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Bronchoscopy in Cystic Fibrosis Infants Diagnosed by Newborn Screening

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Summary. Background: There is evidence of early functional and structural changes in babies with cystic fibrosis (CF) diagnosed on newborn screening (NBS). The aim of the present study was to determine the yield of bronchoalveolar lavage (BAL) microbiology and cytology, and 24 hr pH monitoring in a group of CF infants diagnosed on NBS. Methods: Infants referred to a tertiary pediatric respiratory center between July 2007 and November 2009 underwent surveillance fiber-optic bronchoscopy (FOB), BAL, and insertion of a 24 hr dual pH probe under a single general anesthetic. Results: We studied 33 infants, median age of 100 days (47–215 days) at the time of FOB. In 9 of 33 (27%) bacterial organisms were identified. Seven of the nine patients (78%) were asymptomatic and only one had had a positive cough swab prior to FOB. Neutrophilia was identified in 18/27 (67%) cases with a median of 11% (6–73%). 13/31 (42%) had an abnormal pH study with a pH index $>12\%$. Conclusions: The high yield of microbiology, cytology, and pH probe investigations in NBS infants justifies invasive surveillance. Longitudinal studies to determine if early aggressive treatment results in improved outcome are awaited. **Pediatr Pulmonol. 2011; 46:696–700.**

Key words: cystic fibrosis; newborn screening; bronchoalveolar lavage; gastro-esophageal reflux.

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INTRODUCTION

Cystic fibrosis (CF) newborn screening (NBS) raises the important issue of how to manage asymptomatic infants. There is evidence of early functional,[1] structural,[2,3] and pathological[4,5] changes and even bronchiectasis[3] in NBS CF babies. Reliable culturing of lower airway pathogens is a challenge in infants who do not expectorate. Many centers currently regard bronchoalveolar lavage (BAL) as the golden standard in this age group and its methodology, ethics, and safety are well established.[6,7]

In a cohort study from the pre-NBS era we have previously shown a high prevalence of unsuspected lower airway infection in 25 newly diagnosed CF children. Forty-four percent of asymptomatic children investigated with fiber-optic bronchoscopy (FOB) and BAL at a median age of 1 year had a positive culture. Five children (20%) had a first isolation of *Pseudomonas aeruginosa*.[8]

Gastro-esophageal reflux (GOR) is common in older CF children with a prevalence between 25 and 50%.[9,10] In our pre-screen cohort, 12 out of 21 (57%) children had positive pH studies, only two of them had displayed GOR symptoms.[11] In older children, GOR is associated with reduction in first second forced expired volume (FEV_1)[12–14] and improves with medical treatment.[9] The only available study in NBS diagnosed infants <6 months of age reported the incidence of GOR at 19% defining a pH index of >10 as abnormal.[15]

Given the above data, and in particular the findings in other NBS populations[1–5] we routinely perform FOB, BAL, and pH monitoring in our NBS population. In this manuscript, we report the diagnostic yield of these procedures.

METHODS

Infants diagnosed through the national screening program within our catchment area attend the ward in our specialist center for a 2-day educational visit shortly after diagnosis, usually within 1 month of birth and within

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1 week of diagnosis. A cough swab is obtained and prophylactic flucloxacillin routinely commenced, together with pancreatic enzyme replacement therapy and vitamin supplements in those with pancreatic insufficiency. Children are subsequently invited to attend electively for FOB and pH study under the same general anesthetic at 3–4 months of age. Written parental consent is obtained.

