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Etiology of parapneumonic effusion and pleural empyema in children. The role of conventional and molecular microbiological tests.

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ABSTRACT

Background: An increasing incidence of parapneumonic effusion and pleural empyema (PPE/PE) has been reported in recent studies. As only few data on etiology of PPE/PE in Central Europe have been reported, we undertook a study on the etiology of PPE/PE in children, using both standard culture and molecular techniques.
Methods: This prospective study was conducted between June 2011 and December 2013. Consecutive children with PPE/PE complicating community acquired pneumonia, who required diagnostic/therapeutic thoracentesis were included. Blood and pleural fluid samples for microbiological cultures were collected. Molecular methods were applied to identify *Streptococcus pneumonia*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and respiratory viruses in pleural fluid.
Results: The study group included 64 children, median age 4 (1–15). Seven of 64 (10.9%) blood cultures and 11 of 64 (17.2%) pleural fluid cultures revealed bacterial growth. The most common bacteria detected was *S. pneumoniae* (13 blood and pleural fluid samples from 11/64 (17.2%) children). DNA sequences of typical bacteria were found in 29/64 (45.3%) pleural fluid samples. *S. pneumoniae* was identified in 90% of these samples. The most common serotypes were: serotype 6B in 9/26 (36.6%), 19A in 6/26 (23%), serotype 3 in 3/26 (11.5%), 6A and 23F (both in 2/26 i.e. 7.7%) patients. Molecular methods identified atypical bacteria in 8/58 (13.8%) and respiratory viruses in 12/58 (20.7%) pleural fluid samples.
Conclusions: *S. pneumoniae*, in particular serotype 6B and 19A, is the most common etiologic agent of PPE/PE in Polish children. The use of PCR significantly improves pathogen identification in pleural fluid.

1. Introduction

Community acquired pneumonia (CAP) remains one of the major causes of morbidity in children worldwide [1]. For years, *Streptococcus pneumoniae* has been invariably found the most common etiologic factor of CAP [2]. The introduction of the pneumococcal conjugate vaccine (PCV) had an important impact on both the incidence of CAP and its complication rate. The incidence of CAP has been reported to decline in some well developed countries, where PCV had been widely introduced, but at the same time, an increasing incidence of local complications was noted. Data from the United States collected between 1996 and 2007 showed that all-cause pneumonia hospitalization rate in children younger than 2 years decreased by 33%, and the number of children hospitalized due to community acquired pneumococcal pneumonia decreased even by 61%. However, this decline was associated with 2-fold increase of empyema. This phenomenon may have an important impact on the overall efficacy of CAP treatment [3].

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Parapneumonic effusion and pleural empyema (PPE/PE) are the most common complications of CAP in children, with the annual incidence of hospitalization reaching 12.5 per 100 000 in some regions of the US [4]. *S. pneumoniae* remains the most common etiologic agent of PPE/PE in children, exceeding 60% of cases in some studies [5]. *Streptococcus pyogenes* and *Staphylococcus aureus* were reported as the second and the third most common species identified as the causes of PPE/PE in children. [3,5,6] Nevertheless, the spectrum of causative organisms is much wider and includes *Haemophilus influenzae*, *Streptococcus milleri* group, *Stenotrophomonas maltophila*, anaerobic and atypical bacteria [5–7]. Some viruses are also considered to play a potential role in the pathogenesis of PPE/PE in children [8].

The treatment of PPE/PE is challenging. Proper antibiotic therapy and an early local intervention are the cornerstones of the effective management. Since the results of individual microbiological studies are usually not available at the time of decision making on antibiotic therapy and the positive results of these studies can be expected in only minority of patients [5,6,9], the choice of antibiotics is based on local microbiological data and the results of epidemiological studies. In this context, studies on etiology of PPE and PE have an enormous impact on proper antibiotic treatment in children with local complications of CAP. It has been shown that molecular techniques are significantly more sensitive than standard microbiological cultures. Their use may be associated with even several fold increase in the detection rate of *S. pneumoniae* and other bacteria [5,6,10].

