ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2019, 0, 1–6
doi: 10.1093/mmy/myz052
Advance Access Publication Date: 0 2019
Original Article

Original Article

Evaluation of quantitative real-time PCR and Platelia galactomannan assays for the diagnosis of disseminated *Talaromyces marneffei* infection

Xinlei Li[1,2,†], Yanqing Zheng[1,3,†], Fengyao Wu[3,†], Dongdong Mo[1,†], Gang Liang[4], Rufan Yan[1,5], Jazeer Abdul Khader[1,5], Nianning Wu[3,*] and Cunwei Cao[1,*]

[1]Department of Dermatology and Venereology, the First Affiliated Hospital of Guangxi Medical University, Nanning, P. R. China, [2]Department of Mycology, Yulin Hospital of Dermatology, Yulin, P. R. China, [3]HIV/AIDS Clinical Treatment Center of Guangxi, The Fourth People's Hospital of Nanning, Nanning, P. R. China, [4]School of Preclinical Medicine of Guangxi Medical University, Nanning, P. R. China and [5]Guangxi Key Laboratory of AIDS Prevention and Treatment, Guangxi Medical University, Nanning, P. R. China

*To whom correspondence should be addressed. Nianning Wu, MD, Department of Infection, The Fourth People's Hospital of Nanning, Nanning 530023, P. R. China. Tel: 8610-0771-2637126; E-mail: wunianning62@163.com; Cunwei Cao, MD, PhD, Department of Dermatology and Venereology, The First Affiliated Hospital of Guangxi Medical University, Nanning 530021, P. R. China; Tel: 8610-0771-5356514; E-mail: caocunwei@yeah.net.

†These authors contributed equally to this work.

Received 16 January 2019; Revised 16 April 2019; Accepted 7 May 2019; Editorial Decision 21 April 2019

Abstract

Talaromyces (Penicillium) marneffei is an emerging pathogen that causes significant morbidity and mortality in immunocompromised patients in endemic regions such as southeast Asia. The diagnosis of disseminated *T. marneffei* infection remains challenging in clinical practice. In the study, a well-validated real-time quantitative polymerase chain reaction (qPCR) target region of ITS1-5.8S-ITS2 and a Platelia galactomannan (GM) assay were compared for their diagnostic performance using serum samples from patients with or without human immunodeficiency virus (HIV). The results showed that this novel qPCR method is highly sensitive and specific for *T. marneffei* DNA detection in serum samples, and the limit of detection and species-specificity of qPCR were five copies of DNA and 100%, respectively. For detection in serum samples from 36 talaromycosis patients, the sensitivity of qPCR was 86.11% (31/36), including 20/20 (100%) patients with fungemia and 11/16 (68.75%) patients without fungemia. For the GM assay, the sensitivity was 80.56% (29/36) when the GM optical density cutoff index was $\geq$0.5, including 19/20 (95%) patients with fungemia and 10/16 (62.5%) patients without fungemia. These results indicate that the novel qPCR and GM assays can be used as a valuable tool in the diagnosis of *T. marneffei* infection. Serum samples are convenient hematological specimens for *T. marneffei* DNA quantification. Combining the GM assay and qPCR is more scientific and appropriate for diagnosing *T. marneffei* infection in endemic areas.

Key words: *Talaromyces (Penicillium) marneffei*, diagnosis, quantitative real-time PCR, Platelia galactomannan (GM) assay.

Introduction

The dimorphic fungus *Talaromyces marneffei*, formerly called *Penicillium marneffei*, is an emerging pathogen that is endemic in southeast Asia, including Thailand, southern China, Vietnam, and northeastern India.[1–3] This pathogen can cause disseminated talaromycosis in immunocompromised hosts, such as AIDS/human immunodeficiency virus (HIV) patients and is considered an "AIDS-defining pathogen" in endemic areas.[4] The major manifestations of disseminated talaromycosis are fever, anemia, lymphadenopathy, hepatosplenomegaly, respiratory signs

and skin lesions,[5] which are not specific to or reliable for diagnosing and associated with a high fatality rate.[3] Microbiologic isolation and species identification of the pathogen may be time-consuming; it can take up to 1–4 weeks.[2] Given the rapid, fatal course of this mycosis, improved methods for early diagnosis with subsequent initiation of antifungal therapy are urgently required to significantly improve the mortality rate.

