Respiratory syncytial virus in hospitalized children
A 3-year study

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Summary

Objectives > To assess the prevalence of respiratory syncytial virus (RSV) and other important respiratory viruses in children hospitalized in a pediatric hospital in Paris (France) during a 3-year period (2001 to 2004).

Patients and Methods > The study included all patients aged 8 days to 16 years admitted from the community through the emergency department with bronchiolitis, pneumonia, upper respiratory tract infection, asthma or acute isolated fever and who had nasopharyngeal samples taken for viral identification by immunofluorescence (RSV, influenza, parainfluenza, and adenoviruses).

Results > A virus was found in 464 of 1208 patients with samples taken. RSV was identified in 375 patients, 74% of them younger than 6 months and diagnosed with bronchiolitis. RSV was isolated more often than any other virus, overall and for all diagnoses except "isolated fever," for which influenza was more frequent. In patients aged 24 months or older, influenza and RSV were identified at the same frequency. Overall, influenza virus was found in 53 patients, adenoviruses in 24 and parainfluenza viruses in 11.

Conclusion > RSV was the respiratory virus isolated most often, even in older children, during this 3-year study. The relative rarity of hospitalizations due to parainfluenza viruses is characteristic of this area, compared with some other countries.
Acute infections due to respiratory viruses, especially respiratory syncytial virus (RSV) and influenza and parainfluenza viruses, are a major cause of pediatric hospitalization [1-4]. Their frequency may be underestimated because most studies focus on acute respiratory infections, although infected children may have isolated fever and no respiratory signs. These viruses are observed most often in the youngest children, principally because of bronchiolitis, which leads to hospitalization and testing to identify the virus. Numerous older children are seen on an outpatient basis or briefly hospitalized for less severe conditions, without any viral testing [1-10]. The distribution of viruses may vary from one country to another: for example, the influenza and parainfluenza viruses are an important cause of pediatric hospitalization in the United States [2,3], but are observed less often in Mexico [4]. Acute viral infection is only one aspect of the respiratory conditions due to these viruses. In children, probably more often than in adults, bacterial pneumonia may occur after an initial viral infection, often with few symptoms, two or three weeks later when viral replication is apparently completed [11-13]. Preventive procedures, including vaccination or antibodies, are just beginning to be used. It is important to have regional epidemiologic information covering several years to determine the proportion of these infections according to region in hospitalized and in ambulatory children.

We studied the principal respiratory viruses in a population of children hospitalized for acute respiratory infection, fever with toxic appearance, or asthma for 3 years in a Paris hospital.

What is already known

• Respiratory syncytial virus (RSV) is very frequent in children with bronchiolitis.
• Influenza and parainfluenza viruses 1, 2 and 3 are observed at high rates in North American studies of children hospitalized with acute respiratory infections.

What this article adds

• RSV is observed in children older than 2 years and frequently exacerbates known asthma, requiring hospital admission. After the age of 2 years, it is as frequent as influenza virus in hospitalized children.
• Parainfluenza viruses 1, 2 and 3 are rarely observed in hospitalized children, accounting for only 2.3% of the respiratory viruses isolated in this series, while the figures reach 15-20% in North America.
• Influenza viruses accounted for only 11% of the respiratory viruses isolated: acute influenza is not a major cause of hospitalization, and most children with influenza are treated on an outpatient basis.

Methods

The study included patients aged 8 days to 16 years, hospitalized through the emergency department between 1 January 2002 and 31 December 2004 in the general pediatrics department of Saint-Vincent-de-Paul Hospital for a community-acquired condition and who had nasopharyngeal samples taken by suction for immunofluorescence viral identification. Patients with a respiratory disease were routinely included, that is, those hospitalized for bronchiolitis, asthma, pneumonia, or upper airway infection. Nasopharyngeal samples were also collected from patients hospitalized for apparently isolated acute fever without respiratory signs at admission and were tested with immunofluorescence for respiratory viruses.

Patients were either hospitalized after examination or remained for a maximum of 6 hours in the emergency department of Saint-Vincent-de-Paul Hospital and, after observation, were admitted if they could not go home according to procedures described previously [5,6]. For those with pneumonia or bronchiolitis, hospitalization was based on difficulty bearing the respiratory signs, especially hypoxia measured by transcutaneous oxygen saturation. The criterion for hospitalization for acute asthma was the failure of repeated bronchodilator inhalations in the emergency department, associated with hypoxia [6]. Most children with upper respiratory infections were aged less than 6 months, and hospitalization was due to the associated hypoxia or, in some cases, elevated fever or coughing fits, often paroxysmal. Children hospitalized for apparently isolated acute fever were admitted because of a toxic appearance or inability to bear the fever or its association with pain.

