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Fungal colonization before or after lung transplantation has no negative impact on survival or the development of chronic lung allograft dysfunction

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INTRODUCTION: Long-term survival following lung transplantation (LTx) faces impediments due to chronic lung allograft dysfunction (CLAD), while infections hinder short-term survival. Fungal colonization and invasive fungal infections (IFI) are common within the first year after LTx. There is ongoing debate regarding the impact of such events on CLAD development and mortality. This study aims to investigate this matter further.

METHODS: A total of 134 LTx recipients transplanted between 2011 and 2020 were included. The median follow-up time was 3.9 years. Fungal colonization and IFI were defined according to international consensus guidelines and were noted if present within the first 12 months after LTx.

Abbreviations: A1AD, alpha-1-antitrypsin deficiency; BALF, bronchoalveolar lavage fluid; CF, cystic fibrosis; CI, confidence interval; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CYP3A4, cytochrome P450 3A4; DCM, dilated cardiomyopathy; EORTC, European Organization for Research and Treatment of Cancer; Gram-, gram-negative; Gram+, gram-positive; GvHD, graft versus host disease; HR, hazard ratio; HSV, herpes simplex virus; ISHLT, International Society for Heart and Lung Transplantation; LTx, lung transplantation; MSGERC, Mycoses Study Group Education and Research Consortium; n, number of; PCR, polymerase chain reaction; RA, rheumatoid arthritis; RSV, respiratory syncytial virus; SLE, systemic lupus erythematosus; Spp., species

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RESULTS: Postoperative fungal colonization was found in 101 patients, and 14 patients had an IFI within twelve months of transplantation. Nineteen patients were neither colonized nor infected. Out of the 115 patients with colonization or IFI, 61 patients had growth of a yeast such as *Candida* species (spp.). Fifty-six patients were colonized prior to LTx. Being colonized with fungus before or within the first 12 months post-LTx did not significantly affect survival or CLAD development.
CONCLUSIONS: The results of the current study indicate that fungal colonization either pre-transplantation or within the first 12 months after does not correlate with increased risks of mortality or CLAD development. These findings show that while fungal colonization is a common occurrence in LTx recipients, it does not predispose the patients of the cohort to adverse outcomes.
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Introduction

Lung transplantation (LTx) remains one of the few treatment options for patients with end-stage pulmonary disease, however, survival remains the shortest of all solid organ transplantations.[1–3] A major limitation for long-term survival is the development of chronic lung allograft dysfunction (CLAD), affecting up to 50% of LTx recipients within the first five years.[1] Moreover, LTx recipients are at great risk of infections due to high doses of immunosuppressive drugs, and natural exposure of the graft to the environment.[4,5]

Respiratory tract infections after LTx can cause severe complications, and both bacterial and viral infections have previously been implicated in increased mortality and the onset of CLAD.[2,4,6,7] However, the impact of fungal infections, and fungal colonization, on post-LTx outcomes remains unclear.[8] Opportunistic infections caused by fungi are common in LTx recipients, especially within the first year after transplantation.[7] Fungal infections are often mild but can become invasive and cause complications like tracheobronchitis, pneumonia, mediastinitis, and fungemia.[7,9] In immunocompromised patients, the risk of developing an invasive fungal infection (IFI) is heightened, and it has been suggested that IFI may be able to induce CLAD.[4,7] The fungal families most commonly found in LTx recipients are *Candida* spp. and *Aspergillus* spp., especially the strain *Aspergillus fumigatus*, and approximately 25% of all LTx recipients are colonized by *Aspergillus fumigatus* prior to transplantation.[4,5] The *Aspergillus* spp. is not normally pathogenic in immunocompetent individuals, however, following LTx the mucociliary function is impaired and the patients are subjected to intense immunosuppressive regimens, paving the way for both colonization and IFI.[2,4] The spores of *Aspergillus fumigatus* are small, permitting dissemination into distal parts of the allograft where the fungus has been suggested to establish an ischemic microenvironment through microvascular invasion, thrombosis, and production of antiangiogenetic factors.[2] This causes local inflammation and can theoretically predispose the patients to the development of CLAD. Other fungal infectious agents include *Rhizopus* spp., *Cryptococcus* spp. and *Pneumocystis* spp., among others.[7]

As of today, infectious complications remain one of the largest hurdles to overcome in the efforts of prolonging LTx survival. Fungal colonization and IFI have garnered increased interest lately, but knowledge surrounding the true impact of such cases remains limited. Previous studies have suggested IFI to have a negative impact on LTx outcomes, however it remains unclear whether fungal colonization on its own has similar effects. The current study aims to explore the impact of fungal colonization within the first year after LTx on the two outcomes survival and CLAD development.

