Role of comorbidities in acquiring pulmonary fungal infection in chronic obstructive pulmonary disease patients

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Background Bacteria and viruses have been implicated as a major cause of chronic obstructive pulmonary disease (COPD) exacerbations; however, the potential role of fungal colonization and infection is poorly understood.

Objective The aim of this study was to assess the profile of pulmonary fungal infection among COPD patients with and without comorbidities to determine their prevalence, risk factors, and outcome among those patients.

Patients and methods In this prospective cross-sectional analytic study, different samples (sputum, bronchoalveolar lavage, blood, and others) from 177 COPD patients at risk for pulmonary fungal infection were examined using mycological analysis (direct microscopy and culture). Bronchoalveolar lavage and blood samples were examined using the human 1,3-β-D-glucan and galactomannan ELISA tests.

Results The prevalence of pulmonary fungal infection was significantly higher in COPD patients with comorbidities (77.8%) versus COPD patients without comorbidities (53.1%) ($P < 0.001$), with a predominance of *Candida* and *Aspergillus* spp. in both groups. Mechanical ventilation, corticosteroid therapy, ICU admission, and age were major risk factors for pulmonary fungal infection in COPD patients with comorbidities [$P = 0.012$, odds ratio (ODR) = 2.23; $P = 0.028$, ODR = 1.99; $P = 0.025$, ODR = 1.94; and $P = 0.034$, ODR = 2.60; respectively]. COPD patients with comorbidities had significantly higher mortality rate (12.3%) compared with COPD patients without comorbidities (3.1%; $P < 0.05$). Blood galactomannan antigen was positive in 16 (19.7%) COPD patients with comorbidities versus seven (7.3%) in COPD patients without comorbidities ($P < 0.05$).

Conclusion COPD patients with comorbidities had a higher prevalence of pulmonary fungal infection and higher mortality rate compared with COPD patients without comorbidities. Age, mechanical ventilation, corticosteroid therapy, and ICU admission were independent risk factors for pulmonary fungal infection in COPD patients with comorbidities.

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Introduction

Comorbidities frequently impact chronic obstructive pulmonary disease (COPD) patients and significantly affect the patients' survival, quality of life, and exacerbation frequency [1]. It is believed that chronic inflammatory state in COPD may accelerate the natural history of some comorbidities, and hence COPD is considered as a systemic disorder [2].

Airways of COPD patients are often colonized with potential pathogenic microorganisms [3] and may lead to increased airway inflammation [4]. The potential role of fungal colonization and infection in the pathogenesis of COPD is poorly understood as bacteria and viruses were usually considered as the major cause of COPD exacerbations. *Aspergillus* spp. is the most common fungal genus to cause pulmonary-associated fungal infections in COPD patients [5].

The primary goal of this study was to screen COPD patients with comorbidities and COPD without comorbidities for microbiological and serological pieces of evidence for pulmonary fungal infection to determine the prevalence of pulmonary fungal infection among those patients. The second goal was to identify the risk factor for pulmonary function infection among those patients. The third goal was to investigate the frequency of positive fungal culture and the clinical outcomes among those patients.

Patients and methods

Study design and ethics

This prospective cross-sectional analytic study included 177 COPD patients at risk for pulmonary fungal infection who were admitted in the Chest Department and Respiratory Intensive Care Unit during the period from January 2013 to March 2015. The study was approved by the Faculty of Medicine Ethics Committee, Assiut University. After meeting the inclusion criteria, informed

consent was obtained from all study participants or next of kin according the clinical condition of the patients.

Patients

COPD patients who were eligible for enrollment displayed a combination of the following host factors:.

