# Epidemiology of Invasive Fungal Infections in Lung Transplant Recipients on Long Term Azole Antifungal Prophylaxis

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## Abstract

**Background:** Most lung transplant centers employ some form of antifungal prophylaxis (AF PPX) post-transplantation, although the agent and duration of AF PPX is variable. Data regarding contemporary epidemiology of invasive fungal infections (IFI) in lung transplant recipients (LTR) receiving prolonged azole AF PPX is currently lacking.

**Objective:** To investigate the impact of prolonged azole AF PPX on the epidemiology of IFI and outcomes of LTR at our institution.

**Methods:** The Mayo Clinic (Rochester) transplant database was reviewed for all adult lung transplants performed between 2002 to 2011. Patients diagnosed with proven or probable IFI were included. Survival analyses were performed using Cox proportional hazard modeling.

**Results:** 15 of 91 LTR developed 19 IFI episodes (8 proven, 11 probable) during the 10-yr study period. The cumulative incidence of IFI at 1, 3 and 5 years post-lung transplantation was 16%, 24% and 29% respectively, with the median time to diagnosis of first IFI episode being 245 days. Majority of IFI developed on PPX (n=17 [89%]), and in episodes where AF trough levels were available (n=12 [63%]), 33% (4/12) were found to be sub-therapeutic. *Aspergillus fumigatus* (n=9) was the most commonly isolated fungal organism, followed by *A. versicolor* (n=4), *Fusarium sp.* (n=2), *Candida glabrata* (n=2), *A. terreus* (n=1), *Rhizopus sp.* (n=1) and *Scopulariopsis sp.* (n=1). The most common site of infection was pulmonary (n=11 [58%]), followed by tracheobronchitis (n=4 [21%]), sinusitis, fungemia and intra-abdominal abscess (n=1 each). A higher hazard of mortality was observed in LTR who developed IFI post-transplantation, although this was not statistically significant. (HR: 1.71; 95% CI (0.58-4.05); p-value = 0.27)

**Conclusions:** IFI continue to occur in LTR in spite of prolonged AF PPX. Invasive Aspergillosis (IA) was the most common IFI. Although some of these episodes may be attributed to azole-resistant fungal organisms and sub-therapeutic AF trough levels, reason(s) for development of the remaining IFI remains to be determined.

## Objectives

**Primary Goal**

• To evaluate the impact of AF PPX on the epidemiology, clinical manifestations, and outcomes of IFI in LTR at our institution.

**Secondary Goals**

• To identify predictors of developing IFI in LTR.

• To perform a subset analysis of LTR with proven or probable IA.

## Methods

**Study Design & Methods:** We obtained prior IRB approval for this single center, retrospective cohort study. Electronic chart review and data collection was performed using a standard abstraction form. All lung and heart-lung transplant recipients aged 18 and above who were transplanted at Mayo Clinic (Rochester) between January 2002 and December 2011 were included. Patients who seek care at Mayo Clinic but received their transplants elsewhere were excluded.

**Protocols:** All LTR received either caspofungin (2012 onwards) or IV fluconazole (2001 to 2012) followed by itraconazole (ITC) or voriconazole (VRC) for ≥1 year post-transplantation. Most received lifelong AF PPX. Target trough levels for ITC and VRC were ≥2 µg/mL and ≥1 µg/mL respectively. LTR received induction immunosuppression with OKT-3 (1/2002 – 4/2008) or thymoglobulin (5/2008 – 2/2009), intraoperative methylprednisolone bolus(es) followed by standard maintenance immunosuppression. Screening bronchoscopies were performed at 1,3 & 6 months post-transplantation.

**Case Definitions: Colonization :** ≥ 1 positive fungal culture(s) from a non-sterile site in the absence of clinical or radiographic evidence of invasive disease. **Infection :** Categorized as proven or probable according to the EORTC/MSG 2008 criteria. Possible cases were excluded.

