Itraconazole prophylaxis for invasive *Aspergillus* infection in lung transplantation.

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Abstract: Invasive *Aspergillus* infection (IA) is a significant cause of morbidity in lung transplantation (LT). However, its optimal prophylaxis is unclear. We routinely administer itraconazole (ITCZ) prophylaxis to all patients undergoing LT. In this study, we retrospectively evaluated the duration of prophylaxis and risk factors of IA. Among 30 adult patients who underwent LT, 5 patients developed IA. All patients with IA stopped ITCZ treatment within 1 year. At least 1-year of itraconazole prophylaxis is essential for the prevention of IA. Cytomegalovirus infection, renal replacement therapy and tracheotomy were risk factors for IA.

Key words (5): lung transplantation, itraconazole, invasive *Aspergillus* infection, therapeutic drug monitoring, prophylaxis duration

Introduction:

Invasive *Aspergillus* infection (IA) is a major cause of morbidity and mortality in lung transplantation (LT) patients (1). IA, occurring in 19-49% [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). Clin Infect Dis 2010 Apr 15;50(8):1101-1111]/ [Tofte N, Jensen C, Tvede M, Andersen CB, Carlsen J, Iversen M. Use of prophylactic voriconazole for three months after lung transplantation does not reduce infection with Aspergillus: a retrospective study of 147 patients. Scand J Infect Dis 2012 Nov;44(11):835-841.] LT patients, is responsible for deaths (2)[Felton TW, Roberts SA, Isalska B, Brennan S, Philips A, Whiteside S, et al. Isolation of Aspergillus species from the airway of lung transplant recipients is associated with excess mortality. J Infect 2012 Oct;65(4):350-356.]. Though prophylaxis is crucial, the optimal strategy for preventing IA is uncertain (3). Itraconazole (ITCZ) has been reported to exhibit significant activity against *Aspergillus* spp. and other fungi (4). At Kyoto University Hospital in Japan, we administered ITCZ prophylaxis to all patients undergoing LT. All patients were administered 200–400mg ITCZ as 1% oral solution (Itrizole®, Janssen Pharmaceutica). Serum ITCZ concentrations, i. e. itraconazole and hydroxyitraconazole (ITCZ plus OH-ITCZ) were measured once a week after administration. Therapeutic drug monitoring (TDM) of ITCZ was performed in at least one sample from each patient. There is little consensus on the duration of prophylaxis. In our hospital, the doctor in charge decided the dosing period.

Methods:

In this research, adult LT patients transplanted from 2008 to 2012 at Kyoto University Hospital were retrospectively studied. All lung recipients were followed clinically by a team of physicians (respiratory surgeons, anesthesiologist, infectious disease physicians, cardiologists) They participated in the pre-transplant evaluation and in post-transplant management of patients. Postoperative immunosuppression consisted of triple-drug therapy with cyclosporine or tacrolimus, azathioprine or mycophenolate mofetil, and corticosteroids. [H. Date, M. Aoe, Y. Sano, I. Nagahiro, K. Miyaji, K. Goto et al. Improved survival after living-donor lobar lung transplantation J Thorac Cardiovasc Surg, 28 (2004), pp. 933–940] Episodes of acute rejection were treated with high doses of methylprednisolone.

All patients received routine antifungal prophylaxis with micafungin intravenously and ITCZ enterally following transplantation. After serum ITCZ concentrations reached

500-1000 ng/ml, we stopped administering micafungin. We compared patients suffering IA with patients free from IA with more than 1 year observational time after transplantation.

Sixteen female and 14 male patients (age range 26–62 years, mean 45. 7 years) were included. We defined LT patients with proven, probable or possible IA based on European Organization for the Research and Treatment of Cancer/Mycoses Study Group criteria (5). The classification of Aspergillus infection is based on the type of the host-fungus relationship.[Karnak D, Avery RK, Gildea TR, Sahoo D, Mehta AC. Endobronchial fungal disease: an under-recognized entity. Respiration. 2006;74(1):88–104.]

Among 30 patients, 21 patients underwent double lung transplantation (DLT) and 9 underwent single lung transplantation (SLT). Four patients (13%) acquired cytomegalovirus (CMV) infection, 11 patients (37%) developed acute rejection and 12 patients (40%) underwent tracheotomy. During 5 years, 5 patients developed *Aspergillus* infection (IA group), including 2 patient with proven and 3 with probable (Table 1).