(1) A history of pre-existing COPD and immunosuppression from corticosteroids or other underlying conditions (e.g. diabetes, malnutrition, and liver cirrhosis).
(2) Clinical signs and/or symptoms suggestive of invasive mycosis. The European Organization of the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria for the diagnosis of invasive fungal infection (IFI) was used, which comprised major and minor clinical criteria. The major clinical criterion was the presence of any of the following new infiltrates on computed tomography (CT) imaging: halo sign, air-crescent sign, or cavity within area of consolidation (in the absence of infection by organisms that may lead to similar radiological findings including cavitation, such as *Mycobacterium*, *Legionella*, and *Nocardia* spp.). The minor clinical criteria were as follows: symptoms of lower respiratory tract infection; cough, chest pain, hemoptysis, or dyspnea; physical finding of pleural rub; any new infiltrate not fulfilling the major criterion; pleural effusion; and worsening of respiratory insufficiency despite appropriate respiratory therapy and ventilatory support [6].
(3) One of the following symptoms of lower respiratory tract infection: new sputum secretions, dyspnea, or hemoptysis; pleuritic chest pain; or physical finding of pleural rub in the background of host factor and microbiological criteria.
(4) Fever refractory to at least 3 days of appropriate antibiotics, or fever relapsing after a period of defervescence of at least 48 h while still receiving antibiotics.
(5) Development of new pulmonary infiltrates on chest radiograph.
(6) Any of the following new infiltrates on CT imaging: halo sign, air-crescent sign, or cavity within area of consolidation.
(7) Steroid use: at least 4 mg of methylprednisolone (or equivalent) a day for at least 7 days in the past 3 weeks before admission or during the course of the ICU stay for at least 5 days, or a cumulative dose of at least 250 mg of methylprednisolone (or equivalent) in the past 3 months before enrollment [7,8].
(8) Recipient of any other immunosuppressive treatment.

Exclusion criteria included patients who received systemic antifungal therapy within 3 days before sample collection and patients who refused to participate in the study.

Baseline data

All patients were subjected to clinical and routine laboratory investigations. Radiology, including plain chest radiograph, and high resolution CT of the chest were performed for patients.

Source of the specimen and method of examination according to the site of the lesion involved the following: direct microscopic examination of sputum samples, bronchoalveolar lavage (BAL) samples, and sterile catheter samples; percutaneous ultrasound-guided tissue biopsy; thoracotomy biopsy (if thoracotomy had been done for another cause); culture examination of pleural fluid and blood samples [9]. Sabouraud's glucose agar was routinely used. HiChrome agar was used for the identification of some species of *Candida*. Serologic diagnosis was carried out using the human 1,3-β-D-glycosidase [10] and human galactomannan (GM) ELISA tests [6] of blood and BAL samples.

Procedures

Collection of sputum samples from the patients according to universal precautions [9]

Sputum samples were collected by instructing the patient to cough as deep as possible to expectorate about 5–10 ml in a sterile container, usually early in the morning.

Specimen transport/storage: Each specimen was individually collected in a sealed plastic bag with proper legible labeling in sterile containers and sent to the Mycological Center at the Faculty of Science, Assiut University, for mycological examination within 4 h after collection, or if it was stored in the refrigerator at 4°C it was sent within 12–24 h.

Bronchoalveolar lavage

BAL sample was collected under complete aseptic conditions according to the BTS guideline (2013) [11] with the help of a white light flexible bronchoscope (Pentax Medical FB 18 V G11456; Tokyo, Japan) attached to a light source (Pentax Medical LH-15011; Tokyo, Japan) and a digital camera. Selection of the site for collection of BAL fluid was guided by prior imaging studies and

determination of the disease site. As stated by Jourdain *et al.* [12], no end bronchial suction was performed during the advancement of the bronchoscope to avoid contamination with upper airway flora and the first two samples were discarded. BAL fluid was collected in a sterile container and transported immediately to the mycology laboratory. Serum samples were also collected from the same patients and stored at −20°C for serological analysis. Equipments were used according to BTS guideline (2013) [11] for diagnostic flexible bronchoscopy in adults, including flexible bronchoscope, sterile collection trap, suction tubing, sterile saline, vacuum source, syringe 50 ml, swivel connector, lidocaine 1–2%, supplemental oxygen and monitoring equipment, ECG, pulse-oximetry, and blood pressure cuff. Moreover, we used premedication with bronchodilators and/or warm saline solution for those at risk for bronchospasm.

