Article

Invasive Pulmonary Aspergillosis in Lung Transplant Recipients: Retrospective Clinical Analysis from a Tertiary Transplant Center

Sibel Doğan Kaya[1], Yeşim Uygun Kızmaz[1], Aysu Türkmen Karaağaç[2]

[1]Infectious Diseases and Clinical Microbiology Department, Koşuyolu High Specialization Training and Research Hospital, 34865 Istanbul, Türkiye
[2]Pediatric Health and Diseases Department, Koşuyolu High Specialization Training and Research Hospital, 34865 Istanbul, Türkiye

AIM: Aspergillosis is the most common invasive fungal infection among lung transplant recipients (LTRs). Although its incidence is lower than that of bacterial or viral infections, it poses a similar or even higher mortality rate due to challenges in early diagnosis, limited treatment options, and various complications. Therefore, we aimed to evaluate the pulmonary aspergillosis cases in our tertiary lung transplant center.

METHODS: A retrospective analysis of 146 LTRs was performed. The demographic data, microbiological and histopathological test results, and radiological findings used for Aspergillus identification were recorded.

RESULTS: Aspergillus spp. was detected in 13 of 146 LTRs (9%), mean age 42.5 ± 14.06 years, an average of 18.9 months after lung transplantation. 3 cases (23%) had Aspergillus growth in tissue culture, and 2 (15.4%) showed fungal elements with septal hyaline fibrils in tissue pathology. Aspergillus spp Polymerase chain reaction (PCR) was positive in bronchoalveolar lavage of 8 (61.5%) cases. In addition, 4 (30.7%) cases had relevant tomography findings. The most common pathogens were A. Terreus (21%), A. Fumigatus (14%), and A. Flavus (14%). The mortality rate was 15%.

CONCLUSIONS: LTRs are at high risk of Aspergillus spp infections. Early diagnosis with microbiological, histopathological, and radiological tests, in addition to well-established prevention strategies, prophylaxis, and treatment will provide a better survival rate for patients.

Keywords: fungal infection; lung transplant recipient; invasive pulmonary aspergillosis

Introduction

Aspergillus spp. is a ubiquitous filamentous mold that can cause invasive pulmonary aspergillosis (IPA) by invading the pulmonary vasculature through the septate hyphae, mostly in immunocompromised individuals. IPA remains the most common fungal infection among lung transplant recipients (LTRs), resulting in significant morbidity and mortality [1]. Decreased mucociliary clearance, impaired cough reflex, continuous exposure of the lungs to the environment, and immunosuppression are the main risk factors that contribute to bacterial or fungal infections in LTRs [2]. In most cases of IPA, clinical symptoms are cough, pleuritic chest pain, or fever. Acute respiratory failure secondary to IPA is rare. Tracheobronchial aspergillosis affects almost exclusively LTRs and can lead to airway obstruction, bronchial ulcerations, and pseudomembrane formation. Bronchial anastomosis infection can cause detachment requiring aggressive combined medical and surgical treatment [3, 4]. We conducted this study of IPA cases in our tertiary lung transplant unit to focus on the importance of early diagnosis, prevention, and treatment for improvement of patient survival.

Materials and Methods

A retrospective analysis was performed to identify IPA cases among the 146 patients that received lung transplants in Kartal Koşuyolu Research and Training Hospital between January 2012 and June 2022. The age and gender of the patients, type and indication of lung transplantation, time interval between the lung transplantation and the IPA diagnosis, administered drugs, survival results, and the microbiological, pathological, and radiological findings were recorded from the computer database and clinical records. All patients included in the study were using mycophenolate mofetil, methylprednisolone, and tacrolimus as immunosuppressive drugs after transplantation. IPA diagnosis was made according to the standard recommendations of the American Society of Transplantation and the International Society for Heart and Lung Transplantation [5]. The 'probable' category of pulmonary fungal infection was assigned based on the presence of predisposing factors (prolonged neutropenia or transplantation); new or worsening endobronchial or radiological findings with new positive fungal culture; and/or new positive bronchoalveolar lavage (BAL) antigen testing, in particular galactomannan (GM), a

Correspondence to: Sibel Doğan Kaya, Infectious Diseases and Clinical Microbiology Department, Kosuyolu High Specialization Training and Research Hospital, 34865 Istanbul, Türkiye (e-mail: sibeldogankaya@yahoo.com).

major component of the Aspergillus cell wall, the circulating level of which is indicative of the fungal burden in the host. The "proven" category was assigned to patients who met all clinical, endobronchial, or radiological criteria and also had histology showing fungal hyphae in biopsy tissue (with or without microbiological criteria) [5, 6].