Novel nonculture-based approaches focus on hasten diagnosis, thereby enabling diagnostic-driven rather than empirical antifungal treatment strategies. In recent decades, quantitative polymerase chain reaction (qPCR) has been extensively studied and explored as a tool for detection and identification of *Aspergillus fumigatus* and other pathogenic fungi in clinical samples.[6–8] Previous studies have reported using qPCR to identify *T. marneffei* in whole blood and plasma samples, and the results were encouraging, as qPCR can rapidly detect *T. marneffei* in clinical samples.[9,10] However, the performance of qPCR in serum samples from talaromycosis patients has not yet been evaluated.

Galactomannan (GM) is a heteropolysaccharide composed of a nonimmunogenic mannan core and immunoreactive galactofuranose side chains present in the cell walls of most *Aspergillus* and *Talaromyces* species.[11,12] Studies have shown that detecting the GM antigen in serum is useful for diagnosing talaromycosis in HIV-infected patients;[13] however, GM assay has not been tested in serum samples from talaromycosis patients without HIV coinfection, and its performance has not yet been compared with that of qPCR assays in diagnosing *T. marneffei* infection. The present study aimed to develop a novel serum-based qPCR assay and to compare the sensitivities of qPCR and GM assays in diagnosing *T. marneffei* infection.

Methods

Preparation of fungal and bacterial

The following fungi were used in this experiment: *T. marneffei* type strain FRR2161, which was generously provided by Dr. Alex Andrianopoulos (Department of Genetics, University of Melbourne, Australia), the *T. marneffei* GX1233H, GX1229H, GX1323H, GX1420H, and GX1434H human isolates, *C. glabrata*, *C. tropicalis*, *C. albicans*, *C. parapsilosis*, *C. krusei*, *Cryptococcus neoformans*, *A. flavus*, *A. niger*, *A. fumigatus* (AF293), *A. terreus*, *A. nidulans*, *A. oryzae*, *Rhizopus oryzae*, *Rhizomucor pusillus*, *Scedosporium apiospermum,* and *H. capsulatum*. The assorted bacteria included *Staphylococcus aureus, Streptococcus pneumoniae,* and *Escherichia coli*. The yeast forms of fungus were cultured on brain heart infusion agar (BHA, Becton Dickinson and Company, Franklin Lakes, NJ, USA) at 37°C from 3 to 7 days. The mould forms of fungus were cultured on potato-dextrose-agar (PDA; Becton Dickinson and Company, USA) at 25°C from 4 to 7 days. A portion of mycelial or yeast mat weighing approximately 10–20 mg was harvested with sterile forceps and placed into a sterile 1.5 ml Eppendorf tube with 600 μl of phosphate-buffered saline (PBS; PH = 7.0).

Patient group and samples

A case-control study was performed to anonymously evaluate qPCR and GM assays for talaromycosis diagnosis. The tests were performed retrospectively and had no impact on patient diagnoses or management. Serum samples were collected from 36 culture-proven talaromycosis patients, 45 other invasive fungal infection and 30 healthy volunteers, which patients prior to initiating antifungal therapy between May 2011 and April 2016 at the following major hospitals in Guangxi province: The First Affiliated Hospital of Guangxi Medical University, Nanning, China; The People's Hospital of Bose, Bose, China; and The Fourth Hospital of Nanning, Nanning, China. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University and was conducted in accordance with the Declaration of Helsinki, ethics amendment dated 28/10/2011, approval number KY-E-008.

DNA extraction

Fungal genomic DNA from a pure culture was extracted using a phenol-chloroform method followed by glass bead pulverization after liquid nitrogen frozen.[14] Then A QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA from 200-μl serum samples as described previously.[15] The DNA was eluted in 100 μl of sterile water and stored at −20°C until analysis.

