The Impacts of Antiretroviral and Antifungal Treatments, and Tuberculosis Co-infection on Mortality and Relapse in Patients with *Talaromyces Marneffei* Infection

Thanh Nguyen Tat[1, 2]*, Dat Nguyen Tat[1], Praveen T. Krishnamurthy[3]
[1]Medical Doctor, School of Clinical Research, Texila American University, Guyana
[2]Woolcock Institute of Medical Research, Ho Chi Minh City, Vietnam
[3]Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, The Nilgiris, Ooty-643001, India

Abstract

To date, clinical data on long-term clinical outcomes, including 6-month mortality and relapse in talaromycosis (Tm) patients and impacts of ART and secondary antifungal prophylaxis are still lacking. We conducted a secondary data analysis from 6-month prospective observation of patients with culture-confirmed talaromycosis who participated in the Itraconazole versus Amphotericin B for HIV-associated Talaromycosis (IVAP) trial. The primary outcome was 6-month Tm mortality, while the secondary outcome was relapse. Multivariable Cox proportional hazard models were used to identify predictors of outcomes of interest. The median patient age was 34 years (IQR:30 – 38). The median pre-ART CD4 counts at baseline were 10 (IQR: 5-21) cells/μL. The cumulative 76/435 (17.4%) patients died, and Tm relapse was observed in 18/435 (4.1%) patients. The multivariable analyses showed that strong independent predictors of 6-month Tm mortality included ineffective ART (either absence of ART or ART failure) (HR = 6.26, 95% CI: 3.95 – 9.92, P < 0.001), and TB co-infection (HR =1.98, 95% CI: 1.23 – 3.17; P < 0.01). Induction antifungal treatment with itraconazole versus amphotericin B deoxycholate was significantly associated with Tm death in the univariable model, however, it became insignificant in the multivariable model. In addition, the significant risk factors for Tm relapse were ineffective ART, induction antifungal treatment with itraconazole than intravenous amphotericin B, and shorter duration of itraconazole secondary prophylaxis after completing induction therapy in-hospital (all with significant P-values). Antiretroviral therapy, antifungal treatment and tuberculosis co-infection were main predictors for 6-month Tm fatality as well as relapse.

Keywords: Invasive fungal infections, Mortality, Relapse, Talaromyces marneffei, Vietnam.

Introduction

Talaromyces marneffei (Tm) is a dimorphic fungus that can cause a life-threatening systemic infection in immunocompromised individuals living in or traveling to Southeast Asia and southern China [1, 2]. The Tm mortality on antifungal therapy ranges from roughly 10% to 30% [3-7]. In the IVAP trial, approximately 20% of talaromycosis patients died within six months after admission, and roughly 10% of those who responded successfully to in-hospital intensive antifungal therapy further developed disease relapse or Tm IRIS after discharge [8]. Importantly, talaromycosis was reported to have a high relapse rate of 57% if patients were not maintained on itraconazole for secondary prophylaxis after a period of consolidation therapy (itraconazole 200 mg, orally twice daily

for 12 weeks), and the median time to relapse was 6 months after the induction treatment in a Thai series [9]. Another study in Thailand in 2007, 33 HIV-infected patients with talaromycosis received itraconazole as a secondary prophylaxis against talaromycosis and ART for a median follow-up of 18 months (range, 06-45 months). The incidence rate of talaromycosis relapse was zero case per 641 person-months (95% CI: 0-0.06 cases per person-months), and CD4 cells count increased by 100 cells/μL after 6 months of ART [10]. These studies were limited by either relatively small sample size or retrospective design. Markedly, the IVAP trial demonstrated that disseminated *T. marneffei* infection, disease relapse, HIV co-infections (particularly tuberculosis), as main causes of death among Tm patients during 6 month of study follow up.

Therefore, we conducted this study to study the incidence and risk factors for 6-month mortality and relapse among participants with *Talaromyces marneffei* in the IVAP trial, with focus on studying the impacts of antiretroviral therapy and antifungal treatments, and tuberculosis co-infection in talaromycosis patients. In addition, regarding poorer prognosis in patients with relapse than Tm IRIS, I will evaluate a set of clinical and laboratory features that can be helpful in differentiation between these two disease complications.

