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RESEARCH ARTICLE
KLİNİK ÇALIŞMA

Fungal culture results and clinical effects of bronchoscopy specimens

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ABSTRACT

Fungal culture results and clinical effects of bronchoscopy specimens

Introduction: *Early isolation of the fungal pathogen and early initiation of treatment affect mortality and morbidity rates. The purpose of this study was to reveal the frequency of determination of fungal pathogens in bronchoscopy unit patients.*

Materials and Methods: *The study was designed retrospectively. All patients who underwent bronchoscopy for any reason were enrolled. The patients with suspected fungal infection were divided into three groups after the procedure:*

1) Proven fungal infection,

2) Colonization,

3) Without infection.

Results: *One thousand one hundred and twenty-eight patients were included in the study. Fungal infection was suspected in 188 (16.7%) patients before bronchoscopy. After the examination of the bronchoscopic materials, it was determined that 59 (5.2%) patients had proven fungal infection, 148 (13.1%) patients had colonization, and 921 (81.7%) patients did not have fungal infection. The radiological findings of the patients that were indicative of fungal infection before bronchoscopy were observed as consolidation in 391 (34.7%) and nodule in 413 (36.6%). Fungal growth in bronchoscopic cultures was found in 186 (16.4%) patients, and the most common fungus was Candida albicans (C. albicans) in 110 (9.7%). The treatment was not changed according to the culture results in the patients. No treatment was initiated in the other 108 (98.2%) patients with C. albicans. One hundred and sixty-five (88%) of all fungal growths were detected in the BAL/bronchial lavage fluid. While 29 (45%) of them had not received antifungal treatment before, antifungal treatment was started after bronchoscopy.*

Conclusion: *C. albicans was isolated the most among all bacterial and fungal agents in all patient groups that were immunosuppressed or not at a routine bronchoscopy unit. Diagnostic bronchoscopic sampling should be performed at the early stages of clinically or radiologically suspected fungal illness.*

Key words: *Antifungal agents; bronchoscopic procedures; fungal infection; outcome*

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ÖZ

Bronkoskopi örneklerinin mantar kültür sonuçları ve klinik etkileri

Giriş: *Fungal patojenin erken izolasyonu ve tedaviye erken başlanması mortalite ve morbidite oranlarını etkiler. Bu çalışmanın amacı, bronkoskopi ünitesi hastalarında fungal patojenlerin saptanma sıklığını ortaya koymaktır.*

Materyal ve Metod: *Çalışma retrospektif olarak tasarlandı. Herhangi bir nedenle bronkoskopi yapılan tüm hastalar çalışmaya alındı. İşlem sonrası mantar enfeksiyonu şüphesi olan hastalar üç gruba ayrıldı:*

1) Kanıtlanmış mantar enfeksiyonu,

2) Kolonizasyon,

3) Enfeksiyon yok.

Bulgular: *Bin yüz yirmi sekiz hasta çalışmaya dahil edildi. Bronkoskopi öncesi 188 (%16.7) hastada mantar enfeksiyonundan şüphelenildi. Bronkoskopi öncesi fungal enfeksiyon belirtisi olan hastaların radyolojik bulguları 391'inde (%34.7) konsolidasyon, 413'ünde (%36.6) nodül olarak izlendi. Bronkoskopik materyallerin incelenmesi sonucunda 59 (%5.2) hastada kanıtlanmış mantar enfeksiyonu, 148 (%13.1) hastada kolonizasyon ve 921 (%81.7) hastada fungal enfeksiyon olmadığı belirlendi. Bronkoskopik kültürlerde mantar üremesi 186 (%16.4) hastada saptandı ve en sık görülen mantar 110 (%9.7) hastada Candida albicans (C. albicans) idi. Hastalarda kültür sonuçlarına göre tedavi değiştirilmedi. C. albicans'lı diğer 108 (%98.2) hastada ise herhangi bir tedavi uygulanmadı. Tüm mantar üremelerinin 165'i (%88) BAL/bronş lavajı sıvısında saptandı, 29'u (%45) daha önce antifungal tedavi almamışken, bronkoskopi sonrası antifungal tedavi başlandı.*

Sonuç: *Rutin bronkoskopi ünitesinde immünsüprese olan veya olmayan tüm hasta gruplarında bakteriyel ve fungal etkenler arasında en fazla C. albicans izole edildi. Tanısal bronkoskopik örnekleme, klinik veya radyolojik olarak şüpheli mantar hastalığının erken evrelerinde yapılmalıdır.*