**Statistical Analysis:** Survival analyses were performed with IFI and IA as time-dependent covariates, and association between clinical variables and development of IFI was investigated using Univariate Cox models. Statistical significance was pre-defined as *p*- value <0.05.

## Results

- Baseline demographics and post-transplant characteristics of LTR with and without IFI are summarized in **Table 1.**
- The cumulative incidence of IFI at 1, 3 and 5 years post-transplantation was 16%, 24% and 29% respectively.
- The development of IFI post-transplantation was associated with decreased survival, although this was not statistically significant. (HR: 1.71; 95% CI (0.58-4.05); p-value = 0.27) **[Figure 1(a)]**
- Idiopathic pulmonary fibrosis was significantly associated with increased likelihood of developing IFI post-transplantation (HR: 4.29; 95% CI: 1.15-15.91; p = 0.03). **[Table 2]**
- A total of 19 IFI episodes (8 proven; 11 probable) were identified, with *Aspergillus fumigatus* being the most common causal organism. The most common site of involvement was pulmonary (n=11 [58%]), followed by tracheobronchitis (n=4 [21%]). **[Table 3]**
- Majority of IFI developed on PPX (n=17 [89%]). Of IFI episodes where AF trough levels were available (n=12 [63%]), 33% (4/12) were found to be sub-therapeutic. **[Table 3]**

### Table 1 : Baseline & Post-transplantation Characteristics

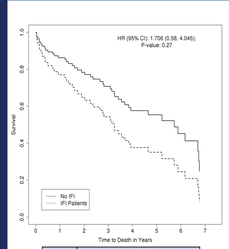
| Patient Characteristic | Without IFI (n = 76) | With IFI (n = 15) |
|---|---|---|
| **Age, years*** | 54.4 (51.5, 57.2) | 59.9 (56.7, 63.3) |
| **Male sex** | 33 (43) | 8 (53) |
| **Ethnicity** | | |
| African-American | 2 (2) | 1 (6) |
| Caucasian | 68 (90) | 13 (87) |
| Other | 6 (8) | 1 (7) |
| **BMI, kg/m²*** | 24.6 (23.7, 25.5) | 26.6 (24.1, 29.1) |
| < 18.5 | 5 (6) | 1 (6) |
| 18.5 to 24.9 | 32 (42) | 4 (27) |
| 25 to 29.9 | 30 (40) | 7 (47) |
| ≥ 30 | 9 (12) | 3 (20) |
| **Diabetes mellitus** | 9 (12) | 3 (20) |
| **Serum creatinine, mg/dL** | 0.8 (0.8, 0.9) | 0.8 (0.7, 0.9) |
| **LAS*** | 41.8 (38.4, 45.1) | 45.5 (35.9, 55.1) |
| **Ventilator at time of transplant** | 3 (4) | 1 (7) |
| **Type of transplant** | | |
| Single lung | 22 (29) | 3 (20) |
| Double lung | 48 (63) | 12 (80) |
| Heart-lung | 6 (8) | 0 (0) |
| **Indications for transplant** | | |
| Alpha-1-antitrypsin deficiency | 9 (12) | 1 (7) |
| COPD | 25 (33) | 3 (20) |
| Congenital | 2 (3) | 0 (0) |
| Cystic fibrosis | 3 (4) | 0 (0) |
| IPF | 16 (21) | 9 (60) |
| Interstitial lung disease | 4 (5) | 0 (0) |
| Other | 13 (17) | 2 (13) |
| Primary pulmonary hypertension | 4 (5) | 0 (0) |
| **Induction Immunosuppression** | | |
| Thymoglobulin | 26 (35) | 6 (40) |
| OKT-3 | 31 (42) | 3 (20) |
| No induction | 17 (23) | 6 (40) |
| **Allograft rejection*** | 1.0 (0.7, 1.4) | 1.5 (0.8, 2.2) |
| 0 | 38 (51) | 3 (21) |
| 1 episode | 18 (24) | 4 (29) |
| 2 episodes | 11 (15) | 5 (36) |
| >3 episodes | 8 (11) | 2 (13) |
| **Bronchiolitis Obliterans Syndrome** | 15 (20) | 1 (7) |
| **Anastomotic stricture(s)** | 3 (4) | 3 (20) |
| **Bronchial stent(s)** | 2 (3) | 2 (13) |

**IFI**, invasive fungal infections; **COPD**, chronic obstructive pulmonary disease; **IPF**, idiopathic pulmonary fibrosis; **ILD**, interstitial lung disease; **LAS**, lung allocation score; **BOS**, bronchiolitis obliterans syndrome.

