

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/367239706>

A Case-Control Study of the Effects of Aspergillus Clinical Phenotypes on Pulmonary Functions in Patients with Cystic Fibrosis

Article in *Pediatric Pulmonology* · January 2023

DOI: 10.1002/ppul.26319

CITATIONS

0

READS

42

22 authors, including:



Halime Nayir Buyuksahin
Hacettepe University

36 PUBLICATIONS 56 CITATIONS

[SEE PROFILE](#)



Nagehan Emiralioğlu
Hacettepe University

218 PUBLICATIONS 679 CITATIONS

[SEE PROFILE](#)



Dilber Ademhan Tural
Hacettepe University

71 PUBLICATIONS 143 CITATIONS

[SEE PROFILE](#)



Beste Özsezen
Hacettepe University

60 PUBLICATIONS 91 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Turkish Thoracic Society Early Career Member Group [View project](#)



UNGEMİ OLGULARINDA HIZLI BİR ÖN TANILAMA TESTİ: POZİTİF KAN KÜLTÜRÜ ŞİŞESİNDEN YAPILAN DOĞRUDAN GERM TUP TESTİNİN DEĞERLENDİRİLMESİ [View project](#)

A case-control study of the effects of *Aspergillus* clinical phenotypes on pulmonary functions in patients with cystic fibrosis

Halime Nayir Buyuksahin MD¹  | Deniz Dogru MD¹  |
 Nagehan Emiralioglu MD¹  | Dilber Ademhan Tural MD¹  |
 Beste Özsezen MD¹  | Birce Sunman MD¹ | Ismail Guzelkaş MD¹ |
 Didem Alboğa MD¹ | Sevtap Arian-Akdagli MD² | Zeynep Sarıbaş MD² |
 Dolunay Gulmez MD² | Alpaslan Alp MD² | Gülşen Hazırolan MD² |
 Özge Soyer MD³ | Bülent Enis Şekerel MD³ | Gözde İnam MD⁴ | İlim Irmak MD⁴ |
 Ebru Damadoğlu MD⁴ | Fuat Kalyoncu MD⁴ | Ebru Yalçın MD¹ |
 Ugur Ozcelik MD¹ | Nural Kiper MD¹

¹Department of Pediatrics, Division of Pulmonology, Hacettepe University Faculty of Medicine, Ankara, Turkey

²Department of Medical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey

³Department of Pediatrics, Division of Allergy and Asthma, Hacettepe University Faculty of Medicine, Ankara, Turkey

⁴Department of Chest Diseases, Hacettepe University Faculty of Medicine, Ankara, Turkey

Correspondence

Halime Nayir Buyuksahin, Department of Pediatric, Division of Pulmonology, Hacettepe University of Medicine, Ankara, Turkey.
 Email: hnayirbuyuksahin@gmail.com

Abstract

Introduction: There are no precise data about the effect of *Aspergillus* infection on lung function other than allergic bronchopulmonary aspergillosis (ABPA) in patients with cystic fibrosis (pwCF). Here, we aimed to determine clinical phenotypes caused by *Aspergillus* spp. using laboratory and immunologic parameters and to compare *Aspergillus* phenotypes in terms of pulmonary function tests (PFT) prospectively.

Methods: Twenty-three pwCF who had *Aspergillus* isolation from respiratory cultures in the last year (case group) and 20 pwCF without *Aspergillus* isolation in sputum (control group) were included. *Aspergillus* immunoglobulin (Ig)-G, *Aspergillus* IgE, *Aspergillus* polymerase chain reaction (PCR), galactomannan, total IgE from blood samples, and *Aspergillus* PCR and galactomannan from sputum, and skin prick test reactivity to *Aspergillus* antigen were used to distinguish different *Aspergillus* phenotypes. Pulmonary functions and frequency of pulmonary exacerbations were evaluated during a 1-year follow-up.

Results: Of 23 pwCF, 11 (47.8%) had *Aspergillus* colonization, nine (39.1%) had *Aspergillus* bronchitis, and three (13%) had ABPA. *Aspergillus* infection was not associated with worse z-scores of forced expiratory volume in the first second (FEV1) ($p = 0.612$), forced vital capacity ($p = 0.939$), and the median FEV 1% decline (0.0%/year vs. -4.7%/year, $p = 0.626$). The frequency of pulmonary exacerbations in the *Aspergillus* infected and noninfected groups was similar.

Conclusion: Although *Aspergillus* spp. Isolation in pwCF was not associated with decreased lung function, a further decline was seen in the ABPA subgroup, and frequent pulmonary exacerbations during the 1-year follow-up.

KEYWORDS

Aspergillus, bronchitis, colonization, cystic fibrosis, pulmonary functions

1 | INTRODUCTION

Respiratory tract infections are important causes of mortality and morbidity in cystic fibrosis (CF) and these infections are often polymicrobial.¹ The importance of bacterial CF pathogens is well known.¹ Fungal infections in patients with CF (pwCF) have increased in recent years. *Aspergillus* species (spp.) are the most common filamentous fungi in CF airways, with prevalence rates of up to 78%.² Moreover, Reece et al. reported that *Aspergillus fumigatus* was likely more prevalent in early CF disease than was currently reported due to proving that molecular methods were superior in detecting *A. fumigatus* in pwCF sputum.³ *Aspergillus* spores are commonly found in the environment, including fertile soils, rotting vegetation, swimming pool water, leaky basements, crawl spaces, beds, and dust from homes.⁴ Spores are inhaled frequently and usually cleared without the development of inflammation or invasion in the lung. However, cystic fibrosis transmembrane conductance regulator (CFTR) has an important role in airway epithelial cell clearance of *Aspergillus* spores, and due to the deficiency of CFTR in pwCF, inhalation of spores results in susceptibility to apoptosis, and aberrant inflammatory responses, and accumulation in the distal airways.⁵ Thus, *Aspergillus* initiates pulmonary inflammation in the airways of pwCF and causes different clinical pictures such as allergic bronchopulmonary aspergillosis (ABPA), *Aspergillus* sensitization, *Aspergillus* colonization, and *Aspergillus* bronchitis.^{6,7} Nevertheless, why pwCF may respond differently to *Aspergillus* is unknown.⁸

