Clinimetric properties of bronchoalveolar lavage inflammatory markers in cystic fibrosis

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ABSTRACT The Standardisation Committee of the European Cystic Fibrosis Society Clinical Trial Network has undertaken the evaluation of clinical end-points for therapeutic interventions regarding their use in multicentre clinical trials in cystic fibrosis (CF). This review of biomarkers in bronchoalveolar lavage (BAL) is part of the group's work.

The aims of this project were: 1) to review the literature on reliability, validity and responsiveness of BAL in patients with CF; 2) to gain consensus of the group on the feasibility of BAL; and 3) to gain consensus on answers to key questions regarding the promotion of BAL to surrogate end-point status.

Assessment of BAL inflammatory markers in the literature indicates that their reliability, validity and responsiveness are adequate for clinical trials. After discussion of the practical characteristics it was concluded that BAL has an attractive validity profile, albeit with limited feasibility. It is particularly applicable to multicentre trials in preschool children with CF and early or mild lung disease. This is the first article to collate the literature in this manner. This provides a rationale to support the use of BAL in early clinical trials in preschool children with CF.

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Review of the literature provides rationale to support the use of BAL in well-designed early-phase clinical trials in CF http://ow.ly/qGInZ

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Introduction

The European Cystic Fibrosis Society Clinical Trial Network (ECFS-CTN) Standardisation Committee is undertaking a rigorous evaluation of outcome measures used in clinical trials in cystic fibrosis (CF). The committee is composed of six groups consisting of researchers with expertise in specific outcome measures: CF transmembrane conductance regulator biomarkers [1], respiratory function, inflammatory markers, anthropometrics, microbiology definitions and chest imaging. This article summarises the work of the inflammatory markers group on inflammatory markers obtained *via* bronchoalveolar lavage (BAL) and is one of a series of documents from the six groups.

Outcome measures can be classified as clinical end-points, surrogate end-points or biomarkers. Clinical end-points reflect how a patient feels, functions or survives and detect a tangible benefit for the patient [2, 3]. A surrogate end-point is a laboratory measurement used to predict the efficacy of therapy [2, 3] when direct measurement of clinical effect is not feasible or practical. Surrogate end-points may shorten the period of follow-up required; however the link between the surrogate end-point and long-term prognosis must be proven. Forced expiratory volume in 1 s (FEV_1) is still the only accepted surrogate outcome for the European Medicines Agency and the US Food and Drug Administration. With regard to their use as outcome measures, inflammatory markers obtained *via* BAL are currently considered to be ''biomarkers''. A biomarker is defined as ''a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic response to a therapeutic intervention'' [2, 3]. Biomarkers are mainly used to explore proof-of-concept for a specific compound. Some are currently being considered for ''promotion'' to the status of surrogate end-point. A full description of the classification of outcome measures is provided in the first document in the series of articles from the ECFS-CTN Standardisation Committee [1].

BAL inflammatory markers that may be considered, depending on the clinical or research goal [4, 5], include total and differential cell counts (in particular neutrophils), elastase and its complexes with inhibitors (for example neutrophil elastase (NE)-α1-antitrypsin) [6, 7], pro- and anti-inflammatory cytokine/chemokine levels (interleukin (IL)-8 and tumour necrosis factor (TNF)-α), levels of eicosanoids and leukotrienes, biomarkers of oxidative stress (such as 3-chlorotyrosine) and the inflammatory state of epithelial cells recovered from the airways. For the purpose of the present report the working group focused on BAL polymorphonuclear (PMN) neutrophil count or percentage, NE, IL-6 and IL-8.

To gain acceptance by researchers and licensing bodies, an end-point must have a body of supporting evidence: acceptable clinimetric properties such as reliability, validity and responsiveness to treatment, and sufficient feasibility and safety (table 1). Clinimetric properties and feasibility are population- and situation-dependent, therefore data cannot readily be extrapolated to the CF population from other disease populations. It is necessary to provide an evidence base from CF specifically. It is also necessary to consider

TABLE 1 Clinimetric properties of biomarkers

	Definition	Justification of importance
Reliability	Degree to which a measurement is consistent and free from error	Important to quantify error (systematic and random) so that true changes can be discerned from changes due to normal fluctuations
Validity		
Concurrent validity	Degree to which a test correlates with a ''gold standard'' criterion test which has been established as a valid test of the attribute of interest	The gold standard outcome measures are often not feasible; therefore, it is important to know how an alternative outcome measure compares to the gold standard, and how different outcome measures compare
Convergent validity	Degree to which a test correlates with another test which measures the same attribute	
Discriminate validity	Degree to which a test differentiates between groups of individuals known to differ in the attribute of interest	It is important to know the ability of outcome measures to discriminate between different groups
Predictive validity	Degree to which an attribute can be predicted using the result of a predictor test/or degree to which prognosis can be predicted	
Responsiveness	Degree to which a test changes in response to an intervention known to alter the attribute of interest	Important attribute of tests used in clinical practice or research to assess treatment benefit (*e.g.* to identify improvements in response to an intervention)

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subgroups of patients, for example age range and clinical severity may be important. The intervention is also an important factor when considering the responsiveness of an outcome measure.

The aims of this study were: 1) to review the literature on clinimetric properties (*e.g.* reliability, validity and responsiveness) of BAL inflammatory markers in patients with CF; 2) to gain consensus of the group on the feasibility of BAL; and 3) to gain consensus on answers to key questions regarding the promotion of BAL inflammatory markers to surrogate end-point status.

Methods

An exhaustive literature search was conducted in MEDLINE, Allied and Complementary Medicine (AMED) and Embase using a combination of keywords: ''bronchoalveolar lavage'' or ''pulmonary inflammation'' or ''airway inflammation'' or ''lung inflammation'' or ''neutrophil'' or ''polymorphonuclear'' or ''PMN'' or ''interleukin'' or ''IL-6'' or ''IL-8'' or ''cytokine'' or ''epithelial lining fluid'' or ''inflammatory marker'', and ''cystic fibrosis''. The search was limited to full text articles in the English language, with no limits on year of publication. A bibliography search was also conducted of all included articles and other relevant literature such as reviews and editorials.

For clinimetric properties, data were extracted and tabulated for reliability, validity, correlation with other outcome measures, responsiveness and reference values. Definitions are given in table 1. To evaluate feasibility, data were extracted and tabulated on the proportion of attempts that were successful and reasons for excluding tests. An expert panel also discussed the following topics at several face-to-face meetings (Venice, Italy: November 17 and 18, 2010; Hamburg, Germany: June 9, 2011; Rotterdam, the Netherlands: March 23 and 24, 2012) and reached consensus on each: risk involved, cost, ease of performance, ease of administration, time to administer, equipment and space needed, and applicable age group. Specific advantages and limitations of BAL were also discussed.

