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Serial anti-tuberculous immune responses during the follow-up of patients with tuberculous pleurisy

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Abstract

Little is known about the decay kinetics of interferon (IFN)-γ response and its influencing factors in tuberculous pleurisy. We enrolled thirty-two patients with tuberculous pleurisy prospectively and followed up at month 0, 6, and 9, at which time peripheral venous blood was drawn for interferon gamma release assay (IGRA) by means of QuantiFERON-TB Gold In-Tube (QFT-GIT). Demographic and clinical data were captured. To identify significant predictive factors influencing the IFN-γ response, multiple linear regression analyses were performed. Percentage of CD4+, CD8+, Vγ2Vδ2 T cells and Treg cells were measured by flow cytometry. The percentage of QFT-GIT-positive patients at baseline, month 6 and month 9 were 96.9% (30/32), 90.6% (29/32) and 84.4% (27/32), respectively. Quantitative IFN-γ response at baseline were significantly correlated with symptom duration ($P = .003$, $R^2 = 0.261$) and age ($P = .041$, $R^2 = 0.132$). Besides, the decreases of the IFN-γ response at month 6 and month 9 were positively correlated with the IFN-γ level at baseline. The dynamic tendency of the percentages of Treg cells was similar to the IFN-γ responses at each time-point. Quantitative IFN-γ response could be influenced by host immune status, instead of disease burden and anti-tuberculosis treatment. IGRA is probably not a useful biomarker of treatment efficacy in tuberculous pleurisy.

Abbreviations: ADA = adenosine deaminase, BCG = Bacillus Calmette-Guerin, DOT = directly observed treatment, ELISA = enzyme-linked immunosorbent assay, EPTB = extrapulmonary TB, FBS = fetal bovine serum, HIV = human immunodeficiency virus, ICS = intracellular cytokine staining, IFN = interferon, IGRA = interferon gamma release assay, IQR = interquartile range, MTB = *Mycobacterium tuberculosis*, OD = optical density, PBMCs = peripheral blood mononuclear cells, PBS = phosphate-buffered saline, QFT-GIT = QuantiFERON-TB Gold In-Tube, RBC = red blood cell, SD = standard deviation, TB = tuberculosis.

Keywords: biomarker, interferon gamma release assay, serial testing, tuberculosis, tuberculous pleurisy

1. Introduction

The interferon gamma release assay (IGRA) has been used as one of the representative tests to diagnose tuberculosis (TB) infection.[1,2] The cellular response to *Mycobacterium tuberculosis* (MTB) is assessed by measuring the level of interferon gamma (IFN-γ) released from peripheral blood lymphocytes after stimulation with MTB-specific antigens. Preclinical studies have shown a relationship between the bacillary load and the magnitude of MTB antigen-specific IFN-γ responses,[3] which led to a postulation that a decrease in the magnitude of antigen-specific IFN-γ responses measured by IGRA can be used as a biomarker of treatment success.

Editor: Babak Abdinia.

BYZ and ZMY contributed equally to this work.

Written informed consent was obtained from all the participants.

This study was conducted according to the recommendations described by the Declaration of Helsinki and was approved with written consent by the Ethics Committee of Huashan Hospital, Fudan University with the approval number of 2011-247.

The present study was partly supported by the National Natural Science Foundation of China (No. 81501359 and No. 81671553), Wuxi Science and Technology Development Fund Project (CSE31N1713) and Wuxi Health and Family Planning Committee Youth Project (Q201765).

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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How to cite this article: Zhang By, Yu Zm, Yang Ql, Liu Qq, Chen Hx, Wu J, Wang S, Shao Ly, Weng Xh, Ou Qf, Gao Y, Zhang Wh. Serial anti-tuberculous immune responses during the follow-up of patients with tuberculous pleurisy. Medicine 2020;99:2(e18367).

Received: 20 May 2019 / Received in final form: 3 November 2019 / Accepted: 13 November 2019

http://dx.doi.org/10.1097/MD.0000000000018367

A systematic review had been conducted to evaluate the kinetics of IGRA responses during the treatment of TB, and concluded that due to large degree of variation of IGRA responses in each individual that IGRA was unlikely to be useful for monitoring anti-tuberculosis treatment in clinical practice.[4] However, little is known about its role on monitoring extrapulmonary TB (EPTB). Tuberculous pleurisy is one of the most common forms of EPTB in developing countries.[5] Thus, we studied the anti-tuberculous immune responses during the course of treatment in tuberculous pleurisy to explore the factors influencing the fluctuation of IFN-γ response.

