DIAGNOSTIC MODEL OF TALAROMYCOSIS IN HIV/AIDS PATIENTS

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ABSTRACT

Introduction: Talaromycosis (formerly penicilliosis), one of the most important opportunistic infections in Southeast Asia, is a common cause of HIV-AIDS-associated death. The current diagnosis of *Talaromyces marneffei* relies on culture. As microbiological results are often late, taking up to 14 days for pathogens isolation, the mortality rate is high. Serum Mp1p ELISA test was used to determine an earlier diagnosis of Talaromycosis. The research objectives were: (1) to determine the cut-off value, sensitivity, and specificity of the Mp1p ELISA test in AIDS patients; (2) to develop diagnostic models (with and without Mp1p ELISA test) of talaromycosis in AIDS patients. **Methods:** A descriptive cross-sectional study. HIV-infected patients aged ≥18 years with TCD4 <100 cells/mm^3 admitted to Ward E, Hospital for Tropical Diseases from June 2017 to July 2019 were recruited. Data analysis using SPSS 25.0 software. A receiver operating characteristic (ROC) curve and Youden index on the ROC curve was used to determine the cut-off value. The area under the ROC curve (AUC) and the 95% confidence intervals (CIs) were used to determine the discrimination power between cases and controls. Chi-square test, odds ratio (OR), and 95% confidence interval were used to find associations among variables. After multivariate analysis, the model of talaromycosis diagnosis was built by Monogram in R.3.6.3 software. **Results:** 533 patients meeting the inclusion criteria were enrolled. 78.4% were male; the median age was 34 years (IQR: 29-40), and 77.5% were <40 years-old groups. 70 patients diagnosed with Talaromycosis were compared with other opportunistic infected participants (OIs).. The maximum Youden index=0,856 in correlation with the 0,22 OD cut-off value of serum Mp1p presents the highest sensitivity and specificity, with SEN = 88.6% and SPE = 97.0%, respectively. Two models (with and without serum Mp1p) were built based on the adjusted OR of risk factors. Two models based on living areas, skin lesions, low platelet, and Mp1p result. The two models have high accuracy in diagnostic talaromycosis. The SEN and SPEC of the model without Mp1p were 68.6% and 98.1%, and the SEN and SPEC of the model with Mp1p were 85.7% and 98.7%. **Recommendation:** Serum Mp1p ELISA as well as Diagnostic models of talaromycosis are considered as novel tools for early diagnosis of Talaromycosis in AIDS patients.

Keywords: *HIV/AIDS, TCD4, Talamomyces marneffei, Talaromycosis, Mp1p ELISA, cut-off value, diagnostic model.*

I. INTRODUCTION

Talaromycosis, an invasive fungal infection caused by *Talaromyces marneffei (T.marneffei)*, formerly *Penicillium marneffei*, ranks the third opportunistic infections (OIs) in Asian countries, after tuberculosis and Cryptococcosis. This is a disseminated infection that occurs predominantly in individuals whose TCD4 count is below 100 cells/mm^3. According to Thuy Le et al (2011), the mortality rate can be up to 20%; one of the major causes of this mortality rate is late diagnosis and treatment [6]. The common symptoms of diseases consist of prolonged fever, weight loss,

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hepatosplenomegaly, lymphadenopathy, and anemia. However, these symptoms are not always presented; only range 50 – 60% [1],[2],[4]. Moreover, these symptoms are non-specific and can be indistinguishable from those of disseminated tuberculosis, other systemic mycoses like *Cryptococcus* species, or other infections due to intracellular pathogens such as *Salmonella* species. Skin lesions with central-necrotic papules on the face, trunk, and extremities, the most specific characteristic, occur in the late stage of talaromycosis with a percentage of 40 – 70%. Therefore, it is uncertain to diagnose the disease only based on clinical symptoms. Up till now, a definitive diagnosis of Talaromycosis is mostly based on the isolation of the organism from culture of specimens such as blood, and skin lesions. However, the cultural result cannot be immediate; usually, it returns in 4 – 5 days but sometimes takes up to 14 days. In a recent talaromycosis treatment trial in Vietnam, 38 of 573 (6.6%) patients died before the culture became positive [3]. The rate of isolation varies from the specimen; the rate in blood and skin lesion is 76% and 84%, respectively [4]. That means 15 – 20% of talaromycosis patients cannot be diagnosed based on routine specimens. In such cases, bone marrow culture is required; but this technique can only be done at the big hematology centers [8]. For the above reason, a new test or model is needed to diagnose talaromycosis.