At admission, all the children underwent nasopharyngeal suction to take samples to test by immunofluorescence to identify any viruses, according to techniques previously described [5,6]. We tested for RSV, influenza viruses A and B, parainfluenza viruses 1, 2 and 3, and the respiratory adenoviruses. Other examinations required by the patient’s condition were also performed. Blood cultures were performed routinely for children with apparently isolated fever or pneumonia.

The definitions used in this study have been described earlier [5,6,10]. The diagnosis of pneumonia was based on the presence of a focus of consolidation, even minimal, on chest radiography [5]. Bronchiolitis met the standard clinical definitions in pediatrics: rapid-onset acute respiratory failure, fever, and sibilant rhonchi in a young child [10]. In our department, asthma is diagnosed in children younger than 2 years when they have at least two prior episodes of clinically important wheezing, except during bronchiolitis, which induced a visit to a physician or emergency department. If the episode occurred immediately after bronchiolitis, with no clear interval, the patient was excluded.

Results

During the 3 years (1 January 2002 through 31 December 2004), 5063 children aged 8 days to 16 years were hospitalized in the department, 58% of them between 1 November and 30 April of each year. In all, 1250 hospitalized children met our inclusion criteria. Immunofluorescence to identify respiratory viruses was performed for 1208 children (42% girls and 58% boys), accounting for 25.7% of all admissions and 97% of the patients meeting the other inclusion criteria. Their mean age was 24.8 months and the median 9 months; 489 (40.5%) were younger than 6 months, 376 (31.1%) aged 6 months to 2 years, and 342 (28.4%) older than 2 years.

Tables I and II summarize the conditions explored and the viruses identified by immunofluorescence. During these 3 years, immunofluorescence tested for respiratory viruses for 90% of all patients admitted with one of these 5 diagnoses (bronchiolitis, asthma, pneumonia, upper respiratory infection, or isolated fever). More than 80% of the patients who had immunofluorescence viral testing of their nasopharyngeal secretions also had a chest radiography.

Immunofluorescence identified one of the viruses we tested for in 464 of 1212 patients, and the distribution differed strongly according to age: 63% of the patients younger than 6 months were positive, 28.5% of those aged 6 months to 2 years, and 14.6% of those older than 2 years. RSV was isolated in 375 patients, 74% of them younger than 6 months. Influenza virus A was isolated in 53, while only one patient, a 2-month-old with rhinitis, was found positive for influenza B. In all, respiratory adenoviruses were isolated in 24 children, parainfluenza virus 1 in 3, and parainfluenza virus 3 in 8; no children were positive for parainfluenza virus 2. Immunofluorescence identified a double infection in 3 children: RSV and parainfluenza 3 in a 1-month-old, and parainfluenza 3 associated with adenovirus in a 4-year-old and with influenza A in a 5-year-old boy.

Most (43/52 or 82.7%) of the children hospitalized for upper respiratory infections were younger than 6 months; the main viruses were RSV (23% of patients) and influenza (13%). Six of these children had laryngitis (croup) and only two were positive for one of the viruses sought – one has RSV, the other parainfluenza 3.

A virus was observed in 56% of the 574 children hospitalized for bronchiolitis. In 95% of cases, it was RSV. This proportion did not change when a pulmonary focus was associated with bronchiolitis, and chest radiography was performed for 85% of the children at admission. A 12-day-old child hospitalized for RSV bronchiolitis had a blood culture at admission that was positive for *Escherichia coli* with the K1 antigen, probably involving maternal-fetal infection that appeared late.