2. Materials and methods

2.1. Study population

A total of 50 patients with tuberculous pleural effusion from Wuxi Infectious Diseases Hospital were enrolled prospectively between January 2011 and December 2011. Thirty-two patients were enrolled within 2 weeks of treatment commencement and successfully followed up at month 0, 6, and 9, at which time peripheral venous blood was drawn for IGRA and Immunofluorescence staining. Data on patient demographics, comorbidities, Bacillus Calmette-Guerin (BCG) scar, human immunodeficiency virus (HIV) serology, bacteriological status and radiological and histopathological findings, biochemical examinations of pleural fluids were captured. Individuals were excluded if they had been diagnosed with chronic hepatitis B virus or hepatitis C virus infection, or had received immune modulator treatments.

2.2. Diagnosis

Patients were diagnosed with tuberculous pleurisy as either

1) positive results for a TB culture or acid-fast bacilli from the sputum or pleural fluid;
2) histological analysis of the pleural tissue showing granulomatous inflammation with or without caseous necrosis or multinucleated giant cells; or
3) pleural effusion comprising lymphocyte-dominant exudates with adenosine deaminase concentration >40 U/L or positive IGRA and a positive treatment response to 9-month anti-tuberculosis therapy, and no alternative diagnosis for the effusion other than TB was appropriate.[6]

2.3. IGRA

QuantiFERON-TB Gold In-Tube (QFT-GIT) test was performed according to the manufacturer's instructions (QFT-GIT, Cellestis Ltd., Carnegie, Australia). Briefly, a 3 mL of whole blood was collected from each participant and aliquoted into three tubes (TB-specific antigen, mitogen and nil tubes, respectively). The samples were incubated at 37°C in a humidified 5% CO_2 incubator for 24 hours. The tubes were centrifuged at 3000 rcf for 10 minutes on the second day, and the supernatant was collected and stored at 4°C until the IFN-γ assay was performed using an enzyme-linked immunosorbent assay (ELISA). The optical density (OD) of each test was read using a 450 nm filter with a 620 nm reference filter using the ELISA plate reader. The results were interpreted as positive, negative or indeterminate using the QFT-GIT analysis software developed by the company (QFT-GIT, Cellestis Ltd., Carnegie, Australia). If IFN-γ secretion in response to TB antigen was ≥0.35 IU/mL after subtracting the nil control, the sample was considered positive. If the value was <0.35 IU/mL, it was considered negative. If the negativity was associated with a poor phytohemagglutinin response (i.e., IFN-γ secretion in response to mitogen was <0.5 IU/mL), it was considered an indeterminate or invalid result for QFT-GIT. Subjects with IFN-γ secretion >8.0 IU/mL in the nil control samples were also considered indeterminate for QFT-GIT.

2.4. Immunofluorescence staining and flow cytometric analysis

Peripheral blood mononuclear cells (PBMCs) were isolated and analyzed by phenotyping and intracellular cytokine staining (ICS) at the biocontainment laboratory. Cells were isolated from heparin-anticoagulated blood by density gradient sedimentation using Lympholyte-H (Cedarlane Laboratories Ltd, Ontario, Canada). For cell-surface staining, 100 μL of anticoagulated blood was treated with red blood cell (RBC) lysis buffer and washed twice with 5% fetal bovine serum (FBS)-phosphate-buffered saline (PBS) prior to staining. PBMCs were stained with up to 5 Abs (conjugated to FITC, PE, allophycocyanin, pacific blue, and PE-Cy7) for at least 10 minutes at room temperature. After staining, the cells were fixed with 2% formaldehyde-PBS prior to analysis on a BD FACS Aria flow cytometer (BD Bioscience, San Diego, CA). Lymphocytes were gated based on forward-scatter and side-scatter properties; at least 20,000 gated events were analyzed using the Flowjo 7.6.2 Software (Stanford University, Stanford, CA). The following mouse anti-human mAbs were used: Vγ2 (7A5) and Vδ2 (15D) (Thermo Scientific, Rockford, MD); CD3 (SP34, SP34–2), CD8 (RPA-T8), CD25 (M-A251) and CD4 (OKT4) (BD Bioscience, San Diego, CA). The secondary Ab (PE-conjugated goat anti-mouse IgG; Beckman Coulter, Marseille, France) was used for indirect staining.[7] Intracellular staining for Foxp3 protein was performed by using fixation and permeabilization buffers provided by the Foxp3 kit (eBioscience, San Diego, CA) according to the manufacturer's instructions.