Recently, a new biology marker, Mp1p – a Mannoprotein which is a major part of fungal cells and has many functions like determining cell shape, supporting growth, and shape change, allowing intercellular attachment, covering voids in the cell wall; as well as closely related to bacterial virulence, has been researched. The Mp1p ELISA was positive in 15/20 (75.0%) of culture-confirmed talaromycosis patients and negative in 537/540 (99.4%) of control patients, demonstrating excellent analytical and clinical specificities [7]. This result was not convincing because the number of talaromycosis is too small compared to the number of control group and there are only 15/540 patients in the control group are invasive fungal infections. The Mp1p ELISA has also been shown to be more sensitive than blood culture in store specimens (in 372 culture-proven talaromycosis cases, sensitivity was 86.3% for the Mp1p ELISA and 74% for blood culture) and is highly specific (98.1% specificity in 338 healthy controls and 179 patients without HIV, but with other infections)[5]. However, the cut-off value of the test has not been defined clearly (0.5 OD or 0.2 OD)

To shorten the diagnostic time and to improve talaromycosis outcome, the research aims to: (1) determine the cut-off, sensitivity, and specificity of the Mp1p ELISA test in AIDS patients; (2) develop and validate models (with and without Mp1p ELISA test) in diagnosis talaromycosis in AIDS patients.

II. MATERIALS AND METHODS

2.1 Study design: Cross-sectional observation study

2.2 Sample size: 533 HIV/AIDS patients admitted to Ward E, Hospital of Tropical Diseases (HTD) from 6/2017 to 7/2019.

2.3 Inclusion Criteria: (1) HIV patients ≥ 18 years old; (2) TCD4 count below 100 cells/mm^3.

2.4 Exclusion Criteria: (1) History of talaromycosis or *Cryptococcus neoformans* meningitidis; (2) use of anti-fungal drugs, (3) pregnancy.

2.5 Data collection: age, gender, address, BMI, route of HIV transmission, history of

ART, lymphocyte count, hemoglobin concentration, transaminases (AST, ALT), TCD4 count, history of cotrimoxazole prophylaxis, tuberculosis prophylaxis, and Mp1p result. The collection period was from 6/2017 to 7/2019.

Talaromycosis is defined based on the results of blood cultures, skin lesions, bone marrow, or body fluids.

2.6.Data collection tools: The information was collected by questionnaire and medical records.

2.7.Measurements: Mp1p was performed by the Sandwich ELISA technique at the time when the patient enrolled in the study. Mp1p was detected by attaching polyclonal antibodies (PAbs) to the Mp1p antigen at the bottom of the microplate. Then, the specimen was placed on the surface. If Mp1p is present in the specimen, it will be detected by polyclonal antibodies (PAbs). This antigen-antibody complex can be detected using the biotin-bound Mp1p monoclonal antibodies (MAbs). This antibody can be bound to the staining enzyme streptavidin horseradish peroxidase (HRP), allowing the presence of Mp1p to be determined in patient samples.

2.8 Data analysis: The SPSS 20.0 software was undertaken. Descriptive statistics were used to report variables related to the demographic, clinical, and subclinical data of patients.

A receiver operating characteristic (ROC) curve was generated, which displayed all sensitivity and specificity pairs for different OD cutoff points using GraphPad Prism 8.0. The assay cutoff was determined based on the Youden index on the ROC curve, which maximizes true positives and minimizes false positives. The discrimination power between cases and controls was determined by calculating the area under the ROC curve (AUC) and the 95% confidence intervals (CIs). The point estimates and the 95% CIs for sensitivity and specificity were calculated based on the reference standard

Analytic statistics with a Chi-square test, odds ratio (OR), and 95% confidence interval were used to compare 2 group: talaromycosis and control. After multivariate analysis, the model of diagnosis talaromycosis was built by Monogram in R.3.6.3 software.