Narrative answers to four key questions were prepared, discussed and agreed by the group. 1) Do BAL inflammatory markers have the potential to become surrogate outcome measures? 2) For what kind of therapeutic trial are BAL inflammatory markers appropriate? (Therapeutic aim, phase of trial, target population, number of patients involved and number of sites involved.) 3) Within what timeline can change be expected and what treatment effect can be considered clinically significant? 4) What are the most needed studies to further define BAL inflammatory markers in patients with CF and to explore their potential as a surrogate outcome measures? The consensus of the group is presented in the current article.

Results

Why use BAL inflammatory markers in clinical trials in CF?

Flexible bronchoscopy with BAL provides a regional measurement of lung inflammation and infection within the respiratory tract, including the small airways and alveoli [8]. Inflammation has been shown to be present early in CF lung disease before changes are detected by traditional pulmonary function techniques such as spirometry [9]. The ability to identify early airway inflammation and infection in these ''silent years'' is of great importance for investigating new therapies in infants and young children.

Being the most direct measurement of infection and inflammation of the lower airways and distal lung, BAL has greatly contributed to the understanding of the pathophysiological process in CF, especially in infants and young children. Lung secretions from patients with CF contain large concentrations of TNF-α and interleukins/chemokines (IL-8, IL-6, IL-1, *etc.*) [5]. All of these cytokines share a common characteristic: their synthesis is promoted by nuclear factor-κB, which is activated by cellular interaction with bacteria, bacterial products and proinflammatory cytokines. IL-8 is a chemoattractant of PMN cells and its mRNA increases with osmotic stress, which is a part of the pathogenesis of CF [10, 11]. Cell counts and mediators reflect endobronchial inflammation. PMN cell infiltration induces the release of oxidants and proteases, leading to lung tissue degradation. CF lungs may be primed for inflammation, and bacterial and/or viral infection may potentiate such inflammation [12]. Inflammation with increased numbers of PMN cells is seen in BAL from the first few weeks of life, independent of clinical signs and symptoms. In early disease, BAL changes represent a preclinical indicator of possible underlying (but clinically silent) inflammation [13–15]. A substantial proportion of infants diagnosed with CF after detection by newborn screening have active pulmonary inflammation, 30% have detectable NE activity, 20% have pulmonary infection and 80% have evidence of structural lung disease on chest computed tomography (CT) at 3 months of age [9].

Together with chest CT, BAL inflammatory markers have been used as clinical end-points in pathophysiological studies (Australian Respiratory Early Surveillance Team for CF) [9], in clinical trials of tobramycin inhaled solution [16–18] and in clinical trials of recombinant human (rh)DNase [19–21]. It has also been used to direct therapy against *Pseudomonas aeruginosa* infection (Australasian CF Bronchoalveolar Lavage) [22]. However, no soluble marker of inflammation has been studied in a

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comprehensive, longitudinal manner so as to be validated as a ''gold standard'' for assessing the inflammatory response [23, 24].

Clinimetric properties of BAL inflammatory markers

Reliability

To the best of our knowledge, short-term reproducibility has not been studied in CF. One study of 60 BAL samples from intubated non-CF children in the intensive care unit showed satisfactory short-term reproducibility of cell counts in blind nonbronchoscopic BAL, except for lymphocytes [25] (table E1). In noninfected infants with CF, some insight regarding long-term reproducibility has been provided by repeat BAL conducted 6–18 months apart, indicating a certain degree of variability related to an ongoing inflammatory process [26]. Similarly, PAUL *et al.* [19] showed that the PMN cell percentage and NE in pooled BAL samples significantly increased over time (up to 36 months) in children with CF not treated by rhDNase.

Although considered the gold standard for sampling lower respiratory secretions, BAL may produce variable results due to regional heterogeneity of inflammation [23, 27]. PMN cell count and percentage and NE activity are generally greater in upper-lobe BAL fluid (BALF) in adults [27]. Upper- *versus* lower-lobe differences were more pronounced in subjects with better preservation of lung function [27].

Concurrent validity

Due to the localised nature of enhanced inflammation in CF, respiratory secretions from the lower airways are considered by many researchers to be the optimal source of inflammatory cells and soluble mediators [5]. BAL is considered the best method for obtaining samples from the lower respiratory tract (especially in very young children who are too young to successfully undergo sputum induction with hypertonic saline), thus yielding the most accurate measure of the infectious and/or inflammatory process in the CF airway lumen [24]. However, it has been shown that the inflammatory patterns and responses to infective stimuli in the airway lumen and the airway wall of children with CF are distinct and compartmentalised, and thus BAL and endobronchial biopsy provide different but complementary information [28]. In contrast to the neutrophil dominated inflammation present in the airway lumen, the bronchial mucosa is characterised by the recruitment and accumulation of lymphocytes [28].

Convergent validity

In young pre-adolescent children, there is a good correlation ($r>0.55$) between BAL PMN cell count/percentage and IL-8 (five out of five studies [13, 29–32]) or neutrophil elastase (one study) [13]. The correlation was not a constant finding in older patients and adults (three out of four studies) [27, 33–35] (table E2).

One study reported good correlation ($r^2=0.48$, $p=0.036$) between the inflammatory response (IL-8 and PMN cells) in the upper airway, sampled by nasal washes, and the lower airways, sampled by BAL [36]. The correlation between inflammatory markers obtained *via* BAL and other sampling methods is variable. Two studies showed no correlation between BAL and induced sputum [6, 34], while one study showed excellent agreement between IL-8 recovered from BALF and spontaneously expectorated sputum ($r=0.967$, $p=0.007$) [37]. In contrast to induced sputum, which originates from the airways, BAL samples the bronchial or terminal airways and air spaces (alveoli), according to the fractions analysed (the latter aliquots more likely representing the alveolar region). Induced sputum and BAL thus provide different but complementary data.

Discriminate validity

Discriminate validity of BAL inflammatory markers is shown in tables 2, 3 and 4.

The majority of studies (17 out of 19 studies [6, 26, 31, 33, 35, 37–43, 45–51]) indicate that all four biomarkers are present at significantly higher levels in CF *versus* non-CF patients, the latter consisting mainly of patients undergoing bronchoscopy for other causes such as recurrent lower respiratory tract infection, chronic cough and upper airway obstruction, over a wide age span (from 2 weeks to adult). However, there was no significant difference in BAL IL-8, NE and PMN cells between children with CF and primary ciliary dyskinesia (one study) [45].

Several studies have demonstrated the ability of BAL inflammatory markers to discriminate between groups of patients with CF who have different degrees of lung disease severity. BAL PMN cell percentage (two out of two studies [9, 30]) and NE (one study) [9] are significantly more elevated in symptomatic *versus* asymptomatic children with CF (but not adults (one study) [53]).

