REVIEW

WILEY · PEDIATRIC PULMONOLOGY

Mapping targetable inflammation and outcomes with cystic fibrosis biomarkers

Olivia Giddings MD, PhD[1,2,3] | **Charles R. EstherJr MD, PhD**[2,3] (iD)

[1] Pulmonology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

[2] Pediatric Pulmonology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

[3] Marsico Lung Institute, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Correspondence
Charles R. Esther, Jr MD, PhD, Pediatric Pulmonology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.
Email: charles_esther@med.unc.edu

Abstract

Cystic fibrosis is characterized by an overly exuberant neutrophilic inflammatory response to pathogens and other stimuli that starts very early in disease. The overwhelming nature of this response is a primary cause of remodeling and destruction of the airways, suggesting that anti-inflammatory therapies could be beneficial in CF. However, finding therapies that can effectively reduce the inflammatory response without compromising host defenses remains elusive. New approaches towards mapping inflammatory targets promise to aid in developing novel therapeutic strategies and improve outcomes in individuals with CF.

KEYWORDS
bronchoalveolar lavage, exhaled breath condensate, metabolomics, Sputum

1 | INTRODUCTION

Inflammation in cystic fibrosis (CF) is characterized by a marked and persistent influx of neutrophils into the airways. Despite the overwhelming nature of this inflammatory response, it remains insufficient to eradicate infection, resulting in a vicious cycle of infection, inflammation, and mucus hypersecretion/dehydration that causes progressive remodeling and destruction of the airways. This high degree of airway inflammation is responsible for much of the lung disease in CF, with concentrations of inflammatory biomarkers (particularly neutrophil elastase) the most predictive of disease progression.[1,2] Nevertheless, there are relatively few therapies developed to directly address airway inflammation in CF. This lack of treatment options reflects several challenges in developing effective anti-inflammatory therapies, including difficulties in measuring airway inflammation. This review will summarize the origins of airway inflammation in CF, current options for treatment, and how developments in measuring biomarkers of airway inflammation may lead to a new generation of anti-inflammatory treatments for CF.

1.1 | Origins of CF inflammation

A key finding of studies of early CF lung disease is that airway inflammation begins at or very soon after birth. Neutrophils and

neutrophil elastase can be detected in bronchoalveolar lavage (BAL) in patients diagnosed with CF by newborn screen as early as 3 months of age, and these inflammatory markers correlate with future development of bronchiectasis and gas trapping on CT scan.[1] This increase in inflammation does not appear to be solely a response to infection, since less than half of infants with neutrophil elastase detected in BAL fluid had an active pulmonary infection or history suggesting infection. These observations suggest that inflammation in CF airways is multifactorial (Fig. 1) and can occur even in the absence of an infectious stimulus.

Localized hypoxia in the CF lung could explain the early inflammation seen in the absence of obvious infection.[3] The gene mutated in CF, the cystic fibrosis transmembrane conductance regulator (*CFTR*), encodes a cAMP dependent anion channel that conducts chloride and bicarbonate and regulates the balance of chloride secretion and sodium absorption in the airway.[4] Loss of CFTR channel activity produces a dehydrated airway surface environment where the total mass of salt and volume of water are inadequate to maintain mucus hydration, leading to defects in mucociliary clearance. The resulting thickened mucus and mucus plugging in the small airway create localized areas of hypoxia, which can trigger inflammatory responses[5] including release of cytokines such as IL-1 and activation of the inflammatory cascade via binding to the IL-1 receptor.[6] The resultant increase in inflammation may then worsen hypoxia and
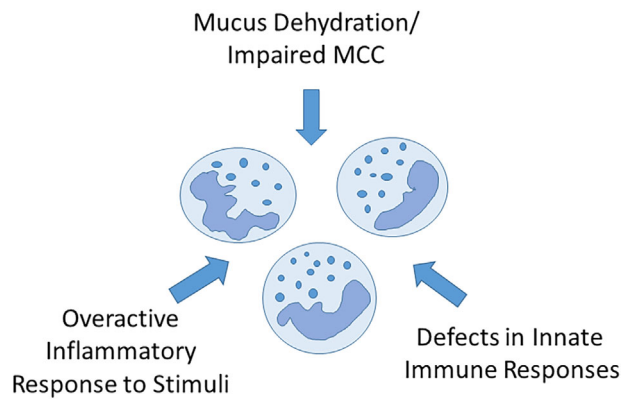
FIGURE 1 Factors that contribute to the excessive inflammatory response in CF

contribute to a niche for anaerobic bacteria, thus further propagating the inflammatory cycle.[3]

While infection may not be required to initiate inflammation in CF, defects in immune responses to pathogens likely contribute to the excessive inflammatory environment. A number of mechanisms of immune dysregulation have been described in CF, including aberrant responses in inflammatory cells such as neutrophils and macrophages as well as altered signaling pathways in airway epithelia. These aspects of immune dysregulation were reviewed in detail in 2015 by Nichols and Chmiel in a previous volume of "Barriers to Normalcy"[7] and will be only briefly summarized here. The mucus dehydration and impaired mucociliary clearance contribute to enhanced inflammatory responses, with failure to clear pathogens out of the airway leading to prolonged stimulation of inflammatory pathways.[8,9] Furthermore, there is evidence that CFTR may play a more direct role in regulation of inflammatory responses. For example, neutrophils isolated from patients with CF tend to undergo necrotic rather than apoptotic responses, releasing additional pro-inflammatory molecules such as High Mobility Group Box 1 (HMGB1) protein and metalloproteases.[10,11] There is also evidence that CFTR is involved in the acidification of phagosomes and bacterial killing in both neutrophils and macrophages.[12,13] Similarly, CF macrophages and monocytes also demonstrate defective immune response.[14]

