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Original article

A novel method for rapid detection of *Streptococcus pneumoniae* antigens in blood

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ABSTRACT

In this study, we used "RAPIRUN® *Streptococcus pneumoniae* HS (otitis media/sinusitis) (RAPIRUN-HS)," a rapid *S. pneumoniae* antigen detection kit, to investigate methods for detecting *S. pneumoniae* antigens in blood of 32 bacterial pneumonia patients. We simultaneously performed PCR to detect *S. pneumoniae* in blood samples. The results of these tests were compared based on pneumonia severity, determined using the Pneumonia Severity Index (PSI) score classification. Four *S. pneumoniae* PCR-positive patients of the six severe pneumococcal pneumonia patients (PSI risk class IV/V) also tested positive using RAPIRUN-HS. Twenty-four mild to moderate pneumonia patients (PSI risk class I–III) were *S. pneumoniae* PCR-negative; of these, 21 tested negative using RAPIRUN-HS. The pneumococcal pneumonia patients testing positive using RAPIRUN-HS had low leukocyte counts and elevated C-reactive protein and procalcitonin levels, indicating that RAPIRUN-HS results were correlated with pneumonia severity. The time course evaluations of the laboratory tests for severe pneumococcal pneumonia patients showed that RAPIRUN-HS and *S. pneumoniae* PCR yielded positive results earlier than the changes in procalcitonin and IL-6. Thus, concomitant pneumococcal bacteremia was strongly suspected in patients testing positive using RAPIRUN-HS. In conclusion, RAPIRUN-HS may be useful for determining whether to admit patients into hospitals and selecting the appropriate antimicrobial agents.

1. Introduction

Streptococcus pneumoniae is the most frequently detected pathogen in cases of community-acquired pneumonia and lower respiratory tract infection and has high morbidity and mortality rates worldwide. Pneumococcal infection has a high incidence and can become more severe. Therefore, confirming the presence of *S. pneumoniae* as early as possible and selecting the appropriate treatment are crucial for improving the prognosis and preventing drug-resistant bacteria development. Treatments are planned according to pathogenic bacteria test results. Culture tests are the gold standard for detecting and identifying pathogenic bacteria in pneumonia; however, culture tests results require longer time. Therefore, rapid diagnostic agents for detecting antigens have recently been adopted.

Bacteremia occurs concomitantly with pneumonia at approximately 10% frequency and can occur in >60% pneumococcal pneumonia cases [1,2]. Because of the high risk of worsening the prognosis in concomitant bacteremia patients, it is important to rapidly detect bacteria in blood [1,2]. However, blood culture test, which are the gold standard for bacteremia, is poor in detecting pathogenic bacteria [3,4]. Molecular methods, including polymerase chain reaction (PCR), are also used to detect bacteremia pathogens. Real-time PCR shows significantly higher detection rate than culture-based methods [5,6]. The high detection rate of real-time PCR may result in overdiagnosis, although pneumococcal DNA has not been detected in blood from a healthy population [7]. Molecular methods are also used in clinical application for other specimens such as cerebrospinal fluid and pleural fluid [8–11];

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however, these tests are expensive and technical skills are required. Therefore, a diagnostic agent that can rapidly, inexpensively, and easily detect bacteria in blood is required. "RAPIRUN® *S. pneumoniae* HS" (otitis media/sinusitis; Otsuka Pharmaceutical, Tokyo, Japan; RAPIRUN-HS) was developed as a *S. pneumoniae* antigen detection kit to rapidly and easily detect *S. pneumoniae* from middle ear fluids and otorrheal and nasopharyngeal secretions and has been used since the end of 2011. RAPIRUN-HS detects *S. pneumoniae* capsular antigens and common cell wall and cell membrane antigens and also all serotypes of *S. pneumoniae* [12].

Here we evaluated the clinical utility of detecting *S. pneumoniae* antigens in blood using RAPIRUN-HS based on results obtained by real-time PCR, and subsequently determined the associations between the test results and pneumonia severity and biochemical examination findings.

2. Patients and methods

The study comprised 32 pneumonia patients visiting the Japanese Red Cross Nagasaki Genbaku Isahaya Hospital between March 2009 and July 2012. Pneumonia was defined based on clinical symptoms, radiographs (chest X-ray and computerized tomography), and biochemical examinations. If *S. pneumoniae* was detected predominantly in the sputum of patients by culture, the patients were classified into pneumococcal pneumonia group. Consequently, 20 pneumonia patients were diagnosed with pneumococcal pneumonia. Mean age of the 32 patients was 63.0 (range: 23–97) years; they comprised 19 men and 13 women. Twenty-three patients had underlying diseases of respiratory organs; of these, 12 had asthma, 5 had chronic obstructive pulmonary disease (COPD), 2 had influenza, 2 had obsolete pulmonary tuberculosis, 2 had respiratory disorders, 2 had diabetes, 2 had high blood pressure, 2 had interstitial pneumonia, 1 had lung cancer, 1 had aftereffects of cerebral hemorrhage, 1 had heart failure, and 1 had renal insufficiency. Nine patients had no underlying diseases. Pneumonia severity was classified according to the Pneumonia Severity Index (PSI) score described in the guidelines recommended by the Infectious Diseases Society of America and the American Thoracic Society. The patients were not questioned regarding their age, sex, or whether they were taking antimicrobial drugs. Sputum, urine, and blood were collected from the patients. This study was approved by the Institutional Review Board of the Ethical Committee of the Hospital. Written informed consent was obtained either from the patients or a legal representative.

