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CONCISE ARTICLE

Persistence of *Streptococcus pneumoniae* urinary antigen excretion after pneumococcal pneumonia

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Abstract The aim of this study was to determine the duration of *Streptoccocus pneumoniae* antigen excretion in urine after pneumococcal pneumonia. Urinary antigen detection remained positive in nonconcentrated urine in 18 (52.9%) of the 34 patients in the first month after pneumonia diagnosis. In 12 of these positive cases, the test was still positive in the second month, in six patients after 4 months, and in two cases 6 months after the diagnosis of pneumonia. Using concentrated urine, antigenuria remained positive in all patients for at least 3 months, with antigen detected in three cases more than one year later. We did not observe a relation between age, gender, immunosuppression, underlying diseases, pneumonia severity, positive blood culture, or X-ray presentation and longer-term antigenuria excretion. However, the small number of patients evaluated is a limitation for statistical analysis. In order to correctly analyse a positive urinary antigen test result in patients with pneumonia, it is necessary to know which patients have recently had a previous episode of pneumonia.

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Introduction

Streptococcus pneumoniae is still the most common cause of community-acquired pneumonia [1]. The diagnosis of pneumococcal infection traditionally requires recovery of the microorganism from an uncontaminated specimen [1, 2]. However, blood cultures are positive in only about one fourth of the cases, and prior antibiotic therapy significantly reduces the number of positive blood cultures. In addition, invasive methods used to obtain uncontaminated specimens from foci of infection cannot be systematically and indiscriminately performed [1]. Cultures of expectorated sputum only provide a probable diagnosis because pneumococcal organisms are often carried in the oropharynx. Polysaccharide capsular antigen (PCA) detection by the counterimmunoelectrophoresis (CIE) method is an alternative for the diagnosis of pneumococcal pneumonia, but these methods have failed due to a lack of sensitivity [3].

In order to increase the number of etiologic diagnoses a new immunochromatographic (ICT) test (Binax Now *Streptococcus pneumoniae* Antigen Test, Portland, Maine, USA) was developed for detecting polysaccharide C (PnC)

in urine samples [4–6]. The introduction of a *S. pneumoniae* urinary antigen assay in clinical practice has increased the rate of this etiological diagnosis [7]. The test has proven to be rapid, sensitive, and specific in diagnosing pneumococcal pneumonia in adults [4, 5, 8]. Furthermore, concentrating the urine by selective ultrafiltration may elevate the utility of this test because it increases sensitivity [7, 9]. Urinary antigen excretion may persist in some patients after specific antibiotic therapy, although to date a prospective study has not been reported [10, 11].

Therefore, the aim of this study was to determine the duration of *S. pneumoniae* antigen excretion in urine in patients diagnosed with pneumococcal pneumonia and to analyse the related factors.

Materials and methods

Patients We prospectively studied 186 urine samples from 39 patients diagnosed with pneumococcal pneumonia with a positive urinary antigen by the ICT method in non-concentrated and/or concentrated urine (NCU/CU). In addition, three patients were also diagnosed by blood culture, one by PCA detection by CIE and, in five patients *S. pneumoniae* was also isolated in sputum samples. The following data were collected: age, sex, underlying diseases, immunosuppression status, radiological extension, the pneumonia severity index (PSI), and the antibiotic therapy. Demographic characteristics are summarized in the Table 1.

Table 1 Demographic characteristics of all patients studied (n=39)

Variables	*N* (%)
Gender	
Female	15 (38.5)
Male	24 (61.5)
Age (mean ± standard deviation)	60.2±18.9
Underlying disease	
Asthma	7 (17.9)
Mental disease	5 (12.8)
Heart failure	5 (12.8)
Diabetes	1 (2.6)
Hepatitis C virus infection	2 (5.1)
Tuberculosis	1 (2.6)
None	18 (46.2)
Immunosuppression	
Steroids	2 (5.1)
None	37 (94.9)
X-ray presentation	
Unilobar	22 (56.4)
Bilateral	4 (10.3)
Pleural effusion	6 (15.1)
Unavailable data	7 (17.9)
Pneumonia severity index	
I	2 (5.1)
II	9 (23.1)
III	8 (20.5)
IV	13 (33.3)
Unavailable data	7 (17.9)
Antibiotic therapy	
Amoxicillin-clavulanate	10 (25.6)
Levofloxacin	14 (35.9)
Ceftriaxone + clarithromycin	4 (10.3)
Other combinations	5 (12.8)
Unavailable data	6 (15.4)

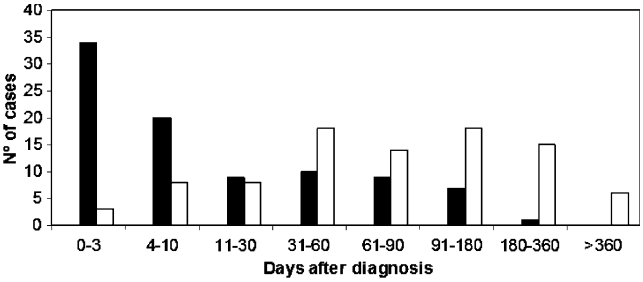

Fig. 1 Distribution of positive (*black*) and negative (*white*) results of *S. pneumoniae* urinary antigen detection in NCU samples collected during the study period

