ORIGINAL RESEARCH

Performance of Interferon-γ Release Assays in Patients with *Mycobacterium kansasii* Infection

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Objective: To evaluate the performance of interferon-γ release assays (IGRAs) in patients with *Mycobacterium kansasii* infection.

Methods: Consecutive patients between May 2012 and June 2021 who had positive for mycobacterial cultures and who underwent IGRAs (T-SPOT.TB or QuantiFERON-TB Gold [QFT-G]) were included in the analysis. The IGRA positivity rates among patients with *M. kansasii* isolates were then calculated. If *M. kansasii* was identified in at least two sputum samples or in sterile samples, *M. kansasii* disease was then diagnosed. Otherwise, colonisation was considered.

Results: During the study period, 54 patients with *M. kansasii* infection underwent T-SPOT.TB (n=48) or QFT-G (n=6) assays. The mean age was 44.1±13.4 years, 85.2% (46/54) were male. Eight patients were diagnosed with *M. kansasii* disease and another 46 patients were considered to have colonisation. Twenty-four patients (T-SPOT.TB, n=23; QFT-G, n=1) were positive for IGRAs, for an overall rate of 44.4% (24/54; T-SPOT.TB, 47.9% [23/48]; QFT-G, 16.7% [1/6]) for IGRAs, 25.0% (2/8) for *M. kansasii* disease, and 47.8% (22/46) for colonisation.

Conclusion: Positive IGRA rates were relatively low in patients with *M. kansasii* infection. More efforts are required to improve the performance of IGRAs in diagnosing *M. kansasii* infection.

Keywords: interferon-gamma release assay, tuberculosis, *M. kansasii*, diagnosis, infection

Introduction

Interferon-γ (IFN-γ) release assays (IGRAs) are designed to identify tuberculosis (TB) infections. Currently, two commercial assays are available; each uses different platforms to measure the IFN-γ production stimulated by early secretory antigenic target (ESAT)-6 and culture filtrate protein (CFP)-10.[1] The QuantiFERON-TB assay (QFT; Qiagen, Hilden, Germany) is an overnight whole-blood enzyme-linked immunosorbent assay (ELISA) for quantitation of IFN-γ in the supernatant, whereas the T-SPOT.TB (Oxford Immunotec, Oxford, UK) measures the number of mononuclear cells secreting IFN-γ via an overnight enzyme-linked immunospot assay. In general, an IGRA is sensitive for detecting TB infection, with a sensitivity of 0.94 for active TB and 0.91 for latent TB infection.[2] However, IGRAs have shown limitations in borderline intervals, as well as in consistency. For example, caution should be taken when interpreting QFT results close to the assay cut-off, where assay conversion/reversion is likely to be observed in serial testing.[3] For Chinese village doctors, the concordance between the T-SPOT and QFT-Plus assays was 88.93% (Kappa coefficient: 0.73), and this remains unsatisfactory.[4]

For many decades, the tuberculin skin test (TST) was the only test available for detecting TB infection. However, the TST has several well-known disadvantages, including cross-reactivity with Bacillus Calmette-Guérin (BCG) and non-tuberculous mycobacteria (NTM), poor adherence due to return for results and wide variations observed in reading the results. IGRAs have recently been recommended in clinical practice and have several advantages for the diagnosis of TB

infection[1,5–7]. Compared to the TST, IGRAs have several advantages, such as being unaffected by BCG vaccination, IGRAs may produce fewer false positive reactions due to exposure to environmental mycobacteria. Compared with the TST, IGRAs are also less time consuming, because only a single patient visit is required and the results can be available within 24 hours.

NTMs are opportunistic pathogens and are widely distributed in the environment. Current, evidence suggests that the isolation rate of NTMs has been increasing in recent years.[8] In addition, human immunodeficiency virus (HIV) infection is a known risk factor for mycobacterial disease (both TB and NTM infections),[9] and its epidemic increases the complexity of the situation regarding NTM diseases. *Mycobacterium kansasii* is one of the most common NTM species, accounting for about 10% of NTM species isolated from clinical samples.[10–12] Until now, the diagnosis of NTM disease has mostly relied on microbiological evidence showing positive at least two separate sputum samples.[13] This diagnostic criterion is very specific and may contribute to a significantly missed diagnosis of *M. kansasii* infection. Therefore, more efforts aimed at improving the clinical and radiologic items of the guidelines would improve the effective diagnosis of NTM disease.[13] NTMs like *M. kansasii* that shares the RD1 region and show expression of ESAT-6 and CFP-10, can give rise to positive IGRA results.[14] Although the IGRA results for *M. kansasii* infection were reported in our previous study, the sample size (n=4) was too small to provide full details of the IGRA performance.[15] Therefore, the present study adopted a larger sample size to evaluate the performance of IGRAs in patients with *M. kansasii* infection and to assess the actual role of IGRAs in detecting *M. kansasii* infections.

