# Use of the QuantiFERON-TB Gold test in Japanese patients with sarcoidosis.

# USE OF THE QUANTIFERON-TB GOLD TEST IN JAPANESE PATIENTS WITH SARCOIDOSIS

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**Abstract**

*Background and Objective: Mycobacterium tuberculosis* has been proposed as a candidate agent for the cause of sarcoidosis. The QuantiFERON-TB Gold test has a higher specificity for detecting *Mycobacterium tuberculosis* infection than the conventional tuberculin skin test. This study aimed to investigate the rate of positive QuantiFERON-TB Gold results in Japanese sarcoidosis patients.

*Patients and Methods:* The QuantiFERON-TB Gold test, an enzyme-linked immunosorbent assay, was used to assess the levels of interferon-gamma resulting from immune responses to *Mycobacterium tuberculosis*-specific antigens, namely early secretory antigen target 6 and culture filtrate protein 10, in 90 Japanese sarcoidosis patients.

*Results:* The QuantiFERON-TB Gold result was positive in 3 of the 90 patients tested.

*Conclusion:* The positivity rate of QuantiFERON-TB Gold was 3.3% in Japanese sarcoidosis patients.

## Introduction

The QuantiFERON-TB Gold (QFT) test, a whole blood interferon-gamma assay, is a new technique for diagnosing *Mycobacterium tuberculosis* infection (TBI) (1,2). Although the tuberculin skin test (TST) is the current standard diagnostic test for detecting latent TBI, it shows low specificity. This probably arises because its purified protein derivative (PPD) is a mixture of mycobacterium antigens that are also present in both bacillus Calmette-Guérin (BCG) strains and non-tuberculous mycobacteria (NTM). Since the antigens in the QFT are early secretory antigen target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are specific to *M. tuberculosis* and absent from BCG vaccine strains and the majority of NTM species, the QFT can detect latent TBI with higher specificity than the conventional TST (3,4).

Sarcoidosis is a systemic granulomatous disorder of unknown etiology (5). Due to the clinical and pathological similarities, mycobacterial infections, especially TBI, have been proposed as candidates for infectious causes of sarcoidosis. However, it remains controversial whether sarcoidosis is caused by *M. tuberculosis*, since numerous studies using currently available molecular techniques have resulted in

positive or negative results (5,6). Furthermore, it is sometimes difficult in practice to distinguish between sarcoidosis and TBI (5). The TST is unsuitable for detecting TBI in sarcoidosis patients due to its anergically-depressed reaction. Burton used interferon-gamma production by alveolar lymphocytes in response to tuberculosis PPD stimulation to distinguish sarcoidosis from TBI (7).

Recently, Drake et al. reported that one ESAT-6 peptide was recognized in 8 of 26 sarcoidosis patients using an enzyme-linked immunospot (ELISPOT) assay (8). The present study aimed to elucidate the rate of positive QFT results in Japanese sarcoidosis patients.

## Methods

### *Patients and controls*

Ninety consecutive patients with sarcoidosis (29 males, 61 females; mean age: 48.7 yr) were included in this study. The diagnosis of sarcoidosis was based on a compatible clinical picture and histological finding of noncaseating granulomas (5). Cases were excluded if they had previously been diagnosed with TBI or had chest

radiographic evidence of healed TBI. None of the patients was under systemic steroid or immunosuppressive therapy. Our Institutional Review Board approved the study protocol and each patient gave written informed consent.

*Sample Collection and TST*

A heparinized peripheral blood sample was collected from each patient. For the TST, 0.1 ml of tuberculin PPD (equivalent to 3 tuberculin units of PPD-S; Nippon BCG Manufacturing, Tokyo, Japan) was injected intradermally and the induration diameter was measured after 48-72 h.

*Whole Blood Interferon-gamma Assay*

Interferon-gamma production in whole blood was measured using the QFT, a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cellestis, Carnegie, Australia). The assay was performed by a blinded investigator according to the manufacturer's instructions. We interpreted the test result as positive if the

concentration of interferon-gamma for either of the antigens was more than 0.35 IU/ml (4,9).

**Results**

All 90 patients had sarcoid lesions in their lungs. TST results were available in 84 patients, among which 2 and 1 patients showed indurations of >10 and 15 mm, respectively. The QFT was performed in all 90 patients, and their levels of interferon-gamma response to phytohemagglutinin were at least 0.5 IU/ml. All individual tests were deemed valid. Among the 90 sarcoidosis patients, the QFT result was positive in 3 patients (3.3%; Table 1). Their specimens were negative for *M. tuberculosis* by acid fast staining, culture and PCR evaluation of lung or skin tissues. During a 1-year follow-up, none of the patients developed TBI. There were no characteristic clinical, radiographic or pathologic differences between the 3 QFT-positive patients and 87 QFT-negative patients.

**Discussion**

In the present study, 3 of 90 sarcoidosis patients showed a positive QFT result. They had no evidence of TBI in microbiological, radiological and pathological examinations. The QFT positivity rate in our Japanese sarcoidosis patients was 3.3%, which was nearly identical to those in healthy non-sarcoidosis subjects (3,4,9-12).

Although there is no definitive method for diagnosing latent TBI, many studies have evaluated the specificity of the QFT in low-risk subjects. Some surveys mainly targeted students, while others contained middle-aged subjects with no identified risks for *M. tuberculosis* exposure (3,4,9-12). In a Japanese survey, the specificity of the QFT for a BCG-vaccinated group was reported to be 98.1% (4). Another Japanese investigation showed that 94% of healthy volunteers were negative for the QFT (9). Currently, the high specificity of the QFT is recognized independently of BCG vaccination status and age in low-risk groups (3,4,9-12). In the present study, 3 patients showed a positive QFT response (3.3%). Compared with previous data targeting various TB-prevalent countries and study populations (3,4,9-13), the rate of positive results was almost identical to those in non-sarcoidosis subjects.

In this study, we cannot provide a link between Japanese sarcoidosis patients

and TBI detected using the QFT, which does not directly imply or exclude the possibility that *M. tuberculosis* causes sarcoidosis. The results of the QFT are reflected by the exposure level and amount of *M. tuberculosis.* If *M. tuberculosis* causes sarcoidosis in a non-infectious fashion and in trace quantities, the release of interferon-gamma may be below the detection limit of the QFT. Moreover, sarcoidosis is an immune disease that may not require continuous antigen exposure. In contrast to our results, surprisingly, Drake et al. reported that one ESAT-6 peptide was recognized in 8 of 26 sarcoidosis patients (8). They performed an ELISPOT assay with ESAT-6 and their original KatG peptide, and found that these mycobacterial antigens induced T-cell responses in the blood of sarcoidosis patients. Therefore, they suggested immunologic links between mycobacteria and sarcoidosis. However, there are many differences between their study and the present study. First, they targeted mainly African-Americans and more than half of their patients received certain immunosuppressive drugs. Second, the ESAT-6 peptides in their ELISPOT assay were different from the seven types of proteins in our commercially available ELISA kit. Improving the sensitivity of the test and studies in various areas and races are required

to clarify the involvement of *M. tuberculosis* in the pathogenesis of sarcoidosis.

**Conflict of interest statement for Respiratory Medicine**

On behalf of all the authors (N. Inui, T. Suda, and K. Chida), I report that all the authors have no conflict of interest, including financial, personal, academic and intellectual issues.

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