Methods

Inclusion and exclusion criteria

The current study includes 134 LTx recipients transplanted at Skåne University Hospital in Lund, Sweden, between the years 2011 – 2020, with the last follow-up date of 2023–06-01. Of the included subjects, a total of 125 underwent double LTx (93%), and 70 were men (52%). A total of 174 patients underwent LTx at this site in the years of inclusion, however patients having undergone re-transplantation, aged below 18 years of age, or who died within 30 days from LTx were excluded. An additional ten patients were excluded due to loss of follow-up (post LTx follow-up was carried out at another hospital). A flowchart of inclusion and exclusion criteria is shown as supplemental Figure 1. Retrospective chart reviews of local medical records were conducted for all patients.

Fungal colonization was defined according to the International Society for Heart and Lung Transplantation's (ISHLT) consensus guidelines, as the presence of fungus in sputum or bronchoalveolar lavage fluid (BALF) detected by culture, polymerase chain reaction (PCR) or galactomannan (cutoff index, serum < 0.1, cutoff index, BALF < 0.5), in the absence of symptoms, radiological and endobronchial changes.[10] Fungal colonization either before LTx or within the first year after was recorded. LTx recipients are all examined with bronchoscopy and BALF samples at 3-, 6-, 9-, 12-, 18-, and 24-months post-transplant and thereafter yearly, plus additional samples in the case if symptoms. The definition of IFI was done according to the European Organization for Research and Treatment of Cancer (EORTC)

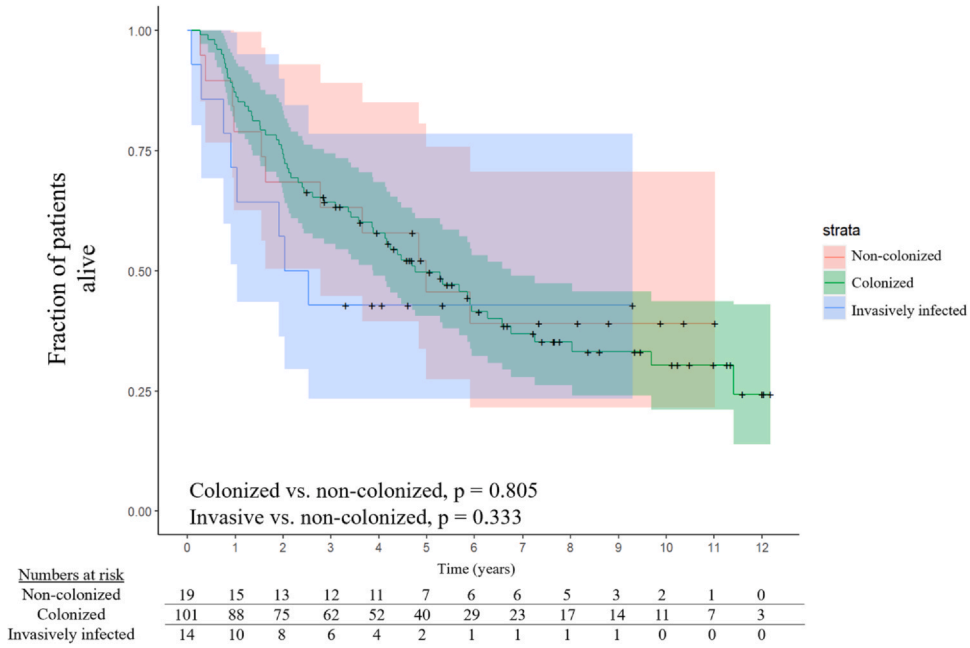

Figure 1 No significant difference in risk of mortality compared between all three subgroups. Analysis of survival comparing the probability of survival with 95% CI between patients with invasive fungal infection, fungal colonization, and no fungal incident within the first 12 months post-LTx. The risk of death was not increased in either subgroup. Numbers at risk table beneath indicates the number of patients alive at each time point. Statistical calculations performed with cox proportional hazards, p-values for HR displayed in figure. *CI: confidence interval, LTx: lung transplantation, HR: hazard ratio.*

