Comparison of nasopharyngeal aspirate and nasal swab specimens for detection of respiratory syncytial virus in different settings in a developing country

L. G. Stensballe1,2, S. Trautner1, P.-E. Kofoed1,3, E. Nante1, K. Hedegaard2, I. P. Jensen4 and P. Aaby1,2

1 Projecto de Saúde de Bandim, Bissau, Guinea-Bissau
2 Department of Epidemiology Research, Danish Epidemiology Science Centre, Statens Serum Institut, Copenhagen, Denmark
3 Department of Paediatrics, Kolding Sygehus, Kolding, Denmark
4 Department of Virology, Statens Serum Institut, Copenhagen, Denmark

Summary

OBJECTIVE To compare detection of respiratory syncytial virus (RSV) for diagnostic purposes using nasopharyngeal aspirate (NPA) and nasal swabs (NS) in different clinical settings in a community study in Guinea-Bissau.

METHOD During 1996–98 paired specimens were obtained from 635 children under 5 years of age (median: 274 days; interquartile range: 144–453 days) with symptoms of lower respiratory infections (LRI). The specimens were analysed by an enzyme-linked immunosorbent assay for RSV antigen in Guinea-Bissau and re-analysed in Denmark using the same assay. The gold standard for RSV antigen detection was defined as any test being positive.

RESULTS RSV antigen was detected in 84 (13%) children, the prevalence being 19% (41/219) among infants aged < 6 months, 12% (22/184) in infants aged 6–11 months, and 9% (21/230) in older children. Sensitivity of antigen detection was higher in NPA (92% in analyses in Guinea-Bissau and 98% in Denmark) than in NS (63% in analyses in Guinea-Bissau, 71% in Denmark). Specificity of RSV antigen detection was equally high in NPA and NS (99–100%). Time since onset of symptoms was significantly shorter in RSV antigen positive than negative samples. Sensitivity did not depend on clinical setting or age of the child.

CONCLUSION Using NS samples was associated with a 27–31% reduction in sensitivity compared with NPA specimens. As NPAs are costly and considered a nuisance by the population, it might be cost-effective in larger epidemiological studies to lose 25–30% in sensitivity but be able to collect samples from a much larger population.

Keywords respiratory syncytial virus, nasopharyngeal aspirate, nasal swab

correspondence Lone Graff Stensballe, Department of Epidemiology Research, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark. Fax: + 45 32 683 165; E-mail: lgn@ssi.dk

Introduction

Respiratory syncytial virus (RSV) is one of the most common respiratory pathogens of infants and young children, causing yearly outbreaks of upper and lower respiratory infection (LRI) in both developed and developing countries (Glezen & Denny 1973; Selwyn 1990). Confirmation of viral aetiology is important before choosing therapy. RSV infection can be rapidly diagnosed with enzyme-linked immunosorbent assay (ELISA) for direct detection of RSV antigen or RSV specific antibodies (McIntosh et al. 1982; Hornslet et al. 1986; Jensen et al. 1997). Earlier studies in developed countries have indicated that the source of specimens influences the likelihood of RSV detection (Hall & Douglas 1975; McIntosh et al. 1982; Treuhaft et al. 1985; Akuwuila et al. 1987; Cruz et al. 1987; Frayha et al. 1989; Michaels et al. 1992). Most of these studies document the better sensitivity of nasopharyngeal aspiration (NPA) and nasal wash compared with nasal swab (NS), nasopharyngeal swab, or...
throat swab techniques. Furthermore, detection of RSV antigen is more common in children with more severe disease and in samples taken as soon as possible after onset of symptoms (Hall et al. 1976; Glezen et al. 1981; Buckingham et al. 2000). To our knowledge, this has not been assessed in a developing country and in different clinical settings. In certain situations, a less sensitive test could be of interest if it were less unpleasant or costly.

The Bandim Health Project in Guinea-Bissau has conducted community studies of LRIs associated with RSV (Stensballe et al. 2000). As part of these studies we compared RSV detection using NPA and NS sampling in different clinical settings.

Subjects and methods

The Bandim Health Study conducts demographic and epidemiological surveillance in several districts in the capital of Guinea-Bissau, including Bandim 1, Bandim 2 and Belem (Aaby 1997). More than 3000 houses in the area are visited monthly to detect new pregnancies and to register newborns as soon as possible after birth. Children under 3 years of age are visited every 3 months to register growth, immunizations, morbidity and survival of the children.

