

An investigation into biomarkers for the diagnosis of ABPA and aspergillus disease in cystic fibrosis

Karen Keown MB, BCH, BAO  | Salome Abbott MD | Boris Kuzeljevic MA |
Jonathan H. Rayment MD | Mark A. Chilvers MD | Connie L. Yang MD 

Division of Respiratory Medicine, BC
Children's Hospital, Vancouver, British
Columbia, Canada

Correspondence

Dr. Connie L. Yang, Division of Respiratory
Medicine, BC Children's Hospital, 4480 Oak
Street, Vancouver, BC V6H 3V4, Canada.
Email: Connie.yang@cw.bc.ca

Abstract

Background: Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease due to *Aspergillus fumigatus* (Af) which occurs in 10% of patients with cystic fibrosis (CF). ABPA is associated with increased morbidity and accelerated lung function decline; however, existing diagnostic criteria are nonspecific and diagnosis remains challenging. As ABPA is driven by Th2 inflammation, the aim of this study was to evaluate exhaled nitric oxide (FE_{NO}), eosinophilic cationic protein (ECP), peripheral eosinophil count, and bronchodilator response (BDR) in patients with CF.

Methods: A prospective observational cohort study of pediatric CF patients in a tertiary center. Patients had a clinical and serologic ABPA assessment, FE_{NO}, serum ECP, peripheral eosinophil count, and assessment of BDR. Patients were stratified into three groups; ABPA, Af sensitized (AFS), and non-ABPA non-Af-sensitized (non-AFS).

Results: A total of 62 patients were included in the study: 13% ABPA, 19% AFS, and 68% non-AFS. Mean FE_{NO} was higher in the ABPA group at 37.8 ppb compared to AFS 15.1 ppb ($P = .05$) and non-AFS 13.7 ppb ($P = .04$). Mean peripheral eosinophil count in ABPA group was also higher at 1000 cells/uL, compared to AFS 221 cells/uL ($P = .03$) and non-AFS 220 cells/uL ($P = .03$). Mean BDR in ABPA group was 13% compared to 5.5% in non-AFS ($P = .01$). Serum ECP was higher in patients with ABPA positive compared to the other groups, although this was not statistically significant.

Conclusion: In children with cystic fibrosis, FE_{NO} and peripheral eosinophil counts are elevated in ABPA compared to those that are just sensitized to *Aspergillus* and may serve as useful diagnostic tests.

KEYWORDS

ABPA, aspergillus sensitization, biomarker, cystic fibrosis, exhaled nitric oxide, peripheral eosinophils, serum eosinophilic cationic protein

1 | INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease due to *Aspergillus fumigatus* (Af). It occurs in approximately 10% of patients with cystic fibrosis (CF) and is associated with accelerated lung function decline, more frequent hospitalizations, and greater CF

morbidity.¹ Making a diagnosis of ABPA is particularly challenging in patients with CF as clinical features are often indistinguishable from bacterial pulmonary exacerbation and laboratory criteria may reflect sensitization and not disease.² This can lead to overdiagnosis of ABPA with subsequent side effects of systemic steroids or underdiagnosis and lack of treatment leading to clinical deterioration.

Patients with *Af* sensitized (AFS) more commonly develop ABPA and the immunologic response to *Af* may represent a spectrum of disease. Findings from small studies suggest poorer lung function in patients with AFS as compared to non-sensitized patients,³⁻⁵ therefore, there is a growing interest in determining the pathological and clinical relevance of AFS. In 2013, a novel classification system based on cluster analysis in adults was proposed with four distinct classes of aspergillus lung disease in CF: ABPA, aspergillus sensitization, aspergillus colonization, and aspergillus bronchitis.⁶ Whilst this classification provides a framework for researching *Af* lung disease, biomarkers to differentiate between these clusters still need to be prospectively validated.