Studies of animal models suggest that defects in innate immunity contribute to the excessive inflammatory responses in CF. CF pigs have decreased bacterial clearance and increased inflammation relative to unaffected litter mates after exposure to bacterial pathogens.[15] CFTR knockout ferrets[16] also show abnormal bacterial clearance and enhanced inflammatory responses.[17] The mechanisms that underlie these defects are not fully defined, though there is evidence that defective bacterial clearance in the CF pig reflects altered airway acidification, likely related to loss of CFTR mediated bicarbonate secretion that alters the efficacy of antimicrobial peptides.[18]

Despite this evidence, the role of altered inflammatory responses directly related to CFTR deficiency (as opposed to secondary effects from defective mucociliary clearance) remains controversial. Systemic

infection remains uncommon in CF despite high airway bacterial loads,[19] raising some questions about the clinical relevance of abnormalities observed in isolated CF inflammatory cells. Studies in animal models must also be interpreted with caution, since no animal model faithfully recapitulates all aspects of human disease. For example, the altered airway pH observed in pigs may not be present in human CF,[20] and airway acidification similar in magnitude to that of the CF pigs has been observed in asthma,[21,22] a disease that is not commonly associated with airway infection.[21,22]

1.2 | Anti-inflammatory therapies in CF

Although the factors that contribute to inflammation in CF are not fully defined, the relevance of inflammation as a therapeutic target is unquestioned.[23] Nevertheless, despite intensive effort, limited therapies are available. Prednisone is perhaps the most canonical anti-inflammatory, and alternate day therapy with prednisone has been shown to increase forced vital capacity (FVC) in treated CF patients compared to placebo.[24] However, chronic use of systemic steroids is contraindicated due to their adverse effects including growth retardation, osteoporosis, cataracts, hyperglycemia and risk of opportunistic infection. High dose ibuprofen is a more targeted anti-inflammatory that has been shown to slow the rate of decline of FEV_1 in two separate double blind, placebo controlled studies,[25,26] and this clinical benefit has been associated with a decrease in neutrophil migration to the lung.[27] Although trials with ibuprofen did not show a significant increase in adverse events between treatment and placebo groups, the perceived risk of gastrointestinal bleeding and renal toxicity coupled with the need to obtain serum levels to minimize these risks has inhibited widespread use of this drug.

The most widely used therapy in CF with anti-inflammatory properties is azithromycin. Interest in azithromycin as a CF therapeutic stemmed from its benefit in diffuse panbronchiolitis,[28] a disease with many similarities to CF, and was thought to possibly relate to its antimicrobial activity against *Pseudomonas aeruginosa* growing in biofilms.[29] Indeed, the initial large study of chronic, low dose azithromycin in CF was targeted towards patients with persistent *Pseudomonas* infection. This study demonstrated that chronic azithromycin treatment led to improvement in FEV_1, a decrease in exacerbations requiring antibiotic therapy, as well as improved quality of life (QOL) scores.[30,31] However, the clinical benefits occurred despite minimal impact on *Pseudomonas* bacterial density, suggesting that a different mechanism of action was responsible. Azithromycin has a number of anti-inflammatory effects, including reduction in neutrophil oxidative burst and increases neutrophil apoptosis.[32,33] In lung macrophages azithromycin also appears to inhibit apoptosis, stimulate phagocytosis of bacteria and cellular debris, as well as skew macrophage cytokine expression toward an anti-inflammatory phenotype.[34] Other anti-inflammatory effects of azithromycin include decreased mucin production with a resultant decrease in mucus viscosity, maintenance of tight junctions between epithelial cells and improvement of the integrity of the epithelial cell layer under inflammatory conditions.[33] These immunomodulatory effects may

underlie the benefits of azithromycin more than its anti-Pseudomonal activity, and a large multi-center study demonstrated clinical benefit of chronic azithromycin in patients who did not have *Pseudomonas* infection.[35]

Given the extensive number of pathways identified as playing roles in CF airway inflammation, it may seem surprising that other anti-inflammatory therapies have not yet been developed. However, an effective anti-inflammatory for CF must manage a careful balancing act, providing sufficient potency to reduce inflammation induced lung damage without interfering with the ability to resolve infection. This balance can be difficult to achieve, as revealed by the Phase II clinical trial of BIIL 284 BS, a promising antagonist of the leukotriene B4 receptor known to play a significant role in CF airway inflammation. This trial was stopped early due to an increase in pulmonary adverse events in those receiving the active drug compared to placebo.[36] Further studies showed that treatment of CF mice with BIIL 284 BS interfered with their ability to resolve *Pseudomonas aeruginosa* respiratory infection.[37] These results showcase the difficulty of balancing a reduction in inflammation while not significantly increasing the bacterial burden with the use of anti-inflammatory therapies and emphasize the need for pre-clinical testing of novel therapeutics.[23]

1.3 | Barriers to anti-inflammatory development: Measuring airway inflammation

The dearth of effective anti-inflammatory therapies represents an ongoing barrier to normalcy in CF and suggests a need to identify new therapeutic targets. Testing of anti-inflammatory therapies and other treatments in CF has become increasingly challenging, as overall improvements in lung function and health make observing changes in traditional endpoints such as lung function or pulmonary exacerbations more difficult to assess without large and expensive trials.[38] However, accurately measuring airway inflammation directly as a marker of therapeutic activity can be difficult. Indeed, most of the major trials of anti-inflammatory therapies described above[24–26,36] did not include an airway inflammation biomarker, with the exception of the azithromycin trial that demonstrated statistically significant though modest changes in neutrophil elastase.[30] Although treatment related reductions in airway inflammation biomarkers were often shown in smaller studies,[27,39] better biomarkers of airway inflammation are clearly needed to identify potential therapeutic targets and serve as surrogate markers of efficacy for clinical trials. This need is particularly great in young children to try and limit inflammation before the onset of lung damage. The challenges in developing better biomarkers reflect limitations in the primary methods to obtain airway samples: sputum collection, bronchoalveolar lavage, and assessments of exhaled breath (Fig. 2 and Table 1).

