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Clinical implications of fungal isolation from sputum in adult patients with cystic fibrosis

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Background/aim: Cystic fibrosis is an autosomal recessive disease with a defect in mucociliary activity that is characterized by recurrent pulmonary infections. Bacterial agents frequently implicated in airway colonization are *Haemophilus influenzae*, *Staphylococcus* spp., and *Pseudomonas* spp. Fungal isolation from sputum is common in adults. However, growth of fungal agent only in sputum culture in patients with cystic fibrosis is insufficient for the diagnosis of fungal diseases. There is limited data about the clinical significance of fungal isolation in sputum cultures. The aim of the study was to investigate the clinical outcomes andsignificance of fungal isolation from sputum samples in adult CF.

Materials and methods: This retrospective study included patients who have been admitted between October 2017 and January 2019 in an adult cystic fibrosis unit. Patients were grouped according to fungal pathogenicity as; fungal disease group, colonization group, and nonisolated group. The data of the last one year, including demographics, clinical data, laboratory, treatment modalities, results of cultured bacteria and fungus from sputum samples, respiratory function parameters, frequency of exacerbation, and hospitalizationwere compared between groups.

Results: A total of 330 sputum samples from 88 adult patients with CF were collected. Patients were divided into 3 groups, the fungal disease group (n = 10, 11.4%), colonization group (n = 49, 55.7%), and nonisolated group (n = 29, 32.9%). Presence of pulmonary exacerbation, number of admissions to emergency department, and the number of positive cultures for bacteria from sputum were higher in the fungal disease group ($p = 0.03$, $p = 0.01$ and $p < 0.001$). The fungal disease group had higher rate of antibiotics by parenteral routethan other groups ($p = 0.001$) whereas lung functions were similar. Use of nutritional supplementation and parenteral antibiotherapy were the factors associated with elevated risk of fungal isolation.

Conclusion: Frequent use of parenteral antibiotics and use of nutritional supplementation were found to be independent risk factors for fungal isolation from sputum in adult CF.

Key words: Adult cystic fibrosis, sputum, fungi

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive multisystemic disease characterized by defective chloride (Cl^-) channel transport and frequent respiratory infections leading to bronchiectasis and bacterial airway colonization later in life. Over the past years, novel therapeutic agents have been developed altering the natural course of the disease and prolonging mean life expectancy of these patients. This improvement in life expectancy has brought an ever growing population of CF patients surviving into adulthood [1,2]. The most important factors that determine the prognosis, mortality, and morbidity of this debilitating disease are chronic bacterial airway infections and respiratory failure secondary to acute exacerbations. Bacterial agents most frequently implicated in airway colonization and infection are *Haemophilus influenzae*, *Staphylococcus* spp. and *Pseudomonas* spp. Previously, several studies have been conducted to shed

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light on the effects of bacterial infections on disease progression [3]. Frequent antibiotic and inhaled steroid use may predispose to orofarengeal and airway fungal colonizations [3–5]. Isolation of fungal species is more common among adult CF patients owing to bronchiectasis and airway colonization in this group that emanates from chronic pulmonary infections [6,7]. *Aspergillus* spp. and *Candida* spp. have high prevalence in adult CF patients (8). *Aspergillus fumigatus* is the most commonly isolated filamentous fungus from adult patients' sputum which varies from 6% to 58% and this variation is explained by forecast different areas, differences in methods of culture and difference in climatic conditions [9,10]. The prevalence of allergic bronchopulmonary aspergillosis (ABPA) is higher in adulthood than childhood (10.1% vs. 8.9%) [11]. This condition is diagnosed by presence of pulmonary infiltrates on chest radiography, detection of immunoglobulin G (IgG) and immunglobulin E (IgE) antibodies specific for *A. fumigatus*, and elevated total IgE [12]. The clinical significance of isolation of *A. fumigatus* in CF patients' sputum other than ABPA is unknown. Recent studies have shown that ABPA is likely to be a prognostic factor for progression of disease and deterioration of lung function [3,13]. *Candida albicans*, on the other hand, is a common yeast that is isolated from sputum in up to 75% of patients with CF [4,9].The importance of *Candida* isolation is unknown, although limited studies have shown that it is related to worsening of lung functions [14,15]. Limited data exists with regards to fungal species and their potential role in CF lung infections. Moreover, there is lack of sufficient evidence to implicate fungal colonization or infection in CF disease progression and clinical outcomes. It is thought that fungal pathogens will achieve increasing significance as there are important causes associated with CF pulmonary infection or deterioration in lung function [16]. Putting the evidence gap into consideration, the aim of the present study was to investigate clinical characteristics, pulmonary functions, and treatment modalities of adult CF patients that had fungi isolated from their sputum samples, as well asthe clinical significance of fungal isolation.