Since ABPA is a Th2-driven process, biomarkers such as peripheral eosinophil count, eosinophilic cationic protein (ECP) and exhaled nitric oxide (FE_{NO}) may be useful markers for ABPA. FE_{NO} is an established clinical test to assess eosinophilic inflammation and steroid-responsiveness in asthma.⁷ It is paradoxically low in cystic fibrosis, however, it has not been studied as a marker of ABPA in CF.⁸ Peripheral eosinophil count has been included in some diagnostic criteria for ABPA, however it has not been found to be a sensitive marker for ABPA diagnosis⁹ and has not been included in the CF Foundation criteria.² Serum ECP is a granular protein released from activated eosinophils. Patients with CF were found to have higher levels of serum and sputum ECP, despite normal peripheral eosinophil counts,¹⁰ indicating that enumeration of eosinophils may not reflect airway inflammatory activity and ECP may be a more sensitive marker of early eosinophilic inflammation. A clinical hallmark of ABPA is the presence of wheezing which may indicate airway hyperreactivity; therefore, a significant change in FEV₁ with bronchodilator may be a useful objective marker of ABPA.

The objective of this study was to determine if FE_{NO}, serum ECP, peripheral eosinophil count or FEV₁ bronchodilator response (BDR) are useful biomarkers in differentiating ABPA from other aspergillus phenotypes in children with cystic fibrosis.

2 | METHODS

2.1 | Study design and patients

We performed a prospective observational cohort study in a tertiary pediatric CF center in Vancouver, British Columbia between December 2012 and May 2014. Participants were age 6 and older, with a confirmed diagnosis of cystic fibrosis by sweat testing and/or genetics. Patients were excluded from the study if they were unable to perform reproducible spirometry or FE_{NO}. This study was approved by the Children's and Women's Research Ethics Board (H13-00676) and informed consent was obtained.

2.2 | Procedures

As part of their clinical care, participants had standard ABPA testing (Total IgE, Aspergillus specific IgE or skin prick testing, aspergillus

precipitins) and a complete blood count (including eosinophil count) during annual bloodwork or if there was a clinical suspicion of ABPA. ECP concentration was determined with an enzyme-linked immunosorbent assay (ImmunoCAP ECP) tested at the same time or within 3 months with future bloodwork. All patients had skin prick testing to a panel of aeroallergens and aspergillus using the Multi-Test II (see the Supporting Information). Spirometry and BDR assessment was performed according to ATS criteria¹¹ and percent predicted values were generated using the Hibbert reference equation.¹² Patients were advised to hold short-acting bronchodilators for 6 hours and long-acting bronchodilators for 24 hours before testing, as per standard laboratory protocol. Exhaled nitric oxide measurements were performed using the NIOX MINO (Aerocrine, Sweden) with subjects exhaling at 50 mL/s against resistance for 10 seconds using standard visual feedback display.¹³

2.3 | Definitions

A diagnosis of new ABPA or ongoing ABPA on treatment, was made by the responsible CF clinician if patients met the classic or minimal diagnostic criteria according to the CFF 2003 consensus statement (Table 1).² *Af* sensitization was diagnosed if either skin prick testing was positive or if Specific *Af* IgE was elevated (greater than 0.35 kU/L) in a patient who did not have a clinical features of ABPA. Diagnosing and treating clinicians were blinded to ECP results but were aware of FE_{NO}, BDR, and peripheral eosinophil count. Atopy was defined by a positive skin prick test (wheal of ≥3 mm than the negative control) to one or more aeroallergen, excluding aspergillus.

TABLE 1 CF Foundation 2003 Consensus Criteria for the Diagnosis of ABPA in patients with cystic fibrosis

Classic criteria
1. Acute or subacute clinical deterioration not attributable to another etiology
2. Serum total IgE concentration 1000 IU/mL (2400 ng/mL), unless the patient is receiving systemic corticosteroids
3. Immediate cutaneous reactivity to Aspergillus (>3 mm) or serum IgE antibody to <i>A. fumigatus</i>
4. Precipitating antibodies to <i>A. fumigatus</i> or serum IgG antibody to <i>A. fumigatus</i>
5. New abnormalities on chest radiography, not cleared with antibiotics and standard physiotherapy
Minimal diagnostic criteria
1. Acute or subacute clinical deterioration not attributable to another etiology
2. Total serum IgE concentration of >500 IU/mL (1200 ng/mL)
3. Immediate cutaneous reactivity to Aspergillus (>3 mm) or serum IgE antibody to <i>A. fumigatus</i>
4. One of the following: (a) precipitins to <i>A. fumigatus</i> or IgG antibody to <i>A. fumigatus</i> ; or (b) new abnormalities on chest radiography, not cleared with antibiotics and standard physiotherapy

Abbreviation: ABPA, allergic bronchopulmonary aspergillosis.