The serum GM, BAL GM antigen, and aspergillus Polymerase chain reaction (PCR) in BAL were examined. The ELITE MGB® Kit (RTS171ING, ELITechGroup S.p.A., Turin, Italy) with *Aspergillus* spp. and the ELITE In-Genius® instrument (F2102-000, ELITechGroup S.p.A., Turin, Italy), a quantitative real-time PCR with a target in 28S rDNA, were used. Aspergillus PCR range >10 copies/mL was considered positive. Serum GM (Platelia Aspergillus Ag® (62794, BioRad Laboratories, Hercules, CA, USA) and BAL GM (index value ≥1) levels were included in the data analysis. Serum GM index >0.5 and BAL GM index ≥1.0 were considered positive [7].

Continuous variables were presented as the median (min–max), and categorical variables were expressed as numbers and percentages. Informed consent was provided by all patients. Koşuyolu High Specialization Training and Research Hospital Ethics Committee approved the study (number 2022110/607 Date: 01.07.2022).

Results

Out of 146 lung transplants performed in our hospital between January 2012 and June 2022, *Aspergillus* spp was detected in 13 (9%) LTRs, comprising 5 females (38%) and 8 males (62%). The median age was 42.5 years (min 16–max 63 years). 12 lung transplants were bilateral (92%) and 1 (8%) was unilateral. The indications for lung transplantation were idiopathic pulmonary hypertension (n: 7, 54%), chronic obstructive pulmonary disease (n: 3, 23%), bronchiectasis (n: 2, 15%), and silicosis (n: 1, 8%). Aspergillus infection was detected an average of 18.9 months after transplantation. The most commonly identified pathogens in the BAL specimens were *Aspergillus terreus* (21%), *Aspergillus fumigatus* (14%), and *Aspergillus flavus* (14%). The mean BAL GM antigen result was 2.93 ng/mL (min: 1.7–max: 4.8) and the serum GM antigen level was 1.66 ng/mL (min: 0.4–max: 4.9). The mean BAL *Aspergillus* PCR value was 1860.875 copies/mL (min: 900–max: 3919). Although *Aspergillus* spp. growth was observed in tissue culture in 3 (23%) IPA cases, and fungal elements with septal hyaline fibrils could be observed in tissue pathology in only 2 (15.4%) patients. Concomitant radiological findings of aspergillosis including halo sign, cavitation, and ground-glass opacity were detected in the High-Resolution Computed Tomography (HRCT) of 4 (30.7%) IPA cases (Table 1). Of 13 LTRs 2 (15%) died in the first 3 months following transplantation.

Discussion

The present study showed that *Aspergillus* spp. infections should be suspected and diagnostic evaluations should be initiated without delay in the lung transplant units, in order to initiate antifungal treatment as soon as possible to increase patient survival. Lung transplantation has become one of the standard treatments providing high survival rates for patients with end stage pulmonary diseases such as emphysema, cystic fibrosis, interstitial pulmonary fibrosis, pulmonary arterial hypertension, bronchiectasis, sarcoidosis, obliterative bronchiolitis, and connective tissue disease [8]. 146 (48.1%) of the 303 lung transplants performed in our country since 2011 were performed in our hospital, which is a tertiary lung transplant center [9]. In our unit, the most common indications for lung transplantation were interstitial pulmonary fibrosis, chronic obstructive pulmonary disease, bronchiectasis, and cystic fibrosis. LTRs are more likely to experience invasive fungal infections, mostly by *Candida* spp. in the early and by *Aspergillus* spp. in the late postoperative period, compared with other solid-organ transplants [1, 10]. The median age of the LTRs in our study was 42.5 years (16–63 years). In a study by Weill D *et al.* [11], the median age of LTRs was 62 years, and 25.5% were older than 65 years. However, age over 65 years is a relative contraindication for lung transplant surgery in many countries.