2.6 Ethics consideration: Ethical approval was achieved by the ethics committee in the biomedical research Hospital of Tropical Disease, Decision No. 08/HĐĐĐ dated Jan 29, 2018. The research ensured the voluntary consent of participants and confidentiality, and the research results were used for research purposes only.

III. RESULTS

In the collection period, 533 HIV/AIDS with TCD4 count below 100 cell/mm^3 patient enrolled in the study. Of these patients, 418 (78.4%) were male, 396 (74.3%) were below 40 years old, and 169(31.7%) had not used ARV.

3.1. Diagnostic value of serum Mp1p ELISA test.

Among 533 patients, there were 70 (13.1%) talaromycosis patients and 463 opportunistic infections (consisting of tuberculosis, cryptococcis, sepsis,...). The median of serum Mp1p in talaromycosis was 2.53 (IQR: 0.76 – 3.01), higher than in the other OIs groups, which was 0.02 (IQR: 0.01 – 0.05), $p < 0.01$.

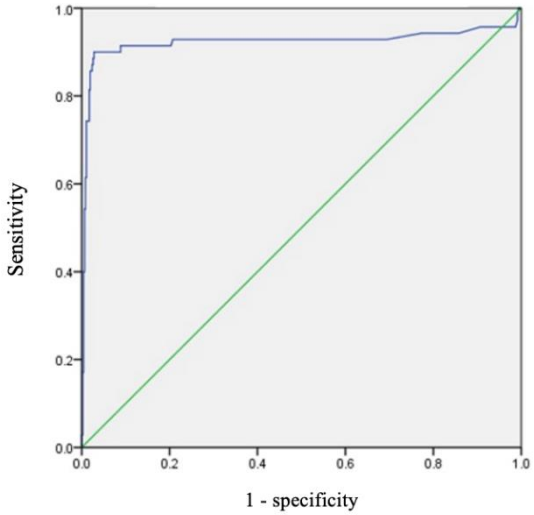

Figure 1. ***The receiver operating characteristic (ROC) of serum Mp1p ELISA test***

Figure 3.1. shows the ROC curve plotting true positives (sensitivity) against false positives (1-specificity). The AUC demonstrated a 93% (95% Confidence Interval (CI): 88 – 98%) accuracy in the discrimination between participants with and those without talaromycosis.

Table 1. ***The cut-off value, sensitivity, and specificity of serum Mp1p ELISA test***

Mp1p	Sensitivity	Specificity	Youden index
0,0015	1,000	0,002	0,002
0,0035	0,971	0,013	- 0,016
0,0025	0,986	0,011	- 0,003
...	...	...	...
0,2095	0,886	0,965	0,851
0,2175	0,886	0,968	0,854
0,2205	0,886	0,97	0,856
0,2225	0,871	0,97	0,841
...	...	...	...
3,61400	0,029	1	0,029
3,75350	0,014	1	0,014

At cut-off 0.22 OD generated by the Youden index, the diagnostic characteristics of the serum Mp1p ELISA test were: sensitivity (SEN) = 88.6%, specificity (SPE) = 97.0%, Youden index = 0,856 (Table 3.1).

3.2. Diagnostic model of talaromycosis in HIV/AIDS patients

a. Diagnostic model of talaromycosis without serum Mp1p ELISA test

After adjustment by multivariate analysis, living in Southeast and Central Highlands region (OR: 4.16, 95%CI: 1.68 – 10.28), skin lesions (OR: 60.09, 95%CI: 22.12 – 163.20) platelet per 1 K/ul-decrement (OR: 1.02, 95%CI: 1.01 – 1.04) were still associated with talaromycosis. Three risk factors were used to develop the diagnostic model.