Methods

This study was conducted at Shandong Provincial Chest Hospital and conformed to the tenets of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Shandong Provincial Chest Hospital. Due to the retrospective nature of this investigation and the anonymous nature of the data collection, written informed consent was waived by that Ethics Committee. Between May 2012 and June 2021, consecutive patients who were positive for mycobacterial culture and underwent IGRAs (T-SPOT.TB or QuantiFERON-TB Gold [QFT-G]) were included in the analysis. The patient data were retrieved from the laboratory information system (Ruimei, Shanghai, China). Other clinical characteristics, such as age, sex and HIV status, were also collected.

Sputum samples were cultured using Löwenstein-Jensen medium method and corresponding quality control was conducted as previously reported.[10] *M. kansasii* was identified using a Mycobacteria Identification Array Kit (CapitalBio, Beijing, China) or by sequencing 16S rDNA.[10] If *M. kansasii* was identified in at least two sputum samples or in sterile samples, *M. kansasii* disease was then diagnosed.[16,17] Otherwise, colonisation was considered.

The T-SPOT.TB and QFT-G IGRAs were performed according to the manufacturer's instructions. A positive T-SPOT. TB test result was defined using a cut-off value of at least 6 spots. For QFT-G results, the manufacturer's indicated cut-off of more than 0.35 IU/mL was considered a positive test result.

Continuous variables were presented as mean ± standard deviation (SD) and categorical variables were described as count and percentages.

Results

During the study period, 25,617 mycobacterial strains were found in the clinical samples. Of these, 1107 (4.3%) strains were identified as NTM species, including 110 strains isolated from outpatients and 997 strains from 828 inpatients. The 997 strains were identified as *M. intracellulare* (n=662, 66.4%), *M. kansasii* (n=111, 11.1%), *M. chelonei* (n=103, 10.3%), *M. fortuitum* (n=39, 3.9%), *M. gordonae* (n=20, 2.0%), *M. avium* (n=20, 2.0%), and others (n=42, 4.2%). A total of 111 *M. kansasii* isolates were collected from 97 inpatients.

Of the 97 patients, 54 underwent T-SPOT.TB (n=48) or QFT-G (n=6). The mean age was 44.1±13.4 years, 85.2% (46/54) of them were male, and 97.6% (40/41) were HIV-negative. Of the 54, 8 were diagnosed with *M. kansasii* disease and the remaining 46 patients were considered to have colonisation. In total, 24 patients (T-SPOT.TB, n=23; QFT-G, n=1) were positive for IGRAs and the positive IGRA rate in patients with *M. kansasii* was calculated as 44.4% (24/54; T-SPOT.TB, 47.9% [23/48]; QFT-G, 16.7% [1/6]). The positivity rates were further calculated as 25.0% (2/8) for *M. kansasii* disease and 47.8% (22/46) for colonisation. Table 1 shows the characteristics of the included patients.

Table 1 The IGRA Results and Clinical Characteristics of Patients with *M. Kansasii* Infection

	IGRA (+)	IGRA (-)	Total
N	24 (44.4%)	30 (55.6%)	54
Age (years)	42.9±10.4	45.1±15.5	44.1±13.4
Male, %	87.5% (21/24)	83.3% (25/30)	85.2% (46/54)
HIV (-)	100% (16/16)	96.0% (24/25)	97.6% (40/41)
Infection			
M. kansasii diseases	2 (8.3%)	6 (20.0%)	8 (14.8%)
Colonization	22 (91.7%)	24 (80.0%)	46 (85.2%)
IGRA results	T-SPOT.TB (n=23), QFT (n=1)	T-SPOT.TB (n=25), QFT (n=5)	T-SPOT.TB (n=48), QFT (n=6)

Abbreviations: IGRA, interferon-gamma release assay; HIV, human immunodeficiency virus; QFT, QuantiFERON-TB Gold.

Discussion

A significant IFN-γ production in response to a high conservation antigen of *M. tuberculosis* was previously observed in individuals with NTM infection.[18] Hence, positive IGRA results are expected among patients with *M. kansasii* infection, as both the ESAT-6 and CFP-10 antigens are found in *M. kansasii*.[19,20] In this study, assessment of the IGRA performance in patients with *M. kansasii* infection yielded an overall positivity rate of 44.4%. Our data suggested that the T cell response to antigens of *M. kansasii* is relatively weak and that the diagnostic value of IGRAs may be limited for *M. kansasii* infection.