Samples were collected on the day of the first medical examination, on the day the patients were first admitted, or the time when they were diagnosed with pneumonia. Some of the collected blood was used for culture test and serum or plasma was separated by centrifugation from blood. The serum was then subjected to biochemical examination and PCR was performed to detect *S. pneumoniae*. *S. pneumoniae* antigens in the blood were also tested. The sputum was subjected to microscopic examination and culture tests to identify the pathogenic bacteria. An aerobic resin bottle was used for blood culture, where blood was cultured for 24–120 h using the BD BACTEC™ 9050 Blood Culture System (Becton Dickinson and Company, NJ, US). The biochemical examination involved measuring brain natriuretic peptide (BNP) in plasma, serum procalcitonin (PCT), and serum interleukin-6 (IL-6) levels in addition to the standard tests such as white blood cell (WBC) counts. BNP measurements were performed using PATHFAST® (LSI Medience Corporation, Tokyo, Japan), according to manufacturer's instructions. PCT and IL-6 were measured using a cobas® e411 (Roche Diagnostics, Basel, Switzerland), according to manufacturer's instructions. Blood and chocolate agar plates were used for sputum culture tests; the sputum was cultured for 24–48 h at 37 °C.

RAPIRUN-HS positivity/negativity was measured using a method different from that described in the package insert. Undiluted serum samples could not be measured using RAPIRUN-HS as serum viscosity prevents sample flow. Therefore, serum samples were diluted 2, 6, and 18 times with the extraction solution supplied with the kit to determine the most suitable dilution ratio for RAPIRUN-HS measurements. The most suitable dilution ratio was then calculated based on the line intensity measured with a densitometer (ATTO, Tokyo, Japan). Suitable proportions of the specimen extract included in the kit and the serum were added to a sample cup, and a test stick was then placed into the cup. The results were obtained after approximately 15 min, as described in the package insert. *S. pneumoniae* real-time PCR (hereafter, PCR) was performed by Kitasato Otsuka Biomedical Assay Laboratories, as described previously [13].

RAPIRUN-HS was used to evaluate correlations between the *S. pneumoniae* PCR results, PCT level, and IL-6 level using the serum obtained from a 71-year-old male patient diagnosed with pneumococcal pneumonia. MedCalc (MedCalc Software, Belgium) was used for statistical processing. The Mann–Whitney U test and Fisher's exact test were used for result analysis.

3. Results

3.1. Patient population

Pathogens detected in sputum culture tests comprised *S. pneumoniae* (22 patients), *Haemophilus influenzae* (12 patients), and *Haemophilus parainfluenzae* (six patients), along with *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Corynebacterium* sp., methicillin-resistant *S. aureus*, *Morganella morganii*, and *Candida albicans*. No pathogen was detected from the blood culture in 20 patients with pneumococcal pneumonia. The patients were classified by pneumonia severity according to their PSI scores: five, risk class I; 15, risk class II; four, risk class III; four, risk class IV; and four, risk class V. Patients with mild pneumonia, classified into risk classes I or II, were treated as outpatients. Patients with moderate pneumonia, classified into risk class III, were hospitalized for a short duration. Patients with severe and very severe pneumonia, classified into risk classes IV or V, were admitted for inpatient treatment. For analysis, the patients were stratified into a Class I–III group comprising 24 patients with mild to moderate pneumonia and a Class IV/V group comprising eight patients with severe to very severe pneumonia.

3.2. RAPIRUN-HS with blood samples

Because all 6 specimens produced the highest line strength at a dilution ratio of 2, the most suitable dilution ratio required that the specimen was mixed with an equal amount of extract solution (Fig. 1). Thus, a ratio of 2 was used in this study. Table 1 shows the correlation between the RAPIRUN-HS and PCR results. Of the 24 patients in the Class I–III group, 14 were classified into the pneumococcal pneumonia group. Among these, three were positive and 11 were negative for blood antigens according to RAPIRUN-HS. Blood PCR performed simultaneously was negative for all patients. Thus, the negative concordance rate for the RAPIRUN-HS and PCR among the pneumococcal pneumonia patients was 78.6%. All 10 patients in the other pathogenic pneumonia group tested negative by both the RAPIRUN-HS test and PCR. Thus, the negative concordance rate of the RAPIRUN-HS and PCR in the other pathogenic pneumonia group was 100%. In contrast, of patients in the Class IV/V group, 6/8 patients showed *S. pneumoniae* predominantly in sputum culture test. Among these, four tested positive by PCR and all tested positive using RAPIRUN-HS. Thus, the positive