

# Characterization of Anti-Interferon- $\gamma$ Antibodies in HIV-Negative Patients Infected With Disseminated *Talaromyces marneffei* and Cryptococcosis

Wen Zeng,<sup>1,\*</sup> Ye Qiu,<sup>1,\*</sup> Shudan Tang,<sup>1,\*</sup> Jianquan Zhang,<sup>1</sup> Mianluan Pan,<sup>1</sup> and Xiaoning Zhong<sup>1</sup>

<sup>1</sup>Department of Respiratory Medicine, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi 530021, China

**Background.** Few reports of *Talaromyces marneffei* (TM) or cryptococcosis infections among HIV-negative patients with high-titer anti-IFN- $\gamma$  autoantibodies (nAIGAs) have been published. We investigated the clinical manifestations of patients with nAIGAs and TM infections.

**Methods.** HIV-negative adults ( $\geq 18$  years) were enrolled if they had disseminated TM infection (group 1; further divided into nAIGAs positive [group 1P] and negative [group 1N]); cryptococcosis (pulmonary cryptococcosis and/or cryptococcosis of the brain) (group 2); pulmonary tuberculosis (group 3); and healthy controls (group 4) with nAIGAs detected. Complete histories, physical examinations, and routine clinical laboratory tests were obtained at baseline.

**Results.** Overall, 88 participants were in the four groups (20, 13, 23, and 32 in groups 1 to 4, respectively). Significant differences occurred between groups with higher nAIGAs titers ( $P < 0.001$ ), and higher total white-cell and absolute neutrophil counts ( $P < 0.001$ ) in group 1. Lungs (90.0%), lymph nodes (60.0%), skin (55.0%), and bones (50.0%) were most common sites of involvement. Significant differences in total white-cell and absolute neutrophil counts occurred between groups 1P and 1N. Patients with recurrent TM infections, particularly group 1P, had higher initial nAIGA titer.

**Conclusions.** Patients with persistent infection who died tended to have positive initial nAIGA titer. It suggests that nAIGAs may play a critical role in the pathogenesis of TM infections, and may be associated with more severe, refractory infection.

**Key words.** anti-interferon- $\gamma$  antibodies; cryptococcosis; HIV-negative patients; talaromycosis.

In recent years, an increasing number and proportion of *Talaromyces marneffei* (TM) infections are being reported in non-HIV-infected patients who had other immunocompromising conditions [1], such as systemic lupus erythematosus [2]. Some had abnormalities of immune genes or high-titer neutralizing anti-interferon- $\gamma$  autoantibodies (nAIGAs) in the peripheral blood. These nAIGAs are increasingly being recognized as a cause of both adult-onset immunodeficiency and increased risk of infections with intracellular pathogens, including *Cryptococcus neoformans*, *Histoplasma capsulatum*, TM, and disseminated salmonellosis. The relationship between nAIGAs and disseminated nontuberculous mycobacterial (NTM) infections have been reported already. Although

nAIGAs recently are being recognized as a mechanism in NTM infection, few reports of TM or cryptococcosis infection (without HIV infection) and nAIGAs have been published. A total of 111 NTM patients with nAIGAs were identified from January 2004 to November 2016 [3, 4]. In all of the patients, coinfection with NTM or other pathogens were reported. All of the patients, except one who was reported in mainland China, had serological evidence of penicilliosis without culture-positive evidence [5–7]. However, no systematic report exists for nAIGAs and TM infections with culture-positive evidence of penicilliosis. We currently do not know whether an association between nAIGAs and TM infection or cryptococcosis exists. There are also no large-scale case series that have focused on their detailed clinical information. Therefore, the purpose of this study was to describe the clinical manifestations, disease course, therapeutic regimens, and outcomes in patients with nAIGAs and TM infection from Guangxi, where there is an epidemic of TM. We also compared these to other pathogens, including *Cryptococcus neoformans* and *Mycobacterium tuberculosis*.

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\*W. Z., Y. Q., and S. T. contributed equally to this manuscript and should be considered co-first authors.

Correspondence: J. Zhang, MD, PhD, Department of Respiratory Medicine, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi 530021, China (jqzhang2002@126.com).

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## METHODS

### Participants and Definitions

All patients were adults (ie, 18 years old and above) who were followed up on account of their infections at First Affiliated Hospital Guangxi Medical University between 2015 and 2018. We enrolled the

participants into 4 study groups: patients with disseminated TM infection (group 1); patients with cryptococcosis (pulmonary cryptococcosis, cryptococcosis of the brain, or both) (group 2); pulmonary tuberculosis (group 3); and healthy controls (group 4). All patients met the following criteria: (1) must be 18 years-old or older and (2) come from mainland China. All patients were HIV negative and previously healthy and had no history of cancer, immunodeficiency, or immune suppression within 4 weeks of enrollment or diagnosis of their infections. Each patient met the diagnostic criteria of the group to which they were assigned. Controls comprised anonymous healthy blood volunteers who were from mainland China. As patients were enrolled, we detected their nAIGAs, collected complete histories, performed physical examinations and routine clinical laboratory tests, and created immunological indices. Data were recorded on standardized case-report forms. We followed up on all patients until December 2018. Only age, sex, race, or ethnic group were recorded for participants in group 4 (the controls) as numbers, age, sex, and race were reasonable matches.