From July 1994 to the onset of the war June 1998 (Aaby et al. 1999), children under 5 years of age from the three study districts, who attended the outpatient clinic of the paediatric department of the national hospital in Bissau as well as the child clinic at the local health centre in Bandim, were screened systematically for LRIs to establish the incidence of RSV. In February 1995, we initiated a community cohort of all newborns in Bandim 1 and Bandim 2. The newborns were visited weekly by field assistants, who interviewed the mothers using morbidity questionnaires and clinically examined the babies for LRI. Sick children identified by a field assistant were referred to the hospital or visited on the same day by a project physician. The identification of LRI was based on an age-adjusted standard for respiratory frequency and chest in-drawings as recommended by World Health Organization (WHO) (Wright & Cutts 1996). There was no RSV diagnostic prior to these studies, and as they were interrupted by the war in 1998, there has not been any in Guinea-Bissau.

Sample collection and storage

From 1996 to 1998, paired specimens were obtained from 635 children with respiratory symptoms. NPA specimens were collected by suction of secretions from one nostril into mucous traps using a nasopharynx suction device (tracheal suction set manufactured by Kirudan, Denmark, for Maersk Medical) connected to a foot pump (Ambu® Twin Pump, Ambu International A/S). The catheter was pushed into the nasopharynx equivalent to the distance from the ear to the nostril of the patient. At this position suction was started while gently rolling and retracting the catheter. Secretions remaining in the catheter were obtained by washing with 5 ml isotonic saline. NS specimens were collected through the other nostril with a cotton-tipped swab (Culture Swab ™ Transport System, manufactured by Copan, Italia SpA, for DIFCO Laboratories). The swab was inserted 2–3 cm into the nostril and rotated thoroughly against the respiratory epithelial surface of the nasal cavity. The swab was then removed and placed in a container adding 5 ml of isotonic saline. Three Guinean physicians and three specially trained field assistants collected the samples.

Laboratory procedures

All specimens were delivered to the laboratory within a few hours and processed in an identical fashion irrespective of whether they derived from NPA or NS samples. Initially, 100 ml of the samples were examined for RSV antigen at the National Public Health Laboratory in Guinea-Bissau (Laboratório Nacional de Saúde Pública, LNSP), with an antigen ELISA developed at Statens Serum Institut in Copenhagen. This antigen test covers both RSV type A and B. All samples were stored at –20 °C and subsequently transported to Copenhagen to be re-analysed by the same ELISA for RSV antigen (Obel et al. 1995). The RSV antigen re-testing served as a quality control of the ELISA test in Guinea-Bissau.

RSV antigen detection and definition of positive and borderline positive samples

As in previous studies and irrespective of the source of specimens, samples were considered positive if the optical density (OD) value was three times higher than the OD value of the unspecific control and higher than the OD of the negative control. In calculating the sensitivity and specificity, a gold standard RSV antigen positive child was defined as testing positive in one or more of the four tests of his or her samples. A sample was considered borderline positive if the OD value was between two and three times the OD value of the unspecific control and higher than the OD of the negative control. There were considerably more borderline values for the NS specimens. Virtually all of these borderline results were antigen positive in the NPA specimens. Including borderlines as positives increased sensitivity of the NS from 43 to 63% in Guinea-Bissau and
from 51 to 71% in Denmark without affecting specificity (data not shown). In further analyses, borderline positive samples were therefore regarded as RSV positives.

**Statistical analyses**

Standard statistical methods for analysing categorical and continuous data were used. Independent groups were compared using either chi-squared test, trend test or *T*-test as appropriate. Paired groups were compared using McNemar’s test. 95% confidence intervals are given throughout.

**Ethical approval and consent**

The Ministry of Health in Guinea-Bissau and the Central Ethical Committee of Denmark (C-1994-27) approved the study. Inclusion of children in the study was based on informed consent of their mother.

**Results**

We collected paired samples from 635 children. Eighty-four samples were RSV antigen positive in at least one test. The prevalence was highest among samples from children under 6 months (18.7%; 41/219) compared with infants aged 6 months to 1 year (12.0%; 22/184), and children aged 1 year and older (9.1%; 21/230) (trend test *P* = 0.003) (Table 1). The prevalence of positive samples was virtually the same for boys (13.5%; 46/342) and girls (12.8%; 37/290). For 531 children, there was information available on the delay between the onset of respiratory symptoms and the collection of the samples (Table 2). Missing information was usually because of the mother being away at the time of the interview. For samples positive according to our gold standard, the mean (SD) interval was 4.3 (2.1) days compared with 6.0 (5.0) days for negative samples, a highly significant difference (*T*-test with correction for unequal variances *P* = 0.0001) (data not shown).

As seen in Table 3, sensitivity obtained with NPA analyses was equally good in Guinea-Bissau (91.7%, CI: 83.6–96.6) and Denmark (97.6%, CI: 91.7–99.7). Nor was there any difference in the sensitivities of NS samples analysed in Guinea-Bissau (63.1%, CI: 51.9–73.4) and Denmark (71.4%, CI: 60.5–80.8).