Anahtar kelimeler: *Antifungal ajanlar; bronkoskpik prosedürler; fungal enfeksiyon; sonuç*

INTRODUCTION

Fungi are divided into two main groups as endemic infections in humans and opportunistic infections developing in immunosuppressed patients. Fungal infections of the lungs are much rarer than bacterial and viral infections. Fungi are generally considered when antimicrobial and antiviral treatments are not a solution for differential diagnosis. However, opportunistic fungal infections have become more visible due to the increase in the number of immunocompromised patients as a result of treatments frequently applied in recent years (1,2). *Candida* spp. and *Aspergillus* spp. are the most frequently isolated microorganisms in immunosuppressed patients (3). While candidiasis is the most common in intensive care units (ICUs) and low-birth-weight infants, aspergillosis is observed more in hematological malignancy and bone marrow transplant patients (4). Early isolation of the agent and early initiation of treatment significantly affect the mortality and morbidity of the diseases. Flexible bronchoscopy is an important procedure in the diagnosis of fungal infections of the lung, as it allows us to directly see and exemplify lesions in the endobronchial system. Flexible bronchoscopy is a relatively safe and minimally invasive method which is generally tolerated well by patients. Diagnosis is made by microbiological and cytopathological examination of the bronchoalveolar lavage fluid, mucosal brushing and mucosal biopsy samples (5,6). Nevertheless, it is a time-consuming process to establish a definitive diagnosis of fungal infection as a result of microbiological and pathological examinations. Early treatment is very important in groups of patients with fungal infections. The presence of endobronchial lesions, which are frequently observed in fungal infections, may guide clinicians towards starting empirical antifungal therapy (7). The most frequently observed endobronchial pathologies in fungal infections may be listed as tracheobronchial stenosis, mucosal edema, mucosal irregularity, mucosal ulcers, yellow-white discharges, white nodular lesions, white patches and gray-black pigmentations (7). Conversely, a fungal infection may be present in patients who do not have these as endobronchial lesions.

In the light of all this information, the purpose of our study was to reveal the frequency of determination of fungal pathogens in bronchoscopy unit patients with minimally invasive pulmonary sampling with various indications and present the characteristics of the cases in which these pathogens are considered as infectious agents and their effects on patient treatments.

MATERIALS and METHODS

The Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guideline was used as a guide for this manuscript (8). Our study was planned as a retrospective examination of the patient records of the Faculty of Medicine, Pulmonary Medicine Clinic, Bronchoscopy Unit.

All patients with and without immunosuppression who underwent bronchoscopy for any reason between January 2014 and December 2018 were included in the study. All bronchoscopy procedures were performed with the patient's signed consent. None of the patients had serious agitation, convulsion, increased intracranial pressure, severe bronchospasm, severe arrhythmia or problems such as hypoxemia (PaO_2< 50mmHg) despite the administration of 100% O_2 or severe pulmonary hypertension that would constitute a contraindication to the bronchoscopy procedure. These data were obtained from the patient's outpatient anamnesis records. Platelet transfusion was applied to patients with comorbidity that would lead to thrombocytopenia, especially hematological malignancy, to provide the minimum count of platelets required before the procedure, since the number of platelets should be minimum 20.000 mm^3 for BAL (bronchoalveolar lavage) and 50.000 mm^3 for biopsy.

The patients consisted of those admitted to our bronchoscopy unit as outpatients and inpatients consulted from the wards.

The patient's demographic data, clinical characteristics, laboratory data, indications for bronchoscopy, comorbid diseases, medications they used, radiological image detected in computed tomography of the thorax before bronchoscopy, bronchoscopy procedure (bronchoalveolar lavage, bronchial lavage, brushing, mucosal biopsy), symptoms of fungal infection before the process, images that may be related to fungal infection in the gross image (nodular lesion, pseudo-membrane, white patches, sticky sputum, etc.) during bronchoscopy, possible fungal species in the samples, other bacterial factors in the samples, pathological diagnosis and the change of treatment based on the result were saved. Samples taken from bronchoalveolar lavage were sent to the microbiology laboratory and studied. Bronchoalveolar lavage and brush samples were centrifuged for bacterial agents and gram staining was done. For culture, they were inoculated on blood agar, chocolate agar, MacConkey, and EMB agar. It was incubated at 35-37 degrees in 5% CO_2 for 48-72 hours. Growths of $>10^4$ in BAL and $>10^3$ in the protected brush were reported with the antibiotic susceptibility test. 10-30% KOH, KOH-DMSO (Dimethyl sulfoxide) or KOH chalcophor white was used for microscopic examination, especially if fungi were considered as agents in the patient. In addition, gram staining for each sample was examined for fungi. If *P. jiroveci* was considered as the causative agent, immunofluorescent staining or Giemsa stain was used. Fungal culture details are studied from samples after each bronchoscopy. Bloody or pus-containing parts of BAL cultures were directly planted. Cultures more than 2 mL were planted after centrifugation (1500-2000 g for 10 min). Highly mucoid specimens were treated with mucolytic agent before planting. Bronchial brush culture was vortexed in a small amount of distilled water/SF, then it is plated for 3-5 minutes on each medium. Samples were plated on SDA (sabouraud dextrose agar) (with cycloheximide, without cycloheximide) medium and incubated at 30 and 35 degrees aerobically for a minimum of four weeks. If *Histoplasma capsulatum* was suspected, cultures were incubated for 6-8 weeks. Galactomannan antigen test was used for rapid diagnostic test from BAL in patients with a pre-diagnosis of *Aspergillus*.

