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Original Article

Role of bacterial infection in the exacerbation of acute or prolonged asthma attack in children

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Abstract

Quantitatively cultured bacteria in sputum sampled from asthmatic children aged 0–14 years was examined to determine whether there is a relationship between asthmatic states and bacterial species present in the respiratory tract. We used cytological examination to improve the specificity of pathogens cultured in sputum. Asthmatic children were divided into three groups: (i) group 1, acute asthma attack (n = 191); (ii) group 2, prolonged asthma attack (n = 68); and (iii) group 3, pneumonia without asthma attack (n = 39). The number of specimens was 212, 75 and 44 for groups 1, 2 and 3, respectively. The number of specimens with pathogenic bacteria present in group 1 decreased with age, from 36.8% in infants under 1 year of age to 8.7% in children over the age of 9 years. The species of bacteria in group 1 were *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and these were distributed evenly in the samples. In group 2, pathogenic bacteria were present in 34.7% of patients (26/75), peaking at over 40% among children between 4 and 8 years of age. The presence of *H. influenzae* was dominant in group 2. The percentage of pathogenic bacteria present in group 3 specimens was 40.9%. The data suggest that there is a significant relationship between the presence of bacteria in sputum and clinical symptoms, such as fever and pneumonic episodes, during acute asthma attacks.

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Introduction

Viral infections of the respiratory tract have been reported to be a factor in the induction of bronchial asthma attacks in children. Improvements in viral diagnostic methods have shown a tendency to corroborate these findings.[1–6] In addition, a variety of respiratory viruses have been found to be associated with the exacerbation of existing asthma conditions. While the link between the presence of viral infections and the exacerbation of asthmatic conditions has been well documented and researched, the link between bacterial infections and the aggravation of asthmatic states is less clear. A key reason for this is that, unlike the diagnosis of viral infections, definitive diagnosis methods for bacterial infection, such as blood cultures or lung centesis, are limited. The presence of bacteria in expectorant may be caused by contamination from oropharyngeal microflora or may be the result of a simple colonization by bacteria. Specific diagnostic techniques for bacterial infections of the respiratory tract in children have not yet been established. However, diagnostic methods for detecting bacterial infections have improved in cases such as antigenemia and antigenuria, which are usually undetectable in normal children,[7,8] and in the measurement of specific antibodies against bacteria in serum.[9–12] By using these advanced methods, the concurrent rate of bacterial infection in cases of pneumonia or lower respiratory tract inflammation along with the presence of viral infection has been found to increase[7,13,14] in comparison with those found in previous reports.[3,15]

We have previously collected respiratory tract secretions from children with asthma and related diseases, used cytological examination and reported the data.[16,17] Furthermore, in the present study we have studied and assessed cultured bacteria from sputum, aspirated or expectorated at the time of hospital admission, from patients who suffered from acute or prolonged childhood asthma. The purpose of the present study was to identify the type of bacteria contained in secretions from the lower respiratory tracts of children with asthma and to determine the role of bacterial infection in the exacerbation of asthma attacks in children.

METHODS

Subjects

Subjects were patients suffering from childhood asthma between the ages of 6 months and 14 years who required hospitalization. Hospitalization and medical treatment took place at Chiba Children's Hospital, Chiba Prefecture, Japan. Patients having past histories of more than two episodes of wheezy distress were diagnosed as having asthma, while patients with other organic diseases were excluded from the present study. Subjects presenting with an episode of temperature rising to above 37.7°C within 3 days before or after their admission were diagnosed as having pyrexia and those having an abnormal shadow in their chest roentgenogram were diagnosed as pneumonic. Chest roentgenograms and sputum were taken at the time of admission. According to their symptoms, patients were divided into three groups and the backgrounds of patients in each group are described in Table 1.

Group 1 (attack group)

Patients had severe or moderate acute wheezy distress and were admitted for the purpose of resolving respiratory obstruction. Of this group, there were some cases presenting concurrent symptoms, such as pyrexia and a slight pneumonia-like shadow in chest X-rays, during asthma attacks. This finding suggested the presence of infections. However, patients with massive pneumonia shadows suffering respiratory distress were not included in the study. Cases with wheezy distress, regardless of presence or absence of infectious symptoms, were classified as group 1 subjects.