2. Materials and methods

This retrospective cohort study was conducted in the Department of Chest Diseases, at the Adult Cystic Fibrosis Unitin the University School of Medicine. The study was approved by the local ethics committee of the University of Medical Faculty Hospital (Ankara–Turkey–22.01.2019/03-25). Ethical approval was obtained in accordance with the Helsinki Declaration. Data were collected from patients' files in the hospital database. Due to the retrospective nature of the study, informed consent was not obtained from the patients for the use of medical data for publication purposes with the approval of the local scientific committee. All patients' ID information was kept confidential.

2.1. Patients

Patients who have been admitted between October 2017 and January 2019 were retrospectively evaluated. The patient population of the study consisted of all adult cystic fibrosis patients (ages 18 and older) who were followed at the study center in the study time period. In all patients, the diagnosis of CF was determined by clinical features associated with positive sweat test (chloride> 60 mmol/L) and/or cystic fibrosis transmembrane regulator (CFTR) mutation analysis. Patients who had a history of lung or liver transplantation were excluded. All sputum samples collected from patients during the preceeding year regardless of whether the presence of pulmonary exacerbation were analyzed for fungi. Bronchoscopy was not performed on any patient for sampling and all samples examined were sputum samples.

Patients were grouped according to fungal pathogenicity as; fungal disease group, colonization group, and nonisolated group.

Fungal colonization was defined as the positive fungal species in ≥1 of sputum cultures without clinical and radiological infection signs and/or acute decline in lung functions. Patients with any fungal isolation who have been evaluated for antifungal treatment, and then who received antifungal treatment were defined as the fungal disease group. The nonisolated group included patients with negative fungal cultures during the previous year.

Decision of the antifungal treatments and the duration of these treatments were decided on the patient basis by discussing the clinical, laboratory and radiological characteristics in the multidisciplinary council (consist of department of pediatric pulmonology, adult chest diseases, and infectious diseases).

2.2. Data

The data were collected electronically from the hospital database. Patient demographics, comorbidities, diagnosis age, follow up period (months) at adult CF center, type of mutation, total IgE level, hemoglobin, leukocyte count, renal and liver function parameters, hemoglobin A1c (HgA1c) level, systemic and/or inhaled corticosteroid use, number of antibiotherapy use, route of antibiotherapy administration (parenteral route), presence of history of antibiotherapy(regardless of antibiotherapy duration),number of cultured bacteria and fungus from sputum samples in the previous year, respiratory function parameters, and frequency of exacerbation and hospitalization were recorded from patients' files. The data obtained was compared between groups.

2.3. Isolation of fungal and bacterial strains

For mycological cultures, sputum samples were plated on Sabouraud dextrose agar (SDA) and incubated at 35 ±

2°C and 25 ± 2°C for 7 days. Identification of the isolated fungal strains on mycological or bacteriological media (5% sheep blood agar, MacConkey agar, chocolate agar with bacitracin, mannitol salt agar, and *Burkholderia cepacia* selective agar) at species/species complex level were performed using conventional mycological methods [17].

All samples were cultured according to the standard diagnostic laboratory protocol, which included testing for bacterial organisms that were identified on the basis of macroscopic and microscopic morphology.

2.4. Statistical analysis

The data were collected from the files of the patients and the operating systems of the hospital and analyzed using theSPSS programme v.23.0 (IBM Corp,Armonk, NY, USA). The normality of variables were examined with the Shapiro–Wilk test, boxplots, and Q-Q plots. Descriptive statistics were shown as the median, the 25th, and 75th percentiles as the normality assumption was not satisfied. Furthermore, for continuous variables independent three groups were compared with Kruskal–Wallis variance analysis, while categorical variables were compared with a chi-square or a likelihood ratio test. The univariate logistic regression models were conducted to specify candidate variables in multiple logistic regression. The variables which are significant at $p < 0.25$ were chosen as variables for multiple logistic regression. Backward elimination was performed with those variables. The results of final logistic regression models were represented with odds ratio (OR), 95% of confidence interval and p-value. The level of statistical significance was set at a p-value < 0.05. All reported p-values are 2-sided.