Serum

Blood samples were collected according to standard laboratory procedures. Serum samples must be uncontaminated with fungal spores and/or bacteria. The samples were transported and stored in sealed tubes [13], unexposed to air. Thereafter, the collected blood samples were allowed to clot for 2 h at room temperature and centrifuged at 2000–3000 rpm for 20 min to remove suspended solids, and then the supernatant was collected. Collected serum samples were stored at −20°C in the refrigerator until used [14].

Evaluation criteria for the diagnosis of fungal infection

We used the EORTC/MSG diagnostic criteria as the reference standard for case definition of invasive aspergillosis (IA), which were classified as definite, probable, or possible [6].

Statistical analysis

Data were recorded to statistical package for the social sciences (version 20.0; IBM Inc., Armonk, New York, USA). Data were described as mean±SD or frequencies and percentage depending on whether they were quantitative or qualitative, respectively. The χ^2-test and the Fisher exact test was used to compare categorical variables. Comparison of quantitative variables between the study groups was made using the Mann–Whitney *U*-test. Univariate and multivariate logistic regression analysis was performed to test for the effect of all important risk factors on the occurrence of fungal infections. *P* values less than 0.05 were considered significant.

Results

During the study period, 177 COPD patients at risk for pulmonary fungal infection fulfilled the inclusion criteria. There were 81 (46%) COPD patients with comorbidities and 96 (54%) COPD patients without comorbidities. The baseline characteristics of COPD patients with comorbidities versus those without comorbidities are shown in Table 1. There was a statistically significant difference ($P<0.05$) as regards the mean age between the two groups; the mean age in COPD patients with comorbidities was 54.6 ± 8.3 versus 57.5 ± 6.5 in COPD patients without comorbidities. There was no statistically significant difference as regards sex between the two groups. There was a statistically significant difference as regards forced expiratory volume in 1 s (FEV1%) predicted between the two groups ($P<0.05$). COPD patients with comorbidities were associated with lower lung function (FEV1%: 45.5 ± 18.1 vs. 53.8 ± 19.5% predicted in COPD patients without comorbidities).

Table 1 Baseline characteristics of chronic obstructive pulmonary disease patients with comorbidities versus chronic obstructive pulmonary disease patients without comorbidities

Variables	COPD with comorbidities (*n* = 81)	COPD without comorbidities (*n* = 96)	*P*-value
Age	54.6 ± 8.3	57.5 ± 6.5	0.012*
Sex			
Male	54 (66.7)	71 (74)	0.289
Female	27 (33.3)	25 (26)	
Length of hospital stay			
<1 week	26 (32.1)	43 (44.8)	0.085
>1 week	55 (67.9)	53 (55.2)	
FEV1% predicted	45.5 ± 18.1	53.8 ± 19.5	0.022*
GOLD class			
I	3 (3.7)	0 (0)	0.267
II	3 (3.7)	1 (1.04)	
III	21 (25.9)	22 (22.9)	
IV	16 (19.7)	17 (17.7)	

Data expressed as *n* (%) or mean ± SD. COPD, chronic obstructive pulmonary disease; FEV1%, forced expiratory volume in 1^0s; GOLD, Global Initiative for Chronic Obstructive Lung Disease. *Statistically significant difference ($P<0.05$).

Among the different comorbid diseases, the common associations included diabetes mellitus (DM), liver cirrhosis, cardiovascular diseases, malignancy, chronic renal failure, and anemia. Diabetes was the most predominant comorbid disease and was recorded in 46 (26.0%) of 177 COPD patients, followed by liver cirrhosis and cardiovascular diseases in 36 (20.3%) and 21 (11.8%) patients, respectively. Malignancy was present in 9% of cases, including bronchogenic carcinoma in 8.5% (15/177 patients) and one case of lymphoma (Table 2).

The current study demonstrated that there was no significant difference in the clinical presentation between the two groups. Chronic cough, dyspnea, wheezes, and cyanosis were present in both groups. Chest pain and hemoptysis were present in a small percentage of both groups (11.6 vs. 8.3 and 6.2 vs. 5.2) in COPD patients with and without comorbidities, respectively.