1.3.1 | Sputum

Historically, assessments of airway inflammation in CF (and other diseases) have been primarily based on analysis of biomarkers in sputum, in part reflecting the long experience and existence of

Sources of Airway Biomarkers
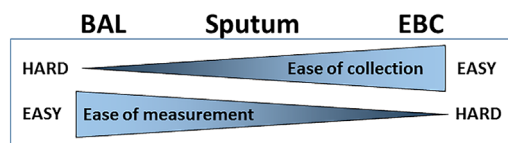
FIGURE 2 Ease of collection and ease of biomarker measurement (including processing steps) are generally inversely related

standardized protocols for this airway sample. Given the intense airway inflammation that characterizes CF, it is no surprise that a multitude of inflammatory biomarkers are elevated in CF sputum, as summarized in several excellent reviews.[2,40] Among these biomarkers, sputum neutrophil elastase has emerged as one of the most predictive, with concentrations of sputum NE most highly correlated with lung function decline in large studies.[2,41,42]

However, the utility of sputum is limited somewhat by the need for specialized procedures to process samples that typically must be performed immediately after collection.[43] Spontaneously expectorated sputum likely arises from more affected regions of the lung, and concentrations of inflammatory markers can be influenced by regional variability in lung disease[39] Furthermore, in general only older patients with more advanced disease can regularly expectorate sputum spontaneously. While sputum induction using hypertonic saline can be utilized to obtain samples from patients who do not spontaneously expectorate, many younger children have difficulty expectorating sputum even after induction.[44,45] Thus, sputum has a limited role in assessing—and by extension treating—airway inflammation in the youngest children.

1.3.2 | Bronchoalveolar lavage fluid

For patients who cannot expectorate sputum, flexible bronchoscopy with BAL is considered the gold standard for airway biomarker assessment.[40] As with sputum, numerous inflammatory biomarkers are elevated in BAL fluid in children with CF including neutrophil counts, neutrophil elastase, pro-inflammatory cytokines such as interleukin-8, and others.[46–51] Several of these inflammatory biomarkers correlate with other aspects of disease severity including infection,[52] radiologic findings,[51,53,54] and infant lung function testing.[46,49] Like sputum, neutrophil elastase represents one of the most informative markers in BAL fluid, with elevated concentrations in infancy predictive of future bronchiectasis.[1,55–57]

Use of BAL fluid as a source of airway inflammation biomarkers is constrained by several limitations, including the time, expertise, and expense needed for the procedure.[58] Furthermore, bronchoscopy requires sedation, which carries both short term risks and increasing concerns about long term adverse outcomes.[59] Due to these limitations, BAL has seen a limited role in clinical trials, though longer term observational studies that include BAL biomarkers such as AREST CF have provided significant insights into early disease.[51,60,61]

TABLE 1 Comparison of airway samples for biomarker measurement

	Advantages	Disadvantages	Region sampled	Inflammatory biomarkers
Sputum	Well established Non-invasive	Requires immediate processing Difficult in young children	Most affected large airways	Cell counts Cytokines/proteins Metabolites
BAL	Can be used in all subjects	Higher risk (anesthesia) Expensive	Targeted smaller airways	Cell counts Cytokines/proteins Metabolites
EBC	Simple to collect Non-invasive	Very low and variable biomarker concentrations	Small airways (may under-sample plugged airways)	Cytokines/proteins Metabolites

1.3.3 | Exhaled breath

Many of the limitations of sputum and BAL can be overcome through use of exhaled breath, which contains both volatile and non-volatile compounds that could serve as inflammatory biomarkers. Exhaled biomarkers are often collected as exhaled breath condensate (EBC), and since collection only requires the subject to exhale through a chilled tube, EBC can be obtained simply and non-invasively even in young children.[62,63] Indeed, a number of airway inflammatory biomarkers that are informative in sputum or BAL fluid are also elevated in EBC from subjects with CF, including inflammatory cytokines,[63–66] 8-isoprostane,[64,67] nitrates,[64,68] leukotrienes,[69] and purines,[49,70] with measures of EBC leukotrienes and purines shown to track changes related to CF exacerbations.[69,70] EBC pH has also been shown to be decreased in subjects with CF and change with treatment of an exacerbation.[71–73] With specialized methods, EBC can even be collected from the youngest children during infant pulmonary function testing (iPFTs).[74–76]

The ease of EBC collection is belied by difficulty in analysis, with EBC being described as "easy on patients" but "hard on scientists."[77] Airway secretions in EBC arise from microaerosols generated during respiration, which represent a very low and highly variable fraction of the fluid volume of the condensate and may under-sample obstructed airways.[78,79] Therefore, extremely sensitive methods are typically needed to assess the low concentrations of most traditional biomarkers found in EBC, which ideally should also include a means to control for variable dilution.[78,80] Our own approach has been to utilize mass spectrometry to measure relevant biomarkers as well as urea as a dilution marker,[81–83] though there are other valid methods.[30,32] Failure to adequately address these challenges impacts the reproducibility and validity of EBC biomarkers and may limit their utility as effective measures of airway inflammation.[84]

Some of the limitations of EBC can be addressed by a focus on volatile biomarkers, which are not dependent on microaerosol generation for incorporation in exhaled breath. Several studies had shown that volatile organic carbon (VOC) profiles are altered in individuals with CF and could serve as inflammatory biomarkers.[85,86] One of the potentially exciting application of VOC profiling in CF is the development of electronic "nose" systems that could provide information on airway inflammation at the point of care.[87,88] However, current methods require sophisticated mathematical modeling to identify complex patterns in the detected VOCs, and

the reproducibility of these signatures and their relationships to specific aspects of airway inflammation have not been established.