Table 2. ***Risk factors of talaromycosis without serum Mp1p (Multivariate analysis)***

Variable	OR (95%CI)	P value
Living in the Southeast or Central Highlands	4.16 (1.68 – 10.28)	0.01
Skin lessions	60.09 (22.12 – 163.20)	<0.001
Platelet per 1 K/ul-decrement	1.02 (1.01 – 1.04)	0.01

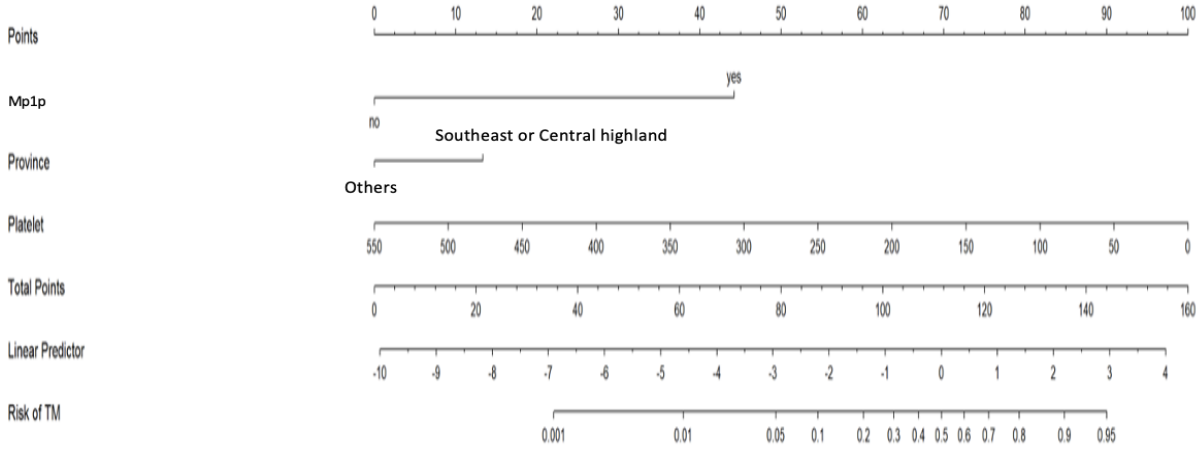

Figure 2. ***Diagnotic talaromycosis model (without serum Mp1p ELISA)***

When using data from this research to validate the model, it showed that the sensitivity and specificity were 68.6% (48/70) and 98.1% (454/463), respectively. The overall accuracy percentage was 94.2% (Table 3.3)

Table 3. ***Validating model diagnostic of talaromycosis (without serum Mp1p ELISA)***

		Predictive talaromycosis		Accuracy percentage
		Yes	No	
Definitive talaromycosis	**Yes**	48	22	68,6
	No	9	454	98,1
Overall accuracy percentage				94,2

To illustrate the model, we have an example. A HIV/AIDS patitent living in the Southeast or Central Highlands region with TCD4 < 100 cells/mm^3 was admitted to the hospital with a skin lesion, and the platelet was 100 K/ul. The total score was 140 (45 for skin lesions, 15 for living in the Southeast or Central Highlands region, and 80 for platelet). So, the risk of talaromycosis was 92%. Because the overall accuracy percentage of the mode was 94.2%, the real risk of talaromycosis was 86.7%.

b. Diagnostic model of talaromycosis with serum Mp1p ELISA test

After combining Mp1p with other factors, there were four risk factors still associated with talaromycosis: living in Southeast and Central Highlands region (OR: 4.42 CI: 1.30 – 14, 99%, skin lesions (OR: 100.93, 95%CI: 12.62 – 808.45) platelet per 1 K/ul-decrement (OR: 1.02, 95%CI: 1.01 – 1.04) and serum Mp1p positive (OR: 451.32, 95% CI:56.29 – 3618.42).

