Acta Oto-Laryngologica

Detection of respiratory viruses in nasopharyngeal secretions and middle ear fluid from children with acute otitis media

Hisakazu Yano; Naohiro Okitsu; Toru Hori; Oshi Watanabe; Tomoko Kisu; Etsuko Hatagishi; Akira Suzuki; Michiko Okamoto; Akira Ohmi; Mitsuko Suetake; Syun Sagai; Toshimitsu Kobayashi; Hidekazu Nishimura

Virus Research Center, Clinical Research Division, Sendai Medical Center, Department of Otolaryngology, Tohoku Rosai Hospital, Department of Virology, Tohoku University Graduate School of Medicine, Department of Otolaryngology, Head and Neck Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan

First Published: 2009

Please scroll down for article
Detection of respiratory viruses in nasopharyngeal secretions and middle ear fluid from children with acute otitis media

HISAKAZU YANO1, NAOHIRO OKITSU2, TORU HORI1, OSHI WATANABE1, TOMOKO KISU1, ETSUKO HATAGISHI1, AKIRA SUZUKI3, MICHIKO OKAMOTO1, AKIRA OHMI1, MITSUKO SUETAKE2, SYUN SAGAI4, TOSHIMITSU KOBAYASHI1 & HIDEKAZU NISHIMURA1

1Virus Research Center, Clinical Research Division, Sendai Medical Center, 2Department of Otolaryngology, Tohoku Rosai Hospital, 3Department of Virology, Tohoku University Graduate School of Medicine and 4Department of Otolaryngology, Head and Neck Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan

Abstract

Conclusions. Our results suggest that various respiratory viruses contribute to the pathogenesis of acute otitis media (AOM). Objective. AOM is one of the most common complications of viral upper respiratory tract infections in children. Recently, the importance of respiratory viruses has been stressed as causative agents of AOM. Subjects and methods. A total of 1092 children ≤10 years old (average age 1.38 years) diagnosed as having AOM between 2002 and 2004 were studied. Bacterial and viral cultures of both nasopharyngeal secretions (NPS) and middle ear fluid (MEF) were performed for all 1092 children. Body temperature, changes of the tympanic membrane, and the number of days from the onset of illness were analyzed. Results. Respiratory viruses were detected in 360 of 1092 NPS specimens, including 157 isolates of respiratory syncytial virus and 88 of influenza virus. Among 1092 MEF specimens, 102 were virus-positive, including 43 for respiratory syncytial virus and 29 for influenza virus. In 75 children, respiratory viruses were detected in MEF. The viral detection rate was higher in children with fever at an early stage of their illness. The tympanic membrane changes associated with viral infection tended to be less severe, while changes were more severe in cases with bacterial infection, especially co-infection with bacteria and viruses.

Keywords: Acute otitis media, respiratory viruses, viral culture

Introduction

Acute otitis media (AOM) is one of the most common diseases of the upper respiratory tract in childhood. Recurrent AOM tends to affect children under 2 years of age, particularly those who have episodes of AOM during the first year of life. Treatment of AOM is the most frequent reason children take antibiotics in the USA [1], and most Japanese physicians also prescribe antibiotics for children with AOM. In the past, it was easy for physicians to treat AOM because the antibiotics were highly effective against the causative bacteria. However, overuse of antibiotics has been an important cause of the emergence of resistant bacteria [2,3], such as penicillin-resistant Streptococcus pneumoniae and β-lactamase non-producing Haemophilus influenzae. The incidence of recurrent AOM has recently increased in Japan, and many children now need hospitalization for treatment with intravenous antibiotics because of severe AOM with persistent purulent otorrhoea [4].

AOM is known to be frequently complicated by viral respiratory tract infection in children, with the main pathogens being respiratory viruses such as respiratory syncytial virus (RSV), influenza virus, parainfluenza virus, and enterovirus [5]. Heikkinen and Chomnaitree estimated that AOM occurs in approximately 20% of children infected with these viruses [6]. Recently, the importance of respiratory
viruses as causative agents of AOM has been emphasized [7], and isolation of respiratory viruses from middle ear fluid (MEF) has been reported [8,9]. It is important to clarify the role of these viruses and to determine the prevalence of viral infection in AOM patients to achieve appropriate use of antibiotics. In the present study, we investigated respiratory viruses in the nasopharyngeal secretions (NPS) and MEF of AOM patients, and also analyzed the clinical features of patients in whom respiratory viruses were detected in NPS and/or MEF.

Subjects and methods

Patients and study design

A total of 1092 children who attended the Department of Otolaryngology of Tohoku Rosai Hospital and were diagnosed as having AOM between January 1, 2002 and December 31, 2004, were studied. The children included 647 boys and 445 girls. They were all ≤10 years old, with the average age being 1.38 ± 1.54 years, and 918 children (84%) were aged ≤2 years. Diagnosis of AOM was done by otolaryngologists and was based on otoscopic detection of fluid in the middle ear associated with bulging, redness, and/or opacity of the tympanic membrane.