1.3.4 | Non airway samples

The high levels of inflammation in the airways of individuals with CF translate into increases in systemic inflammatory biomarkers that could be assessed in serum or plasma, which are relatively easily obtained and analyze. Indeed, a large number of blood inflammatory markers are elevated relative in CF, including C-reactive protein,[89–92] immunoglobulin G,[90,93,94] cytokines,[91] tumor necrosis factor,[95] and transforming growth factor β,[96] many of which are altered with pulmonary exacerbation.[91,92,96,97] However, the potential contribution of non-pulmonary inflammation reduces the specificity of these biomarker for lung disease and limits applicability. There have also been small trials investigating the use of biomarkers in both saliva and urine as a surrogate for lung inflammation.[98,99]

1.3.5 | Imaging

A number of small studies have been done using fluorodeoxyglucose (FDG) PET to quantify lung inflammation and follow response to treatment of CF exacerbations. FDG is concentrated in activated neutrophils which are recruited to sites of inflammation. The degree of inflammation can be estimated by the degree of FDG emission. A study following the kinetics of FDG movement to the lung showed that increased influx into the lung correlated with a more rapid decline in FEV1 over time.[100] Other studies have used FDG PET monitor changes in inflammation during antibiotic treatment of CF exacerbation. Patients underwent FDG PET on days 1 and 14 of treatment and degree of inflammation was determined using standard uptake values (SUV). This group found that over the course of 14 days of IV therapy the max SUV decreased.[101] Regular use of FDG PET CT is limited by radiation exposure, however, as low dose CT protocols improve this may become a useful technique to follow lung inflammation.

1.4 | Novel strategies

Despite all we have learned about the inflammatory pathways involved in CF lung disease, numerous challenges remain in translating these

TABLE 2 Ideal inflammatory pathways to target

Characteristics
1. Involved in early disease
2. Associated with a biomarker that is readily measured in a non-invasive sample
3. Can be altered by a relatively simple pharmaceutical
4. Inhibition reduces inflammation without limiting ability to contain infection

findings into effective anti-inflammatories. Many signaling pathways are not easily amenable to pharmacological inhibition, often requiring biologic antibody based treatments that while effective can be expensive and difficult to administer.[102] Other pathways may be too vital to host defenses to serve as a viable therapeutic targets, as suggested by the outcomes of the BIIL 284 BS study.[36] Therefore, there remains an urgent need to identify new pathways that could serve as viable targets for anti-inflammatory development. The ideal pathway would have defined characteristics, including an involvement in early disease, a readily measurable biomarker of activity, and availability of a relatively simple pharmaceutical treatment. Perhaps most importantly, blockade of this pathway should reduce inflammation without interfering with the ability to resolve infection (Table 2).

Use of 'omics strategies, particularly metabolomics, is well suited towards identifying pathways that meet these criteria. The changes in metabolite patterns associated with disease reflect cellular enzymatic activities, which are attractive as therapeutic targets since they can often be inhibited by small molecule therapeutics.[103] Furthermore, the identified metabolites can serve as biomarkers of pathway activity and drug effects, many of which can be readily measured using standard methods even in non-invasive sample such as EBC.[81,82,104] The potential metabolomics has been demonstrated in several studies that find CF specific metabolite patterns in sputum,[105] BALF,[106–108] blood,[109,110] and even EBC.[111,112]

Metabolomics studies can be particularly informative when interpreted in conjunction with other 'omics evaluations. For example, one of the largest gene wide association studies to date in CF identified associations between disease severity and expression of the gene APIP,[113] which encodes an enzyme involved in the methionine salvage pathway, and metabolites associated with this pathway, including polyamines and free adenine, are associated with neutrophilic inflammation in CF.[107,108,114] Similarly, the lysophosphatidic acid receptor LPAR6 has been linked to CF lung disease in genomic studies,[115] while the lysophospholipid substrates of this receptor are elevated in CF bronchitis.[107,116] While such studies demonstrate the promise of metabolomics to identify biomarkers and therapeutic targets, further study is needed before the potential of these identified pathways is truly known.

2 | CONCLUSIONS

The intense inflammation present in the airways of individuals with CF is one of the most significant causes of progressive lung disease. Until we have a cure for CF, development of effective anti-inflammatories needs to be a priority for the CF research community. New approaches using metabolomics and other strategies to map the inflammatory targets in CF hold promise in development of new therapies.

CONFLICTS OF INTEREST

None declared.