Case notes and computer records of patients referred between July 2007 (when the NBS program was launched in London and the surrounding regions) and November 2009 were reviewed retrospectively. Results of cough swabs obtained during the initial educational visit and any subsequent clinical encounters were recorded. Respiratory symptoms at the time of bronchoscopy were assessed from the clinical records noting parameters such as increased cough, respiratory distress, tachypnea and added respiratory sounds on auscultation. FOB was performed using an Olympus BF-XP40 bronchoscope with 2.8 mm external diameter containing a service channel measuring 1.2 mm (KeyMed, Southend on Sea, Essex, UK) pernasally via an appropriately sized facemask chosen by the anesthetist, with suction only applied once the bronchoscope had passed through the vocal cords. FOB and BAL were carried through according to the same protocol by all operators, instilling three aliquots of 1 ml/kg normal saline into a single lobe, usually the lingula or right middle lobe. Standard microbiological culture, virology immunofluorescence, and neutrophil percentage were performed from pooled BAL samples. Fat laden macrophages (FLMs) were scored as small, moderate, or large numbers without calculating percentage of total macrophages present.[16]

Dual probes simultaneously measuring gastric and distal esophageal acidity were used to maximize sensitivity and specificity. A pH index >12 was used to diagnose pathological reflux in an effort to minimize false positive test results.[17] Parents of patients empirically treated with anti-reflux medication were asked to discontinue this 3 days prior to attendance.

In view of the small numbers, non-parametric Spearman tests were employed to evaluate correlations between pH indices on the one hand and airway neutrophilia and FLMs on the other hand using GraphPad Prism 5.02 software (San Diego, CA). Since this was a service evaluation, ethical approval was deemed not necessary.

RESULTS

Thirty-three children, nineteen (58%) male, underwent FOB, BAL, and 24 hr pH monitoring at a median age of 100 days (47–215 days). The diagnosis of CF had been confirmed with either sweat tests or genotyping at a median age of 25 days (0–80 days). Twelve were homozygous for the $\triangle$F 508 mutation, sixteen heterozygous, and five showed a combination of two other mutations. One set of parents declined FOB. All children were receiving oral flucloxacillin prophylaxis at the time of FOB and this was not discontinued. Thirty-one out of 33 (94%) infants were asymptomatic at the time of FOB. In 9 out of 33 (27%) patients at least one bacterial organism was grown on BAL fluid culture. The organisms identified are shown in Figure 1. All led to a change in antibiotics. Seven of the nine infected patients (78%) were asymptomatic at the time of FOB and only one had a positive cough swab prior to FOB (*Pseudomonas aeruginosa*). All the patients in the non-infected group were asymptomatic and two had prior positive cough swabs (both *Staphylococcus aureus*). All but two patients (infected with *S. aureus* and *P. aeruginosa*, respectively) had normal macroscopic airway appearance. Viral immuno-fluorescence was negative in all patients.

Three children had had culture-positive cough swabs prior to FOB. Two had grown *S. aureus* (66 and 136 days prior to FOB, respectively) and were treated with appropriate courses of antibiotics. Their BAL was sterile. One had grown *P. aeruginosa* and FOB was performed 9 days later following treatment with oral antibiotics to evaluate whether macroscopic airway appearance warranted intravenous eradication. Thick secretions were identified and *P. aeruginosa* identified at BAL, assessment of neutrophil count was not possible due to a sample processing error. The child was treated with intravenous antibiotics for 2 weeks and subsequent cough swabs were negative. Three out of 33 children (9%) showed increased cough following the procedure. None of those children had prior positive cough swab cultures and one of them grew *Haemophilus influenzae* from the BAL. They were all were treated with a 4-week course of oral antibiotics.

Twenty-seven out of 33 (82%) samples were of satisfactory quality for differential cell counts. Three out of the remaining six samples contained predominantly epithelial cells and were not deemed representative of alveolar contents. Three samples were not analyzed owing to processing errors. Four of the samples without differential cell count were infected (two *H. influenzae* and two *P. aeruginosa*). Using an upper limit normal of 4%,[6] 18 patients (67%) had neutrophilia (median 11%, range 6% to 73%). Thirteen (72%) of those were not bacterially infected. All infected patients had raised neutrophil counts, see Figure 2.