Of the patients hospitalized for asthma, immunofluorescence identified viruses in 17/117 patients (14.5%) younger than 2 years and in 16/222 (7.2%) of those older than 2 years; in two thirds of cases, it was RSV. Influenza A virus was isolated in 3.4%

<table>
<thead>
<tr>
<th>Table I</th>
<th>Respiratory viruses isolated during 3 consecutive years in 1212 hospitalized children: distribution according to reasons for hospitalization</th>
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<tbody>
<tr>
<td>RSV</td>
<td>Bronchiolitis</td>
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<tr>
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</tr>
<tr>
<td>299</td>
<td>24</td>
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<tr>
<td>Parainfluenza 1 and 3</td>
<td>4</td>
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<tr>
<td>Influenza A</td>
<td>6</td>
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<tr>
<td>Adenovirus</td>
<td>6</td>
</tr>
<tr>
<td>IF positive (%)</td>
<td>315 (58.7)</td>
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<tr>
<td>IF negative</td>
<td>222</td>
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IF: immunofluorescence; RSV: respiratory syncytial virus.
of the children with asthma younger than 2 years and 1.3% of those older. The same results were observed for pneumonia, associated with asthma or not. Of the 222 children older than 2 years and hospitalized for asthma, we were able to check the vaccination record for 120: only 6 had received an influenza vaccination in the previous 2 years. The virus proportions were identical when a focus of consolidation was associated with the asthma attack.

Of the 136 children hospitalized for isolated community-acquired pneumonia, without sibilant rhonchi, 22 (16.2%) had an acute viral infection diagnosed by immunofluorescence. RSV was most often for those younger than 2 years and influenza A in those older than 2 years; the distribution changed little when pneumonia was observed during either bronchiolitis or asthma (table III). In 2 patients aged 11 and 13 months among the 53 hospitalized for pneumonia (with or without asthma or bronchiolitis) with a virus isolated in the rhinopharynx, a blood culture was positive for Streptococcus pneumoniae, associated with an adenovirus in one case and with RSV in the other. In 125 patients negative for the viruses tested, the blood culture was positive 5 times, for pneumococci in all cases. Finally, 107 children, 63.4% of them younger than 2 years, were hospitalized for apparently isolated fever with a febrile appearance and 52 of them (48.6%) had a positive immunofluorescence, generally to influenza A, less often to an adenovirus or RSV.

The wintertime concentration of respiratory virus epidemics in Paris was especially clear for RSV: 85% of the cases were seen during November, December and January. On the other hand, 44.4% of the hospitalizations for influenza virus infections and 20.8% for adenoviruses were observed during these 3 winter months during the 3 years considered, and the parainfluenza 3 infections were observed in the spring. If we consider the period from 1 November to 30 April, it includes 90% of the RSV infections, 100% of the influenza infections, and 62% of the adenovirus infections. Moreover, 72% of the pneumonia cases and 67% of the asthma attacks were also hospitalized during this period.

### Discussion

This study is the first in France and one of the few in Europe to assess the extent of respiratory viruses as a cause of hospitalization in a large population of children. During this 3-year period, 9.2% of all hospitalized children had one of the respiratory viruses for which we tested. A virus was isolated by immunofluorescence in 42.5% of the children of all ages hospitalized for an acute respiratory infection; this does not include the children with an isolated fever or asthma attack, whom we also routinely tested for viruses, despite the absence of any obvious sign of respiratory infection.

RSV was the predominant virus. It was the cause of 95% of the cases of bronchiolitis positive for one of the viruses sought, and it was not found only in very young children with bronchiolitis. It was a frequent cause of asthma attacks, even in children older than 2 years, thereby confirming a previous series by our group [6]. On the other hand, it is also a frequent cause of viral pneumonia [5]. In children younger than 6 months, hospitalized for pneumonia without bronchiolitis, it was the cause of the pneumonia in half the cases. In older children, RSV is the second leading cause of viral pneumonia, just behind influenza viruses [13-18]. Generally, in hospitalized children older than 2 years, it is observed at the same frequency as influenza viruses. A viral acute fever syndrome, without any respiratory signs, may accompany RSV. In this series, isolated fevers with a febrile syndrome and pain were due principally, as expected, to influenza, but adenoviruses and RSV were also observed.

One of the most important points in this series is the low frequency of parainfluenza viruses compared with the results of pediatric studies in North America [2,4,8]. Few children with acute pharyngitis, usually due to parainfluenza 1 virus [2], were hospitalized during these 3 years. Even though some of the patients with laryngitis were treated on an outpatient basis, the number of children requiring hospitalization was low. In the United States, surveillance of community-acquired viral infections during the 1990s showed that the number of croup cases was almost equal to the number of bronchiolitis cases [2]. In a Mexican study [4], the frequency of parainfluenza viruses in children hospitalized for respiratory infections was almost as great as that of influenza viruses, while in our study we observed five times more influenza than parainfluenza viruses.