REFERENCES

1. Sly PD, Gangell CL, Chen L, et al. Risk factors for bronchiectasis in children with cystic fibrosis. *N Engl J Med.* 2013;368:1963–1970.
2. Mayer-Hamblett N, Aitken ML, Accurso FJ, et al. Association between pulmonary function and sputum biomarkers in cystic fibrosis. *Am J Respir Crit Care Med.* 2007;175:822–828.
3. Montgomery ST, Mall MA, Kicic A, Stick SM, Arest CF. Hypoxia and sterile inflammation in cystic fibrosis airways: mechanisms and potential therapies. *Eur Respir J.* 2017;49.
4. Boucher RC. An overview of the pathogenesis of cystic fibrosis lung disease. *Adv Drug Deliv Rev.* 2002;54:1359–1371.
5. Livraghi-Butrico A, Kelly EJ, Klem ER, et al. Mucus clearance, MyD88-dependent and MyD88-independent immunity modulate lung susceptibility to spontaneous bacterial infection and inflammation. *Mucosal Immunol.* 2012;5:397–408.
6. Fritzsching B, Zhou-Suckow Z, Trojanek JB, et al. Hypoxic epithelial necrosis triggers neutrophilic inflammation via IL-1 receptor signaling in cystic fibrosis lung disease. *Am J Respir Crit Care Med.* 2015;191:902–913.
7. Nichols DP, Chmiel JF. Inflammation and its genesis in cystic fibrosis. *Pediatr Pulmonol.* 2015;50:S39–S56.
8. Boucher RC. On the pathogenesis of acute exacerbations of mucoobstructive lung diseases. *Ann Am Thorac Soc.* 2015;12:S160–S163.
9. Boucher RC. Evidence for airway surface dehydration as the initiating event in CF airway disease. *J Intern Med.* 2007;261:5–16.
10. Rowe SM, Jackson PL, Liu G, et al. Potential role of high-mobility group box 1 in cystic fibrosis airway disease. *Am J Respir Crit Care Med.* 2008;178:822–831.
11. Gifford AM, Chalmers JD. The role of neutrophils in cystic fibrosis. *Curr Opin Hematol.* 2014;21:16–22.
12. Painter RG, Bonvillain RW, Valentine VG, et al. The role of chloride anion and CFTR in killing of Pseudomonas aeruginosa by normal and CF neutrophils. *J Leukoc Biol.* 2008;83:1345–1353.
13. Haggie PM, Verkman AS. Defective organellar acidification as a cause of cystic fibrosis lung disease: reexamination of a recurring hypothesis. *Am J Physiol Lung Cell Mol Physiol.* 2009;296:L859–L867.
14. Bruscia EM, Bonfield TL. Cystic fibrosis lung immunity: the role of the macrophage. *J Innate Immun.* 2016;8:550–563.
15. Stoltz DA, Meyerholz DK, Pezzulo AA, et al. Cystic fibrosis pigs develop lung disease and exhibit defective bacterial eradication at birth. *Sci Transl Med.* 2010;2:29ra31.
16. Sun X, Sui H, Fisher JT, et al. Disease phenotype of a ferret CFTR-knockout model of cystic fibrosis. *J Clin Invest.* 2010;120:3149–3160.
17. Keiser NW, Birket SE, Evans IA, et al. Defective innate immunity and hyperinflammation in newborn cystic fibrosis transmembrane conductance regulator-knockout ferret lungs. *Am J Respir Cell Mol Biol.* 2015;52:683–694.
18. Shah VS, Meyerholz DK, Tang XX, et al. Airway acidification initiates host defense abnormalities in cystic fibrosis mice. *Science.* 2016;351:503–507.

19. Fahy JV, Keoghan MT, Crummy EJ, FitzGerald MX. Bacteraemia and fungaemia in adults with cystic fibrosis. *J Infec*. 1991;22:241–245.

20. McShane D, Davies JC, Davies MG, Bush A, Geddes DM, Alton EW. Airway surface pH in subjects with cystic fibrosis. *Eur Respir J*. 2003;21:37–42.

21. Carpagnano GE, Foschino Barbaro MP, Resta O, et al. Exhaled markers in the monitoring of airways inflammation and its response to steroid's treatment in mild persistent asthma. *Eur J Pharmacol*. 2005;519:175–181.

22. Tseliou E, Bessa V, Hillas G, et al. Exhaled nitric oxide and exhaled breath condensate pH in severe refractory asthma. *Chest*. 2010;138:107–113.

23. Torphy TJ, Allen J, Cantin AM, et al. Considerations for the conduct of clinical trials with anti-inflammatory agents in cystic fibrosis: a cystic fibrosis foundation workshop report. *Ann Am Thorac Soc*. 2015;12:1398–1406.

24. Eigen H, Rosenstein BJ, FitzSimmons S, Schidlow DV. A multicenter study of alternate-day prednisone therapy in patients with cystic fibrosis. Cystic Fibrosis Foundation Prednisone Trial Group. *J Pediatr*. 1995;126:515–523.

25. Konstan MW, Byard PJ, Hoppel CL, Davis PB. Effect of high-dose ibuprofen in patients with cystic fibrosis. *N Engl J Med*. 1995;332:848–854.

26. Lands LC, Milner R, Cantin AM, Manson D, Corey M. High-dose ibuprofen in cystic fibrosis: canadian safety and effectiveness trial. *J Pediatr*. 2007;151:249–254.

27. Konstan MW, Krenicky JE, Finney MR, et al. Effect of ibuprofen on neutrophil migration in vivo in cystic fibrosis and healthy subjects. *J Pharmacol Exp Ther*. 2003;306:1086–1091.

28. Kudoh S, Azuma A, Yamamoto M, Izumi T, Ando M. Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin. *Am J Respir Crit Care Med*. 1998;157:1829–1832.

29. Wozniak DJ, Keyser R. Effects of subinhibitory concentrations of macrolide antibiotics on Pseudomonas aeruginosa. *Chest*. 2004;125:62S–69S; quiz 69S.

30. Saiman L, Marshall BC, Mayer-Hamblett N, et al. Azithromycin in patients with cystic fibrosis chronically infected with Pseudomonas aeruginosa: a randomized controlled trial. *JAMA*. 2003;290:1749–1756.

31. Southern KW, Barker PM, Solis-Moya A, Patel L. Macrolide antibiotics for cystic fibrosis. *Cochrane Database Syst Rev*. 2012;11:CD002203.

32. Culic O, Erakovic V, Cepelak I, et al. Azithromycin modulates neutrophil function and circulating inflammatory mediators in healthy human subjects. *Eur J Pharmacol*. 2002;450:277–289.

33. Parnham MJ, Erakovic Haber V, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. Azithromycin: mechanisms of action and their relevance for clinical applications. *Pharmacol Ther*. 2014;143:225–245.

34. Hodge S, Reynolds PN. Low-dose azithromycin improves phagocytosis of bacteria by both alveolar and monocyte-derived macrophages in chronic obstructive pulmonary disease subjects. *Respirology*. 2012;17:802–807.