Twenty-four hour pH monitoring was successful in 31 out of 33 children, the probe having coiled up in the esophagus of two patients. Ten (32%) had been treated with prokinetics (domperidone) and acid reducing agents (ranitidine or omeprazole) on clinical grounds prior to FOB. These had been discontinued 3 days prior to admission. Thirteen patients (42%) had an abnormal pH study with a pH index greater than 12. In five (38%) of them GOR had been clinically suspected and treated

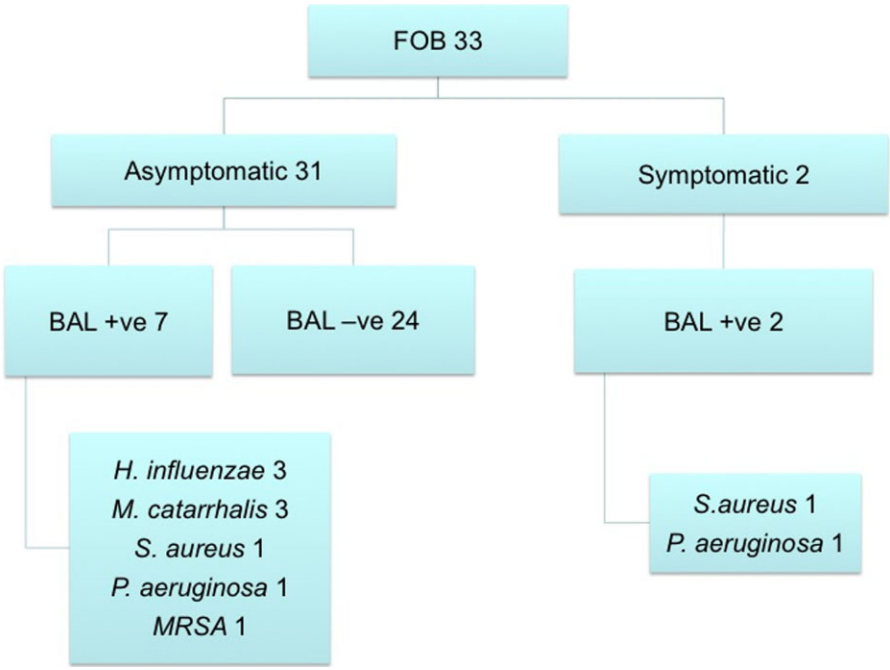

Fig 1. Distribution of pathogens from BAL (n = 33) and relationship with symptoms, 11 isolates in nine patients. Nine organisms grown in seven asymptomatic patients as two were doubly infected with *H. influenzae*/MRSA and *M. catarrhalis*/*S. aureus*, respectively.

empirically prior to the pH study. Five out of 18 (28%) patients with a normal pH index had been treated empirically. Clinical assessment of GOR hence showed a sensitivity of 0.38 (0.15–0.68) and specificity of 0.72 (0.46–0.89). FLM were visually assessed on BAL and categorized as small (4 patients), moderate (7 patients) or large (20 patients) numbers. Spearman testing revealed no significant correlation between pH results and FLM ($P = 0.89$). Treatment for GOR was newly initiated in 12 patients (39%) based on a combination of clinical evaluation, airway appearance, FLM count and pH results. Three patients on empirical anti-reflux treatment showed no evidence of GOR on either pH study or FLM count.

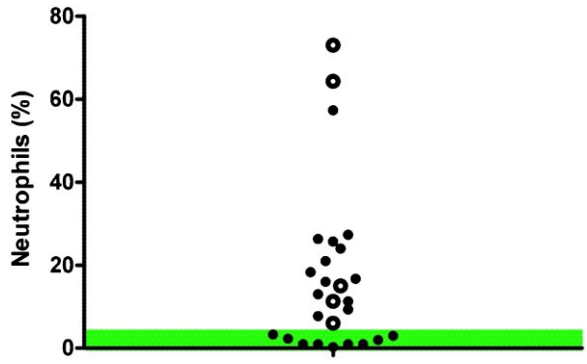

Fig 2. BAL neutrophil counts (n = 27), shaded area shows normal values (<4%), infected patients depicted with open symbols.

Decisions about discontinuation of their anti-reflux treatment were postponed until the following clinic appointment. The number of infants treated for GOR thus rose from 10 (32%) to 22 (71%). There was no correlation between GOR on pH study and airway neutrophilia ($P = 0.52$).

