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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Keywords: chronic obstructive pulmonary disease, comorbidities, pulmonary fungal infection

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 to 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 cavitition, 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 18V 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 50ml, 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 2h 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 χ²-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 1s (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 1s; 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).

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 = 0.036, 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.
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,
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 groups.

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                    |                                 |                                   |         |
| Albicnan                   | 18 (28.6)                       | 20 (39.2)                         | 0.472   |
| Nonalbicnan                | 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=0.5ng/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=10ng/ml) |     |                                   | 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. 1:χ² = 17.255, statistical significant difference (P < 0.01). 2:χ² = 7.544, statistical significant difference (P < 0.05). *Statistical significant difference (P < 0.05).
In contrast, Tutar et al. [23] found that GM with median value 0.540 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].

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.

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### 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 | Standardized |
|-----------------------|-------------------------------|----------------------------------|---------|-----------------------------|--------------|
| Age                   | 54.6 ± 8.3                    | 57.5 ± 6.5                       | 0.012   | 0.4 (0.10–1.66)             | 0.43 (0.120–1.45) |
| < 40 years            | 6 (7.4)                       | 3 (3.1)                          | 0.21    |                             |              |
| ≥ 40 years            | 75 (92.6)                     | 93 (96.9)                        |         |                             |              |
| 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. aUnivariate analysis ODR. bMultivariate analysis ODR. *Statistical significant difference (P < 0.05).
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.

References
1 Pulcha N, Fuhman MA, Hansel NN, Drummond MB, Boyd CM. Impact of comorbidities on self-rated health in self-reported COPD: an analysis of NHANES 2001-2006. COPD 2013; 10:324–332.
2 Divo M, Cote C, de Torres JP, Casanova C, Marin JM, Pinto-Plata V, et al. Comorbidities and risk of mortality in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012; 186:155–161.
3 Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med 2008; 359:2355–2365.
4 O’Donnell R, Breen D, Wilson S, Djukarovic R. Inflammatory cells in the airways in COPD. Thorax 2006; 61:448–454.
5 Bulpa P, Dive A, Sibille Y. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. Eur Respir J 2007; 30:782–800.
6 Pfeffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis 2006; 42:1417–1427.
7 Dutkiewicz R, Hage CA. Aspergillus infections in the critically ill. Proc Am Thorac Soc 2010; 7:204–209.
8 Gamachio-Montero J, Olaechea P, Alvarez-Lerma F, Alvarez-Rocha L, Blanquer J, Galván B, et al. Epidemiology, diagnosis and treatment of fungal respiratory infections in the critically ill patient. Rev Esp Quimioter 2013; 26:173–188.
9 Xavier MO, Oliveira FdM, Severo LC. Chapter 1: laboratory diagnosis of pulmonary mycoses. J Bras Pneumol 2009; 35:907–919.
10 Wright WF, Overman SB, Ribes JA. (1-3)-β-D-glucan assay: a review of its laboratory and clinical application. Lab Med 2011; 42:679–685.
11 Du Rand IA, Blaikley J, Booton R, Chaudhuri N, Gupta V, Khalid S, et al. British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults: accredited by NICE. Thorax 2013; 68(Suppl 1):i1–44.
12 Jourdain B, Joly-Guillou ML, Dombret MC, Calvat S, Trouillet JL, Gibert C, Chastre J. Usefulness of quantitative cultures of BAL fluid for diagnosing nosocomial pneumonia in ventilated patients. Chest 1997; 111:411–418.
13 Stynen D, Goris A, Sarfati J, Latgé JP. A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. J Clin Microbiol 1995; 33:487–500.
14 Latgé JP. Aspergillus fumigatus and aspergillosis. Clin Microbiol Rev 1999; 12:310–350.
15 Pulcha N, Han MK, Martinez CH, Foreman MG, Anzueto AR, Casaburi R, et al. Comorbidities of COPD have a major impact on clinical outcomes, particularly in African Americans. Chronic Obstr Pulm Dis (Milan) 2014; 1:105–114.
16 Dal Negro RW, Bonadiman L, Turco P. Prevalence of different COPD comorbidities in COPD patients by gender and GOLD stage. Multidiscip Respir Med 2015; 10:24.
17 Meersewman W, Lagrou K, Maertens J, Van Wijngaerden E. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. Thorac Inst 2013; 199:498.
18 Názara Otero CA, Baloirot Villar A. The continuum of COPD and cardiorespiratory risk: a global scenario of disease. Clin Invest Arterioscler 2015; 27:144–147.
19 MacNee W, Rabinovich RA, Choudhury G. Ageing and the border between health and disease. Eur Respir J 2014; 44:1332–1352.
20 Jo YS, Choi SM, Lee J, Park YS, Lee SM, Yim JJ, et al. The relationship between chronic obstructive pulmonary disease and comorbidities: a cross-sectional study using data from KNHANES 2010-2012. Respir Med 2015; 109:96–104.
21 Miravitlles M, Price D, Rabinovich RA, Choudhury G. Ageing and the border between health and disease. Eur Respir J 2013; 8:4533–4543.
22 Meersewman W, Vandecasteele SJ, Willmer A, Verbeken E, Peereman WS, Van Wijngaerden E. Invasive aspergillosis in critically ill patients without malignancy. Am J Respir Crit Care Med 2004; 170:621–625.
23 Tutar N, Melan G, Koç AN, Yilmaz I, Bozkurt I, Simsek ZO, et al. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. Multidiscip Respir Med 2013; 8:1–7.
24 Ader F, Naeer S, Le Berre R, Leroy S, Tille-Leblond I, Marquette CH, Dufourch A. Invasive pulmonary aspergillosis in chronic obstructive pulmonary disease: an emerging fungal pathogen. Clin Microbiol Infect 2005; 11:427–429.
25 Agusti C, Rarió A, Filella X, González J, Moreno A, Xaubet A, Torres A. Pulmonary infiltrates in patients receiving long-term glucocorticoid treatment: etiology, prognostic factors, and associated inflammatory response. Chest 2003; 123:488–498.
26 Guineas J, Torres-Narbona M, Gilpón P, Muñoz P, Pozo F, Pérez E, et al. Pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: incidence, risk factors, and outcome. Clin Microbiol Infect 2010; 16:870–877.
27 Leav BA, Fanburg B, Hadley S. Invasive pulmonary aspergillosis associated with high-dose inhaled fluticasone. N Engl J Med 2000; 343:586.
28 Muquim A, Dial S, Menzies D. Invasive aspergillosis in patients with chronic obstructive pulmonary disease. Can Respir J 2005; 12:199–204.
29 He H, Ding L, Li F, Zhan Q. Clinical features of invasive bronchial-pulmonary aspergillosis in critically ill patients with chronic obstructive respiratory diseases: a prospective study. Crit Care 2011; 15:R5.
30 Perfect JR, Cox GM, Lee JY, Kauffman CA, de Repentigny L, Chapman SW, et al. The impact of culture isolation of Aspergillus species: a hospital-based survey of aspergillosis. Clin Infect Dis 2001; 33:1824–1833.