Materials and Methods

Ethics Statement

We used a secondary dataset from IVAP trial, "the Itraconazole versus Amphotericin B for HIV-associated Talaromycosis clinical trial", which was published in NEJM in 2017 [8]. The IVAP trial was approved by the independent ethics committee at each participating hospital, by the Vietnam Ministry of Health, and by the Oxford University Tropical Research Ethics Committee. The study dataset was permitted and provided by the principal investigator. All participants' names had been de-identified and the secondary dataset was manipulated in compliance with the principle in Good Clinical Practice and Helsinki Declaration. Using secondary dataset was causing less than minimal risk to study participants, in this respect the ethical clearance for this study was suggested to be waived off.

Study Setting, Design and Population

All 435 adult patients with culture-confirmed Tm infection who participated in the IVAP trial were included in the analysis. In brief, the IVAP trial recruited adult patients with Tm infection from five referral hospitals across Vietnam between October 2012 and December 2015 [8]. All patients were followed monthly to be assessed for clinical outcomes including deaths and disease relapse during 6 months of study participation. The study flowchart of IVAP participants during 6 months of prospective follow-up is presented in Figure 1

Study Definitions

Talaromycosis was defined as any disease conditions that *Talaromyces marneffei* was isolated from blood, skin scrapings, lymph node, bone marrow and/or anybody fluid samples [3, 8]. Isolation and identification of *Talaromyces marneffei* was performed in accordance with the standard culture techniques.
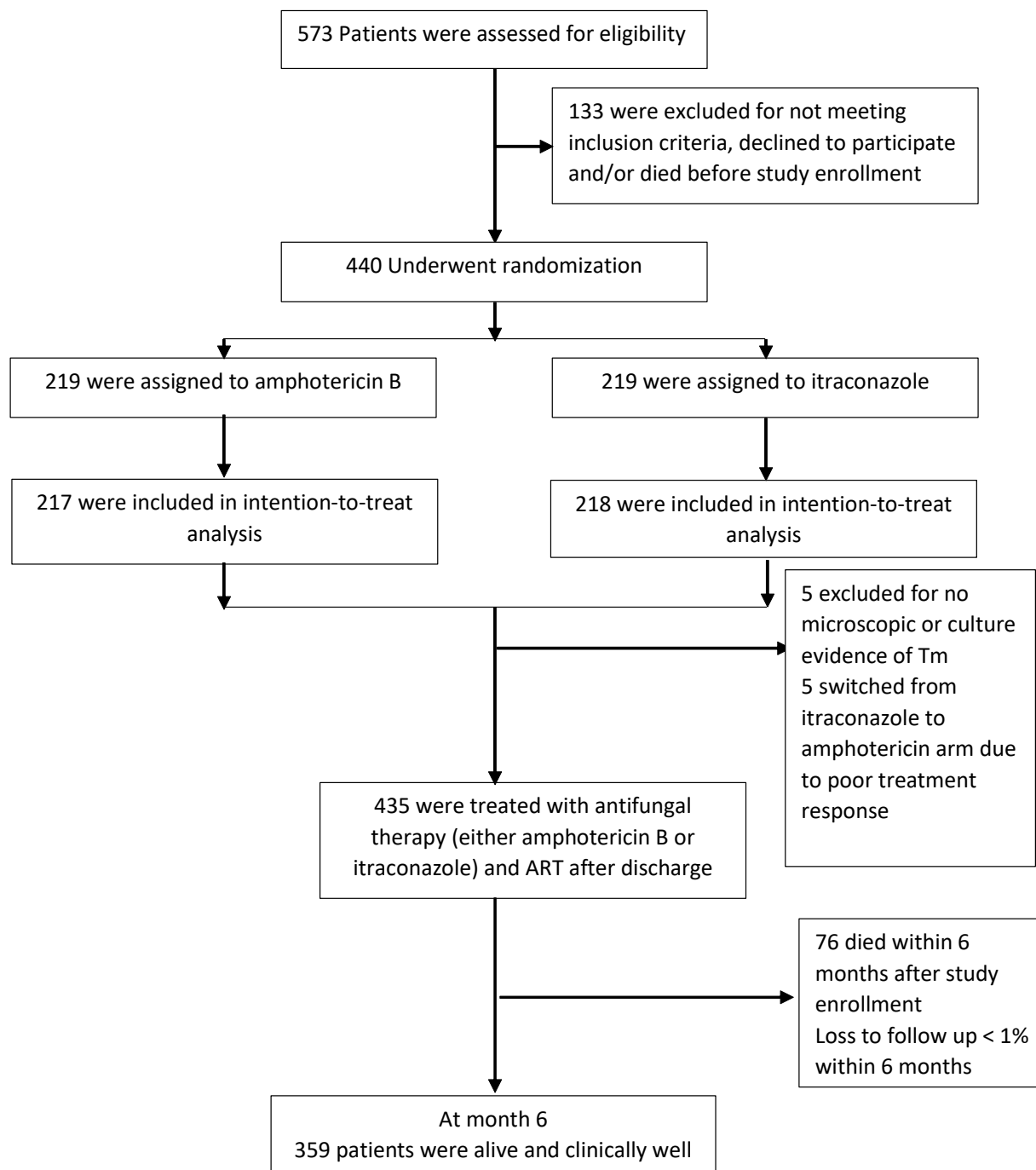
Figure 1. Study Flowchart of Study Participants during 6-Month Follow-up

