Purulent Pericarditis due to *Streptococcus pneumoniae* Diagnosed by Pneumococcal Urinary Antigen Assay and 16S rDNA Sequence of the Pericardial Fluid

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**Abstract**

A 57-year old woman was admitted to our hospital with massive pericardial fluid. Culture of the pericardial fluid was negative, however, Binax NOW® *Streptococcus pneumoniae* urinary antigen test was positive in pericardial fluid. 16S rDNA sequencing and PCR for lyt(A) gene of the pericardial fluid sample confirmed the microbiological diagnosis of *S. pneumoniae*. The patient was treated with surgical drainage and continuous intravenous infusion of penicillin G and its concentration in the serum and pericardial effusion was monitored. Incorporation of molecular methods such as antigen testing and nucleic acid sequencing would benefit the management of infectious diseases especially in culture negative cases.

**Key words:** bacterial pericarditis, pneumococcal urinary antigen test, 16S rDNA sequence, *Streptococcus pneumoniae*

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**Introduction**

Pericarditis, which is defined as an inflammation of the pericardium, presents in various settings and can manifest as acute, subacute and chronic courses. Etiologies vary from infectious causes to noninfectious and the prognosis may also vary according to the causes and the underlying conditions. Among infectious etiologies, tuberculous pericarditis remains important in developing countries and in immunocompromised hosts (1-6). Bacterial pericarditis has become less common due to the advent of broad spectrum antimicrobials but there still exist some cases and the management has become more complicated due to the increase of resistant organisms (7).

Diagnosis can be challenging especially when antimicrobials have been initiated before appropriate culture specimens are taken. We describe a case of pneumococcal pericarditis successfully diagnosed by Binax NOW® pneumococcal urinary antigen assay and 16S rDNA PCR and sequence of the pericardial fluid. Antimicrobial resistance could also be presumed using PCR for penicillin and macrolide-resistant genes.

**Case Report**

A 57-year-old woman experienced a sudden onset of severe back pain and went to a nearby clinic 13 days before admission to our hospital. She received an intramuscular steroid injection in the lumbar area, but her symptoms did not improve. On the following day she felt dyspnea and pain in the upper limbs and went to another hospital where she was found to have fever and swelling in her upper limbs. She was diagnosed as cellulitis and was hospitalized. Intravenous cefotiam was administered, however her symptoms did not improve, therefore she was transferred to our hospital.

On admission she had a fever of 38.6°C, a blood pressure...
of 118/72 mmHg, a pulse rate of 102 beats/min and a respiratory rate of 18 breaths/min. Her chest was clear, but heart sounds were slightly diminished. Friction rubs were not heard even when she leaned forward. Her liver was palpable 3 cm below the right costal margin and both upper limbs were swollen.

Laboratory examinations on admission showed white blood cell counts were 13,200 cells/μL with 11,600 neutrophils/μL, 16.1 mg/dL CRP, 6.3 g/dL TP, 2.3 g/dL albumin, 67 IU/L AST, 65 IU/L ALT, 353 IU/L LDH, 582 IU/L ALP and 165 IU/L \( \gamma \)-GTP. Renal function was found to be normal and blood cultures taken on admission were negative for the presence of bacteria. Polymerase chain reaction (PCR) analysis of blood was negative for \textit{Mycobacterium tuberculosis}. The chest radiograph and CT showed cardio-megaly, with a cardio-thoracic ratio of 59% and left pleural effusion (Figs. 1, 2). The electrocardiogram showed a low voltage in the limb leads as well as an elevation of ST-segment in V2 and V3. The echocardiogram showed a low voltage in the limb leads as well as an elevation of ST-segment in V2 and V3. The echocardiography revealed a massive pericardial effusion and thickened pericardium (Fig. 3). There was no sign of cardiac tamponade on admission. Based on these findings, we made a tentative diagnosis of pericarditis by unknown etiology and started intravenous meropenem (0.5 g every 8 h).

