Rapid detection and clinical features of infants and young children with acute lower respiratory tract infection due to respiratory syncytial virus

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Abstract

During December to the end of February of 2003 and 2004, a total of 282 nasopharyngeal aspirates were obtained from infants and young children admitted to the Buraidah Maternity and Pediatric Hospital, Al-Qassim, Saudi Arabia, and clinically diagnosed as suffering from acute lower respiratory tract infections. The aspirates were tested for the presence of respiratory syncytial virus using direct fluorescein-labeled monoclonal antibody assay. Of the 282 specimens, 128 (45.4%) were found to be positive for respiratory syncytial virus. The most positive specimens came from patients less than one year old (51.3%), and were associated with bronchopneumonia (56.7%) or bronchiolitis (55.4%). Coughing (100%) and tachpnea (98%) were significantly more frequent in infants with respiratory syncytial virus infection, followed by wheezing, crepitation and retraction, each representing 66%. Three deaths were reported. The availability of a rapid viral diagnostic assay will be an important tool for physicians to make more accurate treatment decisions and therefore reduce unnecessary antibiotic usage and hospital stay for the patients.

Introduction

Viruses are the most common cause of lower respiratory tract infection in infants and young children, and are a major public health problem in this age group (Van Woensel et al., 2003). Among these viruses are respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, rhinoviruses, adenoviruses, and the recently identified human metapneumovirus (Hoogen et al., 2001).

World-wide, RSV is the most common cause of acute lower respiratory tract infection (ALRTI) in infants and young children (Simoes, 1999). It causes widespread outbreaks of bronchiolitis and pneumonia in infants and young children (Adcock et al., 1997; Meqdam et al., 1997), and trachiobronchiolitis and upper respiratory tract infection (RTI) in older children and adults (Dagan et al., 1993).

Virtually all children have developed antibodies to RSV by the time they are 3 years old. World-wide, RSV directly or indirectly contributes to the deaths of between 600 000 and 100 000 infants and children under the age of 5 years (Simoes, 1999). In the USA, several hundred infants may die directly from the infection, while the deaths of an additional several thousand may be attributed to RSV-related complications (Polak, 2004).

Although the prevention and treatment of RSV infection is still limited, the antiviral drug ribavirin has been restricted to immunocompromised patients with severe RSV disease (Simoes, 1999). Recently, palivizumab, a humanized monoclonal antibody directed against the F protein of RSV (Sandritter, 2000), and RSV-IGIV (RespiGam), an IgG immune globulin with high concentrations of RSV neutralizing antibody (DeVincenzo et al., 2000), have been used to prevent severe lower respiratory tract infections caused by RSV in high-risk patients.

With the development of rapid viral diagnostic technologies and the availability of effective antiviral therapy, community physicians will be able to make more accurate treatment decisions, reduce unnecessary antibiotic usage (Ipp et al., 2002), and isolate the patients to decrease nosocomial spread (Thomas & Book, 1991). RSV could be diagnosed by rapid noncultural assays within 2 h, either by direct or indirect immunofluorescence assay or enzyme immunoassay, and these assays provide good sensitivity and specificity when compared with tissue culture (Hijazi et al., 1996; Meqdam et al., 1997). The direct immunofluorescence assay is simple and can be performed fairly rapidly. The advantage of this test is its low cost; however, the disadvantage is the need for highly skilled laboratory personnel to interpret the results.
This is the first study from the Al-Qassim area to determine the prevalence of RSV-causing ALRTI using rapid screening and identification direct immunofluorescence assay in association with clinical data, hospitalization, and the use of antibiotics.

Materials and methods

Patients
During the winter seasons of 2003 and 2004, a total of 282 nasopharyngeal aspirates (NPAs) were obtained from infants and young children aged less than 10 years suffering from ALRTI who had been admitted to the Buraidah Maternity and Pediatric Hospital, Al-Qassim, Saudi Arabia. The Buraidah Maternity and Pediatric Hospital is the only specialized hospital in Al-Qassim area. It was established in 2003, and has 255 beds. It provides free medical care, serving about 500 patients per day, and having 12 000 deliveries per year from the Buraidah city population and its suburbs.

