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ORIGINAL ARTICLE

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Rapid optimization of antimicrobial chemotherapy given to pediatric patients with community-acquired pneumonia using PCR techniques with serology and standard culture

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Abstract Children ($n = 117$; mean age 2.4 ± 2.9 years) were diagnosed as having community-acquired pneumonia (CAP) using clinical symptoms, chest X-rays, and hematological data. The causative pathogen was determined using real-time polymerase chain reaction (PCR) (6 bacteria), multiple reverse transcription-PCR (MPCR; 11 viruses), bacterial culture, and serology. The initial chemotherapy was evaluated based on the pathogens identified using PCR. We found 27 viral cases (23.1%), 25 bacterial cases (21.4%), 45 mixed infections with virus and bacteria (38.5%), 10 *Mycoplasma pneumoniae* (8.5%), 7 mixed infections with *M. pneumoniae* and another pathogen (6.0%), 1 *Chlamydophila pneumoniae* (0.9%), and 2 unknown pathogens (1.7%). *Streptococcus pneumoniae* and *Haemophilus influenzae* accounted for 58 (49.5%) and 27 (23.0%) of the cases, respectively. The median values (50%) of the white blood cell count (WBC) and C-reactive protein (CRP) using the box-and-whisker and plot method, respectively, were 11.7 $\times 10^3\,mm^{-3}$ and 1.4 mg/dl in viral infections, 15.6 $\times 10^3\,mm^{-3}$ and 4.8 mg/dl in mixed infections with virus and bacteria, 17.8 $\times 10^3\,mm^{-3}$ and 6.3 mg/dl in bacterial infections, 6.7 $\times 10^3\,mm^{-3}$ and 1.4 mg/dl in *M. pneumoniae* infections, and 21.5 $\times 10^3\,mm^{-3}$ and 6.4 mg/dl in mixed infections with *M. pneumoniae* and other bacterial infections. Sulbactam/ampicillin ($n = 61$), carbapenems ($n = 12$), and ceftriaxone ($n = 7$) were selected for the patients suspected of having bacterial infections alone or mixed infections with bacterial and viruses in accordance with our criteria defined tentatively. For those with *M. pneumoniae* and *C. pneumoniae* infections, azithromycin or minocycline was initially used. Treatments averaged 3–5 days. The empirical chemotherapy was improper in 9.4% of cases in relation to the etiologic agents finally identified. We conclude that rapid and comprehensive identification using PCR can provide optimal antimicrobial chemotherapy for CAP patients.

Key words Community-acquired pneumonia · Child · C-Reactive protein · Antimicrobial chemotherapy

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Introduction

Community-acquired pneumonia (CAP) is one of the most common infections occurring in children.[1–3] CAP is caused by multiple etiologic agents including viruses, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and other agents.[4–6] The rates of these causative microorganisms are quite different depending on many factors including the detection methods, seasonal epidemics, and the antibiotics predominantly used.[7–9]

In Japan, antimicrobial chemotherapy for patients with CAP is begun empirically based on (1) chest X-rays, (2) clinical findings including respiratory status, (3) age, and (4) laboratory tests such as white blood cell count (WBC) and C-reactive protein concentration (CRP). Recently, the guidelines to optimize empirical chemotherapy for CAP patients were published.[10] However, we believe the goal of chemotherapy is to select the most appropriate antibiotic for every patient within a short time after admission, based on laboratory results and immediately determining the causative agent.

Recently, the detection of viruses and bacteria using polymerase chain reaction (PCR) in addition to serological diagnosis has determined etiologic agents with high precision.[11–16] In children with CAP, the determination of the causative pathogen is difficult because of the difficulty in collecting direct clinical samples from alveoli, unlike in

adults. Because the pathogens must be identified using indirect nasopharyngeal samples that have low invasiveness, the physician has to determine whether the isolated microorganism is the etiologic agent or not. Therefore, a system to estimate the causative pathogens using obtainable clinical samples on the day of hospitalization is needed to quickly select the appropriate chemotherapy.

Our aim here was to use a multiplex PCR (MPCR) for viruses in parallel with bacterial detection using real-time PCR[17] in nasopharyngeal samples that were obtained from patients with pediatric CAP. Conventional bacterial cultures using the same samples and serological diagnosis with paired sera from the patient were performed to verify the results of the PCR. The clinical findings and laboratory test results from the patient were compared for every causative pathogen, and the appropriateness of the antimicrobial agents selected empirically according to the clinical findings was evaluated.

Patients and methods

Patients

Pediatric patients with CAP (male: $n = 59$; female: $n = 58$) were admitted to the Pediatric Department of Hakujikai Memorial Hospital, Tokyo, from May 2004 to April 2005. The criteria for hospitalization were: (1) the presence of pulmonary infiltrates found in the chest X-ray, (2) acute respiratory symptoms (e.g., tachypnea), and (3) deterioration of the general clinical state. We excluded patients requiring intensive therapy including artificial ventilation, those with chronic respiratory disease, those with congenital heart disease, those hospitalized with the same disease within a 1-month period, and patients with congenital or acquired immunosuppressive conditions.