Paradoxical Tm IRIS was defined as the "paradoxical" worsening of previously resolved symptoms of Tm infection which occurred in the context of effective antifungal treatment and ART therapy and/or increasing CD4 cell counts and excluded the non-compliance of antifungal and ART regimens [8, 11].

Disease relapse was defined as the recurrence of symptoms of previous Tm infection and a positive fungal culture from any sterile site, which occurred in the context of previously ineffective antifungal treatment and either not yet receiving ART or ART failure [3, 8].

Study Outcomes and Candidate Predictors

The primary endpoint was the development of Tm mortality within six-month follow-up period, while the secondary endpoint was Tm relapse. A set of pre-defined covariates included patient age, sex, the baseline CD4 cell count before starting ART, type of induction fungal therapy (itraconazole versus amphotericin B deoxycholate), antifungal secondary prophylaxis with itraconazole, tuberculosis co-infection and ART status (not on ART, ART failure, and effective treatment on ART). These candidate predictors were pre-determined on the basis of the disease pathogenesis and our clinical knowledge.

Data Collection

We used the de-identified secondary dataset from IVAP trial from October 2012 to December 2015 [8]. The study dataset was permitted and provided by the principal investigator.

Statistical Analysis

Descriptive analyses were performed for baseline characteristics of patients, using means (standard deviation (SD) for continuous variables and absolute count (%) for categorical variables. The complete-case analyses with univariable and multivariable Cox proportional hazards model was used to identify the clinical predictors of 6-month Tm death and relapse, based on the pre-defined sets of covariates. Time-to-event was the time from primary Tm infection diagnosis at study enrolments to death and relapse events.

The backward stepwise model selection was proceeded. Based on the parameters of AIC and BIC, the best predictive model was selected. Additionally, we used the Wilcoxon rank sum test for pairwise comparisons between Tm IRIS and disease relapse to identify important factors which can be used to distinguish those disease conditions. All analyses were conducted with R software, version 4.2.2 [12].

Results

Baseline Characteristics of Participants in IVAP Study

The mean age of patients was 35 years (standard deviation, SD = ± 7.4 years), and 69 % was male sex. The mean CD4 cell count at study enrolment was 22 cells/µL (SD = ± 47). Tuberculosis co-infection was observed in 19.6 % of study participants. A majority of participants (> 90%) were recommended and received ART during 6-month participation. Roughly 12.4 % of patients had the first-line ART failure (clinical, immunological and/or virological criteria). Induction antifungal treatments were randomized by ratio 1:1 with either intravenous amphotericin B or oral itraconazole in the IVAP trial, then all Tm patients would continue secondary itraconazole prophylaxis for 6 months on study participation after hospital discharge. The mean length of itraconazole prophylactic treatment was 5.3 months, (SD = ± 1.8), considering short duration of itraconazole intake among Tm patients experiencing death outcome.