As some differences in etiology of PPE/PE were observed in earlier studies [5,6,9,11], reliable local microbiological and epidemiological data seem to be a prerequisite for the proper choice of antibiotic therapy. To our knowledge no such data have been published for Poland. This issue seems to be particularly important in terms of increasing proportion of Polish children receiving PCV. Thus, we undertook a study aimed at the evaluation of the etiology of PPE/PE in children managed in the referral pulmonary center in Poland. The secondary goals of the study were: 1) comparison of the diagnostic yield of standard culture with molecular techniques in the determination of PPE/PE etiology in children, 2) comparison of microorganisms responsible for local complications of CAP in children vaccinated vs. non-vaccinated with PCV.

2. Material and methods

2.1. Study design

This prospective, observational study included all consecutive children with PPE/PE complicating CAP who were referred to our institution between June 2011 and December 2013, and who required a diagnostic/therapeutic thoracentesis or other more complex pleural intervention. In all patients, blood samples for standard microbiological cultures were collected on the day of admission. Pleural fluid samples were taken between the 1st and 5th day of hospital stay. The diagnostic algorithm used to identify microorganisms in blood and pleural fluid samples is presented in Fig. 1. All children were treated with intravenous antibiotics. The type of local intervention i.e. therapeutic thoracentesis, chest tube drainage or video-assisted thoracoscopic surgery depended on the clinical course of the disease, the results of pleural fluid analysis and pleural space anatomy (the volume and loculation of pleural effusion).

2.2. Patients

Children who met the following inclusion criteria were enrolled: age between 1 month and 18 years, CAP defined according to British Thoracic Society (BTS) guidelines [2], pleural effusion that required at least a diagnostic thoracentesis. The exclusion criteria were as follows: immunodeficiency, pleural effusion caused by diseases other than CAP.

2.3. Microbiological investigations

2.3.1. Culture

Culture of blood samples was processed using the BacT/Alert 3D60 platform (bioMérieux, France). Samples of pleural fluids were plated on Columbia agar with 5% sheep blood, Chocolate agar PolyVitex, MacConkey agar (bioMérieux, France), brain heart infusion broth (Grasso, Poland). The plates (Columbia agar, chocolate agar) were incubated at 37 °C in a 5% CO_2 −atmosphere or under aerobic conditions (MacConkey, BHI broth) for 3 days. Bacterial isolates were identified using standard laboratory methods, including optochin susceptibility and bile solubility testing for *S. pneumoniae*, latex agglutination for group A antigen and bacitracin disk sensitivity for *S. pyogenes*, and latex clumping factor and protein A for *S. aureus*. All other isolates were identified with VITEK 2 system (bioMérieux, France), the isolates' identification was confirmed only when probabilities of identification was ≥92%.

2.3.2. Molecular techniques

Pleural fluid samples were stored in temperature −70 °C for a maximum 6 months before testing.

a) Nucleic acid extraction

DNA was extracted from pleural fluid samples with GeneProof PathogenFree DNA nucleic acid extraction kit (GeneProof, Brno, Czech Republic) or QIAamp DNA Mini Kit (Qiagen, Düsseldorf, Germany). RIBO-sorb (Amplisens, Moscow, Russia) nucleic acid extraction kit was used to isolate viral RNA from pleural fluid samples. Both tests were performed in accordance with the manufacturer's protocols. In case of nucleic acids of *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and all viruses, extraction from every assayed sample was carried out in the presence of internal control.

b) cDNA synthesis

REVERTA-L reverse transcription kit (Amplisens, Moscow, Russia) was used for complementary DNA (cDNA) synthesis from isolated RNA. The reverse transcription procedure was carried out according to the manufacturer's instruction.

c) Polymerase chain reaction (PCR)-based detection of *C. pneumoniae*, *M pneumoniae*

For detection of the DNA of *C. pneumoniae*, *M. pneumoniae* AmpliSens *M. pneumoniae*/*C. pneumoniae* - FRT qualitative PCR kit (AmpliSens, Moscow, Russia) was applied. The test was performed according to the manufacturer's instruction on Rotor-Gene Q instrument (Qiagen, Germany). The real-time hybridization-fluorescence and following amplification conditions were applied: predenaturation 95 °C 5 min, I stage denaturation 95 °C 10 s, annealing 63 °C 30 s, elongation 72 °C 10 s, II stage denaturation 95 °C 10 s, annealing 60 °C 30 s, elongation 72 °C 10 s. The human prothrombin gene fragment was used as an endogenous internal control fragment for extraction and amplification stages. Analysis of results measured in three channels (FAM, ROX, JOE) was performed by the software of Rotor-Gene Q instrument.

d) PCR-based detection of respiratory viruses