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# In-hospital blood collection increases the rate of indeterminate results in interferon-gamma release assays

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***Abstract***

**Background:** The interferon (IFN)-γ release assay (IGRA) has recently been established as a method to evaluate the infection status of tuberculosis instead of the tuberculin skin test. However, indeterminate results can create challenges to interpretation. The IGRA has been available in Japan since 2005, including the recently launched QuantiFERON-TB Gold Plus (QFT-plus) assay.

**Objectives:** The aim of this study was to investigate the clinical features and predictors of indeterminate results by the QFT-plus test in routine practice.

**Methods:** This was a cross-sectional study of 1258 patients. Multivariate logistic regression models were employed to investigate the clinical factors related to indeterminate results by the QFT-plus.

**Results:** Overall, 91.8% of results were found to be conclusive and 8.2% were indeterminate. The QFT-plus indeterminate results were predominantly due to a low level of IFN-γ production by mitogens. Multivariate analysis indicated that an indeterminate result was significantly associated with age, sex, corticosteroid use, autoimmune disease, and inpatient setting.

**Conclusion:** Certain types of individuals are at higher risk of an indeterminate IGRA result. The QFT-plus test for hospitalized patients should be avoided as much as possible, and it is better to perform the test for those patients in outpatient settings.

***Keywords:*** Inpatient setting, Interferon-γ release assay, *Mycobacterium tuberculosis*, QuantiFERON-TB Gold Plus

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## Introduction

Tuberculosis (TB) remains one of the major infectious diseases worldwide.[1] The efficacy of the interferon (IFN)-γ release assay (IGRA) for the detection of TB infection is well established.[2] To determine whether *Mycobacterium tuberculosis* (MTB) infection is present, the IGRA has recently replaced the traditional tuberculin skin test. The IGRA measures IFN-γ produced by T cells in the presence of TB-specific antigens. IGRA results are divided into three categories: positive, negative, and indeterminate. A positive IGRA result is indicative of TB infection but does not distinguish between active and latent TB. Conversely, a negative IGRA result cannot completely deny the possibility of TB infection. Several studies have revealed the efficacy of combining a positive IGRA with clinical risk factors to estimate active TB.[3–5] This permits initiating empirical active TB treatment as soon as possible. Meanwhile, latent TB infection (LTBI) is defined as a positive IGRA result with no evidence of MTB in the smear or culture, regardless of parenchymal abnormalities. It is crucial to provide preventive TB treatment to patients with LTBI at high risk of progression to active TB. Thus, the IGRA test has a prominent

role in clinical practice. The disadvantage of the IGRA is that indeterminate results are present in definite proportions. Some medical conditions that impair immune functionality can potentially reduce IFN-γ responses in the IGRA.[6–9] In addition, technical procedures may influence IGRA variability.[8–12] The IGRA has been available in Japan since 2005. Currently, the newest IGRA, the QuantiFERON-TB Gold Plus (QFT-plus; Qiagen, Hilden, Germany), has been launched. This is the first study to investigate the clinical features and predictors of a QFT-plus indeterminate result in routine practice.

## Materials and methods

### *Population and data collection*

A cross-sectional study was conducted to investigate indeterminate IGRA results in clinical practice at the Showa University Fujigaoka Hospital from April 2019 to April 2020. IGRAs were carried out in outpatients and inpatients, all of whom were suspected of having active or latent TB based on the clinical course and imaging studies. All clinical data were collected from the patients' medical records on the day of IGRA testing. The estimated glomerular filtration rate (eGFR) of each patient was calculated using the following formula: eGFR (ml/min/1.73 $m^2$) = $194 \times$ serum creatinine $^{-1.094} \times$ age $^{-0.287} \times 0.739$ (if female).[13] Chronic renal failure (CKD) was defined as eGFR $<60$ ml/min/1.73 $m^2$. HIV antibody tests were not performed because the HIV infection rate is only 0.001% in Japan, according to a recent national survey.[14] The study protocol was approved by the Institutional Ethics Committee of Showa University (approval no. F2020C104). The requirement to obtain informed consent from the patients was waived because of the retrospective nature of this study.