However, in China, the use of IGRAs has several advantages in the diagnosis of *M. kansasii* infection. First, if anti-TB therapy fails, a positive IGRA result would increase the suspicion of *M. kansasii* infection and provide a rapid diagnosis.[14] Second, *M. kansasii* is usually considered pathogenic, as it readily causes disease.[21] A previous study demonstrated that a single positive culture for *M. kansasii* in the proper context could provide sufficient evidence for treatment initiation.[22] Hence, single isolation and a positive IGRA may be appropriate criteria for the diagnosis of *M. kansasii* disease. Third, *M. intracellulare, M. kansasii* and *M. fortuitum* are the three most common NTM species in China.[8,10] These epidemiological characteristics which were similar to our reports, raise several clinical implications for IGRA identification of *M. kansasii* infection. For example, IGRAs have the ability to discriminate *M. kansasii* infections from infections by other NTMs (such as *M. intracellulare* and *M. fortuitum*, which lack ESAT-6 and CFP-10.[14] A positive IGRA could increase the certainty of a diagnosis of *M. kansasii* infection.

At present, several studies with small sample sizes have been performed to evaluate of IGRA performance in *M. kansasii* infection. Kobashi Y et al evaluated the clinical usefulness of the QFT-2G test in patients with NTM disease and found a positive response rate of 52% for QFT-2G test in 33 patients with *M. kansasii* disease.[23] In a preliminary study conducted in Poland, positive IGRA results were found in 22% (2/9) of patients with *M. kansasii* infection.[24] In a Danish study, only two patients with *M. kansasii* infection were tested using QFT-G, but both were positive for QFT-G.[25] In Japan, a retrospective study showed that patients with *M. kansasii* infection had positive IGRA rates ranging between 25.9% and 33.3%.[26] By contrast, due to the lack of ESAT-6 and CFP-10, the IGRA positivity rate was significantly lower for infection with *M. avium* complex (1–2%),[23,27] which is a major source of NTM infection.[23,28]

The response to ESAT-6 and CFP-10 antigens is weaker in patients with *M. kansasii* infection than in those with TB infection. One reason may be that differences in ESAT-6 and CFP-10 between *M. kansasii* and *M. tuberculosis* have been reported for nucleotide sequences (10–12%) and amino acid sequences (<5%); therefore, these differences may attenuate the response. Indirect evidence has indicated attenuation of the T cell response in TB patients due to the differences in TB-ESAT-6 and *M. kansasii*-ESAT-6.[29] Another reason may be that the production capacity of IFN-γ is lower in NTM patients than in TB patients.[30]

Impairment of the IFN-γ responses dependent on stimulation has been observed in patients with NTM infection,[31] suggesting an immunologic imbalance in NTM infection. Future studies should re-evaluate the optimal cut-off values of IGRAs for the diagnosis of *M. kansasii* infection with respect to CFP-10 and ESAT-6.[32,33] Recently, a highly specific protein that can stimulate specific IFN-γ secretion has been designed for the diagnosis of *M. abscessus* infection. This novel assay has a moderate AUC of 0.773 for diagnosis,[34] suggesting antigens originating from *M. kansasii* could also be used to improve the IGRA results for *M. kansasii* infection.

Our study had several limitations. First, it was conducted in a high-TB burden country, so latent TB infection may affect our results. Second, the distribution of NTM species a geographical variation; therefore, the epidemiological characteristics could have a significant effect on IGRA usefulness. Third, although the study had a small sample size, it remains one of the largest studies compared with others. Fourth, the IGRAs are designed for the evaluation of TB infection; therefore, further studies are required to evaluate the IFN-γ response to antigens from NTM for the diagnosis of NTM diseases.[34] Fifth, two kinds of commercial IGRAs were used in this study, and this could have introduced an additional bias due to the inherent differences between them, such as the TB antigens (T-SPOT.TB assays, RD-1 antigens; QFT assays, TB 7.7 and RD-1 antigens),[35] technical platforms (T-SPOT.TB uses effector T cells; QFT determines IFN-γ levels), specimens used (QFT uses whole blood cells; T-SPOT.TB uses mononuclear cells), indeterminate results (these are more common in QFT),[36] and diagnostic performance.[37,38] Therefore, interpreting the results requires caution.

In conclusion, our data demonstrated that a significant proportion of *M. kansasii* infections generate positive results for IGRAs. Infection by *M. kansasii* could therefore induce IFN-γ production with the TB antigens. However, further studies are required to address this more completely, and to evaluate the IFN-γ responses to *M. kansasii* antigens for the diagnosis of *M. kansasii* infections.

Data Sharing Statement

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Disclosure

The authors have no competing interests in this work.

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