3. Results

During the study period, 88 (97.7%) of the 90 patients admitted to the Adult CF Center were enrolled (Figure) in to the study. Age range of the study population was 18–43 years (median 24), and 39 (44.3%) were female. A total of 330 sputum samples were collected and at least one positive culture for fungi was collected from 159 (48.1%) of them. Patients were divided into 3 groups, the fungal disease group (n = 10, 11.4%), colonization group (n = 49, 55.7%), and nonisolated group (n = 29, 32.9%).

Eighty-four patients (95.5%) have positivity growing with bacterial species and median number of bacterial culture pozitivity was 3 (1–21 range). Fifty-nine patients (67%) had positivity growing with fungal species and median number of fungal culture pozitivity was 2 (1–15 range). Twenty-six (26/59, 44%) of them had fungal growth in only one sputum. Both bacterial and fungal culture positivity were present in 56 (63.6%) patients.

Candida spp. and *Aspergillus* spp. were the most common fungi isolated, corresponding, respectively, to 36 (73%) and 22 (44.9%) in the colonization group, and 8 (80%) and 6 (60%) in the fungal disease group. At species level, *C. albicans* and *A. fumigatus* were the most commonly isolated types (n = 30, 61.25% and n = 21, 42.9%, n = 8, 80%, and n = 5, 50%) (Table 1).

Staphylococcus aureus and *Pseudomonas aeruginosa* were the two most common bacterial species demonstrating no difference between the study groups. The third most common isolate, meticiline resistant *Staphylococcus aureus* (MRSA), had a higher proportion despite a lower number

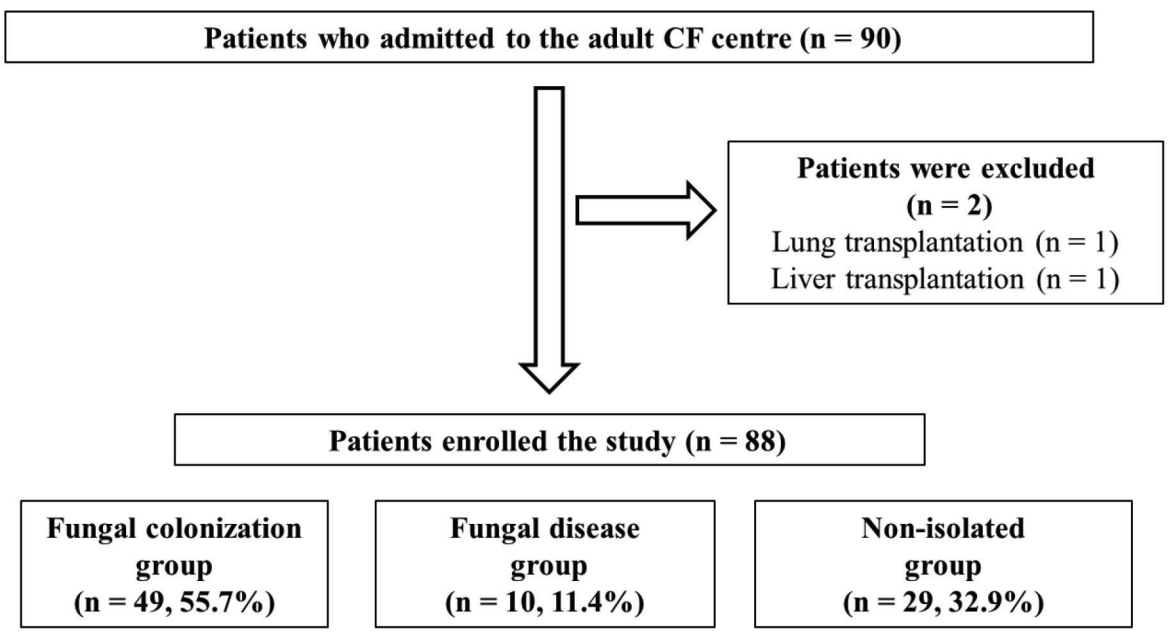

Figure. Flow chart of patients' distribution.