In our study, the rate of pulmonary aspergillosis was 9% and the median time to diagnosis was 18.9 months. The rate and duration of mold infections were moderately low and our universal prophylaxis strategy is consistent with that of previous studies. In addition, the microbiological, histopathological and radiological examination facilities of our lung transplant center, the strict control of hand hygiene practices of healthcare workers, close follow-up of patients, and early diagnosis contributed to these outcomes. A multicenter cohort study has reported a 1-year cumulative incidence of *Aspergillus* spp. and other mold infections in LTRs of 8.6% [1]. In a multicentre cohort study among 1173 North American LTRs, the 12-month cumulative incidence of invasive aspergillosis was 4.1%. Although the highest incidence was reported within one year, most cases emerged within 2 years of transplantation [12]. Neofytos *et al.* [13] followed 13,289 LTRs in the Swiss cohort study and found an incidence of IPA of 8.3%, with a median time to diagnosis of 274 days. In another study, the rate of Invasive Fungal Infections (IFI) was 3.8% at 1 year, 7.6% at 3 years, and 10.1% after 5 years of transplantation [14].

We performed BAL on the 13 LTRs with signs of fungal infection including unexplained fever, increased acute phase reactants, and patchy pulmonary infiltrates despite ongoing medical treatment. The serum/BAL GM level was found above the normal limits in 7 cases (53.8%) and BAL PCR for *Aspergillus* spp. was positive in 8 patients (61.5%). In this study, the fact that our hospital received advanced laboratory examination services from another hospital negatively affected our ability to access the results of the patients and perform the examination on time. We have been performing all the necessary tests in our own laboratory for the last 5 years.

Table 1. General characteristics of the pulmonary aspergillosis cases.

Patient no	Age /gender	Pre transplant diagnosis	Type of lung transplant	Post transplant diagnosis time	Serum/BAL galactomannan level	BAL Aspergillus PCR	Tissue culture	Tissue pathology	Mortality	HRCT
1	63/F	Bronchiectasis	Bilateral	84 months	Neg	Not performed	Neg	Granulation tissue with 45 degree branching hyphaes	Survival	Nodular lesion
2	56/M	COPD	Bilateral	24 months	BAL: Neg Serum: 4.9 ng/mL	Not performed	*Aspergillus fumigatus*	No finding suggestive of fungal infection	Survival	No finding
3	34/F	IPF	Bilateral	3 months	Neg	Not performed	Neg	Fungal elements with septal hyaline fibrils	Ex	Pulmonary infiltrates unexplained with other reasons
4	59/M	IPF	Bilateral	1 month	Neg	900 copies/mL	Neg	No finding suggestive of fungal infection	Survival	No finding
5	39/M	Silicosis	Unilateral	24 months	Neg	Not performed	*Aspergillus flavus*	No finding suggestive of fungal infection	Survival	No finding
6	61/M	COPD	Bilateral	9 months	BAL: 3.2 ng/mL Serum: 1.4 ng/mL	3919 copies/mL	Neg	No finding suggestive of fungal infection	Survival	No finding
7	16/M	IPF	Bilateral	24 months	Neg	Not performed	*Aspergillus tereus*	No finding suggestive of fungal infection	Survival	Cavitation
8	60/F	IPF	Bilateral	1 month	BAL: 1.7 ng/mL Serum: 0.4 ng/mL	3571 copies/mL	Neg	No finding suggestive of fungal infection	Ex	No finding
9	46/M	IPF	Bilateral	9 months	BAL: 4.8 ng/mL Serum: 1.1 ng/mL	2018 copies/mL	Neg	No finding suggestive of fungal infection	Survival	No finding
10	27/F	IPF	Bilateral	9 months	BAL: 4.4 ng/mL Serum: neg	1118 copies/mL *A.Tereus*	Neg	No finding suggestive of fungal infection	Survival	No finding
11	28/F	IPF	Bilateral	36 months	Neg	955 copies/mL *A.Terreus*	Neg	No finding suggestive of fungal infection	Survival	Halo sign
12	34/M	IPF	Bilateral	21 months	BAL: 1.77 ng/mL Serum: neg	1347 copies/mL *A.Fumigatus*	Neg	No findingsuggestive of fungal infection	Survival	Ground glass opacities
13	38/M	COPD	Bilateral	1 month	BAL: 1.73 ng/mL Serum: 0.5 ng/mL	1059 copies/mL *A. Flavus*	Neg	No finding suggestive of fungal infection	Survival	No finding

IPF, Idiopatic pulmonary fibrosis; COPD, Chronic Obstructive Pulmonary Disease; HRCT, High-Resolution Computed Tomography; BAL, bronchoalveolar lavage; Neg, Negative; M, male; F, female; PCR, Polymerase chain reaction.

