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Respiratory virus detection in nasopharyngeal aspirate versus bronchoalveolar lavage is dependent on virus type in children with chronic respiratory symptoms

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ABSTRACT

Background: The comparative yield of respiratory virus detection from nasopharyngeal aspirate (NPA) versus bronchoalveolar lavage (BAL) is uncertain. Furthermore, the significance of virus detection and its relationship to lower airway neutrophilic inflammation is poorly studied.
Objectives: To evaluate the sensitivity, specificity and predictive values of NPA for detecting respiratory viruses in BAL; and to determine the relationship between viruses and lower airway neutrophilia in children with non-acute respiratory illness.
Study design: 150 paired NPA and BAL samples were obtained from 75 children aged <18 years undergoing flexible bronchoscopy for investigation of chronic respiratory symptoms. Viral studies were performed using polymerase chain reaction (PCR). Cellularity studies were performed on BALs. Diagnostic parameters of NPA compared to BAL and associations between viruses and lower airway %neutrophils were evaluated.
Results: NPA had a higher yield than BAL for detection of any respiratory virus (52 versus 38, respectively). NPA had a high sensitivity (92%) and low specificity (57%) for detecting HRV in BAL with poor kappa agreement value of 0.398 (95% CI 0.218–0.578, $p < 0.001$). NPA had a fair sensitivity (69%) and good specificity (90.3%) for detecting HAdV on BAL, kappa agreement was 0.561 (95% CI 0.321–0.801, $p < 0.001$). HAdV positivity on NPA, compared to negativity, was independently associated with heightened airway neutrophilia [mean difference (95% CI): 18 (1,35); $p = 0.042$].
Conclusions: NPA has a higher yield for respiratory virus detection than BAL, however its diagnostic accuracy is dependent on viral species. Adenovirus positivity is associated with significantly heightened lower airway neutrophilia in children with chronic respiratory symptoms.

1. Background

Accurate identification of respiratory pathogens is important in clinical medicine and research. In bacteriology, upper airway sampling can misrepresent the lower airway microbiota [1,2]. In contrast, there is little such comparative data for virology. Nasopharyngeal aspirates (NPA) or nasal washes are regarded as the specimen of choice for detection of upper respiratory tract viruses by polymerase chain reaction (PCR) [3,4]. Upper airway sampling is relatively simple, can be performed at the bedside and is minimally invasive. In contrast, lower airway sampling

Abbreviations: NPA, nasopharyngeal aspirate; BAL, bronchoalveolar lavage; PCR, polymerase chain reaction; RVI, respiratory virus infection; HRV, human rhinovirus; HAdV, human adenovirus; RSV, respiratory syncytial virus; IFAV, human influenza A virus; IFBV, human influenza B virus; HPIV1–3, human para-influenza virus 1–3; hMPV, human metapneumovirus; HBoV, human bocavirus; HCoV – NL63, OC43, 229E, HKU1, human coronaviruses; WUPyV, WU polyomavirus; KIPyV, KI polyomavirus.

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in young children is invasive, requiring a bronchoalveolar lavage (BAL).

Traditionally, BAL has been considered the 'gold-standard' for the microbiological diagnosis (viral and bacterial) of lower respiratory tract infection in children. However, as BAL requires specialist input and is often performed under anesthesia, it is reserved for patients with complex or severe disease, e.g., in the setting of intensive care; immune-compromise or in children with chronic or recurrent respiratory tract symptoms. The disparities in ease of collection of NPA versus BAL, raise two important questions. Firstly, what is the comparative yield of NPA compared to BAL for respiratory virus detection in children? Secondly, what is the significance of a positive NPA with respect to lower airway inflammation? Given the lack of studies addressing these important questions [5,6], we evaluated paired NPA and BAL specimens in 75 children.

2. Objectives

To compare the yield and diagnostic parameters of respiratory virus detection on NPA versus BAL using molecular methods, and to evaluate whether virus positivity on NPA correlates with lower airway neutrophilic inflammation and by inference, active viral pulmonary disease.

3. Study design

The Queensland Children's Health Services Ethics Committee approved the study and written informed consent was obtained from each parent or guardian. Children undergoing flexible bronchoscopy, for any clinical indication, as arranged by their treating physician, were eligible. Samples evaluated in this article were obtained from children recruited to a larger cohort study on chronic cough in children [7]. Caregivers completed a standardized clinical questionnaire on the day of bronchoscopy including current respiratory symptoms and relevant demographics. Children with symptoms of significant acute lower respiratory tract infection, e.g., high fever, tachypnea/shortness of breath, wheeze or rattly chest were deemed, by an anesthetist, to be unfit for anesthesia and excluded from the study.