### *IGRA testing*

Phlebotomists collected blood samples from outpatients, whereas residents or bedside nurses collected samples from hospitalized patients. A QFT-plus test was performed according to the manufacturer's guidelines. The test was composed of four tubes: negative control, mitogen (positive control), and two TB antigens (TB1 and TB2). TB1 antigen contains ESAT-6 and CFP-10 modified to elicit CD4+ T-cell responses, whereas TB2 antigen is linked to a CFP-10 short-chain peptides in addition to TB1 antigens, which initiate CD8+ T-cell responses. Incubation was performed within 16 h of sample collection. The duration of incubation was rigidly defined as the interval between 21 and 22 h. The concentration of IFN-γ in each tube was measured using enzyme-linked immunosorbent assay. Test results were reported as positive when IFN-γ for TB1 minus negative control or TB2 minus negative control was $\geqslant 0.35$ IU/ml in addition to the value of IFN-γ $\geqslant 25\%$ of the negative control. Cases where the mitogen minus negative control was $<0.5$ IU/ml or the negative control was $>8$ IU/ml were defined as indeterminate.

### *Clinical isolates for MTB detection*

Sputum samples were collected from each patient if possible, or curette lavage fluid was collected by bronchoscopy in a probable case of active TB. In brief, curette lavage fluid was obtained by scraping the site of suspected TB lesions with a curette, followed by flushing with 5 ml of saline. Clinical isolates were cultured in mycobacterial growth indicator tubes and in 2% Ogawa solid medium. Active TB was defined as positive MTB culture.

### *Statistical analysis*

All data are expressed as mean ± standard deviation for continuous variables or as percentages for categorical variables. Group mean values were compared using the Mann–Whitney rank-sum test. Pearson's chi-square test or Fisher's exact test was used for the univariate analysis of the association between two categorical variables. The adjusted effects of multiple variables on indeterminate results were evaluated using a logistic regression model, and the findings were presented as odds ratios (ORs) with 95% confidence intervals (CIs). Statistical significance was set at $p<0.05$. All statistical analyses were performed using JMP software version 16.0 (SAS Institute, Cary, NC).

## Results

### *Patient characteristics and QFT-plus results*

As shown in Figure 1, 1315 patients were enrolled in this study. Of these, 2 infants and 55 duplicated patients were excluded. Eventually, 1258 patients, including 1247 Japanese patients (99.1%)