ABPA is a TH2-mediated allergic response to *Aspergillus* characterized by immunoglobulin (Ig)-E elevation, with *Aspergillus* specific IgE elevation and *Aspergillus* skin test positivity.⁴ *Aspergillus* sensitization describes patients who do not meet ABPA criteria but are characterized by elevated IgE against *Aspergillus*.⁹ *Aspergillus* colonization is a term used to describe patients with growth of *Aspergillus* in two or more respiratory tract cultures within the following 12 months who are asymptomatic and do not have an immunologic response. *Aspergillus* bronchitis is characterized by *Aspergillus* growth in sputum cultures, clinical worsening of the patient, poor response to antibiotics, and good response to antifungal therapy. In this group, *Aspergillus*-specific IgE and total IgE are normal, and *Aspergillus*-specific IgG is high.¹⁰ However, an international study showed that diagnostic and therapeutic approaches to *Aspergillus*-related conditions in CF differed among physicians.¹¹

The effect of ABPA on lung functions is well known, but there are no clear data on the effect of other *Aspergillus* infections on lung functions in patients with CF. Previous studies suggested that *Aspergillus* colonization, sensitization, and bronchitis were also associated with decreased pulmonary function tests.^{10,12,13} By contrast, other studies have shown no association between *Aspergillus* infection and lung function decline.^{14–16} We hypothesized that pwCF infected by *Aspergillus* spp. might have worse clinical outcomes

compared with pwCF without *Aspergillus* spp. isolation. Therefore, the primary aim of this study was to determine clinical phenotypes caused by *Aspergillus* spp. using laboratory and immunologic parameters in pwCF. The secondary outcome was to prospectively compare *Aspergillus* phenotypes in terms of pulmonary function tests (PFTs).

2 | MATERIALS-METHODS

2.1 | Study design and ethics

This prospective, case-control, 1-year longitudinal study was conducted from September 2019 to September 2022 and approved by the local ethics committee (Date: September 10th, 2019; Reference No.: GO 19/866); all participants/parents gave written informed consent.

2.2 | Patients and procedures

Participants were aged older than 8 years with a confirmed diagnosis of CF based on consensus guidelines from the CF Foundation.¹⁷ Twenty-three patients who had *Aspergillus* isolation from respiratory cultures constituted the “case group.” Patients with a history of ABPA and those using systemic steroids were excluded from the study. To classify the case group for a spectrum of CF *Aspergillus* disease, *Aspergillus* total IgG, *Aspergillus* IgE, *Aspergillus* polymerase chain reaction (PCR), galactomannan, and total IgE were measured from blood samples, and *Aspergillus* PCR and galactomannan were detected from sputum, and skin prick test reactivity to *Aspergillus* antigen was performed. Twenty age and sex-matched pwCF without *Aspergillus* isolation in sputum in the past year were included as the “control group.” Galactomannan was studied from sputum samples in the control group.

We followed the patients for 1 year, with an evaluation every 6 months. Symptoms, physical examination findings, PFTs, and sputum cultures were measured at the study baseline, midpoint, and end of the study in all patients. Any change in respiratory conditions including a decrease in forced expiratory volume in 1 s (FEV1), a drop of oxygen saturation, new infiltrates on chest X-ray, hemoptysis, increased effort in breathing, weight loss, fever, change in sputum, and increased cough that needed hospitalization for intravenous antibiotic treatment was considered as an acute pulmonary exacerbation (PEX).¹⁸ Data on the baseline demographic characteristics of the children, including age, sex, CF mutations, and related diseases such as exocrine pancreatic insufficiency and CF-related diabetes, and numbers of PEX were noted using medical records.

PFTs were performed using a Vyntus Spiro PC Spirometer (CareFusion). Spirometry measurements were interpreted according to the American Thoracic Society and European Respiratory Society guidelines, and the FEV1 and forced vital capacity (FVC) percentage (%) predicted of pwCF were assessed.¹⁹ Percent-predicted z-scores of FEV1 and FVC were calculated using Global Lung Initiative (GLI) reference equations.²⁰ We also calculated the change in values of parameters from the baseline to the end of the first year. Body mass index (BMI) for age z-scores was calculated using the World Health Organization anthropometric calculator.