and the Mycoses Study Group Education and Research Consortium's (MSGERC) consensus guidelines.[11] All patients with positive BALF galactomannan also had positive fungal cultures for *Aspergillus* spp. All patients were divided into subgroups based on IFI, colonization or no fungal event. Colonized patients were further divided into subgroups depending on the type of colonizing fungus. Rejection in the form of CLAD was defined according to the ISHLT's consensus guidelines.[12] There are currently no international guidelines for the general definition of bacterial and viral infections in cardiothoracic transplant recipients, according to the ISHLT.[13] In this study, bacterial and viral coinfection was defined as positive cultures for bacteria, and positive PCR-tests for viruses, in blood, sputum or BALF, including co-colonization. Infection with CMV was defined according to international consensus guidelines, as evidence of CMV replication found in either BALF or serum, regardless of symptomatology.[14] Patient demographics are presented in Table 1.

Immunosuppressive treatment regimen

Induction therapy administered in conjunction with LTx consisted of intravenously administered methylprednisolone and three doses of thymoglobulin up until 2017. After 2017 the induction therapy consisted of one dose of oral calcineurin inhibitor, intravenously administered methylprednisolone, and thymoglobulin. For maintenance of immunosuppression, a triple treatment regimen consisting of a calcineurin inhibitor, mycophenolate mofetil and steroids was administered.

Antifungal treatment regimens

Prophylactic treatment regimens for fungal infections consisted of three to four weeks of postoperative prophylactic treatment with orally administered nystatin to prevent infection with *Candida* spp. Infection with *Candida* spp. was treated with echinocandins, triazoles or amphotericine B. Lifelong treatment with a combination of trimethoprim and sulfamethoxazole, starting one to two weeks after LTx as prophylaxis for *Pneumocystis jirovecii* was also given. For patients with pre-transplant colonization with *Aspergillus* spp., additional postoperative treatment with a triazole was given for a duration of one month. Infection with *Aspergillus* spp. was treated with oral voriconazole for a period of four to eight weeks if the patient was asymptomatic. Symptomatic patients were treated with intravenous voriconazole for at least eight days, after which oral treatment was given until a total treatment duration of at least 12 weeks was reached. Severe pneumonia caused by the fungus *Pneumocystis jirovecii* was treated with intravenous trimethoprim and sulfamethoxazole until alleviation of symptoms, whereafter oral treatment was continued until a total treatment period of three weeks had been reached. Mild pneumonia with *Pneumocystis jirovecii* was treated orally. Commonly, the dosage of corticosteroids is also increased in the instances of fungal infections.

Statistical analysis

All statistical analyses were performed in RStudio version 2023.12.1, build 402, R version 4.1.2 (2021–11-01).

Table 1 Demographic Data

	Invasive Infection	Colonization	No Fungal Event
N (% of all)	14 (10)	101 (76)	19 (14)
Age at LTx (years), mean (range)	52 (27 – 67)	52 (19 – 68)	50 (19 – 65)
Male sex, n (%)	12 (86)	48 (48)	10 (53)
Type of transplantation			
Single, n (%)	2 (14)	3 (3)	2 (11)
Double, n (%)	11 (79)	98 (97)	16 (84)
Heart and lung, n (%)	1 (7)	0 (0)	1 (5)
Underlying disease			
CF, n (%)	5 (36)	16 (16)	3 (16)
COPD, n (%)	3 (21.5)	35 (34)	6 (32)
Pulmonary fibrosis, n (%)	2 (14)	16 (16)	5 (26)
A1AD, n (%)	1 (7)	16 (16)	1 (5)
Pulmonary hypertension, n (%)	0 (0)	10 (10)	2 (10.5)
Other*, n (%)	3 (21.5)	8 (8)	2 (10.5)
Preoperative fungal colonization, n (%)	8 (57)	42 (42%)	6 (32)
CMV mismatch, n (%)	4 (29)	15 (15)	4 (21)
Coinfection, n (%)	12 (86)	62 (61)	19 (100)
Viral, n (%)	5 (36)	22 (22)	14 (74)
Bacterial, n (%)	10 (71)	55 (54)	19 (100)
CLAD, n (%)	3 (21.5)	32 (32)	6 (32)
Survival (days), mean (range)	1045 (30 – 3397)	1831 (98 – 4448)	1852 (99 – 4026)
Retransplantation, n (%)	0 (0)	9 (9)	1 (5)