2.4 | Statistical analysis

Normally distributed data are expressed as means ($\pm$ SD) and otherwise are expressed as median (range). *P* values of .05 or less were considered statistically significant. Groups were compared to identify any difference in demographics or clinical characteristics. Distribution of data was analyzed using the Kolmogorov-Smirnov test and a one-way ANOVA or the Kruskal Wallis test were used to compare normally and nonnormally distributed data respectively. *Post hoc* analysis of multiple comparisons was performed using the Dunnett T3 test. Statistical analysis was conducted using SPSS 25.

3 | RESULTS

Of the 93 patients 6 years and over followed in the CF clinic during the study, 70 patients were enrolled between October 2012 and May 2014 (Figure 1). Eight were excluded because they did not have aspergillus sensitization testing (Figure 1). FE_{NO} was performed in all 62 patients, serum eosinophils in 57, and BDR in 61. Serum ECP was collected in 46 patients however the test was not completed in 11 due to sampling or processing errors.

Eight patients (12.9%) were diagnosed with ABPA, 12 (19.3%) were AFS, and 42 (67.7%) were ABPA negative and non-sensitized (non-AFS). Of those with ABPA, 3 were new diagnoses at that visit and 5 had been diagnosed with ABPA in the last 3 months and were on treatment with an oral corticosteroid.

More patients with ABPA or Af sensitization were chronically colonized with *Pseudomonas Aeruginosa* ($P < .001$; $P < .001$), on chronic inhaled antibiotic therapies ($P = .001$; $P < .001$) or inhaled corticosteroids ($P < .001$; $P < .001$) compared to those who were not sensitized to Af (Table 2). Those with ABPA had lower FEV₁ (mean 80.7%) than the other groups (AFS 88.8% $P = .01$; non-AFS 93.7% $P = .01$). In addition, patients with a diagnosis of ABPA had the highest frequency of pulmonary exacerbations per year, however,

differences were not statistically significant. More patients with ABPA or AFS were atopic compared to those that were non-AFS ($P = .01$).

Mean FE_{NO} in the ABPA group was 37.8 ± 21.2 ppb, significantly higher than AFS (15.1 ± 10.1 ppb, $P = .05$) and nonsensitized patients (13.7 ± 13.7 ppb, $P = .004$) (Figure 2). Mean peripheral eosinophil count in the ABPA group was 1000 ± 62 cells/uL which was significantly higher than the other groups (AFS 221 ± 140 cells/uL, $P = .03$, non-AFS 220 ± 178 cells/uL, $P = .03$; Figure 2).

Mean BDR was significantly higher in those with ABPA ($13\% \pm 10.1$) than non-AFS ($5.5\% \pm 6.1$; $P = .01$), although not significantly higher compared to those with AFS ($8.7\% \pm 4.5\%$). One of the 49 patients on short-acting beta agonists did not hold it for 6 hours before testing, 11 of the 21 patients on long-acting beta agonists did not hold it for 24 hours ($n = 5$ ABPA, $n = 2$ AFS, and $n = 4$ non-AFS). Serum ECP values were higher in those with ABPA (61.8 mg/L ± 30.5) compared to AFS (AFS 46.8 mg/L ± 25.7) and non-AFS (43.1 mg/L ± 30.4), however, differences were not statistically significant.

4 | DISCUSSION

In this prospectively collected, cross-sectional study we demonstrate that FE_{NO} and peripheral eosinophils are increased in pediatric CF patients with ABPA, compared to those who were Af sensitized and Af nonsensitized. ECP was not significantly different between the three groups. To our knowledge, this is the first systematic assessment of FE_{NO} in this context and suggests that this biomarker may be useful to help diagnose patients with ABPA.