2.3 | Assays of microbiologic studies

Respiratory specimens were cultured on Sabouraud dextrose agar and blood agar plates and were then incubated at $35 \pm 2^\circ\text{C}$ and $30 \pm 2^\circ\text{C}$ for 7 days. Any mold colonies on agar plates were identified using macroscopic and microscopic morphology.²¹ *A. fumigatus* complex isolates were further tested for growth at 50°C to confirm the identification of *A. fumigatus* sensu stricto. Azole susceptibility of *A. fumigatus* isolates was screened using agar screening methodology.²² Antifungal susceptibility of *Aspergillus* isolates other than *A. fumigatus* was tested according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) microdilution assay guidelines.²³

Serum and respiratory samples were aliquoted and stored at -80°C . Sputum samples were homogenized with TE (Tris-EDTA) buffer, before testing with enzyme-linked immunosorbent assay (ELISA) kits. Galactomannan was detected in serum and respiratory samples using ELISA (Euroimmun Medizinische Labordiagnostika AG), and optical density indexes (ODI) > 0.5 were considered positive. Serum *Aspergillus* IgG levels were measured using an ELISA kit from Abnova Corporation, Taiwan, and values with an ODI of greater than 1.1 were considered positive. All ELISA tests were performed and evaluated according to the manufacturers' instructions.

DNA from serum and respiratory specimens was extracted (Genrui Nucleic Acid Extraction Kit) and tested with 28S rRNA primers for *A. fumigatus*, *A. terreus*, and other *Aspergillus* spp. DNA (AsperGenius Multiplex Real-time PCR Kit), according to the manufacturer's recommendations.

In the skin prick test, the diameter of the wheal of an *Aspergillus* allergen extract was measured, and a positive wheal was $\geq 3\text{ mm}$.²⁴ Serum samples were tested for *Aspergillus* IgE using the ImmunoCap assay (Thermo Fisher Scientific).

2.4 | Definitions of CF *Aspergillus* disease

Patients with *Aspergillus* isolation on airway cultures were categorized into four groups according to immunologic and sputum biomarker criteria defined by Baxter et al.²⁵ and Felton et al.⁶ as follows: *Aspergillus* colonization, ABPA, *Aspergillus* sensitization, and *Aspergillus* bronchitis. We used *Aspergillus*-IgG, *Aspergillus*-IgE, total IgE, *Aspergillus* PCR, and sputum galactomannan for this classification.

1. *Aspergillus* colonization: *Aspergillus* PCR was positive.
2. Serologic ABPA: All of the above markers were elevated or positive.
3. *Aspergillus* sensitization: *Aspergillus*-specific IgE and total IgE were elevated but did not fulfill ABPA criteria.
4. *Aspergillus* bronchitis: *Aspergillus*-specific IgG was elevated; *Aspergillus* PCR and sputum galactomannan were positive.

2.5 | Statistical analysis

The sample size was calculated as $\alpha = 0.05$ and $\beta - 1 = 90\%$, with a minimum of 20 patients for each group. Statistical analyses were performed using the SPSS 22 software package (IBM Corp.). The variables suitable for normal distribution were assessed using Shapiro-Wilk tests. Normally distributed continuous variables were analyzed using Student's *t*-test and expressed as mean $\pm$ standard deviation (SD). Non-normally distributed continuous variables were analyzed using the Mann-Whitney U or Kruskal-Wallis tests and expressed as median (interquartile range). Categorical variables are presented as numbers and percentages (%) and analyzed using Pearson's χ^2 test or Fisher's exact test. *p* values of < 0.05 were considered statistically significant. The effect of *Aspergillus* on the change in lung function over time was investigated using repeated measures analysis of variance (ANOVA). Greenhouse-Geisser correction was used when the sphericity assumption was violated.

3 | RESULTS

3.1 | Characteristics of the study population

We included 23 pwCF in the case group and 20 pwCF in the control group. The general characteristics of the study population at baseline are shown in Table 1. The baseline characteristics (age at study and CF diagnosis, sex, CFTR mutations, presence of bronchiectasis, colonization pathogens, chronic CF therapies, CFTR modulators, inhaled glucocorticoid use in the 3 months prior, pulmonary exacerbations in the last year, BMI, and lung function parameters) were similar between the case and control groups. Ten of 23 children (43.4%) in the case group and 8 of 20 (40%) in the control group were colonized by *Pseudomonas aeruginosa* ($p = 0.818$). No participants died or underwent transplantation during the study period. None of the participants had CF-related diabetes.

The case group consisted of 23 pwCF in whom *Aspergillus* had been isolated; 11 (47.8%) of them were diagnosed as having *Aspergillus* colonization, nine (39.1%) had *Aspergillus* bronchitis, and three (13%) had ABPA. None of the patients had *Aspergillus* sensitization. *A. fumigatus* was the most commonly isolated species in our case group at a frequency of 65.2% (15/23). *A. niger*, *A. terreus*, and *A. flavus* were found in the respiratory specimens of three (13%), three (13%), and two (8.7%) patients, respectively. Seventeen percent (4/23) of the patients with *Aspergillus* isolation had positive symptoms at the time of *Aspergillus* detection. Two were diagnosed

TABLE 1 Baseline characteristics of the *Aspergillus* infected (case) and *Aspergillus* noninfected (control) groups.