Summary table of demographic data, divided by invasive fungal infection, fungal colonization or no fungal event. Data are presented as mean, range, number of subjects and percentage of cohort. *Other underlying diseases include sarcoidosis, bronchiectasis, GvHD, SLE, congenital heart defects, chronic pleuritis, RA and scleroderma. *Spp.: species, n: number of, LTx: lung transplantation, CF: cystic fibrosis, COPD: chronic obstructive pulmonary disease, A1AD: alpha-1-antitrypsin deficiency, CMV: cytomegalovirus, CLAD: chronic lung allograft dysfunction, GvHD: graft versus host disease, SLE: systemic lupus erythematosus, DCM: dilated cardiomyopathy, RA: rheumatoid arthritis.*

Balance between groups was achieved with propensity score weighting (PSW), calculated with a linear regression model, including sex, age, mean ischemic time, and underlying pulmonary disease prior to LTx (pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), pulmonary hypertension or alpha-1-antitrypsin deficiency (A1AD)). Variables included in the calculation of propensity scores (PS) were chosen based on factors likely to affect outcomes following LTx.[15] Kaplan Meier curves were fitted to visualize mortality and incidence of CLAD compared between subgroups, with the events being death (death or graft failure leading to retransplantation) or a CLAD diagnosis, and time being recorded in days. Patients who were alive or without a CLAD diagnosis at the end of follow-up were censored. Weighted cox proportional hazards models were fitted to calculate hazard ratios (HR) with 95% confidence intervals (CI) for the risks of death and CLAD development, adjusting for preoperative fungal colonization and underlying pulmonary disease. Data are presented as mean, range, HR with 95% CI, number of subjects (n) and percentage (%). Statistical significance was defined as $p < 0.05$.

Ethical considerations

The study was conducted in accordance with the declaration of Helsinki and is approved by the Swedish Ethical Review Authority with Dnr: 2020–07115 and 2020–01864.

Results

Descriptive results

This study investigates the impact of fungal colonization up to one year after LTx on mortality and CLAD development. The study includes a total of 134 subjects and the mean age in the cohort was 52 (range 19 – 68 years). The median follow-up time was 3.9 years (1419 days, range 30 – 4448). In total, 115 patients were colonized or had an IFI post LTx, and 19 did not. Sixty-one patients were colonized by a yeast or a yeast-like fungus (*Candida* spp., n = 57, *Saccharomyces* spp., n = 1, *Pneumocystis jirovecii*, n = 1, multiple spp. of yeast, n = 2), with a spread in time passed from LTx to positive culture of 0 – 6 months. Nine of these patients also had candidal colonization prior to LTx. This group is henceforth referred to as candidal (n = 61). Fifty-four patients were colonized by mold with 21 cases of *Aspergillus* spp., 29 cases of multiple spp., and four cases of a single other spp. (*Paecilomyces* spp. or *Rhizopus* spp.), this group is henceforth referred to as non-candidal (n = 54). Of the 115 colonized or infected patients, a total of 102 cases (89%) were from patients with respiratory symptoms or abnormal results on chest imaging. For detailed information on the identified fungal spp., see supplemental table 1. The mean time elapsed from LTx to a positive fungal culture was 2.1 months, with 64 of the patients (56%) being colonized or infected already within the first month after LTx. Fourteen patients (12%) had an IFI

(candidal, n = 8, non-candidal, n = 6) which were identified by positive blood cultures or the presence of fungal hyphae or cells in histology. Fifty-six of the total 134 included patients (42%) were colonized by a fungus prior to LTx, of whom eight developed a postoperative IFI, 42 were postoperatively colonized as well and six patients had no postoperative fungal event.