There is only one study examining exhaled NO levels in adult patients at risk of developing ABPA as defined by increased Af specific IgE, IgE greater than 500KU/L (or recent doubling) and history of decreased lung function responsive to oral steroids.¹⁴ In

FIGURE 1 Study flow diagram. ABPA, allergic bronchopulmonary aspergillosis; Af, *Aspergillus fumigatus*; BDR, bronchodilator response; ECP, eosinophilic cationic protein; FE_{NO}, exhaled nitric oxide [Color figure can be viewed at wileyonlinelibrary.com]

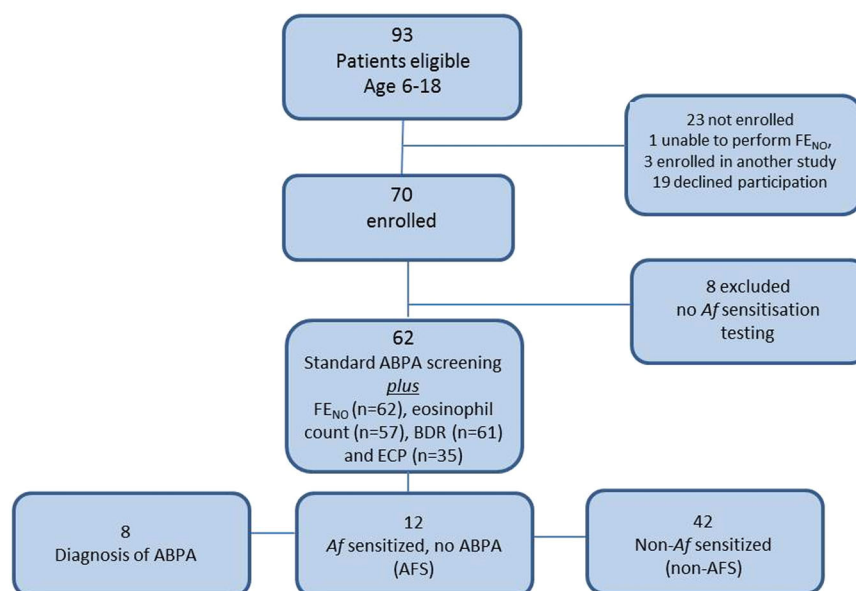


TABLE 2 Patient baseline characteristics

	ABPA N = 8	Sensitized N = 12	Nonsensitized N = 42
Age mean years $\pm$ SD	13.6 $\pm$ 2.8	12.6 $\pm$ 2.0	11.1 $\pm$ 3.4
Male N (%)	4 (50)	6 (50)	19 (45)
F508 homozygous N (%)	3 (37)	6 (50)	24 (57)
F508 heterozygous N (%)	4 (50)	5 (41)	11 (26)
Other/Other N (%)	1 (12)	1 (8)	7 (16)
Atopic ^a N (%)	6 (75)*	6 (50)*	11 (26)
Number of positive skin tests ^b N $\pm$ SD	3.0 $\pm$ 1.5	1.6 $\pm$ 0.81	2.5 $\pm$ 1.7
On inhaled steroids N (%)	8 (100)*	11 (92)*	17 (40)
On oral steroids N (%)	5 (63)	0	0
Chronic inhaled antibiotic N (%)	7 (88)*	11(92)*	16 (38)
<i>P. Aeruginosa</i> colonized ^c N (%)	6 (75)*	8 (66)*	4 (9)
Af isolated in sputum ^d N (%)	3 (37.5)	7 (58.3)	10 (23.8)
Mean FEV1% $\pm$ SD	80.7 $\pm$ 16.0***	88.8 $\pm$ 18.1	93.7 $\pm$ 15.4
Mean exacerbations/year N $\pm$ SD	2.5 $\pm$ 1.6	1.9 $\pm$ 1.5	1.7 $\pm$ 1.6
Mean Total IgE (mcg/L) $\pm$ SD	2385 $\pm$ 1497	500 $\pm$ 623	124 $\pm$ 167

Abbreviation: ABPA, allergic bronchopulmonary aspergillosis.

^a ≥ 1 positive skin test (excluding *A. fumigatus*).

^bExcluding *A. fumigatus*.

^cOver 50% of cultures positive for *P. Aeruginosa* in last 12 mo.

^dPositive sputum culture during study.