Group 2 (prolonged group)

Wheezing was audible through a stethoscope or by ear at the time of the patients' visits, but respiratory distress was not so serious as to disturb daily activity. Patients included in this group suffered from coughing and wheezing during the night, as well as having mild to moderate asthma attacks during the day. These symptoms were present intermittently for more than 10 days.

Table 1 Characteristics of patients and episodes

Group		Age (years)						Total
		< 1	1	2–3	4–5	6–8	> 9	
1	No. cases	16	44	61	36	13	21	191
	Positive RAST	9	34	54	34	11	18	160
	No. episodes	19	47	63	43	17	23	212
	Febrile episodes	12	24	22	15	4	5	82
	Pneumonia	6	8	7	11	2	0	34
2	No. cases	0	3	10	18	17	20	68
	Positive RAST	0	1	9	15	15	19	59
	No. episodes	0	4	10	19	20	22	75
	Febrile episodes	0	1	6	2	3	2	14
	Pneumonia	0	0	1	1	0	0	2
3	No. cases	0	3	11	13	9	3	39
	Positive RAST	0	1	10	13	9	3	36
	No. episodes	0	4	11	16	10	3	44
	Febrile episodes	0	3	8	13	6	1	31
	Pneumonia	0	4	11	16	10	3	44

RAST, radioallergosorbent test.

Group 3 (pneumonic group)

This patient group comprised asthmatic patients who were hospitalized for the treatment of infectious symptoms, such as coughing or pyrexia, without the occurrence of asthma attack. All presented pneumonic shadows in chest roentgenograms. These patients had three or more asthma attacks in their past histories.

All patients in all three groups underwent a variety of medical treatments, including intravenous infusion, bronchodilatation and lung physiotherapy. However, no patient received mechanical intubation. Ultimately, all these patients were released after full remission.

Table 1 shows allergic reactions for the subjects in this study by age. Mites, egg white and milk are considered to be the major allergens in Japan. Table 1 shows the quantity of specific IgE measured by enzyme immunosorbent assay. Measurement of specific IgE was performed according to the manufacturer (Pharmacia CAP-System FEIA®; Pharmacia-Upjohn, Uppsala, Sweden). Measurement results indicate the number of cases that possess specific IgE antibodies to one of the three antigens. For all age groups, the positive rate of IgE occurrence in each age and study group was high. The breakdown by episode was 82 febrile episodes and 34 pneumonic episodes in group 1, 14 febrile episodes and two pneumonic episodes in group 2 and 31 febrile episodes in group 3.

Specimen collection and processing

Both expectorated and aspirated sputa were used as specimens for bacterial culture and microscopic examination. For patients who were capable of expectorating, expectorated sputum was used as a specimen. For infants or young children who were not capable of expectorating, sputum was collected by oropharyngeal suction. In either case, we confirmed that the secretion obtained ascended from the lower respiratory tract during coughing. As described previously,[16] sputum was harvested using a suction tube with a trap. When aspirating sputum, we rubbed the lower portion of the patient's cricoid cartilage to initiate the coughing reflex. The collected secretion was required to contain substantial mucus or purulent mass. If the secretion was small in volume, it was centrifuged at 1600 *g* for 10 min and the sediment collected as a specimen. A portion of the specimen acquired with this method was put on a slide, stained and observed under a microscope. Specimens without the presence of macrophages or specimens containing diffuse massive squamous epithelial cells were excluded from further consideration.

After homogenizing the specimen with the tip of a platinum loop, 0.01 mL was streak-inoculated directly onto trypticase-soy agar plates supplemented with 5% sheep blood and chocolate agar (BBL, Berton Dickinson, Tokyo, Japan) and incubated overnight at 35°C in 7% CO_2. The bacterial species were identified according to the standard method.[18] Briefly, strains of *Streptococcus pneumoniae* were identified on the basis of α-hemolysis on blood agar, optochin susceptibility and bile solubility. Strains of *H. influenzae* were identified by Gram-staining appearance, a requirement for X factor and V factor and hemolysis of horse blood. *Moraxella catarrhalis* was identified using the RapID NH® kit (Innovative Diagnostic System Inc., Noreross, GA, USA). The number of bacteria was semi-quantitatively estimated from the distribution of colonies in the streak culture. When an adequate amount of sample was not available, sampling was done a few hours after transfusion or bronchodilatory treatment. For patients admitted in the evening, sputum was collected before any use of antibiotics and was kept at 4°C overnight. If antibiotics were not used, sputum was collected the following morning. We were unable to identify the type, if any, of oral medications administered prior to hospitalization. However, it was confirmed that no patient was administered antibiotics parenterally.