At the first visit, the body temperature and clinical severity (based on the changes of the tympanic membrane) were evaluated as described previously with some modifications [10]. Tympanic membrane changes were scored to rate the severity of inflammation based on bulging (0, 1, or 2 points), redness (0, 1, or 2 points), and opacity (0 or 1 point) in the patients whose tympanic membranes could be photographed endoscopically. Then the total AOM severity index was calculated as the sum of the above scores. The onset of symptoms, such as fever, otalgia, otorrhea, purulent rhinorrhea, and other respiratory symptoms, was defined as day 1 of the illness.

Specimens

After obtaining informed consent from the parents or guardians, myringotomy or tympanocentesis was performed to alleviate the patient’s aural symptoms and to collect MEF for bacterial and viral culture in all of the patients. NPS samples were also obtained for bacterial and viral culture.

Bacterial and viral culture of both NPS and MEF from all 1092 children was performed at the first visit. Both NPS and MEF specimens were immediately sent for routine bacterial examination and viral culture at the Microbiology Laboratory of Tohoku Rosai Hospital and the Virus Research Center of Sendai Medical Center, respectively. Viral culture was performed as described previously [11]. The NPS and MEF specimens were kept in transport medium, which was Eagle’s minimal essential medium (Sigma, St Louis, MO, USA) with 0.5% gelatin containing 500 units/ml of penicillln G and 500 µg/ml of streptomycin. Each specimen was centrifuged at 4000 g at 4°C for 15 min, and supernatant was inoculated into a tissue culture microplate containing human embryonic fibroblasts, HEp-2, Vero, HMV-II, B95a, and Mardin-Darby canine kidney cells. This microplate system can support the growth of many kinds of viruses, including RSV, influenza viruses, parainfluenza viruses, enteroviruses, adenoviruses, cytomegalovirus, herpes simplex virus, rhinovirus, mumps virus, and measles virus.

A rapid viral antigen test was also performed as an adjunct to diagnosis in the children with suspected influenza virus, RSV, or adenovirus infection based on their clinical presentation. Capilia Flu AB® (Nippon Becton Dickinson Co. Ltd, Tokyo, Japan), Testpack RSV® (Abbott Japan Co. Ltd, Tokyo, Japan), and Check Ad® (Alfresa Pharma Corporation, Osaka, Japan), respectively, were used for detection of influenza virus, RSV, and adenovirus antigens in NPS and/or MEF as described by the manufacturers.

Statistical analysis

The chi-squared test was used for statistical analysis as appropriate.

Results

Bacterial and viral culture

At least 1 of 3 common bacteria, i.e. S. pneumoniae, H. influenzae, or Moraxella catarrhalis, was isolated from 978/1092 NPS samples (90%) and 470/1092 MEF samples (43%). Among the 978 NPS samples, S. pneumoniae was isolated in 617 cases, H. influenzae in 569, and M. catarrhalis in 608. Among the 470 MEF samples, there were 243, 227, and 48 isolates, respectively (Table I). Of the 1092 children analyzed, respiratory viruses were detected in 384 (35%) patients, including 24 who only had positive results from MEF. Among 1092 NPS samples, 382 respiratory viruses were detected (22 samples had two viruses), including 157 isolates of RSV, 88 of influenza virus (60 influenza A, 15 influenza B, and 13 influenza C), 65 of adenovirus, 26 of cytomegalovirus, 21 of parainfluenza virus, 11 of enterovirus, and 9 of rhinovirus. Among the MEF samples, 104 respiratory viruses were detected (2 samples had two viruses), including 43 isolates of RSV, 29 of influenza virus (20 influenza A,
8 influenza B, and 1 influenza C), 13 of adenovirus, 3 of cytomegalovirus, 6 of parainfluenza virus, 5 of enterovirus, and 2 of rhinovirus (Table II).

Of the 384 virus-positive AOM children, the same virus was detected concurrently from both NPS and MEF samples in 73 cases (Table II). Several kinds of viruses were detected from both NPS and MEF samples from the same individual child, and the frequency of concurrent detection of RSV, influenza A virus, and adenovirus was higher than that of other viruses.

In about 28% of NPS and 3% of MEF specimens, both bacteria (S. pneumoniae, H. influenzae, or M. catarrhalis) and respiratory viruses were detected. In 5% of NPS and 7% of MEF specimens, only respiratory viruses were detected without isolation of any bacteria (Figure 1).

The patients in whom RSV or influenza virus was detected in MEF were analyzed for the presence of bacteria (Table III). H. influenzae was most frequently cultured in MEF containing RSV, although this correlation was not statistically significant.