Eighty-one patients (60%) died or experienced graft failure leading to the need for retransplantation within the entire follow-up period, with a total of 21 deaths within the first year after LTx. Of the 101 patients with postoperative fungal colonization, eleven patients died within the first year (11%). Forty-one of all included patients (31%) developed CLAD. There were 25 cases of missing data in the category preoperative fungal colonization.

Survival after LTx is not affected by fungal colonization

Patients with fungal colonization but no IFI (n = 101) were compared to patients without any postoperative fungal event (n = 19). No statistically increased risk of death could be seen for colonized patients (HR = 1.06 (95% CI: 0.60 – 1.90), p = 0.832). When including patients with IFI and comparing all three subgroups, there results remained consistent (HR, IFI = 1.57 (95% CI: 0.63 – 3.93), p = 0.333, HR, colonized = 1.08 (95% CI: 0.57 – 2.06), p = 0.805) (Figure 1). Survival was also compared between patients with and without preoperative fungal colonization, irrespective of postoperative status. No significantly increased risk of death was seen for preoperatively colonized patients (HR = 1.50 (95% CI: 0.82 – 2.75), p = 0.186). A multivariate analysis was carried out, exploring the risk of death depending on preoperative fungal colonization and pulmonary disease prior to LTx, showing no significantly increased risk of death for patients with postoperative fungal colonization (adjusted HR = 1.37 (95% CI: 0.69 – 2.72), p = 0.370). The underlying pulmonary disease CF was a significant protective factor for death (HR for CF = 0.30 (95% CI: 0.09 – 0.95), p = 0.041, HR for COPD = 1.65 (95% CI: 0.66 – 4.08), p = 0.282, HR for pulmonary fibrosis = 1.38 (95% CI: 0.45 – 4.29), p = 0.573, HR for A1AD = 0.76 (95% CI: 0.23 – 2.53), p = 0.654, HR for pulmonary hypertension = 0.31 (95% CI: 0.06 – 1.54), p = 0.153, HR for preoperative fungal colonization = 1.65 (95% CI: 0.80 – 3.39), p = 0.173).

The risk of CLAD is not affected by postoperative fungal colonization

When comparing the risk of CLAD between patients with (n = 101) and without postoperative fungal colonization (n = 19) there was no significantly increased risk for colonized patients (HR = 0.82 (95% CI: 0.38 – 1.77), p = 0.612). When including patients with IFI the results remained consistent, with no significant difference in risk between the subgroups (HR, IFI = 1.01 (95% CI: 0.25 – 4.03), p = 0.994, HR, colonized = 1.04 (95% CI: 0.45 – 2.49), p = 0.932) (Figure 2). Comparing the risk of CLAD between patients with and without fungal colonization prior to LTx similarly showed no significantly increased risk for preoperatively colonized patients (HR = 1.19 (95% CI: 0.61 –