Table 4. ***Risk factors of talaromycosis without serum Mp1p (Multivariate analysis)***

Variable	OR (95%CI)	P value
Living in the Southeast or Central Highlands	4.42 (1.30 – 14.99)	0.02
Skin lesions	100.93 (12.62 – 808.45)	<0.001
Platelet per 1 K/ul-decrement	1.02 (1.01 – 1.04)	0.02
Mp1p	451.32 (56.29 – 3618.42)	<0.001

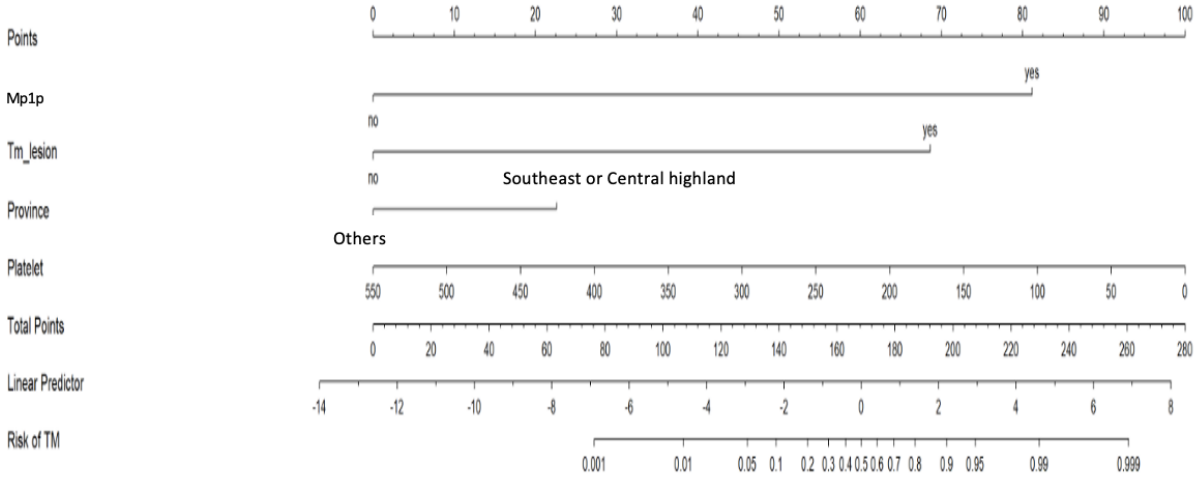

Figure 3. ***Diagnostic model of talaromycosis (with serum Mp1p ELISA)***

The model with serum Mp1p ELISA test had sensitivity and specificity was 85.7% (60/70) and 98.7% (457/463) and the overall accuracy percentage was 94.2% (Table 3.5)

Table 5. ***Validating diagnostic model of talaromycosis (with serum Mp1p ELISA)***

		Predictive talaromycosis		Accuracy percentage
		Yes	No	
Definitive talaromycosis	**Yes**	60	10	85.7
	No	6	457	98.7
Overall accuracy percentage				97.0

Return to the previous case, now the patient has a serum Mp1p ELISA test result. If the result was negative, the new total patient score would be 130, the risk of talaromycosis would be 6% and the real risk of talaromycosis would be 5.82%. In contrast, if the result was positive, the new total patient score would be 250, the risk of talaromycosis would be 99.0% and the real risk of talaromycosis would be 85.7%.

IV. DISCUSSION

By the Youden index, the cut-off was defined at 0.22. This cut-off was similar to the cut-off value in the study of Wang YF et al (2011) (0.22 vs 0.2), but smaller than in the study of N.T.M.Thu et al (2021) (0.22 vs 0.5) [5],[7]. The different ways our and Wang YF ‘s research used “fresh” patient serum, but N.T.M.Thu‘s research was a retrospective study with stored specimens and insufficient clinical data. Therefore, the

cut-off value at 0.2 OD is better to diagnose talaromycosis.