* $P < .05$ in comparison to non-sensitized.

** $P < .05$ in comparison to sensitized.

contrast to our findings, FE_{NO} levels were lower in patients at risk of developing ABPA compared to those who were not at risk. This discordance may be due to a difference in FE_{NO} in subjects with active ABPA vs only being at risk for ABPA. As both studies were cross-sectional it is difficult to test this hypothesis further. In addition, that study involved adult patients with a much lower FEV₁ in comparison to our pediatric population, with some data showing that a lower FE_{NO} is associated with a lower FEV₁ in patients with cystic fibrosis.¹⁵ As the FE_{NO} levels across all the groups in the adult study were lower (1.2–18 ppb) than in our study (5–73 ppb), the variation in FE_{NO} may not be as apparent, regardless of ABPA status.

Peripheral eosinophils are not part of the CF consensus criteria²; however, eosinophil counts over 400 cells/ μ L¹⁶ or 1000 cells/ μ L¹⁷ are included in other diagnostic criteria. A previous study of ABPA in 10 children with cystic fibrosis found that none had an eosinophil count over 1000 cells/ μ L and only 30% (3 of 10) had an eosinophil count over 400 cells/ μ L.⁹ In our study, 75% of our ABPA patients had an eosinophil count over 400 cells/ μ L with 40% having counts over 1000 cells/ μ L, while none of the patients in the other groups had an eosinophil count over 1000 cells/ μ L, suggesting that this could be a specific biomarker for ABPA in children. The explanation for this discordance is not clear however, these findings suggest that peripheral eosinophil count may be a better biomarker for ABPA in children than in adults. Peripheral eosinophils may not be the optimal biomarker in ABPA given that they are not in the compartment of interest, which would be the airways. Given the strong Th2 response with airway eosinophilia and the reported contribution of eosinophil

extracellular traps in the viscous mucous characteristic of ABPA,^{18,19} sputum eosinophils would be a logical biomarker, however, has not yet been studied in CF.

Given the significant difference between groups in peripheral eosinophils that was found in this study, it is surprising that the same results were not seen with serum ECP which has been shown to be a sensitive marker of eosinophil activity.¹⁰ However, as sampling and processing problems resulted in reduced ECP data, these negative findings should be interpreted with caution. Serum ECP has previously to be inversely related to lung function in patients with CF,²⁰ however, the influence of ABPA status was not assessed in that study, therefore further evaluation of ECP is needed to ascertain the role of ECP in the diagnosis of ABPA.

ABPA is more prevalent in patients with CF who have a diagnosis of asthma,¹⁷ however establishing a diagnosis of asthma in patients with CF is challenging.²¹ In this study, BDR was assessed as an objective marker of bronchial hyperreactivity, and those with ABPA were more likely to have a significant BDR than those without ABPA. Those with ABPA were more likely to have taken a long-acting beta agonist in the 24 hours before testing in comparison to the two other groups, so this signal may actually be underestimated. In cystic fibrosis a significant BDR has been documented in up to 40% of patients with episodic variability over time,²² thus the usefulness of a BDR in diagnosing ABPA should be evaluated in a longitudinal study.

Interestingly, elevated Th2 markers (FE_{NO}, peripheral eosinophil count) were seen in all of our patients with ABPA, despite the majority being on treatment with systemic corticosteroids at the time