Patients' Outcomes within 6-month Follow-up

Amongst the total of 435 participants, 76/435 (17.4%) patients died, and 18/435 (4.1%) patients experienced Tm relapse during 6 months of prospective observation in IVAP study. Notably, 06/18 (33%) patients with Tm relapse died of persistent high Tm burden and/or ineffective ART.

Risk Factors for 6-month Mortality in Patients with Talaromycosis

Table 1 presents the univariable and multivariable Cox proportional hazard models to identify risk factors for mortality of Tm patients during 6 months of follow-up. The multivariable Cox proportional hazard analyses showed that strong independent predictors of 6-month Tm mortality included either absence of ART or ART failure (HR = 6.26, 95% CI: 3.95 – 9.92, $P < 0.001$), and TB co-infection (HR

=1.98, 95% CI: 1.23 – 3.17; *P* < 0.01). Induction antifungal treatment with itraconazole was significantly associated with Tm death in univariable model (HR =1.86, 95% CI: 1.17 – 2.96; *P* < 0.01); however, it became insignificant in the multivariable model (HR =1.45, 95% CI: 0.9 – 2.32; *P* = 0.12).

Table 1. Risk Factors for 6-Month Talaromycosis-Associated Mortality

Risk factors	Fatal	Alive	Univariate effect HR (95% CI)	Multivariate effect HR (95% CI)
	(n = 76)	(n = 362)	*P* value	*P* value
Age, years (mean ± SD)	36 (± 7.1)	35 (± 7.4)	1.2 (0.9 – 1.59)	-
			P = 0.22	-
Male sex, n (%)	52 (68)	252 (70)	0.96 (0.59 – 1.56)	-
			P = 0.87	
Baseline CD4 counts, cells/µL (mean ± SD)	25 (± 53)	21 (± 46)	1.01 (0.97 – 1.05)	-
			P = 0.52	
Induction therapy with itraconazole (compared to amphotericin B), n (%)	48 (63)	166 (46)	1.86 (1.17 – 2.96)	1.45 (0.9 – 2.32)
			P < 0.01	*P* = 0.12
Not on ART or the first-line ART failure, n (%)	42 (55)	42 (12)	6.97 (4.43 – 11.0)	6.26 (3.95 – 9.92)
			P < 0.001	*P* < 0.001
Length of secondary itraconazole prophylaxis, months (mean ± SD)	5.2 (± 1.9)	5.4 (±1.7)	0.96 (0.85 – 1.08)	-
			P = 0.45	
Relapse, n (%)	6 (7.9)	12 (3.3)	2.0 (0.87 – 4.61)	-
			P = 0.10	
Tuberculosis co-infection, n (%)	27 (36)	59 (16)	2.46 (1.54 – 3.94)	1.98 (1.23 – 3.17)
			P < 0.001	*P* < 0.01

Summary statistics are mean (± SD) for continuous variables and frequency (%) for categorical variables; HR, hazard ratio; 95% CI, confidence interval

Risk Factors for Talaromycosis Relapse

Table 2 presents the univariable and multivariable Cox proportional hazard models to identify risk factors for disease relapse of patients with *T.marneffei* infection during 6 months of follow-up. The multivariable Cox proportional hazard analyses showed significant predictors of 6-month relapse from talaromycosis, including induction antifungal treatment with itraconazole compared to amphotericin B (HR = 6.58, 95% CI: 1.49 – 29.1, *P* = 0.013), absence of ART or ART failure (HR = 17, 95% CI: 5.97 – 48.2, *P* < 0.001). Longer duration of itraconazole secondary prophylaxis was a protective factor against Tm relapse (HR = 0.81, 95% CI: 0.67 – 0.98, *P* = 0.03).

