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Prevalence and pattern of isolated fungi from bronchoalveolar lavage among patients with lung cancer: a prospective cross-sectional study

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Abstract

Background Fungal colonization or infection has recently been reported in patients with lung cancer, and it is possible that it has a role in the pathogenesis of lung cancer or having an effect on treatment and outcome. Aim of work was to assess the prevalence and pattern of isolated fungi from patients with lung cancer at the time of diagnosis. In this prospective cross-sectional observational study, patients with suspected lung tumors were subjected to fiberoptic bronchoscopy (FOB) for biopsy, bronchoalveolar lavage (BAL) with its culture for fungal growth. After a pathological diagnosis, 100 cases of confirmed lung cancer were entered into the study analysis. The prevalence and type of isolated fungi have been determined and compared to the characteristics of the participants and cell types of lung cancer.

Results Fungi were isolated from 68% of the studied lung cancer cases. The most common isolated fungi were Candida albicans (32%), Aspergillus niger (28%), and Aspergillus fumigatus (8%). Fungi were isolated with a higher frequency in lung cancer cases with the following characteristics: males ($p = 0.008$), current or ex-smokers ($p = 0.002$), and chronic obstructive pulmonary disease (COPD) association ($p = 0.01$). In comparison to lung cancer cases with negative fungal culture, detection of fungal colonization was more associated with increasing severity of clinical presentation: higher grades of dyspnea (grade 1 vs. grade 2, p 0.001), a higher cough score (score 1 versus score 3, p 0.001), a higher chest pain score (score 0 versus score 1, p 0.001), and higher scores of hemoptysis (score 0 versus score 3, p 0.001). Otherwise, no difference was detected regarding age, frequency of comorbidities, chest computed tomography (CT) findings, lung cancer cell type, and staging in lung cancer patients with fungal colonization ($p >$ 0.05).

Conclusion Fungi were isolated in more than two thirds of lung cancer cases at the time of diagnosis with higher frequency among males, smokers, and those having associated COPD. This may negatively affect the response to treatment and prognosis of lung cancer. ClinicalTrials.gov (NCT 05575388).

Keywords Lung cancer, Fungal colonization, Fiberoptic bronchoscopy

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Background

Global cancer statistics estimated the incidence of lung cancer at 2.09 million new cases (11.6% of whole malignancy cases) as well as its related mortality at 1.76 million deaths (18.4% of total cancer deaths), making lung cancer the most common cancer and the etiology of cancer-related death in men and women combined [1].

Lung cancer is frequently complicated by lung infections reducing the effectiveness of oncological treatment and survival, which are detected in 9.5 to 84% of cases [2]. Colonization in lung cancer can occur as a result of a localized bronchial abnormality, such as narrowing or defective mucociliary function, or it can be induced by local inflammatory response caused by co-existing bronchiectasis and COPD [3]. Bronchial colonization plays a key role in developing lung infections in patients with lung malignancy and may harm patients undergoing chemotherapy or surgery during which secretion clearance and cough reflex are decreased [3, 4].

Moreover, studies suggest that chronic pulmonary infection plays an etiologic role in lung carcinogenesis, either acting alone or as a co-factor with tobacco smoke in enhancing lung cancer risk [5, 6].

Many studies analyzed pulmonary infections in patients with lung cancer during their course of treatment [7–9]. Few emerging researches studied the pattern of microbial colonization in lung cancer at the onset of diagnosis and prior to any definite treatment [3, 10] as well as fungal colonization [11, 12].

Screening those cases for fungal colonization of the respiratory tract would characterize patients who needed closer monitoring for the occurrence of potential complications such as acute invasive fungal infection [13].

The present study aimed to detect the prevalence and pattern of isolated fungi from patients with lung cancer at the time of diagnosis before starting chemotherapy or radiotherapy.

Methods

Design and setting of the study

This prospective cross-sectional observational study aimed to assess the prevalence and pattern of isolated fungi from patients with lung cancer at the time of diagnosis. The Institutional Research Board of the Faculty of Medicine, Mansoura University, approved the study protocol (code no.: MS/353) and written informed consents were obtained from patients before enrollment in the study. The protocol was registered in the ClinicalTrials.gov (NCT 05575388).