	<i>Aspergillus</i> infected group (n = 23)	<i>Aspergillus</i> noninfected group (n = 20)	p Value
Number of females, n (%)	18 (78.3)	14 (70)	0.536
Age at study (years), mean (±SD)	14.03 ± 3.22	13.9 ± 3.17	0.966
Age at diagnosis (months), median (IQR)	3 (2–5)	3.2 (2.0–10.7)	0.473
Genotype, n (%)			
F508del/F508del	6 (26.0)	7 (35.0)	0.192
F508 del heterozygous	2 (8.6)	5 (25.0)	
Other/unknown	15 (65.2)	8 (40.0)	
BMI z -score, mean (±SD)	−0.15 ± 1.09	−0.26 ± 1.18	0.757
Pulmonary function tests, mean (±SD)			
FEV1 z-score	−1.46 ± 2.45	−1.28 ± 2.5	0.814
FVC z-score	−1.43 ± 2.01	−1.39 ± 1.84	0.951
Pancreatic insufficiency, n (%)	22 (95.6)	16 (80.0)	0.167
Chronic CF therapies, n (%)			
Dornase alfa	22 (95.6)	19 (95.0)	>0.999
Hypertonic saline	9 (39.1)	4 (20.0)	0.173
Mannitol	0 (0)	1 (5)	0.465
Azithromycin	1 (4.3)	1 (5)	>0.999
Inhaled antibiotics	10 (43.4)	5 (25)	0.205
CFTR modulators used during the study period, n (%)	4 (17.3)	1 (5)	0.351
Inhaled glucocorticoid use in 3 months prior, n (%)	3 (13.0)	0 (0)	0.236
Chronic infection, n (%)			
Methicillin-sensitive <i>Staphylococcus aureus</i>	11 (47.8)	10 (50.0)	0.887
Methicillin-resistant <i>Staphylococcus aureus</i>	7 (30.4)	2 (10.0)	0.142
<i>Pseudomonas aeruginosa</i>	10 (43.4)	8 (40.0)	0.818

Abbreviations: BMI, body mass index; CF, cystic fibrosis; CFTR, CF transmembrane regulator; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

as having ABPA, one had *Aspergillus* colonization, and one had *Aspergillus* bronchitis. There was no significant difference between the *Aspergillus* disease groups regarding symptoms at *Aspergillus* isolation ($p = 0.054$) (Table 2).

3.2 | Laboratory and immunologic parameters

At the sixth month evaluation of the case group, among the 23 tested patients, 14 (60.8%) had no *Aspergillus*, six (26%) had *A. fumigatus*, one (4%) had *A. niger*, and two (8%) had *A. terreus* growth in their respiratory specimens. In the first-year evaluation of the case group, among the 23 tested patients, 18 (78%) had no *Aspergillus*, four (17%)

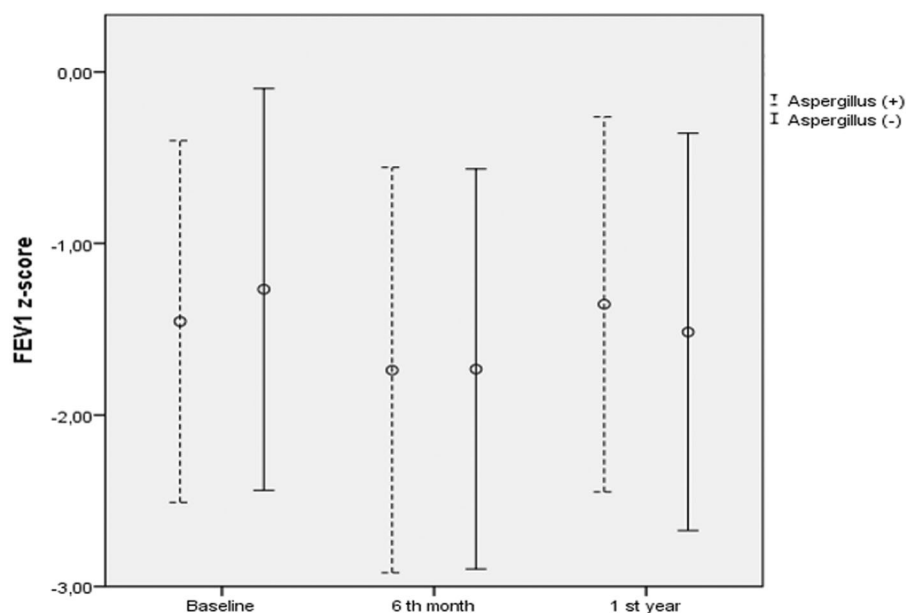
had *A. fumigatus*, and one (4%) had *A. flavus* growth in the respiratory specimens.

There was no significant difference between the case and control groups regarding blood galactomannan ($p > 0.999$). There was no significant difference between the *Aspergillus* phenotypes groups regarding blood *Aspergillus*-IgG ($p = 0.443$), *Aspergillus* PCR, galactomannan ($p = 0.443$), and sputum *Aspergillus* PCR ($p = 0.676$). There was a significant difference between the clinical *Aspergillus* phenotypes originating from ABPA in terms of blood *Aspergillus*-IgE ($p = 0.001$), total IgE greater than 500 IU/mL ($p < 0.001$), and skin prick test reactivity to *Aspergillus* antigen ($p = 0.002$). The sputum galactomannan test was positive in all patients with bronchitis or ABPA and this finding was significantly different from that in patients

TABLE 2 Laboratory and immunologic parameters of CF *Aspergillus* disease.

	<i>Aspergillus</i> colonization (n = 11)	<i>Aspergillus</i> bronchitis (n = 9)	ABPA (n = 3)	p Value
Positive blood sample, n (%)				
<i>Aspergillus</i> -IgG	0 (0)	1 (11.1)	0 (0)	0.443
<i>Aspergillus</i> -IgE	0 (0)	6 (66.6)	3 (100)	0.001^a
<i>Aspergillus</i> PCR	0 (0)	0 (0)	0 (0)	-
Galactomannan	0 (0)	1 (11.1)	0 (0)	0.443
Total IgE (>500 IU/mL)	0 (0)	0 (0)	3 (100)	<0.001^a
Sputum sample, n (%)				
Galactomannan	0 (0)	9 (100)	3 (100)	<0.001^a
<i>Aspergillus</i> PCR	2 (18.1)	1 (11.1)	1 (33.3)	0.676
Skin prick test reactivity to <i>Aspergillus</i> antigen, n (%)	0 (0)	3 (33.3)	3 (100)	0.002^a

Abbreviation: ABPA, allergic bronchopulmonary aspergillosis.