Patients who had immunosuppressive disease, received immunosuppressive therapy, had radiological findings such as cavity, nodule, and halo findings that could be seen in fungal infection, and whose radiological findings did not regress despite antibiotherapy, and who had fever unresponsive to antibiotherapy were considered clinically suspected for fungal infection. The patients with suspected fungal infection (risk factor, clinical criteria and supportive radiological findings) were divided into three groups after the procedure:

1) Proven fungal infection,

2) Colonization,

3) Without infection.

Each case was classified by two pulmonologists by evaluating all data of the patients. In our clinic, pre-diagnoses of fungal infections are confirmed by infectious disease consultations. It is based on the IDSA guide recommendations for the period.

1. Proven fungal infection: It was defined as demonstration of hyphae, yeast, tissue invasion with histo-

pathological, cytopathological or direct microscopic analysis of sterile lung specimens together with the compatible clinical picture.

This description is based on IDSA's Invasive Fungal Disease Consensus Definitions and performed according to infectious diseases consultations. In addition, galactomannan antigen was examined in blood and bronchoalveolar lavage fluids in cases with clinically and radiologically suspected invasive pulmonary aspergillosis (9). In the diagnosis of Aspergillosis; galactomannan antigen detected in plasma, serum, BAL, or CSF (cerebrospinal fluid). Any 1 of the following: 1. Single serum or plasma: ≥1.0, 2.BAL fluid: ≥1.0, 3. Single serum or plasma: ≥0.7 and BAL fluid ≥0.8, CSF: ≥1.0. Cases with histopathology/microscopy negative, galactomannan positive, clinical risk factors and compatible radiology were diagnosed as *Aspergillus* infection (9).

2. Colonization: It was defined as the growth of the fungal agent in samples taken by bronchoscopic procedures without creating any clinical picture.

3. Without infection: Clinically and microbiologically defined as no evidence of the presence of the agent.

Exclusion criteria in the study were patients who underwent bronchoscopic procedure but could not obtain samples for any reason, and patients younger than 18 years of age.

Statistics

The data were analyzed by using IBM SPSS V23. Compatibility with normal distribution was examined with Kolmogorov-Smirnov test. A Chi-squared test was used to compare the categorical variables according to the groups. Independent-samples t-test was used for the normally distributed data, and Mann-Whitney U test was used for the non-normally distributed data in the comparison of the quantitative variables according to the binary groups. The results of the analyses were presented as mean ± standard deviation and median (minimum-maximum) for the quantitative data and frequency (percentage) for the categorical data. The effects of the examined parameters on predicting fungal infection were investigated with a univariate and multivariate logistic regression analysis. ROC analysis was used to calculate the cut-off scores of continues values. The significance level was accepted as $p< 0.05$.

RESULTS

One thousand one hundred and twenty-eight patients who underwent bronchoscopy between the specified dates at our bronchoscopy unit were included in the study. Three hundred and ninety-three (393-34.8%) of the patients were females, seven hundred thirty five (735-65.2%) were males. Mean age of the female patients was 55.55 ± 15.57 years, while that of the male patients was 68.60 ± 15.01 years. Malignancy, interstitial lung disease (ILD), tuberculosis and bacterial infection were the top four most common causes of bronchoscopy. The most common comorbidities were asthma in 159 (14.1%) patients and diabetes mellitus in 149 (13.2%) patients. Seventy (6.2%) of the patients received chemotherapy, 4 (0.4%) received anti-TNF, 76 (6.7%) received corticosteroids, and 63 (5.6%) received other immunosuppressive therapies.