For group 1, disseminated disease was defined as infection in at least 2 noncontiguous and sterile sites. Positive cultures for TM were characterized by dimorphic fungi that grew as a mold at 25°C and as yeast at 37°C. The yeast form of TM was confirmed by cytology and histopathology from tissues and secretions using Periodic acid-Schiff (PAS) staining or Wright's stain that showed a characteristic morphology that included a transverse septum [4]. The group 2 patients were diagnosed with cryptococcosis, based on positive results from one or more of the following: pathogen culture, histopathological analysis, India ink staining, and cytological analysis of clinical specimen cerebrospinal fluid cryptococcal antigen testing [8]. The group 3 patients with pulmonary tuberculosis, who were originally recruited as study controls with mycobacterial disease, had culture-proven tuberculosis or smear-positive results for acid-fast bacilli and appropriate response to direct antituberculous therapy; all of these patients were HIV negative. This study was approved by the First Affiliated Hospital of Guangxi Medical University's Ethical Review Committee (2018.KY-E-096). All participants provided written informed consent separately. The clinical course of the TM-infected patients was divided into the following 3 categories: cured (no recurrence of TM infection for at least 6 months after discontinuation of antifungal therapy), persistent infection, and death. The recurrence of infection was defined by patients' clinical symptoms improving or the pathogen detection being negative after effective treatment and then reappearing with signs of pathogen infection, pathogen detection being positive again, or both. The recurrence of infection included reinfection or recrudescence. Recurrence frequency of TM infection in each patient was also recorded.

#### Anti-interferon- $\gamma$ Autoantibody Assay

The anti-interferon- $\gamma$  (anti-IFN- $\gamma$ ) antibody in the plasma was determined by ELISA kit (USCN Life Science, Inc., Wuhan, China). To do so, first, we added 100  $\mu$ L each of dilutions of standard, blank, and samples into appropriate wells and incubated these for 1 hour

at 37°C. Then, we removed the liquid of each well and added 100  $\mu$ L of detection reagent A. We incubated for 1 hour at 37°C. Next, we used 1 $\times$  wash solution, totally washed 5 times, and removed any remaining wash buffer. Then, we added 90  $\mu$ L substrate solution to each well. These were incubated in the dark for 10–20 minutes at 37°C. Once the first 3 wells of standard wells turned to blue gradient, we gently added 50  $\mu$ L of stop solution to each well. The liquid will turn yellow and finally the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of anti-IFN- $\gamma$  in the sample then is determined by comparing the optical density of the sample to the standard curve.

#### Statistical Analysis

The normal range of anti-IFN- $\gamma$  autoantibody concentration was defined by the 99<sup>th</sup> percentile (for group 3 and 4 patients combined) and was estimated with the use of the log-normal distribution. Outlying concentrations were classified as positive for anti-IFN- $\gamma$  autoantibodies. We used the Luciferase Immunoprecipitation Systems to analyze the data [6].

Data were expressed as median  $\pm$  interquartile range. Differences between groups were compared using the Kruskal-Wallis test or Mann-Whitney test; Dunn tests were used for post hoc comparisons. Chi-square tests or Fisher exact test were used to compare categorical variables. Spearman rank correlation was used for ranked data to measure the dependence of 2 nonparametric variables. Univariable analysis and multivariable analysis for risk factors of infection were used for logistic regression analyses. Univariable analysis and multivariable analysis for risk factors of recurrence used the Kaplan-Meier method and the Cox proportional hazards regression model. Analysis was performed using IBM SPSS Statistics for Windows, version 22 (SPSS Inc, Chicago, Illinois), and  $P < .05$  was considered significant.

## RESULTS

There were 88 persons: 20 patients with disseminated TM infection (group 1); 13 with cryptococcosis (pulmonary cryptococcosis, cryptococcosis of the brain, or both) (group 2); 23 with pulmonary tuberculosis (group 3); and 32 healthy controls (group 4). The participants in group 1 were further grouped into 2 groups: nAIGAs-positive (group 1P) and nAIGAs-negative (group 1N).

Sex and age distribution did not differ significantly between the groups (Table 1). Plasma obtained from all participants were tested for nAIGAs. The distribution of nAIGAs differed markedly across the groups; 55% (11 out of 20) of patients in group 1 had high titer nAIGAs, compared with only 1 patient in group 3 and none in groups 2 and 4 ( $P < .001$ ). The nAIGA titer was higher in group 1 compared to groups 2, 3, and 4 (Figure 1).

White blood cell counts (WBC) and absolute neutrophil counts (N) (17.93[11.30–32.78]  $\times 10^9/L$ , 14.62[8.90–26.65]  $\times 10^9/L$ ) in group 1 were increasing higher than the other groups ( $P < .001$ ).