Table 2. Risk Factors for Relapse from Talaromycosis

Risk factors	Tm relapse	Non-Tm relapse	Univariate effect HR (95% CI)	Multivariate effect HR (95% CI)
	(n = 18)	(n=420)	*P* value	*P* value
Age, years (mean ± SD)	35 (± 7.9)	35 (± 7.4)	1.19 (0.66 – 2.13)	-
			P = 0.56	
Male sex, n (%)	15 (83)	289 (69)	2.27 (0.66 – 7.82)	-
			P = 0.20	
Baseline CD4 counts, cells/µL (mean ± SD)	14 (± 10)	22 (± 48)	0.89 (0.67 – 1.18)	-
			P = 0.42	
Induction therapy with itraconazole (compared to amphotericin B deoxycholate), n (%)	16 (89)	198 (47)	9.24 (2.12 – 40.2)	6.58 (1.49 – 29.1)
			P < 0.01	*P* = 0.013
Length of secondary itraconazole prophylaxis, months (mean ± SD)	4.5 (± 2.3)	5.4 (±1.7)	0.82 (0.68 – 0.99)	0.81 (0.67 – 0.98)
			P = 0.04	*P* = 0.03
Not on ART or the first-line ART failure, n (%)	13 (72)	71 (17)	18.9 (6.73 – 53.1)	17.0 (5.97 – 48.2)
			P < 0.001	*P* < 0.001
Tuberculosis co-infection, n (%)	6 (33)	80 (19)	2.44 (0.91 – 6.49)	-
			P = 0.08	

Summary statistics are mean (± SD) for continuous variables and frequency (%) for categorical variables. HR, hazard ratio and 95% confidence interval

Differentiation between Tm IRIS and Disease Relapse

Table 3 shows the clinical factors which can be used to differentiate Tm IRIS from Tm relapse. On the basis of my clinical knowledge, six routine laboratory tests were selected and used to distinguish both disease conditions. There were statistical differences between Tm relapse and Tm IRIS in the CD4 cell count ($P <$ 0.001), haemoglobin level (P = 0.004), aspartate transaminase, AST (P = 0.02). More specifically, Tm IRIS patients had significantly higher levels of both CD4 cell count and haemoglobin, and normal value of AST compared to those with Tm relapse.

Table 3. Laboratory Features to Differentiate between Paradoxical Tm IRIS and Relapse

Factors	Tm IRIS* (n = 23)	Relapse* (n = 18)	*P* **
CD4 cell count at IRIS/Relapse (cells/µL)	94 (50 - 123)	13 (6 - 30)	< 0.001
White blood cell count (x 10^3 cells/µL)	5.0 (4.4 - 8.3)	5.1 (3.2 - 9.2)	0.68
Haemoglobin level (g/dL)	11.2 (10.3 - 12.6)	8.7 (8.0 - 9.1)	0.004
Platelet cell count (x 10^3 cells/µL)	286 (232 - 341)	263 (40 - 343)	0.38
Aspartate aminotransferase (AST) (U/L)	39 (25 - 55)	169 (62 - 308)	0.02
Alanine aminotransferase (ALT) (U/L)	27 (22 - 44)	54 (25 - 134)	0.28

* Summary statistics is median (interquartile range). ** P values were withdrawn from Mann-Whiney-U tests

Discussion

To our knowledge, this is a large prospective cohort to characterize the long-term clinical outcomes of talaromycosis. Our study cohort originated from the previously published IVAP trial [8]. Participants were closely monitored by well-experienced medical staffs from study team. Our study showed that the 6-month mortality rate of mortality was 17.4% and that of relapse was 4.1%. Although the 30-day in-hospital mortality of talaromycosis patients was well reported, ranging from 10% to 30% with antifungal treatments [3-7, 13]. To date, there are still lacking prospective data for 6-month mortality as well as relapse. The identification of main predictors of 6-month clinical outcomes will aid to design prevention schemes in order to reduce the disease burden.

The major causes of deaths reported in IVAP participants were tuberculosis, disseminated talaromycosis, Tm relapse, ineffective Tm secondary prophylaxis and severe immunosuppression due to either not receiving ART or ART failure. My study clearly showed strong independent predictors of 6-month Tm mortality, including either absence of ART or ART failure (HR = 6.26, 95% CI: 3.95 – 9.92, $P < 0.001$). This places emphasis on restoration of patient's immunity with robustly effective ART as soon as possible after induction antifungal therapy for Tm patients. Notably, the most important concern for rapid ART initiation after disseminated TM infection is Tm IRIS; however, our published report demonstrated that patients with Tm IRIS had a favourable survival outcome [14]. On this account, I recommend commencing ART quickly after hospital discharge. Another critical issue is the increasing number of the first-line ART failure in the Vietnamese HIV-infected population and among cohorts in African countries [15-19]. The WHO has recommended that dolutegravir-based regimens should be the prioritized in all HIV populations [20], and Thanh NT et al. showed the good results from dolutegravir-based ART was effective in terms of rapidly increased CD4 counts and HIV viral suppression, fewer side effects, and improved patient's treatment adherence in the HIV outpatients in Vietnam [19]. Notably, previous studies have revealed that tuberculosis is the most common HIV co-infection with the prevalence of roughly 22 % among HIV-infected patients [3, 19, 21]. Again, in this study TB co-infection was the strong independent predictors of 6-month Tm mortality (HR =1.98, 95% CI: 1.23 – 3.17; $P < 0.01$). TB had closely two-fold of risk of 6-month death among Tm patients.

