

1. Study Purpose and Rationale

The opportunistic mycosis caused by *Penicillium marneffeii* is endemic in South and Southeast Asia and has emerged as a common Human Immunodeficiency Virus (HIV)-associated opportunistic infection, ranking third after tuberculosis and cryptococcal meningitis in Northern Thailand and after *Pneumocystis jirovecii* pneumonia (PCP) and tuberculosis in Hong Kong. It is increasingly diagnosed in immunocompromised individuals in non-endemic regions who have emigrated from or have traveled to Southeast Asia. HIV-infected individuals with CD4 count <100 cells/ μ L are particularly at risk and make up the majority of infections in endemic regions. Untreated disseminated infection is fatal, and case fatality rate in treated individuals is between 10% and 28%. Despite being geographically restricted and despite considerable research efforts to look for its ecological niche thus far, *P. marneffeii*'s natural reservoir and its modes of transmission remain poorly understood.

There are several known risk factors associated with *P. marneffeii* infection. *P. marneffeii* has been isolated from the bamboo rat (*Rhizomys* and *Cannomys* species), but exposure to or consumption of these rats does not correlate with human illness. *P. marneffeii* infection has been associated with male gender and intravenous drug use. Infection rates increase in immunocompromised individuals during periods of heavy rainfall. In particular, soil exposure during the rainy season has been associated with *P. marneffeii* infection. Infection has also increased during times of high humidity. This study will seek to understand the environmental reservoir of *P. marneffeii*, with the hypothesis that outdoor occupational exposures, including variants of soil, water, and organic matter, but also including non-occupational exposures, will lead to higher incidence of illness.

The geographic distribution of *P. marneffeii* infection in Vietnam has not been studied and remains based on anecdotal information. This study will seek to reveal this geographic distribution. This new information may indicate clusters of illness and aid in identifying an environmental reservoir in that local environment. Based on the environmental exposure and geographic information, public health officials and physicians in Vietnam will be able to tailor screening programs and interventions to treat and limit exposure to this illness based on location and exposure risk.

2. Study Design and Statistical Procedures

This is a chart review of previously collected data. Cases (N=230) were consecutively recruited from HIV-infected individuals from inpatient and outpatient HIV clinic at the Hospital for Tropical Disease (HTD) in Ho Chi Minh City, Vietnam who are newly diagnosed with culture-confirmed penicilliosis over a 12 month period. Cases from inpatient and outpatient clinic were recruited to minimize case selection bias towards a milder or more severe group of disease. Since HTD is both a primary and a referral center for HIV care for southern VN, the case population very closely reflects the general patient population with penicilliosis.

HIV-infected controls (N=460) were recruited simultaneously. Control subjects were similar to cases but have never been diagnosed or at the time of recruitment or suspected to have penicilliosis and are simultaneously admitted to the hospital for other conditions or seen in

the clinic for routine or acute care. Controls from inpatient care needed to have a negative blood culture; controls from clinics had a blood culture drawn at enrollment. If a blood culture turned positive for *P. marneffeii*, that subject was enrolled as a case. Two controls were individually matched to each case for sex, 5-year age categories, WHO HIV disease stage and injection drug use status.

All subjects completed a one-to-one estimated 30-minute interview with 2 trained staff. The interviewers were blinded to the disease status of the subjects. All data for subjects has been coded and stored behind 2 secure locks.

Univariate and multivariate logistic-regression models will be used to estimate the odd ratios and associated 95% confidence intervals of exposure variables. Assessment of presence of exposure, duration of exposure and recent/past exposure will be made for all exposure variables. Multivariate models will be created through stepwise elimination of variables of interest from univariate analysis while relevant variables will be retained. Additive and multiple interactions among exposure variables will be evaluated.

Subject address data (province, district, and ward) will be recorded in GIS mapping software. Cluster analysis will be done using Euclidian distance from district and ward-level centroids of subjects' primary addresses. Heat maps created to identify loci of disease.

Power analysis: this study will use an $\alpha = 0.05$ and $\beta = 0.80$. Assuming that controls will have no relation between disease status and environmental exposure and location ($p_1=0.5$), the study will be able to detect a $p_2>0.61$.

3. Study Procedures

There are no procedures in this study as this study will be completely based on chart review.

4. Study Drugs or Devices

There are no study drugs or devices

5. Study Instruments

Data was collected using a questionnaire developed at the Oxford Clinical Research Unit (OUCRU) in Ho Chi Minh City. The questionnaire was written in English and translated into Vietnamese.

6. Study Subjects

As state above, study subjects are HIV-infected adult patients who present or are referred to HTD in Ho Chi Minh City, Vietnam. The study includes both inpatient and outpatient groups. Excluded groups are patients under 18 years old. Cases (N=230) were newly diagnosed with culture-confirmed penicilliosis over a 12 month period.

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the hospital for other conditions or seen in the clinic for routine or acute care. Controls from inpatient care needed to have a negative blood culture; controls from clinics had a blood culture drawn at enrollment. If a blood culture turned positive for *P. marneffeii*, that subject was enrolled as a case. Two controls were individually matched to each case for sex, 5-year age categories, WHO disease stage and injection drug status.

7. Recruitment

There is no current recruitment for this study, as data has already been collected and de-identified. However, these subjects were originally recruited in the inpatient and outpatient settings of HTD in Ho Chi Minh City, Vietnam. They were recruited by direct interaction with investigators after being informed of the study by their treating physician.

8. Informed Consent Process

There will be no informed consent collected during this study as data has been previously collected and de-identified. As it is a chart review, there is minimal risk to the subjects. On initial recruitment and data collection, consent was obtained using consent form requiring signature in Vietnamese which was administered by Vietnamese-speaking members of the investigation team.

9. Confidentiality of Study Data

Initial data collection is on a paper form which is kept in a locked file cabinet behind a locked door. The PI at OUCRU (Dr. Thuy Le) has the key to both the door and cabinet. Data from the paper form is coded for electronic data entry using Microsoft Access. This data file is password-protected. No identifying data is present in the electronic file.

10. Privacy Protections

All personal information recorded in questionnaires is kept secure as described above. All other coded data is password-protected with no identifying information besides subject number.

11. Potential Risks

Risks of this study are minimal as data collection and enrollment have been completed and this study only involves chart review.

12. Data and Safety Monitoring

There is no indication for safety monitoring in this study as risk related to chart review is minimal.

13. Potential Benefits

Benefits to subjects consist of identifying potential risks associated with *P. marneffeii* infection. This may prevent future infection with this fungus. Benefits to other immunocompromised individuals in Southern Vietnam as well as the remainder of Southeast Asia include identifying risks associated with *P. marneffeii* infection and identifying geographic loci of illness.

14. Alternatives

This is not a clinical trial involving therapy.

15. Research at External Sites

Research will be primarily conducted at the Oxford Univ. Clinical Research Unit (OUCRU) and the Hospital for Tropical Disease (HTD) in Ho Chi Minh City, Vietnam. Institutional review at OUCRU has been completed and the project has been approved. This project is funded through OUCRU. All data will be maintained at OUCRU as described above. OUCRU will be the lead institution on this project.

16. Columbia as Lead Institution

CU will not serve as the lead institution.