Identification of the causative pathogens

After informed consent obtained from the child's parents or guardians, blood samples were taken to determine WBC, CRP, and serum antibody titers for several pathogens. Nasopharyngeal samples were also collected to determine the causative pathogens.

Each sample was used for: (1) real-time PCR screening for six bacterial pathogens, (2) multiple-reverse transcription PCR (MPCR) screening for 11 viral pathogens, and (3) conventional bacterial culture. These techniques were performed immediately after collection of the samples at the Laboratory of Molecular Epidemiology for Infectious Agents, Kitasato Institute for Life Sciences.

Real-time PCR

Streptococcus pneumoniae, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Streptococcus pyogenes*, and *Legionella pneumophila* were identified within 1.5 h after using the real-time PCR with the RTI kit[17] (Takara Bio, Kyoto, Japan) and Stratagene Mx3000P (Stratagene, La Jolla, CA, USA). The sensitivity and specificity were high at 95% and 98%, respectively, as compared with standard culture as previously described.[17,18]

Multiple-reverse transcription PCR

For the identification of viruses, an MPCR kit (Maximbio, San Francisco, CA, USA) was used according to the manufacturer's instructions. The MPCR kit identifies seven viruses: respiratory syncytial virus (RSV), adenovirus (Adeno), influenza virus A (FluA), influenza virus B (FluB), and parainfluenza virus-1, -2, and -3 (PIV-1, PIV-2, PIV-3). In addition, four primer sets to identify rhinovirus (Rhino),[19] human metapneumovirus (hMPV),[20] human bocavirus (hBoV),[21] and coronavirus[19] were prepared and the PCRs were performed using the same conditions as used for the MPCR kit. MPCR required 5 h.

Bacterial cultures

Bacterial culture was performed according to the *Manual of Clinical Microbiology*.[22] Serotyping of *S. pneumoniae* was performed using antiserum purchased from Statens Serum Institut (Denmark).

Serological test

Antibody titers against *M. pneumonia*, *C. pneumonia*, RSV, Adeno, FluA and FluB, and PIV-1, -2, and -3 were determined in paired sera from the acute and convalescent phase using the complement fixation (CF) test, hemagglutination inhibition (HI) test, or enzyme-linked immunosorbent assay (ELISA). When a significant rise in antibody titer was noted in the convalescent phase, the corresponding microorganism was considered to be the causative pathogen. A four-fold rise in titer for *M. pneumoniae* and Adeno; for RSV using the CF assay; and for PIV-1, -2, and -3, and FluA and FluB using the HI assay were used as indicators. *Chlamydophila pneumonia* was diagnosed using ELISA and the identified patient had an index value (ID) of 1.35 for IgG in the paired sera.

Clinical criteria to begin antimicrobial chemotherapy

The decision to begin antimicrobial chemotherapy was based on four conditions described previously;[23] clinical course, chest X-ray findings, age, and the laboratory findings. For clinical observations we used: (1) the presence or absence and timing of fever and respiratory symptoms such as tachypnea, wheezing, and retractive breathing; (2) presence or absence of nasal discharge and its properties; and (3) recurrent fever during the period of recovery of common cold-like symptoms. The diagnosis of tachypnea used World Health Organization (WHO) criteria.[24] Chest X-rays were divided into typical pneumonia (segmental or bronchial pneumonia), atypical pneumonia (ground-glass appear-

ance, skip lesion, pleurisy, and segmental), and viral pneumonia (bronchial pneumonia and interstitial shadow).

With regard to age, patients aged 5 years or older were usually thought to have *M. pneumoniae* infection and those aged 4 years or younger to have viral or mixed viral and bacterial infections. Bacterial infection and mixed infection were suspected in patients aged 4 years or younger with a WBC count of $13 \times 10^3\,mm^{-3}$ or greater, or a CRP value of 3.8 mg/dl or greater. These values may be low in some cases in the early stage after onset and the WBC may readily fluctuate for various reasons in infants. However, ultimately the estimation of infection to be bacterial, viral, mycoplasmal, or mixed was determined using a composite of the clinical course, chest X-rays, age, and the laboratory data.

Criteria for selection of antimicrobial agent and treatment period

Of the four parenteral antibiotics of ampicillin–sulbactam (SBT/ABPC), panipenem (PAPM), meropenem (MEPM), and ceftriaxone (CTRX), a single agent was selected for the patients suspected of having a bacterial infection alone or a viral–bacterial mixed infection from four conditions described above. The respective doses were as follows: SBT/ABPC 100–120 $mg\,kg^{-1}\,day^{-1}$ (q.i.d.), PAPM and MEPM 60 $mg\,kg^{-1}\,day^{-1}$ (t.i.d.), and CTRX 40–60 $mg\,kg^{-1}\,day^{-1}$ (b.i.d.). The antibiotics were administered for 3 days after defervescence (37.5°C).