2.5. Statistical analysis

Categorical variables were presented as percentages and were compared using the Chi-squared or Fisher exact tests when appropriate. Continuous variables were summarized as mean and standard deviation (SD) or median and interquartile range (IQR), and the Mann–Whitney *U* test was used to evaluate the group differences. Relationships between study variables were analyzed using Pearson linear correlation. To identify significant predictive factors that influenced the IFN-γ responses, multiple linear regression analysis was performed. SPSS 20.0 (IBMcorp, Chicago, IL) and GraphPad Prism software (version 6.02; GraphPad Software, Inc.) were used to analyze the data and create the artwork. *P* values less than .05 were considered statistically significant.

3. Results

3.1. Study population

During the study period, a total of 32 subjects were enrolled. Of these, 3 were pleural effusion culture-positive for MTB complex, 13 were diagnosed with thoracoscope. Twenty-one had concur-

Table 1

Characteristics of enrolled subjects at baseline.

Factors	N=32
Age, years (mean±SD)	44.0±19.7
Male	25 (78.1%)
Symptom duration, days (median, IQR)	11 (7–37.5)
Clinical features	
Fever	13(40.6%)
Cough	29 (90.6%)
Chest pain	18 (56.2%)
Chest distress	12 (37.5)
Diabetes mellitus	2 (6.3%)
Malignancy	1 (3.1%)
Liver cirrhosis	1 (3.1%)
Unilateral pleural fluid	31 (96.9%)
Concurrent pulmonary tuberculosis	21 (65.6%)
Positive acid-fast bacilli in sputum	8 (25%)
Positive culture of MTB in sputum	6 (18.8%)
Positive culture of MTB in pleural effusion	3 (9.4%)
Diagnosis by thoracoscope	13 (40.6%)
Anti-TB treatment regimen	
HRZE	18
HRE	6
HRtEL	3
Others	5
Blood tests	
WBC count, $\times 10^9$ cells/mL (mean±SD)	6.6±1.8
Neutrophil percentage, (mean±SD)	67.3±8.5
Lymphocyte percentage, (mean±SD)	25.6±9.4
Erythrocyte sedimentation rate (ESR), mm/h, (mean±SD)	44.5±24.0
Pleural effusion analysis	
Lactate dehydrogenase (LDH), U/L, (median, IQR)	580.9 (355.6–760.9)
Adenosine deaminase (ADA), U/L, (median, IQR)	29.8 (26.4–35.0)
Total protein, g/L, (median, IQR)	44.6 (44.1–49.7)

E=ethambutol, H=isoniazid, IQR=interquartile range, L=levofloxacin, MTB=*Mycobacterium tuberculosis*, R=rifampicin, Rt=rifapentine, SD=standard deviation, Z=pyrazinamide.

rent pulmonary tuberculosis. All patients received directly observed treatment (DOT) and completed the treatment. At month 9, only one patient was not resolved with positive sputum smear and culture for MTB. At the time of commencement of anti-tuberculosis treatment, all patients received 30 mg prednisone to suppress the immune responses. The characteristics of the patients at baseline are shown in Table 1.

3.2. Dynamic changes of IGRA at each time point

Of the 32 patients enrolled, only one had negative IGRA result at baseline. The percentage of QFT-GIT-positive patients at baseline, month 6 and month 9 were 96.9% (30/32), 90.6% (29/32) and 84.4% (27/32), respectively, which were not significantly different (P=.094, P=.156, respectively, for comparisons with each time point relative to the baseline).