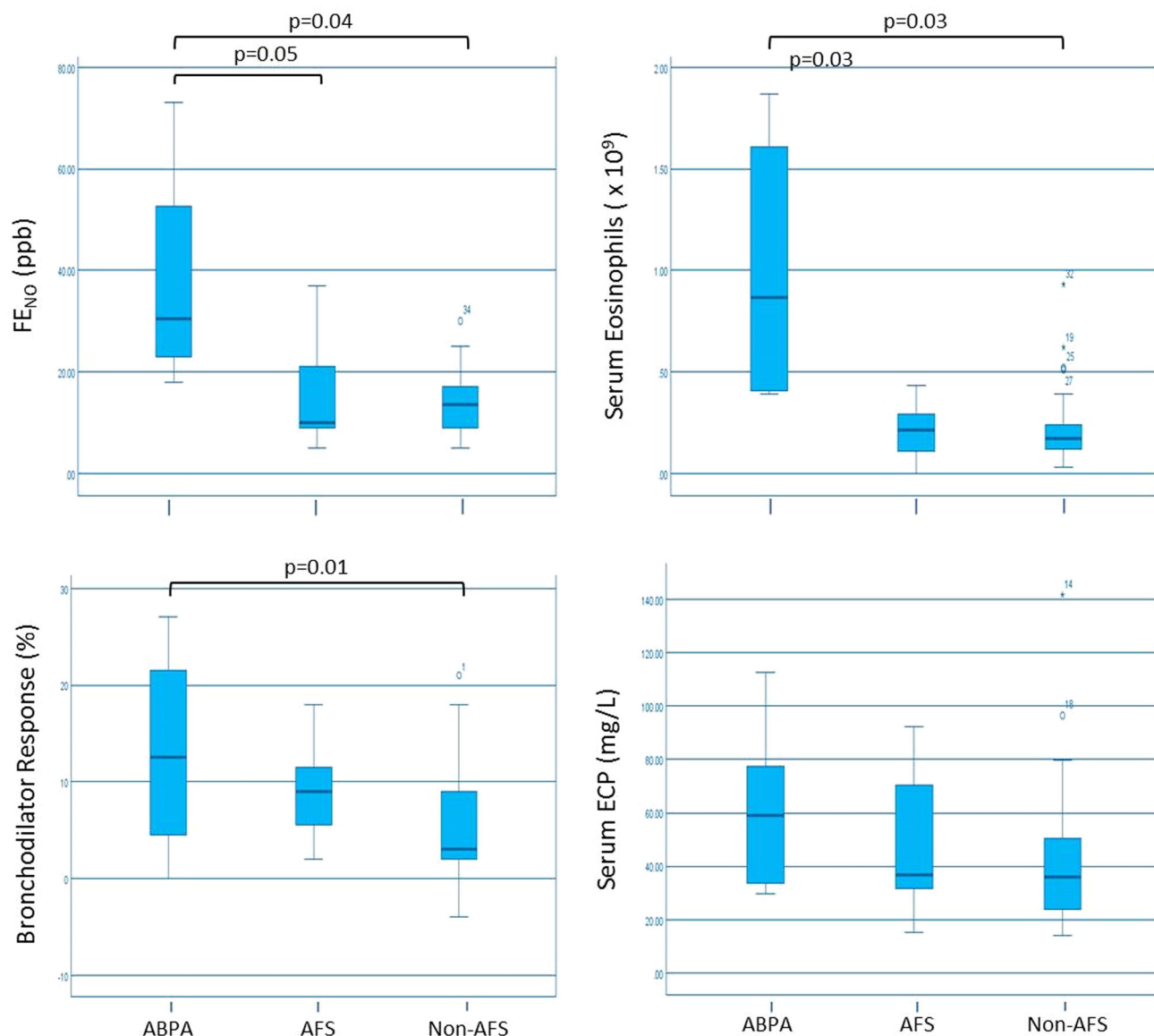


FIGURE 2 Boxplot distributions (25thile, median, 75thile) of biomarkers in ABPA positive, Af sensitized (AFS) and non-Af sensitized (non-AFS). ABPA, allergic bronchopulmonary aspergillosis [Color figure can be viewed at wileyonlinelibrary.com]

of testing. Given that corticosteroids are known to attenuate eosinophilic and Th2 inflammation, our results may underestimate the elevation of these markers, further adding to the validity of our findings. While the effect of corticosteroid on FE_{NO}, peripheral eosinophils and BDR is well recognized in clinical practice, serum ECP is also likely to be suppressed given that it is released by degranulation of active eosinophils. It is not known if ECP is more effected by steroids than other Th2 biomarkers and this may be a contributory factor in the negative findings for ECP in this study, however, numbers are too small to meaningfully compare levels in those treated and not treated with corticosteroid.

The prevalence of Af sensitization and ABPA in our study was 32% and 13%, respectively. Consistent with other studies, our data indicate that Af sensitization, even without ABPA is associated with poorer lung function compared to non-sensitized controls.³⁻⁵ These

findings add to the growing body of evidence indicating Af sensitization is an important clinical entity requiring further research.