Fungal infection was suspected in 188 (16.7%) patients before bronchoscopy.

Radiological findings of all patients who underwent bronchoscopy showed consolidation in 391 (34.7%), cavity in 135 (12%), nodule in 413 (36.6%), cork spheroid in 6 (0.5%) and 6 (0%) halo sign. No patient had a crescent sign, and 14 (1.2%) patients did not have any of these signs. Radiological findings were not normal in any of the patients with proven fungal infection. Consolidation was observed in 29 (49%), cavity in 18 (30%), nodule in 22 (37%), fungus ball in 2 (3.3%) and halo in 4 (6.8%) patients with fungal infection.

One thousand one hundred and five (98%) BAL/bronchial lavage, 176 (15.6%) brushing and 160 (14.2%) mucosal biopsy operations were performed. While lesions that were observed endobronchially and thought to be related to fungal infection were detected as mucosal hyperemia in 51 (4.5%) patients, mucosal irregularity in 45 (4%) patients, white and sticky sputum in 28 (2.5%) patients, white plaques in 19 (1.7%) patients, nodular lesion on the mucosa in 17 (1.5%) patients and mucosal ulceration in 2 (0.2%) patients, pseudo-membrane was not observed in any patient.

Fungal growth in bronchoscopic cultures was found in 186 (16.4%) patients, and the most common fungus was *C. albicans* in 110 (9.7%). One hundred and sixty-five (88%) of all fungal growths were detected in the BAL/bronchial lavage fluid. The most common

Table 1. Fungus and bacteria types of the patients

	Number of patients			Number of patients	
Fungus	**n**	**%**	**Bacteria**	**n**	**%**
Candida albicans	110	9.75	*Pseudomonas aeruginosa*	101	9
Candida albicans	60	5.3	MRSA	20	1.8
Aspergillus fumigatus	3	0.265	MSSA	71	6.3
Aspergillus flavus	-	-	*Klebsiella pneumoniae*	28	2.5
Aspergillus niger	-	-	Enterococci	12	1
Pneumocystis jirovecii	13	1.1	*Acinetobacter baumanii*	15	1.3
Coccidioides immitis	-	-	*Escherichia coli*	25	2.2
Paracoccidioides brasiliense	-	-	*Atypical mycobacterium*	19	1.7
Cryptococcus neoformans	-	-	*Mycobacterium tuberculosis*	74	6.6
Mucorales	-	-	Other	366	32.4
Total Number	186	16.4		731	64.8
			Multiple reproductions of mo	213	18.9

MRSA: Methicillin-Resistant *Staphylococcus aureus*, MSSA: Methicillin-Sensitive *Staphylococcus aureus*, mo: Microorganism.

bacterial agent other than fungi observed in 101 (9%) patients was *Pseudomonas aeruginosa* (Table 1).

Cytopathological examinations revealed *Aspergillus* hyphae in the bronchoalveolar lavage fluid (BAL) of two patients, *Candida* in one patient, and *Pneumocystis jiroveci* hyphae in one patient. Fungal hyphae was detected in BAL of two patients, but the type was not specified. *Aspergillus* hyphae was observed in the mucosal biopsy examination of one patient and the transbronchial biopsy examination of one patient.

After the examination of the bronchoscopic materials, it was determined that 59 (5.2%) patients had a proven fungal infection, 148 (13.1%) patients had colonization, and 921 (81.7%) patients had without fungal infection. In the patients with clinical suspicion of fungal infection before the bronchoscopy procedure, the status of "fungal infection" or "colonization" as the final decision after the procedure is given in Table 2.

There was a statistically significant difference between the distribution of the final decision of fungal infection according to clinical suspicion status ($p< 0.001$). While the final decision was "fungal infection" in 11 (1.2%) of those without clinical suspicion, the final diagnosis decision was "fungal infection" in 48 (25.5%) of those with clinical suspicion. According to the clinical suspicion status, there was no statistically significant difference between the distributions of the final decision of "colonization" ($p> 0.05$). While there was no "colonization" in 157 (83.5%) of those with clinical suspicion, in 31 (16.5%), the "colonization" state was decided. According to culture positivity, there was a statistically significant difference between the distributions of final decisions of "fungal infection" and "colonization" ($p< 0.001$). While the final decision was a fungal infection in 27 (15.3%) of those with culture positivity, the final decision was found to favor "fungal infection" in 32 (3.4%) of the culture-negative ones.