35. Saiman L, Anstead M, Mayer-Hamblett N, et al. Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with Pseudomonas aeruginosa: a randomized controlled trial. *JAMA*. 2010;303:1707–1715.

36. Konstan MW, Doring G, Heltshe SL, et al. A randomized double blind, placebo controlled phase 2 trial of BIIL 284 BS (an LTB4 receptor antagonist) for the treatment of lung disease in children and adults with cystic fibrosis. *J Cyst Fibros*. 2014;13:148–155.

37. Doring G, Bragonzi A, Paroni M, et al. BIIL 284 reduces neutrophil numbers but increases P. aeruginosa bacteremia and inflammation in mouse lungs. *J Cyst Fibros*. 2014;13:156–163.

38. Simpson SJ, Mott LS, Esther CR, Jr., Stick SM, Hall GL. Novel end points for clinical trials in young children with cystic fibrosis. *Expert Rev Respir Med*. 2013;7:231–243.

39. Chmiel JF, Konstan MW, Accurso FJ, et al. Use of ibuprofen to assess inflammatory biomarkers in induced sputum: implications for clinical trials in cystic fibrosis. *J Cyst Fibros*. 2015;14:720–726.

40. Sagel SD, Chmiel JF, Konstan MW. Sputum biomarkers of inflammation in Cystic Fibrosis lung disease. *Proc Am Thorac Soc*. 2007;4:406–417.

41. Sagel SD, Wagner BD, Anthony MM, Emmett P, Zemanick ET. Sputum biomarkers of inflammation and lung function decline in children with cystic fibrosis. *Am J Respir Crit Care Med*. 2012;186:857–865.

42. Waters VJ, Stanojevic S, Sonneveld N, et al. Factors associated with response to treatment of pulmonary exacerbations in cystic fibrosis patients. *J Cyst Fibros*. 2015;14:755–762.

43. Alexis NE, Hu SC, Zeman K, Alter T, Bennett WD. Induced sputum derives from the central airways: confirmation using a radiolabeled aerosol bolus delivery technique. *Am J Respir Crit Care Med*. 2001;164:1964–1970.

44. Ho SA, Ball R, Morrison LJ, Brownlee KG, Conway SP. Clinical value of obtaining sputum and cough swab samples following inhaled hypertonic saline in children with cystic fibrosis. *Pediatr Pulmonol*. 2004;38:82–87.

45. Ordonez CL, Kartashov AI, Wohl ME. Variability of markers of inflammation and infection in induced sputum in children with cystic fibrosis. *J Pediatr*. 2004;145:689–692.

46. Dakin CJ, Numa AH, Wang HE, Morton JR, Vertzyas CC, Henry RL. Inflammation, infection, and pulmonary function in infants and young children with cystic fibrosis. *Am J Respir Crit Care Med*. 2002;165:904–910.

47. Rosenfeld M, Gibson RL, McNamara S, et al. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis*. *Pediatr Pulmonol*. 2001;32:356–366.

48. 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.

49. Esther CR, Jr., Alexis NE, Clas ML, et al. Extracellular purines are biomarkers of neutrophilic airway inflammation. *Eur Respir J*. 2008;31:949–956.

50. Nixon GM, Armstrong DS, Carzino R, et al. Early airway infection, inflammation, and lung function in cystic fibrosis. *Arch Dis Child*. 2002;87:306–311.

51. Stick SM, Brennan S, Murray C, et al. Bronchiectasis in infants and preschool children diagnosed with cystic fibrosis after newborn screening. *J Pediatr*. 2009;155:623–628 e621.

52. Armstrong DS, Hook SM, Jamsen KM, et al. Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. *Pediatr Pulmonol*. 2005;40:500–510.

53. Davis SD, Fordham LA, Brody AS, et al. Computed tomography reflects lower airway inflammation and tracks changes in early cystic fibrosis. *Am J Respir Crit Care Med*. 2007;175:943–950.

54. Mott LS, Park J, Murray CP, et al. Progression of early structural lung disease in young children with cystic fibrosis assessed using CT. *Thorax*. 2012;67:509–516.

55. Tepper RS, Montgomery GL, Ackerman V, Eigen H. Longitudinal evaluation of pulmonary function in infants and very young children with cystic fibrosis. *Pediatr Pulmonol*. 1993;16:96–100.

56. Garratt LW, Sutanto EN, Ling KM, et al. Matrix metalloproteinase activation by free neutrophil elastase contributes to bronchiectasis progression in early cystic fibrosis. *Eur Respir J*. 2015;46:384–394.

57. Sly PD, Brennan S, Gangell C, et al. Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. *Am J Respir Crit Care Med*. 2009;180:146–152.

58. Effros RM, Dunning MB, III, Biller J, Shaker R. The promise and perils of exhaled breath condensates. *Am J Physiol Lung Cell Mol Physiol.* 2004;287:L1073–L1080.

59. DiMaggio C, Sun LS, Ing C, Li G. Pediatric anesthesia and neurodevelopmental impairments: a Bayesian meta-analysis. *J Neurosurg Anesthesiol.* 2012;24:376–381.

60. Frayman KB, Armstrong DS, Carzino R, et al. The lower airway microbiota in early cystic fibrosis lung disease: a longitudinal analysis. *Thorax.* 2017. https://doi.org/10.1136/thoraxjnl-2016-209279 [Epub ahead of print].

61. Fayon M, Kent L, Bui S, Dupont L, Sermet I. European cystic fibrosis society clinical trial network standardisation C. clinimetric properties of bronchoalveolar lavage inflammatory markers in cystic fibrosis. *Eur Respir J.* 2014;43:610–626.

62. Sagel SD. Noninvasive biomarkers of airway inflammation in cystic fibrosis. *Curr Opin Pulm Med.* 2003;9:516–521.

63. Bodini A, D'Orazio C, Peroni D, et al. Biomarkers of neutrophilic inflammation in exhaled air of cystic fibrosis children with bacterial airway infections. *Pediatr Pulmonol.* 2005;40:494–499.