A high rate of atopy of up to 40% has been reported in adults with CF,²³ we found a similar prevalence among our pediatric population at 37%. It is not clear why atopy is more prevalent in CF than the general population, however, as high rates have also been found in non-CF bronchiectasis²⁴ it is likely related to chronic airway inflammation, increased epithelial permeability and greater access for aeroallergens into the bronchial mucosa.²⁵ Similar to findings by Nepomuceno et al,²⁶ we found that ABPA rates were higher amongst atopic than nonatopic patients (26% vs 5.1%), therefore determination of atopic status with closer surveillance for ABPA in this group of patients is warranted in the clinical setting.

The main strength of this study was the prospective nature of the collection of the ABPA biomarkers across a large pediatric CF cohort.

We acknowledge a number of limitations of this study. First, it is a single center study of pediatric patients thus limiting its external validity. Second, the sample size of patients with ABPA was small and not all biomarkers, particularly ECP were assessed in every patient further limiting the sample size for some analyses. A further limitation which we acknowledge is the potentially confounding effect of systemic steroid therapy on these biomarkers. Finally, clinicians were not blinded to the results of FE_{NO}, BDR, and peripheral eosinophil count which may have led to diagnostic review bias.

5 | CONCLUSION

In clinical practice, the diagnosis of ABPA in patients with cystic fibrosis continues to be a challenge due to the lack of sensitive and specific diagnostic tests. This study adds to the literature on biomarkers for ABPA and illustrates that FE_{NO}, serum eosinophils, and BDR are significantly higher in CF patients with ABPA compared to those nonsensitized to *Af*. In addition, FE_{NO} and serum eosinophils are higher in those with ABPA compared to those sensitized to *Af* which makes them attractive markers for differentiating these two entities. Further studies are needed to determine if these biomarkers should be included in future diagnostic criteria for ABPA in cystic fibrosis.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

MC and CY were responsible for study design and patient selection. SA was responsible for all data collection; KK and CY reviewed each case, analyzed data and prepared the final manuscript. BK performed statistical analysis. All authors reviewed the final manuscript and were involved in the decision to submit for publication. CY is the guarantor of the paper.