BAL PMN cell counts (seven out of eight studies) and percentage (seven out of seven studies), NE (four out of five studies) and IL-8 (eight out of 11 studies) have been shown to increase in children infected with

TABLE 2 Discriminate validity of bronchoalveolar lavage (BAL) inflammatory markers between cystic fibrosis (CF) patients and non-CF subjects

First author [ref.]	Aliquot fraction analysed	CF		Non-CF		PMN cell counts $\times 10^3$ cells·mL^{-1}, unless otherwise stated	PMN cells %	Neutrophil elastase	IL-6 $\times 10^3$·mL^{-1}, unless otherwise stated	IL-8 $\times 10^3$·mL^{-1}, unless otherwise stated	Statistical tests	Data presentation
		Subjects n	Age	Subjects n	Age							
Armstrong [26]	F	50 infected	19.1 (1.5–71) months	19	12.4±11.8 months	CF 50 (39–65) *versus* non-CF 8.2 (4.6–15)***	CF 80 (66–90) *versus* non-CF 32 (13–57)***		CF 1153 (754–1762) *versus* non-CF 24 (12–47)***	CF 50 (39–65) *versus* non-CF 8.2 (4.6–15)***	Wald test	Geometric mean (95% CI)
Armstrong [26]	F	28	Infants (age not reported)	10	Infants (age not reported)			CF 11 (8.7–13) *versus* non-CF 2.1 (0.95–4.7) μg·mL^{-1}***			Wald test	Geometric mean (95% CI)
Muhlebach [38]	Not specified	17	25±4 months	25	15±3 months	CF 41.2±7.0 *versus* non-CF 21.8±5.2*			CF 6868±2065 pg·mL^{-1} *versus* non-CF 1434±362 pg·mL^{-1}¶	CF 41.2±7.0 *versus* non-CF 21.8±5.2*	Mann–Whitney U-test	Mean±SEM
Armstrong [39]	F	5 virus infected	Infants (age not reported)	13	Infants (age not reported)	CF 169 (16–1809) *versus* non-CF 7 (2–22)***				CF 1372 (561–3358) *versus* non-CF 32 (12–84)***	ANCOVA	Geometric mean (95% CI)
Khan [31]	P	16	0.48 (0.08–0.97) years	11	1.02 (0.25–2.7) years	CF 673.6±367.9 *versus* non-CF 6.9±1.7+		CF 1.18±0.46 μg·mL^{-1} *versus* non-CF 0 μg·mL^{-1}§		CF 1298±338 pg·mL^{-1} *versus* non-CF 92±25 pg·mL^{-1}***	Independent t-test	Mean±SEM
Kirchner [40]	P	16	10.5 (2–25) months	9 (disease)	11.7 (3–30) months	¶					Kruskal–Wallis	Values not reported
Muhlebach [41]#,¶	Not specified	51	1.8 years (3 weeks–13 years)	50 (disease)	1.0 years (2 weeks–8.3 years)	***	*			***	Independent t-test	Values not reported
Muhlebach [42]#	Not specified	55	3.7 (0.1–13) years	56	2.1 (0.04–9) years	*				*	Independent t-test	Values not reported
Noah [43]	P	15 (infected)	2.4 (0.1–13) years	12 (uninfected)	1.4 (0.1–8.8) years	CF 3358 (100–11 520) *versus* non-CF 10 (1–117)*	CF 84 (16–96) *versus* non-CF 5 (1–51)*		CF 56 (17–681) *versus* non-CF 10 (2–59)*	CF 11 614 (1424–71 100) *versus* non-CF 459 (21–2040)*	Kruskal–Wallis	Median (range)
Noah [43]	P	15 (infected)	2.4 (0.1–13) years	12 (infected)	1.4 (0.1–8.8) years	CF 3358 (100–11 520) *versus* non-CF 229 (1–2976)*	CF 84 (16–96) *versus* non-CF 46 (1–93)*		NS	CF 11 614 (1424–71 100) *versus* non-CF 1860 (18–6224)*	Kruskal–Wallis	Median (range)
MacGregor [44]	P	39	6.4±0.7 years	38	6.1±0.6 years		NS			CF 1308.1±56.4 *versus* non-CF 716±89.9***	Mann–Whitney U-test	Mean±SEM
Hilliard [45]	P	43	6.2 (0.3–16.8) years	7 (PCD)	9.2 (5.7–14.8) years	NS		NS		NS	Nonparametric tests	
Hilliard [45]	P	43	6.2 (0.3–16.8) years	26 (CRD)	4.8 (0.9–15.2) years	CF 0.7 (0.02–23.97)$\times 10^6$·mL^{-1} *versus* non-CF 0 (0–17.91)$\times 10^6$·mL^{-1}***		CF 201 (100–5000) μm·mL^{-1} *versus* non-CF 100 (100–1297) μm·mL^{-1}***		CF 1340 (31–1760) pg·mL^{-1} *versus* 703 (31–1560) pg·mL^{-1}***	Nonparametric tests	Median (range)
Hilliard [45]	P	43	6.2 (0.3–16.8) years	7 (disease control)	5.8 (0.3–16.3) years	CF 0.7 (0.02–23.97)$\times 10^6$·mL^{-1} *versus* non-CF 0 (0–0.01)$\times 10^6$·mL^{-1}***		CF 201 (100–5000) μm·mL^{-1} *versus* non-CF 100 (100–100) μm·mL^{-1}***		CF 1340 (31–1760) pg·mL^{-1} *versus* 64 (31–221) pg·mL^{-1}**	Nonparametric tests	Median (range)

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TABLE 2 Continued

First author [ref.]	Aliquot fraction analysed	CF		Non-CF		PMN cell counts $\times 10^3$ cells·mL^{-1}, unless otherwise stated	PMN cells %	Neutrophil elastase	IL-6 $\times 10^3$·mL^{-1}, unless otherwise stated	IL-8 $\times 10^3$·mL^{-1}, unless otherwise stated	Statistical tests	Data presentation
		Subjects n	Age	Subjects n	Age							
HARRIS [46]	F	29 (during exacerbation)	9.1±12 years	12	6.0±1.4 years	CF 6.1±0.12 $\log_{10}$ *versus* 4.9±0.17 $\log_{10}$***	CF 68.8±3.6 *versus* 16.4±3.8***				Wilcoxon rank sum test	Mean±SEM
DEAN [37]	svBAL, F	5	8 (4–18) years	8 (asthma)	Adults (age not reported)					CF 6618 (1200–11 136) pM *versus* non-CF 302.4 (48–984) pM*	Not reported	Mean (range)
DEAN [37]	svBAL, F	5	8 (4–18) years	6 (PCD)	Children (age not reported)					CF 6618 (1200–11 136) pM *versus* non-CF 302.4 (48–984) pM*^{f}	Not reported	Mean (range)
REEVES [47]	Not specified	15	Children and adolescents	12	8.21±1.22 years						Mann–Whitney U-test	Values not reported
RATJEN [48]	F	105	5–37 years	48	3–15 years		First aliquot CF 52±30 *versus* non-CF 4±5$^{\#\#}$				Mann–Whitney U-test	Mean±SD
RATJEN [48]	P (aliquots 2 and 3)	105	5–37 years	48	3–15 years		Pooled CF 29±23 *versus* non-CF 2±3$^{\#\#\P}$				Mann–Whitney U-test	Mean±SD
REEVES [49]	F	18	19.6±5.2 years	14 (other disease)	50.14± 10.5 years					CF 325±81.96 pg·mg^{-1} *versus* 125.92±43.95 pg·mg^{-1} *	Independent t-test	Mean±SEM
MCGARVEY [6]	F (cytology), P (remaining)	11	22.7±3.8 years	9 (mild asthma)	24.1±3.9 years			NS		NS	ANOVA	
				11 (healthy)	24.3±2.8 years							
BONFIELD [33]	P	22	20±2 years	13	296 years	CF 41±10×10^6·mL^{-1} *versus* non-CF 0.09±0.02×10^6·mL^{-1} *	CF 60±5 *versus* non-CF 1±0.3*	CF 1.9±0.5 µM *versus* non-CF 0 µM*	p=0.06 CF 287±104 *versus* non-CF 0	CF 32 565±9686 *versus* non-CF 0*	Kruskal–Wallis	Mean±SEM
KONSTAN [50]	P	18	20±1 years	23	25±1 years	CF 38±14 (1–222)×10^6·mL^{-1} *versus* non-CF 0.1±0.04 (0–0.6)×10^6·mL^{-1} *					Independent t-test	Mean±SEM (range)
MEYER [35]	P	14 (exacerbation)	17–40 years	8	19–24 years		CF 51.1±5.2 *versus* non-CF 0.3±0.4*	CF 323±127 nM·min^{-1}·mL^{-1} *versus* non-CF 0 nM·min^{-1}·mL^{-1}*			Independent t-test	Mean±SEM
NAKAMURA [51]	Not specified	8	28±2 years	7	29±3 years	CF 101±26 *versus* non-CF 0.4±0.3*		CF 31±10 *versus* non-CF 0*		CF 35±13 *versus* non-CF 0*	Not reported	Mean (range)