Table 1. Fungal and bacterial species isolated from sputum samples in groups.

	Fungal colonization group (n = 49)	Fungal disease group (n = 10)	Non-isolated group (n = 29)	All Patients (n = 88)	p value
Fungi, n (%)					
Candida spp.	36(73.5)	8(80)	NA	44(50)	0.67#
C.albicans	30(61.2)	8(80)	NA	38(43.2)	0.23#
C.parapsilosis	3(6.1)	0	NA	3(3.4)	0.43#
C.glabrata comlex	4(8.2)	0	NA	4(4.5)	0.04#
C.dubliniensis	4(8.2)	0	NA	4(4.5)	0.04#
Aspergillus spp.	22(44.9)	6(60)	NA	28(31.8)	0.39#
A.fumigatus	21(42.9)	5(50)	NA	26(29.5)	0.68#
A.terreus	2(4.1)	0	NA	2(2.3)	0.52#
A.flavus	2(4.1)	1(10)	NA	3(3.4)	0.44#
A.niger	1(2)	0	NA	1(1.1)	0.65#
Penicillium	7(14.3)	0	NA	7(8)	0.007#
Saccharomyces cerevisea	0	1(10)	NA	1(1.1)	0.34#
Cryptococcus albicus	0	1(10)	NA	1(1.1)	0.34#
Bacterium, n(%)					
Staphylococcus aureus	36(73.5)	9(90)	22(75.9)	67(76.1)	0.53*
Pseudomonas aeruginosa	27(55.1)	7(70)	18(62.1)	52(59.1)	0.63*
MRSA	8(16.3)	4(40)	5(17.2)	17(19.3)	0.21*
Stenotrophomonas maltophilia	2(4.1)	0	5(17.2)	7(8)	0.06*
Burcholderia cepacia	4(8.2)	1(10)	1(3.4)	6(6.8)	0.63*
Acromobacteria	4(8.2)	2(20)	0	6(6.8)	0.04*
Nocardia	1(2)	2(20)	0	3(3.4)	0.04*

Chi-square test.
* Kruskal–Wallis test.
MRSA; methicillin resistant Staphylococcus aureus, NA; not assesment.

in the fungal disease group (n = 4, 40% vs. n = 8, 16.3% and n = 5, 17.2%). Acromobacteria and Nocardia were the least isolated microorganisms, but were the ones more frequently seen in the fungal disease group compared to other groups (Acromobacteria; n = 2, 20%, n =4, 8.2%, n= 0 and Nocardia; n = 2, 20%, n = 1, 2%, n = 0, p=0.04, each).

Table 2 summarizes the demographics of the groups as well as the hospital data, laboratory test results, lung function parameters and treatment characteristics. Both groups had similar age, BMI (body mass index) and comorbidities. ABPA was significantly higher in the fungal disease group than other two groups (n = 3, 30%, n = 2, 4.1% and n = 2, 6.8%, p = 0.01). The presence of exacerbation and number of admission to emergency department in the preceeding year were significantly higher in the fungal disease group (presence of exacerbation; n = 9, 90%, n = 22, 44.9%, n = 9, 31%, p = 0.03 and median number of admission to emergency; 3.5[1–4.25], 1 [1–2],1 [1–2], p = 0.01), whereas both the number of exacerbation and hospitalization were similar between all study groups.

Higher C-reactive protein (CRP) and total IgE levels were seen in the fungal disease group (p = 0.02 and p = 0.03). There was no difference in respiratory function parameters between groups. In mutation analysis, the CFTR locus was examined in 57 (64%) patients. In a total of 114 CF chromosomes, 31 different mutations were identified in 105 (92.1%) of the alleles. The most common mutation was F508del 33 (31.4%), the rate for which was similar between groups.

The number of positive cultures for bacteria from sputum in one year was significantly higher in the fungal disease group (8 [5–13.5], 3 [2–5] and 2 [1–3], p<0.001).

Treatment characteristics and outcomes in both groups are shown in Table 3. Patients in the fungal disease group had higher rate of parenteral antibiotherapy than other groups (n = 8, 80%, n = 17, 34.7% and n = 5, 17.2%, p = 0.001); however, both the number and length of antibiotherapy were similar between groups.Enteral nutrition support using high energy feeding solutions with varying content and amount based on individual need was significantly higher in the colonization group than in the nonisolation group (n = 24, 49%, n = 4, 40% and n = 6, 20.7%, p = 0.04). Other maintenance drug use was similar between groups.