While the median of the galactomannan level in the whole group was 0.39 (IQR:0.245-0.70) ng/mL, this value was 0.5 (IQR: 0.28-1.96) ng/mL fungal in the group diagnosed with a fungal infection and 0.355 (IQR: 0.232-0.532) ng/mL in the non-infected group ($p= 0.028$).

The consolidation, cavitary lesion, fungus ball and halo findings were significantly higher in the patients with fungal infection than in the patients without infection (Table 3).

While 29 (45%) of them had not received antifungal treatment before, antifungal treatment was started after bronchoscopy. The treatments initiated were voriconazole in 19 (65.6%) patients, amphotericin B in 1 (3.4%) patient and fluconazole in 9 (31%) patients. While the same drug was continued in 17 (56.7%) of the patients who had received antifungal treatment before, the drugs were changed for 13 (43.3%) of them.

Table 2. The effect of clinical-bronchoscopic suspicion and culture results on diagnosis

		Clinical Suspicion for Fungal Infection		
Final Diagnosis for Fungus		**No**	**Yes**	**p**
Fungal Infection (n= 59)	No	929 (98.8%)	140 (74.5%)	<0.001
	Yes	11 (1.2%)	48 (25.5%)	
Colonization (n= 148)	No	823 (87.6%)	157 (83.5%)	0.167
	Yes	117 (12.4%)	31 (16.5%)	
		Bronchoscopic Suspicion for Fungal Infection		
		No	**Yes**	
Fungal Infection (n= 59)	No	1058 (94.9%)	11 (84.6%)	0.098
	Yes	57 (5.1%)	2 (15.4%)	
Colonization (n= 148)	No	970 (87%)	10 (76.9%)	0.285
	Yes	145 (13%)	3 (23.1%)	
		Culture Positivity for Fungal Infection		
		No	**Yes**	
Fungal Infection (n= 59)	No	919 (96.6%)	150 (84.7%)	<0.001
	Yes	32 (3.4%)	27 (15.3%)	
Colonization (n= 148)	No	951 (100%)	29 (16.4%)	<0.001
	Yes	---	148 (83.6%)	

Table 3. Comparison of radiological findings of groups with and without fungal infection

	Fungal Infection		
CT Findings	**None n= 1069**	**Present n= 59**	**p**
Normal	14 (1.3%)	---	0.381
Consolidation	362 (33.8%)	29 (49%)	**0.012**
Cavity	117 (10.9%)	18 (30%)	**<0.001**
Nodule	391 (36.5%)	22 (37%)	0.831
Fungus Ball	4 (0.4%)	2 (3.3%)	**0.002**
Halo Finding	2 (0.2%)	4 (6.8%)	**<0.001**
Air Crescent Sign	-	-	-

When the patient groups were analyzed in terms of comorbidities, it was observed that the rate of fungal infections was significantly higher in patients with chronic renal failure, HIV, hematological malignancy, organ transplantation and inherited immunodeficiency compared to those without (Table 4).

Ten (17%) of the patients diagnosed with fungal infection had lesions in the endobronchial system that could cause a suspected fungal infection. The incidence of only white plaques among these lesions was significantly higher than the non-infected group (p= 0.037) (Table 5).

Again, looking at the treatments they received, some drugs were significantly associated with the development of fungal infections. These were especially chemotherapy medicines (p< 0.001) in 27 (46.6%) patients and corticosteroids (p< 0.001) in 11 (19%) patients.

We enrolled comorbidities, radiological findings, drugs used by the patients and laboratory parameters in the univariate and multivariate analyses. Among the laboratory parameters, sedimentation, CRP and galactomannan were statistically significant in the fungal infection group (p< 0.001). On the other

Table 4. Comparison of comorbidities of groups with and without fungal infection

	Fungal Infection		
Comorbidities	**None n= 1069**	**Present n= 59**	**p**
Asthma	155 (14.5%)	4 (6.8%)	0.097
DILD	9 (0.8%)	0 (0.0%)	0.483
Chronic Kidney Disease	37 (3.5%)	6 (10.2%)	**0.008**
Diabetes Mellitus	145 (13.6%)	4 (6.8%)	0.145
HIV	3 (0.3%)	2 (3.4%)	**<0.001**
Lung Malignancy	22 (2.1%)	2 (3.4%)	0.474
Hematological Malignancy	61 (5.7%)	31 (52.5%)	**<0.001**
Chronic Liver Disease	9 (0.8%)	0 (0.0%)	0.483
Organ Transplantation	8 (0.7%)	6 (10.2%)	**<0.001**
Inherited Immunodeficiency	0 (0.0%)	1 (1.7%)	**<0.001**
Connective Tissue Disease	49 (4.6%)	1 (1.7%)	0.303

DILD: Diffuse infiltrative lung disease, HIV: Human immunodeficiency virus.

