Evaluation of Quantitative Real-Time PCR and Platelia Galactomannan Assays for Diagnosis of Disseminated *Talaromyces marneffe* Infection

Xinlei Li^{1,3†}, Yanqing Zheng^{1,2†}, Fengyao Wu^{4†}, Dongdong Mo^{1†}, Hao Liang^{2,5}, Gang Liang⁶, Rufan Yan¹, Jazeer Abdul Khader¹, Nianning Wu^{4*}, Cunwei Cao^{1,2*}

1.Department of Dermatology and Venereology, the First Affiliated Hospital of Guangxi Medical University. 2.Guangxi Key Laboratory of AIDS Prevention and Treatment, Guangxi Medical University. 3.Department of Mycology, Yulin Hospital of Dermatology. 4.The Fourth People’s Hospital of Nanning. 5.School of Public Health and Life sciences Institute, Guangxi Medical University. 6.School of Preclinical Medicine of Guangxi Medical University

Background: *Talaromyces (Penicillium) marneffe* is an emerging pathogen that causes significant morbidity and mortality in immuno-compromised patients in endemic regions, such as Southeast Asia. This study compared the diagnostic performance of a well-validated real-time quantitative PCR (qPCR) assay targeting the ITS1-5.8S-ITS2 region and a Platelia galactomannan (GM) assay for the diagnosis of talaromycosis marneffe (TSM) using serum samples from patients with or without HIV.

Methods: Establish a TaqMan qPCR to detect and identify *T. marneffe* DNA coding for ITS1-5.8S-ITS2 region. Thirty-six patients with TSM were enrolled for laboratory diagnosis using qPCR and GM assays. Serum samples were first tested by qPCR and later for the GM antigen using an ELISA assay. qPCR was carried out using an automated quantitative fluorescence PCR cyler, and the results were analyzed using a receiver operating characteristic curve. Sensitivity and specificity of serum qPCR assay were calculated. GM levels were determined using Platelia Aspergillus enzyme immuno-assay. The GM optical density cutoff index was ≥ 0.5 . The sensitivities of both diagnostic methods were compared using Fisher’s test.

Results: A total of 36 patients were enrolled in this study. The mean patient age was 38.64 years (range 1-69 years). Fourteen (38.89%) patients had HIV co-infection, while the other 22 (61.11%) had no detectable underlying diseases. The qPCR system proved to be highly specific for *T. marneffe* with an amplification product of 202 bp (primers and probe show in Table 1). The correlation between Cq value and DNA copy was $Cq = -3.34 \times Lg (\text{copy number}) + 43.22$ ($R^2 = 0.994$; Eff = 99.6%) (Figure 1). Using serum samples from cultureproven TSM patients for the ROC curve as a reference standard, the AUC was 0.931 (95% confidence interval, 0.863-0.998; $p < 0.001$). The optimal Cq value for qPCR was 39 cycles (sensitivity 86.1%; specificity 100%; Youden index 0.861) (Figure 2).

Table 1. Primers and probe for qPCR assay.

Primer and probe	Nucleotide sequences(5'-3')	Position of L37406 (bp)	Tm (°C)
Forward primer	5'-AACCTGATGAAGATGGACTGTCT-3'	175-198	59.0
Reverse primer	5'-AAATGACGCTCGGACAGGC-3'	358-376	59.8
TaqMan probe	FAM-5'AATCTTTGAACGCACATTGCGCCC3'-TAMRA	314-335	68.5

Regions of the specific primers and TaqMan probe, which are located on the *T. marneffe* ITS1-5.8S-ITS2 region.

The results showed that this novel qPCR method is highly sensitive and specific for detection of *T. marneffe* DNA in serum samples; the limit of detection and species specificity were five copies of DNA and 100%, respectively. In assaying serum samples from 36 patients with TSM, the sensitivity of the qPCR method was 86.11% (31/36), including 20/20 (100%) patients with fungemia and 11/16 (68.75%) without fungemia. For GM assay, the sensitivity was 80.56% (29/36) when the GM optical density cutoff index was ≥ 0.5 , including 19/20 (95%) patients with and 10/16 (62.5%) without fungemia.

Table 2. Serum GM OD indices and qPCR cycles of patients with TSM, aspergillosis, or cryptococcosis and healthy volunteers

Patient group	GM OD index		No.(%) with a GM OD index of:				qPCR		No.(%) with a qPCR cycle of:
	range	median	>1.5	>1.0	>0.5	<0.5	range	median	<40 cycles
TSM									
With HIV(n=14)	0.978-7.219	5.731 ^a	13(92.86)	13(92.86)	14(100)	0(0.00)	30.53-37.036	33.244 ^e	14(100)
With fungemia (n=12)	0.978-7.219	5.842	11(91.67)	11(91.67)	12(100)	0(0.00)	31.084-37.036	34.145	12(100)
Without fungemia (n=2)	2.663-5.731	4.197	2(100)	2(100)	2(100)	0(0.00)	30.53-33.225	31.878	2(100)
Without HIV (n=22)	0.154-2.738	0.68 ^a	5(22.73)	6(27.27)	15(68.18)	7(31.82)	33.259-40	35.978 ^e	17(77.27)
With fungemia (n=8)	0.488-2.738	0.895	3(37.5)	4(50)	7(87.5)	1(12.5)	33.259-36.545	34.752	8(100)
Without fungemia (n=14)	0.154-1.609	0.547	2(14.29)	2(14.29)	8(57.14)	6(42.86)	33.569-40	36.908	9(64.29)
All (n=36)	0.154-7.219	1.31 ^{b, c, d}	18(50)	19(52.78)	29(80.56)	7(19.44)	30.53-40	34.835	31(86.11)
Aspergillosis									
Proven (n=24)	0.546-7.409	3.178	20(83.33)	21(87.5)	24(100)	0(0.00)	NT	NT	0(0.00)
Probable (n=10)	0	0	1(10)	2(20)	4(40)	6(60)	NT	NT	0(0.00)
All (n=34)	0.216-7.409	2.311 ^b	21(61.76)	23(67.65)	28(82.35)	6(17.65)	NT	NT	0(0.00)
Cryptococcosis									
Cryptococcal meningitis (n=9)	0.145-1.196	0.221	0(0.00)	1(11.11)	2(22.22)	7(77.78)	NT	NT	0(0.00)
Pulmonary cryptococcosis (n=2)	0.247-0.845	0.546	0(0.00)	0(0.00)	1(50)	1(50)	NT	NT	0(0.00)
All (n=11)	0.145-1.196	0.243 ^c	0(0.00)	1(9.09)	3(27.27)	8(72.72)	NT	NT	0(0.00)
Healthy control (n=30)	0.116-0.82	0.432 ^d	0(0.00)	0(0.00)	3(10)	27(90)	NT	NT	0(0.00)

ap<0.001, GM OD indices for TSM with HIV versus TSM without HIV
bp>0.05 , GM OD indices for TSM versus Aspergillosis
cp<0.001, GM OD indices for TSM versus Cryptococcosis
dp<0.001, GM OD indices for TSM versus Healths
ep<0.001, cycle number for TSM with HIV versus TSM without HIV

Conclusion: These results indicate that a novel qPCR assay and GM assay can be valuable tools in the diagnosis of *T. marneffe* infection. Serum is a convenient hematological sample source for *T. marneffe* DNA quantification. Combining GM and qPCR assays is a more accurate and appropriate method for diagnosis of *T. marneffe* infection in endemic areas.