ROC curve and Youden index allow for comparison of the values of different cut-off values based solely on mathematical optimization of sensitivity and specificity. However, in clinical practice, which cut-off value is more appropriate to apply depends on many other factors. According to N.T.M.Thu et al. (2021), when reducing the cut-off value from 0.5 to 0.2 OD, the sensitivity increased by 4.5% (86.5% to 91%) but the specificity decreased by 8.2% (98.1% to 89.9%). With the rate of talaromycosis in the AIDS population is about 10%, for every 1000 people infected with HIV, decreasing the cut-off point from 0.5 to 0.2 OD will help to diagnose 4.5 more talaromycosis patients, but 74 people without talaromycosis will be over-treated with the antifungal drug amphotericin B, an anti-fungal drug can cause renal failure, and maintain itraconazole for at least 6-12 months. In this case, the cost of over-treating these additional patients must be weighed against the reduced mortality in untreated talaromycosis patients. Therefore, the decision to choose the Mp1p cut-off value depends on the purpose of the Mp1p test: diagnosis of talaromycosis in suspected cases or screening a large group without symptoms.

The sensitivity, and specificity of serum Mp1p ELISA were 88.6%, and 97.0%, respectively; and the AUC demonstrated a 93% (95% CI: 88 – 98%) accuracy in the discrimination between participants with and those without talaromycosis. These results are similar to Wang and N.T.M.Thu's studies [5],[7]. Thereby, serum Mp1p is a promising test in the diagnosis of talaromycosis. Moreover, another advantage of the serum Mp1p test should be emphasized that this technique can shorten the time to diagnosis (6 hours); compared to traditional cultures (which can take up to 10-14 days). Not only that but this test can also be performed in medical center substandard laboratories to perform culture techniques. However, these studies were performed in Vietnam and China, which are considered epidemic areas of talaromycosis; so, it is needed to perform other studies in different areas or populations.

So far, talaromycosis usually has been diagnosed by clinical symptoms such as prolonged fever, weight loss, hepatosplenomegaly, lymphadenopathy, and anemia. However, these symptoms are indistinguishable in many other OIs, especially in the late stage of HIV/AIDS patients. Tables 3.2 and 3.4 show that these symptoms have little value in the diagnosis of talaromycosis in AIDS patients with TCD4 < 100 cells/mm^3. Living areas, skin lesions, low platelet, and especially Mp1p result are more helpful to the diagnosis of talaromycosis.

Both two models (with and without Mp1p) have high value in diagnostic talaromycosis; the overall accuracy percentage was 94.2% and 97%. However, the model without Mp1p has a low sensitivity (68.2%), but a high specificity (98.1%). So, this model should be used to eliminate than diagnosis of talaromycosis. For example, if an AIDS patient with TCD4 counts below 100 cells/mm^3 is admitted because of fever, hepatosplenomegaly, or anemia; but does not live in the Southeast/Central Highland, or other *T.marneffei* epidemic regions, nor does have skin lesions or low platelet. Based on the model, the patient does not look like

talaromycosis, we should do other test to differentiate other OIs. In the model with Mp1p, the sensitivity is higher (85.7% vs 68.2%), and the specificity is similar (98.7% vs 98.1%). The reason is that serum Mp1p ELISA has a strong correlation with talaromycosis, the multivariate-adjusted OR was 451.32 (56.29 – 3618.42), much higher than other risk factors (living areas, skin lesion, and low platelet). So, serum Mp1p should be done in every AIDS patient with TCD4 below 100 cells/mm^3 to screen for talaromycosis.

Figures 3.2 and 3.3 show the contribution of each risk factor in the model (with and without serum Mp1p ELISA). These shall be helpful tools in the diagnosis of talaromycosis. Previously, talaromycosis patients without skin lesions is a challenge for doctors. Nowadays, based on these models, the doctor can use other information such as living areas, low platelet, and especially serum Mp1p to diagnose talaromycosis. Patients should be tested for serum Mp1p if suspected talaromycosis although skin lesions are absent.

These models are new ones. Therefore, it is needed to perform other studies with larger sample sizes in different areas or populations to validate the accuracy of models.

V. CONCLUSION AND RECOMENDATION

Serum Mp1p ELISA with cut-off 0.22 OD is a novel test in diagnostic talaromycosis, so should be applied in clinical practice. Two models (with and without serum Mp1p ELISA) are helpful tools for the diagnosis of talaromycosis in AIDS patients with TCD4 count below 100 cell/mm^3, especially the model with serum Mp1p ELISA.

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