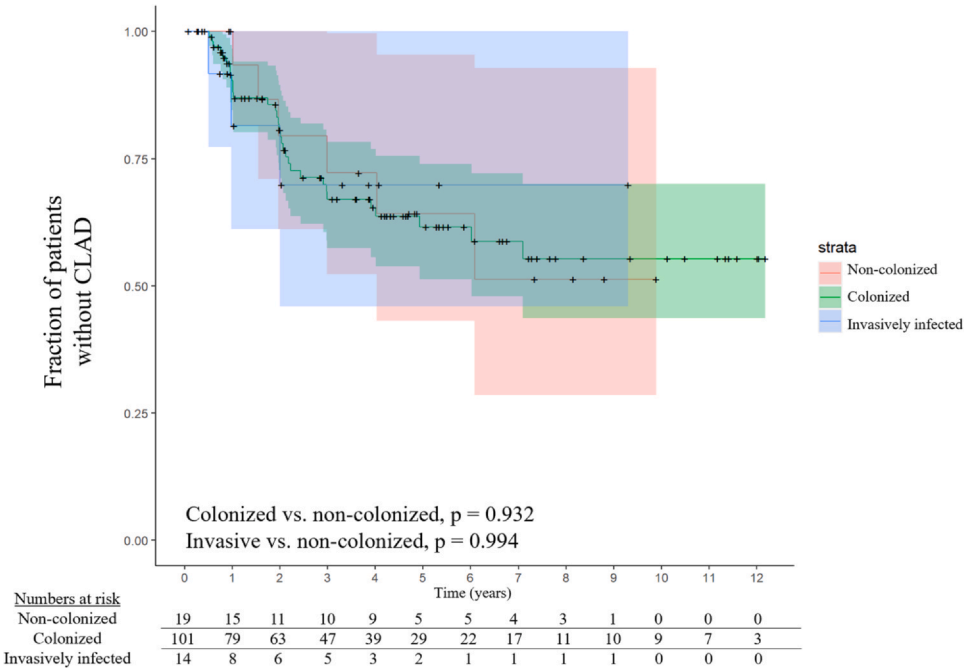

Figure 2 No significant difference in risk of CLAD development compared between three subgroups. Analysis of the CLAD development probability with 95% CI compared between patients with invasive fungal infection, fungal colonization and no fungal incident within the first 12 months post-LTx. The risk of CLAD was not increased in either subgroup. Numbers at risk table beneath indicates the number of patients alive and without CLAD at each time point. Statistical calculations performed with cox proportional hazards, p-values for HR displayed in figure. *CLAD: chronic lung allograft dysfunction, CI: confidence interval, LTx: lung transplantation, HR: hazard ratio.*

Table 2 Bacterial and Viral Coinfections

	Invasive Infection	Colonization	No Fungal Event
N (% of all)	14 (10)	101 (76)	19 (14)
Bacterial coinfection, n (%)	10 (71)	55 (54)	19 (100)
Aerobic, n (%)	7 (50)	33 (33)	14 (74)
Gram- rod, n (%)	7 (50)	28 (28)	13 (68)
Gram+ rod, n (%)	0 (0)	3 (3)	2 (11)
Gram- cocci, n (%)	0 (0)	2 (2)	0 (0)
Anaerobic, n (%)	0 (0)	0 (0)	1 (5)
Gram+ rod, n (%)	0 (0)	0 (0)	1 (5)
Facultative anaerobic, n (%)	6 (43)	32 (32)	17 (89)
Gram- rod, n (%)	4 (29)	8 (8)	6 (32)
Gram+ cocci, n (%)	2 (14)	24 (24)	15 (79)
Viral coinfection, n (%)	5 (36)	22 (22)	14 (74)
CMV, n (%)	5 (36)	17 (17)	14 (74)
Influenza, n (%)	0 (0)	1 (1)	1 (5)
HSV, n (%)	0 (0)	2 (2)	2 (11)
COVID–19, n (%)	0 (0)	2 (2)	0 (0)
RSV, n (%)	0 (0)	1 (1)	0 (0)

Bacterial and viral coinfections in the included patients, divided by invasive fungal infection, fungal colonization and no fungal event. Data are presented as number of patients and percentage of cohort. *LTx: lung transplantation, n: number of, gram-: gram-negative, gram+: gram-positive, CMV: cytomegalovirus, HSV: herpes simplex virus, COVID-19: coronavirus disease 2019, RSV: respiratory syncytial virus.*

2.31), p = 0.612). A multivariate model including the factors preoperative fungal colonization and pulmonary disease prior to LTx was fitted. The model showed no significantly increased risk of CLAD for colonized patients (adjusted HR = 1.08 (95% CI: 0.42 – 2.80), p = 0.879). Only the underlying pulmonary disease COPD turned out to be a significant risk factor (HR for CF = 4.16 (95% CI: 0.68 – 25.54), p = 0.124, HR for COPD = 7.35 (95% CI: 1.11 – 48.88), p = 0.039, HR for pulmonary fibrosis = 2.47 (95% CI: 0.32 – 19.04), p = 0.386), HR for A1AD = 2.93 (95% CI: 0.38 – 22.89), p = 0.305, HR for pulmonary hypertension = 5.08 (95% CI: 0.64 – 40.34), p = 0.124, HR for preoperative fungal colonization = 0.81 (95% CI: 0.30 – 2.22), p = 0.681).