The diagnosis of RSV in NPAs specimens was made using a respiratory direct immunofluorescence assay (DFA) identification kit according to the manufacturer's instructions (ID no. 3137, Chemicon). The kit contains fluorescein-labeled monoclonal antibodies. A virus culture was not done due to the lack of facilities in the laboratory. Information concerning each patient, including their clinical data, age, sex, length of hospitalization and use of antibiotics, was collected by questionnaire.

Specimen collection
NPAs were collected from the patients according to the specimen collection method of Mezieri et al. (1990) as follows: NPAs were collected by the physician using a sterile infant feeding tube (a suction catheter tube with sizes of 6, 8 and 10 Fr, and of 50 cm length) connected to a vacuum pump. The tube was introduced through each nostril yielding about 0.5–1 mL of aspirate. The tip of the tube holding the aspirate was cut and placed into a tightly capped sterile container labeled with the patient’s name or identification number. The container, along with the questionnaire was passed to the laboratory of King Saudi University, Al-Qassim, Saudi Arabia, in a cold box.

Specimen processing
The specimens were processed according to the method described by the manufacturer, with minor modifications as follows. The content of the infant feeding tube was emptied into an Eppendorf tube by flushing with 1 mL of PBS, pH 7.4. The specimen was vortexed for 30 s to dislodge cells from the mucus, and centrifuged at 300–500 g for 5 min using a Labofuge 200 centrifuge (Heraeus, Germany). The supernatant was discarded and the pellet was used for the detection of RSV antigen by DFA.

Preparation of cell pellet for immunofluorescence staining
The cell suspension was prepared from the pellet by washing it twice in PBS, pH 7.4, for 5 min to decrease the mucus viscosity. The sediment was next resuspended in 0.2 mL PBS to make a slightly cloudy suspension. Twenty microliters of cell suspension was spotted per well of an eight-well slide (5 mm diameter). The slide was allowed to air-dry completely, and fixed with chilled (2–8 °C) acetone for 10 min. The slide was air dried after fixation.

Viral identification by direct immunofluorescence staining
To each well we added 20 μL of fluorescein-labeled monoclonal antibodies against RSV. The slide was incubated at 37 °C for 30 min in a humid chamber, and then rinsed thoroughly with PBS-Tween for 10–15 s, directing the PBS-Tween away from the cell spot. The excess solution was shaken off and the slide was mounted in phosphate-buffered glycerol (20–80%, v/v). The slide was examined using a fluorescence microscope (Olympus, Japan) at ×160–200 magnification for the presence of cells exhibiting fluorescence. Detailed examination was carried out at ×400 magnification. A sample containing fewer than 20 epithelial cells was considered inadequate and rejected. RSV infected and uninfected epithelial cells were stained as positive and negative controls, respectively, in each trial. The presence of two or more intact cells exhibiting specific apple-green fluorescence was required for us to consider a specimen positive for RSV antigen. Uninfected cells stained a dull red due to the Evan Blue reagents.

Data analysis
Data analysis was carried out using statistical program SPSS version 10.0 for Windows (SPSS Inc., Chicago, IL.). The differences in proportions were compared using a χ² test.

Results
A total of 282 NPAs specimens were tested for the presence of RSV using a respiratory DFA identification kit.

The age and sex distribution of the positive and negative RSV cases is shown in Table 1. The age of children in this study ranged from birth to 10 years old with a mean of 9 months and a standard deviation of 9.1 months (standard deviation = 2.9 and 3.9 for the age groups <1 years old and 1–2 years, respectively). RSV was strongly associated with patients who were less than 2 years old (47.2%, P = 0.019).
Fig. 1. The frequency of signs and symptoms for 128 children with respiratory syncytial virus infection.

In most published studies, RSV has been a highly seasonal infection. RSV outbreaks in areas with temperate, Mediterranean and desert climates appear mainly during the cold season, whereas in tropical climates with seasonal rainfall, they appear to be associated with the rainy season (Bakir et al., 1994; Weber et al., 1998; Meqdam & Nasrallah, 2000; Djelantik et al., 2003).