As regards chest radiology, the majority of patients showed hyperinflation in both groups of COPD. There was no statistically significant difference between the two groups ($P > 0.05$). There was a statistically significant difference ($P < 0.01$) as regards CT of the chest; nodular and reticulonodular, interstitial shadows, pleural effusion, air crescent, and mass were present only in COPD patients with comorbidities (2.5, 2.5, 4.9, 4.9, and 1.2%, respectively). Moreover, bronchiectatic changes were present in 6.2% of COPD patients with comorbidities versus 21.9% of COPD patients without comorbidities. Normal CT was present in 2.1% of COPD patients without comorbidities (Table 3).

The prevalence of pulmonary fungal infection was significantly higher in COPD patients with comorbidities (77.8%) compared with COPD patients without comorbidities (53.1%) ($P < 0.001$) (Table 4).

Table 2 Comorbid diseases associated with chronic obstructive pulmonary disease in the study group

Comorbid disease[a]	*N* (%)
Uncontrolled DM	46 (26.0)
Chronic renal failure	4 (2.3)
Malignancy	16 (9)
Bronchogenic carcinoma	15 (8.5)
Lymphoma	1 (0.5)
Cardiovascular diseases	21 (11.8)
Coronary heart disease (CHD)	10 (5.6)
Congestive heart failure (CHF)	11 (6.2)
Liver cirrhosis	36 (20.3)
Anemia	10 (5.6)

Data expressed as *n* (%). COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus. [a]The same patient had more than one comorbid disease.

There was no statistically significant difference as regards fungal species ($P > 0.05$) with predominance of *Candida* and *Aspergillus* spp., in both groups. There was a statistically significant difference as regards blood GM, which was positive in 16 (19.7%) COPD patients with comorbidities versus seven (7.3%) COPD patients without comorbidities ($P < 0.05$). However, 1,3-β-D-glucan and BAL GM showed no statistically significant difference between the groups ($P > 0.05$) (Table 5).

The present study revealed that, of 177 COPD patients, there were 61/177 (34.5%) patients at risk for IFI: 41 patients with comorbidities and 20 patients without comorbidities. The prevalence of IFI in COPD patients with comorbidities was significantly higher (41/81; 50.6%) than that in COPD patients without comorbidities (20/96; 20.8%) ($P < 0.001$). Moreover, the proven IFI and probable IFI were significantly higher in COPD patients with comorbidities (4.9 and 46.3% vs. 0 and 15%, respectively) than that in COPD patients without comorbidities ($P < 0.05$). As regards the outcome, COPD patients with comorbidities at risk for pulmonary fungal infection had statistically significantly higher mortality rate (12.3%) compared with COPD patients without comorbidities (3.1%) ($P < 0.05$) (Table 6).

Table 7 summarizes the evaluation of risk factors for fungal infection using univariate analysis and multivariate analysis in COPD patients with comorbidities versus COPD patients without comorbidities. Mechanical ventilation, corticosteroid therapy, ICU admission, and age were major risk factors for pulmonary fungal infection [$P = 0.012$, odds ratio (ODR) = 2.23; $P = 0.028$, ODR = 1.99; $P = 0.025$, ODR = 1.94; and $P = 0.034$, ODR = 2.60; respectively]. Neutropenia was found in only one patient with COPD with comorbidities, and hence it was difficult to evaluate it as a risk factor for fungal infection in this study. Multivariate analysis showed that age, mechanical ventilation, corticosteroids therapy, and ICU admission were independent risk factors associated with pulmonary fungal infection in COPD patients with comorbidities versus COPD patients without comorbidities.

Discussion

This study was designed to assess the profile of fungal infection among COPD patients with and without comorbidities to determine the prevalence, risk factors, and outcome of pulmonary fungal infection among those patients.