Patients had an IFN-γ median response at baseline and month 6 of 3.97 vs 1.65 IU/ml (P<.001) and a median decrease of −1.48 (−0.39 to −3.12). At month 9, the IFN-γ median response significantly increased to 3.91 IU/ml (P=.040, for comparison to month 6) and showed a median increase of 0.99 (−0.34 to 5.92). The changes were similar to those between baseline and month 9 (P=.928) (Fig. 1).

3.3. A relationship between clinical characteristics and IFN-γ response of patients at different time points

As shown in Figure 2, quantitative IFN-γ response at baseline were negatively correlated with symptom duration (P=.003, R^2=0.261) and age (P=.041, R^2=0.132), but not with white blood cell counts and adenosine deaminase (ADA) in plasma, nor with the total cell count, protein or ADA in pleural fluid. After multiple regression analysis, the inter-relationship between IFN-γ response with symptom duration (B=−0.98, P<.001) and age (B=0.98, P<.001) remained, indicating that IFN-γ response were influenced by disease course and immune condition of the patients (Table 2).

We further investigated the relationship between different factors and changes of IFN-γ responses. Both changes of IFN-γ responses at month 6 and month 9 were correlated with IFN-γ response at baseline (Fig. 3). The stronger the IFN-γ responses at baseline were, the higher the decreases of the IFN-γ response at month 6 and month 9 were.

3.4. The changes of different T cell subsets at each time point

Since IFN-γ responses were closely correlated to the host immune status, we further investigated the distribution of T cell subsets at different time points. PBMCs of 17 patients at baseline, 16 patients at month 6 and 5 patients at month 9 were

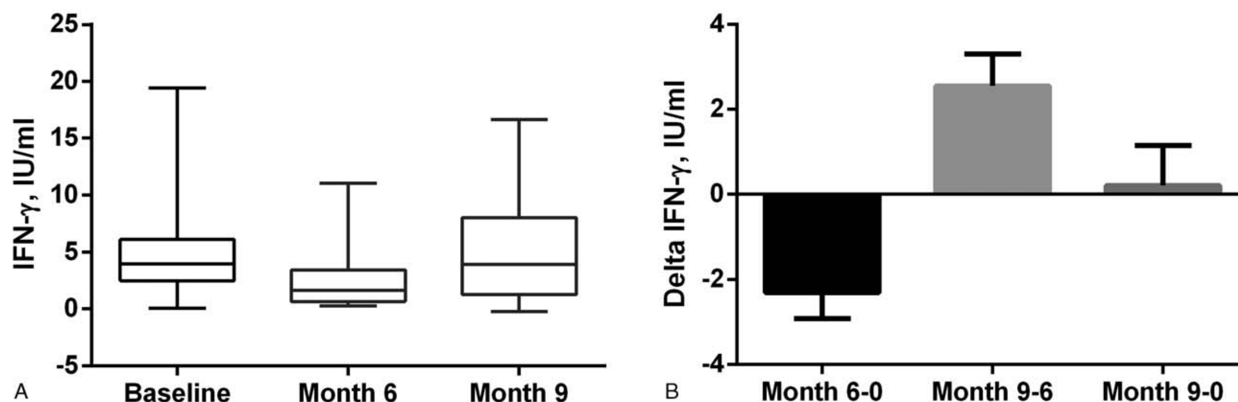

Figure 1. Longitudinal interferon gamma (IFN-γ) responses at different time-points. (A) The mean IFN-γ levels (antigen minus nil tube) (IU/mL) measured by the QuantiFERON-TB Gold In-tube (QFT-IT) assay were 3.97, 1.65 and 3.91 IU/mL at baseline, month 6 and month 9, respectively. These levels declined significantly from baseline to month 6 (P<.001), but not from baseline to month 9 (P=.928). (B) A mean decrease of IFN-γ levels from month 6 to baseline was −2.31, a mean increase of 2.55 from month 9 to month 6.