**Table 1. Clinical Characteristics of the 88 Participants**

Characteristic	Group 1	Group 2	Group 3	Group 4	P Value
	N = 20	N = 13	N = 23	N = 32	
Age (yr)	59.5(45.8,67)	53(47,54)	44(27,55)	49(40.3,58.3)	.270
Male sex (no.)	11	7	18	14 (77.8)	.086
nAIGAs positive no. (%)	11 (55) <sup>a,b,c</sup>	0 (0)	1 (4.3)	0 (0)	<.001
nAIGA titers (ng/ml)	10063.3 <sup>a,b,c</sup> (3799.7,21392.5)	2568.5 (1072.9,3427.9)	455.5 (379.5,1053.9)	647.8 (612.1,820.0)	<.001
WBC ( $\times 10^9/L$ )	17.93(11.30,32.78) <sup>a,b</sup>	9.47(7.06,12.21)	8.10(5.88,9.69)	ND	<.002
N ( $\times 10^9/L$ )	14.62(8.90,26.65) <sup>a,b</sup>	5.58(3.39,6.36)	5.60(2.05,6.92)	ND	<.001
L ( $\times 10^9/L$ )	2.21(1.08,3.18)	1.90(1.45,2.43)	1.44(1.15,1.83)	ND	.912
IgG (g/l)	23.60(13.00,30.20) <sup>a</sup>	10.58(9.50,124.74)	15.81(10.97,18.26)	ND	.003
IgA (g/l)	2.17(1.70,3.33) <sup>b</sup>	1.68(1.36,3.22)	3.01(2.57,4.33)	ND	.015
IgM (g/l)	0.95(0.75,1.15)	1.37(0.84,1.58)	1.07(0.83,1.53)	ND	.448
T cell (%)	68.10(56.19,75.40)	69.60(61.45,77.95)	72.10(67.70,74.85)	ND	.674
CD4+ T cell (%)	33.05(29.25,42.10)	45.43(30.8,49.2)	38.65(29.18,43.88)	ND	.231
CD8+ T cell (%)	26.50(20.68,34.90)	23.29(20.75,28.35)	27.30(18.55,32.08)	ND	.712

<sup>a</sup>Groups 1 and 2,  $P < .0083$

<sup>b</sup>Groups 1 and 3,  $P < .0083$

<sup>c</sup>Groups 1 and 4,  $P < .0083$

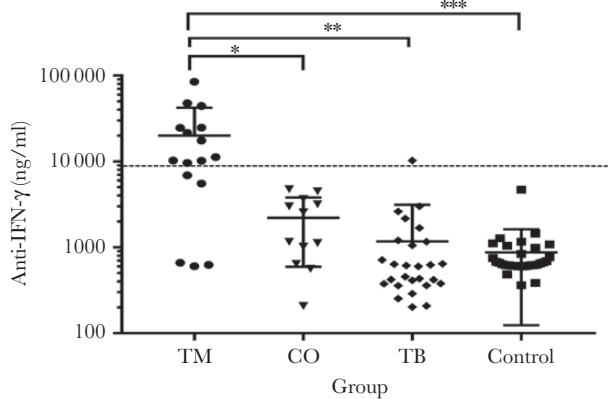
Data are presented as median (25th–75th percentile),  $P < .05$ . Normal range: IgG: 8–18g/l, IgA: 2.01–2.69g/l, IgM: 0.84–1.32g/l. T cell%: 64.20%–78.50%, CD4+: 30.1%–40.4%, CD8+: 20.7%–29.4%.

CD4% indicates CD4+ cell percentage; CD8%, CD8+ cell percentage; Ig, serum immunoglobulin; L, absolute lymphocyte count; N, absolute neutrophil count; nAIGAs, anti-IFN- $\gamma$  autoantibodies; ND, not done; T cell%, T lymphocyte cell percentage; WBC, white cell count.

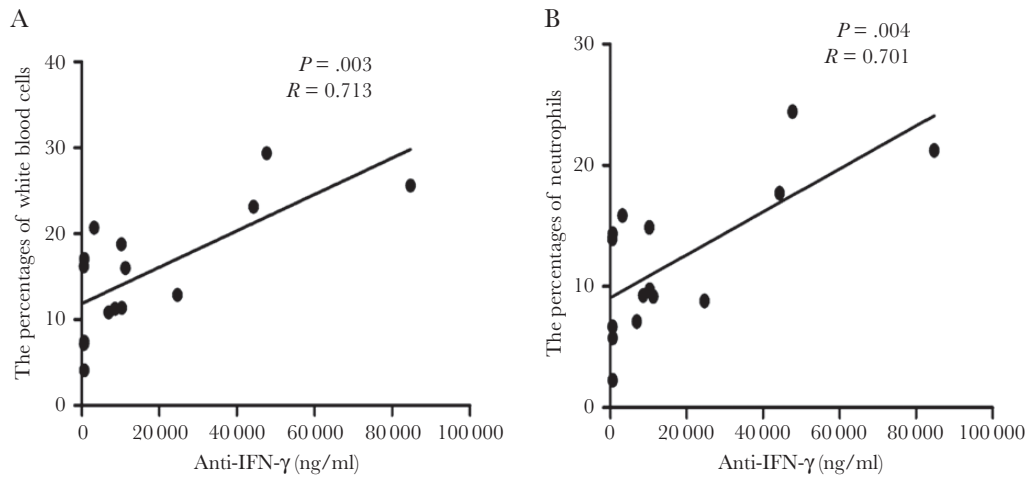
Lymphocyte (L) phenotyping was performed for all patients. The total CD4+ and CD8+ T-lymphocyte counts in groups 1, 2, and 3 patients were similar and were within normal ranges in all groups. All of the participants with the exception of the healthy controls were evaluated for total immunoglobulins (IgA, IgG, and IgM). The level of IgG was disproportionately higher in group 1 patients than in group 2 ( $P = .003$ ). Although IgA was higher in group

3 than in the other 2 groups ( $P = .008$ ), IgM was similar in all 3 groups. We examined the relationship between nAIGAs titer and WBC, N, L, total T-cell, CD8+, and CD4+ T-cells. Statistically significant difference was observed between nAIGAs titer and WBC counts and between nAIGAs titer and N (Figure 2).

