease in a large university hospital setting. Unfortunately, we did not divide results into strong and weak positivity. Recently, new, more specific methods have been developed to differentiate patients with and without pneumococcal infection[23].

There are some limitations to this study. First, it was retrospective in nature. Second, we reviewed only positive SPUAT results. Therefore, we did not estimate the specificity or negative predictive value. Third, we did not include enough serological tests or virological cultures to determine the cause of CAP.

In conclusion, we demonstrated that a positive SPUAT result had a low positive agreement results with conventional cultures, suggesting that the value of a positive SPUAT result in etiology determination may be limited under actual clinical conditions. Further research is needed to delineate the possible effects of prior antibiotic administration on the false-positive rate.

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=국문초록=

한 대학병원에서 폐렴구균 소변항원검사 양성 결과의 분석

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배경: 폐렴알균 소변 항원 검사(*Streptococcus pneumoniae* Urinary Antigen Test)는 지역사회 획득 폐렴의 가장 흔한 원인인 폐렴알균 폐렴의 빠른 진단을 위해 소변 내 polysaccharide C를 검출하는 방법으로 개발되었다. 본 연구자들은 본 검사의 양성 결과의 임상적 의의를 확인하기 위해, 지역사회 획득 폐렴이 의심되어 단일 대학병원의 응급실을 통해 방문해서 시행된 폐렴알균 소변 항원 검사 결과를 후향적 연구를 통하여 평가하였다.

방법: 2007년에서 2008년까지 단일 대학병원의 응급실을 통해 입원한 1,143명의 폐렴 환자의 폐렴알균 소변 항원 검사 결과 중 123건의 양성 결과를 본원의 검사정보시스템에서 추출하였다. 본 검사에서 양성을 보인 모든 환자들에 대해 의무기록을 면밀히 조사하였다.

결과: 폐렴알균 소변 항원 검사에서 양성을 보인 123명의 폐렴 환자 중 24명의 환자들은 병원 획득성 폐렴으로 분류되었으며, 99명의 환자가 지역사회 획득 폐렴으로 추정되었다. 9.1%의 환자에서 객담 및 혈액 배양 검체에서 폐렴알균이 검출되었다. 23.2% 환자에서는 배양 검체에서 다른 세균성 폐렴 원인 미생물이 검출되어, 12.1% 환자에서는 요도 감염 또는 오염으로 고려되는 미생물들이 검출되어, 35.3%의 양성결과들은 위양성으로 고려되었으며, 이러한 미생물들을 교차반응을 일으킬 수 있는 원인으로 분류하였다. 55.6% 환자에서는 배양 검사에서 음성결과를 보였으며, 이 중 일부는 배양 검사가 음성인 폐렴알균 폐렴의 가능성을 시사하였다.

결론: 본 연구를 통해 지역사회 획득 폐렴의 가장 흔한 원인인 폐렴알균 폐렴의 빠른 진단을 위한 폐렴알균 소변 항원 검사의 양성 결과들이 기존 미생물 배양검사법과 낮은 일치도를 보임이 확인되었다. 그러므로, 대학병원의 실제 임상상황에서 폐렴알균 소변 항원 검사의 양성 결과를 통한 원인균의 판정은 제한점이 있음이 시사된다. **[대한임상미생물학회지 2010;13:14-18]**

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