RESULTS

Bacterial culture results

Table 2 shows the presence of pathogenic bacteria in secretions of the lower respiratory tract cultured in excess of 10^4 colony forming units (CFU)/mL. The number of specimens with at least one positive pathogenic bacterial culture present was 43/212 (20.3%) in group 1, 26/75 (34.7%) in group 2 and 18/44 (40.9%) in group 3. Pathogenic bacterial counts were, for the most part, 10^6 CFU/mL or at least 10-fold greater than counts for the non-pathogenic varieties, such as *Neisseria* and *Streptococcus viridans*. The number of specimens in which one of the pathogenic bacteria satisfied this criteria was between 72 and 79% in all three groups. Five cases of multiple bacterial infections were observed in group 1, three cases in group 2 and five cases in group 3.

Figure 1 shows the incidence of infection, counted by bacterial species. *Haemophilus influenzae*, *Moraxella catarrhalis* and *S. pneumoniae* were commonly cultured. One case of *Streptococcus pyogenes* was found, but

Table 2 Presence of pathogenic bacteria in sputum

Group		Age (years)						Total
		< 1	1	2–3	4–5	6–8	≥ 9	
1	No. episodes	19	47	63	43	17	23	212
	> 10^5 CFU/mL or dominant	5	11	6	8	2	2	34
	10^4–10^5 CFU/mL	2	2	3	2	0	0	9
2	No. episodes	0	4	10	19	20	22	75
	> 10^5 CFU/mL or dominant	0	0	2	3	9	4	18
	10^4–10^5 CFU/mL	0	1	1	5	0	1	8
3	No. episodes	0	4	11	16	10	3	44
	> 10^5 CFU/mL or dominant	0	0	4	5	3	1	13
	10^4–10^5 CFU/mL	0	0	2	2	1	0	5

Concentrations of pathogenic bacteria in the sputum greater than 10^4 CFU/mL are shown. Dominant, presence of pathogenic bacteria in concentrations at least ten times greater than non-pathogenic bacteria. CFU, colony forming units.