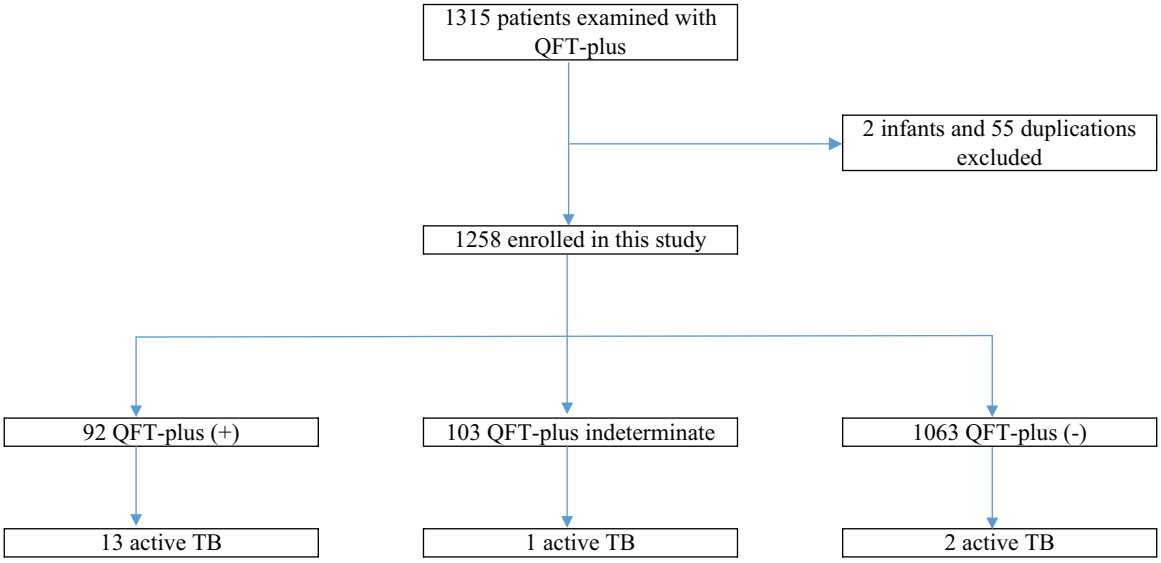


**Figure 1.** Recruitment flowchart of the study cohort.
QFT-plus, QuantiFERON-TB Gold Plus; TB, tuberculosis.

(Supplementary Table), were statistically analyzed. The study cohort included no drug users or homeless individuals. The patient characteristics are shown in Table 1. There were 688 (54.7%) males and 570 (45.3%) females with ages ranging from 10 to 96 years. Overall, 47 (3.7%) were receiving corticosteroids (at least 10 mg/day of prednisolone) for various indications, and 185 (14.7%) had diabetes mellitus. There were 400 (31.8%) patients with various malignancies (Supplementary Table) and 364 (28.9%) with CKD. There were 90 (7.2%) patients with autoimmune disease (Supplementary Table), of whom 10 (10 of 90; 11.1%) received at least 10 mg/day of prednisolone and 30 (30 of 90; 33.3%) received immunosuppressants. There were 310 (24.6%) inpatients and 948 (75.4%) outpatients.

Of the 1258 patients, positive, indeterminate, and negative IGRA results were identified in 92 (7.3%), 103 (8.2%), and 1063 (84.5%) patients (Figure 1). Of the indeterminate cases, 102 had positive control failure and 1 had high IFN-γ level in the negative control.

### *Statistical analysis of IGRA status and clinical features*

In univariate analyses, QFT-plus indeterminate results were significantly associated with age, sex, body mass index (BMI), corticosteroid use, diabetes mellitus, and autoimmune disease. In addition, the difference in QFT-plus indeterminate results between inpatients and outpatients was especially evident, as indicated in Table 1. However, there was no significant association between indeterminate results and malignancies or CKD.

Multivariate analyses were employed to control for potential confounding effects of these variables. Table 2 presents the logistic regression models of factors related to indeterminate results by QFT-plus. The frequency of indeterminate results increased significantly with age (OR, 1.0181; 95% CI, 1.0018–1.0346). QFT-plus indeterminate results were frequently associated with male sex (OR, 2.0001; 95% CI, 1.244–3.2161), corticosteroid use (OR, 2.4238; 95% CI, 1.1191–5.2495), autoimmune disease (OR, 3.8475; 95% CI, 1.9334–7.6566), and inpatients (OR, 8.6461; 95% CI, 5.3991–13.8457). BMI, diabetes mellitus, malignancies, and CKD were not significantly associated with QFT-plus indeterminate results.

### *Retesting with QFT-plus*

Of the 55 duplicates, 6 had indeterminate results in the initial QFT-plus test. Table 3 shows the results of retests for the QFT-plus. Although two resulted in indeterminate results once again, two were determined as positive and two as negative. The interval between the initial and second tests

**Table 1.** Association of each variable with patients examined by QFT-plus.

| | Total | Determinate | Indeterminate | *p*-value |
|---|---|---|---|---|
| No. (%) | 1258 | 1155 | 103 | |
| Age | | | | |
| Mean ± SD | 67.9 ± 15.9 | 67.5 ± 15.9 | 72.3 ± 14.8 | 0.0021* |
| Sex | | | | |
| Male | 688 | 618 (49.1) | 70 (5.6) | 0.0047* |
| Female | 570 | 537 (42.7) | 33 (2.6) | |
| BMI (kg/m$^2$) | | | | |
| Mean ± SD | 21.3 ± 3.9 | 21.4 ± 3.8 | 20.4 ± 4.8 | 0.0025* |
| Receiving corticosteroids[a] | | | | |
| Yes | 47 | 34 (2.7) | 13 (1.1) | < .0001* |
| No | 1211 | 1121 (89.1) | 90 (7.1) | |
| Diabetes mellitus | | | | |
| Yes | 185 | 162 (12.9) | 23 (1.8) | 0.0226* |
| No | 1073 | 993 (78.9) | 80 (6.4) | |
| Malignancies | | | | |
| Yes | 400 | 369 (29.3) | 31 (2.5) | 0.6991 |
| No | 858 | 786 (62.5) | 72 (5.7) | |
| CKD | | | | |
| Yes | 364 | 334 (26.5) | 30 (2.4) | 0.9643 |
| No | 894 | 821 (65.3) | 73 (5.8) | |
| Autoimmune disease | | | | |
| Yes | 90 | 74 (5.9) | 16 (1.3) | 0.0006* |
| No | 1168 | 1081 (85.9) | 87 (6.9) | |
| Inpatient or outpatient | | | | |
| Inpatient | 310 | 238 (18.9) | 72 (5.7) | <0.0001* |
| Outpatient | 948 | 917 (72.9) | 31 (2.5) | |