Twenty-five patients without IA were assigned as the control group (C group).

The demographic data of the patient were analyzed and described using mean and median for continuous variables. Comparisons of continuous data were made using the non-parametric Mann–Whitney test. Two-value comparisons of categorical data were made using the chi-square test or Fisher's exact test. A P value of < 0.05 was considered statistically significant.

Results: Five patients developed IA during observational period. IA patients all suffered invasive *Aspergillus* tracheobronchial infections, which was the most common form of IA [ScientificWorldJournal. 2011; 11: 2310–2329. Tracheobronchial Manifestations of Aspergillus Infections]. All cases had no obvious other organ involvement. The lung was the primary focus of IA.

All the 5 IA cases occurred more than 6 months after surgery. The median time to onset after transplantation in the present study was 307 days. Case 1-3 and 5 developed IA after discontinuing ITCZ prophylaxis. However, case 4 developed IA despite ITCZ administration, whose concentration was more than 1000 ng/ml at the time of IA onset. Under careful monitoring, immunosuppressants trough level were within limits of protocol [H. Date, M. Aoe, Y. Sano, I. Nagahiro, K. Miyaji, K. Goto et al. Improved survival after living-donor lobar lung transplantation J Thorac Cardiovasc

Surg, 28 (2004), pp. 933–940] over an entire period.
Graft of case 2 was loss because of IA and she had a re-transplantation. Case 3 was died because of chronic rejection, gastroparesis and combination of various factors. IA cause directly leading to death in case 5.
During prophylaxis, mean ITCZ concentrations were 878 ± 632 ng/ml in the IA group and 1059 ± 871 ng/ml in the C group (Table 2). There was no significant difference between IA group and C group.

Discussion:
IA occurred 5 patients (16.7%) which rate is relatively low. [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). Clin Infect Dis 2010 Apr 15;50(8):1101-1111]/ [Tofte N, Jensen C, Tvede M, Andersen CB, Carlsen J, Iversen M. Use of prophylactic voriconazole for three months after lung transplantation does not reduce infection with Aspergillus: a retrospective study of 147 patients. Scand J Infect Dis 2012
According to a previous study, IA developed a median of 96 days after transplantation, with 51% occurring within 90 days and 72% occurring within 180 days after transplantation (6). In our hospital, we think that antifungal prevention is partly effective, and as a consequence, the onset rate is comparatively low and the onset time is late. Among 30 patients in this study, 9 patients were administrated ITCZ for more than 1 year. None of them (9 patients) were diagnosed with IA. Twenty one patients stopped ITCZ administration within 1 year and 5 (24%) patients developed IA.
We suppose at least 1 year prophylaxis is necessary. Because of a small sample size and short observation period, further investigation is needed to decide adequate duration of prophylaxis.
Next, we examined the risk factors of IA in LT patients using univariate analysis (Table 2). Factors associated with IA development were CMV infection, renal replacement therapy and tracheotomy. Because of the small sample size, there was no significant difference, but advanced age and acute rejection tended to be associated with IA development (Table2), which was compatible with a previous report (7). SLT did not suppose a significant risk for IA in our study. It is probably because none of our patients had airway colonization with *Aspergillus* before transplantation (data not shown) and were not diagnosed with cystic fibrosis, which is a predictive factor of *Aspergillus* colonization (8).
It is convincible that *Aspergillus* was acquired after stopping ITCZ because all the 5 IA

patients never had positive sputum or BAL cultures for *Aspergillus* spp. before developing IA and their lungs are constantly exposed to *Aspergillus* spores in ambient air. On the other hand, Considering that *Aspergillus* spp. were generally isolated from 35% of LT recipients and anastomotic region is a vulnerable site, [[Felton TW, Roberts SA, Isalska B, Brennan S, Philips A, Whiteside S, et al. Isolation of Aspergillus species from the airway of lung transplant recipients is associated with excess mortality. J Infect 2012 Oct;65(4):350-356.]] another possible mechanism is distant activation of disease, as a result to ineffective early prophylaxis. Either, both or more mechanism are responsible for IA, but that is not confirmed. Further studies are required to investigate potential mechanisms of IA after LT.