Patients

This study included 100 consecutive patients with central bronchial carcinoma between April 2018 and October 2020. It was carried out at interventional pulmonology unit of chest medicine department in collaboration with radiology, microbiology and pathology departments, Faculty of Medicine, Mansoura University.

Inclusion and exclusion criteria

Patients presented to the chest medicine outpatient clinic with clinical and radiological manifestations suspicious of lung cancer, according to Hollings and Shawn [14], were investigated by FOB for both the presence of malignancy and fungal infection. Patients diagnosed as lung cancer were eligible for analysis and enrollment in the study. Exclusion criteria were the presence of neutropenia with an absolute neutrophil count < 1000/μL (equivalent to < 1.0 × 109/L) [15] and patients on systemic corticosteroids therapy or those on chemotherapy or radiotherapy or antifungal treatment.

Data collection and clinical assessment

The following data were recorded: age, sex, and smoking history, comorbidities including COPD, hypertension (HTN), diabetes mellitus (DM), ischemic heart disease, bronchial asthma (BA)] and previous malignancy. Also, blood leukocyte and neutrophil counts were registered.

Patients were evaluated for the presence of toxemic symptoms, compressive manifestations, and severity score of each of the following: dyspnea from 0 to 4 [16], cough from 0 to 5 [17], chest pain from 0 to 5 [18] and hemoptysis, which was scored 1 if the amount of blood expectorated in 24 h was < 30 ml, 2 (30–100 ml), 3 (> 100–600 ml), and 4 (> 600 Ml) [19].

The radiological findings of post-contrast chest CT were revised and reported: description of the lesion, either nodule, mass, cavity, consolidation, or ground-glass opacity, size and site of the lesion, and if there was associated lobar or total lung collapse, hilar, and mediastinal lymphadenopathy assessment [20] and whether there was pleural effusion.

At FOB, no endobronchial suction was applied during introduction of the scope to avoid contamination with upper airway flora. BAL was performed as described by Jourdain et al. [21] using 120 mL of 0.9 % saline solution at room temperature in 20 mL aliquots after discarding the first aliquot. BAL fluid was collected in two sterile containers and transported immediately, one sample to mycology laboratory and the other to cytology lab.

Biopsies were taken from endobronchial tumors with forceps or cyoprobe. Tissue samples were preserved in formalin 10% for histopathological examination of the tumor, cell typing with hematoxylin and eosin (H&E) and immunostaining when needed as well as staining for fungi in lung tumor biopsies with Grocott-Gomori methenamine silver stain if H&E staining was insufficient

to reveal fungal elements in tissue. The results of fungal cultures in cases positive for lung cancer were included in the study. Cytological and histopathological results of lung cancer were classified according to World Health Organization [22]. Lung cancer was staged according to The 8th Edition Lung Cancer Stage Classification [23]. As regards N staging, it was done clinically based on the CT findings.

Mycological processing

BAL was stained by fungal staining with lactophenol stain as well as a culture on Sabouraud dextrose agar. The resultant fungal growth was classified into mild growth (up to 10^2/ml), moderate growth (< 10^2–10^4>/ml) or heavy growth (> 10^4–10^6>/ml).

Diagnostic criteria for fungal infection [24]

This study applied the criteria for differentiating fungal colonization from invasive disease in lung cancer patients with fungal-positive BAL cultures in accordance with the recommendations of the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) [24]. Since the criteria for diagnosis did not prioritize tissue culture over tissue histopathology and/or did not require tissue culture, we relied solely on the histopathology and did not apply the tissue culture for fear of exhausting the specimens taken for lung cancer diagnosis. The case would be classified as Definite Invasive Fungal disease" in the presence of septate branching hyphae in tissue histopathology accompanied by evidence of associated tissue damage in the case of invasive pulmonary aspergillosis or Candida species showing pseudohyphae or true hyphae for invasive candidiasis.