However, three days following admission, her condition worsened with severe dyspnea, high fever, low blood pressure, pulsus paradoxus and sinus tachycardia, thus she was diagnosed as having cardiac tamponade and emergent pericardiocentesis was performed. Pericardiocentesis yielded a total 2,500 mL of yellow, purulent fluid with a white blood cell count of 103,900 cells/μL consisting of 98% neutrophils, 5.5 g/dL total protein and 8 mg/dL glucose. No microorganisms were observed by Gram or Ziehl-Nielsen staining. The cultures of pericardial fluid were negative for bacteria including mycobacteria.

In addition to these conventional clinical microbiological tests, we performed a urinary antigen test (Binax NOW\textsuperscript{®} \textit{Streptococcus pneumoniae}) and 16S rDNA PCR and sequence of the pericardial fluid sample. For bacterial DNA detection, DNA was extracted from 100 μL of pericardial fluid using Isoplant II (Nippon Gene). DNA amplification was performed by PCR using Univ 1N (5'-AGTTTTGATCM TGGCTCAGGACGA-3') and Univ 2N (5'-AAGGAGGTTG WTCGARCCGGA-3') primers. PCR conditions were as following, 95°C-10 min as preincubation, 35 cycles (95°C-30 sec, 55°C-15 sec, 72°C-90 sec), and 72°C-5 min as final extension. Positive PCR product was sequenced using the PCR primers with Univ 1N, 350F (TACGGGAGGCAGCAG), 910F (TCAAAGGGAATTGACGGGGGC), Univ 2N, 1109R (AGGGTTGCGTCCGTTG), and 520R (AAGGAGGTGWT CSARCCGCA), Big Dye Terminator cycle sequence kit (v 1.1, Applied Biosystems), and ABI Prism 3100 genetic analyzer ( Applied Biosystems). The urinary antigen test of the pericardial fluid was positive and 16S rDNA PCR and sequence yielded ≥99% identity to \textit{S. pneumoniae} by Blast search. The PCR for the \textit{lytA} gene was also positive in the pericardial fluid. According to these findings, we diagnosed...
that the pathogen responsible for the pericarditis was \textit{S. pneumoniae}. PCR analysis was positive for \textit{pbp2X} and \textit{ermB} and negative for \textit{pbp1A}, \textit{pbp2B}, and \textit{mefE}. These results indicated that \textit{S. pneumoniae} had an immediately elevated minimal inhibitory concentration (MIC) against penicillin and highly elevated MIC against macrolide (8). Following these results, we decided that pseudomonal coverage was unnecessary and changed meropenem to ceftiraxone (2 g every 12 h) instead of penicillin G. However, her fever persisted and the amount of pericardial effusion did not decrease, therefore, we performed surgical drainage 14 days after admission. We also considered the possibility of drug fever due to ceftiraxone, therefore we switched ceftiraxone to penicillin G (24 million units per 24 hours, continuous infusion) based on the recent change in the breakpoint of nonmeningeal pneumococci by CLSI, assuming that the MIC of this strain against penicillin G would be less than 4 μg/mL because pneumococci with an MIC above 2 μg/mL are rare in Japan even if they had mutations of the \textit{pbp} genes (9). Immediately after the surgical drainage and the switch of antimicrobials, fever resolved and the erythrocyte sedimentation rate and CRP level gradually declined. Her swollen limbs (probably due to pericarditis) improved and 23 days after admission, the pericardial drainage tube was removed. The MRI of the lower spine showed no evidence of abscess formation and her lumbar pain (probably related to lumbar spinal stenosis) also improved. Thirty-one days after admission, we obtained both serum and pericardial fluid through the drainage catheter and measured the concentration of penicillin G (PCG). The concentration of penicillin G in the serum taken at three different time points (8 am, 12 pm and 4 pm) was 13.7, 17.8 and 23.8 μg/mL, respectively, and that of the pericardial fluid taken at 12 pm was 6.8 μg/mL. Forty-nine days after admission, antimicrobials were stopped and eighty days after admission, she was transferred to another hospital for further evaluation of constrictive pericarditis. At the time of transfer, the CRP level was below 1.0 mg/dL and ESR level was below 10 mm/hour.