All four biomarkers are present at higher levels in patients with CF. All samples are BAL, unless otherwise stated. PMN: polymorphonuclear; IL: interleukin; F: first; P: pooled; svBAL: small volume BAL; NS: not significant; PCD: primary ciliary dyskinesia; CRD: chronic respiratory disease. $^{\#}$: [42] includes patients previously reported in [41]; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ¶: $p<0.005$; $^{+}$: $p=0.002$; §: $p=0.03$; ^{f}: $p=0.02$; $^{\#\#}$: $p<0.0001$.

TABLE 3 Discriminate validity of bronchoalveolar lavage (BAL) inflammatory markers between groups of patients with cystic fibrosis (CF) with different phenotypes

First author [ref.]	Aliquot fraction analysed	Group 1		Group 2		PMN cell counts $\times 10^3$ cells·mL^{-1}, unless otherwise stated	PMN cells %	Neutrophil elastase	IL-6 $\times 10^3$·mL^{-1}, unless otherwise stated	IL-8 $\times 10^3$·mL^{-1}, unless otherwise stated	Statistical tests	Data presentation
		Subjects n	Age	Subjects n	Age							
SLY [9]	F (microbiology), P (aliquots 2 and 3)	48 asymptomatic	Infants	9 symptomatic	Infants	NS	Group 1 14.5 (9.0–27.1) *versus* Group 2 39.7 (28.3–65.0)#	Group 1 100 (100–143) ng·mL^{-1} *versus* Group 2 100 (100–2025) ng·mL^{-1}#		NS	Independent t-tests	Median (IQR)
SLY [9]	F (microbiology), P (aliquots 2 and 3)	45 uninfected	Infants	12 infected	Infants	Group 1 31.3 (11.3–76.3) *versus* Group 2 169 (72.2–208.5)#	Group 1 13.5 (8.3–26.1) *versus* Group 2 35.7 (25.3–44.7)¶	Group 1 100 (100–100) ng·mL^{-1} *versus* Group 2 350 (100–1330) ng·mL^{-1}+		Group 1 260 (128–630) pg·mL^{-1} *versus* Group 2 770 (420–2120) pg·mL^{-1}§	Independent t-tests	Median (IQR)
ARMSTRONG [13]	svBAL, P	18 infected	2.6±1.1 months	10 noninfected, nonsymptomatic	2.6±1.1 months	Group 1 153 (52–449) *versus* Group 2 15 (8–29)***		Group 1 78 (52–94)% *versus* Group 2 0 (0–31)%***		Group 1 1112 (582–2126) *versus* Group 2 24 (9–63)***	Independent t-test	Mean (95% CI)
SLY [9]	F (microbiology), P (aliquots 2 and 3)	24 no antistaphylococcal prophylaxis	3.6 (3.4–4.8) months	33 antistaphylococcal prophylaxis	3.6 (2.8–4.7) months	NS	NS	NS		NS	Independent t-tests	
ARMSTRONG [26]	F	28 infected	Infants (age not reported)	7 pristine 17 uninfected	Infants (age not reported)				Group 1 11 (8.7–13) pg·mL^{-1} *versus* Group 2 pristine 2.2 (0.48–10) pg·mL^{-1} and uninfected 2.2 (1.8–2.6) pg·mL^{-1} ***		Wald test	Geometric mean (95% CI)
ARMSTRONG [26]	F	50 infected		13 pristine		Group 1 562 (407–777) *versus* Group 2 pristine 127 (76–210) and uninfected 106 (67–168)***	Group 1 50 (39–65) *versus* Group 2 pristine 8.4 (5.2–14) and uninfected 7.1 (4.4–12)***	Group 1 80 (66–90)% *versus* Group 2 pristine 0 (0–25)% and uninfected 20 (6–44)% of patients with free NE activity***		Group 1 1153 (754–1762) pg·mL^{-1} *versus* Group 2 pristine 32 (15–72) pg·mL^{-1} and uninfected 49 (23–101) pg·mL^{-1}***	Wald test	Geometric mean (95% CI)
ROSENFELD [52]	Not specified	31 ($\geqslant 10^5$ CFU·mL^{-1})	⩽15 months at study entry	29 ($<10^5$ CFU·mL^{-1})	⩽15 months at study entry	NS				NS	Generalised estimating equation	
ROSENFELD [52]	Not specified	31 ($\geqslant 10^5$ CFU·mL^{-1})	⩽15 months at study entry	30 no pathogen	⩽15 months at study entry	Group 1 1145 (580–2264) *versus* Group 2 114 (63–206)***				Group 1 1802 (1104–2942) *versus* Group 2 427 (269–679)+¶	Generalised estimating equation	Geometric mean (95% CI)
ROSENFELD [52]	Not specified	Not specified *P. aeruginosa* positive	⩽15 months at study entry	Not specified other pathogen	⩽15 months at study entry	NS					Generalised estimating equation	
NIXON [53]	F	20 symptomatic	20.7±10.6 months	34 asymptomatic	15.7±9.7 months		NS	NS		NS	Generalised estimating equation	