64. Robroeks CM, Rosias PP, van Vliet D, et al. Biomarkers in exhaled breath condensate indicate presence and severity of cystic fibrosis in children. *Pediatr Allergy Immunol.* 2008;19:652–659.

65. Robroeks CM, Jobsis Q, Damoiseaux JG, et al. Cytokines in exhaled breath condensate of children with asthma and cystic fibrosis. *Ann Allergy Asthma Immunol.* 2006;96:349–355.

66. Bodini A, D'Orazio C, Peroni DG, et al. IL-8 and pH values in exhaled condensate after antibiotics in cystic fibrosis children. *Int J Immunopathol Pharmacol.* 2007;20:467–472.

67. Lucidi V, Ciabattoni G, Bella S, Barnes PJ, Montuschi P. Exhaled 8-isoprostane and prostaglandin E(2) in patients with stable and unstable cystic fibrosis. *Free Radic Biol Med.* 2008;45:913–919.

68. Dressel H, Muller F, Fischer R, et al. Independent information of nonspecific biomarkers in exhaled breath condensate. *Respiration.* 2010;80:401–409.

69. Carpagnano GE, Barnes PJ, Geddes DM, Hodson ME, Kharitonov SA. Increased leukotriene B4 and interleukin-6 in exhaled breath condensate in cystic fibrosis. *Am J Respir Crit Care Med.* 2003;167:1109–1112.

70. Esther CR, Jr., Boysen G, Olsen BM, et al. Mass spectrometric analysis of biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol.* 2009;296:L987–L993.

71. Ojoo JC, Mulrennan SA, Kastelik JA, Morice AH, Redington AE. Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. *Thorax.* 2005;60:22–26.

72. Carpagnano GE, Barnes PJ, Francis J, Wilson N, Bush A, Kharitonov SA. Breath condensate pH in children with cystic fibrosis and asthma: a new noninvasive marker of airway inflammation? *Chest.* 2004;125:2005–2010.

73. Tate S, MacGregor G, Davis M, Innes JA, Greening AP. Airways in cystic fibrosis are acidified: detection by exhaled breath condensate. *Thorax.* 2002;57:926–929.

74. Moeller A, Franklin P, Hall GL, Horak F, Jr., Wildhaber JH, Stick SM. Measuring exhaled breath condensates in infants. *Pediatr Pulmonol.* 2006;41:184–187.

75. Muller WG, Morini F, Eaton S, Peters M, Jaffe A. Safety and feasibility of exhaled breath condensate collection in ventilated infants and children. *Eur Respir J.* 2006;28:479–485.

76. Patel K, Davis SD, Johnson R, Esther CR, Jr. Exhaled breath condensate purines correlate with lung function in infants and preschoolers. *Pediatr Pulmonol.* 2013;48:182–187.

77. Hunt J. If it smells like a duck, it might be an asthma subphenotype. *Am J Respir Crit Care Med.* 2007;175:975–976.

78. Effros RM, Biller J, Foss B, et al. A simple method for estimating respiratory solute dilution in exhaled breath condensates. *Am J Respir Crit Care Med.* 2003;168:1500–1505.

79. Effros RM, Hoagland KW, Bosbous M, et al. Dilution of respiratory solutes in exhaled condensates. *Am J Respir Crit Care Med.* 2002;165:663–669.

80. Effros RM, Dunning MB, 3rd, Biller J, Shaker R. The promise and perils of exhaled breath condensate. *Am J Physiol Lung Cell Mol Physiol.* 2004;287:L1073–L1080.

81. Esther CR, Jr., Olsen BM, Lin FC, Fine J, Boucher RC. Exhaled breath condensate adenosine tracks lung function changes in cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol.* 2013;304:L504–L509.

82. Esther CR, Jr., Boysen G, Olsen BM, et al. Mass spectrometric analysis of biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol.* 2009;296:L987–L993.

83. Esther CR, Jr., Jasin HM, Collins LB, Swenberg JA, Boysen G. A mass spectrometric method to simultaneously measure a biomarker and dilution marker in exhaled breath condensate. *Rapid Commun Mass Spectrom.* 2008;22:701–705.

84. van Horck M, Alonso A, Wesseling G, et al. Biomarkers in exhaled Breath condensate are not predictive for pulmonary exacerbations in children with cystic fibrosis: results of a one-Year observational study. *PLoS ONE.* 2016;11:e0152156.

85. Robroeks CM, van Berkel JJ, Dallinga JW, et al. Metabolomics of volatile organic compounds in cystic fibrosis patients and controls. *Pediatr Res.* 2010;68:75–80.

86. Montuschi P, Paris D, Melck D, et al. NMR spectroscopy metabolomic profiling of exhaled breath condensate in patients with stable and unstable cystic fibrosis. *Thorax.* 2012;67:222–228.

87. Paff T, van der Schee MP, Daniels JM, et al. Exhaled molecular profiles in the assessment of cystic fibrosis and primary ciliary dyskinesia. *J Cyst Fibros.* 2013;12:454–460.

88. Joensen O, Paff T, Haarman EG, et al. Exhaled breath analysis using electronic nose in cystic fibrosis and primary ciliary dyskinesia patients with chronic pulmonary infections. *PLoS ONE.* 2014;9:e115584.

89. Ngan DA, Wilcox PG, Aldaabil M, et al. The relationship of systemic inflammation to prior hospitalization in adult patients with cystic fibrosis. *BMC Pulm Med.* 2012;12:3.

90. Levy H, Kalish LA, Huntington I, et al. Inflammatory markers of lung disease in adult patients with cystic fibrosis. *Pediatr Pulmonol.* 2007;42:256–262.