In group 1, 11 patients had high anti-IFN- $\gamma$  autoantibodies titer. Sex and age distribution did not differ significantly between groups 1P and 1N (Table 2). The time period between onset and diagnosis was 6 months in both groups 1P and 1N. Of 20 patients, 11 were misdiagnosed as tuberculosis, followed by bacterial pneumonia, lung cancer, and lymphoma. Lungs were the most common sites of involvement (90.0%), followed by lymph nodes (60.0%), skin (55.0%), and bones (50.0%). There were no between-group differences in hemoglobin, fever, cough or expectoration, dyspnea, lymphadenopathy, hepatosplenomegaly, underweight, maculopapule or skin nodules, and osteolysis. The WBC counts were highly increased in group 1P (25.60[17.40–34.35]  $\times 10^9/L$ ). The mean neutrophil count was highly increased in group 1P (21.25[12.32–28.35]  $\times 10^9/L$ ). C-reactive protein (CRP) and erythrocyte sedimentation rate were highly increased in groups 1P and 1N. Chest high resolution computed tomography (HRCT) indicated pulmonary involvement, including nodules, infiltrates, pleural effusion, and cavitation. We also compare the date between groups 1P, 1N, and group 2. The nAIGA titers were higher in group 1P compared to groups 1N and group 2. The L, CD4+, and CD8+ T-lymphocyte counts, IgM, and IgA in the different groups showed no difference, and both were within normal ranges. IgG was lower in group 2 compare to the other 2 groups. We analyzed the risk factor of TM infection in group 1, 2, and 3; it showed that the ratio of actual value to cut-off value of nAIGAs was an independent risk factor of TM infection (Table 3, Figure 3).



**Figure 1.** Anti-Interferon- $\gamma$  Autoantibody Concentration in 88 Participants, According to Study Groups. Anti-interferon- $\gamma$  autoantibodies were measured with the use of Luciferase Immunoprecipitation Systems. The dashed line is the estimated 99<sup>th</sup> percentile for the combined control groups of patients with pulmonary tuberculosis (group 3) and healthy controls (group 4) and was estimated with the use of the log-normal distribution. Participants with concentrations exceeding the 99<sup>th</sup> percentile were classified as autoantibody-positive. The dotted line shows that the titer was 9583.21ng/ml. Quantitative data were expressed as the median(interquartile range). ( $P < .0083$ ; \* $P = .002$ , \*\* $P < .001$ , \*\*\* $P < .001$ . CO indicates cryptococcosis; nAIGA, neutralizing anti-interferon- $\gamma$  autoantibodies; TB, pulmonary tuberculosis TM, *Talaromyces marneffei*).



**Figure 2.** Correlations Between (a) the nAIGA Titers and WBC and (b) the nAIGA Titers and N. N indicates absolute neutrophil count; nAIGA, neutralizing anti-interferon- $\gamma$  autoantibodies; WBC, white cell count.

**Table 2. Clinical Characteristics of the 20 Participants With TM Infection**

Characteristic	Group 1P	Group 1N	Group 2	P Value
	N = 11	N = 9	N = 13	
Age (yr)	63(35.5,68)	59(51,63)	-	.610
Male sex (no.)	6	5	-	.960
nAIGA titers (ng/ml)	215076 <sup>a,b</sup> (10758.6,34530.5)	661.3 (627.1,5551.8)	2568.5 (1072.9,3427.9)	.001
Disease course (month)	6.0	6.0	-	.470
WBC ( $\times 10^9/L$ )	25.60(17.40,34.35)	11.07(7.40,16.42)	-	.020
N ( $\times 10^9/L$ )	21.25(12.32,28.35)	9.25(6.70,14.35)	-	.025
L ( $\times 10^9/L$ )	2.44(2.11,3.28)	1.33(1.07,2.34)	-	.149
Hemoglobin (g/L)	90.0(77.25,98.2)	91.4(76.25,98.45)	-	.430
C-reactive protein (mg/L)	131.7(95.05,177.7)	102.66(55.75,192)	-	.619
ESR (mm/1h)	88(82.25,96.75)	75.5(58.5,97.75)	-	1.000
IgG (g/l)	23.60(13,28.5) <sup>#</sup>	21.85(13.16,30.72)	10.58(9.5,124.74)	.009
IgA (g/l)	2.16(1.89,3.33)	2.23(1.67,2.33)	1.68(1.36,3.22)	.687
IgM (g/l)	1.01(0.9,1.15)	0.91(0.77,1.01)	1.37(0.84,1.58)	.374
T cell (/L)	1714.5(1088.75,1961.5)	998.5(751.5,1204.75)	1458(960.5,1659.5)	.206
CD4+ T cell (/L)	817(528.5,1036)	45.43(30.8,49.2)	871(586,1136)	.206
CD8+ T cell (/L)	496(448.25,644.75)	394(281.25,660.75)	459(403.5,544)	.293
T cell (%)	70.9(56.45,76.45)	68.1(56.19,75.4)	69.6(61.45,77.95)	.661
CD4+ T cell (%)	33.2(31.15,41.9)	33.05(29.75,41.85)	45.43(30.8,49.2)	.335
CD8+ T cell (%)	27.9(22.65,33.65)	26.5(21.63,33.34)	23.29(20.75,28.35)	.603
Fever (n)	9	7	-	.820
Cough or expectoration	9	9	-	.480
Dyspnea	4	5	-	.390
Skin lesion	7	5	-	.710
Lymphadenopathy	1	0	-	1.000
Hepatosplenomegaly	6	5	-	.960
Underweight	6	5	-	.960
Osteolysis	6	4	-	.650
Outcome				.415
-cure	1	3	-	
-persistent infection	7	4	-	
-death	3	2	-	

<sup>a</sup>Groups 1 and 2,  $P < .017$

<sup>b</sup>Groups 1 and 3,  $P < .017$

Data are presented as median (25th–75th percentile),  $P < .05$ . Normal range: IgG: 8–18 g/l, IgA: 2.01–2.69 g/l, IgM: 0.84–1.32 g/l. T cell%: 64.20%–78.50%, CD4+: 30.1%–40.4%, CD8+: 20.7%–29.4%.