Table 2. Comparison of clinical characteristics of groups.

	Fungal colonization group (n = 49)	Fungal disease group (n = 10)	Non-isolated group (n = 29)	p value*
Sex, n (%) Female	22 (44.9)	6 (60)	11 (37.9)	0.47
Age, median (min-max)	24 (21–27,5)	24,5 (20.75–26.25)	23 (20–25.5)	0.7
BMI, mean (SD)	20 (18.5–22.5)	20.5 (16.75–22.5)	21 (19–25)	0.47
Age of diagnosis (month), median (min-max)	12 (3–144)	18 (2.75–63)	9 (3–150)	0.85
Follow up duration (month), median (min-max)	36 (12–48)	36 (15–51)	36 (12–48)	0.15
Additional diseases, n(%) DM Asthma Hepatobiliary disorder Chronic sinusitis GERD Osteoporosis Pancreatic insufficiency	 6 (12.2) 6 (12.2) 8 (16.3) 10 (20.4) 6 (12.2) 7 (14.3) 45 (91.8)	 2 (20) 4 (40) 7 (70) 0 0 1 (10) 10 (100)	 1 (3.4) 4 (13.8) 9 (31) 4 (13.8) 4 (13.8) 3 (10.3) 28 (96.6)	 0.22 0.13 0.002 0.11 0.27 0.84 0.37
ABPA, n (%)	2 (4.1)	3 (30)	2 (6.8)	0.01
Hospital data				
History of pulmonary exacerbation, n (%)	22 (44.9)	9 (90)	9 (31)	0.03
Pulmonary exacerbation(≥2), n (%)	8 (53.3)	4 (66.7)	4 (21.1)	0.05
Hospitalization/year, median (min-max)	1 (1–2)	1 (1–3)	1 (1–2.5)	0.95
Admission to emergency department/year, median (min-max)	1 (1–2)	3.5 (1–4.25)	1 (1–2)	0.01
Laboratory and respiratory function parameters, median (min-max)				
Eosinophil (%)	1.6 (1.15 –2.5)	1.35 (0.6–215)	1.3 (0.65–2.15)	0.38
Hemoglobin (g/dL)	14 (13–15)	13.4 (12.7–14.2)	14.8 (13.8–15.8)	0.05
BUN (mg/dL)	12.2 (9.9–15.2)	10.15 (7.34–14.55)	12.4 (10.15–16)	0.2
Creatinin (mg/dL)	0.67 (0.56–0.73)	0.5 (0.41–0.74)	0.66 (0.54–0.76)	0.22
AST (IU/mL)	20 (17–26.5)	18.5 (17–21.75)	22 (19–26.5)	0.11
ALT (IU/mL)	18 (13–26.5)	15.5 (10.75–20.75)	20 (15.5–27.5)	0.21
Total bilirubin (mg/dL)	0.5 (0.38–0.68)	0.41 (0.35–1.71)	0.48 (0.4–0.68)	0.63
CRP (µg/dL)	0.89 (0.4–2.29)	1.73 (1.41–5.47)	1 (0.45–2.2)	0.02
Total IgE (µg/mL)	50.4 (15.05–153)	156 (74.6–454)	58.5 (18.5–144)	0.03
HgA1c (%)	5.6 (5.1–6.1)	5.8 (5.3–6.5)	5.7 (5.3–6)	0.31
FEV1 (%)	60 (39.5–85.5)	47.5 (32.75–82.25)	72 (43–88)	0.36
FEV1/FVC ratio	70 (62.5–77.5)	82.5 (63.25–93.25)	70 (65–81.5)	0.21
Positive bacterial cultures/year, median (min-max)	3 (2–5)	8 (5–13.5)	2 (1–3)	0.00

* Kruskal–Wallis test

BMI; body mass ındex, CRP; C-reactive protein, AST; aspartate aminotransferase, ALT; alanin aminotransferase, GERD; gastroesophageal reflux disease, ABPA; allergic bronchopulmonary aspergillosis, BUN; blood urea nitrogen, HgA1c; hemoglobin A1c, FEV1; forced expiratory volume in 1 s, FVC; forced vital capacsity.