*Data are presented as number of patients (percentage), unless indicated otherwise.

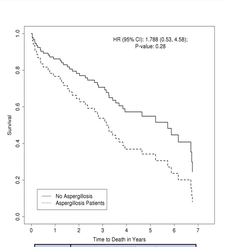
†Indicates values in mean (standard deviations)

### Figure 1 : Patient Survival (Time-Dependent Cox Models)




| | Survival (95% CI) | |
|---|---|---|
| Time | No IFI | IFI |
| 1 year | 86.3 (79.6 – 93.6) | 77.0 (58.9 – 100) |
| 3 year | 70.8 (61.5 – 81.5) | 54.2 (30.8 – 95.4) |
| 5 year | 55.4 (44.3 – 69.2) | 35.3 (14.0 – 88.5) |

**Figure 1(a) : Effect of IFI on overall survival of LTR**




| | Survival (95% CI) | |
|---|---|---|
| Time | No Aspergillosis | Aspergillosis |
| 1 year | 86.1 (79.4 – 93.5) | 76.6 (56.9 – 1) |
| 3 year | 70.7 (61.4 – 81.4) | 53.8 (28.7 – 1) |
| 5 year | 54.9 (43.9 – 68.6) | 34.2 (11.8 – 99.4) |

**Figure 1(b) : Effect of IA on overall survival of LTR**

### Table 2 : Risk Factors for IFI

| Clinical variables | Hazard Ratio | 95% confidence interval | P- value |
|---|---|---|---|
| **Age, years** | 1.07 | 0.99 – 1.14 | 0.08 |
| **Indication for Surgery (Reference group : COPD)** | | | |
| Idiopathic pulmonary fibrosis | 4.29 | 1.16 – 15.91 | 0.03 |
| **BMI** | 1.14 | 0.99 – 1.31 | 0.06 |
| **BMI Levels (reference 18.5 to 24.9)** | | | |
| => 30 | 2.67 | 0.59 – 12.02 | 0.20 |
| **Lung Allocation Score (LAS)** | 1.03 | 1.00 – 1.06 | 0.11 |
| **Baseline Immunosuppressant (Reference group : no induction)** | | | |
| OKT-3 | 0.17 | 0.04 – 0.70 | 0.01 |
| **Pre-transplant recipient airway fungal colonization** | 1.14 | 0.41 – 3.16 | 0.80 |
| **Positive donor bronchus culture at time of transplant** | 1.00 | 0.22 – 4.42 | 1.00 |
| **Positive recipient bronchus culture at time of transplant** | 1.00 | 0.13 – 7.64 | 1.00 |
| **Undetectable first AF trough level** | 1.97 | 0.67 – 5.76 | 0.22 |
| **Time to first therapeutic AF trough level** | 2.33 | 0.51 – 10.58 | 0.27 |
| **First allograft rejection** | 1.16 | 0.35 – 3.81 | 0.81 |
| **CMV infection** | 0.94 | 0.12 – 7.45 | 0.96 |
| **Nadir serum IgG level** | 1.00 | 0.99 – 1.00 | 0.83 |