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TABLE 3 Continued

First author [ref.]	Aliquot fraction analysed	Group 1		Group 2		PMN cell counts $\times 10^3$ cells·mL^{-1}, unless otherwise stated	PMN cells %	Neutrophil elastase	IL-6 $\times 10^3$·mL^{-1}, unless otherwise stated	IL-8 $\times 10^3$·mL^{-1}, unless otherwise stated	Statistical tests	Data presentation
		Subjects n	Age	Subjects n	Age							
Nixon [53]	F	15 infected	22.8±6.7 months	39 uninfected	15.9±10.9 months		Group 1 59 (47–70) *versus* Group 2 28 (21–35) ***	NS		Group 1 641 (305–1350) *versus* Group 2 253 (158–406)##¶	Generalised estimating equation	Mean and geometric mean (95% CI)
Dakin [54]	F	8 infected	23 months	14 uninfected	23 months		90.8% $>10^5$ pathogens *versus* 24.1% $<10^5$ pathogens¶¶			4600 pg·mL^{-1} $>10^5$ pathogens *versus* 1627 pg·mL^{-1} $<10^5$ pathogens++	Independent t-test/ ANOVA	
Brennan [30]	F (microbiology), P (aliquots 2 and 3)	9 infected	1.55 (1.11–2.51) years	15 uninfected	1.58 (1.12–2.91) years	667 (58–5342) infected *versus* 60 (39–91) noninfected‡	52 (38–81)% infected *versus* 17.3 (9–29)% noninfected ***			NS	Not reported	Median (IQR)
Gutierrez [55]	F	6 ($\geqslant 10^5$ CFU·mL^{-1})	35.3±21.3 months	24 ($<10^5$ CFU·mL^{-1})	22.1±13.3 months		*** (right middle lobe) NS (lingula)			§§ (right middle lobe) ¶¶ (lingula)	Independent t-test	
Armstrong [39]	F	5 virus-infected	6.5±5.3 months	22 noninfected	2.6±1.5 months	169 (16, 1809) virus infected *versus* 16 (8, 34) noninfected***				1372 (561, 3358) virus-infected *versus* 47 (21, 108) noninfected***	ANCOVA	Geometric mean (95% CI)
Brennan [30]	F (microbiology), P (aliquots 2 and 3)	14 asymptomatic	<6 years	11 symptomatic	<6 years	Group 1 60.3 (38.45–90.55) *versus* Group 2 667.58 (64.3–5341.48)‡‡,¶	Group 1 16.5 (10.5–26.5) *versus* Group 2 53 (49.67–80.5)***			NS	Not reported	Median (IQR)
Sagel [56]	P	59 *P. aeruginosa* positive	6 months–6 years	31 no pathogens	6 months–6 years	Group 1 5.6±0.7 $\log_{10}$·mL^{-1} *versus* Group 2 4.8±0.6 $\log_{10}$·mL^{-1}***	Group 1 49.3±25.9 *versus* Group 2 22.1±14.4***	Group 1 41% of patients *versus* Group 2 0% of patients with NE detected in BAL***	NS	Group 1 3.1±0.6 pg·mL^{-1} *versus* Group 2 2.3±0.6 pg·mL^{-1}***	Linear regression	Mean±SD
Sagel [56]	P	21 *P. aeruginosa* negative, other pathogen present	6 months–6 years	31 no pathogens	6 months–6 years	Group 1 59±0.3 $\log_{10}$·mL^{-1} *versus* Group 2 5.6±0.3 $\log_{10}$·mL^{-1}***	Group 1 33.6±17.6 *versus* Group 2 22.1±14.4*	NS	NS	Group 1 2.8±0.7 pg·mL^{-1} *versus* Group 2 2.3±0.6 pg·mL^{-1}**	Linear regression	Mean±SD
Sagel [56]	P	59 *P. aeruginosa* positive	6 months–6 years	21 *P. aeruginosa* negative, other pathogen present	6 months–6 years	NS	Group 1 33.6±17.6 *versus* Group 2 22.1±14.4+	NS	NS	NS	Linear regression	Mean±SD
Sagel [57]	P	44 nonmucoid *P. aeruginosa*	6 months–6 years	15 mucoid *P. aeruginosa*	6 months–6 years	Group 1 5.9±0.6 $\log_{10}$·mL^{-1} *versus* 5.5±0.7 $\log_{10}$·mL^{-1}# ***	Group 1 62.7±22.1 *versus* Group 2 44.5±25.7### ¶¶¶	NS	NS	NS	Linear regression	Mean±SD
Sagel [56]	P	Not specified *S. aureus* present	6 months–6 years	Not specified *S. aureus* not present	6 months–6 years				+	***	Linear regression	Values not reported

TABLE 3 Continued

First author [ref.]	Aliquot fraction analysed	Group 1		Group 2		PMN cell counts $\times 10^3$ cells·mL^{-1}, unless otherwise stated	PMN cells %	Neutrophil elastase	IL-6 $\times 10^3$·mL^{-1}, unless otherwise stated	IL-8 $\times 10^3$·mL^{-1}, unless otherwise stated	Statistical tests	Data presentation
		Subjects n	Age	Subjects n	Age							
Hilliard [45]	P	31 exacerbation	6.2 years	12 clinically well	6.2 years	Group 1 1.0×10^6·mL^{-1} *versus* Group 2 0.4×10^6·mL^{-1}*					Nonparametric tests	Median
Muhlebach [41]	Not specified	Not specified *P. aeruginosa* positive	Mean 7.6 years	Not specified other pathogen	Mean 1.9 years	NS				NS	Independent t-test	
Armstrong [15]	Not specified	14 infected	Children (age not reported)	20 noninfected	Children (age not reported)	Group 1 259 (70–968) $\times 10^6$·L^{-1} *versus* 17 (8–36) $\times 10^6$·L^{-1}***				Group 1 2013 (1164–3784) ng·L^{-1} *versus* Group 2 26 (13–51) ng·L^{-1}***	Independent t-test	Mean (95% CI)
Noah [43]	P	15 infected	<13 years	10 uninfected	<13 years	Group 1 3358 (100–11 520) *versus* Group 2 291 (25–952)*	Group 1 84 (16–96) *versus* Group 2 40 (4–62)*		NS	NS	Kruskal–Wallis	Median (range)
Regamey [28]	P	36 during exacerbation		10 stable		Group 1 970 (287–1584) *versus* Group 2 222 (21–380)¶¶¶					Independent t-test	Median (IQR)
Regamey [28]	P	13 *Aspergillus* present		13 *Aspergillus* not present		NS					Independent t-test	
Regamey [28]	P	11 *P. aeruginosa* positive		15 *P. aeruginosa* negative		NS					Independent t-test	
Bonfeld [33]	P	17 *P. aeruginosa* positive	Adolescents and adults (age not reported)	5 *P. aeruginosa* negative	Adolescents and adults (age not reported)					NS	Kruskal–Wallis	
Kirchner [40]	P	7 older patients	Mean (range) 18.6 (13.5–31) years	16 younger patients	Mean (range) 10.5 (2–25) months	NS					Kruskal–Wallis	

Biomarkers are present at higher levels in patients with more severe lung disease. All samples are BAL, unless otherwise stated. PMN: polymorphonuclear; IL: interleukin; F: first; P: pooled; svBAL: small volume BAL; IQR: interquartile range; NS: not significant; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; NE: neutrophil elastase. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; #: $p=0.0001$; ¶: $p=0.027$; +: $p=0.003$; §: $p=0.036$; ƒ: $p=0.04$; ##: $p=0.03$; ¶¶: $p=0.01$; ++: $p=0.0001$; §§: $p=0.05$; ƒƒ: $p= 0.002$; ###: $p=0.02$; ¶¶¶: $p=0.004$.