Aspergillus spp. is a ubiquitous organism mostly found in sputum or BAL samples. 78% of invasive aspergillosis is limited to the lungs. Due to the immunosuppressive drugs, decreased mucociliary motility, colonization, and other risk factors, mold infections most commonly affect LTRs [15]. In airway specimens of LTRs, *Aspergillus* spp. can be detected in 25%–30% of cases [16]. In the diagnosis of IPA infection among LTRs, the sensitivity of BAL GM ranges from 67%–100%, while the specificity ranges from 89%–93% [17].

In several studies the most frequently isolated species from clinical specimens are *A. fumigatus*, *A.flavus*, *A. niger*, and *A.terreus*. In the present study, the most commonly isolated pathogens from BAL specimens were *A. terreus* (21%), followed by *A. fumigatus* (14%) and *A. flavus* (14%).

In our study, HRCT findings detected in 4 patients (30.7%) were halo sign (n: 1, 25%), nodular lesions (n: 1, 25%), cavitation (n: 1, 25%), and undefined patchy infiltration (n: 1, 25%).

The HRCT features of IPA have comparable specificity and sensitivity to commonly used serum tests. Therefore, HRCT analysis can help detect IPA at an early stage, guide clinical intervention, and reduce mortality and morbidity from IPA [18]. Bilateral bronchial wall thickening and tree-patterned centrilobular opacities in the bud were reported as the most common radiological findings in the LTR cohort of Gazzoni *et al.* [19].

There is no well-established consensus on the prevention strategies, use of antifungal prophylaxis, choice of antifungal drug(s), route of administration, and duration of treatment in the majority of lung transplant centers. One of the most widely used preventive strategies against the development of IPA is antifungal prophylaxis. Other strategies include targeted prophylaxis and preventive therapy [20].

We administered voriconazole and inhaled liposomal amphotericin B, both universally used for antifungal prophylaxis [21], for at least 4 weeks during hospitalization. Oral maintenance therapy of voriconazole was continued for 6 months after discharge. Antifungal treatment was chosen according to renal or hepatic status and drug-drug interactions.

The mortality rate of *Aspergillus* infection in the LTRs varies according to the clinical presentation, ranging from 23%–29% in patients with tracheobronchitis, to as high as 67%–82% in patients with IPA [22]. In a recent study, survival has increased to 78% [23]. In the present study, 2 patients died (15%) and the survival rate was 85%.

Conclusions

Lung transplant recipients are at high risk of opportunistic infections, especially of those *Aspergillus* spp. Early diagnosis using microbiological, histopathological, and radiological diagnostic tests, in addition to close follow-up, well-established prevention strategies, antifungal prophylaxis, and treatment will provide a better survival rate for these patients.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Author Contributions

Concept—SDK, YUK, ATK; Design—SDK, YUK ; Supervision—SDK, YUK; Data Acquisition and Processing—SDK, YUK; Analysis and Interpretation—SDK, YUK; Literature Search—SDK, YUK; Writing Manuscript—SDK, YUK, ATK; Critical Reviews—SDK, YUK, ATK. All authors revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The Ethical Committee of Koşuyolu High Specialization Training and Research Hospital Ethics Committee approved the study the present study protocol number (2022110/607 Date: 01.07.2022). The consent of the patients was taken prior to the writing of the manuscript. The study is in accordance with the Declaration of Helsinki.

Acknowledgment

Not applicable.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, *et al*. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America. 2010; 50: 1101–1111.

[2] Mattner F, Fischer S, Weissbrodt H, Chaberny IF, Sohr D, Gottlieb J, *et al*. Post-operative nosocomial infections after lung and heart transplantation. The Journal of Heart and Lung Transplantation: the Official Publication of the International Society for Heart Transplantation. 2007; 26: 241–249.

[3] Fernández-Ruiz M, Silva JT, San-Juan R, de Dios B, García-Luján R, López-Medrano F, *et al*. Aspergillus tracheobronchitis: report of 8 cases and review of the literature. Medicine. 2012; 91: 261–273.

[4] Humar A, Michaels M, AST ID Working Group on Infectious Disease Monitoring. American Society of Transplantation recommendations for screening, monitoring and

reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2006; 6: 262–274.

[5] Husain S, Mooney ML, Danziger-Isakov L, Mattner F, Singh N, Avery R, *et al*. A 2010 working formulation for the standardization of definitions of infections in cardiothoracic transplant recipients. The Journal of Heart and Lung Transplantation: the Official Publication of the International Society for Heart Transplantation. 2011; 30: 361–374.