Acute infections of the lower respiratory tract constitute one of the major causes of morbidity and mortality among the pediatric population of developing countries (Vieira et al., 2003). They are more frequent and serious within the first months of life (Berman, 1991). The importance of RSV was probably underestimated in earlier studies which did not use immunofluorescence or antigen detection as one of the diagnostic methods, as RSV is a fragile virus (Weber et al., 1998). Comparing several community-based studies in developing countries performed with immunofluorescence (Hortal et al., 1990; Tupasi et al., 1990; Vathanophas et al., 1990; Forgæ et al., 1991; Sutmoller et al., 1995) with those performed without the use of immunofluorescence (Kloene et al., 1970; Ota & Bang, 1972; Sutmoller et al., 1983), the percentage of RSV isolates doubled from 23% to 44% in the studies that used immunofluorescence.

In most studies, RSV was found to be the predominant viral cause of acute RTI in childhood, being responsible for 27–90% of hospitalized cases (Berman, 1991; Meqdam et al., 1997; Weber et al., 1998; Meqdam & Nasrallah, 2000; Abdul Wahab et al., 2001; Tsolia et al., 2002; Vieira et al., 2003). In the present study, RSV was identified in 128 (45.4%) of 282 children with ALRTI. There was a similarity between this finding and that described in studies performed in

### Table 1. Age and sex distribution of 128 children positive for respiratory syncytial virus

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total no. of children (M, F)</th>
<th>%</th>
<th>No. of positive children (M, F)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>195 (110, 85)</td>
<td>69.1</td>
<td>100 (58, 42)</td>
<td>51.3</td>
</tr>
<tr>
<td>1–2</td>
<td>59 (34, 25)</td>
<td>20.9</td>
<td>20 (12, 8)</td>
<td>33.9</td>
</tr>
<tr>
<td>≥ 2</td>
<td>28 (17, 11)</td>
<td>10.0</td>
<td>8 (6, 2)</td>
<td>28.6</td>
</tr>
<tr>
<td>Total</td>
<td>282 (161, 121)</td>
<td>45.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M, male; F, female.

### Table 2. Clinical observations in 128 children positive for respiratory syncytial virus

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Total no. (%) of children</th>
<th>No. (%) of positive children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchiolitis</td>
<td>130 (46.1)</td>
<td>72 (55.4)ab</td>
</tr>
<tr>
<td>No bronchiolitis</td>
<td>152 (53.9)</td>
<td>56 (36.8)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>5 (1.8)</td>
<td>5 (100)c</td>
</tr>
<tr>
<td>No pneumonia</td>
<td>277 (98.2)</td>
<td>123 (44.4)</td>
</tr>
<tr>
<td>Bronchopneumonia</td>
<td>120 (42.6)</td>
<td>68 (56.7)bc</td>
</tr>
<tr>
<td>No bronchopneumonia</td>
<td>162 (57.4)</td>
<td>60 (37.0)</td>
</tr>
<tr>
<td>Other respiratory infection</td>
<td>4 (1.4)</td>
<td>2 (50.0)</td>
</tr>
<tr>
<td>No other respiratory infection</td>
<td>278 (98.6)</td>
<td>126 (45.3)</td>
</tr>
<tr>
<td>Duration of hospitalization (days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–4</td>
<td>174 (61.7)</td>
<td>82 (47.1)</td>
</tr>
<tr>
<td>5–8</td>
<td>98 (34.8)</td>
<td>36 (36.7)</td>
</tr>
<tr>
<td>&gt; 8</td>
<td>10 (3.5)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Received antibiotics</td>
<td>Yes</td>
<td>170 (60.3)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>112 (39.7)</td>
</tr>
</tbody>
</table>

Degree of significance: abP = 0.002, bP = 0.014, cP = 0.001, dP = 0.017.

Table 2 shows the clinical observations from 128 children positive for RSV. RSV infection was significantly associated with bronchopneumonia (56.7%, P = 0.001) and bronchiolitis (55.4%, P = 0.002). Of the infected children, 47% and 36.7% were hospitalized for 1–4 days and 5–8 days, respectively. Of the patients positive for RSV, 87 received at least one type of antibiotic (P = 0.017).