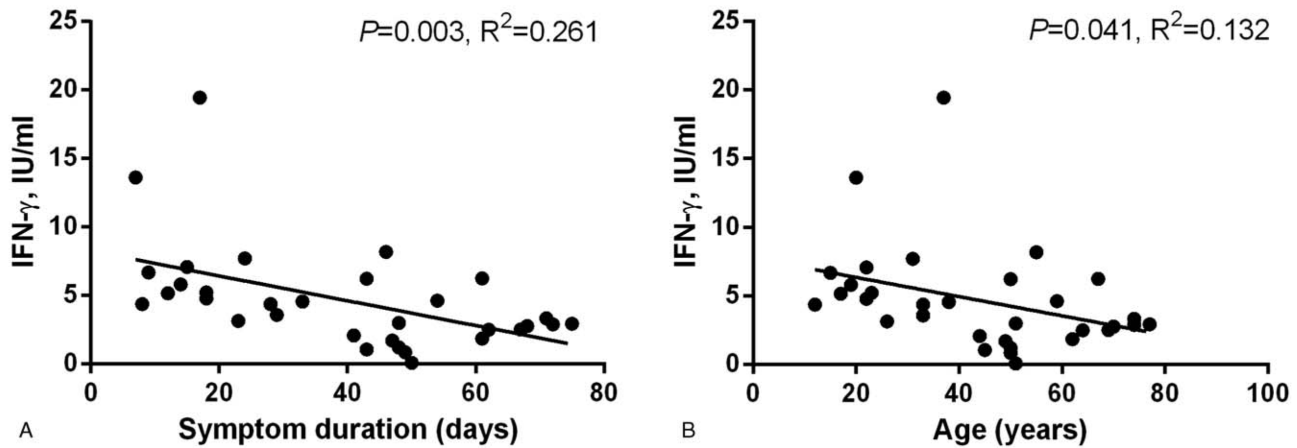

Figure 2. Inverse relationship between quantitative IFN-γ response and symptom duration or age in multiple linear regression analysis (A symptom duration, B age). Quantitative IFN-γ response at baseline were significantly negatively correlated with symptom duration ($P=.003$) (A) and age ($P=.041$) (B).

Table 2

Relationship between symptom duration and age according to multiple linear regression analysis.

Variables	Partial regression coefficient	SE	Standardized partial regression coefficient	*t*	*P*
Constant	0.08	0.42		0.20	.84
Symptom duration	−0.98	0.03	−5.24	−29.62	<.001
Age	0.98	0.04	4.78	27.01	<.001

SE=standardized error.

successfully isolated. Whole blood cell surface staining, intracellular cell staining and flow cytometry were performed to detect the percentage of peripheral blood CD3+CD4+ T cells, CD3+CD8+ T cells, Vγ2Vδ2 T cells and Treg (CD4+CD25high+Foxp3+) cells. Percentages of Foxp3+ Treg cells at baseline decreased significantly at month 6 (11% vs 5.5%, $P=.048$). Compared with the values at month 6, the percentages of Treg cells increased at month 9 (5.5% vs 54.9%, $P=.002$). This dynamic tendency was similar to that of the IFN-γ responses at each time point. There was no difference among different time points in CD3+CD4+ T cells, CD3+CD8+ T cells and Vγ2Vδ2 T cell subsets (Fig. 4 and Supplementary Figure 1, http://links.lww.com/MD/D558).

4. Discussion

To our knowledge, our research is one of few studies evaluating serial IGRA as a potential tool to monitor treatment in patients with active tuberculous pleurisy. This is also one of few studies to address factors that influenced quantitative changes of IFN-γ responses. Furthermore, our study also investigated the different T lymphocytes subsets at different time points during anti-tuberculosis treatment, so that we could understand the underlying mechanisms of dynamic changes of IFN-γ responses.

We confirmed that the value of QFT-GIT was associated with symptom duration and age by multiple linear regression analysis. This reflected that dynamic changes of quantitative IFN-γ responses was similar to the changes of Treg cells, indicating that