CD4% indicates CD4+ cell percentage; CD8%, CD8+ cell percentage; Group 1P, anti-interferon- $\gamma$  autoantibody positive; Group 1N, anti-interferon- $\gamma$  autoantibody negative; ESR, erythrocyte sedimentation rate; Ig, serum immunoglobulin; L, absolute lymphocyte count; N, absolute neutrophil count; nAIGAs, anti-IFN- $\gamma$  autoantibodies; T cell%, T lymphocyte cell percentage; WBC, white cell count.

**Table 3. Results of Univariable Analysis and Multivariable Analysis for Risk Factor of TM Infection (n = 56)**

Variable	Univariable analysis		Multivariable analysis	
	P	P	OR	95% CI
Age (yr)	.0813	-	-	-
Sex (male)	.2823	-	-	-
Relative nAIGA titer	.0017	.0379	9.3105	(1.1330–76.5082)
WBC ( $\times 10^9/l$ )	.0012	.3127	0.5701	(0.1915–1.6971)
IgG (g/l)	.0031	.1055	1.1549	(0.9701–1.3749)
CD4+ T cell (%)	.1231	-	-	-
Neutrophils ( $\times 10^9/l$ )	.0011	.2441	1.9696	(0.6295–6.1627)
Lymphocytes ( $\times 10^9/l$ )	.4423	-	-	-

Relative nAIGA titer indicates the ratio of actual value to cut-off value of nAIGA; CD4%, CD4+ cell percentage; Ig, serum immunoglobulin; nAIGAs, anti-IFN- $\gamma$  autoantibodies; OR, odds ratio; WBC, white blood cell.

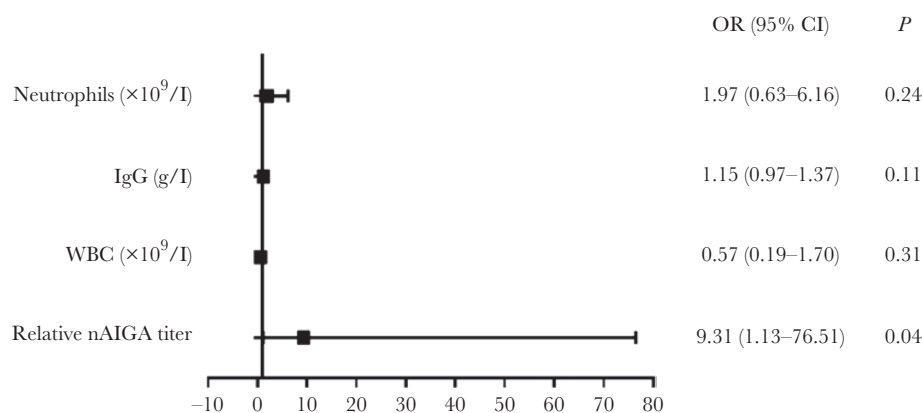
Three patients in group 1P had both nontuberculous mycobacterial and TM infection together. Furthermore, or herpes zoster virus were also isolated from the 3 patients. One patient in group 1N had positive sputum for mycobacterium with antiacid staining for acid-fast bacilli.

Despite intensive treatment, more than half of the patients (11 out of 19, 55.0%) had a persistent TM infection and required long-term antifungal therapy (Table 2). The distribution of the initial nAIGA titers according to the frequency of recurrence of TM infections and patient outcomes are shown in Figure 4. Most cases (55%) had an initial positive nAIGA titer, and they were divided into 3 groups (1–2-, 2–5-, and 5–10-fold positive nAIGA cut-off titer differential points) (Figure 4a). A higher proportion of patients with recurrent episodes, particularly those with >3 episodes, had initial nAIGA titers that were positive (Figure 4b). Patients with persistent TM infection tended to have higher initial nAIGA titers than the cured cases (Figure 4c). It also showed that the ratio of actual value to cut-off value of nAIGAs was TM recurrence (Table 4; Figure 5). Various combinations of antifungal drugs were used to suppress