The febrile period varied considerably in patients with viral and bacterial mixed infections, so the antibiotic was withdrawn 1–2 days after defervescence where we judged the antibiotic had been effective against the bacterial pathogen. For patients suspected of having *M. pneumoniae* or *C. pneumoniae* infection from the four conditions, azithromycin (AZM) or minocycline (MINO) was used. We did not use antimicrobial agents for patients where viral infection alone was strongly suggested from the four conditions described above.

Criteria for etiological classification

Table 1 shows tentative criteria for the etiological classification in CAP patients. Seven categories are as follows: (1) bacterial, (2) mixed infection of viral and bacterial, (3) viral, (4) mycoplasmal, (5) mixed infection of mycoplasmal (chlamydial) and bacterial, (6) mixed infection of mycoplasmal (chlamydial) and viral, and (7) unknown that could not identify any etiological agent.

Statistical analysis

Statistical analysis of the difference in clinical findings in relation to the causative pathogens was performed using the Fisher's exact test. WBC and CRP values on the day of admission were analyzed using the box-and-whisker plot method. The lower hinge, median, and upper hinge of the box corresponded to the 25%, 50%, and 75% percentiles, respectively; half of the cases were included in the box. The dotted line in each box is 1.5 times the quartile deviation.

Results

Patients

One hundred and seventeen CAP cases were 59 male and 58 female patients during May 2004 to April 2005. The

Table 1. Tentative criteria for the etiological classification of pediatric community-acquired pneumonia (CAP)

Diagnosis	Criteria
Bacterial	(i) CRP ≧3.8 mg/dl or WBC ≧$13 \times 10^3\,mm^{-3}$ (ii) Blood culture positive (iii) *S. pneumoniae* and/or *H. influenzae* ≧10^4 CFU in nasopharyngeal (iv) Inflammation findings of PMN in nasopharyngeal (v) Antibacterial agent was effective
Mixed infection Viral–bacterial	In addition to the above (i)–(iv) criteria for "Bacterial" (i) Significant rise of virus antibody (ii) PCR positive for virus
Viral	(i) Significant rise of virus antibody (ii) PCR positive for virus (iii) Antibacterial agent was not effective (iv) Improved without an antibacterial agent
Mycoplasmal (chlamydial)	(i) Significant rise of antibody (ii) PCR positive for *M. pneumoniae* (*C. pneumoniae*)
Mixed infection Mycoplasmal (chlamydial)–bacterial	In addition to (i)–(iv) criteria for "Bacterial" (i) Significant rise of antibody against *M. pneumoniae* (*C. pneumoniae*) (ii) PCR positive for *M. pneumoniae* (*C. pneumoniae*)
Mixed infection Mycoplasmal (chlamydial)–viral	In addition to the two criteria for "Mycoplasmal" (i) Significant rise of virus antibody (ii) PCR positive for virus
Unknown	Not detected any etiologic agent

CRP, C-Reactive protein; WBC, white blood cell count; CFU, colony-forming units; PMN, polymorphonuclease leukocyte; PCR, polymerase chain reaction

patients' ages were <1 year for 26 cases (22.2%), 1–2 years for 49 (41.9%), 3–5 years for 30 (25.6%), and ≥6 years for 12 cases (10.3%).

As described previously, the decision to admit was determined using chest X-rays (i.e., segmental pneumonia, bronchial pneumonia, atypical pneumonia, or interstitial shadows), acute respiratory symptoms, and deterioration in their general state. Fifty-eight cases (49.6%) had previously received oral antimicrobial agents within the 2 weeks prior to their hospitalization.

Viral pathogens

MPCR with 11 viruses were correlated with serological test results (Table 2). All PCR positive cases for RSV, Adeno, FluA, FluB, PIV-1, and PIV-3 showed significantly high antibody titers to the corresponding virus. The specificity of PCR for these agents was 100%; however, the sensitivity of the PCR was 63.6%–100% for viral infections alone and only 21.1%–75.0% for mixed infections. Simultaneous infections with two viruses with Adeno and RSV or hMPV and PIV-3 were identified in two patients each.

The cumulative positive cases determined by serology and PCR were 23 RSV (19.7%) cases, 23 PIV1-3 (19.7%), 13 Adeno (11.1%), 9 FluA (7.7%), 9 FluB (7.7%), 6 Rhino (5.1%), and 3 hMPV (2.6%) cases. No cases having Corona and hBoV infection were identified.