BMI, body mass index; CKD, chronic renal failure; QFT-plus, QuantiFERON-TB Gold Plus; SD, standard deviation.
[a]More than 10 mg of prednisone per day.
*$p < 0.05$ was considered significant.

for the two positive conversions was 9 months in Case 1 and 2 months in Case 2. There were some differences between the two patients with persistent indeterminate results and the four with new conclusive results. The indeterminate results were duplicated in Cases 5 and 6, both of whom were hospitalized for long periods because of advanced cancer.

**Table 2.** Logistic regression models of indeterminate results by QFT-plus.

| | Category | OR | 95% CI | *p* |
|---|---|---|---|---|
| Age | | 1.0181 | 1.0018–1.0346 | 0.0285* |
| Sex | Male/female | 2.0001 | 1.244–3.2161 | 0.0042* |
| BMI (kg/m$^2$) | | 0.9611 | 0.9068–1.0186 | 0.1806 |
| Receiving corticosteroids[a] | Yes/no | 2.4238 | 1.1191–5.2495 | 0.0247* |
| Diabetes mellitus | Yes/no | 1.3537 | 0.7796–2.3504 | 0.2821 |
| Malignancies | Yes/no | 0.805 | 0.4933–1.3135 | 0.3852 |
| CKD | Yes/no | 0.6828 | 0.4129–1.1291 | 0.1371 |
| Autoimmune disease | Yes/no | 3.8475 | 1.9334–7.6566 | 0.0001* |
| Inpatient or outpatient | Inpatient/outpatient | 8.6461 | 5.3991–13.8457 | <0.0001* |

BMI, body mass index; CI, confidence interval; CKD, chronic renal failure; IGRA: interferon-γ release assay; OR, odds ratio; QFT-plus, QuantiFERON-TB Gold Plus.
[a]More than 10 mg of prednisone per day.
*$p < 0.05$ was considered significant.

### *Correlation between QFT-plus and active TB*

Table 4 shows patients with active TB in this cohort. The QFT-plus was performed at the time of diagnosis in all patients with active TB. There were 12 positive, 1 indeterminate, and 3 negative results by QFT-plus. Of the three negative cases, none were retested using QFT-plus and all three were culture-positive for MTB with a negative smear. Cases 10 and 19 had no history of TB, and their CT images showed a cavity in the right upper and right lower lobe, respectively. Case 18 was diagnosed with small-cell lung carcinoma (SCLC) and active TB. In the indeterminate case (Case 1), the patient was hospitalized due to severe pneumonia during the period and developed active TB after 9 months in the hospital. The CT images on admission indicated emphysematous blebs in the bilateral upper lobe, in addition to pulmonary infiltration. However, once the patient recovered from the respiratory failure, he progressively suffered from chronic cough and the bilateral infiltration did not completely disappear despite antibiotic administration for common bacterial infection. Compared with outpatients, the levels of IFN-γ with mitogen were significantly decreased in the inpatient setting (Supplementary Figure). All patients received standard TB therapy for 6 months.

## Discussion

In this study, the correlation between indeterminate IGRA results and clinical features was analyzed. Overall, 91.8% were found to be determinate and 8.2% were indeterminate results. Multivariate analysis indicated that indeterminate results were significantly associated with age, sex, corticosteroid use, and autoimmune disease. Indeterminate results in this study were predominantly derived from low IFN-γ reaction with mitogen, consistent with previous studies.[12,15] In fact, IFN-γ is produced by T-cells and natural killer cells, all of which weaken with age.[16,17] Systemic corticosteroids impair T-cell immune response, and autoimmune diseases can involve cell-mediated immune deficiency in addition to the administration of corticosteroids or immunosuppressants. Thus, host immunosuppression can promote a high rate of indeterminate results in QFT-plus. According to previous studies, the sex-based difference of indeterminate results in IGRA remains controversial.[7,15,18,19] The current study indicated that the rate of indeterminate results was significantly higher in males than in females. This may be related to race, ethnicity, or age distribution. Indeed, the age composition of this cohort was higher than that reported in other studies.[7,15,18,19]