DNA amplification and detection

The primers and TaqMan fluorogenic probe were designed to be highly specific for the ITS1-5.8S- ITS2 rDNA of *T. marneffei* (accession No.L37406.1), which was not examined in earlier studies. The primer and probe sequences are shown in Table 1. The standard curve was prepared as described previously.[16] Ten-fold serial dilutions of the positive recombinant plasmid standard

Table 1. Primers and probe for qPCR assay.

Primer and probe	Nucleotide sequences(5′-3′)	Position of L37406 (bp)	Tm (°C)
Forward primer	5′-AACCCTGATGAAGATGGACTGTCT-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. marneffei* ITS1-5.8S-ITS2 region (accession no. L37406.1).

(the final concentrations were 5×10^7, 5×10^6, 5×10^5, 5×10^4, 5×10^3, 5×10^2, 5×10^1, and $5 \times 10^\circ$ copies per reaction) were utilized in the qPCR amplification following optimal procedures. To test the sensitivity and detection rate, 10^7, 10^4, 10^1, and 5 copies per reaction were detected.

The qPCR included 10 μl of 2 × TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA, USA), 1 μl (5 μM) of each primer, 1 μl (5 μM) of the TaqMan probe, 2 μl of RNase-free water (Qiagen), and 5 μl of the template. The reaction was performed as follows: 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds, 60°C for 1 minute, and annealing and extension at 60°C for 1 minute. The qPCR was performed in an automated quantitative fluorescence PCR cycler (ABI 7500), and the amplification curve was analyzed based on exponential amplification and the C_q value, which was dose-dependent on the positive standard.

GM antigen detection

GM levels were determined using the Platelia *Aspergillus* enzyme immunoassay (Bio-Rad, Marnesla-Coquette, France) according to the manufacturer's instructions. Three different optical density (OD) cutoff indexes for the GM antigen level were used for the diagnosis of *T. marneffei*, including 0.5, 1.0, and 1.5, as recommended by Huang et al.[13]

Statistical analysis

The interquartile ranges of the serum GM and qPCR assays were calculated for patients and negative controls, and differences were analyzed using the Mann-Whitney test. Sensitivity and specificity were calculated to evaluate the performance of the serum qPCR assay. The sensitivities of both diagnostic methods were compared using Fisher exact test.

Results

Characteristics of the study population

Thirty-six patients, including 8 females and 28 males, were enrolled for laboratory diagnosis using qPCR and GM assays; the mean patient age was 38.64 years (range 1–69 years). Fourteen (38.89%) patients had HIV coinfection, while the other 22 (61.11%) patients had no detectable underlying diseases. Among the 36 patients, 20 had a positive blood culture (12 HIV-positive and 8 HIV-negative patients), and 16 were blood-culture negative but positive for the culture of other samples (2 HIV-positive and 14 HIV-negative patients). The clinical manifestations and medical histories of the patients are shown in Table 2. Control blood samples were obtained from 30 healthy volunteers, 34 invasive aspergillosis (IA) patients, including 22 proven and 12 probable cases based on the EORTC/MSG criteria,[17] and 11 cryptococcosis patients (two with cryptococcal pneumonia and nine with cryptococcal meningitis).

Table 2. Baseline characteristics and clinical data of 36 talaromycosis patients enrolled for serum GM and qPCR testing.

	Talaromycosis patients		
Characteristic	All ($n = 36$)	with HIV ($n = 14$)	without HIV ($n = 22$)
Age[mean(range)]	37.14 (1–69)	38.64 (23–51)	36.18 (1–69)
Sex(female/male)	8/28	1/13	7/15
With fungemia	20	12	8
No. (%) of culture-positive specimens[a]			
Blood	20 (55.56%)	12 (85.71%)	8 (36.36%)
Bronchoalveolar lavage	4 (11.11%)	0 (0)	4 (18.18%)
Skin biopsy	15 (41.67%)	2 (14.29%)	13 (59.00%)
Sputum	2 (5.56%)	2 (14.29%)	0 (0)
Skin-pur	8 (22.22%)	1 (7.14%)	7 (31.82%)
Lymph node	3 (8.33%)	0 (0)	3 (13.64%)