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TABLE 4 Discriminate validity of bronchoalveolar lavage (BAL) inflammatory markers

First author [ref.]	Aliquot fraction analysed	Subjects	Age	Participants	Results	Statistics
Khan [31]	P	16	0.08–0.97 years	Neutrophil count PMN cell count above controls (n=11) PMN cell count overlapping controls (n=5)	Lower S_{pO_2} in group with elevated PMN cell count (mean±SEM 92.8±0.9 *versus* 96.6±0.7, p=0.0097) Weight-for-age NS Respiratory rate NS	Independent t-test
Sagel [57]	P	111	6 months–6 years	Neutrophil elastase	Wisconsin chest computed tomography score NS Lower Shwachman scores in group with detectable free neutrophil elastase activity (63.5±8.8 *versus* 69.7±5.5, p<0.0001)	Not reported
Hull [58]	F	32		Presence of inflammation (PMN cell count and IL-8) Inflammation (n=13) No inflammation (n=19)	Higher lipid hydroperoxide concentration in group with detectable inflammation (geometric mean 97.7 *versus* 21.9 μM, p<0.05) Higher γ-GT in group with detectable inflammation (geometric mean 104.2 *versus* 20.2 U·L^{-1}, p<0.05)	Independent t-test

Data are presented as n, range or mean±SD, unless otherwise stated. Patients grouped by level of inflammation (from BAL biomarkers) differ in clinical presentation (including oxidative stress). P: pooled; F: first; PMN: polymorphonuclear; S_{pO_2}: arterial oxygen saturation measured by pulse oximetry; NS: nonsignificant; IL: interleukin; γ-GT: gamma glutamyl transpeptidase.

P. aeruginosa, *Staphylococcus aureus* and/or viruses *versus* no pathogens [9, 13, 26, 30, 39, 43, 52–54, 56]. However, BAL biomarkers are similar in the presence of *P. aeruginosa versus* other pathogens [41, 52, 57]. In one study, PMN cell count and percentage were increased in the presence of a mucoid-type *versus* nonmucoid *P. aeruginosa* [57]. Clinical presentation was also shown to differ between patients grouped according to the level of BAL inflammatory markers [31, 56, 58] (table 4).

Taken together, among the five biomarkers evaluated, PMN cell count and percentage appear to be the most consistently discriminant. BAL IL-8 is less consistently increased and NE and IL-6 appear to be of a much lesser utility in older children and adults.

Predictive validity

To the best of our knowledge, no study has looked specifically at the predictive value of BAL biomarkers in CF.

Correlation with other outcome measures

In older children and adults, BAL NE is significantly inversely correlated to FEV_1 (two out of two studies [45, 59]). BAL PMN cell percentage is most constantly (but moderately) associated to the other measures of lung function, such as respiratory system compliance [54], tissue elastance [30], forced expiratory flow at 25–75% of forced vital capacity [32] or lung distension [32]. Lung function impairment is not a good marker of BAL IL-8 levels (significant correlation in only two out of five studies [30, 32, 52, 54, 59]).

BAL biomarkers have been compared with radiological images obtained from CT scanning, deemed the best means of evaluating early structural lung damage. In 57 infants (median age 3.6 months), radiological evidence of structural lung disease was common, with 46 (80.7%) having an abnormal CT: 11 (18.6%) had bronchial dilatation, 27 (45.0%) had bronchial wall thickening and 40 (66.7%) had gas trapping [9]. As shown in table E3, the inflammatory marker most associated with structural lung disease is free NE activity, which has been significantly associated with presence and extent of bronchial dilatation (OR 10.9 and 10.8, respectively), presence and extent of bronchial wall thickening (OR 9.4 and 4.3, respectively) and extent of airway trapping (OR 3.9) [9]. No significant association was found between free NE activity and presence of air trapping.

BAL biomarkers are not consistently correlated with clinical status (respiratory rate and arterial oxygen saturation (S_{aO_2})), anthropometric parameters and Shwachman score (six studies [27, 29, 31, 37, 52, 60]).

Responsiveness

BAL inflammatory markers have been demonstrated to be responsive to a number of interventions in patients with CF (table 5).

TABLE 5 Responsiveness of bronchoalveolar lavage (BAL) inflammatory markers

First author [ref.]	Aliquot fraction analysed	Subjects n	Age	Intervention	Results					Statistical test Presentation of data
					PMN cell counts	PMN cell %	NE	IL-6	IL-8	
Response to treatment with antibiotics										
Armstrong [26]	F	13	22.1 (14–44) months	Eradication of infection Follow-up BAL at 6–18 months	Decreased T1 56.6% T2 21.2%		Decreased T1 13.6 μg·mL^{-1} T2 8.5 μg·mL^{-1}		Decreased T1 1782 pg·mL^{-1} T2 307 pg·mL^{-1}	Mean
Noah [18]	Not specified	15	14–133 months	TIS (inhaled 300 mg twice daily, 28 days) (n=6) *versus* ceftazidime and tobramycin (*i.v.*, 14 days) (n=9) Follow-up BAL at 4–6 weeks	p=0.02 Inhaled +0.9 (0.6–5.4) Systemic -0.26 (0.03–2.26)	NS Inhaled +5.4 (-11–15.3) Systemic -7.0 (-34.5–19.1)		NS	NS	Mann–Whitney U-test Median (range) ratio post *versus* pre
Gibson [17]	Not specified	21	6 months–6 years	TIS (inhaled, 28 days) *versus* placebo Follow-up BAL at day 28	NS			NS	NS	Not reported
Gibson [16]	Not specified	9	6 months–6 years	TIS (28 days) Follow-up BAL at day 56	NS	NS		NS	NS	One-sample t-test
Gibson [16]	Not specified	9	6 months–6 years	TIS (28 days) Follow-up BAL at day 84	NS	p=0.01 T1 60.5 (29.4) T2 38.8 (18.2)		NS	NS	One-sample t-test
Gibson [16]	Not specified	12	6 months–6 years	TIS (56 days) Follow-up BAL at day 112	NS	p=0.01 T1 53.4 (13.5) T2 35.4 (20.3)		NS	NS	One-sample t-test
Gibson [16]	Not specified	8	6 months–6 years	TIS (28 days) Follow-up BAL at day 112	NS	NS		NS	NS	One-sample t-test
Meyer [61]	P	11	17–40 years	*i.v.* antibiotics for respiratory exacerbation BAL start and end of 2 weeks admission	NS		p=0.017			Not reported
Acquisition of infection										
Armstrong [26]	F	8	20 (13–50) months	No infection in first BAL (T1) but present in second Follow-up BAL at 6–18 months (T2)	Increased T1 9.7% T2 55%		Increased T1 3.3 μg·mL^{-1} T2 10.3 μg·mL^{-1}		Increased T1 88 pg·mL^{-1} T2 1206 pg·mL^{-1}	Mean
Armstrong [13]	F	9	27±12 months	No infection in first BAL, but present in second Follow-up BAL at 6–18 months		NS	p<0.05 Fold change 2.0 (95% CI 1.2–3.2)		p<0.05 Fold change 2.3 (95% CI -1.06–5.2)	ANCOVA
DNase										
Ratjen [21]	F, P (2 and 3)	29 Treated group	5–25 years	Treated group (DNase inhaled 2.5 mg once daily, 18 months) *versus* untreated group (no DNase) Follow-up BAL at 18 months		NS				Unpaired t-test
		19 Untreated group	5–25 years							