^aStatistical significance was between colonization and ABPA groups.**FIGURE 1** Forced expiratory volume in 1 s (FEV1) z-score change over time for *Aspergillus* infected and noninfected patients.

who were colonized ($p < 0.001$). Laboratory and immunologic parameters according to CF *Aspergillus* disease are shown in Table 2.

3.3 | Lung function and pulmonary exacerbations

Baseline z-scores of FEV1 and FVC did not differ between the case and control groups. Similarly, z-scores of FEV1 and FVC did not differ significantly between the two groups over time, including baseline, at the sixth month, and then by the end of the first year ($p = 0.612$ and $p = 0.939$, respectively). When repeated measures for the FEV1-z score were adjusted for covariates, including CFTR modulator therapy and pulmonary exacerbation, we found no significant

difference over time ($p = 0.121$ and $p = 0.604$, respectively). In addition, the median FEV1% decline in 1 year (shown as Δ FEV1%) did not differ significantly between the case and control groups (0.0%/year vs. -4.7%/year, $p = 0.626$). The change of FEV1 z-scores over time for *Aspergillus* infected and noninfected pwCF is shown in Figure 1. The median cumulative number of pulmonary exacerbations by the end of the 1 year did not differ in the *Aspergillus* infected and noninfected groups (1 [0–4] vs. 0.5 [0–3], $p = 0.591$). We found no significant difference in exacerbation frequency between the case and control groups ($p = 0.147$) (Table 3).

Regarding baseline and intertemporal follow-up lung function between the *Aspergillus* phenotype groups, there were no significant differences in the z-scores of FEV1 and FVC values at the end of the

TABLE 3 Annual pulmonary exacerbations for *Aspergillus* infected and noninfected patients.

	<i>Aspergillus</i> infected group (n = 23)	<i>Aspergillus</i> noninfected group (n = 20)	p Value
In the prior year of study, median (min-max)	0 (0-2)	0 (0-2)	0.708
In the 1-year follow-up period, median (min-max)	0 (0-2)	0 (0-3)	0.147

TABLE 4 Change in lung functions over time in the study population.

	<i>Aspergillus</i> infected group (n = 23)	<i>Aspergillus</i> uninfected group (n = 20)	<i>p</i> Value	Cystic fibrosis <i>Aspergillus</i> disease			
				Colonization (n = 11)	<i>Aspergillus</i> bronchitis (n = 9)	ABPA (n = 3)	<i>p</i> Value
FEV1 z-score (mean ± SD)							
Baseline	-1.46 ± 2.45	-1.28 ± 2.52	0.612	-2.34 ± 2.12	-0.26 ± 2.61	-1.78 ± 2.14	0.309
Sixth month	-1.67 ± 2.64	-1.68 ± 2.40		-2.16 ± 2.03	-0.86 ± 3.47	-2.80 ± 2.60	
First-year	-1.35 ± 2.52	-1.51 ± 2.47		-2.16 ± 1.97	-0.00 ± 2.77	-2.44 ± 2.52	
FVC z-score (mean ± SD)							
Baseline	-1.43 ± 2.01	-1.39 ± 1.84	0.939	-2.18 ± 1.85	-0.38 ± 2.07	-1.72 ± 1.19	0.287
Sixth month	-1.62 ± 2.22	-1.66 ± 1.96		-2.00 ± 1.67	-1.05 ± 3.17	-2.59 ± 1.35	
First-year	-1.36 ± 2.07	-1.27 ± 1.92		-2.20 ± 1.79	-0.17 ± 2.18	-1.85 ± 1.29	
FEV1 % annual change (median IQR)	0.0 (-12.5 to 10.6)	-4.7 (-12.9 to 8.7)	0.626	-5.8 (-14.4 to 25.6)	0.0 (-2.5 to 9.4)	-11.7 (-12.1 to 0.04)	0.629

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

first year ($p = 0.309$ and $p = 0.287$, respectively). The annual decline in Δ FEV% was numerically greater in the ABPA group compared with the *Aspergillus* colonization and *Aspergillus* bronchitis group, but these did not reach statistical significance ($p = 0.629$). Changes in lung function over time in the study population are shown in Table 4.

Six of 11 children (54.5%) in the *Aspergillus* colonization group were co-colonized with *P. aeruginosa*. Co-colonization with *Aspergillus* and *P. aeruginosa* was not associated with reduced lung function compared with *Aspergillus* alone in terms of z-scores of FEV1 ($p = 0.853$) and Δ FEV1% at the end of the first year ($-9.15\%/year$ vs. $3.7\%/year$, $p > 0.999$). Likewise, the cumulative number of annual pulmonary exacerbations in the *Aspergillus* colonization group with or without *P. aeruginosa* by the end of the year did not reach significance (median: 1 (min:0, max:2)/year vs. median: 1 (min:0, max:4)/year, $p > 0.999$).

4 | DISCUSSION

In this study, we used laboratory and immunologic parameters including sputum galactomannan, blood *Aspergillus*-IgE, total IgE higher than 500 IU/mL, and skin prick test reactivity to *Aspergillus* antigen to distinguish aspergillus phenotypes. We demonstrated that *Aspergillus* infected and noninfected pwCF had similar pulmonary functions including z-scores of FEV1 and FVC and median FEV1% decline by the end of the first year. Although *Aspergillus* phenotypes

had similar z-scores of FEV1 and FVC over time, the FEV1% decline was greater in the ABPA group. The frequency of pulmonary exacerbations in the *Aspergillus*-infected and noninfected groups was similar.