[a]Some of the patients were positive for multiple specimen types

Sensitivity and specificity of the qPCR assay

The qPCR system proved to be highly specific for both the yeast and hyphal forms of *T. marneffei* with an amplification product of 202 bp. None of the 16 fungal pathogen samples or the 10 bacterial samples tested were identified by these primers and probe. Control blood samples from healthy volunteers and patients with IA and cryptococcosis also tested negative according to the qPCR assay. Thus, the analytical specificity of this method was 100%. Using serial 10-fold dilutions of *T. marneffei* DNA in water showed that the qPCR limit of detection was 5 copies per microliter of DNA (80% detection rate), while the analytical sensitivity was 10 copies of DNA (100% detecting rate). The correlation between the C_q value and DNA copy number was calculated as $C_q = -3.34 \times \text{Lg (copy number)} + 43.22$ ($R^2 = 0.994$; Eff = 99.6%) (Fig. 1).

Quantitation of DNA in clinical samples

Among the 36 talaromycosis cases, all the blood culture-positive samples were also positive according to the qPCR assay (20/20). The C_q value (median, range) of qPCR was 34.24 cycles (31.08–37.04). Additionally, the qPCR method detected 11 of 16 blood-culture negative samples (68.75%). The C_q value (median, range) was 36.41cycles (30.53–40). These results indicated high sensitivity of the qPCR method for *T. marneffei* detection directly from serum samples, and more positive samples were detected by qPCR (31/36; 86.11%) versus conventional blood culture (20/36; 55.56%). The median C_q value for serum qPCR detection among talaromycosis patients with HIV infection was 33.24 cycles (range 30.53–37.04), which was lower than that obtained for patients without HIV (35.98, range 33.26–40; Mann-Whitney test $P < .05$). The diagnostic sensitivity and specificity for talaromycosis based on qPCR for ITS1-5.8S-ITS2 were 86.11% and 100%, respectively.

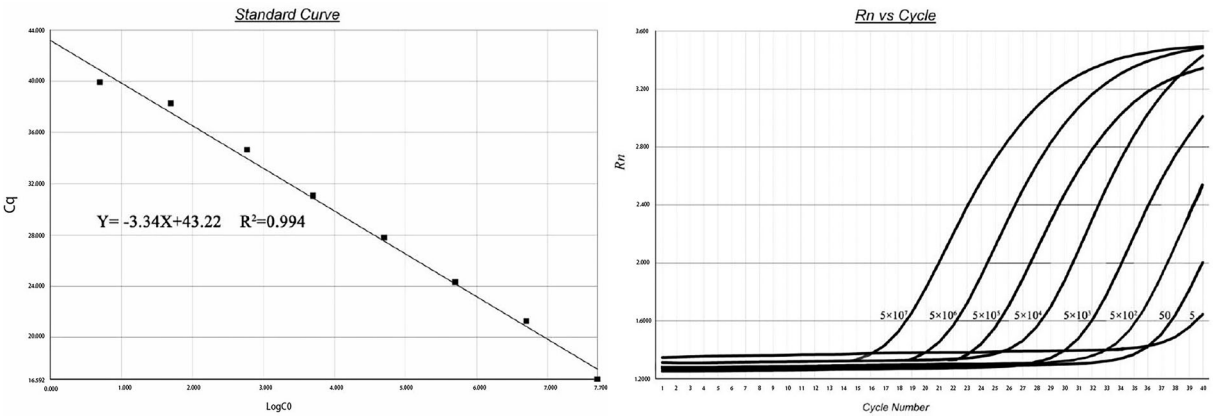

Figure 1. Standard Curve and Rn vs Cycle. (a) Linear regression of the crossing point values vs. log10 concentration of T. marneffei ITS1-5.8S-ITS2 region plasmids. All the samples were performed in triplicate. The correlation between Cq value and DNA copy was Cq=−3.34×Lg (copy number) +43.22 (R2=0.994; Eff=99.6%). (b) Amplification plots of T. marneffei ITS1-5.8S-ITS2 region plasmids with dilution concentrations from 5×107 to 5×100copies per reaction, showing increase in the fluorescence emission of the reporter dye relative to the reference dye.

