Egyptian Journal of Medical Microbiology Volume 34 / No.4 / October 2025 437-443 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Prevalence of Invasive Pulmonary Fungal Infections among Chronic Obstructive Pulmonary Disease Patients in Mansoura University Hospitals

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Key words:
COPD; AECOPD; IPFIs

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ABSTRACT

Background: *COPD is a progressive lung disease marked by chronic respiratory symptoms and persistent airflow restriction. Exacerbations are acute worsening of symptoms that indicate disease progression and are linked to higher morbidity and death. COPD affects roughly 300 million individuals worldwide, with an estimated prevalence of 12.2%.* ***Objectives:*** *This study aimed to estimate the prevalence of invasive pulmonary fungal infections (IPFIs) among COPD patients, identify the most common fungal agents, and assess the diagnostic performance of pan-fungal PCR and serum β-D-glucan (BDG).* ***Methodology:*** *Forty-five patients with acute exacerbation of COPD (AECOPD) with clinical suspicion of IPFIs were included. Bronchoalveolar lavage was performed for fungal culture, blood samples were collected for BDG measurement using ELISA and pan-fungal PCR, and transbronchial biopsies were obtained for histopathological analysis.* ***Results:*** *The prevalence of IPFIs using fungal culture as the reference method was 68.9%. Aspergillus spp. were the most common identified fungi, with Aspergillus fumigatus being the most common species (35.6%). Serum BDG testing showed good diagnostic performance, with an AUC of 0.810 and an optimal cutoff value of 114 pg/mL. Fungal culture demonstrated a sensitivity of 100%, specificity of 35.9%, PPV of 19.3%, and NPV of 100% when compared to histopathology.* ***Conclusion:*** *fungal culture is a sensitive method for detecting IPFIs, while histopathology, though specific, may miss some infections. BDG also appears to be a useful biomarker in diagnosing IPFIs among COPD patients.*

INTRODUCTION

COPD is an ongoing, debilitating lung disease and currently represents the third leading cause of death globally [1]. It imposes a significant burden on healthcare systems through both direct medical costs and loss of productivity. Acute exacerbations of COPD contribute substantially to morbidity, mortality, and disease progression. Annually, between 22% and 40% of COPD patients have at least one exacerbation, while 9% to 16% have frequent exacerbations [2].

Exacerbations are generally managed as a single clinical entity with bronchodilators, antibiotics, and systemic corticosteroids. These treatment strategies, while often effective and are not without complications, repeated courses of antibiotics promote antimicrobial resistance and dysbiosis, while systemic corticosteroids can impair host immunity, predisposing patients to opportunistic infections, including IPFIs [3]. Of an estimated 5 million fungal species, approximately 300 are pathogenic to humans, with around 20 causing the majority of serious infections. Notable pathogens involve Aspergillus fumigatus, Candida spp, and Cryptococcus neoformans, among others. These fungi can cause respiratory and systemic infections in COPD patients, who may be particularly vulnerable due to impaired local defenses and immunosuppressive therapy[4].

IPFIs in COPD patients are often underdiagnosed due to nonspecific symptoms and limitations of conventional diagnostics, leading to delayed treatment and worse outcomes. This study aimed to determine the prevalence of IPFIs throughout COPD exacerbations, identify the main fungal pathogens, and evaluate the diagnostic accuracy of pan-fungal PCR and serum BDG compared to culture and histopathology [5].

METHODOLOGY

The study's protocol was approved by the Institutional Review Board (IRB), Faculty of Medicine, Mansoura University (code number: MD.22.06.655).

This observational cross-sectional study was conducted over a period of twenty-four months (August 2022 to July 2024). A total of 45 patients suffered from acute exacerbation COPD and were suspected of IPFI attended the Chest Medicine Department, Mansoura University Hospitals for bronchoscopy (Bronchoscopic unit, MUHs).

The patients enrolled in the study were aged between 42 and 78 years old, with a mean age of 60.89 ± 9.54 years. They were 35 males and 10 females, with a notable predominance of rural residency. Smoking was a prevalent risk factor, with 38 patients identified as smokers and only 7 as nonsmokers.

All patients included in the study met the diagnostic criteria for COPD exacerbation as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD)[6].