**Table 3.** QFT-plus retests in patients with indeterminate results at the initial examination.

| | Age | Sex | First | | | | | Second | | | | | BMI (kg/m²) | Receiving corticosteroids[a] | Diabetes mellitus | Malignancies | CKD | Autoimmune disease |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Nil | TB1 | TB2 | Mitogen | QFT-plus | Status | Nil | TB1 | TB2 | Mitogen | QFT-plus | Status | | | | | | |
| Case 1* | 71 | Male | <0.05 | <0.05 | <0.05 | 0.27 | Indeterminate | In-patient | 0.2 | 3.74 | 4.07 | 9.15 | (+) | Inpatient | 20.1 | (–) | (–) | (–) | (–) | (–) |
| Case 2 | 72 | Male | <0.05 | 0.07 | 0.1 | 0.22 | Indeterminate | In-patient | 0.1 | 0.37 | 0.62 | 6.91 | (+) | Outpatient | 29.4 | (–) | (–) | (–) | (+) | (–) |
| Case 3 | 82 | Female | 0.06 | <0.05 | <0.05 | 0.29 | Indeterminate | Out-patient | 0.1 | <0.05 | <0.05 | 1.74 | (–) | Outpatient | 13.7 | (+) | (–) | (–) | (+) | (+)[b] |
| Case 4 | 85 | Male | <0.05 | <0.05 | <0.05 | 0.4 | Indeterminate | Out-patient | 0.1 | <0.05 | <0.05 | 4.13 | (–) | Outpatient | 15.8 | (–) | (–) | (–) | (–) | (–) |
| Case 5 | 88 | Male | 0.07 | <0.05 | <0.05 | 0.43 | Indeterminate | In-patient | <0.05 | <0.05 | <0.05 | 0.14 | Indeterminate | Inpatient | 20.6 | (–) | (–) | (+)[c] | (–) | (–) |
| Case 6 | 64 | Female | <0.05 | <0.05 | <0.05 | <0.05 | Indeterminate | In-patient | <0.05 | <0.05 | <0.05 | <0.05 | Indeterminate | Inpatient | 20.1 | (–) | (+) | (+)[d] | (–) | (–) |

BMI, body mass index; CKD, chronic renal failure; QFT-plus, QuantiFERON; TB, tuberculosis.
[a]More than 10 mg of prednisone per day.
[b]Antiphospholipid antibody syndrome.
[c]Laryngeal cancer.
[d]Pancreatic cancer.
*Identical patient in Table 4.

Indeterminate results were found most frequently in inpatients than in outpatients, according to the multivariate logistic regression models. Indeterminate results in QFT-plus have been reported in 2.5–4.2% of tests in Western countries,[12,19] but the rates in TB-epidemic countries have ranged up to 10%.[7,20,21] TB morbidity among the study population should therefore be considered, particularly for QFT-plus, since indeterminate results occur more frequently with QFT-plus than with previous-generation IGRAs.[12] The current prevalence of TB in Japan is up to threefold higher compared with other industrialized countries; however, the morbidity is rapidly decreasing.[22] Especially in outpatients, 3.3% (31 of 948) of results were indeterminate, consistent with the rates found in Western countries.[12,19] In contrast, 23.2% (72 of 310) were found to have indeterminate results in the inpatient setting. Hence, the high rate of indeterminate results in the current study was predominantly caused by QFT-plus results in hospitalized patients. The explanations for this finding could be technical issues at the time of blood collection or post-collection steps. Several studies have discussed the association of IGRA indeterminate with blood volume, shaking the tube, pre-incubation time, and incubation duration.[8,11,12] The manufacturer's guidelines recommend a blood volume of 0.8–1.2 ml per tube; however, smaller volumes will increase the IFN-γ reaction with TB antigen.[10] In addition, shaking each tube homogeneously and simultaneously improved both the sensitivity and specificity in the IGRA.[10,15] Another study revealed decreased indeterminate results of one-tube collection for the QFT-plus, compared to separately collecting four tubes.[12] Likewise, indeterminate results were detected more frequently in QFT-plus than in previous-generation IGRAs,[12] probably because more tubes were collected for QFT-plus. Limiting the pre-incubation time could improve IGRA performance, but the maximum time was limited to less than 1 h.[8] In the current study, blood samples from outpatients and inpatients were collected by phlebotomists and residents/bedside nurses, respectively. However, deficient and excess blood volumes were rigidly excluded, and pre-incubation time was regulated within 16 h according to the manufacturer's guidelines. The incubation time was constant regardless of the inpatient or outpatient setting. QFT-plus indeterminate results were likely due to a low level of IFN-γ production by mitogens in this study. The technical difference between phlebotomists and residents or