The following two requirements must also be met by fungal-positive BAL cultures in order to be identified as probable invasive fungal infections: (1) Host factors, which include one of the following: recent history of neutropenia (500 neutrophils/mm^3) for 110 days; receiving an allogeneic stem cell transplant; prolonged use of corticosteroids at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent; treatment with other known T cell immunosuppressants; or inherited severe immunodeficiency; and (2) radiological findings, such as dense, well-circumscribed lesion(s) with or without a halo sign, air-crescent sign, or cavity all of the aforementioned host factors had been considered and excluded before including the patients in the study.

Fungal respiratory tract colonization was the diagnosis. When a patient did not meet the host requirements and merely had fungal-positive BAL cultures, the three criteria required for a diagnosis of presumptive invasive fungal disease were not met.

Statistical analyses

Data were analyzed with SPSS software (SPSS: An IBM Company, version 16, IBM Corporation, Armonk, NY, USA). Categorical data were presented as numbers (percentages). For data with normal distribution, descriptive statistics were used to calculate mean ± standard deviation. For data with non-normal distribution, medians and ranges were used. The two groups (patients with lung cancer and fungal colonization and patients with lung cancer without proven fungal colonization) were compared with chi-square test or Fisher exact test for categorical data as appropriate; independent-samples *t* test for data with normal distribution and Mann–Whitney test for data with non-normal distribution. *P* values ≤ 0.05 were considered statistically significant.

Results

Characteristics of the studied patients

The studied 100 patients were 68 males (68%) and 32 females (32%), with a mean age of 57.18 ± 10.47 years. Twenty-six patients (26%) were ex-smokers, and 39 (39%) were smokers. The clinical characteristics of the patients are recorded in Table 1. Radiological presentation and the total leucocyte count with its differential of the studied cases are shown in Table 2; the most common detected abnormality was mass in 80% of cases.

Table 1 Clinical characteristics of the studied cases

Variable	Total cases (100)*
Co morbidities	No, (%)
• No comorbidities	38 (38%)
• Hypertension (HTN)	11 (11%)
• Diabetes mellitus (DM)	15 (15%)
• Both HTN and DM	21 (21%)
• Ischemic heart disease	4 (4%)
• Previous malignancy	3 (3%)
• COPD	62 (62%)
Clinical presentations:	No, (%)
• Dyspnea	97 (97%)
• Cough	94 (94%)
• Hemoptysis	84 (84%)
• Chet pain	74 (74%)
• Fever	39 (39%)
Severity of the disease:	Median (range)
• Cough score (0–5)	2 (0–3)
• Dyspnea grade (1–5)	2 (0–3)
• Chest pain score (0–5)	1 (0–3)
• Hemoptysis score (0–4)	3 (0–4)

Abbreviations: *COPD* chronic obstructive lung disease, *HTN* hypertension, *DM* diabetes mellitus

*The cumulative number was not identical to the total number of the studied cases as some patients had more than one presentation and comorbidity

Table 2 Laboratory and radiological criteria of the studied patients

Variables	Total cases (100)*	
CT data	no (%)*	
• Mass	80 (80%)	
• Cavity	8 (8%)	
• Collapse	8 (8%)	
• Bilateral nodules	6 (6%)	
• Ground glass opacities	8 (8%)	
• Consolidation	6 (6%)	
• Thoracic lymph node	98 (98%)	
• Pleural effusion	23 (23%)	
Chest CT distribution of lung cancer	no (%)*	
• Right upper lobe	23 (23%)	
• Right middle lobe	35 (35%)	
• Right lower lobe	15 (15%)	
• Left upper lobe	22 (22%)	
• Left lower lobe	8 (8%)	
• Bilateral	6 (6%)	
Total laboratory results:	Mean ± SD	Range:
• WBCS ($\times 10^3$)	10.02 ± 4.11	(2.6–21.5)
• Lymphocytes (%)	25.77 ± 10.70	(4.5–56)
• Neutrophils (%)	75.00 ± 82.29	(36.05–81.3)
• Eosinophils (%)	1.01 ± 0.89	(0.01–4)