Bacterial pathogens

The bacteria suspected to be the causative pathogens was determined by standard culture and real-time PCR for six pathogens: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Streptococcus pyogenes*, and *Legionella pneumophila* (Table 3). *Streptococcus pneumoniae* was suspected in 47 (40.2%) cases, *H. influenzae* in 16 (13.7%), *M. pneumoniae* in 17 (14.5%), *C. pneumoniae* in 1 (0.9%), and *Moraxella catarrhalis* in 1 case (0.9%). Mixed infections with both *S. pneumoniae* and *H. influenzae* were suspected in 11 (9.4%) cases. In total, *S. pneumoniae* and *H. influenzae* CAP infection was found in 58(49.5%) and 27(23.0%) cases, respectively.

In the patients suspected of having an infection caused by *S. pneumoniae* and *H. influenzae*, the real-time PCR results were all positive with early threshold cycles of 15–25 that indicated more than 1000 CFU per sample. Direct bacterial count showed more than 10^4 CFU per sample using standard culture and was found 83.3% and 84.6% of the time, respectively, for *S. pneumoniae* and *H. influenzae*.

Seventeen cases identified as *M. pneumoniae* infection showed single (ten cases; 8.5%) and mixed (seven cases; 6.0%) infections with other microorganisms. Fourteen (82.3%) of these cases were identified rapidly by real-time PCR.

Table 2. Viruses identified as etiologic agents by PCR and/or serologic test results in CAP patients

Virus	No. of patients[a]	Viral infection		Mixed infection[b]	
		Serological positive	PCR positive	Serological positive	PCR positive
Respiratory syncytial virus	23 (19.7)	11	7 (63.6)	12	6 (50.0)
Adenovirus	13 (11.1)	9	8 (88.9)	4	3 (75.0)
Influenza virus A and B	9 (7.7)	3	3 (100)	6	4 (66.7)
Parainfluenza viruses 1-3	23 (19.7)	4	4 (100)	19	4 (21.1)
Human metapneumovirus	3 (2.6)	ND	0	ND	3
Rhinovirus	6 (5.1)	ND	1	ND	5
Total	77 (65.8)	27	23	41	25

Data shown in parentheses are percentages
ND, Not determined
[a] Percentage for the total number of patients
[b] Mixed infections means viral and bacterial coinfections

Table 3. Bacterial pathogens suspected as etiologic agents with high probability in pediatric patients with CAP

Bacterial pathogens	No. of patients[a]	Bacterial infection alone	Mixed infection with viruses	Mixed infection with *M. pneumoniae*
Streptococcus pneumoniae	47 (40.2)	18	27	2
Haemophilus influenzae	16 (13.7)	3	12	1
S. pneumoniae and *H. influenzae*	11 (9.4)	4	5	2
Moraxella catarrhalis	1 (0.9)		1	–
Mycoplasma pneumoniae	17 (14.5)	10	2	–
Chlamydophila pneumoniae	1 (0.9)	1	–	–
Total	88 (75.2)[b]	36	47	5

[a] Percentages given in parentheses for the total number of 117 patients
[b] Total cases excluded mixed infection of *M. pneumoniae* and other bacteria

Chlamydophila pneumoniae infection was identified in only one (0.9%) case using ELISA serology and PCR. In the 58 isolates of *S. pneumoniae*, 12 isolates belonged to capsule serotype 6B, 8 to 19F, 7 to 6A, 14 to 23F, 2 to serotype 19A, and 15 to other types.

Etiologic agents and distribution by patient age

The etiologic agents found in the 117 patients are shown in Fig. 1. Bacterial infection caused by *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* was identified in 27 (21.4%) of the patients, viral-mixed and bacterial-mixed infection in 45 (38.5%), *M. pneumoniae* infection in 10 (8.5%), *M. pneumoniae* and viral mixed infections in 2 (1.7%), *M. pneumoniae* and bacterial mixed infections in 5 (4.3%), *C. pneumoniae* in 1 (0.9%), and viral infections in 27 (23.1%) cases. Infections where etiologic agent was unknown occurred in only 2 (1.7%) of all the patients examined here.

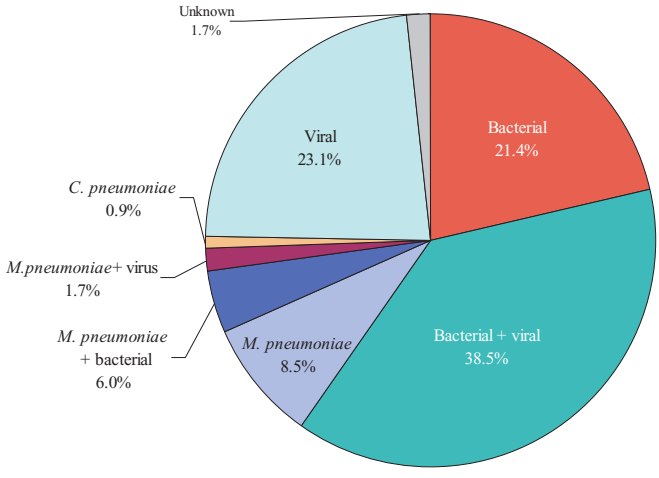

Fig. 1. Pathogens suspected with high probability as the etiologic agents in 117 hospitalized children with community-acquired pneumonia

The rate of individual pathogens in the patients distributed by age is shown in Fig. 2. Viral infection and mixed infection with viruses and bacteria were the most frequent among children 6 months to 3 years of age, bacterial infection alone occurred in those aged 1–6 years, and *M. pneumoniae* occurred in those aged 3 years or older.