Table 3 Radiological manifestations of chronic obstructive pulmonary disease patients at risk for pulmonary fungal infection

Variables	COPD with comorbidities ($n=81$)	COPD without comorbidities ($n=96$)	*P*-value
Chest radiography			
Normal	0 (0)	2 (2.1)	0.353
Hyperinflation	67 (82.7)	86 (89.6)	
Consolidation	2 (2.5)	1 (1)	
Collapse	2 (2.5)	1 (1)	
Mass	2 (2.5)	0 (0)	
Cavities	3 (3.7)	1 (1)	
Bronchiectatic changes	4 (4.9)	3 (3.1)	
Destroyed lung	0 (0)	1 (1)	
Pleural effusion	1 (1.2)	0 (0)	
Others	0 (0)	1 (1)	
CT of the chest			
Normal	0 (0)	2 (2.1)	0.001**
Hyperinflation	52 (64.2)	65 (67.7)	
Consolidation	5 (6.2)	1 (1)	
Mass	1 (1.2)	0 (0)	
Cavities	6 (7.4)	2 (2.1)	
Nodular and reticulonodular	2 (2.5)	0 (0)	
Interstitial shadows	2 (2.5)	0 (0)	
Bronchiectatic changes	5 (6.2)	21 (21.9)	
Destroyed lung	0 (0)	2 (2.1)	
Pleural effusion	4 (4.9)	0 (0)	
Pneumothorax	0 (0)	2 (2.1)	
Mediastinal lesions	0 (0)	1 (1)	
Air crescent	4 (4.9)	0 (0)	

Data expressed as *n* (%). COPD, chronic obstructive pulmonary disease; CT, computed tomography. **Statistical significant difference ($P<0.01$).

Table 4 Fungal culture results in the study group

Culture results	COPD with comorbidities ($n=81$)	COPD without comorbidities ($n=96$)	*P*-value
Culture-positive	63 (77.8)	51 (53.1)	<0.001†
Culture-negative	18 (22.2)	45 (46.9)	

COPD, chronic obstructive pulmonary disease. †$\chi^2=11.647$, statistically significant difference ($P<0.01$).

As regards comorbid diseases in this study, the common associations included DM, liver cirrhosis, cardiovascular diseases, malignancy, chronic renal failure, and anemia. Diabetes was the most predominant comorbid disease and was recorded in 26.0% of patients, followed by liver cirrhosis and cardiovascular diseases in 20.3 and 11.8% patients, respectively. However, bronchogenic carcinoma was recorded in 8.5% of cases. The current study revealed that COPD patients with comorbidities were associated with significantly lower mean age and lower FEV1% predicted compared with COPD patients without comorbidities; however, there was no statistically significant difference as regards sex.

Multiple comorbidities may coexist in individuals with COPD and play an important role in determining clinical outcomes in COPD, even after control of the confounding factors [15,16]. Although, COPD patients have normal pulmonary defense mechanisms against *Aspergillus* spp., such as the ingestion of conidia (the ends of some hyphae are rounded and could be confused with yeast, but that are instead called conidia or spores) by pulmonary macrophages and killing of hyphae by neutrophils. However, there many factors that predispose to colonization and infection with *Aspergillus* spp., including structural changes in lung architecture with the formation of bullae, and the common use of long-term steroid treatment (even inhaled steroids) increases host susceptibility by reducing oxidative killing of the organism by pulmonary macrophages and increases its linear growth by 30–40%. Moreover, comorbidity factors such as diabetes, alcoholism, and malnutrition may further enhance the risk for pulmonary fungal infection in COPD patients [17].

Recent studies reported that COPD may affect the function of other organs, including the heart, vasculature, muscles, liver, gastroenteric apparatus,

Table 5 Fungal culture and serology in the study group

Variables	COPD with comorbidities ($N=81$)	COPD without comorbidities ($N=96$)	*P*-value
Fungal species			
Candida	30 (47.6)	29 (56.9)	0.096
Aspergillus	19 (30.1)	16 (31.4)	
Penicillium	2 (3.1)	1 (2)	
Fusarium	0 (0)	2 (3.9)	
Combined	5 (7.9)	0 (0)	
Others	7 (11.1)	3 (5.9)	
Candida			
Albican	18 (28.6)	20 (39.2)	0.472
Nonalbican	12 (19)	9 (17.6)	
Aspergillus			
Fumigatus	4 (6.3)	6 (11.8)	0.283
Nonfumigatus	15 (23.8)	10 (19.6)	
Serological diagnosis			
Positive GM (cutoff index = 0.5 ng/ml)			
GM (BAL)	18 (22.2)	14 (14.6)	0.188
GM (blood)	16 (19.7)	7 (7.3)	0.014*
Positive 1,3-β-D-glucan (cutoff index = 10 ng/ml)	14 (17.3)	12 (12.5)	0.370

Data expressed as *n* (%). BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; GM, galactomannan ELISA assay.
*Statistical significant difference ($P<0.05$).