*The cumulative number was not identical to that of the studied cases as some patients present with more than one radiological pattern in CT chest and tumor involved more than one lobe of the lung

Table 3 Bronchoscopic findings among the studied cases

Abnormality	Total cases (100)*
• Paralyzed left vocal cord	4 (4%)
• Tracheal mass	3 (3%)
• Endobronchial mass	60 (60%)
• Abnormal nodular mucosal infiltration	25 (25%)
• Bronchial stenosis (extraluminal compression)	2 (2%)
• No abnormality detected	7 (7%)
• Broad carina	9 (9%)
Anatomical site	Total cases (100)* *
• Main bronchi	Right 10, left 9 (19%)
• Upper lobe bronchus	Right 53, left 21 (74%)
• Lower lobe bronchus	Right 70, left 25 (95%)
• Lingula	5 (5%)
• Bronchus intermedius	14 (14%)
• Middle lobe bronchus	13 (13%)

*The cumulative number was not identical to the number of the studied cases as some patients present with more than one abnormality in fiberoptic bronchoscopy

**The cumulative number was not identical to that of the studied cases as tumor site appeared in more than one segment of the lung

Bronchoscopic findings of the studied cases

Endobronchial mass was the most common bronchoscopic finding in 60% of cases, followed by abnormal nodular mucosal infiltration in 25%. The right bronchial tree was more affected than the left side; the right lower lobe was the most commonly affected site, accounting for 70% of the studied cases, followed by the right upper lobe bronchus in 53% of the studied cases (Table 3).

Cell type and TNM staging of the studied cases

Adenocarcinoma was detected in 71% of all the studied cases, followed by small cell lung in 15% and squamous cell carcinoma in 11%. Other findings were 2 cases with large cell carcinoma and one case with a carcinoid tumor. Seventy percent of our cases were diagnosed at TNM stage IV. 20% were classified as TNM stage III, 7% as stage II and 3% as stage I (Table 4).

Sixty-nine patients had M1 disease: 23 cases with associated malignant pleural effusion were pathologically diagnosed; 6 cases had bilateral nodules, and 40 cases had extra-thoracic metastasis.

Table 4 Cell type and TNM staging of the studied cases

Variable	Total cases (no = 100)
Cell type:	
Adenocarcinoma	71 (71%)
Squamous cell carcinoma	11 (11%)
Large cell carcinoma	2 (2%)
Small cell lung cancer	15 (15%)
Carcinoid tumor	1 (1%)
Stage	
I	3 (3%)
II	7 (7%)
III	20 (20%)
IV	70 (70%)
T	
T1	1 (1%)
T2	19 (19%)
T3	28 (28%)
T4	52 (52%)
N	
N0	4 (4%)
cN1	18 (18%)
cN2	67 (67%)
cN3	11 (11%)
M	
M1	69 (69%)

c clinical

Pattern of fungal growth in BAL cultures of the studied cases

Fungi were isolated in BAL cultures of 68% of the studied cases (Table 5). The most commonly isolated fungi were aspergillus niger (28%), aspergillus fumigatus (8%), and candida *Albicans* (32%). Microscopic examination of tissue specimens revealed the presence of dichotomous branching hyphae with frequent septations with a diameter ranging from 2.5 to 4.5 μm in only 6 patients. Fig. 1 shows one of the studied lung cancer cases that had associated fungal colonization.

Table 5 Fungal culture results among the studied cases

Variables	Total cases (100)
Culture fungal isolates	68 (68%)
Aspergillus fumigatus moderate growth	4 (4%)
Aspergillus fumigatus heavy growth	4 (4%)
Aspergillus niger moderate growth	10 (10%)
Aspergillus niger heavy growth	18 (18%)
Candida mild growth	9 (9%)
Candida moderate growth	4 (4%)
Candida heavy growth	19 (19%)
Pathological diagnosis (hyphae)	6(6.0%)

Analysis of studied lung cancer cases with isolated fungi in cultures

We compared lung cancer patients with fungal isolation (Table 6 and 7). It showed that fungal isolation was detected with a higher frequency in males ($p = 0.008$), current or ex-smokers ($p = 0.002$), and in patients with COPD ($p = 0.01$).