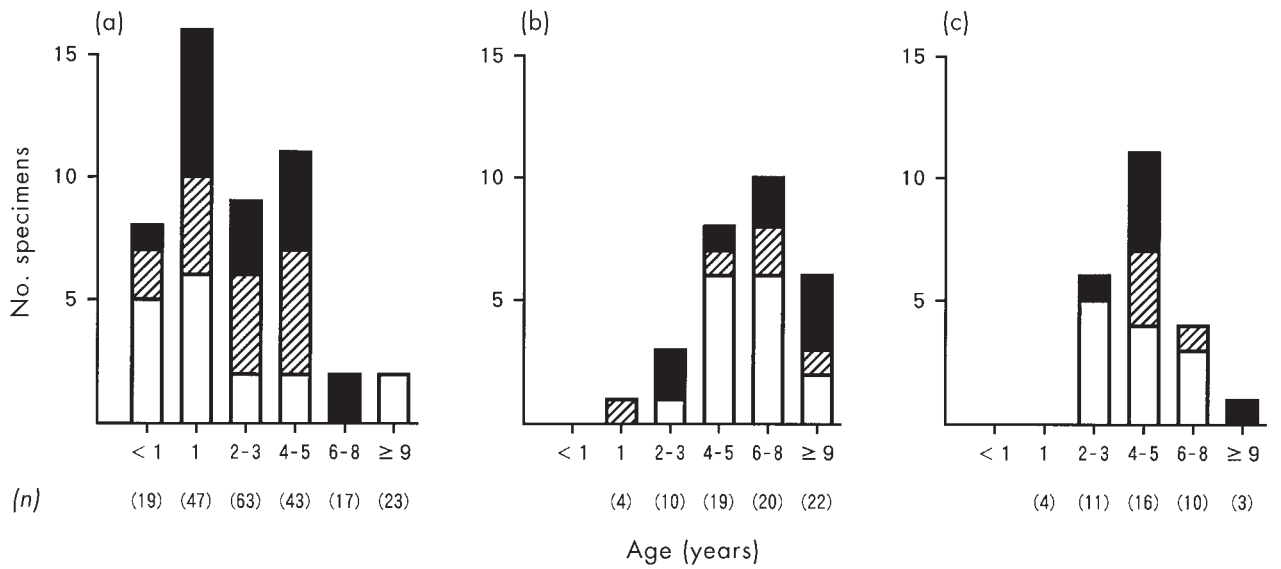

Fig. 1 Bacterial species in sputum taken from asthmatic children in (a) group1, (b) group 2 and (c) group 3. (□), *Haemophilus influenzae*; (▨), *Moraxella catarrhalis*; (■), *Streptococcus pneumoniae*.

excluded from further investigation in this study. There were 17 cases of *H. influenzae* infection, 15 cases of *M. catarrhalis* infection and 16 cases of *S. pneumoniae* infection in group 1. In groups 2 and 3, the respective numbers of infections with these bacteria were 15, five and eight cases and 12, four and six cases. The prevalence of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* was even in group 1 samples.

Figure 2 shows the isolation rate of bacterial-positive specimens as the number of positive specimens as a percentage of the total number of examined specimens in each of the three groups. In group 1, the isolation rate declined as patients became older, decreasing from 36.8% in infants under the age of 1 year to 8.7% in children over the age of 9 years. In group 2, the isolation rate exceeded 40% among patients between 4 and 8 years of age. Because the number of subjects aged 1 year and below in group 3 was too small, this age group was omitted in Fig. 2. In group 3, bacterial infection was observed consistently in 30–50% of patients.

Next, we attempted to determine a correlation between the number of febrile episodes and bacterial colonization in the lower respiratory tract in each patient group. Table 3 shows the relationship between the presence of bacteria and febrile or afebrile conditions in the three patient groups. In group 1, the positive bacterial rate in the febrile cases was 24 of 82 cases (29.3%), but in afebrile cases was 19 of 130 cases (14.6%). A statistically significant difference between these two groups was observed (P = 0.0098). Similarly, we tried to find a