Table 3. Serum GM OD indices and qPCR cycles of patients with talaromycosis, aspergillosis, or cryptococcosis and healthy volunteers.

	GM OD index		No. (%) with a GM OD index of:				qPCR		No. (%) with a qPCR cycle of:
Patient group	Range	Median	>1.5	>1.0	>0.5	<0.5	Range	Median	<39 cycles
Talaromycosis									
With HIV (*n* = 14)	0.98–7.22	5.73[a]	13 (92.86)	13 (92.86)	14(100)	0(0.00)	30.53–37.04	33.24[e]	14 (100)
With fungemia (*n* = 12)	0.98–7.22	5.84	11 (91.67)	11 (91.67)	12(100)	0(0.00)	31.09–37.04	34.15	12 (100)
Without fungemia (*n* = 2)	2.66–5.73	4.20	2 (100)	2 (100)	2(100)	0(0.00)	30.53–33.22	31.88	2 (100)
Without HIV (*n* = 22)	0.15–2.74	0.68[a]	5 (22.73)	6 (27.27)	15(68.18)	7(31.82)	33.26–39	35.98[e]	17 (77.27)
With fungemia (*n* = 8)	0.49–2.74	0.89	3 (37.50)	4 (50.00)	7(87.50)	1(12.5)	33.26–36.55	34.75	8 (100)
Without fungemia (*n* = 14)	0.15–1.61	0.55	2 (14.29)	2 (14.29)	8 (57.14)	6 (42.86)	33.56–39	36.91	9 (64.29)
All (*n* = 36)	0.15–7.22	1.31[b,c,d]	18 (50.00)	19 (52.78)	29 (80.56)	7 (19.44)	30.53–39	34.83	31 (86.11)
Aspergillosis									
Proven (*n* = 24)	0.55–7.41	3.18	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 (=34)	0.22–7.41	2.31[b]	21 (61.76)	23 (67.65)	28 (82.35)	6 (17.65)	NT	NT	0 (0.00)
Cryptococcosis									
Cryptococcal meningitis (*n* = 9)	0.15–1.20	0.221	0 (0.00)	1 (11.11)	2 (22.22)	7 (77.78)	NT	NT	0 (0.00)
Pulmonary cryptococcosis (=2)	0.25–0.85	0.55	0 (0.00)	0 (0.00)	1 (50)	1 (50)	NT	NT	0 (0.00)
All (*n* = 11)	0.15–1.20	0.24[c]	0 (0.00)	1 (9.09)	3 (27.27)	8 (72.72)	NT	NT	0 (0.00)
Healthy control (*n* = 30)	0.12–0.82	0.43[d]	0 (0.00)	0 (0.00)	3 (10)	27 (90)	NT	NT	0 (0.00)

[a] $P < 001$, GM OD indices for talaromycosis with HIV versus talaromycosis without HIV.
[b] $P > .05$, GM OD indices for talaromycosis versus aspergillosis.
[c] $P < .01$, GM OD indices for talaromycosis versus cryptococcosis.
[d] $P < .001$, GM OD indices for talaromycosis versus health.
[e] $P < .01$, cycle number for talaromycosis with HIV versus talaromycosis without HIV.
NT, none detected.

GM antigen detection

GM antigen detection was performed in serum samples collected from the same study population. The serum GM OD index levels of 36 talaromycosis cases and 75 controls are shown in Table 3. Of the 36 talaromycosis patients, 29/36 (80.56%) had a GM OD index ≥0.5 (range 0.15−7.22), including 19/20 (95%) patients with fungemia and 10/16 (62.5%) without fungemia. The median OD index was significantly higher for the 36 talaromycosis patients (1.31) than those for the 11 patients with cryptococcosis (0.24) ($P < .01$) and the 30 healthy controls (0.43) ($P < .001$) (Table 3). Among the talaromycosis patients, fungemic patients had a higher median OD index than patients without fungemia (3.29 vs 0.60; $P < .001$). Because most of the fungemic talaromycosis patients were co-infected with HIV