### Table II. Viruses detected from nasopharyngeal secretions (NPS) and middle ear fluid (MEF) specimens (n = 1092).

<table>
<thead>
<tr>
<th>Viruses</th>
<th>NPS</th>
<th>MEF</th>
<th>Both NPS and MEF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory syncytial</td>
<td>157</td>
<td>43</td>
<td>33</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>60</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>15</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Influenza C virus</td>
<td>13</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Adenovirus†</td>
<td>65</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>26</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Parainfluenza virus</td>
<td>21</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>11</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>9</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Measles virus</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mumps virus</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>382§</td>
<td>104§</td>
<td>73</td>
</tr>
</tbody>
</table>

*No. of cases with concurrent virus detection from NPS and MEF within the same individual child.
†Isolated by culture and/or detected by rapid detection kit.
‡Including 22 cases in whom two viruses were detected.
§Including two cases in whom two viruses were detected.

### Table I. Bacteria (Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis) detected in nasopharyngeal secretions and middle ear fluid specimens from 1092 children with acute otitis media (AOM).

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Nasopharyngeal secretions</th>
<th>Middle ear fluid</th>
<th>Both NPS and MEF%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae</td>
<td>617 (57)</td>
<td>243 (22)</td>
<td>33</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>569 (52)</td>
<td>227 (21)</td>
<td>16</td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>608 (56)</td>
<td>48 (4)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>978 (90)</td>
<td>470 (43)</td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

Although AOM was once generally considered to be a bacterial infection, it has gradually become evident that respiratory viruses play a considerable role in its pathogenesis [6]. In the present study, we performed viral detection using both NPS and MEF specimens from 1092 children with AOM and about one-third of them were found to be infected with respiratory viruses. Various respiratory viruses were found in 10% of the MEF samples from these patients and 70% of them were the only pathogens. This suggests that respiratory viruses can be the causative organisms of AOM. These respiratory viruses detected from MEF in our study were mainly RSV, influenza virus, adenovirus, parainfluenza virus, and enterovirus, and the virus most frequently identified from NPS and MEF specimens was RSV. This result was similar to previous studies reported in different populations and localities [9,12].

We previously reported that human metapneumovirus (hMPV), first isolated from children with upper respiratory tract infections in 2001 [13], was detected in NPS and MEF samples from children...
with AOM [14]. We demonstrated that hMPV was associated with pediatric AOM and was found in 8 (7.8%) of 102 NPS specimens and 3 (2.9%) of 126 MEF specimens from children with AOM by RT-PCR. The prevalence of viral infection shows seasonal variation, but the duration of our previous study was only a few months. Therefore, we cannot directly compare the detection rate of respiratory viruses in this study with that of hMPV in the previous study, but hMPV may be considered a common pathogen of AOM. Recently, new respiratory viruses such as human bocavirus [15] and NL63 [16] have been detected in children with acute respiratory tract infections in addition to hMPV. Although neither bacteria nor viruses were detected in MEF specimens from 50% of our AOM patients, such new viruses may be involved.

We previously reported on the occurrence of AOM in patients with RSV infection. In that study, we examined the tympanic membranes of all children with RSV infection and AOM was found in 52% of them, with the rate increasing to 73% among children ≤ 2 years old. Although the respiratory viruses detected in AOM patients varied, the virus most frequently identified from NPS and MEF specimens was RSV in this study, so RSV is considered to be the most important viral cause of AOM. Vesa et al. mentioned that the seasonal pattern of upper respiratory infection incidence due to RSV coincided with the peaks of AOM occurrence and RSV was most often found at the time of a diagnosis of AOM [12]. In addition, Heikkinen et al. also reported that RSV was the most common virus detected in AOM patients and that it had a strong

Table III. Correlation between detection of viruses and bacteria in middle ear fluid specimens.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Respiratory syncytial virus (n = 43)</th>
<th>Influenza A virus (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae</td>
<td>3 (7)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>7* (16)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>2 (5)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*No significant differences for the comparison with influenza A virus (Fisher’s exact test).

with AOM [14]. We demonstrated that hMPV was associated with pediatric AOM and was found in 8 (7.8%) of 102 NPS specimens and 3 (2.9%) of 126 MEF specimens from children with AOM by RT-PCR. The prevalence of viral infection shows seasonal variation, but the duration of our previous study was only a few months. Therefore, we cannot directly compare the detection rate of respiratory viruses in this study with that of hMPV in the previous study, but hMPV may be considered a common pathogen of AOM. Recently, new respiratory viruses such as human bocavirus [15] and NL63 [16] have been detected in children with acute respiratory tract infections in addition to hMPV. Although neither bacteria nor viruses were detected in MEF specimens from 50% of our AOM patients, such new viruses may be involved.