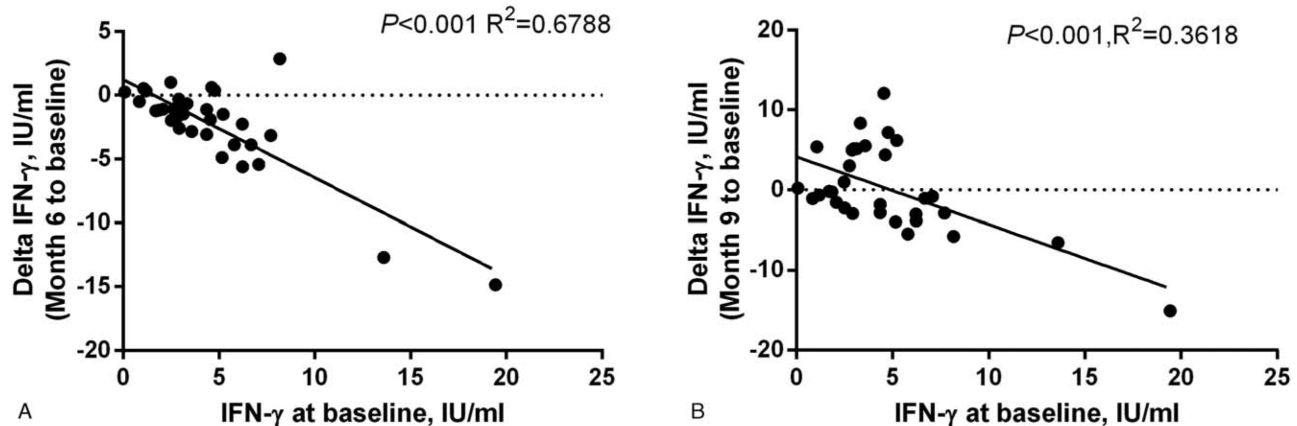

Figure 3. Relationship between quantitative IFN-γ response at baseline and changes of IFN-γ responses at different time points in multiple linear regression analysis (A: Month 6 to baseline; B: Month 9 to baseline). (A). Changes of IFN-γ responses from month 6 to baseline were negatively correlated with IFN-γ response at baseline ($P<.001$). (B) Changes of IFN-γ responses from month 9 to baseline were also negatively correlated with IFN-γ response at baseline ($P<.001$).