According to these guidelines, an exacerbation of COPD is characterized as an acute increase in symptoms beyond the usual day-to-day variation, necessitating changes in therapy such as the initiation of systemic glucocorticoids, antibiotics, or supplemental oxygen. The primary clinical features defining exacerbation included increased dyspnea, increased sputum volume and or viscosity, and increased sputum purulence, as outlined by the Anthonisen criteria[7]. Additional associated symptoms that were commonly observed among the patients included tachypnea, chest discomfort, fatigue, sleep disturbances, cyanosis, and deterioration in pulmonary function.

IPFIs in our COPD patients were classified according to EORTC/MSGERC, Bulpa and ICU Algorithm[8,9,10] into 6 proven, 30 probable and 9 possible IPFIs (All our patients were symptomatic as they had exacerbation of COPD. So, no colonization cases were reported).

BAL samples were obtained from each patient and processed in the Mycology Unit of the Medical Microbiology Department at the Faculty of Medicine, Mansoura University. Between 4 to 7 milliliters of BAL were centrifuged at 3000 rpm for 20 minutes[11]. The supernatant fluid was separated for other potential investigations, while the sediment was used for direct microscopic examination and fungal culture. Direct microscopy was performed using a 10% potassium hydroxide (KOH) preparation under light microscopy at both low (10x) and high (40x) magnifications to detect fungal elements [12]. For culture, a 75 µL aliquot of the sediment was inoculated onto Sabouraud's dextrose agar (SDA).

Macroscopic features such as colony color, texture, and pigmentation on both recto and verso sides of the plates were noted [13]. Microscopic features of the colonies were examined using lactophenol cotton blue (LPCB) stain to evaluate structural elements such as conidiophores, spores, and hyphae for molds such *as Aspergillus*, *Penicillium*, *Mucor*, and *Fusarium*, identification [12]. For Candida species, additional tests such as the germ tube test and CHROMagar Candida medium were used for species-level identification. [12,14]

Transbronchial lung biopsy samples were processed in a private pathological laboratory by an experienced pathologist. Tissues were fixed with 10% buffered formalin, embedded in paraffin blocks, and sectioned into 5 µm slices. To detect fungal elements in tissue and confirm an invasive fungal infection, these slices were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) [15].

Eight millimeters of venous blood were drawn from each patient. Of this, 6 mL were placed in EDTA-containing tubes and stored at −20°C for molecular analysis by pan fungal polymerase chain reaction, and 2 mL were placed in tubes without anticoagulant, and then centrifuged at 5000 rpm for 5 minutes to separate the serum. The serum was transferred aseptically to sterile Eppendorf tubes and stored at -20^{0}C for beta-D-glucan (BDG) testing using enzyme-linked immunosorbent assay (ELISA) [16].

For molecular detection, DNA was extracted from whole blood using the Quick-DNA Miniprep Plus Kit (Zymo Research, USA).

Human beta-globin validates the existence of amplified DNA in the sample, rules out PCR inhibitors, and ensures DNA extraction is successful.

For serological testing, serum BDG levels were measured using a double-antibody sandwich ELISA kit supplied by (Shanghai Korain Biotech Co, Ltd, China).

Table 1 Primers used for detection of the highly conserved region of the fungal 18S rRNA gene by panfungal PCR[17]

Target	Primer sequence (5/ - 3/)	Amplicon size (bp)
18S rRNA gene	**forward:** ATTGGAGGG CAAGTCTGGTG **reverse**: CCG ATC CCT AGT CGG CAT AG	450

Table 2 Primers used for detection of β-globin gene by PCR[18]

Target	PCR gene	Primer sequence (5/ - 3/)	Amplicon size (bp)
β-globin gene	β-glob-F	GAAGAGCCAAGG ACA GGT AC	268
	β-glob-PC04R	CCACTTCATCCA CGT TCA CC	

Statistical analysis

The statistical analysis was performed using SPSS version 24. The Kolmogorov-Smirnov test was used to determine whether the data was normal. Qualitative data were presented as numbers and percentages, and associations were examined using the Chi-square test or, as needed, Fisher's exact and Monte Carlo tests. Quantitative data were reported as mean ± SD for normally distributed variables and median (Min-Max) for non-normal data. Depending on the distribution of the data, the independent t-test or Mann-Whitney test was used to compare groups. The sensitivity and specificity of diagnostic instruments were assessed using ROC curve analysis. A p-value < 0.05 indicated statistical significance.

RESULTS

The patients aged between 42 and 78 years (mean age 60.89 ± 9.54 years), included 35 males and 10 females, 31 patients were living in rural areas while 14 patients were living in urban ones, 38 patients were smokers while 7 patients were nonsmokers table 3.