The determination of *Aspergillus* phenotypes allows for the establishment of the natural history of aspergillosis in CF and the management according to different manifestations. In 2013, Baxter et al.²⁵ conducted a study to reclassify aspergillosis in CF via two new methods of *Aspergillus* detection, including sputum galactomannan and real-time PCR in 146 patients, and showed that galactomannan antigen assays in sputum-differentiated patients with simple colonization from those with a more infective spectrum of disease. On the other hand, although sputum *Aspergillus* PCR was detected as positive in ABPA and *Aspergillus* bronchitis, positive or negative test results were obtained in patients with *Aspergillus* colonization. In our study, whilst sputum galactomannan was positive in those with *Aspergillus* bronchitis and ABPA, it was negative in all *Aspergillus* colonized groups. However, *Aspergillus* PCR was positive or negative in all phenotypes such as *Aspergillus* IgG. In addition, serum samples were tested for *Aspergillus* PCR and it was negative in all phenotypes and not directive in the grouping. The CF Foundation Consensus Conference recommended screening total serum IgE levels intermittently and if the level was greater than 500 IU/mL, further testing should be performed for ABPA.²⁶ In our study, all patients with ABPA had IgE levels higher than 500 IU/mL. Elevated specific IgE to *A. fumigatus* and *Aspergillus* skin tests are known not to be specific but

sensitive markers for ABPA.²⁷ Likewise, in this study, even though the *Aspergillus* skin test and blood *Aspergillus*-IgE were positive in some in the *Aspergillus* bronchitis group, it was positive in all patients with ABPA.

Harun et al.¹⁵ analyzed the lung function of 79 pwCF who had positive *Aspergillus* cultures from bronchoalveolar lavage (BAL) samples at age of 5 years and showed that there was no association between positive *Aspergillus* BAL cultures on FEV1% predicted for that age or with lung function decline between ages 5 years and 14 years. Vrankrijker et al.¹⁴ conducted a study to assess the effects of *A. fumigatus* colonization on lung function including 259 pwCF without ABPA and demonstrated that *A. fumigatus* colonization had no negative effect on the course of lung function over 5 years. Similarly, McMahon et al.²⁸ prospectively compared *Aspergillus* colonized and non-colonized groups and revealed no difference in FEV1 decline. Recently, a study from the Swedish CF registry showed that by comparing baseline with 2 years, there was no statistical support for a difference in either FEV1 or FVC decline in patients colonized by *Aspergillus* as compared with noncolonized groups.²⁹ On the other hand, Noni et al.³⁰ showed that patients with *Aspergillus* colonization had significantly lower FEV1% than the noninfected group throughout the 7-year period. Similarly, in another study, pwCF with *Aspergillus* colonization had a significantly lower mean FEV1%.¹² Recently, a study from the German CF registry showed that patients with *Aspergillus* colonization were significantly associated with lower lung function.³¹ In our study, by the end of the first year, both FEV1 and FVC z-scores and FEV1% decline in the *Aspergillus* spp.-infected group were not different from those in the noninfected group. A possible reason why *Aspergillus* phenotypes did not show a different prognosis might be that *Aspergillus* isolation was not detected again in the 1-year follow-up period in our study.

Blomquist et al.²⁹ observed no significant increase in median hospitalization days between *Aspergillus* colonized and non-colonized groups. In contrast, Amin et al.¹² showed that *Aspergillus* infection was an independent risk factor for hospital admissions in pwCF. In our study, the *Aspergillus* spp.-infected group was not different from the noninfected group in terms of hospitalization.

Kraemer et al.¹³ and Fainardi et al.³² demonstrated that pwCF with ABPA had poorer lung function. In our study, there was no difference between the z-scores of FEV1 and FVC values at the end of the first year among clinical *Aspergillus* phenotypes, most likely due to the small sample size. However, the FEV1% decline was greater in the ABPA group.

P. aeruginosa is an important cause of mortality and morbidity in pwCF and is strongly associated with an accelerated decrease in lung function.³³ There is a complex bidirectional relationship between *P. aeruginosa* and *A. fumigatus*.³⁴ Although co-infection of *P. aeruginosa* and *Aspergillus* is expected to result in a worse disease phenotype, conflicting results have been reported in the literature. For example, an Irish registry analysis showed co-colonization with *P. aeruginosa* and *A. fumigatus* was associated with a significant decline in FEV1 as compared with both non-colonized patients and those who were colonized with only *P. aeruginosa*. However, they also showed that co-infection was not associated with increased hospitalization when

compared with both chronic *P. aeruginosa* and *A. fumigatus* alone.³⁵ Conversely, a United Kingdom registry analysis demonstrated that there was no additional impact of *Aspergillus* co-infection on FEV1 within *P. aeruginosa*-infected groups; however, the authors also showed that co-infection of *Aspergillus* and *P. aeruginosa* was associated with a higher IV antibiotic requirement compared with *P. aeruginosa* alone.³⁶ In our study, co-colonization with *Aspergillus* and *P. aeruginosa* was not associated with reduced lung function compared with *Aspergillus* alone in terms of z-scores of FEV1 and change in FEV1% at the end of the first year. Furthermore, the number of pulmonary exacerbations did not differ in the co-colonization group compared with the group without *P. aeruginosa*.