**Table 4.** QFT-plus results of patients with active TB.

| | Age | Sex | QFT-plus | | | | | Status | TB smear | TB culture | BMI (kg/m$^2$) | Receiving corticosteroids[a] | Diabetes mellitus | Malignancies | CKD | Autoimmune disease |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Nil | TB1 | TB2 | Mitogen | Result | | | | | | | | | |
| Case 1* | 71 | Male | <0.05 | <0.05 | <0.05 | 0.27 | Indeterminate | Inpatient | (+) | (+) | 20.1 | (–) | (–) | (–) | (–) | (–) |
| Case 7 | 89 | Female | 3.0 | 2.08 | 2.04 | 1.92 | (+) | Inpatient | (+) | (+) | 20.1 | (–) | (–) | (+)[b] | (+) | (–) |
| Case 8 | 90 | Male | <0.05 | 0.19 | 0.37 | 2.95 | (+) | Inpatient | (–) | (+) | 15.5 | (–) | (–) | (+)[c] | (+) | (–) |
| Case 9 | 69 | Male | 0.26 | 0.94 | 0.58 | 0.4 | (+) | Inpatient | (–) | (+) | 19.9 | (–) | (–) | (–) | (+) | (–) |
| Case 10 | 53 | Male | 0.08 | 0.19 | 0.23 | 7.2 | (–) | Outpatient | (–) | (+) | 20.4 | (–) | (–) | (–) | (–) | (–) |
| Case 11 | 90 | Female | 0.06 | 0.99 | 0.52 | 6.05 | (+) | Outpatient | (–) | (+) | 23.6 | (–) | (–) | (+)[d] | (+) | (–) |
| Case 12 | 88 | Male | 0.17 | 2.82 | 2.11 | 7.55 | (+) | Outpatient | (+) | (+) | 13.8 | (–) | (–) | (–) | (–) | (–) |
| Case 13 | 68 | Female | <0.05 | 1.5 | 2.84 | 7.16 | (+) | Outpatient | (+) | (+) | 21.9 | (–) | (–) | (–) | (–) | (–) |
| Case 14 | 48 | Male | 0.06 | 0.56 | 0.54 | 4.93 | (+) | Outpatient | (–) | (+) | 24.1 | (–) | (–) | (–) | (–) | (–) |
| Case 15 | 63 | Male | 1.09 | 5.16 | 5.59 | 6.65 | (+) | Outpatient | (+) | (+) | 22.6 | (–) | (–) | (–) | (–) | (–) |
| Case 16 | 61 | Male | 0.07 | 2.14 | 2.21 | 7.26 | (+) | Outpatient | (–) | (+) | 27.8 | (–) | (–) | (–) | (+) | (–) |
| Case 17 | 31 | Male | <0.05 | 0.31 | 0.48 | 8.76 | (+) | Outpatient | (–) | (+) | 20.8 | (–) | (–) | (–) | (–) | (–) |
| Case 18 | 73 | Male | <0.05 | <0.05 | <0.05 | 8.8 | (–) | Outpatient | (–) | (+) | 25.9 | (–) | (+) | (+)[e] | (–) | (–) |
| Case 19 | 50 | Male | 0.05 | <0.05 | <0.05 | 9.97 | (–) | Outpatient | (–) | (+) | 24.6 | (–) | (–) | (–) | (–) | (–) |
| Case 20 | 84 | Male | 0.14 | 0.57 | 0.43 | >10.00 | (+) | Outpatient | (+) | (+) | 22.4 | (–) | (–) | (–) | (–) | (–) |
| Case 21 | 24 | Male | 5.35 | 4.46 | 4.68 | 4.44 | (+) | Outpatient | (+) | (+) | 25.9 | (–) | (–) | (–) | (–) | (–) |