Table 5. Endobronchial lesion properties of groups with and without fungal infection

	Fungal Infection		
Bronchoscopic Findings	**None n= 1069**	**Present n= 59**	**p**
Ulcer	2 (0.2%)	0 (0.0%)	1.000
Nodular Lesion	17 (1.6%)	0 (0.0%)	0.329
Pseudo-membranes	-	-	-
White Patches	16 (1.5%)	3 (5.1%)	**0.037**
Sticky Mucus	26 (2.4%)	2 (3.4%)	0.645
Mucosal Hyperemia	46 (4.3%)	4 (6.8%)	0.650
Mucosal Irregularity	44 (4.1%)	1 (1.7%)	0.560

hand, sedimentation was excluded from the multivariate analyses due not having a good AUC-ROC score for the cut-off value (AUC-ROC 0.590 (95% CI: 0.493–687), p= 0.067). For CRP, 3.95 mg/dL was determined as the cut-off value (AUC-ROC 0.764 (95% CI: 0.706–822), p< 0.001). For galactomannan, 0.415 ng/mL was determined as the cut-off value (AUC-ROC 0.635 (95% CI: 0.507–764), p= 0.028). The results of the univariate analyses for predicting fungal infection are shown in Table 6. Multivariate analyses were performed with parameters which were statistically significant in the univariate analyses (Table 7). Although the Omnibus test scores were p< 0.001, the R-squared values were 0.347 (34.7%) in model summary, percentage correction was 93.8% before the multivariate analyses and increased minimally (93.9%) after the multivariate model.

DISCUSSION

Diagnosing fungal infections is quite difficult, especially in cases where the focus is considered to be the lung. Flexible bronchoscopy is an important sampling method in identifying potential pathogens in selected patients (10). This study aimed to reveal the frequency of detection of fungal pathogens and the characteristics of the cases in which these pathogens were considered as infectious agents in patients undergoing invasive pulmonary sampling with various indications. Fungal infection was diagnosed in only 59 (5.2%) of the 1128 patients who underwent bronchoscopy with the most common pre-diagnoses of malignancy, diffuse interstitial lung disease, tuberculosis and bacterial infection in our study. The pre-diagnosis of fungal infection was present in 188

Table 6. Univariate analyses for predictingfungal infection

Parameters		OR	95% CI	p
Laboratory	CRP higher (3.95 mg/dL)	6.549	3.607 - 11.89	**<0.001**
	Galactomannan (higher 0.415 ng/dL)	1.925	0.826 - 4.49	0.129
Thorax CT	Consolidation	1.634	0.964 - 2.77	0.068
	Cavitary lesion	0.990	0.44 - 2.225	0.980
	Nodule	0.688	0.386 - 1.225	0.204
	Fungus ball	3.669	0.422 - 31.917	0.239
	Halo sign	3.669	0.422 - 31.917	0.239
Lesion in Bronchoscopy	White plaque	1.007	0.132 - 7.672	0.995
	Sticky white sputum	1.408	0.326 - 6.077	0.647
	Mucosal hyperemia	0.359	0.049 - 2.642	0.314
	Mucosal irregularity	1.824	0.631 - 5.273	0.268
Comorbidities	Asthma	0.429	0.153 - 1.2	0.107
	Renal diseases	3.158	1.276 - 7.811	**0.013**
	Diabetes mellitus	0.463	0.165 - 1.298	0.143
	HIV	12.468	2.043 - 76.102	**0.006**
	Pulmonary Malignancy	1.670	0.383 - 7.276	0.495
	Hematologic malignancy	18.295	10.319 - 32.436	**<0.001**
	Organ failure	15.014	5.029 - 44.829	**<0.001**
	Connective tissue disease	0.359	0.049 - 2.645	0.315
Medical Drug	Chemotherapy	20.132	11.091 - 36.543	**<0.001**
	Corticosteroid	3.540	1.755 - 7.139	**<0.001**
	Other immunosuppressives	2.892	1.308 - 6.392	**0.009**

Table 7. Multivariate analyses to predict fungal infection

Parameter		OR	95% CI	p
Laboratory	CRP	3.700	1.891 - 7.237	**<0.001**
Comorbidities	Renal diseases	2.971	1.002 - 8.807	**0.05**
	HIV	53.153	6.465 - 437.08	**<0.001**
	Hematologic malignancy	3.271	1.276 - 8.384	**0.014**
	Organ failure	3.976	1.013 - 15.602	**0.048**
Medical Drug	Chemotherapy	5.693	2.139 - 15.150	**<0.001**
	Corticosteroid	2.571	1.007 - 6.561	**0.048**
	Other immunosuppressives	1.188	0.409 - 3.453	0.751