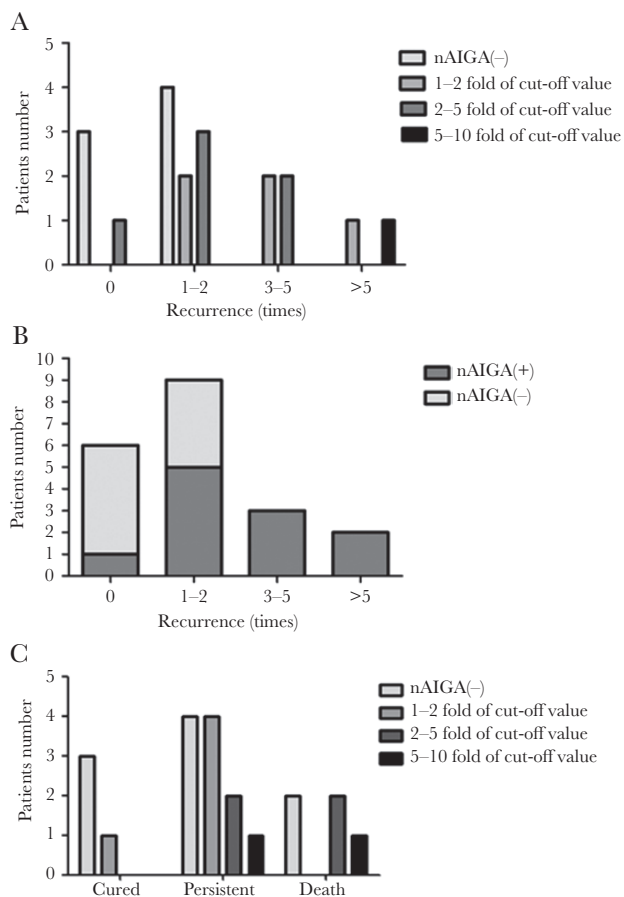
TM infection. They included amphotericin B, fluconazole, voriconazole, and caspofungin. The 3 patients who were infected with both NTM and TM were treated with antitubercular and antifungal drugs, but they had recurrence within 3 years. All 3 patients with persistent infections experienced at least 1 episode of recurrent disease (range: 1–9 times; median: 2.8 times). The 3 patients that were treated with effective antimycobacterial drugs also experienced recurrent episodes of TM or NTM. Three patients died in group 1P; 2 refused hospitalization for further treatment after recurrence and then died at home; and 1 died of multiple organ dysfunction despite use of antifungal therapy. Two patients died in group 1N; 1 died outside the hospital before the culture results were obtained; and 1 patient's death resulted from disseminated intravascular coagulation and multiple organ failure, despite antifungal therapy.

## DISCUSSION

Immunodeficiency due to nAIGAs is an emerging adult-onset immunodeficiency syndrome, associated with severe or disseminated infections, caused by NTM, nontyphoidal salmonella, *Burkholderia* sp., TM, *Cryptococcus neoformans*, *Histoplasma capsularum*, and varicella zoster virus in non-HIV-infected patients. The immunodeficiency may be caused by the high-titer serum nAIGAs. The condition increasingly has been reported in Asians, including Filipinos, Thais, Vietnamese, Japanese, and Chinese residing in Hong Kong and Taiwan, and less commonly in other ethnic groups. In addition, there were just 2 cases from mainland China [5, 7, 9]. The fact that nearly all of the patients identified to date were Asian-born implicates host genetic factors, environmental exposure, or more likely, both. Our description is the first largely culture-positive evidence of penicilliosis that may be due to nAIGAs in ethnic Chinese patients born in mainland China. It signifies the importance of clinical awareness and availability of laboratory diagnostic tests in enhancing our understanding of the true incidence and possibly the predisposing genetic factors of



**Figure 3.** The Univariable Analysis and Multivariable Analysis for Risk Factor of TM Infection. Relative nAIGA titer indicates the ratio of actual value to cut-off value of nAIGA; Ig, serum immunoglobulin; nAIGA, neutralizing anti-interferon- $\gamma$  autoantibodies; TM = *Talaromyces marneffe*; WBC, white blood cell.



**Figure 4.** A, Different multiples of cut-off titers of nAIGA in frequency of recurrent TM infection. B, Patients with recurrent TM infections, particularly those with initial nAIGA positive. C, Patients with persistent infection and death tended to have a higher initial nAIGA titer. nAIGA indicates neutralizing anti-interferon- $\gamma$  autoantibodies; TM, *Talaromyces marneffei*.

**Table 4. Results of Univariable Analysis and Multivariable Analysis for Risk Factor of TM Recurrence (n = 18)**

Variable	Univariable analysis	Multivariable analysis		
	P	P	HR	95% CI
Age (yr)	.0015	.3861	1.0297	(0.9638–1.1000)
Sex (male)	.6105	-	-	-
Relative nAIGA titer	.0007	.0309	1.5773	(1.0429–2.3854)
WBC ( $\times 10^9/l$ )	.0010	.5578	1.7000	(0.2883–10.0246)
CD4+ T cell (%)	.0022	.8113	1.0101	(0.9301–1.0970)
Neutrophils ( $\times 10^9/l$ )	.0007	.5221	0.5572	(0.0930–3.3398)
Lymphocytes ( $\times 10^9/l$ )	.0004	.4846	0.3100	(0.0116–8.2717)

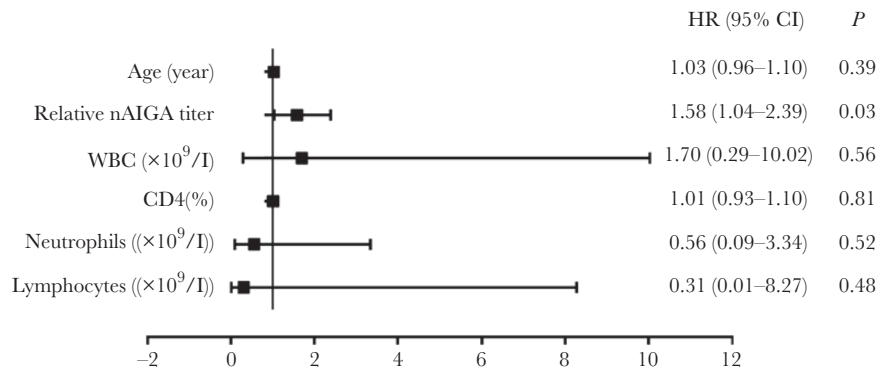
Relative nAIGA titer indicates the ratio of actual value to cut-off value of nAIGA; CD4+, CD4+ cell percentage; Ig, serum immunoglobulin; nAIGAs, anti-IFN- $\gamma$  autoantibodies; HR, hazard ratio; 95% CI, 95% confidence interval.

