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Keywords:	Cystic fibrosis, Newborn screening, Bronchoalveolar lavage, Gastro-oesophageal reflux

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Bronchoscopy in NBS infants with CF

For Peer Review

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 hour pH monitoring in a group of CF infants diagnosed on NBS.

Methods

Infants referred to a tertiary paediatric respiratory centre between July 2007 and November 2009 underwent surveillance fibre-optic bronchoscopy (FOB), BAL, and insertion of a 24 hour dual pH probe monitoring under a single general anaesthetic.

Results

We studied 33 infants, median age of 100 days (47 to 215 days) at the time of FOB. In 9 of 33 (27%) bacterial organisms were identified. 7 of the 9 patients (78%) were asymptomatic and only 1 had had a positive cough swab prior to FOB. Neutrophilia was identified in 18/27 (67%) cases with a median of 11% (6% to 73%). 13/31 (42%) had an abnormal pH study with a pH index greater than 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.

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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 centres currently regard bronchoalveolar lavage (BAL) as the gold 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 twenty-five newly diagnosed CF children. 44% of asymptomatic children investigated with fibre-optic bronchoscopy (FOB) and BAL at a median age of one year had a positive culture. Five children (20%) had a first isolation of *Pseudomonas aeruginosa*. (8)

Gastro-oesophageal reflux (GOR) is common in older CF children with a prevalence between 25 - 50%. (8;9) In our pre-screen cohort, 12 out of 21 (57%) children had positive pH studies, only two of them had displayed GOR symptoms. (10) In older children, GOR is associated with reduction in first second forced expired volume (FEV_1) (11-13) and improves with medical treatment. (9) The only available study in NBS diagnosed infants less than six months of age reported the incidence of GOR at 19% defining a pH index of greater than 10 as abnormal. (14)

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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.

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METHODS

Infants diagnosed through the national screening program within our catchment area attend the ward in our specialist centre for a two day educational visit shortly after diagnosis, usually within one month of birth and within one 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 anaesthetic at three to four 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, tachypnoea 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 anaesthetist, 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

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lingula or right middle lobe. Standard microbiological culture, virology immunofluorescence and neutrophil percentage were performed from pooled BAL samples. Fat laden macrophages were scored as small, moderate or large numbers without calculating percentage of total macrophages present. (15)

Dual probes simultaneously measuring gastric and distal oesophageal acidity were used to maximise sensitivity and specificity. A pH index greater than 12 was used to diagnose pathological reflux in an effort to minimise false positive test results. (16) Parents of patients empirically treated with anti-reflux medication were asked to discontinue this three 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 fat laden macrophages on the other hand using GraphPad Prism 5.02 software (San Diego, California, USA). Since this was a service evaluation, ethical approval was deemed not necessary.

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RESULTS

Thirty-three children, nineteen (58%) male, underwent FOB, BAL, and 24 hour pH monitoring at a median age of 100 days (47 to 215 days). The diagnosis of CF had been confirmed with either sweat tests or genotyping at a median age of 25 days (0 to 80 days). Twelve were homozygous for the ΔF 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 thirty-three (94%) infants were asymptomatic at the time of FOB. In nine out of thirty-three (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 *Staphylococcus aureus* and *Pseudomonas 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 *Staphylococcus aureus* (66 and 136 days prior to FOB respectively) and were treated with appropriate courses of antibiotics. Their BAL was sterile. One had grown *Pseudomonas aeruginosa* and FOB was performed 9 days later following treatment with oral antibiotics to evaluate whether macroscopic airway appearance warranted

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intravenous eradication. Thick secretions were identified and *Pseudomonas 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 two weeks and subsequent cough swabs were negative. Three out of thirty-three 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 four week course of oral antibiotics.

Twenty-seven out of thirty-three (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 analysed owing to processing errors. Four of the samples without differential cell count were infected (two *Haemophilus influenzae* and two *Pseudomonas aeruginosa*). Using an upper limit normal of 4%, (6) eighteen 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.

24 hour pH monitoring was successful in thirty-one out of thirty-three children, the probe having coiled up in the oesophagus 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 three 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 empirically prior to

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the pH study. Five out of eighteen (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). Fat laden macrophages (FLM) were visually assessed on BAL and categorised as small (four patients), moderate (seven patients) or large (twenty patients) numbers. Spearman testing revealed no significant correlation between pH results and FLM (p=0.89). Treatment for GOR was newly initiated in twelve 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. 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).