(16.7%) of all patients before bronchoscopy. Among these patients with a pre-diagnosis of fungal infection, 48 (25.5%) were diagnosed with fungal infection. This may be explained by the fact that fungal infections are not clinically, radiologically and laboratory-specific, and they are often difficult to distinguish from bacterial infections. Considering the general population, pulmonary fungal infections are seen less frequently than bacterial and viral respiratory infections. However, with the increasing number of immunocompromised patients in recent years, the incidence of pulmonary fungal infections has increased. On the other hand, increased awareness of these infections and laboratory methods developed for diagnosis are increasingly related (11).

In opportunistic fungal infections (except for cryptococcal pneumonia in HIV patients), fungal cultures of bronchoalveolar lavage (BAL) fluid are neither specific nor sensitive. Moreover, since fungi such as *Aspergillus* spp., the *Mucorales* and *Candida* spp. may be found in the flora of the gastrointestinal tract when the clinical condition of the patient is not clear, there may be difficulties in distinguishing between infection and colonization (12). In our study, fungal growth was observed in a total of 177 (15.6%) patients among the bronchoscopic samples of 1128 patients. The most common fungal agent was *C. albicans* in 110 (9.75%) patients. In a study, the most common microorganism in the bronchoscopic lower respiratory tract samples of 488 patients has been found as *Candida non-albicans* (13). In a study conducted in Italy, 40% of candidemia attacks have been found to be related to *C. albicans*, followed by *C. parapsilosis* (23%), *C. glabrata* (15%), *C. tropicalis* (9%) and other species (13%) (14). Unlike our study, the high rates in the aforementioned study may be evaluated as the study population being composed of intensive care patients. *Candida* pneumonia may rarely develop with invasion of *Candida* species into the lung parenchyma. Candidiasis in the lung occurs in two forms: primary pneumonia is the aspiration of oropharyngeal secretions containing *Candida*, secondary pneumonia is pneumonia caused by hematogenous dissemination of candidiasis in immunocompromised patients (15).

Invasive fungal infections are associated with increased morbidity and mortality, and most *Candida* spp. develop depending especially on *C. albicans* (16). In fact, detection of *Candida* species in respiratory isolates is generally not important in nonclinical patients. Therefore, it will be correct to focus on *Candida* infection after evaluating all patients in terms of clinical features and other possible factors. The clinical history of patients is the most important parameter suggesting the diagnosis of fungal infection. When the patients applied to our bronchoscopy unit with many different indications in terms of comorbidities, it was observed that the fungal infection rates were significantly higher in the patients with chronic renal failure, HIV, hematological malignancy, organ transplantation and inherited immunodeficiency. Fungal infections were higher in the group receiving chemotherapy and corticosteroids. Similarly, Garnacho-Montero J et al. have defined neutropenia <500mg/m^3, hematological malignancy, lung transplantation, allogeneic stem cell transplant for fungal infections in critically ill patients as a high-risk group, and prolonged steroid therapy and systemic immunosuppressive therapies as a medium risk group (17).

In our study, 165 (88%) of all fungal growths were detected in the BAL/bronchial lavage fluids. It should be remembered that fiberoptic bronchoscopy is not a sterile procedure, and it can often detect organisms transmitted from the upper respiratory tract. As a result, all fungal agents that reproduce after routine bronchoscopy do not cause infection, and specific treatment is not started. In our study, only 27 (15%) of 177 patients with fungal culture positivity were diagnosed with fungal infection. On the other hand, only 32 (3.4%) of patients with culture-negative results were decided for fungal infection with clinical and radiological findings. The decision of fungal infection was made in 48 (25.5%) patients with clinical suspicion of fungal infection before the procedure. With these results, it may be stated that the decision of fungal infection made by the physician by evaluating the patient holistically with complementary clinical, radiological and laboratory parameters is at least as important as the results of the bronchoscopic culture. In our study, isolation of *C. albicans*, which is the fungus most frequently cultured, from the sputum, tracheal aspirate, BAL and even lung tissue, often represents colonization rather than infection (18). Therefore, clinical criteria have been developed for the diagnosis of invasive infection caused by *Candida* spp. Currently accepted diagnostic criteria require a positive blood culture, a positive culture from a sterile site (outside urine, sinuses or respiratory tract) or a histologically positive biopsy specimen. *Candida* spp. isolation only from BAL samples does not show invasive infection alone even in people with immune suppression (19). Another tool to diagnose fungal infection, especially aspergillosis, is antigen assays such as galactomannan and beta-D-glucan (20). In our study, the BAL galactomannan values of the patients were found to be significantly higher in the group diagnosed with fungal infection than the group that was not (p= 0.007). However, *Aspergillus* fumigatus was observed in only 3 (0.3%) of the patients, and no other growth agent from *Aspergillus* spp. was detected. Invasive pulmonary aspergillosis caused by *A. fumigatus* often occurs in patients with immunodeficiency. It is especially common in patients with hematological malig-