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TABLE 5 Continued

First author [ref.]	Aliquot fraction analysed	Subjects n	Age	Intervention	Results					Statistical test Presentation of data
					PMN cell counts	PMN cell %	NE	IL-6	IL-8	
Paul [19]	F, P (2 and 3)	46 Treated group	11.3±5.1 years	Treated group (DNase inhaled, 36 months) *versus* untreated group (no DNase) Follow-up BAL at 18 and 36 months		Increase more pronounced in untreated group ($p<0.005$) than the treated group ($p<0.01$)	Increased in untreated group ($p<0.007$) but remained stable in treated group		Increased in untreated group ($p<0.02$) but remained stable in treated group	Nonparametric repeated measures analyses of variance
		39 Untreated group	12.2±4.4 years							
Ratjen [20]	F, P (2 and 3)	13 Treated group	6–18 years	Treated group (DNase inhaled 2.5 mg once daily, 18 months) *versus* untreated group (no DNase) Follow-up BAL at 18 months		Decreased in treated group -15 (-40–58) Increased in untreated group 20 (-49–48) NS between groups				Median (range) Mann–Whitney U-test
		10 Untreated group	6–18 years							
rSLPI										
McGarvey [6]	P	16	27±2 years	rSLPI (nebuliser 100 mg, twice daily, 1 week) Follow-up BAL 12 after the last aerosol			p<0.03 Average decrease 68%			Not reported

All samples are BAL unless stated. PMN: polymorphonuclear; NE: neutrophil elastase; IL: interleukin; F: first; NS: not specified; P: pooled; T1: first paired (range: 6–18 months) BAL sample; T2: second paired (range: 6–18 months) BAL sample; TIS: tobramycin inhalation solution; rSLPI: recombinant secretory leukocyte protease inhibitor.

Studies have demonstrated the responsiveness of BAL inflammatory markers to acquisition of infection and to treatment with antibiotics. In long-term longitudinal studies in infants, the acquisition of bacterial infection is accompanied by a greater than two-fold increase in BAL PMN cell count, NE and IL-8 [13, 26]. A decrease in NE (one study in adults [61]) was observed 2–6 weeks after *i.v.* antibiotics. Long-term studies show that inhaled antibiotics (tobramycin inhalation solution) inconsistently decrease PMN cell percentages or counts (one out of three studies [16–18]), but do not influence IL-6 or IL-8 (none out of three studies [16–18]). Repeat BAL performed up to 18 months after eradication of infection in young infants was accompanied by a sustained reduction in NE and IL-8 [13, 26].

Treatment with inhaled rhDNase has been shown to attenuate lung inflammation over a 3-year study period, with significant decreases in PMN cell percentage, NE and IL-8 in the treated group, contrasting with no change in the placebo group [19]. Nonsignificant data observed in some smaller studies may be due to small sample size [20, 21]. In adults, NE is significantly decreased 12 h following recombinant secretory leukoprotease inhibitor therapy [62].

The range of detectable change is variable, depending on the parameters studied. A floor effect may be present for certain markers, *e.g.* nondetectable levels of IL-8 [9]. However, there is no ceiling effect regarding absolute number of inflammatory cells or soluble markers (multi-log increases may be observed), although it may be possible to observe almost 100% PMN cells in BALF, *i.e.* PMN cells being the sole BAL cell subtype.

Reference values

BAL inflammatory biomarker levels in infants, older children and adults with and without CF are shown in table E4. A review including studies conducted in Australia, USA and Europe indicated "normal" values of BAL PMN cells, IL-8 and NE in young children and infants with CF [63]. The mean reported values for BAL PMN cells are 17.3% [30] or 134×10^3 cells·mL^{-1} [13] in noninfected, nonsymptomatic infants. In such infants, NE is undetectable and IL-8 levels are very low (24 pg·mL^{-1}) [13]. Values vary according to whether the first *versus* pooled BALF aliquots are analysed. Neutrophil dominated airway inflammation is more pronounced in the first (mean $\pm$ SD 52 $\pm$ 30%), mainly bronchial, BAL sample *versus* the pooled second and third BALF aliquots (mean $\pm$ SD 29 $\pm$ 23%), suggesting that sequential analysis of BALF may have a higher sensitivity to detect early inflammatory changes in CF patients [48]. In stable adults with CF and chronic bacterial infections, PMN cell count is $\geqslant 200 \times 10^3$ cells·mL^{-1}, PMN cells exceed 37.5% of total cell counts, with detectable levels of NE and IL-8 [27].

Feasibility and safety

Two studies included in the review reported success rates [13, 19] (table E5). One study reported a 78% success rate for a one-time BAL with loss of data due to insufficient BALF recovery [13]. One longitudinal study reported a dropout rate of 48–58% due to an unwillingness to repeat bronchoscopy [19].

Eight studies out of those included in this review were found to describe short-term (hours or days) adverse events related to BAL procedures [6, 8, 22, 50, 52, 54, 64, 65] (table E6). Reported adverse events were mainly mild, for example mild fever, worsening of cough, mild respiratory distress or stridor. There is a small risk of severe adverse events due to BAL, for example severe bronchoconstriction has been reported [52]. Bronchoscopy also increases the risk of contamination of the lower airways. In adults, the rate of complications may be greater (three out of 11 patients experienced serious adverse events following BAL in one study [6, 64]).

The large long-term (years) lavage studies have been conducted in CF patients only and no normal controls were studied in parallel [19, 21, 22]. The Australian CF BAL study indicates that the long-term tolerance of repeated BAL (initiated prior to the age of 6 months until the age of 5 years) is satisfactory. In this study, CF controls were treated similarly but without lavage. At the age of 5 years, eight (10%) out of 79 children in the BAL-directed therapy group and nine (12%) out of 76 in the standard therapy group had *P. aeruginosa* in final BAL cultures ($p=0.73$). Mean total CF-CT scores for the BAL-directed therapy and standard therapy groups were 3.0% and 2.8%, respectively ($p=0.74$). No long-term side-effects were reported in the paper.