Clinical characteristics according to etiologic agents

Table 4 shows the differences in clinical findings among patients classified into groups according to the etiologic agents: viral infections (n = 27, Group A), viral-mixed and bacterial-mixed infections (n = 45, Group B), bacterial infections (n = 25, Group C), *M. pneumoniae* infections (n = 10, Group D), and *M. pneumoniae* and bacterial mixed infections (n = 5, Group E).

The following clinical findings on admission were classified according to the respective criteria: (1) chest X-ray findings, (2) with/without asthma, (3) respiration rate, (4) WBC, and (5) CRP. They were analyzed statistically using the Fisher's exact test. As expected, significant differences were noted in chest X-rays among the five groups with signs of interstitial pneumonia being frequently found in patients with viral infection, segmental pneumonia in those with bacterial infections, bronchial or segmental pneumonia in those with mixed infections, and with atypical pneumonia in patients having *M. pneumoniae* infections.

Viral infection and viral and bacterial mixed infections were more frequent in children with asthma compared with other infections. With regard to respiratory rate, tachypnea was not observed in *M. pneumoniae* infections. WBC and CRP differed significantly between viral and bacterial infection, and between bacterial or mixed and *M. pneumoniae* groups.

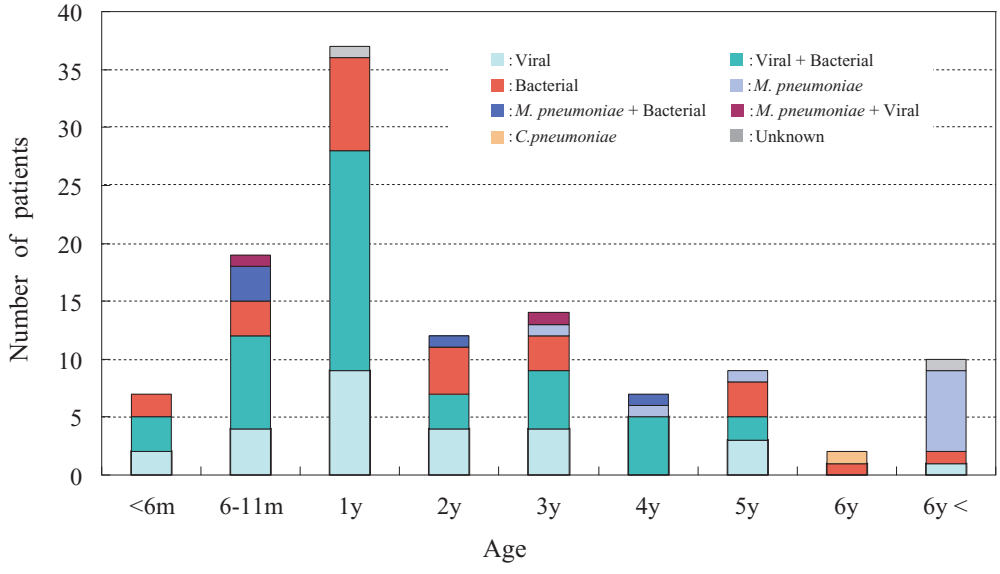

Fig. 2. Causative pathogens in 117 hospitalized children with community-acquired pneumonia distributed by age

Table 4. Characteristics of some clinical findings among 112 hospitalized children with CAP classified into etiologic agents