Table 6 Invasive fungal infection and patient's outcome in chronic obstructive pulmonary disease patients with comorbidities versus chronic obstructive pulmonary disease patients without comorbidities

Variables	COPD with comorbidities (n = 81)	COPD without comorbidities (n = 96)	P-value
Invasive fungal infection			
Noninvasive	40 (49.4)	76 (79.2)	<0.001†
Invasive	41 (50.6)	20 (20.8)	
Proven	2 (4.9)	0 (0)	0.023††
Probable	19 (46.3)	3 (15)	
Possible	20 (56.1)	17 (85)	
Discharge status			
Improved	68 (83.9)	85 (88.5)	0.034*
Discharged on request	3 (3.7)	8 (8.3)	
Died	10 (12.3)	3 (3.1	

COPD, chronic obstructive pulmonary disease. †$\chi^2=17.255$, statistical significant difference ($P<0.01$). ††$\chi^2=7.544$, statistical significant difference ($P<0.05$). *Statistical significant difference ($P<0.05$).

kidney, and brain; it is frequently associated with various disorders [18] and accelerates lung aging [19]. Several comorbidities frequently predominate, particularly in elderly patients, but the relationship linking their prevalence to patients' sex and COPD severity is still unclear [20,21].

This study reported that there was no significant difference in the clinical presentation between the two groups. As regards chest radiograph, there was no statistically significant difference between the two groups. However, there was a statistically significant difference as regards CT of the chest between the two groups, and normal CT was present in 2.1% of COPD patients without comorbidities. Nodular and reticulonodular, interstitial shadows, pleural effusion, air crescent, and mass were present only in COPD patients with comorbidities (2.5, 2.5, 4.9, 4.9, and 1.2%, respectively). Meersseman *et al.*[22] found that the characteristic clinical and radiological findings such as the halo sign or the presence of serum GM are usually prominent in neutropenic patients but not helpful in the diagnosis of aspergillosis in COPD patients.

In the present study, the prevalence of pulmonary fungal infection was significantly higher in COPD patients with comorbidities (77.8%) compared with COPD patients without comorbidities (53.1%). However, there was a predominance of *Candida* and *Aspergillus* spp., in both groups, with no statistically significant difference. There was statistically significant difference as regards blood GM, which was positive in 16 (19.7%) COPD patients with comorbidities versus seven (7.3%) COPD patients without comorbidities. However, 1,3-β-D-glucan and BAL GM showed no statistically significant difference between the two

Table 7 Risk factors of pulmonary fungal infection in chronic obstructive pulmonary disease patients with comorbidities versus chronic obstructive pulmonary disease patients without comorbidities

Risk factors	COPD with comorbidities ($n=81$)	COPD without comorbidities ($n=96$)	*P*-value	ODR (95% CI) Unstandardized[a]	Standardized[b]
Age	54.6±8.3	57.5±6.5	0.012*		
<40 years	6 (7.4)	3 (3.1)		0.4 (0.10–1.66)	0.43 (0.120–1.45)
≥40 years	75 (92.6)	93 (96.9)	0.21		
Sex					
Male	54 (66.7)	71 (74)	0.289	1.42 (0.74–2.72)	1.51 (0.68–2.18)
Female	27 (33.3)	25 (26)			
Drugs					
Antibiotic					
Yes	68 (84)	73 (76)	0.193	1.65 (0.77–3.5)	1.73 (0.79–3.11)
No	13 (16)	23 (24)			
Corticosteroids					
Yes	50 (61.7)	43 (44.8)	0.025*	1.99 (1.09–3.63)	2.01 (1.10–2.96)
No	31 (38.3)	53 (55.2)			
Immunosuppressive					
Yes	14 (17.3)	17 (17.7)	0.941	0.97 (0.44–2.11)	1.03 (0.18–2.22)
No	67 (82.7)	79 (82.3)			
ICU admission					
Yes	37 (45.7)	29 (30.2)	0.034*	1.94 (1.05–3.60)	1.98 (1.15–3.12)
No	44 (54.3)	67 (69.8)			
Length of hospital stay					
<1 week	26 (32.1)	43 (44.8)	0.085	1.72 (0.93–3.18)	0.62 (0.29–1.16)
>1 week	55 (67.9)	53 (55.2)			
Mechanical ventilation					
Yes	25 (30.9)	16 (16.7)	0.028*	2.23 (1.09–4.56)	2.31 (1.61–3.72)
No	56 (69.1)	80 (83.3)			
Neutropenia					
Yes	1 (1.2)	0 (0)	0.43	3.60 (0.14–89.49)	3.71 (0.61–51.32)
No	80 (98.8)	96 (100)			

