

The Evaluation of the Sputum Antigen Kit in the Diagnosis of Pneumococcal Pneumonia

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Abstract

Objective A previously developed sputum antigen detection kit for *Streptococcus pneumoniae* enabled the early diagnosis of pneumococcal pneumonia using sputum samples. We conducted a prospective study to compare the sensitivity of the sputum and urinary antigen kits.

Methods Pneumonia patients who were treated from April 2014 to September 2015 were recruited for the present study. Patients with pneumococcal pneumonia who could not participate in the prospective arm of the study were analyzed in the retrospective arm.

Results Nine of the 69 participants in the prospective study had pneumococcal pneumonia. The sputum antigen kit results correlated well with the sputum culture results. The sensitivity of the sputum antigen kit was 88.9% (8/9), which was higher than that of the urinary antigen kit (5/9; 55.6%). When patients from the retrospective arm of the study were included, the sensitivity of the sputum culture was 93.5% (29/31), which was significantly higher than that of the urinary antigen kit (19/31; 60.6%). False positives were obtained using the sputum antigen kit in four cases. Three of the four false positives were suspected to have resulted from the administration of antibiotics prior to the use of the kit; the remaining case likely occurred due to a false reaction to *S. milleri*-induced pyothorax.

Conclusion Collectively, our findings suggest that the sputum antigen kit has a higher sensitivity for detecting *S. pneumoniae* than the urinary antigen kit. However, the prior administration of antibiotics can render the sputum culture results negative or lead to a false-positive result.

Key words: *Streptococcus pneumoniae*, sputum antigen test, urinary antigen test

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Introduction

Pneumonia is the third leading cause of death in Japan (1), and the fourth leading cause of death in the world (2). Mortality due to pneumonia is on the increase, which makes it a relevant disease, even in present times. *Streptococcus pneumoniae* is considered one of the most important causative agents of pneumonia (3). It is the most frequent causative agent of community-acquired pneumonia and influenza virus-related lethal pneumonia (4). The early diagnosis of pneumococcal pneumonia is crucial for enabling the use of appropriate antibiotics, as well as in reduc-

ing the abuse of broad-spectrum antibiotics.

A urinary antigen test for *S. pneumoniae* (urinary antigen kit) was released and made commercially available in 2005. This kit enabled the rapid diagnosis of pneumococcal pneumonia prior to the administration of antibiotic therapy. The sputum antigen test for *S. pneumoniae* (sputum antigen kit) was released in 2010. This kit also enabled the rapid diagnosis of pneumococcal pneumonia using sputum samples.

In Japan, either the sputum antigen kit or the urinary antigen kit is used, since the insurance system only covers the use of a single antigen detection kit for the diagnosis of pneumococcal pneumonia. Both the urinary and sputum antigen kits are known to yield false-positives and false-

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negative results. Accordingly, the selection of the appropriate antigen kit and the careful interpretation of the results is of the utmost importance.

We conducted a prospective study of pneumonia patients in order to compare the sensitivity of the urinary and sputum antigen kits in the detection of *S. pneumoniae*. We also retrospectively analyzed the diagnostic efficacy of the kits in the patients who could not participate in the prospective arm of the study in order to evaluate the validity of results from the prospective study.

Materials and Methods

We conducted a two-armed study with a prospective arm (Study A) using sputum and urinary antigen kits, and a retrospective arm (Study B) to analyze all of the patients who were diagnosed with pneumococcal pneumonia and compare the sensitivity of the sputum culture antigen kit with that of the urinary antigen kit. Both studies were approved by the institutional review board of our hospital.

Study A: A prospective study was conducted of the patients who were admitted to our hospital during the daytime for the treatment of pneumonia. The inclusion criteria were as follows: patients who were scheduled to undergo sputum culture and a urinary antigen test in routine medical practice and who provided written informed consent. Culture tests other than sputum culturing (e.g., blood culture, pleural fluid culture) were performed at the physician's discretion and were not essential. For these patients, the sputum antigen test was conducted using the same sputum sample that was used in the bacterial culture. Patients were recruited from April 2014 to September 2015. The patients of the prospective arm were recruited from patients who were hospitalized in the daytime, given the shortage of staff at night.