We previously reported on the occurrence of AOM in patients with RSV infection. In that study, we examined the tympanic membranes of all children with RSV infection and AOM was found in 52% of them, with the rate increasing to 73% among children ≤ 2 years old. Although the respiratory viruses detected in AOM patients varied, the virus most frequently identified from NPS and MEF specimens was RSV in this study, so RSV is considered to be the most important viral cause of AOM. Vesa et al. mentioned that the seasonal pattern of upper respiratory infection incidence due to RSV coincided with the peaks of AOM occurrence and RSV was most often found at the time of a diagnosis of AOM [12]. In addition, Heikkinen et al. also reported that RSV was the most common virus detected in AOM patients and that it had a strong

Table III. Correlation between detection of viruses and bacteria in middle ear fluid specimens.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Respiratory syncytial virus (n = 43)</th>
<th>Influenza A virus (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae</td>
<td>3 (7)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>7* (16)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>2 (5)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*No significant differences for the comparison with influenza A virus (Fisher’s exact test).

Figure 1. Pattern of detection of bacteria (three common bacteria, namely Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis) and respiratory viruses in children with acute otitis media. *Includes cases in which the other bacteria were isolated.

Figure 2. Relationship between body temperature and the detection of bacteria and viruses in nasopharyngeal secretions and middle ear fluid. (A) Detection rate of bacteria (Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis). (B) Detection rate of respiratory viruses.
ability to invade the middle ear [9], while Simoes et al. demonstrated that immunoglobulin enriched with RSV-neutralizing antibodies could decrease the incidence of AOM [17]. This suggests that vaccination against RSV may have the potential to prevent AOM, and the use of RSV vaccine in the future may be promising.

Jiang et al. mentioned that RSV infection significantly enhances the attachment of H. influenzae to respiratory tract epithelial cells [18]. Even though no statistically significant association was observed in our study, H. influenzae was cultured more often from MEF containing RSV. Therefore, when we see AOM patients with RSV infection, we should pay attention to the possible occurrence of secondary H. influenzae infection. Similarly, there are some reports that antecedent influenza virus infection leads to S. pneumoniae infection causing AOM, sinusitis, and pneumonia [19]. In our study, no statistically significant association was observed between the detection of S. pneumoniae and influenza A virus. In Japan, rapid detection kits for influenza virus and anti-influenza therapy are commonly available [20]. Because cases of influenza may be treated early after diagnosis by rapid detection kit, this might have prevented us from detecting patients with secondary S. pneumoniae infection.

There have been few reports about the clinical features of AOM patients in whom respiratory viruses are detected in MEF. In this study, we evaluated the relationship between the detected pathogens and the clinical severity of AOM judged from the changes of the tympanic membrane. We found that viral infection was associated with less severe AOM, while bacterial infection – especially combined bacterial and viral infection – tended to be associated with more severe AOM. Viruses are known to induce the production of cytokines and other inflammatory mediators at sites of infection, and some studies have shown that the concentrations

Figure 3. Detection of bacteria and viruses over time after the onset of AOM. NPS, nasopharyngeal secretions; MEF, middle ear fluid.

Figure 4. Pathogens and clinical severity of AOM judged from the tympanic membrane changes in 132 cases. The tympanic membrane changes were scored as follows: bulging (0, 1, or 2 points), redness (0, 1, or 2 points), and opacity (0 or 1 points). The total severity index was calculated as the sum of these scores.
of inflammatory mediators (such as leukotriene, IL-8, and histamine) are higher in MEF containing both bacteria and viruses than in MEF with bacteria alone [6]. This supports our finding that co-infection with bacteria and viruses tends to be associated with severe AOM, and indicates that viruses in MEF have an important role in the etiology and pathogenesis of AOM.

Antibiotic therapy may be unnecessary for AOM when viruses are the only pathogens. We found that the detection rate was independent of the body temperature for common bacterial agents (S. pneumoniae, H. influenzae, and M. catarrhalis). In contrast, the viral detection rate increased in patients with high fever. These results show that the simple presence of a high fever is not an appropriate indication for antibiotic administration and that it is essential to determine whether each case of AOM is bacterial or viral.

We also analyzed the relationship between the detection rate of bacteria and viruses over time from the onset of illness. As a result, the viral detection rate was only high in samples collected at the early stage of illness, especially within 7 days of the onset. This suggests that respiratory viruses might play an important role in the early stage of AOM, and that we should consider the possibility of viral infection when we see AOM patients with high fever soon after the onset of illness.

Acknowledgements
We are grateful to all patients and families for participation in this study. We also thank Yoko Ito and Fumiko Chiba (Virus Research Center, Clinical Research Division, Sendai Medical Center) for technical assistance in viral isolation. This work was supported in part by Grant-in-Aid for Young Scientists (B) no. 17791153 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References