### Table 3 : Clinical Characteristics of LTR with IFI

| Patient no. | Transplant type | Microorganism | Type of IFI | Onset[a] | AF PPX | AF trough level[b] | Outcome |
|---|---|---|---|---|---|---|---|
| 1. | DL | *A. fumigatus* | Tracheobronchial (TB) | 15 | ITC | 0.6 | Alive |
| 2. | DL | *A. fumigatus* | Pulmonary | 33 | VRC | 1.6 | Alive; Stricture |
| 3. | SL | *A. fumigatus* | Pulmonary | 104 | VRC | 3.8 | Alive; Stricture |
| 4. | DL | *A. fumigatus* | Pulmonary | 361 | ITC | 2.9 | Alive |
| 5. | SL | *A. versicolor* | Pulmonary & chest wall abscess | 139 | ITC | N/A[c] | Died |
| 6. | DL | *A. versicolor* | Pulmonary | 667 | VRC | N/A | Alive |
| 7. | SL | *A. fumigatus; A. versicolor* | Pulmonary | 610 | ITC | 0.3 | Died |
| 8. | DL | (i) *A. terreus; A. versicolor* | Pulmonary | 205 | ITC | 1.1 | Died |
| | | (ii) *A. fumigatus* | Empyema | 254 | VRC | 1.5 | |
| 9. | DL | (i) *A. fumigatus* | TB & Pulmonary | 582 | ITC | N/A | Died |
| | | (ii) *Scopulariopsis sp.* | TB | 1373 | PSC | 569 | |
| 10. | DL | *C. glabrata* | Candidemia | 37 | ITC | N/A | Alive |
| | | *A. fumigatus* | TB | 129 | No AF PPX[e] | | |
| | | *A. fumigatus* | Pulmonary | 237 | No AF PPX | | |
| 11. | DL | *C. glabrata* (polymicrobial) | Intra-abdominal abscess | 1075 | ITC | 3.1 | Died |
| 12. | DL | *Fusarium sp.* | Sinusitis | 1388 | ITC | N/A | Alive |
| 13. | DL | *Fusarium sp.* | Pulmonary | 1542 | ITC | 2.6 | Alive |
| 14. | DL | *Rhizopus sp.* | Pulmonary | 27 | VRC | <0.1 | Alive |
| 15. | DL | n/a[d] | Pulmonary | 363 | ITC | 1.9 | Alive |

**SL**, single lung; **DL**, double lung; **ITC**, itraconazole; **VRC**, voriconazole; **PSC**, posaconazole

[a] Onset, measured in number of days from the time of lung transplantation

[b] Antifungal trough level (measured in µg/mL for itraconazole and voriconazole, and ng/mL for posaconazole), if obtained within 2 weeks leading to time of diagnosis of invasive fungal infection.

[c] N/A : antifungal trough level not available

[d] n/a : diagnosis made based on positive BAL galactomannan; culture was negative.

[e] No AF PPX : patient was not on antifungal prophylaxis at the time of diagnosis.

## Discussion

- The 1-yr cumulative incidence (CI) of IFI in LTR at our institution appears to be higher compared to national average reported in the TRANSNET study (16% vs 8.6%) **[reference no. 1]**
- Compared to a prior study conducted at our institution, the 1, 3, and 5-yr CI of IFI in LTR remains unchanged, in spite of a more aggressive approach to AF PPX. **[reference no. 2]**
- We observed that prolonged azole AF PPX resulted in an epidemiologic shift in that none of our LTR developed endemic fungal or Cryptococcal infections, and both cases of invasive Candidiasis were caused by non-albicans *Candida sp.*
- Azole resistance was overall uncommon at our institution. Only two isolates : *Aspergillus fumigatus* (patient no. 3) and *Candida glabrata* (patient no. 10) were proven to be vori- (MIC=4) and panazole-resistant respectively.

## Conclusions

- In the era of azole AF PPX, IFI continue to occur in LTR, with invasive Aspergillosis (IA) being the most common IFI.
- Although some of these episodes may be attributed to azole-resistant fungal organisms and sub-therapeutic AF trough levels, reason(s) for development of the remaining IFI remains to be determined.
- A multi-center, prospective randomized controlled trial is needed to define the optimal agent and duration of AF PPX in LTR.

## References

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