Invasive fungal infection increases the risk of CLAD

When comparing the patients with IFI (n = 14) to patients with fungal colonization (n = 101), no statistically significant increase in the risk of death was seen in invasively infected patients (HR = 1.17 (95% CI: 0.36 – 3.79), p = 0.791). However, a significantly increased risk of CLAD development could be seen (HR = 2.57 (95% CI: 1.32 – 5.02), p = 0.006).

Outcomes are not affected by fungal genus

Previous research has suggested a difference in outcomes following IFI depending on fungal genus. Therefore, additional analyses were performed comparing postoperatively colonized candidal patients to non-candidal patients. The analyses showed no significant differences in the risks of mortality or CLAD development (HR mortality, non-candidal = 1.53 (95% CI: 0.91 – 2.55), p = 0.106, HR CLAD, non-candidal = 0.99 (95% CI: 0.48 – 2.03), p = 0.982).

Coinfections do not impact outcomes

Eighty-four of the 134 included patients (63%) had positive bacterial cultures at the time of fungal colonization, or within the first 12 months post LTx in the cases of no postoperative fungal event, and thirty-six patients (27%) had positive viral cultures. In total, there were 41 separate cases of viral coinfections present in the current cohort, and within those cases there were 40 cases of CMV and HSV combined. In addition, there were five cases of influenza, COVID-19, and RSV in total. One patient may have more than one strain of virus at a time. Among the 101 postoperatively colonized patients 55 had a bacterial coinfection and 22 had a viral coinfection. In the subgroup with IFI (n = 14), ten patients had a bacterial coinfection, and five had a viral coinfection. Of the patients without any fungal event after LTx (n = 19), all patients had at least one positive bacterial culture within the first year after LTx and 14 patients had at least one positive viral culture (Table 2). Comparing the risks of death and CLAD development between patients with and without postoperative fungal colonization, adjusting for bacterial and viral coinfections showed no significant impact of coinfections on outcomes (supplemental results, supplementals). For detailed information on all occurring coinfecting agents, see supplemental table 2 (supplementals).

Discussion

For patients with end-stage pulmonary disease, LTx remains the only definitive treatment option. However, survival is short, in part due to frequent postoperative infections, which together with rejection are one of the leading causes of death after LTx. In fact, both bacterial and viral infections post transplantation have been shown to negatively impact outcomes.[4,7,16] Additionally, a large proportion of LTx recipients are colonized by fungus either before or after transplantation, although the information on how this affects LTx recipients over time is sparse. While some studies have suggested a connection between fungal infections and poorer outcomes, there is also research which does not show such a correlation.[7,17–22] Moreover, existing studies are often limited by a small cohort or a sole focus on IFI.[8,23–27]

This study investigates the implications of fungal colonization in LTx recipients. Yeasts are to be expected in the normal flora of the respiratory tract. While prophylactic

antifungal medications are commonly used, their potential risks and true clinical implications remain unclear.[28] In this cohort 42% of the patients were colonized by fungus prior to LTx, and 87% were colonized after. Over half of the included patients presented with positive fungal cultures within one month after LTx, and the mean time from LTx to colonization or IFI was 2.1 months. Despite large fractions of colonized patients, no significant impact of fungal colonization on death or CLAD development could be seen. The analyses regarding CLAD and mortality were also conducted excluding the four cases of non-candidal yeast colonization, and the results remained consistent. We did, however, see a significant negative impact of IFI on the risk of CLAD. Of the patients with IFI after LTx, only around 50% were colonized before transplantation, reducing the risk of bias from previous fungal colonization influencing outcomes.

On this topic, a study by Chong *et al.*, including 91 LTx recipients with IFI caused by yeasts or molds, showed an increased mortality rate in infected compared to non-infected patients.[16] Two larger studies, including patients infected only by molds, showed three-month and one-year mortality rates of 22% and 44% respectively.[26,29] Furthermore, a retrospective study including 161 LTx recipients showed a connection between IFI caused by both molds and yeasts and poorer outcomes.[25] The follow-up time in the current study reaches a median of 3.9 years and the results show a cumulative mortality rate of 60%, with a low one-year mortality rate of 16%. Within the group of colonized patients this number is even lower at 11%, highlighting the importance of separating fungal colonization and IFI as two different entities. The number of published studies which investigate the impact of fungal colonization alone is low, and these studies are often limited by the exclusion of yeasts and yeast-like fungi. One such study is the retrospective study of 201 LTx recipients by Weight *et al.*, showing increased risks of death and rejection for patients with non-candidal colonization. In summary, results within this field have been incohesive, with some studies suggesting a negative impact of fungal colonization, while others do not see such a correlation.[18,22,23,30,31]