Our study has several limitations. The small sample size and single-center study design limit the generalizability of our results. Although our hospital is the largest CF center in our country,³⁷ the small sample size can be explained by the unexpected coinciding of the study period time with the COVID-19 pandemic, during which outpatient admission was reduced for all patients.³⁸ The lack of evaluating chest imaging is also a limitation of our study because it has been shown that *Aspergillus* spp. is associated with structural lung disease.^{15,39,40} On the other hand, the strength of our study is its prospective longitudinal design.

In conclusion, although *Aspergillus* spp. isolation in pwCF was not associated with decreased lung function and frequent pulmonary exacerbations during the 1-year follow-up, a further decline was seen in the ABPA subgroup. The results of the present study need to be confirmed by larger-scale studies.

AUTHOR CONTRIBUTIONS

Halime Nayir Buyuksahin: Conceptualization; writing – original draft; methodology. **Deniz Dogru:** Conceptualization; writing – original draft; methodology; writing – review and editing. **Nagehan Emiralioglu:** Conceptualization. **Dilber Ademhan Tural:** Conceptualization. **Beste Özsezen:** Validation. **Birce Sunman:** Validation. **Ismail Guzelkas:** Methodology. **Didem Alboga:** Methodology. **Sevtap Arikan-Akdagli:** Writing – review and editing. **Zeynep Saribas:** Writing – review and editing. **Dolunay Gulmez:** Writing – review and editing. **Alpaslan Alp:** Writing – review and editing. **Gülşen Hazirolan:** Writing – review and editing. **Özge Soyer:** Writing – review and editing. **Bülent Enis Sekerel:** Supervision; writing – review and editing. **Gözde Inam:** Methodology. **Ilm Irmak:** Writing – review and editing. **Ebru Damadoglu:** Writing – review and editing. **Fuat Kalyoncu:** Writing – review and editing. **Ebru Yalçın:** Writing – review and editing. **Ugur Ozcelik:** Writing – review and editing. **Nural Kiper:** Writing – review and editing.

ACKNOWLEDGMENTS

This project was funded by Hacettepe University Scientific Research Project Coordination Department (Project number: THD-2020-18554).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Halime Nayir Buyuksahin  <http://orcid.org/0000-0002-6909-7993>