Table 3 Sociodemographic characteristics of the studied patients

Demographic data	The studied patients (no=45)
Age (Years)	
Mean± SD	60.89±9.54
Min-Max	42-78
Sex	
Male	35 (77.8%)
Female	10 (22.2%)
Residence	
Urban	14 (31.1%)
Rural	31 (68.9%)
BMI (Kg/m2)	22.74 ± 2.56
Mean (± SD)	(18.2-29.4)
Min-Max	
Underweight	5 (11.1%)
Normal	32 (71.1%)
Overweight	8 (17.8%)
Smoking status	
Current smoker	23 (51.1%)
Former smoker	15 (33.3%)
Nonsmoker	7 (15.6%)
Smoking index (pack /year)	
Median	32.5
Min- Max	(0-80)
Type of smoking	
Cigarette	30 (78.9 %)
Shisha	4 (10.5 %)
Both	4 (10.5 %)

According to the GOLD classification for COPD exacerbation severity, 9 patients (20%) were categorized as having mild COPD (GOLD 1), 15 patients (33.3%) had moderate COPD (GOLD 2), 19 patients (42.2%) had severe COPD (GOLD 3), and only 2 patients (4.4%) were classified as having very severe COPD (GOLD 4). Based on the EORTC criteria for diagnosing IPFIs, 6 patients (13.3%) were identified as having proven IPFIs, 30 patients (66.7%) were classified as probable cases, and 9 patients (20%) were considered possible cases table 4.

Table 4 Classification groups for the studied patients

Classification groups	The studied patients (no=45)
GOLD stage (exacerbation grade)	
GOLD 1(Mild)	9 (20.0%)
GOLD 2 (moderate)	15 (33.3%)
GOLD 3 (severe)	19 (42.2%)
GOLD 4 (very severe)	2 (4.4%)
EORTC criteria	
Proven	6 (13.3%)
Probable	30 (66.7%)
Possible	9 (20.0%)

GOLD: Global Initiative for Chronic Obstructive Lung Disease, EORTC criteria: European Organization for Research and Treatment of Cancer

Fungal culture on SDA showed that *aspergillus* spp. as the most prevalent fungal pathogen isolated from COPD patients, either as a single pathogen (26.7%) or in combination with other fungi (33.3%). Mixed fungal infections were notably common, with 33.3% of samples showing dual fungal growth, most frequently *Aspergillus* spp. mixed with *Candida* spp. (24.4%) table 5.

Table 5 Fungal culture of BAL samples of the studied patients on SDA

Fungal culture of BAL samples	The studied patients (no=45)
Positive culture:	**31 (68.9%)**
• ***Aspergillus spp,***	12 (26.7%)
• ***Candida spp,***	4 (8.9 %)
• **Combined fungal growth**	**15 (33.3%)**
Aspergillus+ Candida	11 (24.4%)
Aspergillus+ Mucor	2 (4.4%)
Aspergillus+ Penicillium	1 (2.2%)
Aspergillus+ Fusarium	1 (2.2%)
Negative culture:	**14 (31.1%)**

Aspergillus spp. were implicated in fungal infections in 27 out of 45 patients (60%), either as single or mixed infections. Among these, 12 patients (26.6%) had single-species *Aspergillus* infections, with *Aspergillus fumigatus* being the most frequently isolated species, detected in 9 patients (20%). Other isolated *Aspergillus* spp. included *Aspergillus flavus* in 2 patients (4.4%) and *Aspergillus terreus* in 1 patient (2.2%). Mixed infections involving *Aspergillus* and other fungal species were observed in 15 patients (33.3%). These included combinations of *A. fumigatus* with *Candida* in 6 patients (13.3%), *A. flavus* with *Candida* in 5 patients (11.1%), *A. flavus* with *Mucor* in 2 patients (4.4%), *A. fumigatus* with *Penicillium* in 1 patient (2.2%), and *A. niger* with *Fusarium* in 1 patient (2.2%). Pure *Candida* infections were identified in 4 patients (8.9%), all caused by *Candida albicans.* Additionally, mixed infections involving both *Candida* and *Aspergillus* species were found in 11 patients (24.4%) table 6.