Study B: To compensate for the pneumococcal patients who could not participate in the prospective arm (mainly due to admission at night), we collected data on the pneumococcal pneumonia patients retrospectively. The bacteriological information of the patients' medical records were analyzed and patients with positive *S. pneumoniae* cultures (sputum, lavage under bronchoscope, pleural effusion, and blood) or for whom a urinary antigen test was positive were selected. From these, we focused on the patients who were admitted to our hospital with pneumococcal pneumonia. The information of the patients in this retrospective arm was collected during the same period as Study A (from April 2014 to September 2015) in order to complement the shortage of patients with pneumococcal pneumonia in Study A. The results in Study B included the results from 9 patients with pneumococcal pneumonia who participated in Study A.

A definitive diagnosis of pneumonia requires inflammation (WBC >8,500/ μ L, C-reactive protein (CRP) >0.30 mg/dL, or a body temperature of $\geq 37.0^\circ$) and infiltration on chest radiography or chest CT. The causative bacteria of pneumonia were defined by a score of 2+ (equivalent to about 10^6 to 10^7 colony forming unit (CFU)/mL) in a spu-

tum culture, the detection of bacteria from sputum with the phagocytosis of bacteria in gram staining, or the detection of bacteria from the culture of a sterile site (e.g., pleural effusion and blood). Pneumococcal pneumonia was defined as the detection of *S. pneumoniae* with a score of 1+ using the semi-quantitative method, based on the detection limit of the sputum antigen kit (10^5 CFU/mL; equivalent to approximately 1+ to 2+ with the semi-quantitative method). Mycoplasma pneumonia was diagnosed by the loop-mediated isothermal amplification method using nasopharyngeal swabs.

In both arms of the study, we collected information regarding the patients' bacterial culture (sputum culture) and urinary antigen test results, and their history of antibiotic treatment prior to their admission. The urinary antigen test was performed using the BinaxNOW[®] (Alere Medical, Tokyo, Japan) assay, and the sputum antigen test was performed using the Rapirun[®] *Streptococcus pneumoniae* (Otsuka Pharmaceutical, Japan) assay, according to the respective manufacturer's instructions. The chi-squared test was used to compare the differences between the two kits. The Excel-toukei 2012 software program (Social Survey Research Information, Japan) was used to perform the statistical analysis.

Results

We obtained consent from 72 pneumonia patients in study A; three patients were excluded because they could not produce sputum. The final sample population for the prospective arm consisted of 69 patients. Table 1 summarizes the characteristics of the participants in Studies A and B. Study A included 69 participants (median age at study entry, 78 years; male, n=48 [70.0%]). The severity of pneumonia was evaluated using the A-DROP (4) system. The proportions of patients with mild, moderate, severe, and very severe pneumonia were 15.9%, 60.9%, 18.8%, and 4.3%, respectively. There were nine patients with pneumococcal pneumonia in Study A. The characteristics of these patients were similar those of the whole study population of Study A.

In Study B, *S. pneumoniae* was isolated from 54 patients during the study period. Thirteen patients who were treated as outpatients were excluded from the analysis. One patient in whom *S. pneumoniae* was isolated by bronchoscopy was also excluded, since the patient did not meet the diagnostic criteria described in the method. Nine hospitalized patients who did not undergo the urinary antigen test were excluded from the analysis, as the diagnostic efficacy of the kits could not be compared for these patients. Thus, the final study population of study B included 31 patients (isolated from sputum, n=28; isolated from blood and sputum cultures, n=1; isolated from blood culture, n=1; isolated from pleural effusion, n=1). Seventeen of the patients had a positive result using the urinary antigen kit, but did not have a positive *S. pneumoniae* culture. These were considered to be false-positive results and are listed in Table 2. The study population of Study B included 31 participants (median age, 81

Table 1. Character of the Patients in Each Study.

	Study A	SPP in Study A	SPP in retrospective	SPP in Study B
Number	69	9	22	31
Age, years (Range)	78 (23 - 99)	77 (64 - 90)	84 (49 - 95)	81 (49 - 95)
Male / Female (Male %)	48 / 21 (70.0 %)	7 / 2 (77.8 %)	12 / 10 (54.5 %)	19 / 12 (61.3 %)
A-DROP				
Mild (%)	11 / 69 (15.9 %)	1 / 9 (11.1 %)	1 / 22 (4.5 %)	2 / 31 (6.5 %)
Moderate (%)	42 / 69 (60.9 %)	6 / 9 (66.7 %)	12 / 22 (54.5 %)	18 / 31 (58.1 %)
Severe (%)	13 / 69 (18.8 %)	2 / 9 (22.2 %)	6 / 22 (27.3 %)	8 / 31 (25.8 %)
Very severe (%)	3 / 69 (4.3 %)	0 / 9 (0%)	3 / 22 (13.6 %)	3 / 31 (9.7 %)