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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 two to three months prior to FOB. Whilst only two out of thirty-three patients grew *Staphylococcus aureus* six cultured other "early" CF pathogens such as *Haemophilus influenzae* and *Moraxella catarrhalis* (three each). Although upper airway contamination cannot be excluded, this constellation invites speculation about the contribution of staphylococcal prophylaxis. Prospective randomised controlled trials would be necessary to clarify this relationship. Only 6% of patients in the current study grew *Pseudomonas 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 twenty-two (41%) uninfected children had normal cytology, whereas thirteen (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 analysing BAL from "healthy" controls, many of whom display upper airway pathology which might

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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 thirteen (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 thirteen out of eighteen (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 oesophagitis was responsible for airway neutrophilia. (24) Despite using a relatively high threshold to diagnose pathological reflux, 42% had abnormal oesophageal pH indices. Although our

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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 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 (Wainwright C, personal communication) but the five 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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Figure 1: Distribution of pathogens from BAL (n=33) and relationship with symptoms, 11 isolates in 9 patients. 9 organisms grown in 7 asymptomatic patients as 2 were doubly infected with H. influenzae/ MRSA and M. catarrhalis/ S. aureus respectively.

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

For Peer Review

Reference List

(1) Linnane BM, Hall GL, Nolan G, Brennan S, Stick SM, Sly PD, Robertson CF, Robinson PJ, Franklin PJ, Turner SW, Ranganathan SC; AREST-CF. Lung function in infants with cystic fibrosis diagnosed by newborn screening. Am J Respir Crit Care Med 2008 Dec 15;178(12):1238-44.

(2) Linnane B, Robinson P, Ranganathan S, Stick S, Murray C. Role of high-resolution computed tomography in the detection of early cystic fibrosis lung disease. Paediatr Respir Rev 2008 Sep;9(3):168-74.

(3) Stick SM, Brennan S, Murray C, Douglas T, von Ungern-Sternberg BS, Garratt LW, Gangell CL, De Klerk N, Linnane B, Ranganathan S, Robinson P, Robertson C, Sly PD; AREST-CF. Bronchiectasis in infants and preschool children diagnosed with cystic fibrosis after newborn screening. J Pediatr 2009 Nov;155(5):623-8.

(4) Armstrong DS, Grimwood K, Carzino R, Carlin JB, Olinsky A, Phelan PD. Lower respiratory infection and inflammation in infants with newly diagnosed cystic fibrosis. BMJ 1995 Jun 17;310(6994):1571-2.

(5) Sly PD, Brennan S, Gangell C, de Klerk N, Murray C, Mott L, Stick SM, Robinson PJ, Robertson CF, Ranganathan SC. Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. Am J Respir Crit Care Med. 2009 Jul 15;180(2):146-52.

(6) Midulla F, de Blic J, Barbato A, Bush A, Eber E, Kotecha S, Haxby E, Moretti C, Pohunek P, Ratjen F; ERS Task Force. Flexible endoscopy of paediatric airways. Eur Respir J 2003 Oct;22(4):698-708.

(7) de Blic J, Midulla F, Barbato A, Clement A, Dab I, Eber E, Green C, Grigg J, Kotecha S, Kurland G, Pohunek P, Ratjen F, Rossi G. Bronchoalveolar lavage in children. ERS Task Force on bronchoalveolar lavage in children. European Respiratory Society. Eur Respir J 2000 Jan;15(1):217-31.

(8) Hilliard TN, Sukhani S, Francis J, Madden N, Rosenthal M, Balfour-Lynn I, Bush A, Davies JC. Bronchoscopy following diagnosis with cystic fibrosis. Arch Dis Child 2007 Oct;92(10):898-9.

(9) Malfroot A, Dab I. New insights on gastro-oesophageal reflux in cystic fibrosis by longitudinal follow up. Arch Dis Child 1991 Nov;66(11):1339-45.

(10) Brodzicki J, Trawinska-Bartnicka M, Korzon M. Frequency, consequences and pharmacological treatment of gastroesophageal reflux in children with cystic fibrosis. Med Sci Monit 2002 Jul;8(7):CR529-CR537.

(11) Sukhani S, Hiliard TN, Francis J, Rosenthal M, Balfour-Lynn I, Bush A, Davies JC. Flexible bronchoscopy and oesophageal pH monitoring in children newly diagnosed with cystic fibrosis. Arch Dis.Child 91[Suppl I], A50-A54. 2006. Ref Type: Abstract

(12) Navarro J, Rainisio M, Harms HK, Hodson ME, Koch C, Mastella G, Strandvik B, McKenzie SG. Factors associated with poor pulmonary function: cross-sectional analysis of data from the ERCF. European Epidemiologic Registry of Cystic Fibrosis. Eur Respir J 2001 Aug;18(2):298-305.