Contemporaneous NPA and BAL sampling was performed under general anesthesia. NPA was collected first, using a disposable catheter connected to a mucous trap. Dry nasopharyngeal suction, via both nares, was performed, followed by suction of 2–3 ml of sterile normal saline directly into the suction catheter to rinse through remaining contents.

Bronchoscopic BAL was then performed using standardized methods as per European Respiratory Society guidelines [8]. Sterile normal saline, in three aliquots of 1 ml/kg (maximum 20 ml), was instilled into the most affected area, or right middle lobe in patients with generalized disease. To minimize upper airway contamination, suction through the bronchoscope was avoided until the tip had entered the distal airways. The first aliquot was used for microbiological testing, the second and third were pooled for cellularity studies.

Viral studies were undertaken using real-time PCR techniques, as described previously [9–13], to detect 16 respiratory virus types and subtypes. These included human rhinoviruses (HRV), human adenoviruses (HAdV), respiratory syncytial virus (RSV), human influenza A virus (IFAV), human influenza B virus (IFBV), human para-influenza virus 1–3 (HPIV1–3), human metapneumovirus (hMPV), human bocavirus (HBoV), human coronaviruses (HCoV – NL63, OC43, 229E, HKU1), WU polyomavirus (WUPyV) and KI polyomavirus (KIPyV). BAL specimens were refrigerated immediately and processed within 24 h. NPA specimens were stored at −70 °C prior to processing.

We defined positive bacterial culture as growth of $\geq 10^5$ colony-forming units (cfu)/ml [10] of any of the following pathogens on BAL: *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* or Enterobacteriaceae. Total cell count and differential profile (minimum 400 cells counted) were performed on BAL as described previously [14].

3.1. Statistical analyses

Statistical analyses were carried out using IBM SPSS (v20, San Francisco, USA). Medians and inter-quartile ranges were reported as data were non-normally distributed. Kappa analyses were used to assess levels of agreement between sampling techniques. Comparisons of categorical variables were performed using Pearson's χ^2 test or Fisher exact test if any category had an expected value of <5. Mann–Whitney *U* test was used for two group comparisons and Kruskal–Wallis test for >2 groups comparisons of continuous variables. Multiple linear regression was employed to adjust for virus co-detection and bacterial infection. A two-tailed *p*-value <0.05 was considered statistically significant. Sensitivity, specificity, positive and negative predictive values and likelihood ratios were manually calculated.

4. Results

Between February 2010 and May 2012, paired NPA and BAL samples were obtained from 75 children (median age = 33 months, IQR 16, 69; male $n = 50$, 66.7%). One-third of children ($n = 25$, 33.3%) were exposed to household cigarette smoke. Chronic cough (≥4 weeks) was reported in 45 participants (60%) at time of bronchoscopy. The major diagnoses within the cohort included: protracted bacterial bronchitis ($n = 31$, 41%) and bronchiectasis ($n = 17$, 23%); the remaining participants had either no specific diagnosis ($n = 22$, 29%) or a congenital airway abnormality ($n = 5$, 7%). The most common viruses detected on NPA were HRV ($n = 44$) and HAdV ($n = 15$). In regard to seasonality, HAdV positivity on BAL was more likely to occur in spring or summer than winter or autumn ($p = 0.004$) using chi-square analysis. HRV detections followed a bimodal distribution with peak detections occurring in spring and autumn.

4.1. Comparison of yield on paired NPA and BAL samples

Of the 75 paired NPA and BAL samples, 52 (69.3%) children had one or more virus detected from NPA and 38 (50.7%) had one or more virus from BAL. When considering detection of any virus, 32 (42.7%) NPA-BAL pairs were concordant PCR-positive, 17 (22.7%) pairs were concordant PCR-negative and 26 (34.7%) pairs had discordant PCR results. Of the discordant pairs, most ($n = 20$, 76.9%) were PCR positive on NPA but negative on BAL. Detection rates varied among the different viruses (Table 1). Most marked was the difference between NPA and BAL for HRV. Using positive PCR on BAL as an arbitrary gold standard, we calculated sensitivity, specificity, positive and negative predictive values and likelihood ratios (Table 1). HRV and HAdV were the most common viruses detected from NPA and BAL. Levels of agreement between NPA and BAL varied among the different viruses. The agreement between NPA and BAL for HRV was poor, kappa = 0.398 (95%CI 0.218, 0.578, $p < 0.001$) but good for HAdV at 0.561 (95%CI 0.321, 0.801, $p < 0.001$).

4.2. Relationship between virus positivity and BAL neutrophilia

The median lower airway %neutrophils were significantly higher in children who were NPA-positive for any virus compared to