All patients had CAP, which was defined as the presence of an illness lasting for at least 2 days. Chest radiography was performed at the time of admission that showed a radiographic pulmonary infiltrate that was at least segmental or present in one lobe, consistent with pneumonia, and not preexisting. Clinical features were consistent with an acute lower respiratory tract infection including two or more of the following: new or increasing cough, sputum production, pleuritic chest pain, dyspnea, pulmonary consolidation by physical examination; and one or more constitutional symptoms including temperature >37.8°C, sweating, altered mental status, aches and pains, headaches, coryza, or sore throat.

Inclusion criteria The inclusion criteria were: (1) patients diagnosed with pneumococcal pneumonia by PnC antigen detection by ICT in nonconcentrated and/or concentrated urine, and (2) possibility of follow-up in the hospital's outpatient department and periodical urine collection.

Exclusion criteria Patients with a previous episode of pneumococcal pneumonia during the past year, and patients diagnosed with chronic obstructive pulmonary disease (COPD) were not included in the study.

Sample collection For monitoring the excretion of pneumococcal antigen during the pneumococcal pneumonia and after finishing a curative therapy, four serial urine samples were collected. The first sample was taken at diagnosis (within the initial 72 hours), the second sample between the fourth and tenth day, the third after 30 days, and the fourth

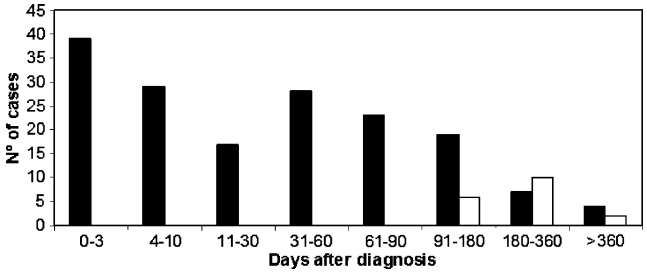

Fig. 2 Distribution of positive (*black*) and negative (*white*) results of *S. pneumoniae* urinary antigen detection in CU samples collected during the study period

after 60 days. In 31 patients, a longer follow-up was performed in order to pinpoint negativity of the test.

Sample treatment Urine specimens were collected and frozen at –20°C until use and thawed immediately before being tested by ICT assay. Ten millilitres of each urine sample were boiled for 5 minutes and then centrifuged at 1,000 g for 15 minutes, a procedure that has been reported to reduce nonspecific reactions. Samples were also concentrated 25-fold by centrifugal ultrafiltration (Amicon Ultra-4; Millipore Corporation, Bedford, MA).

PnC detection ICT was performed according to the instructions of the manufacturer. The results were read visually after 15 minutes. The test was interpreted according to the presence or absence of visually detectable pink-to-purple coloured lines. ICT results were blindly read by two independent trained persons.

Statistical analysis Data collected from each patient were introduced in a database and analysed by means of SPSS statistical software for Windows (SPSS version 15.0; SPSS Inc., Chicago, IL, USA). Categorical variables were analysed by univariate analysis using the Chi-square method or Fisher's exact test. Comparison of continuous variables was performed by using the Student's t-test. Differences were considered significant when the p value was less than 0.05.

Results and discussion

S. pneumoniae urinary antigen detection remained positive in NCU in 18 (52.9%) of 34 patients in the first month after

Table 2 Analysis of the influence of different variables on the persistence of antigenuria excretion in patients with NCU studied for 60 days and patients with CU samples studied 150 days after diagnosis

Variables	Antigen excretion in NCU			Antigen excretion in CU		
	n<60 days (%)	*n*>60 days (%)	*P*	*n*<150 days (%)	*n*>150 days (%)	*P*
Gender						
Female	7 (58.3)	5 (41.7)	0.788	7 (63.6)	4 (36.4)	0.353
Male	12 (63.2)	7 (36.8)		12 (80)	3 (20)	
Age						
<65 y	5 (31.3)	11 (68.8)	0.379	8 (61.5)	5 (38.5)	0.185
>65 y	8 (53.3)	7 (46.7)		11 (84.6)	2 (15.4)	
Underlying disease						
Yes	12 (70.6)	5 (29.4)	0.242	10 (71.4)	4 (28.6)	0.838
No	7 (50)	7 (50)		3 (25)	9 (75)	
Immunosuppression						
Yes	1 (50)	1 (50)	0.627	1 (50)	1 (50)	0.702
No	18 (62.1)	11 (37.9)		18 (75)	6 (25)	
X-ray presentation						
Unilobar	12 (63.2)	7 (36.8)	0.647	11 (68.8)	5 (31.3)	0.197
Bilateral	1 (100)	0 (0)		-	-	
Pleural effusion	2 (50)	2 (50)		4 (100)	0 (0)	
Blood culture						
Yes	1 (100)	0 (0)	0.419	18 (72)	7 (28)	0.536
No	18 (60)	12 (40)		1 (100)	0 (0)	
PSI						
I-III	15 (65.2)	8 (34.8)	0.667	14 (70)	6 (30)	0.517
IV	1 (50)	1 (50)		1 (100)	0 (0)	
Antibiotic therapy						
Amoxicillin-clavulanate	6 (75.2)	2 (25)	0.372	4 (57.1)	3 (42.9)	0.413
Levofloxacin	6 (54.5)	4 (45.5)		7 (87.5)	1 (12.5)	
Ceftriaxone + clarithromycin	2 (100)	0 (0)		2 (66.7)	1 (33.3)	