Data expressed as *n* (%) or mean±SD. CI, confident interval; COPD, chronic obstructive pulmonary disease; ODR, odds ratio. [a]Univariate analysis ODR. [b]Multivariate analysis ODR. *Statistical significant difference ($P<0.05$).

groups. In contrast, Tutar *et al.* [23] found that GM with median value 0.54^{0}ng/ml was positive in nine patients (81.8%) and 1,3-β-D-glucan was examined in five patients and was positive in three (60%) of them.

The current study revealed that the prevalence of IFI in COPD patients with comorbidities was significantly higher (50.6%) than that in COPD patients without comorbidities (20.8%). Moreover, the proven and probable IFI was significantly higher in COPD patients with comorbidities (4.9 and 46.3%, respectively) versus 0 and 15% in COPD patients without comorbidities. In contrast, Ader *et al.* [24] reported that among COPD patients who were admitted in the ICU with acute respiratory distress, 13 cases were diagnosed as having IA and the only risk factor for IFI was corticosteroid treatment. Hence, IA should be suspected in COPD patients receiving steroid treatment who have extensive pulmonary infiltrates [24]. It has been demonstrated that *Aspergillus* spp. is the most frequent microorganism causing pulmonary infiltrates in patients receiving long-term steroid treatment [25]. Moreover, Ader *et al.* [24] reported that COPD was diagnosed in 26 (1.3%) of 1941 patients with IA.

As regards the outcome, this study demonstrated that COPD patients with comorbidities at risk for pulmonary fungal infection had statistically significantly higher mortality rate (12.3%) compared with COPD patients without comorbidities (3.1%). As regards risk factors for pulmonary fungal infection in COPD patients with comorbidities versus COPD patients without comorbidities, age, mechanical ventilation therapy, corticosteroid therapy, and ICU admission were major risk factors for pulmonary fungal infection in COPD patients with comorbidities, with a statistically significant difference. However, antibiotic therapy was prominent in both groups, with no statistically significant difference. Similarly, in recent years, it has been shown that corticosteroid use plays a significant role in terms of increasing the rate of invasive pulmonary aspergillosis (IPA) incidence in COPD cases [26]. In a retrospective study, it was shown that steroid use of over 700 mg in total within the last three months in COPD patients

increased the risk for IPA [13]. Moreover, it has been stated by other authors that inhaled steroids are a risk factor for IPA [27].

Moreover, using three or more antibiotics for 10 days was a risk factor for IPA development in COPD [28,29]. Another study found that four cases had used antibiotics in the last 3 months and a history of antibiotic use was the only risk factor for pulmonary fungal infection in one case that did not receive systemic steroid treatment [25]. In another study on 1209 patients with a positive respiratory culture for *Aspergillus* spp., Perfect *et al.* [30] showed that COPD, corticosteroid use, and DM were the three main risk factors for fungal colonization.

Conclusion

The prevalence of pulmonary fungal infection was significantly higher in COPD patients with comorbidities (77.8%) compared with COPD patients without comorbidities. There was predominance of *Candida* and *Aspergillus* spp., in both groups. Age, mechanical ventilation, corticosteroids therapy, and ICU admission were independent risk factors associated with pulmonary fungal infection in COPD patients with comorbidities. COPD patients with comorbidities at risk for pulmonary fungal infection had higher mortality rate compared with COPD patients without comorbidities.

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Conflicts of interest

There are no conflicts of interest.

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