According to the clinical scores among the studied cases, fungi were more frequently isolated from lung cancer patients with higher scores of dyspnea (grade 2 vs. grade 1, p 0.001), higher scores of cough (score 3 versus score 1, p 0.001), higher scores of chest pain (score 1 versus score 0, p 0.001), and higher scores of hemoptysis (score 3 versus score 0, p 0.001).

Otherwise, no difference was detected regarding age, frequency of comorbidities, CT chest findings, lung tumor pathology, and staging in patients with fungal colonization ($p > 0.05$).

Discussion

This prospective cross-sectional observational study aimed at determining the prevalence and pattern of isolated fungi from patients with lung cancer at the time of diagnosis before starting chemotherapy or radiotherapy. The patients were investigated by FOB for BAL cytology and fungal culture, forceps biopsy of endobronchial

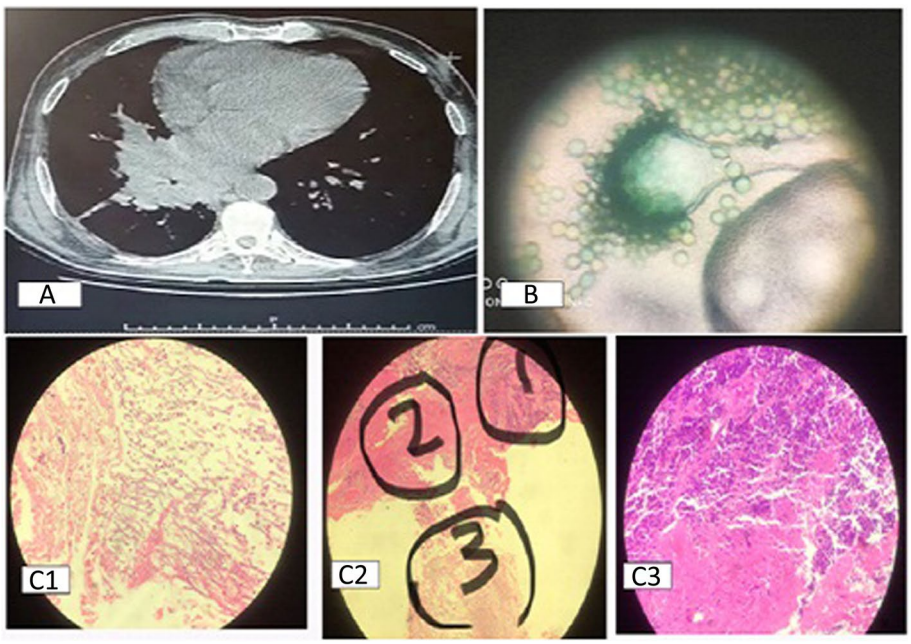

Fig. 1 Male patient 58 years old, current smoker, COPD patient, presented with dyspnea grade 2, cough score 3, chest pain score 2, hemoptysis score 3. **A** Computed tomography (CT) of the chest revealed right hilar mass. **B** Culture of bronchalveolar lavage (BAL) on Sabouraud dextrose agar: Asp fumigatus moderate growth. **C** Pathology with hematoxylin and eosin (H&E) revealed small cell lung cancer with hyphae, C 1 is close picture to fungal hyphae septated and branching; C 2: small cell lung cancer with hyphae (1-tumor cells, 2-areas of necrosis, 3-fungal colonies) and C 3 is close picture for tumor cells above small cell carcinoma (above) and esoniphilia area of necrosis (below)