**Discussion**

Purulent pericarditis has become a rare clinical entity since antimicrobials became available. However, there are still some cases reported and they are associated with high mortality especially when the diagnosis is delayed (1-6). Purulent pericarditis caused by resistant organisms such as methicillin-resistant \textit{Staphylococcus aureus} is also becoming an emerging problem (6, 10, 11).

Approximately 40-50% of purulent pericarditis is caused by Gram-positive pathogens and \textit{S. pneumoniae} is the most common. Introduction of antibiotics decreased the frequency of pneumococcal pericarditis from over 80% to around 25% (2, 4, 12). A recent report by Levy et al showed much less frequency of pneumococcal pericarditis. They performed a systematic analysis including 16S rDNA sequencing of pericardial fluid samples obtained during a seven-year period (1998-2005) from 106 patients with large pericardial effusions. Most of the cases were due to neoplasms and 21 cases (19.8%) were due to infectious pericarditis. \textit{S. pneumoniae} was found in only two patients (13). Nevertheless, considering the recent increase of drug resistant \textit{S. pneumoniae}, pneumococcal pericarditis should still be listed at the top of the differential diagnosis.

In the present case, the culture result of the pericardial fluid revealed no organisms, presumably because intravenous cefotiam had been already administered. However, urinary antigen test (Binax NOW®) and molecular analysis with PCR and sequencing of 16S rDNA successfully enabled us to diagnose the causative organism of pericarditis.

The pneumococcal urinary antigen assay is an \textit{in vitro} rapid immune chromatographic assay for the detection of \textit{S. pneumoniae} antigen in the urine of patients with pneumococcal pneumonia and in the cerebral spinal fluid (CSF) of patients with pneumococcal meningitis (14). However, there have been several reports showing successful diagnosis of pneumococcal infections using Binax NOW® with specimens other than urine and CSF. These include nasopharyngeal secretions, middle ear effusions, bone tissue, pleural fluids and blood cultures (15-17). As far as we know, our report is the first case that used the pneumococcal urinary antigen test for the diagnosis of pneumococcal pericarditis using pericardial fluid. A recent study testing pleural fluids in children with empyema reported that the urinary antigen assay had a high sensitivity and specificity, similar to PCR analysis of 16S rDNA (18). The urinary antigen assay has advantages over PCR and sequencing in that the test is easy to perform and results are available in a short time.

The 16S rDNA sequencing has become important in clinical microbiology and it has contributed to the classification of microorganisms and diagnosis of causative organisms which cannot be cultured for some reasons (19). The sensitivity and specificity are generally high, although this technique has not become common because it is time consuming and labor intensive for most clinical microbiology laboratories. This assay should not necessarily be performed in all cases, however in selected cases such as culture negative cases due to prior antimicrobial therapy, this might play an important role. Levy et al reported that they found one pericardial fluid specimen that was positive for \textit{S. pneumoniae} by 16S rDNA sequencing from among 106 frozen samples retrospectively tested (13).

In the present case, we could obtain the result during the course of treatment and practically use the result for the diagnosis and treatment.

The management of pericarditis involves a combination of surgical drainage along with intravenous administration of appropriate antimicrobials. The penetration of antimicrobials from serum to the pericardium is reported to be excellent (20). The present results indicate that the PCG concentration ratio of pericardial effusion/serum was about 30-40%. The concentration of PCG in the pericardial fluid (6.8 μg/mL) was above the MIC of ordinary penicillin-resistant
S. pneumoniae (1-2 μg/mL) which is prevalent [MBS1] in Japan (9). The increase of antibiotic-resistant organisms may lead to reemergence of uncommon infectious diseases which once were conquered by antibiotics. Nevertheless, the advances in molecular diagnostic tests such as PCR and rDNA sequencing may enable us to correctly diagnose the pathogens and even estimate their antimicrobial resistance. Furthermore, the technique of monitoring concentrations of antimicrobials may help us to perform logical use antimicrobials.

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References


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