(13) Gustafsson PM, Fransson SG, Kjellman NI, Tibbling L. Gastro-oesophageal reflux and severity of pulmonary disease in cystic fibrosis. Scand J Gastroenterol 1991 May;26(5):449-56.

For Peer Review

(14) Stringer DA, Sprigg A, Juodis E, Corey M, Daneman A, Levison HJ, Durie PR. The association of cystic fibrosis, gastroesophageal reflux, and reduced pulmonary function. Can Assoc Radiol J 1988 Jun;39(2):100-2.

(15) Heine RG, Button BM, Olinsky A, Phelan PD, Catto-Smith AG. Gastro-oesophageal reflux in infants under 6 months with cystic fibrosis. Arch Dis Child 1998 Jan;78(1):44-8.

(16) Collins KA, Geisinger KR, Wagner PH, Blackburn KS, Washburn LK, Block SM. The cytologic evaluation of lipid-laden alveolar macrophages as an indicator of aspiration pneumonia in young children. Arch Pathol Lab Med 1995 Mar;119(3):229-31.

(17) Vandenplas Y, Goyvaerts H, Helven R, Sacre L. Gastroesophageal reflux, as measured by 24-hour pH monitoring, in 509 healthy infants screened for risk of sudden infant death syndrome. Pediatrics 1991 Oct;88(4):834-40.

(18) Armstrong DS, Hook SM, Jamsen KM, Nixon GM, Carzino R, Carlin JB, Robertson CF, Grimwood K. Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. Pediatr Pulmonol 2005 Dec;40(6):500-10.

(19) Ratjen F, Rietschel E, Griese M, Ballmann M, Kleinau I, Döring G, Reinhardt D, Paul K. Fractional analysis of bronchoalveolar lavage fluid cytology in cystic fibrosis patients with normal lung function. Bronchoalveolar lavage for the evaluation of anti-inflammatory treatment (BEAT) study group. Eur Respir J. 2000 Jan;15(1):141-5.

(20) Konstan MW, Hilliard KA, Norvell TM, Berger M. Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild lung disease suggest ongoing infection and inflammation. Am J Respir Crit Care Med 1994;150:448-454.

(21) Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. Am J Respir Crit Care Med 1995;151:1075-1082.

(22) Gern JE, Rosenthal LA, Sorkness RL, Lemanske RF. Effects of viral respiratory infections on lung development and childhood asthma. J Allergy Clin Immunol. 2005 Apr; 115(4):668-74.

(23) Sacco O, Silvestri M, Sabatini F, Sale R, Moscato G, Pignatti P, Mattioli G, Rossi GA. IL-8 and airway neutrophilia in children with gastroesophageal reflux and asthma-like symptoms. Respir Med 2006 Feb;100(2):307-15.

(24) Chang AB, Cox NC, Faoagali J, Cleghorn GJ, Beem C, Ee LC, Withers GD, Patrick MK, Lewindon PJ. Cough and reflux esophagitis in children: their co-existence and airway cellularity. BMC Pediatr 2006;6:4.

For Peer Review

(25) Mussaffi H, Fireman EM, Mei-Zahav M, Prais D, Blau H. Induced sputum in the very young: a new key to infection and inflammation. Chest 2008 Jan;133(1):176-82.

(26) Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Lancet. 2005 Jan 8-14;365(9454):130-4.