Category		$n = 112$	Group A viral ($n = 27$)	Group B mixed ($n = 45$)	Group C bacterial ($n = 25$)	Group D mycoplasmal ($n = 10$)	Group E mycoplasma and bacterial ($n = 5$)	Fisher's exact test
Chest X-ray finding	Interstitial	21	21 (100)[a]	0	0	0	0	
	Bronchial	38	5 (13.1)	27 (71.0)	6 (15.8)	0	0	$P < 0.001$
	Segmental	47	0	18 (38.3)	19 (40.4)	6 (12.8)	4 (8.5)	
	Atypical	6	1 (16.7)	0	0	4 (66.7)	1 (16.7)	
Asthma	–	88	14 (15.9)	35 (39.8)	24 (27.2)	10 (11.4)	5 (5.7)	$P < 0.001$
	+	24	13 (54.2)	10 (41.7)	1 (4.2)	0	0	
Respiratory rate	20–29	17	6 (35.3)	2 (11.8)	0	8 (47.0)	1 (5.9)	
	30–39	32	8 (25.0)	13 (40.6)	8 (25.0)	2 (6.3)	1 (3.1)	$P < 0.001$
	40–49	26	4 (15.4)	14 (53.8)	7 (26.9)	0	1 (3.8)	
	≥50	37	9 (24.3)	16 (43.2)	10 (27.0)	0	2 (5.4)	
WBC (mm^{-3})	$<10 \times 10^3$	26	11 (42.3)	6 (23.1)	0	9 (34.6)	0	
	$10–15 \times 10^3$	31	8 (25.8)	13 (41.9)	8 (25.8)	1 (3.2)	1 (3.2)	$P < 0.001$
	$\geq 15 \times 10^3$	55	8 (14.5)	26 (47.3)	17 (30.9)	0	4 (7.3)	
CRP (mg/dl)	<1.9	21	15 (71.4)	2 (9.5)	0	4 (19.0)	0	
	1.9–3.8	24	3 (12.5)	13 (59.1)	4 (16.7)	4 (16.7)	0	
	3.8–5.7	23	3 (13.0)	13 (56.5)	6 (26.1)	0	1 (4.3)	$P < 0.001$
	≥5	44	6 (13.6)	17 (38.6)	15 (34.1)	2 (4.5)	4 (9.1)	

[a]The values in parentheses show percentage in each category

WBC and CRP values compared with causative pathogens

WBC and CRP values of 112 patients on the day of admission were plotted according to the respective causative pathogens (Figs. 3 and 4). Patients with an unclear causative pathogen ($n = 2$), *C. pneumoniae* ($n = 1$), and mixed infections with *M. pneumoniae* and virus ($n = 2$) were excluded. The data were analyzed using the box-and-whisker plot method where each box encompasses 50% of the cases.

As shown in Fig. 3, the median WBC values in the patients with viral, viral and bacterial, bacterial, *M. pneumoniae*, and *M. pneumoniae* and bacterial infections were 11.7×10^3, 15.6×10^3, 17.8×10^3, 6.7×10^3, and $21.5 \times 10^3\,mm^{-3}$, respectively. In patients with defined viral and bacterial mixed infections, the 50% box of WBC values was located between those of the viral and bacterial cases.

As shown in Fig. 4, the median values of the CRP in patients with viral, viral and bacterial, bacterial, *M. pneumoniae*, and *M. pneumoniae* and bacterial infections were 1.4, 4.8, 6.3, 1.4, and 6.4 mg/dl, respectively. The 50% box of cases having bacterial infection show clearly the highest CRP values and do not overlap with the box in cases with *M. pneumoniae* infection. The median CRP value in the case of mixed infections with virus and bacteria was intermediate between the viral and bacterial infections similar to the WBC.

Evaluation of antimicrobial agents selected empirically

The relation between the empirically selected antibiotic for 117 cases and the causative pathogens is shown in Table 5. The decision to use antimicrobials and the selective criterion for the initial treatment were as outlined in Patients and methods. Antibiotics were used in 97 patients (82.9%), namely SBT/ABPC in 61 (52.1%); CTRX in 8 (6.8%); a carbapenem, either MEPM or PAPM, in 12 (10.3%); AZM in 10 (8.5%); MINO in 3 (2.6%); and SBT/ABPC and AZM in 2 (1.7%) patients. No antibiotic was used in 20 (17.0%) patients.

The initially selected antibiotic was retrospectively considered inappropriate in 9 patients with viral infection (Adeno, 4; RSV, 3; InfA, 1; PIV-3, 1), 1 with *M. pneumonia* and viral mixed infection, and 1 patient with an undetermined causative pathogen. Out of the 117 cases, antimicrobials were inappropriately used in 11 (9.4%) cases.

Clinically, defervescence was achieved within 24 h after admission in all the patients with bacterial infections. The average duration of antimicrobial treatment was 3–5 days. The duration of antibiotic therapy was also 4 days in four patients in whom *S. pneumoniae* or *H. influenzae* was isolated from blood cultures taken at admission.

After the termination of treatment, no patient experienced a relapse or was refractory to treatment. However, we experienced four cases having recurrent fever whose hospitalization was slightly prolonged, but this was not due to relapse of pneumonia and all of them recovered spontaneously without further antimicrobial use.