Out of the 45 COPD patients involved in the study, 31 cases were diagnosed with IPFIs (6 cases had proven IPFIs and 25 cases had probable IPFIs) using fungal culture as the reference method. This corresponds to a prevalence rate of 68.9%, table 7

Table 6: Identification of fungal growth isolated from the studied patients up to species level

Identification of fungal growth up to species level	The studied patients (no=45)
***Aspergillus* species:**	12 (26.6%)
Aspergillus fumigatus	9 (20 %)
Aspergillus Flavus	2 (4.4%)
Aspergillus terreus	1 (2.2%)
***Aspergillus species* mixed with other fungi:**	***15 (33.3%)***
Aspergillus fumigatus + Candida albicans	2 (4.4%)
Aspergillus fumigatus+ Candida glabrata	2 (4.4%)
Aspergillus fumigatus+ Candida tropicalis	1 (2.2%)
Aspergillus fumigatus+ Candida krusei	1 (2.2%)
Aspergillus fumigatus+ penicillium	1 (2.2%)
Aspergillus flavus+ Candida albicans	3 (6.7%)
Aspergillus flavus+ Candida glabrata	1 (2.2%)
Aspergillus flavus+ Candida tropicalis	1 (2.2%)
Aspergillus flavus + Mucor	2 (4.4%)
Aspergillus niger+ fusarium	1(2.2%)
Total *Aspergillus* species	**27(60%)**
Candida albicans	**4 (8.9 %)**
***Candida species mixed* with *aspergillus*:**	**11 (24.4%)**
Candida albicans+ aspergillus fumigatus	2 (4.4%)
Candida albicans+ aspergillus flavus	3 (6.7%)
Candida glabrata+ aspergillus fumigatus	2 (4.4%)
Candida glabrata+ aspergillus flavus	1(2.2 %)
Candida tropicalis + aspergillus fumigatus	1(2.2%)
Candida tropicalis + aspergillus flavus	1(2.2%)
Candida krusei+ aspergillus fumigatus	1(2.2%)
Total *Candida* species	**15 (33.3%)**

Table 7 The prevalence of IPFIs among the studied COPD patients based on fungal culture

Total number of patients	Number of patients with IPFIs	Prevalence (%)
45	31	68.9%

The median levels of serum BDG were 427, 110.5, 66 pg/ml for proven, probable and possible IPFIs respectively with statistically significant difference between the 3 groups (**P≤ 0.001**). Beta D glucan was positive in all cases of proven, 93.3% of probable IPFIs and was negative in 6.7% patients of probable and all patients of possible IPFIs with statistically significant difference between the groups (**P≤ 0.001**) figure 1

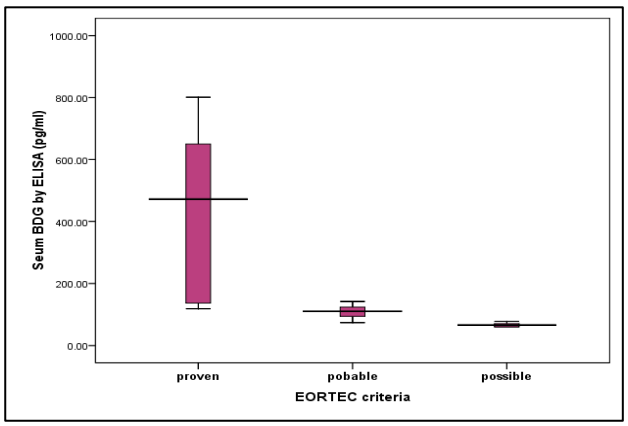

Fig. 1: Box plot for median BDG levels of the studied patients.

Pan fungal PCR from blood samples was positive in 5 cases (83.3%) with proven and 6 cases (20.0%) of probable IPFIs respectively while no cases with possible IPFIs showed positive PCR results. There was a statistically significant difference between proven and probable, proven and possible IPFIs regarding PCR (P1=0.006, P2=0.002) but there was no statistically significant difference between probable and possible IPFIs figure 2

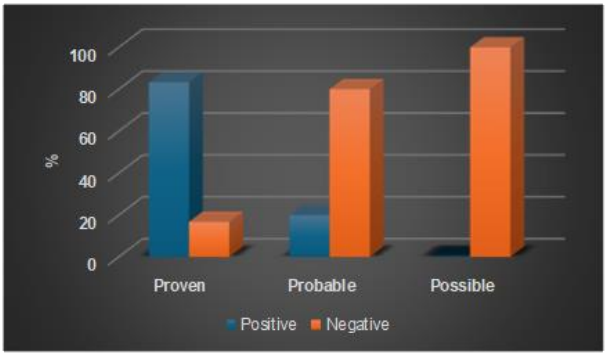

Fig. 2: Comparison of PCR results between different categories of IPFIs in the studied patients