91. Nixon LS, Yung B, Bell SC, Stuartâ€,Elborn J, Shale DJ. Circulating immunoreactive interleukin-6 in cystic fibrosis. *Am J Respir Crit Care Med.* 1998;157:1764–1769.

92. Bell Bowerman, Nixon Macdonald, Elborn Shale. Metabolic and inflammatory responses to pulmonary exacerbation in adults with cystic fibrosis. *Eur J Clin Invest.* 2000;30:553–559.

93. Proesmans M, Els C, Vermeulen F, De Boeck K. Change in IgG and evolution of lung function in children with cystic fibrosis. *J Cyst Fibros.* 2011;10:128–131.

94. Wheeler WB, Williams M, Matthews WJ, Jr, Colten HR. Progression of cystic fibrosis lung disease as a function of serum immunoglobulin G levels: a 5-year longitudinal study. *J Pediatr.* 1984;104:695–699.

95. Norman D, Elborn JS, Cordon SM, et al. Plasma tumour necrosis factor alpha in cystic fibrosis. *Thorax.* 1991;46:91–95.

96. Harris WT, Muhlebach MS, Oster RA, Knowles MR, Clancy JP, Noah TL. Plasma TGF-beta(1) in pediatric cystic fibrosis: potential biomarker of lung disease and response to therapy. *Pediatr Pulmonol.* 2011;46:688–695.

97. Lagrange-Puget M, Durieu I, Ecochard R, et al. Longitudinal study of oxidative status in 312 cystic fibrosis patients in stable state and during bronchial exacerbation. *Pediatr Pulmonol.* 2004;38:43–49.

98. Patel N, Belcher J, Thorpe G, Forsyth NR, Spiteri MA. Measurement of C-reactive protein, procalcitonin and neutrophil elastase in saliva of COPD patients and healthy controls: correlation to self-reported wellbeing parameters. *Respir Res.* 2015;16:62.

99. Dickerhof N, Turner R, Khalilova I, et al. Oxidized glutathione and uric acid as biomarkers of early cystic fibrosis lung disease. *J Cyst Fibros*. 2017;16:214–221.

100. Chen DL, Ferkol TW, Mintun MA, Pittman JE, Rosenbluth DB, Schuster DP. Quantifying pulmonary inflammation in cystic fibrosis with positron emission tomography. *Am J Respir Crit Care Med*. 2006;173:1363–1369.

101. Amin R, Charron M, Grinblat L, et al. Cystic fibrosis: detecting changes in airway inflammation with FDG PET/CT. *Radiology*. 2012;264:868–875.

102. Shaughnessy AF. Monoclonal antibodies: magic bullets with a hefty price tag. *Bmj*. 2012;345:e8346.

103. Hopkins AL, Groom CR. The druggable genome. *Nat Rev Drug Discov*. 2002;1:727–730.

104. Patel K, Davis SD, Johnson R, Esther CR, Jr. Exhaled breath condensate purines correlate with lung function in infants and preschoolers. *Pediatr Pulmonol*. 2013;48:182–187.

105. Yang J, Eiserich JP, Cross CE, Morrissey BM, Hammock BD. Metabolomic profiling of regulatory lipid mediators in sputum from adult cystic fibrosis patients. *Free Radic Biol Med*. 2012;53:160–171.

106. Wolak JE, Esther CR, Jr., O'Connell TM. Metabolomic analysis of bronchoalveolar lavage fluid from cystic fibrosis patients. *Biomarkers*. 2009;14:55–60.

107. Esther CR, Jr., Turkovic L, Rosenow T, et al. Metabolomic biomarkers predictive of early structural lung disease in cystic fibrosis. *Eur Respir J*. 2016;48:1612–1621.

108. Esther CR, Jr., Coakley RD, Henderson AG, Zhou YH, Wright FA, Boucher RC. Metabolomic evaluation of neutrophilic airway inflammation in cystic fibrosis. *Chest*. 2015;148:507–515.

109. Laguna TA, Reilly CS, Williams CB, Welchlin C, Wendt CH. Metabolomics analysis identifies novel plasma biomarkers of cystic fibrosis pulmonary exacerbation. *Pediatr Pulmonol*. 2015;50:869–877.

110. Joseloff E, Sha W, Bell SC, et al. Serum metabolomics indicate altered cellular energy metabolism in children with cystic fibrosis. *Pediatr Pulmonol*. 2014;49:463–472.

111. de Laurentiis G, Paris D, Melck D, et al. Metabonomic analysis of exhaled breath condensate in adults by nuclear magnetic resonance spectroscopy. *Eur Respir J*. 2008;32:1175–1183.

112. Sofia M, Maniscalco M, de Laurentiis G, Paris D, Melck D, Motta A. Exploring airway diseases by NMR-based metabonomics: a review of application to exhaled breath condensate. *J Biomed Biotechnol*. 2011;2011:403260.

113. Corvol H, Blackman SM, Boelle PY, et al. Genome-wide association meta-analysis identifies five modifier loci of lung disease severity in cystic fibrosis. *Nat Commun*. 2015;6:8382.

114. Grasemann H, Shehnaz D, Enomoto M, Leadley M, Belik J, Ratjen F. L-ornithine derived polyamines in cystic fibrosis airways. *PLoS ONE*. 2012;7:e46618.

115. O'Neal WK, Gallins P, Pace RG, et al. Gene expression in transformed lymphocytes reveals variation in endomembrane and HLA pathways modifying cystic fibrosis pulmonary phenotypes. *Am J Hum Genet*. 2015;96:318–328.

116. Guerrera IC, Astarita G, Jais JP, et al. A novel lipidomic strategy reveals plasma phospholipid signatures associated with respiratory disease severity in cystic fibrosis patients. *PLoS ONE*. 2009;4:e7735.