SPP: *S. pneumoniae* pneumonia**Table 2. Comparison of the Bacteriological Profile between Study A and Study B.**

	Study A	Study B
Total number	69	unknown
Number of pneumococcal pneumonia cases	9	31
Positive sputum culture	8	29
Sputum antigen test (positive / negative)	8 / 1	unknown
Urinary antigen test (positive / negative)	5 / 4	19 / 12
False positive in sputum antigen test	4	unknown
False positive in urinary antigen test	5	17
Sensitivity of sputum culture	88.9% (8/9)	p=0.11 93.5% (29/31)
Sensitivity of urinary antigen	55.6% (5/9)	60.6% (19/31)

years; male, n=19 [61.3%]) with pneumococcal pneumonia. The proportions of patients with mild, moderate, severe, and very severe pneumonia were 6.5%, 58.1%, 25.8%, and 9.7%, respectively. The severity in these participants tended to be greater than that in Study A, and was influenced by the greater severity observed in the retrospective arm.

Table 3 summarizes the causative bacteria in Study A. A diagnosis of *M. pneumoniae* was made when the loop-mediated isothermal amplification method yielded a positive result. The causative bacteria were confirmed in 26 of the 69 patients (37.7%); *S. pneumoniae* was detected in 9 patients (34.6%), and *S. aureus*, *H. influenzae*, *M. catarrhalis*, *S. agalactiae*, and *E. coli* were confirmed in 4, 3, 2, 2, and 2 patients, respectively.

Table 4 shows the sensitivity and specificity of the two kits in Study A. The sensitivities of the sputum antigen kit and the urinary antigen kit were 88.9% (8/9) and 55.6% (5/9), respectively. The sensitivity of the sputum antigen kit tended to be superior, although the difference was not statistically significant due to the small number of patients with pneumococcal pneumonia. The specificity of the sputum an-

tigen kit was 93.3% (56/60), and was similar to that of the urinary antigen kit (55/60; 91.7%).

Table 5 summarizes the rates of false-positive results for the 9 patients with pneumococcal pneumonia. Four patients had false-positive results with the sputum antigen kit, and 5 had false-positive results with the urinary antigen kit. Three of the 4 patients who had false positives with the sputum antigen kit had been treated with antibiotics prior to admission. The fourth patient was complicated with *S. milleri*-induced pyothorax. None of the patients for whom false positives were obtained using the urinary antigen kit had received antibiotics prior to admission. However, one patient had previously been diagnosed with pneumococcal pneumonia (eight months prior to admission).

Table 2 summarizes the sputum culture and urinary antigen test results of the pneumococcal pneumonia patients from Studies A and B. The results of both studies were similar. The sensitivity of sputum culture in Study B was 93.5% (29/31), which was significantly higher than the sensitivity of the urinary antigen test (19/31; 61.3%; p=0.01).

Table 3. Causative Bacteria of Pneumonia in Study A.

Causative bacteria	Number of cases (%)
<i>S. pneumoniae</i>	8 9 (34.6%)
<i>S. pneumoniae</i> + <i>H. influenzae</i>	1
<i>S. aureus</i>	4 (15.4%)
<i>H. influenzae</i>	3 (11.5%)
<i>M. catarrhalis</i>	2 (7.7%)
<i>S. agalactiae</i>	2 (7.7%)
<i>E. coli</i>	2 (7.7%)
<i>K. pneumoniae</i>	1 (3.8%)
<i>E. cloacae</i>	1 (3.8%)
<i>Achromobacter xylosoxidans</i>	1 (3.8%)
<i>Mycoplasma pneumoniae</i>	1 (3.8%)
Total	26 (100%)

Discussion

The present study had prospective (Study A) and retrospective (Study B) arms. Study A evaluated the utility of the sputum antigen kit, while Study B evaluated the limitations and utility of sputum culture and the urinary antigen test in the diagnosis of pneumococcal pneumonia. Our findings suggest that the sputum antigen kit is superior to the urinary antigen kit in diagnosing the causative bacteria in patients with pneumococcal pneumonia. We also gained important insights regarding the false-positive results obtained with the sputum antigen kit.