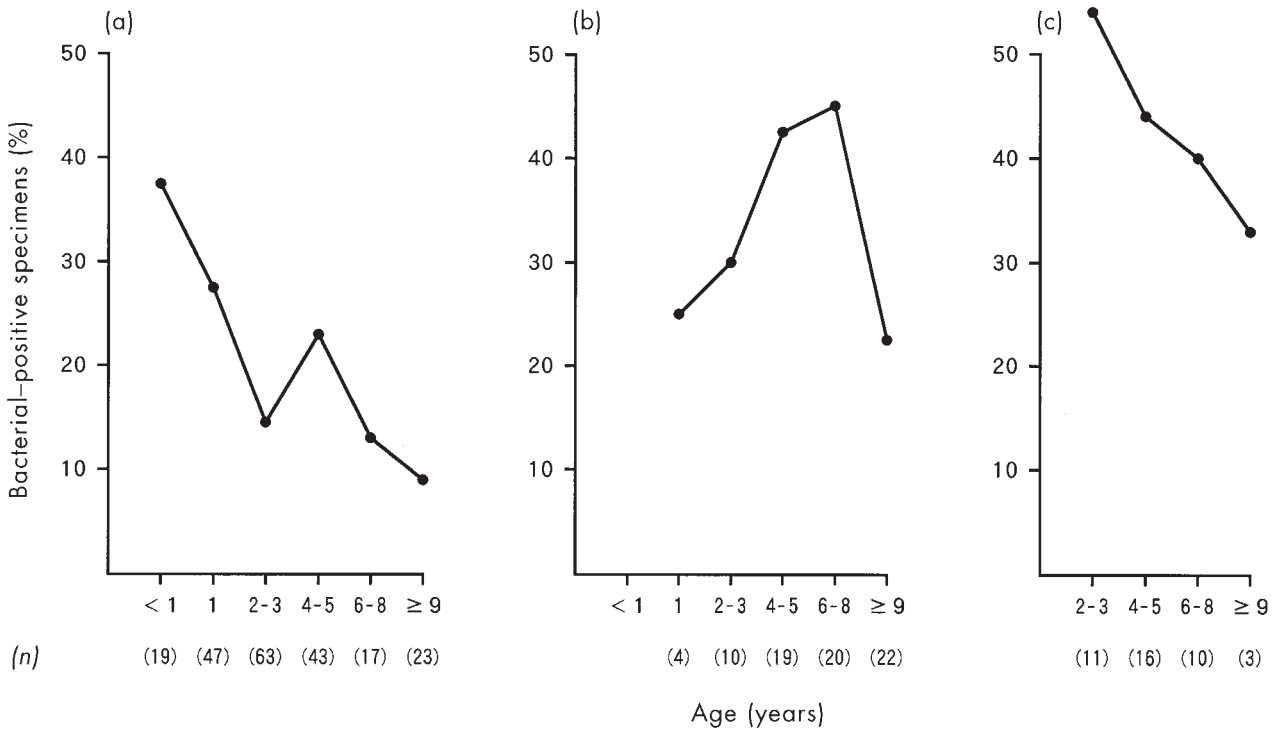

Fig. 2 Percentage of bacterial-positive specimens in the total number of specimens taken from asthmatic children in (a) group 1, (b) group 2 and (c) group 3.

Table 3 Relationship between the presence of bacteria and febrile conditions or pneumonic episodes

	Bacteria +	Bacteria –	Total
Group 1 (attack)			
Febrile	24	58	82
Afebrile	19*	111	130
Total	43	169	212
Pneumonia +	12	22	34
Pneumonia –	31†	147	178
Total	43	169	212
Group 3 (pneumonic)			
Febrile	16	15	31
Afebrile	2‡	11	13
Total	18	26	44

* $P = 0.0098$ compared with febrile cases in the presence of bacteria; † $P = 0.0175$ compared with pneumonic cases in the presence of bacteria; ‡ $P = 0.0257$ compared with febrile cases in the presence of bacteria.

correlation between pneumonic episodes and the presence of pathogenic bacteria. In group 1, the frequency of pathogenic bacteria in pneumonic cases was 12 of 34 cases (35.3%), but in non-pneumonic cases was 31 of 178 cases (17.4%). A statistically significant difference was observed between these figures ($P = 0.0175$). In group 3, the positive bacterial rate in pneumonic cases was 16 of 31 cases (51.6%), but in afebrile cases was two of 13 cases (15.4%). A statistically significant difference was observed between these two groups ($P = 0.0257$).

Relationship between pathogenic bacteria and C-reactive protein

Figure 3 shows the relationship between C-reactive protein (CRP) and the presence of pathogenic bacteria. In group 1, CRP values were determined and the correlation between the presence of pathogenic bacteria and CRP values was examined. Values for CRP were higher when pathogenic bacteria were present in all age groups. For the age group 6 months to 3 years, the mean value of CRP in children without pathogenic bacteria was 0.87, whereas that of children with pathogenic bacteria was 2.88. In the age group from 4 to 14 years, the mean value of CRP in children without pathogenic bacteria was 1.58, whereas that of children with pathogenic bacteria was 4.39. A significant difference in CRP levels was observed between patients with bacteria pathogens and those without, in both the 0–3 year ($P = 0.0056$) and the 4–14 year ($P = 0.0013$) age groups.