Multivariate logistic regression identified nutritional supplementation (CI: 1.17–11.96, p = 0.01), parenteral antibiotherapy (CI: 1.27–12.63, p = 0.02) in the preceeding one year as factors that significantly elevated risk of fungal isolation in patients who had fungal growth on culture in comparison to those without any fungal isolation (Table 4).

Table 3. Treatment characteristics.

	Fungal colonization group (n = 49)	Fungal disease group (n = 10)	Non-isolated group (n = 29)	p value*
Antibiotherapy/year, median (min-max)	1 (1–3)	2 (2–4)	1.5 (1–2.25)	0.08
Parenteral antibiotherapy, n (%)	17 (34.7)	8 (80)	5 (17.2)	0.001
Maintenance drug use, n (%)				
Dornase α	43 (87.8)	10 (100)	23 (79.3)	0.12
Inhaled colistin	3 (6.1)	1 (10)	1 (3.4)	0.73
Inhaled bronchodilatator	18 (36.7)	5 (50)	10 (34.5)	0.67
Inhaled corticosteroid	3 (6.1)	0	2 (6.9)	0.53
ICS+LABA	11 (22.4)	7 (70)	5 (17.2)	0.68
Inhaled mannitol	4 (8.2)	1 (10)	0	0.12
Inhaled antibiotic	20 (40.8)	6 (60)	15 (51.7)	0.43
Nutritional supplementation	24 (49)	4 (40)	6 (20.7)	0.04
Multivitamin	40 (81.6)	9 (90)	19 (65.5)	0.15

* Kruskal–Wallis test.
ICS+LABA;inhaled corticosteroid and long acting beta2 agonist combination.

Table 4. Multivariate logistic regression analysis: comparison of patients with fungus isolated (colonization/disease) and non-isolated group.

Risk factor	Beta	Standard error	Odds ratio	Confidence interval (%95)	p value
Use of parenteral antibiotics	1.38	0.57	3.86	[1.27–12.63]	0.02
Nutritional supplementation	1.36	0.54	3.75	[1.17–11.96]	0.01
Hemoglobin	–0.46	0.19	0.70	[0.43–0.91]	0.10
FEV1	–0.002	0.01	0.99	[0.70–1.24]	0.55

FEV1; forced expiratory volume in 1 s.

4. Discussion

This study is the first to investigate the effects of sputum fungal isolation on clinical characteristics of adult patients with CF from a tertiary care center in Turkey. The primary finding was that several clinical and laboratory variables were related to fungal infection, including history of exacerbation, admission to emergency department with acute clinical deterioration, ABPA, number of positive cultures for bacteria, and history of parenteral antibiotherapy use in the preceedingyear. While the presence of fungi in the sputum of CF patients had generally been considered clinically of less important during the past decade, recent availability of improved culture methods lead to more frequent isolation of fungal species from sputum samples, and they are increasingly coming to the attention of clinicians [8,10,16]. Recent studies have pointed to the evidence that fungal colonization increases with age. This may be explained by association of severe disease with exacerbations secondary to bacterial infectionsthat require antibiotic use [3,7,18–20]. However, growth of fungal agent only in sputum culture in CF patients is not sufficient for the diagnosis of fungal diseases. In the past decade, Chotirmall et al. presented that *C. albicans* colonization is related with increased exacerbations requiring inpatient treatment and FEV1 decline. In the same study, isolation of *Candida spp.* from the first sputum sample was linked to a subsequent high rate of hospitalization which was independent of the frequency of antibiotherapy or exacerbation rates [14]. A retrospective clinical study showed that *Aspergillus* isolated from airways was associated with an increased risk of pulmonary exacerbations requiring hospital admission and with a lower FEV of 1%. The authors of this study also highlighted that the presence of Aspergillus was an independent risk factor for hospitalization [13]. In the same study, majority of fungal infections caused by *Aspergillus* and *Candida* were associated with presence of pulmonary exacerbation and emergency admissions as reported by

previous studies albeit there was no association for fungal colonization. Fungal isolation was clinically inclined to, although not associated with exacerbation frequency. Fungal colonization or fungal infection did not affect the number of hospitalizations during the preceeding year.