(12/20), a higher median OD index was found in HIV-infected patients than that in patients without HIV infection (5.73 vs 0.68; $P < .001$). Of the 7/36 talaromycosis patients with OD indices below 0.5 (19.44%), only one patient had fungemia and his GM OD index was close to 0.5 (GM OD index 0.49); the other six patients had *T. marneffei* isolated from lymph node aspirates, sputum, or skin biopsy samples.

The median OD index of the serum samples from the 36 talaromycosis patients was not significantly different from that of the 34 IA patients in the study (1.31 vs 2.31; $P > .05$) (Table 3). Three of the 30 healthy controls (10.0%) had an elevated OD index of 0.5. Throughout a 6-month follow-up, none of the 30 healthy volunteers developed invasive fungal infections or lung lesions. The diagnostic sensitivity of the talaromycosis GM assay was 80.56%.

Comparison of qPCR and GM assays

High concordance was observed between the qPCR assay and ELISA GM antigen test in diagnosing *T. marneffei* infection. Of the 36 talaromycosis patients, 31 tested positive according to qPCR, while 29 of these 31 patients were also positive based on the GM assay, generating an observed agreement of 93.54% (29/31). Both the qPCR assay and GM ELISA were highly sensitive in detecting talaromycosis patients with fungemia (20 vs 19). However, in patients without fungemia, qPCR was more sensitive than the GM test, with 11/16 (68.75%) positive cases compared to 10/16 (62.5%) positive GM cases. These results indicate that qPCR was more sensitivity than the GM enzyme immunoassay (86.11% vs 80.56%; $P < .05$; Fisher exact test) in diagnosing *T. marneffei* infection in serum samples.

Discussion

Diagnosing *T. marneffei* infection remains challenging partly due to a clinical presentation that is frequently nonspecific but also due to a limited range of accepted nonculture-based diagnostic assays that allow rapid and early detection of the pathogen.

qPCR techniques have been extensively studied and explored as tools for detecting and quantifying pathogenic fungi in clinical samples,[18,19] it has also shown great potential for the diagnosis of *T. marneffei* infection. Pornprasert et al.[9] developed a qPCR assay targeting the 5.8S rDNA that had 100% specificity and the assay could successfully detect *T. marneffei* DNA in whole blood samples from 60% (12/20) of patients. Hien et al.[10] developed a qPCR assay targeting the MP1 gene encoding a cell wall protein specific to *T. marneffei*. The sensitivity and specificity of the assay in plasma samples from talaromycosis patients were 70.4% and 100%, respectively. In our study, the primers and probe were designed to target the ITS1-5.8S-ITS2 region and were tested for analytic sensitivity and specificity using clinically related pathogenic fungi and bacteria and serial 10-fold dilutions of *T. marneffei* DNA, with results of 10 copies and 100%, respectively, indicating the applicability of the qPCR assay.

Since serum and blood are sterile, samples are easy to obtain noninvasively from patients and have been widely used in qPCR assays.[20,21] Our study showed that use of serum for PCR determination had several advantages, such as the ability to use the same sample for GM and DNA detection, the ease of storing sera as frozen samples, and straightforward DNA extraction since automated commercial kits can be used.[21] Bernal et al.[22] compared the performances of qPCR assays with both serum and blood samples from a large number of IA patients, and the results indicated that qPCR performed very similarly in both blood and serum samples; therefore, this group recommended serum as the best type of hematological sample to use for *Aspergillus* DNA detection. In the study, we developed a specific qPCR technique to detect *T. marneffei* DNA in serum samples from culture-proven talaromycosis patients. The results showed that the serum samples were positive according to qPCR in 20/20 of the patients with *T. marneffei* fungemia and in most of the patients (11/16) who were blood culture-negative but whose cultures of other specimens were positive. Overall, the sensitivity of the qPCR assay was 86.11% in detecting clinical talaromycosis patients. The results of our study indicated that the novel qPCR assay had a higher sensitivity than blood culture and that sera are convenient hematological samples for *T. marneffei* DNA quantification.