BMI, body mass index; CKD, chronic renal failure; QFT-plus, QuantiFERON; TB, tuberculosis.
[a]More than 10 mg of prednisone per day.
[b]Colorectal cancer.
[c]Esophageal cancer.
[d]Breast cancer.
[e]Small cell lung cancer.
*Identical patient in Table 3.

bedside nurses could, therefore, be based on shaking. In fact, data from the current study indicated that the IFN-γ reaction with mitogen in active TB cases was significantly lower in inpatient settings than in outpatient settings, suggesting the importance of the mixing procedure. The QFT-plus test for hospitalized patients should be avoided as much as possible, and it is better to perform the test for those patients in outpatient settings.

Conversion from indeterminate results in retesting by IGRA has been reported.[12,21,23,24] Of the six patients with indeterminate results on the initial examination, four were converted into a determinate result (two positive and two negative). The two persistent indeterminate results were found in patients with advanced cancer who needed long-term hospitalization. Malignancy was not associated with indeterminate IGRA results in this study, but prolonged cancer-bearing condition might generate immunological influence including IFN-γ production. Several retests for QFT-plus should be performed for an indeterminate result.

The current study indicated 16 cases of active TB. Of these, the three with negative IGRAs were all identified in the outpatient setting. Active TB was suspected in these patients regardless of the IGRA result based on the patients' symptoms and imaging findings and all were ultimately culture-positive. Cases 10 and 19 were not elderly – around 50 years old. Active TB was strongly suspected during the first visit because these patients had characteristic findings of primary TB on imaging studies. Because of the high risk of transmission in the past, the morbidity of MTB infection is relatively high among the elderly population. The estimated rate of existing TB in people around 50 was only 5%, whereas the rate in people over 70 was up to 40% in Japan.[22] The window period for incipient TB is supposed to be a few months after infection, and positive conversion in IGRA occurs several months after infection.[24] Hence, the QFT-plus negative results at initial examination in Cases 10 and 19 could be due to the early incubation phase. On the contrary, Case 18 was a 73-year-old man diagnosed with active TB in combination with SCLC. Malignancies have been reported as individual susceptibility factors for the increased probability of active TB from LTBI,[3,4] suggesting post-primary TB and technical error at the initial QFT-plus examination.

This study had several limitations. First, the study was carried out at a single facility, which might have reduced the generalizability of the findings. Second, HIV antibody tests were not performed despite HIV infection being an independent risk factor for an indeterminate IGRA result. Third, only six patients with indeterminate results at the initial phase had a repeated QFT-plus test. The sample size was quite small, and we could not perform statistical analysis for these issues.

## Conclusion

The present study demonstrates the prediction of QFT-plus indeterminate results in routine practice. It may be useful to perform several investigations of QFT-plus in case of an indeterminate result at initial examination, especially in elderly patients, male patients, patients undergoing corticosteroid therapy, patients with autoimmune disease, and in inpatients. The mixing procedure may be a critical factor for the indeterminate results in QFT-plus. In the future, education should be designed with this issue in mind.

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## Author contributions

**Yuki Osakabe:** Data curation; Formal analysis; Investigation; Methodology; Project administration; Writing – original draft.

**Fumihiro Yamaguchi:** Conceptualization; Formal analysis; Investigation; Writing – review & editing.

**Ayako Suzuki:** Conceptualization; Data curation; Resources; Software; Visualization.

**Haruka Kitano:** Data curation; Investigation.

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**Shohei Shimizu:** Data curation; Investigation.

**Akira Fujishima:** Data curation; Investigation.

**Yusuke Sikama:** Funding acquisition; Supervision; Writing – review & editing.

## Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Statement of Ethics

The study protocol was approved by the Institutional Ethics Committee of Showa University (approval no. F2020C104). The requirement for obtaining informed consent from patients was waived because of the retrospective nature of this study.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Supplemental material

Supplemental material for this article is available online.

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