Talaromycosis relapse was frequently observed complication among Tm patients alongside with tuberculosis, which considerably accounted for main causes of Tm deaths during first six months after induction antifungal treatment [8]. On this account, identifying the significant predictors of Tm relapse will aid to figure out proactive interventions to prevent Tm relapse and to improve the quality of life of patients. This study shows that absence of ART or ART failure, induction fungal treatment with itraconazole compared to amphotericin B deoxycholate and length of itraconazole secondary prophylaxis were the significant independent predictors for 6-month Tm relapse. These results indicate that suboptimal fungicidal activity on *T.marneffei* fungal load oral itraconazole (compared to intravenous amphotericin B), shorter duration of secondary antifungal prophylactic treatment and patient's immune suppression (absence of ART and/or ineffective ART) are the main mechanisms for developing of disease relapse. Notably, tuberculosis is pathologically an important risk factor for Tm relapse; however, this study revealed no statistical association between tuberculosis and Tm relapse. Therefore, commencing ART quickly after hospital discharge and longer duration of itraconazole secondary prophylaxis are recommended to prevent disease relapse.

In clinical practice, clinicians will certainly encounter the challenging situation of how to distinguish between patients with Tm IRIS and those with Tm relapse with high concern about high mortality (33%) observed in Tm relapse [14]. Hence, it is important to distinguish these disease complications because the patients with relapse need prompt awareness and early management. My study shows that the routine laboratory tests including haemoglobin, platelet cell counts, liver transaminases and CD4 cell counts are of significance to differentiate Tm relapse from Tm IRIS. Notably, these routine blood tests are easily conducted and inexpensive. Our study has several limitations. Firstly, using a secondary dataset to perform a post-hoc analysis is considered a primary limitation. Secondly, there was a certain percentage of missing data due to patient loss to follow-up, regarding that these parameters were significant predictors of the final Tm mortality and relapse risk models.

Conclusion

This study highlights that antiretroviral therapy, antifungal induction and prophylaxis treatments, and tuberculosis co-infections were the main predictors for 6-month Tm fatality as well as relapse. The routine laboratory tests including full blood count, liver transaminase and CD4 count were of significance to distinguish disease relapse with Tm IRIS, considering the poor outcome of Tm relapse.

Conflicts of Interest

All authors declare no conflict of interest.

Financial Support and Sponsorship

The study was self-funded.

Acknowledgements

We are grateful to all study participants. We especially thanked Dr. Le to provide the full dataset for us to conduct this study.

References

[1] Supparatpinyo K, Khamwan C, Baosoung V, Nelson KE, Sirisanthana T. Disseminated *Penicillium marneffei* infection in southeast Asia. Lancet 1994; 344:110-3.

[2] Vanittanakom N, Cooper CR Jr, Fisher MC, Sirisanthana T. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. Clin Microbio Rev. 2006; 19:95-110.

[3] Le T, Wolbers M, Chi NH, Quang VM, Chinh NT, Lan NP, et al. Epidemiology, Seasonality, and Predictors of Outcome of AIDS-Associated *Penicillium marneffei* Infection in Ho Chi Minh City, Viet Nam. Clin Infect Dis. 2011; 52:945–952.

[4] Larsson M, Nguyen LH, Wertheim HF, Dao TT, Taylor W, Horby P, et al. Clinical characteristics and outcome of Penicillium marneffei infection among HIV-infected patients in northern Vietnam. AIDS Res Ther. 2012; 9:24.