Deniz Dogru  <http://orcid.org/0000-0001-9931-9473>

Nagehan Emiralioğlu  <http://orcid.org/0000-0002-1405-8401>

Dilber Ademhan Tural  <http://orcid.org/0000-0002-0334-6336>

Beste Özsezen  <http://orcid.org/0000-0002-0052-8361>

REFERENCES

- Blanchard AC, Waters VJ. Microbiology of cystic fibrosis airway disease. *Semin Respir Crit Care Med*. 2019;40(6):727-736.
- Poore TS, Meier M, Towler E, et al. Clinical characteristics of people with cystic fibrosis and frequent fungal infection. *Pediatr Pulmonol*. 2022;57(1):152-161.
- Reece E, McClean S, Grealley P, Renwick J. The prevalence of *Aspergillus fumigatus* in early cystic fibrosis disease is underestimated by culture-based diagnostic methods. *J Microbiol Methods*. 2019;164:105683.
- Sunman B, Ademhan Tural D, Ozsezen B, Emiralioğlu N, Yalcin E, Özçelik U. Current approach in the diagnosis and management of allergic bronchopulmonary aspergillosis in children with cystic fibrosis. *Front Pediatr*. 2020;8:582964.
- Chaudhary N, Datta K, Askin FB, Staab JF, Marr KA. Cystic fibrosis transmembrane conductance regulator regulates epithelial cell response to *Aspergillus* and resultant pulmonary inflammation. *Am J Respir Crit Care Med*. 2012;185(3):301-310.
- Felton IC, Simmonds NJ. *Aspergillus* and cystic fibrosis: old disease - new classifications. *Curr Opin Pulm Med*. 2014;20(6):632-638.
- Emiralioğlu N, Doğru D, Doğan Ö, et al. Diverse clinical characteristics of *Aspergillus* growth in patients with cystic fibrosis. *Turk J Pediatr*. 2020;62(4):560-568.
- Jones AM, Horsley A, Denning DW. What is the importance of classifying *Aspergillus* disease in cystic fibrosis patients? *Expert Rev Respir Med*. 2014;8(4):389-392.
- King J, Brunel SF, Warris A. *Aspergillus* infections in cystic fibrosis. *J Infect*. 2016;72(suppl):S50-S55.
- Shoseyov D, Brownlee KG, Conway SP, Kerem E. *Aspergillus* bronchitis in cystic fibrosis. *Chest*. 2006;130(1):222-226.
- Hong G, Desai S, Moss RB, Eschenhagen P, Quon BS, Schwarz C. Clinician variability in the diagnosis and treatment of *aspergillus fumigatus*-related conditions in cystic fibrosis: an international survey. *J Cyst Fibros*. 2022;21(1):136-142.
- Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. *Chest*. 2010;137(1):171-176.
- 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(11):1211-1220.
- de Vrankrijker AMM, van der Ent CK, van Berkhout FT, et al. *Aspergillus fumigatus* colonization in cystic fibrosis: implications for lung function? *Clin Microbiol Infect*. 2011;17(9):1381-1386.
- Harun SN, Wainwright CE, Grimwood K, Hennig S. *Aspergillus* and progression of lung disease in children with cystic fibrosis. *Thorax*. 2019;74(2):125-131.
- Hong G, Alby K, Ng SCW, et al. The presence of *Aspergillus fumigatus* is associated with worse respiratory quality of life in cystic fibrosis. *J Cyst Fibros*. 2020;19(1):125-130.
- Farrell PM, White TB, Ren CL, et al. Diagnosis of cystic fibrosis: consensus guidelines from the cystic fibrosis foundation. *J Pediatr*. 2017;181s:S4-S15.e1.
- Waters V, Ratjen F. Pulmonary exacerbations in children with cystic fibrosis. *Ann Am Thorac Soc*. 2015;12(suppl 2):S200-S206.
- Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319-338.
- Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J*. 2012;40(6):1324-1343.
- Walsh TJ, Hayden RT, Larone DH. *Larone's Medically Important Fungi: A Guide to Identification*. John Wiley & Sons; 2018.
- Guinea J, Verweij PE, Meletiadis J, et al. How to: EUCAST recommendations on the screening procedure E.Def 10.1 for the detection of azole resistance in *Aspergillus fumigatus* isolates using four-well azole-containing agar plates. *Clin Microbiol Infect*. 2019;25(6):681-687.
- Subcommittee on Antifungal Susceptibility Testing of the ESCMID European Committee for Antimicrobial Susceptibility Testing, Rodriguez-Tudela JL, Donnelly JP, et al. EUCAST technical note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds. *Clin Microbiol Infect*. 2008;14:982-984.
- Heinzerling L, Mari A, Bergmann KC, et al. The skin prick test - European standards. *Clin Transl Allergy*. 2013;3(1):3.
- Baxter CG, Dunn G, Jones AM, et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. *J Allergy Clin Immunol*. 2013;132(3):560-566.e10.
- 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.
- Hutcheson PS, Knutsen AP, Rejent AJ, Slavin RG. A 12-year longitudinal study of *Aspergillus* sensitivity in patients with cystic fibrosis. *Chest*. 1996;110(2):363-366.
- McMahon MA, Chotirmall SH, McCullagh B, Branagan P, McElvaney NG, Logan PM. Radiological abnormalities associated with *Aspergillus* colonization in a cystic fibrosis population. *Eur J Radiol*. 2012;81(3):e197-e202.
- Blomquist A, Inghammar M, Al Shakirchi M, et al. Persistent *Aspergillus fumigatus* infection in cystic fibrosis: impact on lung function and role of treatment of asymptomatic colonization-a registry-based case-control study. *BMC Pulm Med*. 2022;22(1):263.
- Noni M, Katelari A, Dimopoulos G, Doudounakis SE, Tzoumaka-Bakoula C, Spoulou V. *Aspergillus fumigatus* chronic colonization and lung function decline in cystic fibrosis may have a two-way relationship. *Eur J Clin Microbiol Infect Dis*. 2015;34(11):2235-2241.
- Düesberg U, Wosniok J, Naehrlich L, Eschenhagen P, Schwarz C. Risk factors for respiratory *Aspergillus fumigatus* in German cystic fibrosis patients and impact on lung function. *Sci Rep*. 2020;10(1):18999.
- Fainardi V, Sodini C, Deolmi M, et al. Clinical impact of *Aspergillus fumigatus* in children with cystic fibrosis. *Microorganisms*. 2022;10(4):739.
- Nayir Buyuksahin H, Yalçın E, Emiralioğlu N, et al. The effect of *Pseudomonas aeruginosa* eradication regimens on chronic colonization and clinical outcomes in pediatric patients with cystic fibrosis. *Pediatr Int*. 2022;64(1):e15249.
- Keown K, Reid A, Moore JE, Taggart CC, Downey DG. Coinfection with *Pseudomonas aeruginosa* and *Aspergillus fumigatus* in cystic fibrosis. *Eur Respir Rev*. 2020;29(158):200011.
- Reece E, Segurado R, Jackson A, McClean S, Renwick J, Grealley P. Co-colonisation with *Aspergillus fumigatus* and *Pseudomonas aeruginosa* is associated with poorer health in cystic fibrosis patients: an Irish registry analysis. *BMC Pulm Med*. 2017;17(1):70.

36. Hughes DA, Archangelidi O, Coates M, et al. Clinical characteristics of *Pseudomonas* and *Aspergillus* co-infected cystic fibrosis patients: a UK registry study. *J Cyst Fibros*. 2022;21(1):129-135.
37. Dogru D, Çakır E, Şişmanlar T, et al. Cystic fibrosis in Turkey: first data from the national registry. *Pediatr Pulmonol*. 2020;55(2):541-548.
38. Nayir Buyuksahin H, Emiralioglu N, Ademhan Tural D, et al. Impact of COVID-19 on pediatric pulmonology healthcare practice. *Pediatr Pulmonol*. 2021;56(9):2811-2817.
39. Breuer O, Schultz A, Garratt LW, et al. *Aspergillus* infections and progression of structural lung disease in children with cystic fibrosis. *Am J Respir Crit Care Med*. 2020;201(6):688-696.
40. Lv Q, Elders BBLJ, Warris A, Caudri D, Ciet P, Tiddens HAWM. *Aspergillus*-related lung disease in people with cystic fibrosis: can

imaging help us to diagnose disease? *Eur Respir Rev*. 2021;30(162):210103.

How to cite this article: Nayir Buyuksahin H, Dogru D, Emiralioglu N, et al. A case-control study of the effects of *Aspergillus* clinical phenotypes on pulmonary functions in patients with cystic fibrosis. *Pediatr Pulmonol*. 2023;1-9. doi:10.1002/ppul.26319