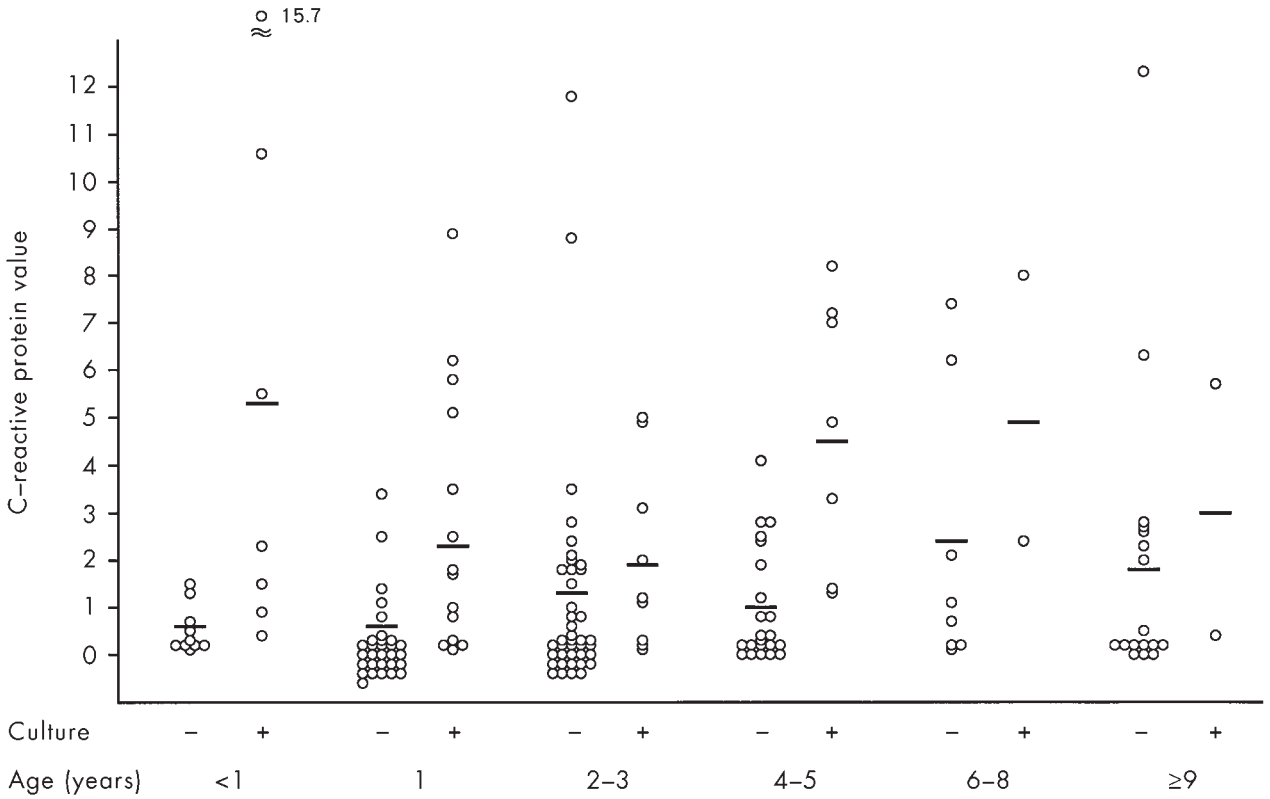

Fig. 3 C-reactive protein (CRP) values in cultures positive and negative for pathogenic bacteria in each age group. (—), average CRP value.

Discussion

The mechanism by which viral infections induce respiratory tract obstruction is still unknown. From recent studies using polymerase chain reactions for the detection of virus, viral infections are observed in 80% of cases with asthma attacks and in 80% of cases in the decrease of peak expiratory flow rate.[6] With advances in definitive diagnosis of bacterial infection, an increased rate of bacterial infection has been detected among children with inflammation in the lower respiratory tract.[7–14] On the basis of these facts, investigating the possible presence of bacterial infection at the time of asthma attack seems quite important. From results obtained in the present study, only three major species of bacteria were identified in culture: *H. influenzae*, *S. pneumoniae* and *M. catarrhalis*, which are frequently seen in lower respiratory tract infections in children[19–21] and chronic obstructive pulmonary disease in adults.[22]

Furthermore, we found some correlation between age and pathological condition in children with asthma. Young patients with asthma are often hospitalized with acute obstruction of the respiratory tract, concurrent with pyrexia and a slight pneumonic shadow on chest X-rays, suggestive of respiratory tract infection. In the present study, pathogenic bacteria were found in the highest frequency among infants (group 1). However, the isolation rate of pathogenic bacteria declined as the age of patient's increased. In contrast with patients in group 1, the isolation rate of pathogenic bacteria remained high among older children in group 2 during prolonged asthmatic states. These results suggest that bacterial infection correlates not only with age, but also with asthmatic condition. The presence of pathogenic bacteria was also at a high rate among children with pneumonia in group 3. The causes of pneumonia have been reported to be respiratory viruses and *Mycoplasma pneumoniae* among children.[23,24] We assume that secondary bacterial infection occurs immediately after primary inflammation of the lower respiratory tract due to non-bacterial pathogens. Once the oropharynx is colonized, viral infection may lead to bacterial multiplication. Under these conditions, colonized pathogenic bacteria in the upper respiratory tract are thought to spread towards the lower respiratory tract. Horn *et al.* isolated more rhinovirus in the lower respiratory tract than in the upper respiratory tract.[3]