DISCUSSION

The main finding of this study is that in our mostly asymptomatic NBS cohort 27% of patients had unsuspected positive BAL cultures. All infected patients and 67% of the whole group had evidence of airway inflammation manifest by airway neutrophilia. Other studies have prospectively and comprehensively evaluated airway infection and inflammation in NBS diagnosed infants[1,3] but few have examined cohorts as young as ours (median age 100 days).[4,5] Our findings are novel in that we routinely prescribe flucloxacillin prophylaxis 2–3 months prior to FOB. Whilst only 2 out of 33 patients grew *S. aureus* six cultured other ''early'' CF pathogens such as *H. influenzae* and *Moraxella catarrhalis* (three each). Although upper airway contamination cannot be excluded, this constellation invites speculation about the contribution of staphylococcal prophylaxis. Prospective randomized controlled trials would be necessary to clarify this relationship. Only 6% of patients in the current study grew *P. aeruginosa* compared to 20% in Hilliard's older symptom-diagnosed cohort.[8]

There has been debate as to whether the CF airway is intrinsically pro-inflammatory in the absence of infection. Nine out of 22 (41%) uninfected children had normal cytology, whereas 13 (59%) were neutrophilic. We used 4% as upper limit of normal for neutrophil differential counts. There is a paucity of literature addressing normal BAL differential counts in infancy. Most data are obtained by analyzing BAL from ''healthy'' controls, many of whom display upper airway pathology which might itself skew results. Moreover, varying dilution methods are used and studies inconsistently discard the first aliquot containing a higher proportion of neutrophils.[7] Had we used a higher upper limit of normal such as e.g., 15%, we would have still found 13 (39%) non-infected neutrophilic patients. We cannot tell whether these infants had previous infections, but the finding of CF patients with normal airway cytology supports the findings of Armstrong et al.[18] that ''pristine'' CF is not necessarily associated with neutrophilic inflammation.

There are a number of weaknesses in this study. Performing BAL in one rather than multiple lobes might have led to underestimation of infection. Although bacterial counts were not available we consider the presence of any pathogens in BAL fluid to be of relevance, in keeping with previous publications.[19–21] Viral studies used immunofluorescence rather than PCR, and thus viral pathogens may have been missed. Indeed, there is evidence that neutrophil activation may follow viral infection[22] and a more rigorous identification strategy might have provided an explanation in 13 out of 18 (72%) non-bacterially infected neutrophilic patients. However, the presence of these would not have triggered any therapeutic change on the basis of current protocols. In non-CF children with asthma-like symptoms, those with GOR had a higher proportion of airway neutrophils than controls.[23] The authors postulated a pathogenic role of IL-8 in the recruitment and activation of neutrophils as a response to aspiration of gastric contents. Other studies suggest that bacterial infection rather than esophagitis was responsible for airway neutrophilia.[24] Despite using a relatively high threshold to diagnose pathological reflux, 42% had abnormal esophageal pH indices. Although our data did not suggest any associations between pH index or FLM and airway neutrophilia, future longitudinal studies should incorporate different monitoring protocols for GOR and quantify the effect of treatment, particularly in asymptomatic patients, on airway infection and inflammation.

The study was not designed to compare microbiological yield from BAL with cough swabs. Many of the cough swabs were obtained weeks prior to BAL and a cough swab closer to the time of BAL might have revealed the identified pathogen. Sputum induction supported by physiotherapy and pharyngeal suction, safely, and successfully performed in infants of a similar age[25,26] may have provided a less invasive and possibly clinically useful alternative route of sampling the lower airway. The main limitation of the study is the lack of outcome data. Such a study is ongoing in Australasia, comparing bronchoscopic guided therapy with standard monitoring. Recruitment is complete (C. Wainwright, personal communication) but the 5-year follow-up period is still ongoing.

The high yield of microbiology, cytology, and pH probe investigations in NBS infants suggests that invasive surveillance FOB should be considered as a routine. Early identification and aggressive treatment of occult pulmonary infection and subclinical GOR are expected to delay decline in lung function, but longitudinal studies are awaited to confirm this hypothesis.

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