Discussion

Identification of the causative pathogens in children with CAP is not always easy because sputum or bronchoalveolar lavage (BAL) samples cannot be obtained as routinely performed in adults. To diagnose children, the causative pathogen is suspected from the history, chest X-ray, and blood examination data while taking into account the patient's age, and then the antibiotic is selected empirically. Recently

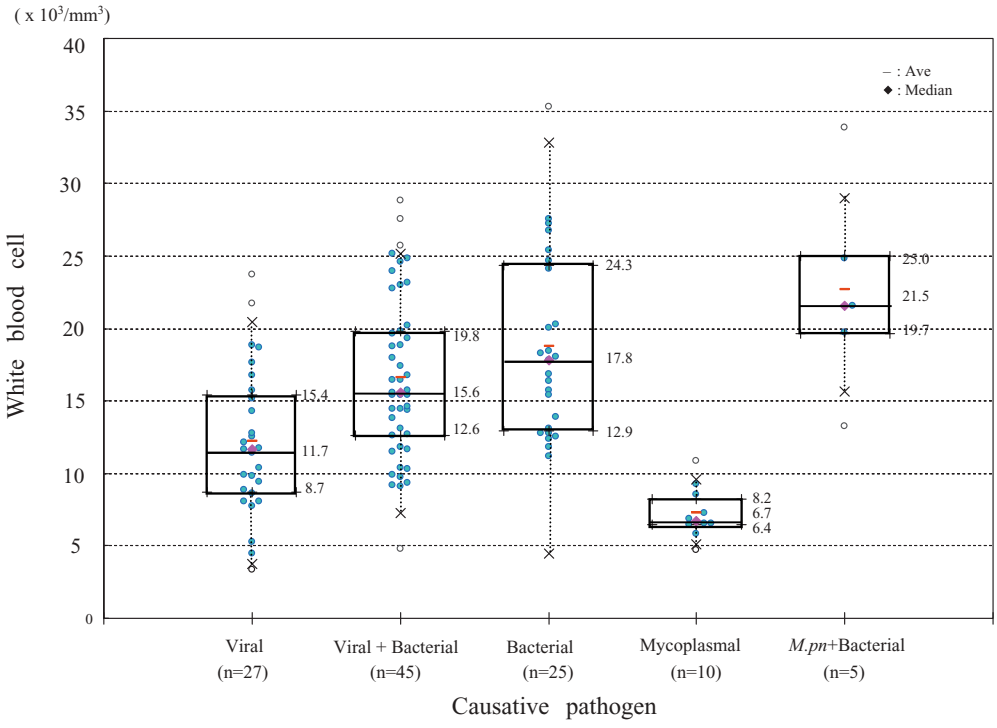

Fig. 3. Characteristics of white blood cell counts analyzed by box-and-whiskers plot method by causative pathogens

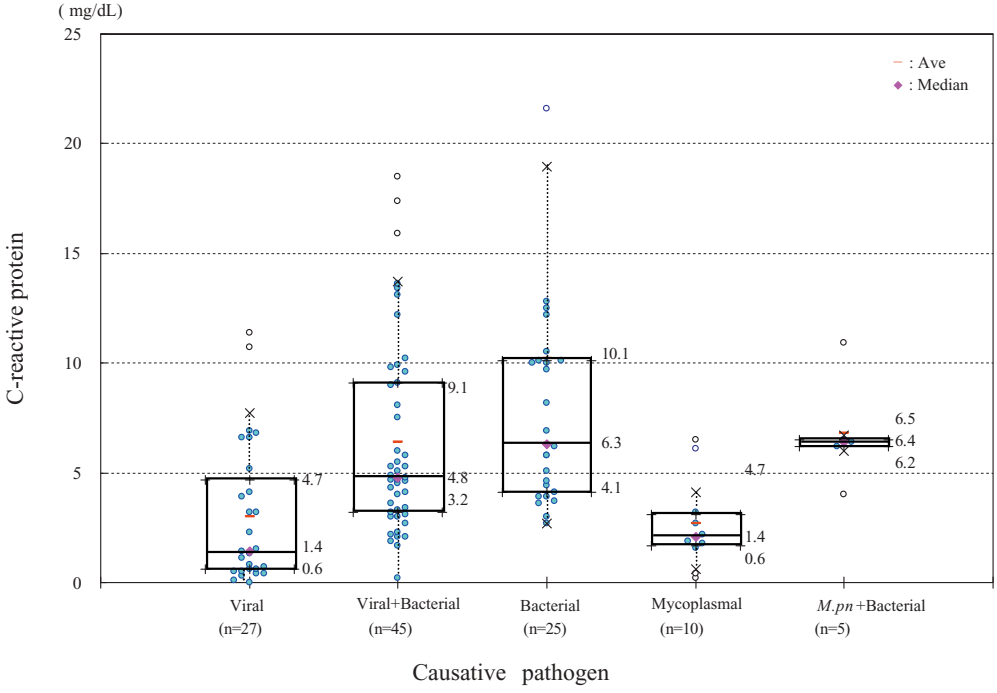

Fig. 4. Characteristics of C-reactive protein values analyzed by the box-and-whiskers plot method by causative pathogens

in Japan,[10] the guidelines for the treatment and management of respiratory tract infection in pediatric patients have been proposed to promote optimal chemotherapy, and similar guidelines are found in other countries.[25,26] However, the incidence rate of pathogenic microorganisms in pediatric CAP in other countries likely differs due to many factors such as the health insurance system, vaccination programs, kinds of antibiotics predominantly used, and population density. There are published studies of the use of PCR methods to determine the pathogens in CAP,[11–16] and the simultaneous detection of both bacteria and viruses is expected to enhance the accuracy of CAP diagnosis.