Table 6 Analysis of studied lung cancer cases with fungal colonization

Variable	Negative culture No. = 32	Positive culture No. = 68	*P* value
Age (mean ± SD)	58.97 ± 11.26	56.51 ± 10.00	0.275
Sex, no (%)			
• Male	16 (50%)	52 (76.5%)	0.008
• Female	16 (50%)	16 (23.5%)	
Smoking, no (%)			
• Non smoker	19 (59.4%)	16 (23.5%)	0.002
• Ex-smoker	6 (18.8%)	20 (29.4%)	
• Current smoker	7 (21.9%)	32 (47.1%)	
Co morbidities, no (%)			
• No comorbidities	10 (32.25%)	28 (40.5%)	0.458
• Hypertension (HTN)	5 (16.1%)	6 (8.7%)	
• Diabetes mellitus (DM)	5 (16.1%)	10 (14.5%)	
• Both HTN and DM	6 (19.4%)	15 (21.7%)	
• Ischemic heart disease	0 (0.0%)	4 (5.8%)	
• Previous malignancy	2 (6.5%)	1 (1.4%)	
• COPD	14 (43.8%)	48 (70.6%)	0.010
Clinical presentation severity			
Dyspnea grade median (range)	1 (0–3)	2 (1–3)	< 0.001
• Cough score median (range)	1 (0–3)	3 (2–3)	< 0.001
• Chet pain score median (range)	0 (0–2)	1 (0–3)	< 0.001
• Hemoptysis score median (range)	0.5 (0–1)	3 (1–4)	< 0.001
• Fever, no (%)	14 (43.8%)	25 (36.8%)	0.504

lesions for histopathological examination of the cell type of the tumors, and inspection if there were any fungal elements.

The role of infection as a cause of lung cancer is still being debated. Dysbiosis of the microbiome is characterized by a reduction in symbiotic bacteria and an increase in pathogenic bacteria. Lung microbiota dysbiosis may promote lung cancer development by releasing cancer-promoting bacterial metabolites and inducing host inflammatory pathways [25, 26]. Moreover, little is known about the role of the airway fungal microbiota in lung cancer pathogenesis [12]. Also, identification of potentially pathogenic organisms colonizing the lower respiratory tract in patients with lung cancer is important as this may increase the risk of lung infections in the natural course of lung cancer that can restrain the effect of oncological treatment and affect their survival [2].

Laroumagne et al. [10] found that bronchial colonization in lung cancer was significantly associated with lower survival ($p = 0.04$). Also, they observed that colonization was associated with 1.61 times (95% CI 0.93–2.82; $p = 0.09$) more bronchopulmonary infections per patient compared to those who were not colonized.

Based on revised definitions of invasive fungal disease from the European Organization for Research [24], we ruled out the presence of invasive infection in the studied cases because there was no evidence of tissue damage or invasion accompanying hyphae detected in the examined biopsies and the 68 cases were defined as colonization.

The current study demonstrated that not all positive BAL cultures (68 cases) showed fungal detection in histopathology (6 cases), and this can be explained by the fungus present in the cultures being a colonizer in the patient, the pathologic specimen not being thoroughly examined with adequate special stains, or the fungal elements being missed as the biopsy was taken away from tissues containing viable fungi [27]. This raises the importance of BAL in approaching fungal infection.

Fungal colonization reported in our study is consistent with studies that analyzed the profile of fungal strains colonizing the respiratory tract in patients with lung cancer at the time of diagnosis and before any specific treatment by taking BAL samples to microscopy and culture for fungal agents, and they detected aspergillus colonization with higher frequencies than candida [12, 13]. However, Laroumagne et al. [10] found Candida albicans in 42.9% and Aspergillus fumigatus in 6.2% of 210 consecutive patients with lung cancer who underwent FOB.