the condition in Chinese population. It was not until 2010 when the association between anti-IFN- $\gamma$  autoantibodies and TM infection was described among 8 Chinese patients living in Hong

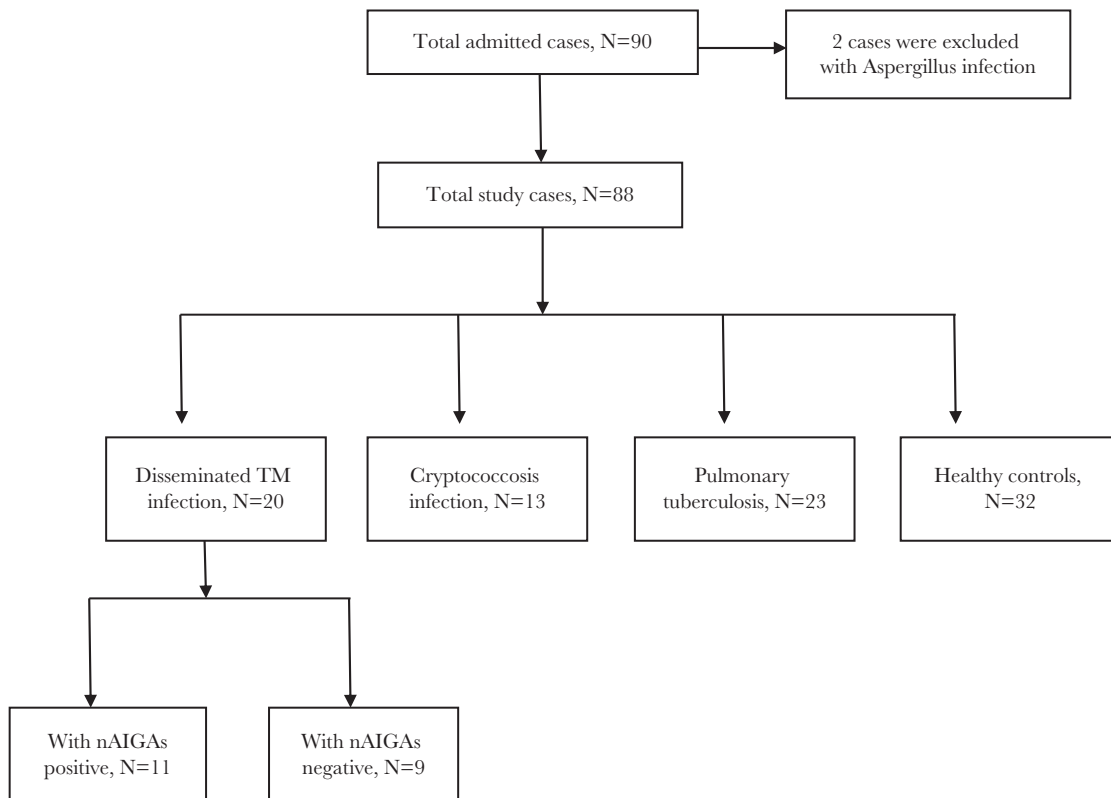
Kong [5]. Most of the TM infections with nAIGAs were described in case reports, and most of these were caused by nontuberculous mycobacteria infections [5–7, 10]. With a 55% positive result in our study and the nAIGA titers that were highly increased in group 1, there is need to be aware of the presence of nAIGAs in HIV-negative patients with TM infection, especially among those that are from an epidemic area. This should be an important cause of concern about infection caused by TM or other opportunistic pathogens in HIV-negative patients.

Cryptococcosis has long been considered one of the top 3 AIDS-defining opportunistic infections, alongside tuberculosis and TM. *Cryptococcus* coinfecting patients, who also were positive for nAIGAs, were infected with disseminated nontuberculous mycobacterial [8]. However, none of our patients infected with *Cryptococcus* were nAIGAs-positive. There was no significant correlation between nAIGAs and cryptococcal infection. The nAIGA titers also increased in group 2, but not as much as in group 1P. This result suggests that TM may infect a host with a poor immune system, or there may be other immune-deficiency states in an infected host with *Cryptococcus*. The anti-granulocyte-macrophage colony-stimulating factor autoantibodies may be associated with some cases of cryptococcosis in otherwise immunocompetent patients [7, 8]. When nAIGAs titer is positive in cryptococcal-infected patients, it might indicate coinfection with other pathogens, such as NTM and TM. High nAIGAs titer was detected in 4% (1 out of 23) of patients in group 3, which is in accordance with previous reports [6]. Therefore, if a patient with positive acid-fast stain for mycobacteria infection without culture-positive evidence may have poor therapeutic response, despite regular antituberculous treatment, test the nAIGA titer. When it is positive, NTM infection should be considered.