[6] Sherif R, Segal BH. Pulmonary aspergillosis: clinical presentation, diagnostic tests, management and complications. Current Opinion in Pulmonary Medicine. 2010; 16: 242–250.

[7] Imbert S, Meyer I, Palous M, Brossas JY, Uzunov M, Touafek F, *et al*. *Aspergillus* PCR in Bronchoalveolar Lavage Fluid for the Diagnosis and Prognosis of Aspergillosis in Patients With Hematological and Non-hematological Conditions. Frontiers in Microbiology. 2018; 9: 1877.

[8] Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, *et al*. A consensus document for the selection of lung transplant candidates: 2014–an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. The Journal of Heart and Lung Transplantation: the Official Publication of the International Society for Heart Transplantation. 2015; 34: 1–15.

[9] Global Observatory on Donation and Transplantation. 2022. Available at: https://www.transplant-observatory.org/ (Accessed Date: 12 July 2022)

[10] Iversen M, Burton CM, Vand S, Skovfoged L, Carlsen J, Milman N, *et al*. Aspergillus infection in lung transplant patients: incidence and prognosis. European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology. 2007; 26: 879–886.

[11] Weill D. Lung transplantation: indications and contraindications. Journal of Thoracic Disease. 2018; 10: 4574–4587.

[12] Doligalski CT, Benedict K, Cleveland AA, Park B, Derado G, Pappas PG, *et al*. Epidemiology of invasive mold infections in lung transplant recipients. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2014; 14: 1328–1333.

[13] Neofytos D, Chatzis O, Nasioudis D, Boely Janke E, Doco Lecompte T, Garzoni C, *et al*. Epidemiology, risk factors and outcomes of invasive aspergillosis in solid organ transplant recipients in the Swiss Transplant Cohort Study. Transplant Infectious Disease: an Official Journal of the Transplantation Society. 2018; 20: e12898.

[14] Chang A, Musk M, Lavender M, Wrobel J, Yaw MC, Lawrence S, *et al*. Epidemiology of invasive fungal infections in lung transplant recipients in Western Australia. Transplant Infectious Disease: an Official Journal of the Transplantation Society. 2019; 21: e13085.

[15] Fortún J, Martín-Dávila P, Moreno S, De Vicente E, Nuño J, Candelas A, *et al*. Risk factors for invasive aspergillosis in liver transplant recipients. Liver Transplantation: Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2002; 8: 1065–1070.

[16] Mehrad B, Paciocco G, Martinez FJ, Ojo TC, Iannettoni MD, Lynch JP, 3rd. Spectrum of Aspergillus infection in lung transplant recipients: case series and review of the literature. Chest. 2001; 119: 169–175.

[17] Clancy CJ, Jaber RA, Leather HL, Wingard JR, Staley B, Wheat LJ, *et al*. Bronchoalveolar lavage galactomannan in diagnosis of invasive pulmonary aspergillosis among solid-organ transplant recipients. Journal of Clinical Microbiology. 2007; 45: 1759–1765.

[18] Wu J, Zhang T, Pan J, Zhang Q, Lin X, Chang L, *et al*. Characteristics of the Computed Tomography Imaging Findings in 72 Patients with Airway-Invasive Pulmonary Aspergillosis. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2021; 27: e931162.

[19] Gazzoni FF, Hochhegger B, Severo LC, Marchiori E, Pasqualotto A, Sartori APG, *et al*. High-resolution computed tomographic findings of Aspergillus infection in lung transplant patients. European Journal of Radiology. 2014; 83: 79–83.

[20] Neoh CF, Snell GI, Kotsimbos T, Levvey B, Morrissey CO, Slavin MA, *et al*. Antifungal prophylaxis in lung transplantation–a world-wide survey. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2011; 11: 361–366.

[21] Bhaskaran A, Mumtaz K, Husain S. Anti-Aspergillus Prophylaxis in Lung Transplantation: A Systematic Review and Meta-analysis. Current Infectious Disease Reports. 2013; 15: 514–525.

[22] Singh N, Paterson DL. Aspergillus infections in transplant recipients. Clinical Microbiology Reviews. 2005; 18: 44–69.

[23] Steinbach WJ, Marr KA, Anaissie EJ, Azie N, Quan SP, Meier-Kriesche HU, *et al*. Clinical epidemiology of 960 patients with invasive aspergillosis from the PATH Alliance registry. The Journal of Infection. 2012; 65: 453–464.