In our study population, there was no association between lung function and either fungal colonization or disease. Current evidence is conflicting in that, while several studies support our findings [5,10,19,21] others report an association with lower FEV1 [13–15,22]. In a cross sectional study by Milla et al., there was no association between *A. fumigatus* colonization and lung function. These findings have been shown to be due to demographic characteristics such as age and sex, which were related to disease severity [7]. A recent systematic review reported that two studies [23,24] found significant association with worse FEV1 in patients with CF and impaired glucose tolerance [25]. In our study, presence of CF related diabetes mellitus and HgA1c levels were similar between the study groups. When these results are taken into consideration, our findings are not significant and this can be explained by demographic characteristics and other confounder factors affecting FEV1.

ABPA, a hypersensitivity reaction to *A. fumigatus* antigens and/or *Aspergillus* isolated from sputum, is an important complication of CF leading to deterioration of lung function [22]. There is no certain recommendation about antifungal therapy in CF patients with ABPA. Although it has been indicated an advantageous approach that it may lead to reduced doses of corticosteroids, comprehensive randomized controlled studies are needed[26].Among our 7 patients diagnosed with ABPA, *A. fumigatus* have been isolated in 2 of them, *C. albicans*in 2, and both fungi in 2 patients, and no fungal isolation receiving in 2 patients. One of the them received systemic steroid and four received antifungal treatment.

The most important causative agents of respiratory exacerbations in CF patients, who are known to have rich diversity of fungal and bacterial microorganisms, are bacterial organisms, especially *Pseudomonas* species in adults [27]. Although there are opposing views [28–30] such as bacterial species suppressing fungal growth in culture, it is known that the use of broad spectrum antibiotics is a predisposing risk factor that potantiatesfungal pathogenicity in these patients [22,31]. In our study, the positive correlation between the number of positive cultures for bacteria from sputum samples and fungal infection is in agreement with this observation.

Staphylococcus aureus and *Pseudomonas aeruginosa* were the two most common species isolated from the sputum of patients, being recovered from 76.1% and 59.1% of the patients, respectively. This prevalence rates are similar to other reports but more studies reported *Pseudomonas* as the most commonly isolated species in adult CF patients [9,32]. The equilibrium between lung microbioma may be impaired in patients from whom *P. aeruginosa* is isolated, resulting in a spiralling of fungal isolation, as reported by some authors. On the other hand, a number of studies suggested the use of wide spectrum antibiotics for bacterial infections. This approach may explain this finding and that fungal colonization/ infection risk is reduced with antipseudomonal antibiotic use [33]. At this point, the question arises whether it is bacterial coinfection or suppression of bacterial growth by antibiotics that explains the excess in fungal isolation. The answer explaining this cause-effect relationship, thus far, remains elusive. In our study, isolation of MRSA and *P. aeruginosa* and parenteral antibiotic use were in excess in the fungal disease group. However, it is not clear whether the decision on starting antifungal treatment was influenced by high rate of exacerbation and bacterial isolation in this group. In other words, it is not clear which came first, the chicken or the egg?

Among isolated fungi, *Candida* and *Aspergillus* showed the highest prevalance in patients, representing 50 and 31%, respectively. The most frequently isolated *Candida* species when all patient groups are taken into consideration were *C. albicans* (43%), *C. glabratacomplex* (4.5%), *C. dubliniensis* (4.5%), and *C. parapsilosis* (3.4%). While *A. fumigatus* (29.5%), *A. flavus* (3.4%), *A. terreus* (2.3%), and *A. niger* (1.1%) comprised the most common *Aspergillus* species. This distribution is similar to findings in several other studies [4,9,10,35].

CF patients frequently receive antibacterial agents through several routes of administrations including orally, parenterally and via aerosols for the treatment of exacerbations and bacterial eradication. Various studies conducted have reported different results regarding the treatment characteristics and effects on fungal pathogenicity. To our knowledge, one extensive study was conducted in the form of a retrospective cohort study comprising 16,096 patients from the CF Foundation Patient Registry with a follow-up period of 6 years. This study reported that chronic therapies, including inhaled, oral and intravenous antibiotics, macrolides, and inhaled corticosteroids were also associated with an increased risk of persistent Aspergillus isolation. However, when applied to the adult subpopulation, only oral steroid use become a significant risk factor for persistant *Aspergillus*[22]. In a large multicenter (69 centers) double-blinded placebo-controlled study incoorporating 595 CF patients, inhaled tobramycin users had a significantly high rate of *Aspergillus* isolation [35].Another study by Bargon et al. on adult CF patients, found that prolonged oral and inhaler antibiotic treatment as well as administration via both routes predisposed to *Aspergillus* colonization, a