Both the sputum antigen kit and the urinary antigen kit detect *S. pneumoniae* using an immunochromatographic assay. However, the key difference is in the bacterial components they detect. The sputum antigen kit detects C-polysaccharide on the bacterial cell wall, while the urinary antigen kit detects the bacterial capsular antigen. The kits also differ in that the sputum antigen kit reacts to *S. intermedius* infection at $>7 \times 10^7$ CFU/mL, while the urinary antigen kit reacts to *S. mitis* infection. The sputum antigen kit also reacts to *Micromonas micros* infection at $>6 \times 10^8$ CFU/mL. *S. pneumoniae* colonization in the oral cavity, which occasionally occurs in children, can lead to a false-positive reaction with the sputum antigen kit, while the diagnostic efficacy of the urinary antigen kit is low in the early phase of infection (5). Obtaining a positive signal takes time because the kit detects a specific antigen, which must find its way into the urine from the local site of infection via systemic circulation. In contrast, the sputum antigen kit can detect the antigen in the early phase of infection because the antigen is detected directly at the site of infection.

It is important to first note how the sputum antigen kit results are related to the sputum culture results. In the present study, a score of 1+ (almost equivalent to approximately 10^3 to 10^5 CFU/mL of *S. pneumoniae*) or higher was obtained in the sputum cultures of all of the patients who tested positive for *S. pneumoniae* using the sputum antigen kit (Table 1). A pre-marketing study also reported consistency between the

Table 4. Sensitivity and Specificity of Sputum and Urinary Antigen Kit in Study A.

	Sensitivity	Specificity
Sputum antigen	88.9% (8/9)	93.3% (56/60)
Urinary antigen	55.6% (5/9)	91.7% (55/60)

sputum antigen kit and sputum culture results (6). A positive sputum antigen kit result requires 10^5 CFU/mL of *S. pneumoniae* (1+ to 2+ in a semi-quantitative sputum culture).

Study A was a prospective study that assessed the sensitivity and specificity of the two kits. The sensitivity of the sputum antigen kit was 88.9%, while that of the urinary antigen kit was 55.6%, indicating that the sputum antigen kit had superior sensitivity; however, the difference was not statistically significant. In the retrospective arm (Study B), sputum culture showed significantly superior sensitivity to the urinary antigen kit (Table 2). Based on these results, we conclude that the sputum antigen kit has greater sensitivity than the urinary antigen kit. The pre-marketing study by Ehara et al. (6) and the multicenter pre-marketing study conducted by Fukushima et al. (7) also reported the higher sensitivity of the sputum antigen kit, which is consistent with the results of our post-marketing clinical study. Interestingly, the sensitivity of the urinary antigen kit in those studies (5, 7), and the present study was lower than that of a different study, which reported a sensitivity of approximately 80% (6). This difference may be attributed to the fact that our study and the study of Fukushima et al. mainly relied on the diagnosis of pneumococcal pneumonia by sputum culture and because our study population mainly consisted of patients with mild to moderate disease severity. The sensitivity of the urinary antigen kit reportedly increases with increased disease severity (8).

The specificity of the sputum antigen kit was 93.3%, which was similar to that of the urinary antigen kit (91.8%). False positives were observed with both kits. Specifically, false-positives were obtained with the sputum antigen kit in four cases in Study A. The pre-administration of antibiotics may have rendered the culture results negative, since antibiotic treatment was initiated prior to hospital admission in three of the four patients with false-positive results. The quality of sputum, as assessed using Geckler's classification, was 3-5 in these three patients, which was similar to that of the patients with sputum antigen-positive pneumococcal pneumonia who had not received antibiotics. In fact, a sputum smear detected gram-positive diplococcus, which is suggestive of *S. pneumoniae*, in one patient (patient 16 in Table 5), despite the patient having a negative sputum culture result. Mukae et al. (9) reported that sputum cultures become negative most quickly after the administration of antibiotics, followed by the sputum antigen kit and the urinary antigen kit. Sputum cultures essentially become negative within three days of the administration of antibiotics. Patient 10 was complicated by *S. milleri*-induced pyothorax (which includes *S. intermedius*), which is thought to have caused

Table 5. Bacteriological Details of the Pneumococcal Pneumonia, Sputum Antigen False Positive Cases, and Urinary Antigen False Positive Cases in Study A.