Huang et al.[13] showed that the GM antigen assay may facilitate an earlier diagnosis of *T. marneffei* infection in HIV-infected patients in endemic areas. In our study, we evaluated the efficacy of the GM antigen assay in both HIV-infected and HIV noninfected talaromycosis patients. After comparing the Mann-Whitney test for different OD cutoff indices, we suggest that 0.5 may be the optimal cutoff OD index for the GM antigen assay in talaromycosis diagnosis, which is in accordance with the cutoff OD index in the talaromycosis study of Huang et al., with a sensitivity of 80.56% (29/36). In the study, most of the patients infected with *T. marneffei* had a GM OD index above 0.5, indicating that *T. marneffei* infection should be considered in the differential diagnosis of patients with elevated GM antigen levels in endemic areas.

Notably, significantly high GM OD indices were observed in most of the patients with *T. marneffei* fungemia, which were higher than those in the patients without *T. marneffei* fungemia; this result was observed not only in HIV-infected patients but also in HIV-noninfected patients. This result is consistent with the findings of Huang et al.[13] in HIV-infected patients. Serum GM levels may be lower in patients with airway IA than those in patients with angio-invasive pulmonary aspergillosis.[23,24] Moreover, studies have shown that *T. marneffei* is more likely than *Aspergillus* spp. to be isolated from blood samples of HIV-infected patients.[25,26] Taken together, our data imply that a significantly high GM OD index in a patient residing

in a *T. marneffei*-endemic area should lead one to suspect a probable fungemic *T. marneffei* infection, particularly in an HIV-infected patient.

To our knowledge, the efficacies of qPCR and GM assays in detecting *T. marneffei* infection had not yet been compared in clinical patients. In our study, we found that the sensitivities of the qPCR and GM assays were 88.89% and 80.56%, respectively. Most of the talaromycosis patients who were diagnosed via qPCR assay were also diagnosed via GM assay, but three GM-negative patients were diagnosed with the serum qPCR test. The sensitivity of the serum qPCR assay was higher than that of the GM assay ($P < .05$).

