ELSEVIER
Journal of Cystic Fibrosis xx (2017) xxx – xxx
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Original Article

Pooling of bronchoalveolar lavage in children with cystic fibrosis does not adversely affect the microbiological yield or sensitivity in detecting pulmonary inflammation

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Received 30 August 2017; revised 26 October 2017; accepted 26 October 2017
Available online xxxx

Abstract

Background: Bronchoalveolar lavage (BAL) is a potentially useful outcome measure for clinical trials in children with CF but its use is limited by variations in approach internationally. We sought to determine if pooling adversely affected the diagnostic properties of BAL.
Methods: Children undergoing bronchoscopy for clinical reasons were included. A multi-step study protocol ensured BAL was collected and analysed both separately and as a pooled fluid.
Results: Eighty-five children (53 CF, 32 control) were recruited. There was a high level of concordance between pooled and non-pooled samples in terms of organism identification (76%). There was good agreement (Bland Altman) between the two methods in terms of detection of inflammation independent of centre, microbiological concordance or disease status. Bi-directional variability in IL-8 levels between pooled and non-pooled samples was seen. Free neutrophil elastase (NE) was detected in 4 cases in pooled lavage when absent in non-pooled lavage. Levels of interleukin-8 (IL-8) were similar between the two groups with pooled samples showing a greater spread of values.
Conclusions: Pooling of BAL in children does not negatively impact on either the detection of pulmonary infection or inflammation or the observed relationship between infection and inflammation. Intra-patient variability in BAL IL-8 levels suggests regional differences in inflammation.

Keywords: Cystic fibrosis; Infection; Inflammation; Children; Bronchoalveolar lavage; Pooling

1. Background

CF lung disease begins early in life, and often in the absence of clinical symptoms [1]. The development of structural lung disease such as bronchiectasis is not reversible, therefore the

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https://doi.org/10.1016/j.jcf.2017.10.016
1569-1993/

ultimate goal of clinicians involved in the care of children with CF is the prevention of lung disease rather than treatment of established disease. This will necessitate the increasing involvement of infants and young children in interventional trials of new therapies [2]. This concept was at the heart of a European Respiratory Society Research Seminar in Rotterdam, the Netherlands in 2012, which focused on developing and standardising robust outcome measures for clinical trials in young children with CF [3]. This study was devised to provide evidence for standardisation of BAL procedures for use in clinical trials.

Current methods for collection and processing of BAL in children vary considerably between centres, and little structured guidance on standard approaches is available given the dearth of primary evidence in this field [4]. There are many different indications for BAL (airway disease, interstitial disease, diffuse or focal disease), and from a clinical point of view, the specific indication should inform the approach to the procedure. BAL is usually performed by instilling 2–3 sequential aliquots of normal saline and suctioning after each. Sequential BAL samples are thought to represent first the proximal (large airway), and then more distal (small airway and alveoli) airspaces.

The majority of BAL procedures (independent of the indication for bronchoscopy) in children, including those with CF, are aimed at detecting airspace infection and/or inflammation [5], without specific regard to the exact location. For this indication, some centres use first lavages for microbial culture, and subsequent lavages from a single lobe (most commonly right middle lobe) for inflammatory markers, with other centres pooling lavage fluid from some or all lobes for both tests. The volume of fluid used for lavage varies considerably. Pooling of sequential BAL samples from different lobes can produce a large volume homogenous sample with greater proximal airway representation. Most centres use a lavage volume based on the patient's weight in an effort to ensure a stable epithelial lining fluid concentration [6], however the number and location of lavages, and how the lavages are used are variable. For the purposes of this study, the use of the pooled first lavages (right and left) for microbiology and the second lavage on the right for inflammatory markers was held as the gold standard.