As outlined above, the problem of IFI and fungal colonization in LTx recipients has been approached in different ways. Some believe that infections caused by the likes of *Aspergillus* spp. and other molds are more difficult to manage and constitute a greater risk compared to yeasts like *Candida* spp.[4,32,33] On the other hand, the significant dangers of candidemia have led to others advocating the inclusion of yeasts in studies on fungal infections.[34] The results of the present study showed no significantly increased risk of death or CLAD development for patients colonized with non-candidal fungi. This is corroborated by a large multi-center study by Law *et al.*, showing a lack of association between airway colonization with *Aspergillus* spp. and the development of bronchiolitis obliterans syndrome.[22]

There are many factors influencing the outcomes after LTx, including coinfections, single LTx, and preoperative fungal colonization.[4,28,35,36] In this project, multivariate analyses including the abovementioned factors showed no association with poor outcomes. They did however reveal the underlying condition CF to act as a protective factor for the risk of death, something that is likely explained by the low age and lack of comorbidities in these patients.[37] On a similar note, the emergence of COPD as a significant risk factor for the development of CLAD may be explained by a greater age and poorer health status of these patients, and research on the topic states CLAD as the leading cause of death following LTx due to COPD.[38] Off note, it is to be expected that increasing amounts of positive fungal cultures are identified post LTx due to the routine follow-up appointments of this patient category. However, the spread of time passed from LTx to positive fungal culture makes it unlikely that the bulk of the cases would be purely due to increased frequency of testing.

Although these results differ somewhat from previous findings, they also add a new layer to the matter, showing that fungal colonization by fungi of any genus does not negatively impact the outcomes after LTx, which has to our knowledge not been demonstrated before. Furthermore, the differing results can likely be explained by the inclusion of both yeasts and molds, and the separation of colonization and IFI in the current paper. The relatively small sample sizes do introduce some uncertainty, as well as the fact that all patients are treated with prophylactic antifungal medications, which one could argue introduces a level of uncertainty as to whether the lack of impact is due to treatment or an actual finding that colonization does not significantly impact outcomes negatively. However, as prophylactic treatment is a standard of care in most clinics, it is not likely to specifically affect the current cohort, but it is important to consider the potential effects of differences in antifungal prophylaxis on outcomes in retrospective studies. Furthermore, patients with active fungal infections are treated for the infection first, before they are put on the waitlist. Further studies at multiple centers and larger cohorts should be encouraged.

Conclusions

The current study indicates that survival after LTx remains unaltered by fungal colonization prior to or within the first 12 months post-LTx. Similarly, the risk of CLAD development does not appear to be significantly influenced by fungal colonization. These findings provide insights into the effects of fungal colonization as its own entity on the outcomes following LTx, showcasing the need for further research which separates fungal colonization and IFI. Additionally, the results suggest that postoperative fungal colonization may not pose as significant of a threat as previously believed.

Ethics approval and consent to participate

The current study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (Dnr: 2020–07115 and 2020–01864).

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CRediT authorship contribution statement

Conceptualization, S.L.; design of work, S.L. and R.I.; data acquisition, E.B. and F.S.; data analysis and statistical work, E.B.; interpretation of data, E.B., S.L., F.O., H.L. and H.A.; writing process, E.B., F.S., F.O., H.L., H.A. and S.L.; revision process, E.B., F.O., F.S., H.L., H.A., R.I. and S.L.; approval of article, E.B., F.S., A.N., H.A., R.I., H.L., F.O. and S.L.; resources, S.L. visualization, E.B.; supervision, S.L. and F.O.; funding acquisition, S.L. All authors have read and agreed to the published version of the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplemental information files. Scripts used for statistical analyses are available upon request.

Appendix A. Supporting information

Supplemental data associated with this article can be found in the online version at doi:10.1016/j.jhlto.2025.100225.

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