the diagnosis of pneumonia. In 12 of these positive cases, the test was positive in the second month, in six patients after 4 months, and in two cases 6 months after the diagnosis of pneumonia.

Using concentrated urine, the antigen remained positive in all patients at least 3 months, with antigen detected in three cases more than one year after diagnosis. Figures 1 and 2 show the distribution of positive and negative results in the different samples collected within the period of study.

We did not observe statistically significant differences regarding age, gender, immunosuppression, underlying diseases, PSI score, positive blood culture, X-ray presentation, and antibiotic therapy between patients with longer-term antigenuria excretion (>60 days in NCU, or >150 days in CU) (Table 2).

S. pneumoniae urinary antigen detection has become a more commonly used procedure to diagnose pneumococcal pneumonia in adults because of its high sensitivity and specificity [7, 4–6, 12]. However, pneumococcal urinary antigen persistence after resolution of pneumonia may limit its diagnostic value in patients with previous pneumonia due to *S. pneumoniae* [13].

Murdoch et al. [10], using NCU, detected pneumococcal urinary antigen in 38 of the 80 samples collected at follow-up after admission to the hospital, with a median interval after onset of symptoms of 49 days (range, 33–89). The longest duration of positivity of test results for any patient was 89 days after onset of symptoms. Moreover, Marcos et al. [11] detected PnC antigen by the ICT test 1 month after diagnosis of pneumococcal pneumonia in 16 of 23 patients (69.5%).

Interestingly, in our experience the X-ray presentation or the presence of underlying diseases was not related to longer pneumococcal urinary antigen excretion. In our study longer-term antigen excretion has been obtained in patients with a PSI between I and III. The amount of antigen excretion in the acute phase has been described as a marker of severity [14]. However, according to our data, there is no relationship between persistence in the urine after the resolution of pneumonia and severity.

The main limitation of this study is that we evaluated a small number of patients; therefore, the power to detect a statistically significant difference is low. Despite this limitation, our study provides relevant data related to variables involved in prolonged excretion of urinary *S. pneumoniae* antigen after a pneumococcal pneumonia.

Longer-term antigen excretion after a pneumonia case has been described for *Legionella* infection [15, 16]. In a previous study [16], we detected that antigenuria became negative in the first 2 months after diagnosis of pneumonia in about the 75% of cases, with detection of longer persistence in 10% of patients. However, in *Legionella* infection, the more prolonged excretion was associated with pharmacological immunosuppression and the persistence of fever for more than 72 hours. In our study population, where the number of immunosuppressed patients was low, the longer-term excretion has been obtained in immunocompetent patients.

The longer-term excretion of urinary antigen in intracellular pathogen infection, as *Legionella* [16] or *Leishmania* [17], has been attributed to the persistence of the microorganism or the specific antigens at an intracellular level, even following resolution of the infection. However, in the case of prolonged excretion of pneumococcal urinary antigen, the reason could be related to the amount of antigen present in the lung. It has been described in a previous study that in pneumococcal pneumonia the lung may contain up to 2 g or even more of capsular polysaccharide [18]. Obviously, the release of large amounts of antigen from the lung to the bloodstream and their total clearance from the human body will require a long time.

Concentration of the pneumococcal antigen present in the urine for diagnosing pneumococcal pneumonia in adults has been shown to increase the sensitivity of the tests without affecting the specificity [7, 11]. This procedure increases the antigen concentration 25-fold [9]; therefore, the antigenuria will be detectable longer than when NCU is used.

In summary, the persistence of *S. pneumoniae* antigenuria following diagnosis of pneumococcal pneumonia is normal and can be prolonged, especially if CU is used. In order to properly interpret a positive urinary antigen test result as being related to the present episode, it is necessary to know which patients have recently had a previous episode of pneumonia.

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Competing interest None of the investigators have any financial interest in or a financial conflict with the subject matter or materials discussed in this manuscript. None of the Scientific Societies, neither Binax Inc. (Portland, Maine, USA) had a role in the study design, conduct, collection, management, analysis, or interpretation of the data, or preparation, review, or approval of the manuscript.

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