Case	S. A.	U. A.	Geckler	Sputum culture	Antibiotics	Note
18	+	+	5	<i>S. pneumoniae</i> 2+	-	
20	+	+	3	<i>S. pneumoniae</i> 3+	-	
30	+	+	5	<i>S. pneumoniae</i> 3+	-	
48	+	+	3	<i>S. pneumoniae</i> 1+	-	
61	+	+	5	<i>S. pneumoniae</i> 3+	-	
2	+	-	2	<i>S. pneumoniae</i> 2+	-	
59	+	-	5	<i>S. pneumoniae</i> 2+	-	
67	+	-	5	<i>S. pneumoniae</i> 2+ <i>H. influenzae</i> 3+	-	
44	-	-	3	N. D.	-	*1
10	+	-	Hemo	<i>E. cloacae</i> 2+	-	*2
16	+	-	3	N. D.	CAM	
19	+	-	5	N. D.	SBT/CPZ	
26	+	-	4	N. D.	AZM	
7	-	+	1	<i>S. agalactiae</i> 3+	-	
22	-	+	3	<i>E. coli</i> 3+	-	
27	-	+	1	N. D.	-	
31	-	+	5	N. D.	-	*3
47	-	+	3	<i>S. aureus</i> 2+	-	

Case 18 - Case 44: pneumococcal pneumonia case

Case 10 - Case 26: false positive in sputum antigen test

Case 7 - Case 47: false positive in urinary antigen test

S. A. : sputum antigen test

U. A. : urinary antigen test

Hemo: hemospitum

N. D.: not detected

*1: *S. pneumoniae* was grown from blood culture.

*2: Case 10 was complicated with pyothorax from *S. milleri*.

*3: Case 31 had a previous history of pneumococcal pneumonia within eight months of using the kit.

the false-positive reaction with the sputum antigen kit. Our results regarding the false-positive reactions obtained with the sputum antigen kit are informative, given the general lack of information on this aspect in the literature.

Five patients had positive results with the urinary antigen kit and negative sputum antigen and sputum culture results in Study A. These cases were considered to be false positives, mainly because we could not detect *S. pneumoniae* from their culture samples (regardless of the positivity of cultures for other bacteria in case 7, 22, and 47). We did not confirm the positive or negative conversion of the urinary antigen kit results in these cases. Patient 31 had a previous history of pneumococcal pneumonia eight months prior to the present study; this was suspected to be the cause of the false-positive reaction. There were as many as 17 false-positive reactions in Study B. Since two of the 17 patients received antibiotics prior to admission, it is possible that the sputum cultures yielded false-negative results. However, 15 patients did not receive antibiotics prior to the sputum culture test. Thus, caution must be exercised when relying solely on the urinary antigen kit to diagnose pneumococcal pneumonia. However, our results do not provide sufficient evidence to prove that these patients did not have pneumo-

coccal pneumonia. Additional studies will be needed to further assess the reasons underlying cases in which the urinary antigen kit yielded a positive result, while sputum culture yielded a negative result.

The present study is associated with several limitations. First, the diagnosis of pneumococcal pneumonia mainly depended on the results of sputum culture. Moreover, we did not routinely conduct blood culture tests for our patients. In cases in which a negative sputum culture and a positive blood culture were detected could be interpreted as non-pneumococcal pneumonia. Such cases could have overestimated the sensitivity of the sputum antigen kit and underestimated the sensitivity of the urinary antigen kit. Second, the sputum culture and the antigen tests were only performed at the time of hospital admission. Repeated antigen tests during treatment may to a certain extent have addressed the issue of false positives. Third, patients with suspected pneumococcal pneumonia in the retrospective arm of the study (study B) may have been tested with the urinary antigen kit, which could have introduced bias.

In summary, the results obtained using the sputum antigen kit were better correlated with the sputum culture results, and sputum culture showed greater sensitivity than the uri-

nary antigen kit. This suggests that the sputum antigen kit is more effective for diagnosing pneumococcal pneumonia. However, the limitations include potential false positives in patients who receive antibiotics prior to testing, and those who are infected with a different type of *Streptococcus* bacteria, which is often seen in patients with pyothorax and aspiration pneumonia. Our findings also revealed that the urinary antigen kit had a high false-positive rate. These results suggest that the appropriate selection of antigen detection kits and the careful interpretation of test results are required for clinicians who engage in the treatment of pneumonia.

The authors state that they have no Conflict of Interest (COI).

Authorship

S. Ikegame performed the study and wrote the paper. T. Nakano, J. Otsuka, M. Yoshimi, and Y. Tao participated in the execution of the study. T. Matsuo and M. Kubota performed the bacteriological examinations. S. Takata designed and supervised the study.

Ethics

This study was approved by the Institutional Review Board of Fukuokahigashi Medical Center.

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