Demonstration of a rise in specific antibodies against bacteria is strong evidence that an organism is a significant pathogen and not just a colonizer. Even using the quantitative culture of bronchoalveolar lavage fluid (BALF), or protected BALF (P-BALF) with a protected catheter does not guarantee that oropharyngeal contamination can be avoided completely. Much literature has set bacterial counts of more than 10^3–10^4 CFU/mL as a cut-off point for sample use.[25–30] Both BALF and P-BALF have been reported to be highly reliable if the cut-off point is set at above 10^4 CFU/mL. Kirkpatrick and Bass examined bacterial culture of BALF and P-BALF specimens from normal adults.[29] Bronchoalveolar lavage fluid is frequently contaminated by oropharyngeal bacterial flora, but quantitation revealed less than 10^4 CFU/mL in all specimens. Both P-BALF and BALF require bronchoscopical operation. Avital *et al.* have examined whether or not oropharyngeal suction can be substituted for BALF in the pediatric area.[31] They reported that the concordance of pathogenic bacterial species between specimens collected by oropharyngeal suction and by BALF was 89%, while the specificity was as high as 94%. Ramsey *et al.* conducted a study of patients with cystic fibrosis, comparing oropharyngeal cultures with the bacterial cultures of bronchial secretion collected with a bronchoscope.[32] They reported that the concordance was excellent for *Pseudomonas aeruginosa* and *Staphylococcus aureus* among cystic fibrosis patients and that oropharyngeal culture was effective in anticipating pathogenic bacteria in the lower respiratory tract. However, pathogenic bacteria infection could not be ruled out, even if the oropharyngeal culture was negative.

It is well known that cell differentiation by microscope can increase the degree of specificity in evaluating pathogenic bacteria.[33–37] In bacterial exacerbation of asthma, there is increased exfoliation of bronchial epithelial cells from the damaged bronchial mucosa and an increase of neutrophils in response to this injury. As a result, microscopic findings should show the minimal presence of squamous epithelial cells, an abundant existence of neutrophils and bacterial phagocytosis by neutrophils. An increase in bacterial flora without these cellular changes being noted in sputum should suggest that only bacterial colonization is present. Alveolar macrophages have been used as markers for the secretions of the lower respiratory tract in the present study. In expectorated sputum, a lump of macrophages is often found in the mass of neutrophils and eosinophils, while in aspirated sputum, macrophages can sometimes be found only after searching over several fields at a magnification of 100×. However, in the present study, we observed the presence of many neutrophils. We cannot deny the contamination of saliva in sputum but, given the timing of specimen collection, we believe that the majority of secretions originated in the lower respiratory tract. While saliva specimens often included *H. influenzae*, numbers of *H. influenzae* were rather limited.[38]

The present study encountered two problems: (i) adequate information was not available about non-bacterial pathogens in the respiratory tract; and (ii) any antibiotic medications taken before specimen collection were not identified. The true incidence of bacterial infection may be higher than that detected. Kalin *et al.* reported that pneumococci were isolated from 52% of pretreatment samples, but from only 8% of samples after treatment.[37] However, we believe our study differs because oral drug compliance may not be high at asthma attack and oral antibiotic-resistant bacterial strains are increasing in Japan. We found a significant relationship between the presence of bacteria in sputum specimens and febrile or pneumonic episodes. The mean value of CRP was high in cases with positive bacterial culture and may indicate that pathogenic bacteria are not only colonizers but also contribute to the aggravation of symptoms. While research on the inflammatory mechanisms of asthma attack is progressing, bacterial infection seems to be one of the factors that should be considered to induce allergic inflammation.

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