QuantiFERON-TB® Gold Plus as a potential tuberculosis treatment monitoring tool

To the Editor:

Novel approaches to controlling tuberculosis (TB) are required, especially in light of the growing number of multidrug-resistant pathogens [1]. The results of sputum smears and culture are often insensitive or slow to detect treatment failure and acquired drug resistance. The lack of biomarker assays for treatment monitoring is listed among the main requirements for next generation assays, as identified among the global TB community [2].

Current US Food and Drug Administration-approved interferon-γ release assays (IGRAs) indirectly detect TB infection, and are based on interferon-γ (IFNγ) responses to $CD4^+$ T-cell stimulating antigens. These antigens cannot distinguish between latent and active TB, despite a significant difference in TB burden, and show inconsistent responses to active and latent TB treatment. Although several studies have shown a general decline in IFNγ values, many have demonstrated that these values never go below the positive threshold, and in some studies, these values show a paradoxical increase [3]. Therefore, current IGRAs are not recommended to determine treatment efficacy or for treatment monitoring.

The new generation IGRA, QuantiFERON-TB Gold Plus (QFT-Plus) contains novel CD8 specific antigens in a second antigen tube (QFT-Plus Tube 2, TB2) designed specifically to stimulate both $CD8^+$ T-cells and $CD4^+$ T-cells. This complements the first antigen tube (QFT-Plus Tube 1, TB1), which contains ESAT-6 and CFP-10 peptides that target cell-mediated immune responses from $CD4^+$ T-helper lymphocytes [4, 5]. Prior studies using flow cytometry have demonstrated increased differential activity of $CD8^+$ T-cells in active TB, and a functional decline in $CD8^+$ T-cell activity that correlates with bacterial clearance during treatment [6–8]. To test the hypothesis that QFT-Plus can be a potential treatment monitoring tool and surrogate marker assay for TB specific $CD8^+$ T-cell responses, patients with microbiologically confirmed TB were tested at three time points during treatment, and evaluated for CD4 and CD8 antigen IFNγ responses.

Patients with pulmonary TB were enrolled prospectively from the National Hospital Organization Hokkaido Medical Center in Sapporo, Japan. Five patients had concurrent pleuritis. The present study included adult patients (aged ⩾18 years) with TB confirmed by culture, who received less than 12 days (average 5.4 days) of internationally recommended anti-TB drugs.

Patients on immunosuppressive therapy were excluded. Bacille Calmette–Guérin vaccination status, and demographic, clinical, and microbiological data (including drug resistance) were collected, as well as other clinical information throughout treatment. Patients were tested with QFT-Plus at 0, 3, and 6 months from the initiation of standard anti-TB treatment. Whole blood samples were processed and the results were interpreted according to the manufacturer's instructions. IFNγ values were quantified by ELISA and converted to international units per millilitre ($IU·mL^{-1}$), using a standard curve constructed from the QFT-Plus analysis software. Test results were interpreted according to the manufacturer's criteria [5]. Positivity of a single antigen tube (TB1 or TB2) defined a positive result. Quantitative values of TB1 and TB2 were analysed separately. To evaluate the $CD8^+$ T-cell response, the difference in quantitative values between TB2 and TB1 was evaluated (TB2-TB1). The $CD4^+$ T-cell response was represented by TB1 quantitative values. The data were analysed using non-parametric statistical analysis and Dunn's multiple comparison tests. Ethical approval was obtained at our institution and written informed consent was obtained from all study participants.