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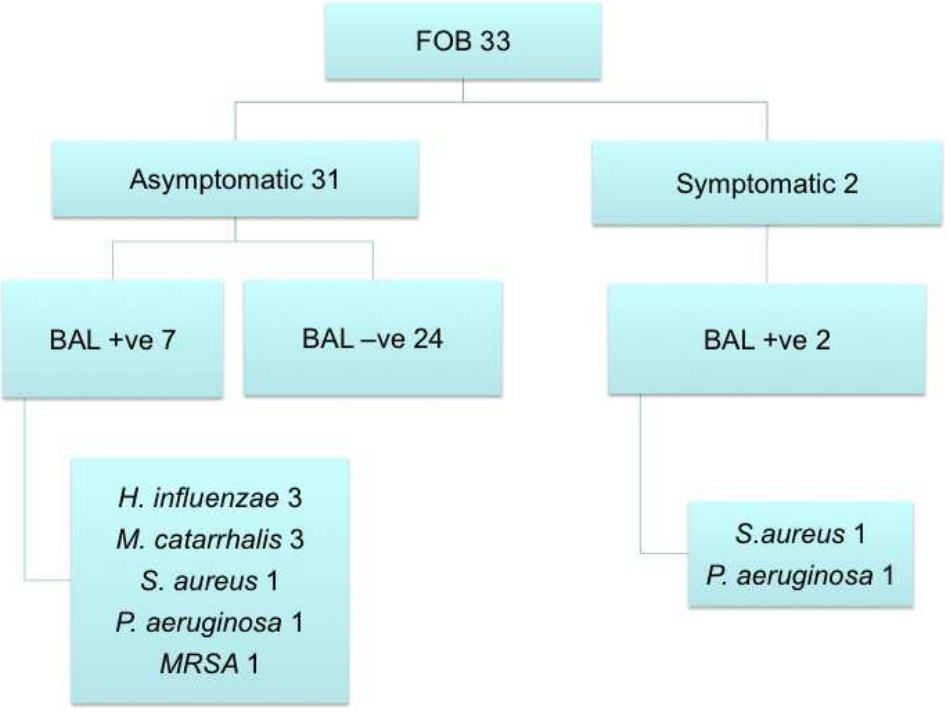

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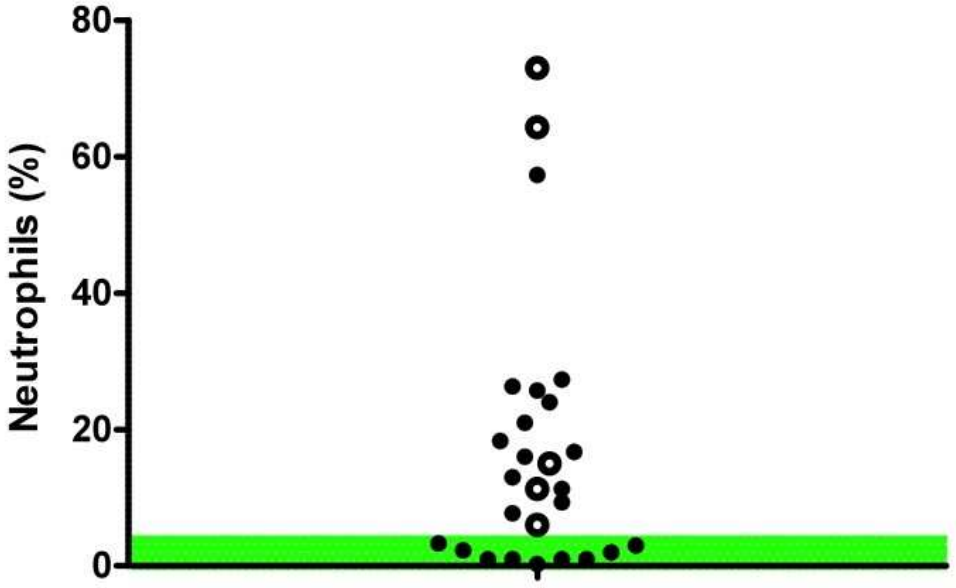

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Methods

Infants referred to a tertiary paediatric respiratory centre between July 2007 and November 2009 underwent surveillance fibre-optic bronchoscopy (FOB), BAL, and insertion of a 24 hour dual pH probe monitoring under a single general anaesthetic.

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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 centres currently regard bronchoalveolar lavage (BAL) as the gold standard in this age group and its methodology, ethics and safety are well established. (6;7)

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intravenous eradication. Thick secretions were identified and *Pseudomonas 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 two weeks and subsequent cough swabs were negative. Three out of thirty-three 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 four week course of oral antibiotics.

Twenty-seven out of thirty-three (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 analysed owing to processing errors. Four of the samples without differential cell count were infected (two *Haemophilus influenzae* and two *Pseudomonas aeruginosa*). Using an upper limit normal of 4%, (6) eighteen 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.

24 hour pH monitoring was successful in thirty-one out of thirty-three children, the probe having coiled up in the oesophagus 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 three 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 empirically prior to

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the pH study. Five out of eighteen (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). Fat laden macrophages (FLM) were visually assessed on BAL and categorised as small (four patients), moderate (seven patients) or large (twenty patients) numbers. Spearman testing revealed no significant correlation between pH results and FLM (p=0.89). Treatment for GOR was newly initiated in twelve 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. 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).