Here our aim was to identify bacteria and viruses using PCR within a short time frame and to evaluate the PCR results in relation to clinical findings. As previously described,[17] DNA/RNA samples were extracted from clini-

Table 5. Retrospective interpretation for dosing of antimicrobial agents for 117 patients with CAP

Causative pathogen	No. of patients	Not used	Antibiotics used					
			SBT/ampicillin	Ceftriaxone	Panipenem or meropenem	Azithromycin	Minocycline	Azithromycin + SBT/ampicillin
Viral alone	27	18	6[a]	1[a]	0	1[a]	1[a]	0
Viral + bacterial	45	0	32	6	7	0	0	0
Bacterial alone	25	0	19	1	5	0	0	0
Mycoplasma pneumoniae alone	10	0	0	0	0	8	2	0
Mycoplasma pneumoniae + bacterial	5	0	4	0	0	0	0	1
Mycoplasma pneumoniae + viral	2	1	1[a]	0	0	0	0	0
Chlamydophila pneumoniae alone	1	0	0	0	0	1	0	0
Unknown	2	1	0	0	0	0	1[a]	0
Total	117	20 (17.1%)	62 (53.0%)	8 (6.8%)	12 (10.3%)	10 (8.5%)	4 (3.4%)	1 (0.8%)

SBT, Sulbactam
[a]Antibiotics were used improperly

cal specimens using the EXTRAGEN II kit and bacterial detection was performed using real-time PCR; MPCR was performed for viral detection. Although, the results for bacterial analysis by the real-time PCR constructed by us can be rapidly obtained within 1.5 h, MPCR for viruses requires 5 h. In the near future, it is anticipated we will also be able to accomplish viral identification with real-time PCR.

We used nasopharyngeal samples as a source to identify the causative pathogens. These materials are appropriate to identify viruses, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*, but this sample type is questionable for *Streptococcus pneumoniae* and *Haemophilus influenzae* because they can be present in normal individuals. A positive result for *S. pneumoniae* and *H. influenzae* by real-time PCR should be carefully considered as to whether it indicates their causal relation with the infection or not where the physician should consider the chest X-rays, clinical symptoms, clinical laboratory findings such as WBC and CRP, bacterial amounts, and inflammation findings of leucocytes in nasopharyngeal samples.

The rate of viruses and bacteria identified in this study as the causative pathogens was similar to the data reported by Michelow et al.[12] and Juvèn et al.,[5] although the rate of *H. influenzae* differed. This was presumably because Hib vaccination has not been approved in Japan[27] where there are cases of pneumonia due to Hib and nontypable *H. influenzae*. Three CAP cases with Hib having positive blood cultures and positive real-time PCR were found among our 117 cases. *Chlamydophila pneumoniae* was detected in only one patient possibly because there were few children older than 6 years in this study.

As for the relation between the diagnosis of CAP and blood examination test, although some studies have only concluded that CRP and WBC can provide useful information for pneumococcal pneumonia,[28–31] these values are used by Japanese pediatricians as useful references in addition to routine chest X-ray to diagnose pneumonia. In clinical practice, we have observed these values do not fluctuate for about half a day after the onset of bacterial infection. In addition, in the cases of adenovirus infection, and virus or *M. pneumoniae* infection in school-age children, the values of WBC and CRP are relatively high. The physician must be mindful of these kinds of exceptional cases; however, as shown in our data, significant differences in these values are found and are correlated to the causative pathogens. At present, we believe the time from onset to presentation in hospital is relatively uniform in Japan as compared with other countries because of our universal health insurance system.

In this study, the antimicrobial agent that was empirically selected was found to have been inappropriately administered to 9.4% of the 117 cases. Using our techniques, we found the treatment for most cases was completed within 3–5 days. In general, the dosing period recommended in the guidelines is 7–10 days; however, we consider this is somewhat long because the antibiotics acted against the causative bacteria within a shorter period. Of the 58 strains of *S. pneumoniae*, 25 were Penicillin resistant streptococcus pneumonia (PRSP), and none of the patients experienced a relapse after treatment with SBT/ABPC and a carbapenem antibiotic (data not shown).

Viral and bacterial mixed infections were identified in 40.2% of the cases in addition to viral infection in 23.1%. Thus, involvement with the viral-related cases was 74 (63.2%) cases in total. Furthermore, cases relating to virus infections may be present, which could not be demonstrated when 5 days or more had elapsed after onset. Timing of acquiring the clinical samples is also very important to demonstrate the causative viruses.

In the future, we expect comprehensive and rapid identification of the causative pathogens outlined here will become routine in clinical practice.

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