Peer Review File

Article Information: https://dx.doi.org/10.21037/jtd-23-601

Reviewer A

This is an article regarding fungal infection after lung transplant. As described, IFD, one of the common opportunistic infections is a significant concern of lung transplants. The included patients were covered with early caspofungin following azole antifungals. However, the prolonged group was not covered with triazole prophylaxis (line 126-130). Is this true? If that, 3 groups did not receive same prophylaxis against fungal infection. This weakens the main conclusion of this study. In addition, the author needs to show 3 groups received the same level of immunosuppression to say the prolonged group is better than others. There is no data regarding average drug level or compliance to drug, only disclosed the immunosuppressive protocol. This is unbelievable whether only NAB is the best prophylaxis for IFD among combination prophylaxis. The author should explain underlying reason for this remarkable finding.

Reply: Thanks for pointing out this issue. We have checked the original version of our manuscript and found out this is an error introduced during the proofreading process. The triazoles were universally used in all patients after lung transplantation at our hospital.

Changes in the text: The erroneously introduced text “who received a short or medium course of NAB” has been removed.

minor

1. There is a lack in baseline characteristics, especially related to postoperative infection or IFDs. Preoperative respiratory colonization can affect postoperative respiratory infection or colonization (J Thorac Dis. 2022 Jun; 14(6): 1900–1908.). However, this data do not show the detail of recipient's preoperative severity, donor culture or other cormobid condition, real drug level of immunesuppressant.

   Reply: Thanks for the suggestion. We added respiratory colonization at 6 months pre-transplant, preoperative severity, and diabetes in Table 1.

   In terms of donor culture, the respiratory secretion cultures were positive in 5% of the donors, and the most common pathogens were *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, and Streptococcus pneumoniae.* All tissue cultures of donor bronchial stumps were negative.

   The majority of the lung transplant recipients were elderly people (mean age around 55 years). Pre-transplant comorbidities were common in our patients, such as hypertension, coronary heart disease, diabetes mellitus, mild osteoporosis, and hyperlipidemia. However, our previous study did not find a significant association between comorbidities and postoperative IFD *(Chunrong Ju, et al. Epidemiology and Prognosis of Invasive Fungal Disease in Chinese Lung Transplant Recipients. Front Med (Lausanne) 2021; 8: 718747. doi: 10.3389/fmed.2021.718747.)* Therefore, these data are not provided in this article. Given that preoperative diabetes mellitus is generally considered a high-risk factor for

fungal infections, this data is added in the revised manuscript.

The drug levels of immunosuppressants were closely monitored in each patient after transplantation to achieve the ideal drug level. And the target level of each patient was individualized considering age, time elapsed since transplantation, immune status, infections, and rejection. According to our protocols, all the recipients received a standardized immunosuppressive scheme including an induction therapy and a triple immunosuppression maintenance therapy. The latter was consisted of a calcineurin inhibitor (cyclosporin A or tacrolimus), mycophenolic acid (MPA), and oral prednisolone. Generally, tacrolimus was administered twice daily at a dose of 0.075 mg/kg ideal body weight to achieve serum levels of 13–17 ng/ml during the first month, 12–16 ng/ml during the second month, 11–15 ng/ml during the third month, and 10–14 ng/ml between the 4th month and 1 year. The area under the curve of MPA was maintained at the range of 30-60 mg h/l. Methylprednisolone was administered at a dose of 500 mg at induction. Oral/injected steroids were titrated to 15 mg daily by 1 week and then maintained at 0.25 mg/kg body weight after that. The induction therapy was prescribed to part of the recipients, based on their specific conditions.

Changes in the text: The newly added data are in Table 1 (line 411).

2. The author said, primary endpoint is the post-transplant 1-year incidences of lung IFDs and airway fungal colonization (line 149-150). However, airway fungal colonization has not been fully described in result part (3.2 IFD).
   Reply: Thanks for the suggestion.
   Changes in the text: The newly added data are in Table 2 (line 419).

Reviewer B

Line 71-73: 22-year old reference.
DOI: 10.1097/TP.0000000000003717 ampho-B prophylaxis for at least 3 months suggested.
Reply: Thanks.
Changes in the text: We have deleted the original sentence and added a new sentence citing the suggested reference (line 86-88).