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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 two to three months prior to FOB. Whilst only two out of thirty-three patients grew *Staphylococcus aureus* six cultured other "early" CF pathogens such as *Haemophilus influenzae* and *Moraxella catarrhalis* (three each). Although upper airway contamination cannot be excluded, this constellation invites speculation about the contribution of staphylococcal prophylaxis. Prospective randomised controlled trials would be necessary to clarify this relationship. Only 6% of patients in the current study grew *Pseudomonas 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 twenty-two (41%) uninfected children had normal cytology, whereas thirteen (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 analysing BAL from "healthy" controls, many of whom display upper airway pathology which might

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Deleted: : There is a very young median age (100 days) and we routinely prescribe flucloxacillin prophylaxis two to three months prior to FOB. The relative absence of *Staphylococcus aureus* and rather large number of infants infected with other "early" CF pathogens such as *Haemophilus influenzae* and *Moraxella catarrhalis*

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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 thirteen (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.

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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 thirteen out of eighteen (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 oesophagitis was responsible for airway neutrophilia. (24) Despite using a relatively high threshold to diagnose pathological reflux, 42% had abnormal oesophageal pH indices. Although our

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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 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 (Wainwright C, personal communication) but the five year follow up period is still ongoing.

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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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Figure 1: Distribution of pathogens from BAL (n=33) and relationship with symptoms, 11 isolates in 9 patients. 9 organisms grown in 7 asymptomatic patients as 2 were doubly infected with H. influenzae/ MRSA and M. catarrhalis/ S. aureus respectively.

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

Reference List

(1) Linnane BM, Hall GL, Nolan G, Brennan S, Stick SM, Sly PD, Robertson CF, Robinson PJ, Franklin PJ, Turner SW, Ranganathan SC; AREST-CF. Lung function in infants with cystic fibrosis diagnosed by newborn screening. Am J Respir Crit Care Med 2008 Dec 15;178(12):1238-44.

(2) Linnane B, Robinson P, Ranganathan S, Stick S, Murray C. Role of high-resolution computed tomography in the detection of early cystic fibrosis lung disease. Paediatr Respir Rev 2008 Sep;9(3):168-74.

For Peer Review

(3) Stick SM, Brennan S, Murray C, Douglas T, von Ungern-Sternberg BS, Garratt LW, Gangell CL, De Klerk N, Linnane B, Ranganathan S, Robinson P, Robertson C, Sly PD; AREST-CF. Bronchiectasis in infants and preschool children diagnosed with cystic fibrosis after newborn screening. J Pediatr 2009 Nov;155(5):623-8.

(4) Armstrong DS, Grimwood K, Carzino R, Carlin JB, Olinsky A, Phelan PD. Lower respiratory infection and inflammation in infants with newly diagnosed cystic fibrosis. BMJ 1995 Jun 17;310(6994):1571-2.

(5) Sly PD, Brennan S, Gangell C, de Klerk N, Murray C, Mott L, Stick SM, Robinson PJ, Robertson CF, Ranganathan SC. Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. Am J Respir Crit Care Med. 2009 Jul 15;180(2):146-52.

(6) Midulla F, de Blic J, Barbato A, Bush A, Eber E, Kotecha S, Haxby E, Moretti C, Pohunek P, Ratjen F; ERS Task Force. Flexible endoscopy of paediatric airways. Eur Respir J 2003 Oct;22(4):698-708.

(7) de Blic J, Midulla F, Barbato A, Clement A, Dab I, Eber E, Green C, Grigg J, Kotecha S, Kurland G, Pohunek P, Ratjen F, Rossi G. Bronchoalveolar lavage in children. ERS Task Force on bronchoalveolar lavage in children. European Respiratory Society. Eur Respir J 2000 Jan;15(1):217-31.

(8) Hilliard TN, Sukhani S, Francis J, Madden N, Rosenthal M, Balfour-Lynn I, Bush A, Davies JC. Bronchoscopy following diagnosis with cystic fibrosis. Arch Dis Child 2007 Oct;92(10):898-9.

(9) Malfroot A, Dab I. New insights on gastro-oesophageal reflux in cystic fibrosis by longitudinal follow up. Arch Dis Child 1991 Nov;66(11):1339-45.