Group consensus on feasibility

The cost of a bronchoscopy/BAL procedure varies across Europe. Self-reported costs by ECFS investigators are in the region of €100–213 per procedure, excluding the cost of the hospitalisation. Costs may also differ according to mediator(s) analysed and analysis technique used (estimated costs range from €18 for differential counts (including preparation in the UK), €8 (Australia) to €38 (including preparation, UK) for a cytokine assay such as IL-8, and €4 (Australia) per sample for NE). The cost may be doubled if the

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values are very high and the assay has to be repeated on diluted samples (in ~10–20% of samples). Moreover, analysing the different fractions of the lavages will increase cost and effort.

In experienced hands, BAL is a relatively easy procedure. The actual time the patient will have to bear the bronchoscope in the lower airways may be limited to a few minutes. In children, general anaesthesia increases the patient's tolerance of the procedure.

Extensive training is mandatory, with clear protocols, in order to reduce the risk of lower airway contamination and to reduce variability in results. North American Therapeutics Development Network standard operating procedures are available for sampling and processing of BAL. There is a need for specialised laboratories and trained technologists to process the samples. Some methodological issues can arise, for example concentrations of IL-8 are higher with cytokine bead analyses [66] compared to ELISA.

In infants and children, it is recommended that BAL be performed under general anaesthesia in circumstances where full anaesthetic monitoring and recovery are available (fully equipped operating theatres). In adults, bronchoscopy is generally conducted under local anaesthesia with or without sedation. A total of 30–45 min is generally required to conduct the whole procedure. Paediatric bronchoscopes come in different sizes. Beyond the neonatal period, a flexible bronchoscope with an external diameter of 3.5–3.7 mm is recommended up to the age of 9 years. Thereafter, 4.6–4.9-mm diameter bronchoscopes are more appropriate [67].

The requirement for a combination of well-adapted equipment, experienced personnel (pulmonologists, anaesthetists, nurses, and laboratory technicians and practitioners) and fully functional operating theatre limits availability of this procedure to tertiary care medical centres.

Advantages and limitations of BAL inflammatory markers

BAL offers the advantages of being able to be performed in any age group, and for its ability to directly sample the same area of the lung for studies that require repeat sampling (as is commonly done with intervention studies). Of note, BAL has been extensively studied in children (>333 procedures in 107 infants in one study [65]), but not adults, in particular in the presence of very altered lung function (FEV_1 <40%) [13, 22, 26, 52, 65]. In this latter subgroup of patients, the volume recovered may be decreased and the risk of the procedure increased. It is noteworthy that inflammatory markers may change with age (*e.g.* IL-8 increases with age), requiring placebo controls for long-term studies.

The invasive character of BAL limits its repeatability in clinical practice and research. Lavage of multiple lobes [8] or all lobes may be appropriate in some studies, and in studies involving serial lavages over time, these lavages should be performed in the same lobe. In addition, BAL techniques may vary to some degree between centres. Sources of variability include type of anaesthesia/sedation, introduction of bronchoscope *via* nose *versus* laryngeal mask airway, bronchoscope size (which determines the extent of the lavaged lung areas), lavage aliquot size and number, and lobe(s) lavaged [25]. In order to decrease such variability, a European BAL standard operating procedure has been developed, and is available on request from the ECFS-CTN (ECFS-CTN@uzleuven.be).

One major factor that has the potential to cause difficulty for combining data from different studies is the dilution of the lung specimen. There is general agreement that correction for dilution is extremely difficult, if not impossible. Unlike urine, where markers can be corrected for dilution using urinary creatinine concentrations, no such marker of BAL dilution exists. Both urea and albumin have been used in attempts to correct for dilution but neither is suitable. As discussed in the European Respiratory Society Task Force report [67], there are major drawbacks with both, especially the fact that the concentrations of both urea and albumin increase in lung disease. For surveillance of inflammation in CF reporting the following is recommended: 1) the volume returned as a percentage of that instilled [63, 67]; 2) total cell count [63, 67]; and 3) differential cell counts, *i.e.* macrophages, neutrophils, lymphocytes and other cell types, as the number of cells [63] and percentage of cell types [67] per millilitre of BALF.

Noncellular components, such as cytokines, inflammatory markers and NE should be reported as the concentration per millilitre of BALF. In addition, where the marker is only detectable once the body's defence has been overwhelmed, *e.g.* unbound NE detected in an activity assay, then this should be reported both as a binary variable (detectable or not) and as the concentration per millilitre of BALF [63].

Group Consensus: The "Four Key Questions"

Question 1: does this outcome have the potential to become a surrogate outcome?

Biomarkers of inflammation are particularly clinically and biologically relevant in CF. However, although it may be a useful surrogate marker, BAL is limited by its invasive nature.

Question 2: for what kind of therapeutic trial is this outcome appropriate? (Therapeutic aim, phase of trial, target population, trial duration, number of patients involved, number of sites involved)

Because of its invasive nature and expense, BAL is best suited for early phase clinical trials (phases 1 and 2), conducted in very specialised centres. BAL cultures have been shown to be an informative primary end-point for trials of antimicrobial therapies as well as anti-inflammatory therapies. BAL may be applied in short-term (weeks) as well as long-term (years) studies in preschool children.

Question 3: within what timeline can change be expected? What treatment effect can be considered clinically significant?

A minimum of 1 month may be sufficient for specific anti-inflammatory biomarkers and therapeutic agents. However, it is to be expected that generally biomarkers of inflammation will change over months rather than weeks. There are insufficient data and appropriate clinical outcome studies (*e.g.* using sensitive markers such as CT scans or sufficient duration) to confidently determine clinically significant levels of improvement in inflammatory markers.

Question 4: what are the most needed studies to further define this outcome parameter in CF patients and its potential to be a surrogate marker?

Further studies on the standardisation and reproducibility of this technique are warranted. Importantly, the original BAL routine of pooling second and third aliquots for inflammatory markers (initially established to investigate interstitial lung disease) should be investigated in CF. Furthermore, it may be important to determine which marker(s) are most informative, to show that changes in BAL characteristics are accompanied by modifications in the natural history of the disease (inflammation and/or infection) (*i.e.* longitudinal studies correlating change in biomarkers to a change in clinical status), to evaluate the validity of biomarkers at different stages of disease, among larger numbers of carefully phenotyped subjects with CF of varying ages, genotype, colonisation status and to perform ''multimodal'' measurement studies and explore the influence of multimodal anti-inflammatory therapies.

Conclusion

This document provides an overview of the work of the ECFS-CTN Standardisation Committee on BAL inflammatory markers. Assessments of BAL inflammatory markers in the literature indicate that their reliability, validity and responsiveness are adequate for clinical trials. The practical characteristics were discussed and it was concluded that the use of BAL in clinical research should be limited due to its invasive nature. It is particularly applicable to early-phase clinical trials (phases 1 and 2), conducted in very specialised centres. It is also applicable to multicentre trials in preschool children with early or mild lung disease. This is the first article to collate the literature in this manner and provides a rationale to support the use of BAL in well-designed early-phase clinical trials in CF.

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