Optimal prophylaxis strategies for lung transplant recipients remain poorly defined.
Different drugs are used throughout the world [Husain S, Zaldonis D, Kusne S, Kwak EJ, Paterson DL, McCurry KR. Variation in antifungal prophylaxis strategies in lung transplantation. Transplant Infectious Disease. 2006;8(4):213–218]
Echinocandins, nebulized amphotericin B, oral voriconazole, or itraconazole, alone or in combinations ar included. [Husain S, Zaldonis D, Kusne S, Kwak EJ, Paterson DL, McCurry KR. Variation in antifungal prophylaxis strategies in lung transplantation. Transplant Infectious Disease. 2006;8(4):213–218][Zaragoza R, Pemán J, Salavert M, et al. Multidisciplinary approach to the treatment of invasive fungal infections in adult patients. Prophylaxis, empirical, preemptive or targeted therapy, which is the best in the different hosts? *Therapeutics and Clinical Risk Management*. 2008;4(6):1261–1280]
The echinocandins may be potentially effective agents for prevention of IA, but their dosage form, intravenous injection, is not suitable after leaving hospital. Recent study showed that use of prophylactic voriconazole for LT patients does not reduce IA and its side effect is troublematic.[Tofte N, Jensen C, Tvede M, Andersen CB, Carlsen J, Iversen M. Use of prophylactic voriconazole for three months after lung transplantation does not reduce infection with Aspergillus: a retrospective study of 147 patients. Scand J Infect Dis 2012 Nov;44(11):835-841.] Now that the use of aerosolized medications may significantly reduce their toxic systemic effects and potential drug interactions, they are an attractive option [Solé A. Invasive fungal infections in lung transplantation: role of aerosolised amphotericin B. International Journal of Antimicrobial Agents. 2008;32(2):S161–S165]. We began to administer nebulised amphotericin B with ITCZ. We will evaluate combination therapy of nebulized amphotericin B and ITCZ including therapy duration.

In conclusion, IA remains a devastating disease in lung transplant patients. We need to reconsider the duration of ITCZ prophylaxis, we think that a prophylactic administration for at least 1 year after transplantation would be reasonable especially to patients considered to be at high risk of IA. CMV infection, renal replacement therapy and tracheotomy were risk factors for IA.

References

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Pt. no.	Age,gender	Diagnosiss	rejection	RRT	tracho tomy	CMV infection	Days of ITCZ	Onset(POD)	Survival	Lung Transplant type
1	35,F	UIP	+	-	-	+	101	668	alive	bilateral LDLT
2	55,F	IPF	+	-	+	+	166	300	alive	bilateral LDLT
3	58,F	BO after HSCT	-	-	+	-	63	363	died	bilateral LDLT
4	61,M	IPF	+	-	+	+	186	186	alive	bilateral LDLT
5	48,M	COPD	-	+	+	-	7	315	died	unilateral(R) DDLT

Table 1: Clinical characteristics of the 4 patients suffering IA

The clinical characteristics of the 4 patients suffering IA are listed in Table 1.

Pt. no. : Patient number, M: Male, F: female; R: right, BO: bronchiolitis obliterans; HSCT: hematopoietic stem cell transplantation, IPF: idiopathic pulmonary fibrosis, COPD: Chronic Obstructive Pulmonary Disease, RRT: renal replacement therapy, POD: postoperative day, LDLT: Living donor lung transplantation, DDLT: deceased donor lung transplantation

*: Case 3 developed IA though he was administered ITCZ at that time. He quitted ITCZ after diagnosis.

Table 2：Comparison of Invasive *Aspergillus* infection group to Control group

	IA group (5)	C group(25)	P value
Age(years):Mean	51. 4	44. 2	0. 19[a]
Gender: Male	2(40%)	12(48%)	0. 74
Transplantation type: DLT	4(80%)	17 (68%)	0. 59
Immunosupression before operation	2(40%)	10(40%)	1
acute rejection	3(60%)	8 (32%)	0. 23
CMV infection	3(60%)	1(4%)	0. 00071
RRT	1(20%)	0(0%)	0. 023
tracheotomy	4(80%)	8(32%)	0. 046
ITCZ use more than 6 months	1(20%)	14(56%)	0. 14
ITCZ use more than 1 year	0(0%)	9(36%)	0. 11
Duration mean	96. 2(7-166)	424(35-1556)	0. 13[a]
ITCZconcentration	878 ± 632 ng/ml	1059 ± 871 ng/ml	0. 61[a]

CMV infection, RRT and tracheotomy were risk for IA.

IA:Invasive *Aspergillus* infection

DLT:double lung transplant,

RRT: renal replacement therapy

CMV: Cytomegalovirus

a Mann-Whitney test