(10) Brodzicki J, Trawinska-Bartnicka M, Korzon M. Frequency, consequences and pharmacological treatment of gastroesophageal reflux in children with cystic fibrosis. Med Sci Monit 2002 Jul;8(7):CR529-CR537.

(11) Sukhani S, Hiliard TN, Francis J, Rosenthal M, Balfour-Lynn I, Bush A, Davies JC. Flexible bronchoscopy and oesophageal pH monitoring in children newly diagnosed with cystic fibrosis. Arch Dis.Child 91[Suppl I], A50-A54. 2006. Ref Type: Abstract

(12) Navarro J, Rainisio M, Harms HK, Hodson ME, Koch C, Mastella G, Strandvik B, McKenzie SG. Factors associated with poor pulmonary function: cross-sectional analysis of data from the ERCF. European Epidemiologic Registry of Cystic Fibrosis. Eur Respir J 2001 Aug;18(2):298-305.

(13) Gustafsson PM, Fransson SG, Kjellman NI, Tibbling L. Gastro-oesophageal reflux and severity of pulmonary disease in cystic fibrosis. Scand J Gastroenterol 1991 May;26(5):449-56.

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For Peer Review

(14) Stringer DA, Sprigg A, Juodis E, Corey M, Daneman A, Levison HJ, Durie PR. The association of cystic fibrosis, gastroesophageal reflux, and reduced pulmonary function. Can Assoc Radiol J 1988 Jun;39(2):100-2.

(15) Heine RG, Button BM, Olinsky A, Phelan PD, Catto-Smith AG. Gastro-oesophageal reflux in infants under 6 months with cystic fibrosis. Arch Dis Child 1998 Jan;78(1):44-8.

(16) Collins KA, Geisinger KR, Wagner PH, Blackburn KS, Washburn LK, Block SM. The cytologic evaluation of lipid-laden alveolar macrophages as an indicator of aspiration pneumonia in young children. Arch Pathol Lab Med 1995 Mar;119(3):229-31.

(17) Vandenplas Y, Goyvaerts H, Helven R, Sacre L. Gastroesophageal reflux, as measured by 24-hour pH monitoring, in 509 healthy infants screened for risk of sudden infant death syndrome. Pediatrics 1991 Oct;88(4):834-40.

(18) Armstrong DS, Hook SM, Jamsen KM, Nixon GM, Carzino R, Carlin JB, Robertson CF, Grimwood K. Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. Pediatr Pulmonol 2005 Dec;40(6):500-10.

(19) Ratjen F, Rietschel E, Griese M, Ballmann M, Kleinau I, Döring G, Reinhardt D, Paul K. Fractional analysis of bronchoalveolar lavage fluid cytology in cystic fibrosis patients with normal lung function. Bronchoalveolar lavage for the evaluation of anti-inflammatory treatment (BEAT) study group. Eur Respir J. 2000 Jan;15(1):141-5.

(20) Konstan MW, Hilliard KA, Norvell TM, Berger M. Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild lung disease suggest ongoing infection and inflammation. Am J Respir Crit Care Med 1994;150:448-454.

(21) Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. Am J Respir Crit Care Med 1995;151:1075-1082.

(22) Gern JE, Rosenthal LA, Sorkness RL, Lemanske RF. Effects of viral respiratory infections on lung development and childhood asthma. J Allergy Clin Immunol. 2005 Apr; 115(4):668-74.

(23) Sacco O, Silvestri M, Sabatini F, Sale R, Moscato G, Pignatti P, Mattioli G, Rossi GA. IL-8 and airway neutrophilia in children with gastroesophageal reflux and asthma-like symptoms. Respir Med 2006 Feb;100(2):307-15.

(24) Chang AB, Cox NC, Faoagali J, Cleghorn GJ, Beem C, Ee LC, Withers GD, Patrick MK, Lewindon PJ. Cough and reflux esophagitis in children: their co-existence and airway cellularity. BMC Pediatr 2006;6:4.

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Deleted: . (21) . Armstrong DS, Grimwood K, Carzino R, Carlin JB, Olinsky A, Phelan PD. Lower respiratory infection and inflammation in newly diagnosed cystic fibrosis. BMJ 1995;310:1571-1572.¶

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For Peer Review

(25) Mussaffi H, Fireman EM, Mei-Zahav M, Prais D, Blau H. Induced sputum in the very young: a new key to infection and inflammation. Chest 2008 Jan;133(1):176-82.

(26) Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Lancet. 2005 Jan 8-14;365(9454):130-4.

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For Peer Review