38 patients with confirmed TB were enrolled from October 2013 to January 2015. 36 patients or 95% of the cohort were QFT-Plus positive. 22 were male and 16 were female, with a mean±SD age of 66±19.3 years (29–94 years). One patient exhibited resistance to streptomycin, and another, resistance to ethambutol; however, no patient showed multidrug-resistant disease. All patients had received 6–9 months of treatment and showed clinical improvement during treatment, based on microbiological conversion, chest radiographs, and symptomatic improvement. No patient showed treatment failure and all were considered cured. Refer to table 1 for IFNγ quantitative and qualitative results (IU·mL^{-1}). TB1 values at 0, 3 and 6 months were 6.40±8.92, 2.56±3.28 and 2.33±3.06, respectively. Significant differences (p<0.05) in quantitative values were noted from initiation of treatment to 3 months, but not between 3 and 6 months. Quantitative values for TB2 were 8.98±16.25, 4.50±7.53 and 3.23±4.95 at 0, 3 and 6 months, respectively. A significant decline was observed between 0 and 3 months, with a nonsignificant decline in the latter half of treatment. Results from the difference between TB2 and TB1 as the surrogate for an isolated CD8 response were as follows: 2.58±8.45, 1.93±5.12 and 0.91±2.85 at 0, 3 and 6 months, respectively, with a nonsignificant decline between 0 and 3 months, and a significant decline between 3 and 6 months. Significant overall decline from the initiation of treatment to 6 months was observed in both antigen tubes and the surrogate CD8 response. Qualitative change from a positive to negative result during treatment occurred in nine out of 35 (26%), five out of 36 (14%) and five out of 36 (14%) positive results for TB1, TB2, and TB1 or TB2, respectively.

The IFNγ values of TB2 compared to those of TB1 were higher at all time points, indicating the presence of CD8 responses throughout treatment of active TB. Consistent with the results of the recent study reported by BARCELLINI et al. [9], higher quantitative values were observed in TB2 compared to TB1 at diagnosis (31 out of 38) (82%). In addition, this trend continued through month 3 of treatment (32 out of 38) (84%), and declined at 6 months (26 out of 38) (68%). The largest and most statistically significant decreases of IFNγ responses observed during treatment of active TB in both TB1 and TB2 antigen tubes (p<0.05) occurred during the first 3 months of treatment, but was not significant in the latter half, in contrast to the significant decline of the surrogate CD8 response during the latter half of treatment. This finding suggests that both CD4$^+$ and CD8$^+$ T-cell activity declines during early effective treatment, correlating with greater bacterial clearance during the first 3 months. However, further containment of infection is more effectively measured by CD8 responses thereafter. The lack of a substantial decline in CD4 antigen responses of TB1 in the latter half of successful treatment might be due to the different role of CD4$^+$ T-cells in the maintenance of cellular immunity, similar to that in latent TB infection (LTBI) during which the bacterial burden is limited [10].

The proportion of subjects with a qualitative test reversion (positive to negative QFT-Plus result) was less than 15% during successful TB treatment, and points to a need for cautious use of test reversion as a marker of efficacy. The lack of reversion evidently did not correlate with the other patients who were successfully cured. The smaller proportion of reversion in TB2 (14%) is not surprising, because TB2 contains both CD4 and CD8 antigens and the responses observed represent T-cell activity throughout treatment. The maintenance of cellular immunity by CD4$^+$ T-cells during active and latent TB infection might partially explain the low qualitative reversion rates observed both in the present and previous studies, making prior IGRAs unreliable biomarker tests for treatment monitoring and efficacy [3].

The findings of the present study are consistent with those of flow cytometry and ELISPOT biomarker studies, and show that the CD8$^+$ T-cell response declines with anti-TB treatment [7]. Furthermore, the present study provides the first evidence for QFT-Plus as a potential monitoring aid during treatment. Limitations of the study include the small size of the cohort, leading to a lack of comparison between patients

TABLE 1 Interferon-γ test quantitative and qualitative results

	0 months	3 months	6 months
Quantitative data			
TB1 (surrogate CD4$^+$ T-cell response) IU·mL^{-1}	6.40±8.92	2.56±3.28*	2.33±3.06
TB2 (CD4 and CD8 response) IU·mL^{-1}	8.98±16.25	4.50±7.53*	3.23±4.95
TB2-TB1 (surrogate CD8$^+$ T-cell response) IU·mL^{-1}	2.58±8.45	1.93±5.12	0.91±2.85*
Qualitative data			
TB1 positive/negative/indeterminate	35/2/1	31/7/0	26/11/1
TB2 positive/negative/indeterminate	36/1/1	32/6/0	32/5/1
TB1 or TB2 positive/negative/indeterminate	36/1/1	32/6/0	32/5/1

Quantitative data are presented as mean±SD. TB1: QFT-Plus Tube 1; TB2: QFT-Plus Tube 2. *: p<0.05 compared to prior value.

with and without treatment failure, and absence of culture conversion. Additionally, the impact of treatment duration on the results during the first 2 weeks of treatment is unknown. Further studies are needed to corroborate the present results on a larger scale and characterise which patients would benefit most.

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