ORCID

Karen Keown  <http://orcid.org/0000-0002-6095-9585>

Connie L. Yang  <http://orcid.org/0000-0003-2060-3290>

REFERENCES

- Maturu VN, Agarwal R. Prevalence of *Aspergillus* sensitization and allergic bronchopulmonary aspergillosis in cystic fibrosis: systematic review and meta-analysis. *Clin Exp Allergy*. 2015;45:1765-1778.
- Stevens DA, Moss RB, Kurup VP, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis--state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis*. 2003;37(Suppl 3):S225-S264.
- Wojnarowski C, Eichler I, Gartner C, et al. Sensitization to *Aspergillus fumigatus* and lung function in children with cystic fibrosis. *Am J Respir Crit Care Med*. 1997;155:1902-1907.
- Nicolai T, Arleth S, Spaeth A, Bertele-Harms RM, Harms HK. Correlation of IgE antibody titer to *Aspergillus fumigatus* with decreased lung function in cystic fibrosis. *Pediatr Pulmonol*. 1990;8:12-15.
- Kraemer R, Deloséa N, Ballinari P, Gallati S, Cramer R. Effect of allergic bronchopulmonary aspergillosis on lung function in children with cystic fibrosis. *Am J Respir Crit Care Med*. 2006;174:1211-1220.
- Baxter CG, Dunn G, Jones AM, et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. *J Allergy Clin Immunol*. 2013;132:560-566.e10.
- Kharitonov SA, Yates D, Robbins RA, Barnes PJ, Logan-Sinclair R, Shinebourne EA. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet*. 1994;343:133-135.
- Ho LP, Innes JA, Greening AP. Exhaled nitric oxide is not elevated in the inflammatory airways diseases of cystic fibrosis and bronchiectasis. *Eur Respir J*. 1998;12:1290-1294.
- Laufer P, Fink JN, Bruns WT, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *J Allergy Clin Immunol*. 1984;73:44-48.
- Koller DY, Gotz M, Eichler I, Urbanek R. Eosinophilic activation in cystic fibrosis. *Thorax*. 1994;49:496-499.
- Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26:319-338.
- Hibbert ME, Lannigan A, Landau LI, Phelan PD. Lung function values from a longitudinal study of healthy children and adolescents. *Pediatr Pulmonol*. 1989;7:101-109.
- Dweik RA, Boggs PB, Erzurum SC, et al. An Official ATS Clinical Practice Guideline: interpretation of exhaled nitric oxide levels (FeNO) for clinical applications. *Am J Respir Crit Care Med*. 2011;184:602-615.
- Lim AYH, Chambers DC, Ayres JG, Stableforth DE, Honeybourne D. Exhaled nitric oxide in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Respir Med*. 2003;97:331-336.
- de Winter-de Groot KM, van der Ent CK. Nitric oxide in cystic fibrosis. *J Cyst Fibros*. 2005;4:25-29.
- Alexander SAK, Anderson AK, Balfour-Lynn I. Clinical Guidelines: Care of Children with Cystic Fibrosis. London: Royal Brompton and Harefield NHS Foundation Trust; 2017.
- Geller DE, Kaplowitz H, Light MJ, Colin AA. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *Chest*. 1999;116:639-646.
- Kita H. Eosinophils: multifaceted biological properties and roles in health and disease. *Immunol Rev*. 2011;242:161-177.
- Ueki S, Hebisawa A, Kitani M, Asano K, Neves JS. Allergic bronchopulmonary aspergillosis-a luminal hypereosinophilic disease with extracellular trap cell death. *Front Immunol*. 2018;9:2346.
- Koller DY, Gotz M, Wojnarowski C, Eichler I. Relationship between disease severity and inflammatory markers in cystic fibrosis. *Arch Dis Child*. 1996;75:498-501.
- Balfour-Lynn IM, Elborn JS. CF asthma: what is it and what do we do about it. *Thorax*. 2002;57:742-748.
- Pattishall EN. Longitudinal response of pulmonary function to bronchodilators in cystic fibrosis. *Pediatr Pulmonol*. 1990;9:80-85.
- Becker JW, Burke W, McDonald G, Greenberger PA, Henderson WR, Aitken ML. Prevalence of allergic bronchopulmonary aspergillosis and atopy in adult patients with cystic fibrosis. *Chest*. 1996;109:1536-1540.
- Ozturk S, Tozkoparan E, Karaayvaz M, et al. Atopy in patients with bronchiectasis: more than coincidence. *Tohoku J Exp Med*. 2006;208:41-48.
- Pang J, Chan HS, Sung JY. Prevalence of asthma, atopy, and bronchial hyperreactivity in bronchiectasis: a controlled study. *Thorax*. 1989;44:948-951.

26. Nepomuceno IB, Esrig S, Moss RB. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *Chest*. 1999;115:364-370.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Keown K, Abbott S, Kuzeljevic B, Rayment JH, Chilvers MA, Yang CL. An investigation into biomarkers for the diagnosis of ABPA and aspergillus disease in cystic fibrosis. *Pediatric Pulmonology*. 2019;1-7.
<https://doi.org/10.1002/ppul.24465>

Supplemental materials

Methods

Aeroallergens tested

Cat hair; cat pelt; dermatophagoides Pteronyssinus; dermatophagoides Farinae; mould mix (Alternaria, heminthesporium, cladosporium, penicillium); Feathers; Dog; alder; birch; Pacific grass mix; coast weeds; western tree mix; aspergillus fumigatus

Test was considered positive if wheal was ≥ 3 mm than the negative control

Skin prick testing protocol (Multi test II ®)

1. Clean both forearms with alcohol
2. Place plastic skin test into allergen wells*
3. Place firmly on each arm, and rock from side to side a few times, remove
4. Dry arm by placing paper towel on arm, by pressing, do not mix allergens
5. Read histamine at 5 minutes
6. Read rest of allergens at 15 minutes
7. Histamine of 3mm or more is a valid test
8. Atopy is when any one of the other allergens is 3mm or more than histamine
9. Read wheal in largest diameter not the flare