The elevated WBC and N in the general circulation had been used as markers of infection. In this study, all group 1 patients had higher CRP levels and increased WBC counts that were within normal range. The WBC and N counts were increased in patients with nAIGAs in our study. These were consistent with previous research reports [11]. It showed that the higher the titers, the higher the leukocyte and neutrophil counts. As nAIGAs suppress the activity of IFN- $\gamma$ , they had a negative effect on the development and differentiation of N, which led to increased N. Yet, nAIGAs titers had no effect on L counts. However, this finding may be related to the small sample size; further research is needed. The increased CRP and WBC levels indicated the presence of inflammation in these patients. We also found that the total CD4+ and CD8+ T cell counts were not significantly changed in all TM-infected patients. Therefore, immunodeficiency symptoms and repeat infection in patients with nAIGAs were not due to panleukopenia or a reduction in the number of phagocytes but may affect cell function or be related to chemokines. Cell-mediated immunity plays a central role in the eradication of infectious diseases, and nAIGAs were shown to neutralize IFN- $\gamma$  activity in the body, leading to



**Figure 5.** The Univariable Analysis and Multivariable Analysis for Risk Factor of TM Recurrence. Relative nAIGA titer indicates the ratio of actual value to cut-off value of nAIGA; CD4%, CD4+ cell percentage; nAIGA, neutralizing anti-interferon- $\gamma$  autoantibodies; TM = *Talaromyces marneffe*; WBC = white blood cell.



**Figure 6.**

immunodeficiency [12]. Isotypes and subtypes of anti-IFN- $\gamma$  antibodies appear to be heterogeneous. IgG1 and IgG4 were found to be the most frequent subtypes in the population [13]. Although reports on adult-onset immunodeficiency related to anti-IFN- $\gamma$  antibodies continue to be on the rise, it was normal in our study. The amount of immunoglobulin is related more to the pathogens than the nAIGAs. The mechanism initiating the production of anti-IFN- $\gamma$  antibodies, however, remains unknown. The lymph nodes, lungs, skin, liver, and spleen were the most common sites of organ involvement by TM. Osteolysis and elevated WBC and N

counts were seen mostly in our groups, similar to those in the HIV-negative patients. For groups 1P and 1N, there were no significant differences in age, sex, clinical symptoms, humoral immunity, or cellular immunity. Therefore, nAIGAs detected in TM infections were associated with adult-onset immunodeficiency, which is defined as the most insidious immunodeficiency syndrome and which often is overlooked. Why was there TM infection when the antibodies were negative? We conjecture that the nAIGAs-negative patients with disseminated opportunistic infections may have another underlying disorder or anti-interferon- $\gamma$  autoantibodies

that may resolve completely. It is unlikely that a single mechanism underlies all cases of adult-onset immunodeficiency.

In this study, patients with positive antibodies relapsed more frequently. In the presence of high titers, which are continuously detected in the blood, even a sensitive treatment regime may remain ineffective; although, there also may be infection with new opportunistic pathogens [14]. For TM infections, relapse is very likely during the treatment process, especially in nAIGAs-positive hosts. Many antifungal drugs are effective against TM. The preferred drug is amphotericin B and its liposome. Although initial treatment with and fluconazole can be effective [15, 16], fluconazole readily becomes resistant, so it is not the drug of first choice. Various adjuvant therapies have been used to improve the clinical outcome of patients with nAIGAs, such as B-cell depletion therapy, including rituximab (RTX), which has attracted much attention in recent years [17, 18]. None of our patients received these therapies. Furthermore, these adjuvant therapies have their own problems. For example, concerns regarding the usage of RTX include its high cost, recovery of the nAIGA titer at the end of RTX therapy, and associated side effects. Based on our limited experience, chemotherapy with subsequent autologous stem cell transplantation is another option to overcome the undesired effects of nAIGAs. In the future, a more inexpensive, more durable, and safer adjuvant therapy likely will be required to modify the effects of nAIGAs.

The present study has some limitations. First, the number of cases was limited. However, our study provides the first detailed clinical picture of patients with TM infection and nAIGAs. Second, some patients were still being followed up at the time of writing, and data, such as the number of relapses, length of illness, and prognosis, are currently unavailable. Thus, we did not detect cytokines and the titer changes in antibodies after treatment. A further long-term study is required to elucidate the relationship between the dynamic changes in antibodies and the change in patient conditions. Finally, adjuvant therapies were not used in our patients, and the efficacy of these agents could not be evaluated based on the present results. Despite these limitations, our results still offer valuable clinical information on patients with TM infection and nAIGAs for physicians dealing with such cases.

## CONCLUSIONS

A common etiology in Guangxi patients with TM infection with or without other opportunistic infections is nAIGA-associated immunodeficiency. When treating patients with unusual clinical presentations of TM infection, including dissemination or frequent recurrences, physicians should be aware of the presence of nAIGAs, particularly in patients with history of NTM or salmonellosis. The adherence to

medical advice and adjuvant therapy is necessary to improve the clinical outcome of patients with nAIGAs. Additional investigations are required to elucidate the trigger of nAIGAs, and there is need to develop a more effective treatment strategy for these patients.

## Notes

**Author Contributions.** WZ conceived and designed the study, acquired, analysed, and interpreted the data; drafted the manuscript. JZ conceived of the study, critically revised the manuscript for important intellectual content. YQ analysed and interpreted the data. ST acquired and analysed the data and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. MP agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. XZ gave final approval of the version to be published. All authors read and approved the final manuscript.

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