DISCUSSION

The present study reported a 68.9% prevalence of IPFIs among acute exacerbation COPD patients based on fungal culture, aligning closely with Zhou et al.[1] and Kim et al. [20], who reported prevalence rates of 70% and 66%, respectively. Higher prevalence was observed in studies by Huber et al. [21] and Verberne et al. [22], at 80% and 75%, respectively. These elevated rates may be explained by more severe patient conditions, ICU admissions, mechanical ventilation, or increased exposure to hospital-acquired infections. In contrast, Fahmy et al.[23] and Ryu et al.[24] reported lower prevalence rates of 45% and 35%, respectively, likely due to differences in geographic location, environmental exposures, clinical profiles, or antifungal treatment practices.

In this study, *Aspergillus fumigatus* was the most common fungus isolated either alone (20%) of cases or in combination with other fungi (15.6%). This result aligns with several studies, such as Chen et al. [25] and Wessolossky et al. [26], who similarly reported a high prevalence of *Aspergillus fumigatus* (60% and 70.1%) respectively. In contrast, Mohamed et al. [27] found a higher prevalence of non-fumigatus *Aspergillus* species. In the present study, *candida albicans* was the most common *candida* species (20%) which was isolated alone from (8.9%) and in combination with *aspergillus* from (11.1%) of patients. This result coincides with the findings of Han and Meng [28] and Mohamed et al. [27] showed a higher prevalence of *Candida albicans* (60%).

The unique aspect of our research lies in its identification of the prevalence of proven IPFIs across different COPD exacerbation groups, which sets it apart from previous studies. This study and the one by Mahmoud et al.[29] shared similar findings regarding the higher prevalence of IPFIs among patients with positive fungal cultures compared to those with negative cultures. Both studies showed a significant statistical difference between the groups, with the majority of patients with positive cultures having probable IPFIs.

This study highlighted the utility of serum BDG as a biomarker for diagnosing IPFIs in COPD patients. BDG testing was highly effective in identifying proven IPFIs (100% positive) and most probable cases (93.3% positive), with much lower positivity in possible IPFIs showing a notable difference between the groups ($P \leq 0.001$). Median BDG levels were significantly higher in proven cases (427 pg/ml) compared to probable (110.5 pg/ml) and possible cases (66 pg/ml) with a statistically significant difference across these groups ($P \leq 0.001$). These results align with previous research by Theel et al. (2013) [(30)] who reported positive BDG results in 75% of proven IPFIs and 63.6% of probable IPFIs and median BDG levels of 261.5 pg/ml for proven and 98 pg/ml for probable cases with a statistically significant difference between the groups ($P \leq 0.001$).

In the current study, pan fungal PCR showed higher positivity in proven (83.3%) and probable (20.0%) cases, with no positive results in the possible IPFI group, and statistically significant differences between the proven and probable, as well as between the proven and possible groups. In contrast, Chen et al. [25] found higher positivity in probable (90%) and proven (58.3%) IPFIs, with no significant difference between the groups, and also included respiratory samples for PCR testing.

The current study identified 25 culture-positive cases despite negative histopathological diagnoses, and this suggests that histopathology might fail to detect fungal infections in some instances, particularly if the fungal burden is low or the sample is not well-

preserved. This finding opposes Zhang et al.[31], who reported 24 culture-negative cases despite positive histopathological diagnoses.

CONCLUSIONS

The prevalence of IPFIs among COPD patients based on fungal culture was 68.9%.

Aspergillus fumigatus was the most prevalent fungal pathogen, with mixed fungal infections commonly observed in proven cases. Culture techniques are more sensitive than histopathological examination in detecting invasive pulmonary fungal infections (IPFIs) among COPD patients emphasizing the effectiveness of culture methods in identifying infections, even with sparse fungal elements.

Recommendation

Use of BDG as a screening tool: Given the association between elevated BDG levels and the severity of IPFIs, incorporating BDG testing into routine screening protocols during exacerbations of COPD could help identify at-risk patients early and allow for prompt intervention.

Disclosure statement: the authors reported no potential conflict of interest.

Submission declaration: The manuscript has not been published elsewhere and has not been submitted simultaneously for publication elsewhere.

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