There is an important bias in our study, because the research for respiratory viruses was performed only by immunofluorescence, which limits the viruses that can be identified. Rhinoviruses require PCR (polymerase chain reaction) and thus are not considered here: They may contribute to the induction of asthma attacks and are found rather frequently in young children hospitalized for respiratory infections associated with other viruses. Metapneumoviruses were not sought routinely in these children, hospitalized from 2002-2004. The introduction of reliable and reproducible PCR is recent, and the results of studies in several...
places show that these viruses concern 5-10% of children hospitalized for respiratory infection [19-21]. It is probable that they play an important role in the onset of bronchiolitis, especially after the age of 6 months, and in exacerbating asthma attacks. Generally, the absence of PCR prevents identification of viruses not specifically sought and especially underestimates the importance of co-infections to several viruses. Immunofluorescence uses respiratory mucosa cells from nasopharyngeal samples carrying the viruses sought: the specific antibodies show the viruses predominant in these cells and neglect the others, present in lower quantities. This is especially true for rhinoviruses, which are moderately pathogenic alone, but which promote bacterial or viral mucosal superinfection.

During the 3 years of this study, influenza epidemics in France and Europe were of intermediate intensity, comparable to preceding years [22]. The frequency of hospitalization for influenza of children without respiratory signs, regardless of age, is important, as habitually reported in pediatrics. It is highly probable that using immunofluorescence to search for viruses in the upper airways underestimates the importance of influenza among causes of pneumonia and probably also of asthma. During these 3 years, no culture-proven case of pneumococcal pneumonia was associated with the discovery of an influenza virus by immunofluorescence of rhinopharyngeal samples. Several studies have shown that pneumococci infections peak in children 2 or 3 weeks later than influenza infections [11-13]. An American pediatric study showed that a large number of children hospitalized for severe pneumococcal infections had had serologically-proven influenza infections 2 to 4 weeks earlier, compared with controls from close family and friends [13]. We reported identical results for Paris in a serologic study: 40% of the children older than 2 years with pneumococcal pneumonia and 20% of those with mycoplasma pneumonia had had influenza recently [11]. Immunofluorescence testing thus underestimates the real importance of influenza virus in the causation of pneumonia. The figure of 6% of immunofluorescence tests positive for influenza is identical to that of a previous series [15]. It shows only that acute influenza infections are directly associated with the cause of pneumonia, but it does not take into account the reality of the role of the influenza virus in the onset of pneumonia: the virus promotes the subsequent onset of bacterial infection, and the search for acute viral infection largely underestimates this role.

The data are less clear for asthma. Only seven children, hospitalized for asthma attacks, had immunofluorescence positive for influenza A. Few had been vaccinated. Nonetheless, more than 60% of the patients with asthma attacks were hospitalized during the winter months. Even though the other viruses, in particular rhinoviruses and RSV, play a role, we might conclude that an initial and apparently benign viral infection, including influenza, may contribute to a subsequent asthma attack by its inflammation of the respiratory mucosa. It is nonetheless true that the number of patients hospitalized for asthma at the same time as they have acute influenza is very low in this study, contrary to the rates for RSV infection. While we do not question the universal guidelines about vaccination against influenza of children with asthma [22-28], we note that 2 studies [26-27] have shown that influenza A virus does not play as great a role as commonly attributed to it in asthma exacerbations in children. The absence of testing for rhinoviruses and metapneumoviruses also underestimates the importance of viral infections in exacerbations of asthma attacks [19-21]. This study considered only hospitalized children. Only a study of children examined in the emergency department, regardless of whether they are admitted, can show the proportion of acute viral infections in childhood diseases that are sufficiently serious to lead to a hospital visit. Only viruses usually sought by immunofluorescence were identified. This study, nearly exhaustive in its viral testing, shows the importance of RSV at all ages; it dominated among the children younger than 2 years, but was also frequent after this age, a finding that suggests that its role is probably underestimated in adults [29]. It showed that the parainfluenza viruses and, to a lesser extent, influenza are rarer in children hospitalized in France than in North America. It is therefore important that regional studies be available from different places to facilitate the development of locally appropriate preventive measures in the future, when they become available.

Conflicts of interest: none

References

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