Ali et al. [11] studied the prevalence of *Aspergillus* infection in 45 patients with bronchogenic carcinoma

Table 7 Analysis of the association between tumor radiological findings, pathology, staging, and fungal colonization

	Negative fungallung infections No. = 32	Positive fungallung infections No. = 68	*P* value
CT chest findings			
• Mass	26 (81.3%)	54 (79.4%)	0.830
• Cavity	3 (9.4%)	5 (7.4%)	0.728
• Collapse	3 (9.4%)	3 (4.4%)	0.330
• Military pattern	2 (6.3%)	4 (5.9%)	0.942
• Ground glass opacities	2 (6.3%)	6 (8.8%)	0.658
• Consolidation	1 (3.1%)	7 (10.3%)	0.218
• Mediastinal lymhadenopathy	31 (96.8%)	67 (98.6%)	0.581
• Pleural effusion	7 (19.4%)	16 (24.6%)	0.854
Cell type			0.640
• Adenocarcinoma	23 (71.9%)	48 (70.6%)	
• Squamous cell carcinoma	5 (15.6%)	6 (8.8%)	
• Large cell carcinoma	0 (0.0%)	2 (2.9%)	
• Small cell lung cancer	4 (12.5%)	11 (16.2%)	
• Carcinoid tumor	0 (0.0%)	1 (1.5%)	
Stage			0.992
• I	1 (3.1%)	2 (2.9%)	
• II	2 (6.3%)	5 (7.4%)	
• III	6 (18.8%)	14 (20.6%)	
• IV	23 (71.9%)	47 (69.1%)	

by BAL direct microscopy and culture. Additionally, they measured Aspergillus galactomannan (GM) antigen in serum and BAL samples. Culture positivity was detected in 69.5% of antigen-positive patients.

The diagnostic accuracy of serum and BAL GM in patients with reduced immunity suspected of having invasive aspergillosis is reasonable (sensitivities and specificities in the range of 0.80), which is higher than the 30 to 50% sensitivity of cultures of the respiratory samples, but cultures have the advantage of susceptibility testing and growth of other molds, such as Mucorales, which do not give positive GM or (1–3)-b-D-glucan (BDG) results [28, 29].

Apostolou et al. [30] studied the presence of bacterial and fungal microflora in surgically removed lung tissue of patients with lung cancer by using PCR methods and special primers; Mycoplasma strains were identified in all samples, followed in descending frequency by Staphylococcus epidermidis, Streptococcus mitis, Bacillus strains, Chlamydia, Candida, Listeria, and Haemophilus influenzae.

PCR is another extremely accurate way to diagnose invasive fungal infection by amplifying fungal DNA in formalin-fixed paraffin-embedded tissue or BAL fluid and serum with sensitivities and specificities reaching 90%. However, PCR is available only in highly specialized laboratories and cannot be used as a routine clinical test [29, 31].

Our study revealed that the frequency of male sex, current or ex-smoking, and COPD were significantly higher for studied lung cancer patients with positive fungal colonization versus patients with negative fungal growth.

In agreement with our results, Laroumagne et al. [10] analyzed the association between the patients' characteristics and microbial colonization [bacterial and fungal pathogens] in 210 lung cancer patients, and they found that aged lung cancer patients [mean age 61.9, ($p = 0.02$) with COPD ($p = 0.008$) were significantly more frequently colonized. They also found that males were more at risk for colonization than females but without statistical significance (81.2% versus 18.8%. $p = 0.14$). On the other hand, Carpagnano et al. [12] found no difference according to sex, smoking habit, pack-years, time since quitting smoking when analyzing lung cancer patients with fungal colonization versus those with negative fungal culture ($p > 0.05$).

In comparison to lung cancer cases with negative fungal culture, detection of fungal colonization was more common with increasing severity of clinical presentation: higher grades of dyspnea (grade 1 in patients with lung cancer without proven fungal colonization vs. grade 2 in patients with lung cancer and fungal colonization, p 0.001) and a higher cough score [score 1 in those without fungal colonization versus score 3 in patients with fungal colonization, p 0.001]. This could be explained by the higher frequency of colonization in those who were smokers and/or had COPD.

Impairment of mucociliary clearance either by smoking or secondary to increased luminal mucus in COPD promotes adhesion of pathogenic organisms to the airway epithelia, affects the function of pulmonary host defense cells and encourages microbial colonization [32, 33].