finding that was later reproduced by Vrankrijker et al. in a cohort study that also included adult patients [10,19]. Factors affecting *Candida* colonization examined by Noni et al., revealed the independent effect of the duration of both inhaled antibiotic and inhaled corticosteroid use on *Candida* colonization, but they did not find an association with annual intravenous antibiotic courses [5]. In contrast, a retrospective study from France found that the number of antibiotic use via oral, inhaled or intravenous routes in 1and 5 years had no effect on *Aspergillus* spp. isolation [36]. Similarly, a longitudinal study concluded there is no association between recurrent broad spectrum antibiotherapy and *Candida* colonization [37]. On the other hand, a prospective observational study of 26 adult patients with CF reported that the use of short term intravenous antibiotics targeting *Pseudomonas* reduced the presence of *Aspergillus* in sputum [33]. Our current study concluded that antibiotic use via intravenous route was associated with fungal disease but not fungal colonization. Multivariate analysis confirmed that intravenous route of administration may both be predictive risk factors for fungal colonization increasing fungal pathogenicity risk approximately three-fold. Our results with results of previous studies, showed that duration and fequency of antibiotic use, oral or inhaled, did not affect either colonization or isolation of fungus [22,36]. Maintanence use of inhaled steroids, bronchodilators or a combination of both were not associated with fungal colonization or infection in the adult population.

Increased energy needs due to chronic pulmonary infections and excessive respiratory effort as well as insufficient energy intake and malabsorbtion are important causes of malnutrition in CF patients [38]. A recent study indicated that poor nutritional status was an independent risk factor for ABPA [36]. Prior studies have shown that comorbidities such as liver failure and inadequate nutrition are often associated with fungal airway colonization [39,40]. In accordance with this, we demonstrated that fungal isolation was associated with nutritional supplementation. Nutritional support in CF patients may be a predictor of fungal colonization. At our center, enteral supplementation with high-energy adult feeding solutions to cover daily nutritional requirements in excess of 20% is afforded to CF patients in need. Additionaly, early enteral nutrition support is provided to all patients with inadequate oral intake, even if they have normal BMI. This may explain the observed lack of association between BMI and fungal isolation. A recent retrospective study that included 257 patients identified concominant fungal and bacterial colonization to be independently associated with liver disease in CF patients [41]. In this study, fungal colonization was significantly higher in patients with hepatobiliary disease as opposed to other comorbidities. However, there are limitied studies and more epidemiological research is needed to investigate pathogens and to clarify the predisposing factors.

One important limitation of this study is that delineating fungal colonization and fungal disease is challenging. Whereas previous studies defined fungal colonization as the presence of fungal species in ≥50% of sputum cultures collected in one year independent of clinical findings, in our study, we used the term to refer to fungal growth not associated with any findings suggestive of infection. This approach may have accounted for the discrepancy between our results and those reported by other authors. Another restriction of our study was the fact that ABPA patients were included in our study because fungal isolation is not a mandatory criterion for ABPA diagnosis. As such, ABPA and elevated IgE levels were more common in the fungal disease group. Being a single-center study on a homogenous population obviously had implications on the rate of fungal isolation since this is also affected by ethnic and regional factors. However, ours is the first established center that deals with adult CF patients that has the largest number of patients in Turkey, the number of which is expected to grow up in coming years. Lastly, the sample size was insufficient for the evaluation of the distribution of certain variables betweenstudy groups and the data of antifungal treatments could not be evaluated due to the retrospective nature of the study.

In conclusion, frequent use of parenteral antibiotics and use of enteral feeding supplements were found to be independent risk factors for fungal colonization or disease in adult CF patients. It seems that antibiotic use leads to fungal overgrowth; however, it is not clear which patients would benefit from antifungal treatment. Studies including larger patient population and expanded data on antifungal treatment outcomes are needed to clearly understand the clinical importance of fungal isolation.

Conflict of interest
No financial support has been taken. All authors declared that there is no conflict of interest.

Informed consent
The committee from whom received ethical approval, did not deem the patient's informed consent necessary since the study was retrospective.

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