[5] Son VT, Khue PM, Strobel M. Penicilliosis and AIDS in Hai Phong, Vietnam: Evolution and predictive factors of death. Med Mal Infect. 2014; 44:495-501.

[6] Kawila R, Chaiwarith R, Supparatpinyo K. Clinical and laboratory characteristics of penicilliosis marneffei among patients with and without HIV infection in Northern Thailand: a retrospective study. BMC Infect Dis. 2013; 13:464.

[7] Hu Y, Zhang J, Li X, Yang Y, Zhang Y, Ma J, et al. *Penicillium marneffei* infection: an emerging disease in mainland China. Mycopathologia. 2013; 175:57-67.

[8] Le T, Kinh NV, Cuc NTK, Tung NLN, Lam NT, Thuy PTT, et al. A Trial of Itraconazole or Amphotericin B for HIV-Associated Talaromycosis. N Engl J Med. 2017; 376:2329-2340.

[9] Supparatpinyo K, Perriens J, Nelson KE, Sirisanthana T. A controlled trial of itraconazole to prevent relapse of *Penicillium marneffei* infection in patients infected with the human immunodeficiency virus. N Engl J Med. 1998; 339:1739-43.

[10] Chaiwarith R, Charoenyos N, Sirisanthana T, Supparatpinyo K. Discontinuation of secondary

prophylaxis against penicilliosis marneffei in AIDS patients after HAART. AIDS. 2007; 21:365-7.

[11] Thanh NT, Vinh LD, Liem NT, Shikuma C, Day JN, Thwaites G, Le T. Clinical features of three patients with paradoxical immune reconstitution inflammatory syndrome associated with *Talaromyces marneffei* infection. Med Mycol Case Rep. 2016; 19:33-37.

[12] The R Project for Statistical Computing, version 4.2.2, 2023. Available at https://www.r-project.org/. Accessed and cited on 8 August 2023.

[13] Jiang J, Meng S, Huang S, Ruan Y, Lu X, Li JZ, et al. Effects of Talaromyces marneffei infection on mortality of HIV/AIDS patients in southern China: a retrospective cohort study. Clin Microbiol Infect. 2019; 25:233-241.

[14] Thanh NT, Thu NTM, Ly VT, Guy T, Le T. Paradoxical Immune Reconstitution Inflammatory Syndrome associated with Talaromyces marneffei Infection among ART-Naïve Patients. The Asia Pacific AIDS & Co-infections Conference 2019 (APACC) June 27-29, 2019, Hong Kong.

[15] Assemie MA, Alene M, Ketema DB, Mulatu S. Treatment failure and associated factors among first line patients on highly active antiretroviral therapy in Ethiopia: a systematic review and meta-analysis. Glob Health Res Policy. 2019; 4:32.

[16] Hunt GM, Dokubo EK, Takuva S, de Oliveira T, Ledwaba J, Dube N, et al. Rates of virological suppression and drug resistance in adult HIV-1-positive patients attending primary healthcare facilities in KwaZulu-Natal, South Africa. J Antimicrob Chemother. 2017; 72: 3141-3148.

[17] Boender TS, Kityo CM, Boerma RS, Hamers RL, Ondoa P, Wellington M, et al. Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa. J Antimicrob Chemother. 2016; 71:2918-27.

[18] Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM. Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. Lancet Infect Dis. 2010; 10 :155-66.

[19] Thanh NT, Dat NT. Prevalence of HIV-associated Co-infections and Clinical Characteristics among HIV/AIDS Outpatients in the Context of Dolutegravir Roll-out Program in Vietnam. Texila International Journal of Public Health 2022.doi:10.21522/TIJPH.2013.10.02.Art021.

[20] World Health Organization. Transition to new antiretroviral drugs in HIV programmes: clinical and programmatic considerations. Geneva: 2017. [Internet]. [Accessed and cited on 9 August 2023]. Available from: http://apps.who.int/iris/bitstream/10665/255887/1/WHO-HIV-2017.23-eng.pdf?ua=1.

[21] Mitku AA, Dessie ZG, Muluneh EK, Workie DL. Prevalence and associated factors of TB/HIV co-infection among HIV Infected patients in Amhara region, Ethiopia. Afr Health Sci. 2016; 16:588-95.