Also, this study observed that severity of chest pain is higher in lung cancer cases with fungal colonization (p 0.001). Patients with invasive pulmonary aspergillosis may present with pleuritic chest pain if there is vascular invasion by aspergillus hyphae causing thromboses and small pulmonary infarcts [31]. Although in our cases, there was no microscopic evidence of tissue invasion by the detected hyphae, we mentioned before that the specimen taken might be away from the site of tissue invasion, and this highlights the necessity of finding other ways to prove mycological evidence of infection besides culture and biopsy, like using GM antigen index

defined as > 0.5 in plasma/serum and/or GM antigen > 0.8 in BAL according to recent revised definitions of invasive fungal infection [34].

Our results showed increased severity of hemoptysis in the studied lung cancer cases with positive fungal colonization. Although hemoptysis may result from bronchogenic carcinoma, endobronchial aspergilloma might have been implicated in hemoptysis by stimulation of vascular endothelial growth factor (VEGF) [35, 36]. The new vessels associated with chronic inflammatory or tumor pro-angiogenesis are usually thin-walled and fragile and thus prone to rupture into the airways, causing hemoptysis [37].

Our results showed that the mean age of the 68 patients who had positive fungal growth was comparable to the 32 patients with negative fungal growth (56.25 ± 10 versus 59.26 ± 11.33, $P = 0.185$). Both groups are also comparable as regarding the frequency of comorbidities ($p = 0.458$), total leucocyte count and its differentiation [$p = 0.227$], the detected CT chest abnormalities ($p = 0.705$), tumor pathology ($p = 0.694$), and staging ($p = 0.694$).

The results of our study were matched with most of the studies analyzing microbial colonization in lung cancer and found no statistically significant association between culture-positivity and the presence of any of the risk mentioned above factors ($p > 0.05$) [12, 14, 15].

Establishing specific radiologic criteria suggestive of fungal infection in lung cancer cases is difficult as sometimes fungal infection can present with radiological features such as lung nodules or masses indistinguishable from bronchogenic carcinoma [38].

This study's limitations include the dependency on BAL fungal culture only without accompanying it with using BAL and serum GM and PCR for aspergillus detection or serum (1–3)-b-D-glucan (BDG) for candidiasis. The second limitation of this study is not culturing part of the tissue biopsy for fear of exhausting the sample and not leaving enough portions suitable for diagnosing the tumor. Also, because of this, we could not determine the species of the aspergillus hyphae found in the tissue biopsy. The third limitation was that we did not examine surgically removed lung tumors as the small biopsies taken by FOB may not target the site of fungal infiltration and give false-negative results. The fourth limitation is that we did not study the impact of fungal colonization of lung cancer patients on their survival. The last limitation is the small sample size used in our study.

We suggest the need for additional multicenter research on large sample size to evaluate the prevalence by using antigen detection like GM additionally and to determine the exact role of fungal colonization in lung cancer from 2 points; if it has a carcinogenic effect and also if it predisposes to active infection affecting prognosis and survival.

Conclusion

In conclusion, our prospective study showed that fungi were isolated from 68% of studied patients with lung cancer with higher frequency among males, smokers, and those having associated COPD. The severity of hemoptysis was more in those with fungal colonization.

Abbreviations

BA	Bronchial asthma
BAL	Bronchoalveolar lavage
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
DM	Diabetes mellitus
EORTC/MSG	European Organization for Research and Treatment of Cancer/Mycosis Study Group
FOB	Fiberoptic bronchoscopy
GM	Galactomannan
H&E	Hematoxylin and eosin
HTN	Hypertension
VEGF	Vascular endothelial growth factor

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Authors' contributions

El-B. M.K. contributed in the conception and design of the study. A. A., A. R. E., A. E. E., and R. A. E. contributed in the acquisition of data, analysis and interpretation of data. El-B. M.K. and R. A. E.: contributed in drafting the article, revising it critically for important intellectual content. El-B. M.K. contributed in final approval of the version to be submitted.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The Institutional Research Board of the Faculty of Medicine, Mansoura University approved the study protocol (code no.: MS/353) and written informed consents were obtained from patients before enrollment in the study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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