It would be desirable for this very common clinical indication to have a standardised approach to BAL which, aside from the obvious benefit in terms of clinical trials outcome measures, would also help considerably to facilitate multi-centre collaborative research into disease mechanisms and biomarkers of lung disease. The aim of this study was to assess the microbiological yield of pooled BAL compared to first lavages, and to determine whether pooling of BAL adversely affected the detection of inflammatory markers or their relationship to infection compared to the second lavage on the right.

2. Methods

All bronchoscopies were clinically indicated and were performed under general anaesthesia with a flexible bronchoscope. The detailed study protocol (see supplementary information) aimed at comparing the two commonest BAL practices involved two individual lavages on both the right and left sides with samples collected individually and then a component pooled resulting in four key samples: Two microbiology samples - pooled first lavages from right and left, and all four samples pooled, and two inflammation samples – second lavage from the right side and all four lavages pooled. Samples were transported to the laboratory on ice and processed as per study protocol (see supplementary information). Microbiological cultures were processed in the individual hospitals clinical microbiology laboratories. Given variations in reporting across centres and at times within centres, culture results translated into a semi-quantitative scale as follows: + (Scanty/0–20 cfu), ++ (Light/20–100 cfu), +++ (Moderate/100–1000 cfu), ++++ (Heavy/>1000 cfu). BAL IL-8 and NE were measured centrally at one laboratory (EF, PS). IL-8 levels were determined using a commercial AlphaLISA assay kit in accordance with the manufacturers' instructions (Human Interleukin 8 AlphaLISA kit AL224C, Perkin Elmer, Melbourne, Australia).

Neutrophil elastase was measured by an enzymatic assay. The kinetics of substrate hydrolysis catalyzed by human neutrophil elastase of specific substrate *N*-methoxysuccinyl-ala-ala-pro-val *p*-nitroanilide (Sigma-Aldrich Pty. Ltd. Sydney, Australia) were monitored by an increase in absorbance using a BMG FLUOstar Omega plate reader. The enzymatic activity of the sample was compared to a Human Neutrophil Elastase standard (Sigma-Aldrich Pty. Ltd. Sydney, Australia).

Research ethics approval was obtained from the institutional review boards of each of the individual hospitals and parents/carers provided informed consent for the use of samples for research; age appropriate assent was obtained from children. Descriptive statistics were used for clinical information and microbiology results. The primary method of analysis for establishing agreement between the pooled and non-pooled methods was the Bland-Altman plot. Histograms were used to compare the spread of IL-8 values between groups and receiver operator curves (ROC) were used to compare the sensitivity and specificity of the two methods to detect active infection.

3. Results

Eighty-five subjects (mean 4.6 years [SD 3.1 years]) including 53 with CF, (4.9 years [2.9]) and 32 controls (4.1 years [3.3] $p = 0.28$) undergoing clinically indicated bronchoscopy and BAL were prospectively recruited to the study. Indications for bronchoscopy were varied and can be seen in Table 1. Mean BAL return (for 2 × 1 ml/kg lavages) was 37 [9.2] % (mean [SD]) for the first lavages and 66 [19.7] % for the second lavages. Mean total pooled lavage return was 50% or 2.01 [0.44] ml/kg body weight. Of 198 culture results in 85 patients, 151 (76%) showed concordance between the first and pooled lavages in terms of identification of organisms. Of the 47 instances where culture results differed, 14 cases (7.1%) involved a recognised pathogen: these were detected from the first but not pooled sample in 8 cases [*P. aeruginosa* × 1, *H. influenza* × 3, *S. pneumonia* × 4] and from the pooled but not first lavages in 6 cases [*P. aeruginosa* × 2, *S. maltophilia* × 1, *H. influenza* × 1, *S. pneumonia* × 1, *M. catarrhalis* × 1] ($p = 0.57$). In the other

Please cite this article as: McNally P, et al, Pooling of bronchoalveolar lavage in children with cystic fibrosis does not adversely affect the microbiological yield or sensitivity in detecting pulmonary inflammation, J Cyst Fibros (2017), https://doi.org/10.1016/j.jcf.2017.10.016