nancies, hematopoietic stem cells or solid organ transplants. Severe chronic neutropenia, high dose steroid use and impaired cellular immune responses are also among the risk factors. *A. fumigatus* is shown as the most common species of *Aspergillus* that reproduces in a wide series of 5589 diseases with hematopoietic stem cell transplantation (21). In our study, only one of the *Aspergillus* species, which was detected in the mixed patient population in the bronchoscopy unit, reproduced in one hereditary immunodeficiency patient, the other was detected in one patient with diabetes mellitus, and the third was in a patient without a comorbid status. Antifungal treatment was changed in one of these patients according to the bronchoscopy results, new antifungal treatment was started in the other patient, and the other was recommended to continue the current antifungal treatment. With this and other results of the study, flexible bronchoscopy appears to be very useful in clinical practice for invasive pulmonary sampling. So, to what extent does this tool direct the diagnosis and treatment of patients? As a result of our study, it was observed that while 29 (45%) patients had not been receiving antifungal treatment before, antifungal treatment was initiated after bronchoscopy, and while 17 (56.3%) of the patients who previously received antifungal treatment continued with the same drug, the drugs that were given were not changed in 13 (43.3%) of the patients. The treatments initiated were voriconazole in 19 (65.6%) patients, Amphotericin B in 1 (3.4%) patient and fluconazole in 9 (31%) patients. To initiate targeted therapy, we think that the effect of bronchoscopic sampling, which would be performed in immunosuppressed patient groups early (without developing respiratory failure), is important in the control of the disease and in survival. Radiological findings, which have an important role in the diagnosis of fungal infections, should also strengthen the early bronchoscopy decision. In our study, in thoracic tomography, findings of consolidation, cavitary lesion, fungus ball and halo sign were found to be significantly higher in patients with a diagnosis of fungal infection than in patients without fungal infection. In a relatively large study, the most common radiological finding was micronodular appearance (43%), and the second was consolidation (26%) (22). In another major study involving patients with and without neutropenia, it was observed that specific radiological findings (nodules and cavitation) were rare in lung tomography, and consolidation, ground-glass opacification and pleural effusion were more common (23).

The limitations of our study: Since the patients were retrospectively screened, the patient outcomes after follow-up were not recorded. Secondly, the low number of immunosuppressed patients indicated that there were not enough invasive sampling decisions in this patient group. Thirdly, the lack of an immunosuppressed patient group caused a low rate of fungal varieties that could be seen frequently as an agent in such cases. Finally, since the data were collected retrospectively, there was a possibility of data loss.

CONCLUSION

As a result, our study covered a wide range of patients involving routine procedures at a bronchoscopy unit and makes an important contribution to the limited data in this field. *C. albicans* was isolated the most among all bacterial and fungal agents in all patient groups that were immunosuppressed or not at a routine bronchoscopy unit. However, with this result, it was seen that there was no change in the treatment of the patients with *C. albicans*. Flexible bronchoscopy is an important diagnostic tool for isolating fungal agents. The fungus that does not cause disease in the normal patient population may cause progressive clinical conditions in immunosuppressed patients. For this reason, in our opinion, not an absolute result of the study but as an observation, diagnostic bronchoscopic sampling should be performed at the early stages of clinically or radiologically suspected fungal illness.

The most important limitation of our study is that it is a retrospective study. In retrospective studies, there may be difficulties in accessing some patient data. We believe that large-scale prospective studies on this subject will be beneficial because the data it will provide is very valuable.

Ethical Committee Approval: This study approval was obtained from KTÜ Faculty of Medicine Scientific Research Ethical Committee (Decision No: 2018/324, Date: 14.01.2019).

CONFLICT of INTEREST

The authors declare that they have no conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: All of authors

Analysis/Interpretation: All of authors

Data acqusition: All of authors

Writing: All of authors

Clinical Revision: All of authors

Final Approval: All of authors

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