Compared with the sensitivity of the NPA specimens, NS samples were 31% [1 – (63.1%/91.7%)] less sensitive when analysed in Guinea-Bissau and 27% [1 – (71.4%/97.6%)] in Denmark (McNemar’s test *P* < 0.001 in both cases). The difference between sensitivities of NPA and NS was the same when analysed in Guinea-Bissau (29%, CI: 19–38%) or Denmark (26%, CI: 16–36%).

Specificity was high for both NPA and NS specimens (Table 3). No significant differences were found. Analysed according to setting, specificities remained high, the lowest occurring in NS specimens from the health centre analysed in Guinea-Bissau (97.3%, CI: 93.8–99.1).

There was no significant difference in sensitivity for NS according to age [data not shown, Denmark: *P* = 0.99 (chi-square test); Guinea-Bissau: *P* = 0.54 (chi-square test)]. For samples analysed in Guinea-Bissau and stratified

### Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of RSV antigen positives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
</tr>
<tr>
<td>Age &lt; 6 months</td>
<td>24/127  (18.9%)</td>
</tr>
<tr>
<td>6 months ≤ age &lt; 1 year</td>
<td>11/100  (11.0%)</td>
</tr>
<tr>
<td>Age ≥ 1 year</td>
<td>11/115  (9.6%)</td>
</tr>
<tr>
<td></td>
<td>46/342  (13.5%)</td>
</tr>
</tbody>
</table>

The final column and row include two children with unknown age and three children with unknown gender, respectively.

### Table 2

<table>
<thead>
<tr>
<th>Days after onset of symptoms</th>
<th>0–1 day</th>
<th>2 days</th>
<th>3 days</th>
<th>4 days</th>
<th>5 days</th>
<th>6 days</th>
<th>7 days</th>
<th>8–30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive samples/total samples</td>
<td>1/18, 5.6%</td>
<td>3/26, 11.5%</td>
<td>13/98, 13.3%</td>
<td>4/58, 6.9%</td>
<td>2/36, 5.6%</td>
<td>1/25, 4%</td>
<td>1/48, 2.1%</td>
<td>1/36, 2.8%</td>
</tr>
</tbody>
</table>
according to setting, the loss in sensitivity using NS instead of NPA was 27% [1 – (68%/94%)] for samples collected in a clinical setting (hospital and health centre) and 36% [1 – (57%/89%)] for samples collected in the community. This difference was not significant (P^0.49).

Discussion

Comparing NS with NPA or nasal wash, previous studies from developed countries (Hall & Douglas 1975; McIntosh et al. 1982; Treuhaft et al. 1985; Ahluwalia et al. 1987; Cruz et al. 1987; Frayha et al. 1989; Michaels et al. 1992) have found between 9 and 65% reduction in sensitivity, the large variation presumably being because of the limited size of many studies and variation in how the swab specimens were collected. In a developing country, Guinea-Bissau, the reduction in sensitivity of NS specimens compared with NPA specimens for RSV antigen detection by ELISA was 25–30%. Specificity only differed slightly, NS being the most specific. However, in comparing the two techniques, it should be taken into consideration that NS requires a vacuum system, a suction catheter, a mucus trap, and a transport medium, whereas NS only needs a swab and a transport medium. In our opinion and the opinion of the mothers, the NS was also less traumatic to the children. NS samples are much easier and therefore cheaper to collect, while the NPA technique usually requires more time and better trained personnel. In epidemiological studies or vaccination trials where repeated samples have to be taken, some reduction in sensitivity could be acceptable if it were outweighed by facility, lower cost, and better acceptability among parents. Using NS one might be able to cover a much larger population and/or collect samples more frequently.

The proportion of borderline positive values to positive values was higher in NS than NPA specimens. This might be because of differences in the quantity of specimens, as the much smaller amount of nasal secretion obtained by NS was also diluted with 5 ml isotonic saline. Besides, samples are not routinely microscoped before an ELISA procedure and the cellular context of the samples is unknown. As the catheter used for NPA covers a larger area of airway epithelium, it is likely that the content of RSV infected cells is higher in NPA than NS samples.

To test whether severity of disease would influence the sensitivity of antigen detection, the data were analysed according to the setting in which the samples had been collected and according to the age of the children as primary infection in the youngest cases are usually most severe. Children going to hospital or the health centre were presumed to be more severely ill than cases only identified during home visits. But neither setting nor age had any major effect on the sensitivity.

In 2001 prices a tracheal suction set with catheter was nine times as costly as a swab (17/1.8 DKr) without freight. The usefulness of the less sensitive NS depends on considerations of costs, ease of collection, and acceptability of repeated sampling. These considerations may be important in studies needing a large number of RSV cases.

Acknowledgements

The project received financial support from the Council for Development Research, Denmark and WHO, Geneva.
References


Selwyn BJ (1990) The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries. Reviews in Infectious Diseases 12 (Suppl. 8), S870–S888.


© 2002 Blackwell Science Ltd