The frequency of the signs and symptoms of the 128 children positive for RSV is shown in Fig. 1. Cough and tachypnea were the most frequent, occurring in 100% and 98% of the children respectively, followed by fever (81%), wheezing, crepitation, and retraction, each representing 66%.

### Discussion

Respiratory syncytial virus is the most important respiratory pathogen of infants and young children, causing disease throughout the world (Hijazi et al., 1995; Bakir et al., 1998). Little is known about the epidemiology of RSV infection in developing countries (Wilczynski et al., 1994).
developed and developing countries, where RSV is considered to be the principal viral agent of ALRTI.

The age distribution of RSV infection in developing countries is similar to that observed in developed ones. An estimated 75% of hospitalized children with RSV infection are less than 1 year old (Glezen et al., 1981). Our results showed that the majority of children (90.1%) were less than 2 years old. Of these, 47.2% ($P = 0.019$) were infected with RSV.

In most studies, males were more commonly affected than females and the male-to-female ratio was between 1.5 and 1.8 (Glezen, 1977; Tsolia et al., 2002). This male preponderance corresponds to the generally higher incidence of ALRTI of any etiology in boys (Reeves et al., 1985).

Bronchiolitis and pneumonia are the most common manifestations of viral lower respiratory tract infection in infants. Differentiation between these clinical syndromes is difficult, as their definition is not based on standardized clinical criteria (Ruuskanen & Orge, 1993). In one study, bronchiolitis accounted for 50% of cases and pneumonia for 37% (Hall, 1985), and in another pneumonia for 34.6% of cases and bronchiolitis only 16% (Misra et al., 1990). In our study, the major clinical syndromes associated with RSV infection were bronchopneumonia (56.7%, $P = 0.001$) and bronchiolitis (55.4%, $P = 0.002$). Viral infections usually start with rhinorrhea, cough and fever. After one or two days the lower respiratory tract may become involved, with signs of respiratory distress, including tachpnea, retraction and cyanosis in severe cases (Van Woensel et al., 2003). In one study, cough (100%) and wheezing (80%) were the major clinical symptoms associated with RSV bronchiolitis in children (Dagan et al., 1993). In our study, patients tested positive for RSV were more likely to have cough (100%), tachpnea (98%) and fever (81%).

Mortality from ALRTI is difficult to assess from published data. Only a few deaths have been reported and a number of studies stated that there had been no mortality from RSV during the course of the study. Of all the 282 patients with ALRTI studied during the RSV season there were three deaths from respiratory failure which were positive for RSV. The age of the infant deaths were less than 3 months old, two with bronchopneumonia and one with bronchiolitis. The overall case fatality rate was 1% and 2.3% for the RSV-positive patients. It is possible that a vaccine trial with an effective vaccine will be able to show how much mortality and morbidity is attributable to RSV (Weber et al., 1998).

In one study, 61.1% of children received antibiotics, in spite of the fact that 34.7% of them had a laboratory test confirming a viral aetiology (Edwards et al., 1985). In other studies, 43.4% and 45% of RSV-positive children received antibiotics after admission (Hijazi et al., 1995; Adcock et al., 1997). This study showed that 87 (51.2%, $P = 0.017$) patients positive for RSV out of 170 (60.3%) received at least one type of antibiotic after admission. The prescribing of antibiotics may be due to viral illnesses being mistaken with bacterial infection (Byington et al., 2002), or due to the fact that the Buraidah Maternity and Pediatric Hospital has no facilities for confirming viral infection.

The availability of a rapid viral diagnostic assay will be important for decreasing the use of unnecessary and costly antibiotic therapy and in reducing the length of hospital stay for patients. Further advanced tests for the detection and typing of RSV, using PCR for example, is advisable for better appraisement, to draw conclusions and give recommendations.

The introduction of a safe and effective RSV vaccine in developing countries would be critical in preventing the long-term morbidity associated with severe RSV infection, as well as in the prevention of acute infection.

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**References**


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