Our data showed that this novel qPCR method is highly sensitive and specific for detecting *T. marneffei* DNA in serum samples and can be used as a valuable tool to diagnose *T. marneffei* infection. GM detection using an *Aspergillus* enzyme immunoassay is a useful test for the diagnosis of talaromycosis with fungemia. However, combining the GM and qPCR tests is more accurate and appropriate for early diagnosis of *T. marneffei* infection in endemic areas.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (no. 81571971 and no. 81271804) and Natural Science Foundation of Guangxi province of China (2017GXNSFAA198004 and AB18221017). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Supparatpinyo K, Khamwan C, Baosoung V, Sirisanthana T, Nelson K. Disseminated *Penicillium marneffei* infection in southeast Asia. *Lancet*. 1994;344:110–113.
2. Duong TA. Infection due to *Penicillium marneffei*- an emerging pathogen: review of 155 reported cases. *Clin Infect Dis*. 1996;23:125–130.
3. Hu Y, Zhang J, Li X et al. *Penicillium marneffei* infection: an emerging disease in mainland China. *Mycopathologia*. 2013;175:57–67.
4. Maniar J, Chitale A, Miskeen A, Shah K, Maniar A. *Penicillium marneffei* infection: an AIDS-defining illness. *Indian J Dermatol Venereol Leprol*. 2005;71:202.
5. Vanittanakom N, Cooper CR, Fisher MC, Sirisanthana T. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. *Clin Microbiol Rev*. 2006;19:95–110.
6. Ostrosky-Zeichner L. Invasive mycoses: diagnostic challenges. *Am J Med*. 2012;125:S14–S24.
7. Hope W, Walsh T, Denning D. Laboratory diagnosis of invasive aspergillosis. *Lancet Infect Dis*. 2005;5:609–622.
8. Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. *Lancet Infect Dis*.2009;9:89–96.
9. Pornprasert S, Praparattanapan J, Khamwan C et al. Development of TaqMan real-time polymerase chain reaction for the detection and identification of *Penicillium marneffei*. *Mycoses*. 2009;52:487–492.
10. Hien HTA, Thanh TT, Thu NTM et al. Development and evaluation of a real-time polymerase chain reaction assay for the rapid detection of *Talaromyces marneffei* MP1 gene in human plasma. *Mycoses*. 2016;59:773–780.
11. Latge J-P, Kobayashi H, Debeaupuis J-P et al. Chemical and immunological characterization of the extracellular galactomannan of *Aspergillus fumigatus*. *Infect Immun* 1994;62:5424–5433.
12. Mennink-Kersten MA, Donnelly JP, Verweij PE. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. *Lancet Infect Dis*. 2004;4:349–357.
13. Huang Y-T, Hung C-C, Liao C-H, Sun H-Y, Chang S-C, Chen Y-C. Detection of circulating galactomannan in serum samples for diagnosis of *Penicillium marneffei* infection and cryptococcosis among patients infected with human immunodeficiency virus. *J Clin Microbiol*. 2007;45:2858–2862.
14. Gealt MA, Sheir-Neiss G, Morris NR. The isolation of nuclei from the filamentous fungus *Aspergillus nidulans*. *J Gen Microbiol*. 1976;94:204–210.
15. Challier S, Boyer S, Abachin E, Berche P. Development of a serum-based Taqman real-time PCR assay for diagnosis of invasive aspergillosis. *J Clin Microbiol*. 2004;42:844–846.
16. Lin JC, Xing YL, Xu WM et al. Evaluation of galactomannan enzyme immunoassay and quantitative real-time PCR for the diagnosis of invasive pulmonary aspergillosis in a rat model. *J Microbiol Biotechnol*. 2014;24:1044–1050.
17. De Pauw B, Walsh TJ, Donnelly JP et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008;46:1813–1821.
18. Li Y, Gao L, Ding Y et al. Establishment and application of real-time quantitative PCR for diagnosing invasive aspergillosis via the blood in hematological patients: targeting a specific sequence of *Aspergillus* 28S-ITS2. *BMC Infect Dis*. 2013;13:255.
19. Buitrago M, Berenguer J, Mellado E, Rodriguez-Tudela J, Cuenca-Estrella M. Detection of imported histoplasmosis in serum of HIV-infected patients using a real-time PCR-based assay. *Eur J Clin Microbiol Infect Dis*. 2006;25:665–668.
20. White PL, Mengoli C, Bretagne S et al. An evaluation of *Aspergillus* PCR protocols for testing serum specimens. *J Clin Microbiol*. 2011;49:2842–2848.
21. Maaroufi Y, Heymans C, De Bruyne J-M et al. Rapid detection of *Candida albicans* in clinical blood samples by using a TaqMan-based PCR assay. *J Clin Microbiol*. 2003;41:3293–3298.
22. Springer J, Morton CO, Perry M et al. Multicenter comparison of serum and whole-blood specimens for detection of *Aspergillus* DNA in high-risk hematological patients. *J Clin Microbiol*. 2013;51:1445–1450.
23. Hidalgo A, Parody R, Martino R et al. Correlation between high-resolution computed tomography and galactomannan antigenemia in adult hematologic patients at risk for invasive aspergillosis. *Eur J Radiol*. 2009;71:55–60.
24. Reichenberger F, Habicht J, Gratwohl A, Tamm M. Diagnosis and treatment of invasive pulmonary aspergillosis in neutropenic patients. *Eur Respir J*. 2002;19:743–755.
25. Kami M, Tanaka Y, Kanda Y et al. Computed tomographic scan of the chest, latex agglutination test and plasma (1AE3)-beta-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: a prospective study of 215 patients. *Haematologica*. 2000;85:745–752.
26. Gallant JE, Ko AH. Cavitary pulmonary lesions in patients infected with human immunodeficiency virus. *Clin Infect Dis*. 1996;22:671–682.