124: was anastomosis necrosis taken into consideration? Screening bronchoscopy is discussed in lines 143-146, but they do not seem to have influence on the duration of ampho-B?
Reply: Thank you for your good question. Anastomosis necrosis was also taken into consideration of the duration of NAB treatment. If a patient suffers from anastomosis necrosis, he was suggested to be on long-term NAB treatment because anastomosis necrosis is a very higher risk factor on IFD post-lung transplantation, based on our previous studies (*Chunrong Ju, et al. Epidemiology and Prognosis of Invasive Fungal Disease in Chinese Lung Transplant Recipients. Front Med (Lausanne) 2021; 8: 718747. doi: 10.3389/fmed.2021.718747. Ju C, et al. Antifungal prophylactic effectiveness and intrapulmonary concentrations of voriconazole versus posaconazole in lung transplant recipients. Med Mycol. 2022 Sep*

2;60(9):myac041. doi: 10.1093/mmy/myac041.).
Changes in the text: "Anastomotic necrosis" was added in line 135.

135: what about ISHLT 2010 consensus?
Reply: Thanks for the suggestion.
Changes in the text: We have added this citation in line 164.

173: very high spread 2 weeks to 3 months. Please report median and IQR of duration of ampho B in this group.
Reply: Thanks for the suggestion.
Changes in the text: The required data have been added (line 204-205).

178: repetition.
Reply: Thanks for the suggestion.
Changes in the text: We have deleted the repeated sentence.

186: medium-course group: did you mean, infection in spite of ampho-B, or did these patients have IPA at 3-6 months? because they were not on ampho-B then, so we cannot deem this a "breakthrough" infection. Please rephrase for clarity.
Reply: Thanks for the suggestion. A breakthrough infection was defined as an IFD that occurred during NAB treatment in our study. To eliminate the ambiguity, we have revised the sentence.
Changes in the text: The first sentence was revised as "In the prolonged-course NAB group, no breakthrough IFDs occurred throughout the NAB treatment." (line 215-216)

What about infections after 6 months? You analysed the whole year? would be interesting to see if there is a difference across the groups after 6 months.
Reply: Thanks for the suggestion. Similar to the 1-year IFD incidence, the 6-month data also show significant differences between the three groups.

Table. Post-transplant 6-months incidences of lung IFDs

	s-NAB (n=106)	m-NAB (n=132)	p-NAB (n=95)	P-value
Overall IFDs, n (%)	37 (34.9)	19 (14.4)	5 (5.3)	<0.001
Candidiasis, n (%)	20 (18.9)	4 (3.0)	0 (0)	
Aspergillus, n (%)	12 (11.3)	13 (9.8)	1 (1.1)	
Cryptococcosis, n (%)	1 (0.9)	0 (0)	0 (0)	
Pneumocystis jiroveci pneumonia, n (%)	3 (2.8)	1 (0.8)	2 (2.1)	
Mucormycosis, n (%)	1 (0.9)	1 (0.8)	2 (2.1)	

IFDs: invasive fungal diseases; s-NAB: short-course nebulized amphotericin B (<2 weeks); m-NAB: medium-course nebulized amphotericin B (2 weeks to 3 months); p-NAB: prolonged-course nebulized amphotericin B (>3 months)

236: incorrect, 2 weeks to 3 months.
Reply: Thanks for the suggestion.
Changes in the text: This sentence has been revised as "……was discontinued at 3 months after transplantation". (line 270-271)

240: what about prolonged group?
Reply: Thanks for the suggestion.
Changes in the text: The median time to Aspergillus spp. infection was 10 months in the prolonged-course NAB group, which has been added in line 276.

General: I would recommend further statistical analysis. Can you perform cumulative incidence curves of breakthrough IFDs?
Reply: Thanks for the suggestion. We defined breakthrough IFDs as infections that occurred during NAB treatment. However, the duration of NAB treatment differed significantly between the three groups, which resulted in different observation time window for the IFD infections as defined as breakthrough IFDs. This means that longer NAB treatment may reduce the risk of IFD but also increase the observation time, which may bias the final results. A more meaningful data is the post-transplant 1-year incidences of IFDs. Therefore, we believe further analysis of the incidence of breakthrough IFDs may not be accurate to reflect the nature of our data.

Reviewer C

This is a paper with important information. The methodology is adequate. The conclusion is pertinent with the results.
It is well written.

Reply: Thank you for your comments.