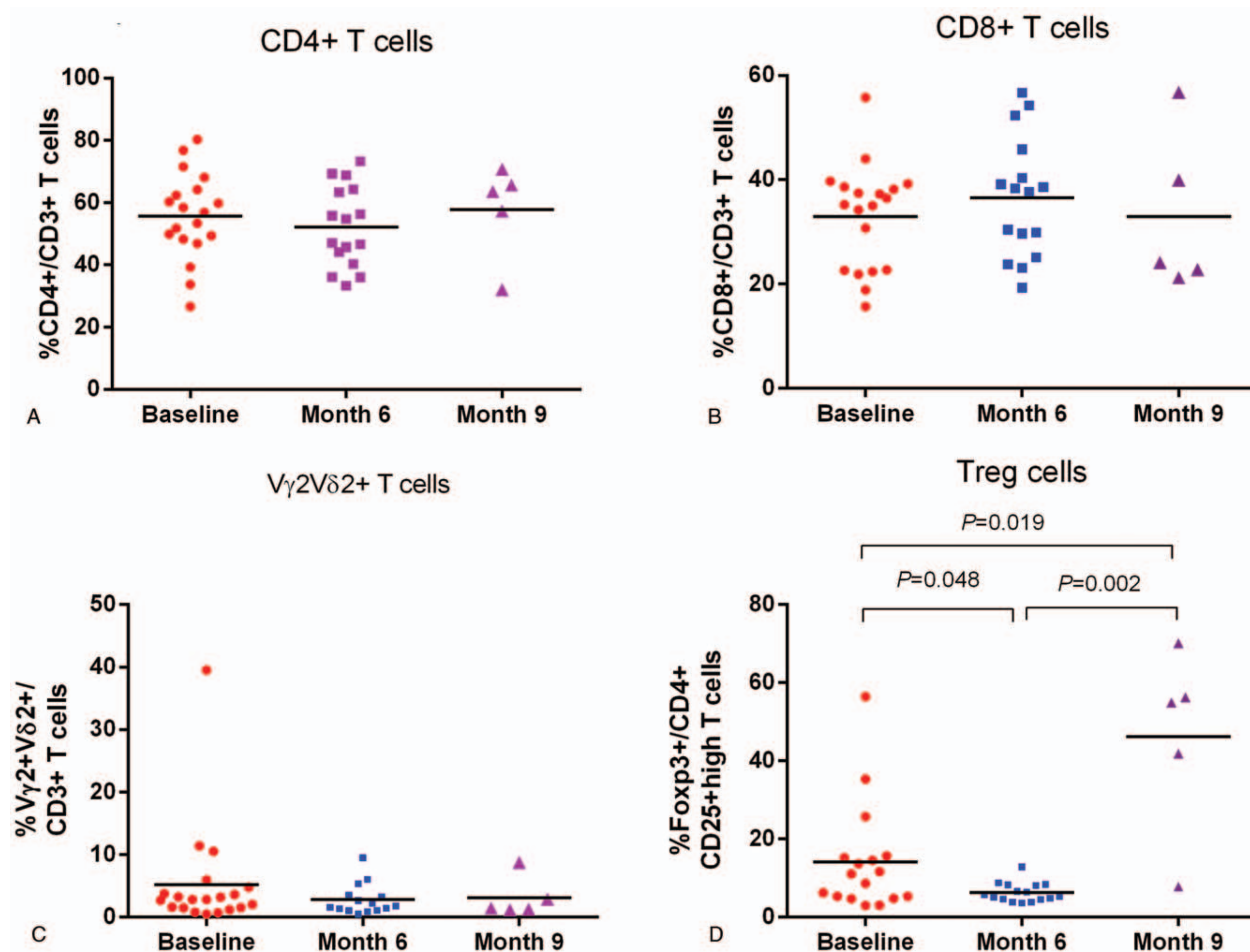

Figure 4. T cell subset distributions in participants at different time points. The short transverse lines represent mean. Treg, regulatory T cells. The percentage of CD3+CD4+ T cells (A), CD3+CD8+ T cells (B) and Vγ2Vδ2 T cells (C) showed no significant difference at baseline, month 6 and month 9. But the percentages of Foxp3+ Treg cells at baseline decreased significantly at month 6 (11% vs 5.5%, $P=.048$). Compared with values at month 6, the percentages of Treg cells increased at month 9 (5.5% vs 54.9%, $P=.002$). The short transverse lines represent mean.

quantitative IFN-γ responses reflected host immunological changes rather than immunomodulatory effects of anti-tuberculous drugs as well as disease burden.[8]

Our result showed that 96.9% (30/32) patients were positive in IGRA at baseline, which was a little higher than previously reported results.[9–13] This could be a result of high prevalence of tuberculosis infection in China.[14] Quantitative value of IFN-γ responses declined at month 6 after treatment, which was consistent with previously results,[4] however, it increased at month 9. This could in part due to the use of corticosteroids. Corticosteroids were prescribed routinely to control the inflammatory reaction in patients with tuberculous pleurisy for at least 2 weeks and tapered gradually with low dose of prednisone for a month. The use of corticosteroids could last as long as 3 months, which resulted in a significant negative influence on IFN-γ release.[8,15,16]

Substantial studies had engaged in the investigation to evaluate whether MTB antigen-specific quantitative IFN-γ releases could be a biomarker to monitor the anti-tuberculosis treatment. However, the results were contradictory with each other. And recently, Vanessa Clifford et al conducted a meta-analysis to elucidate this issue. They concluded that quantitative IFN-γ responses generally fall during treatment for TB, and the large degree of variation in results between individual in each study means that IGRAs are unlikely to be useful for monitoring anti-tuberculosis treatment in clinical practice for any individual patient.[4] Besides, our results also showed that although almost all the patients were cured clinically, most of them remained positive at the end of treatment, which added the leverage that IGRA is not suitable to monitor the treatment.

In this case, our study aimed to see the variables which could related to the IFN-γ responses. Our results showed that age had a significant effect on IFN-γ response, consistent with the observation that mean IFN-γ concentration increased with age.[13] Besides, IFN-γ decreased with the disease course, indicating that chronic course of the disease resulted in the decay of IFN-γ. Ifedayo et al conducted a clinical trial and observed a significant decline in qualitative and quantitative IGRA results with time in latent tuberculosis infection which was not influenced by isoniazid therapy. Quantitative IFN-γ response decline spontaneously with time, therefore the reversion seemed to be caused by natural decline of T cell response.[17]

Previous study had noticed that reversion were slightly more frequent in those with an initial result close to the cut-off

point,[18] indicating that baseline IFN-γ responses somehow influence the fluctuation of IFN-γ responses. We noticed that the stronger the IFN-γ responses at baseline were, the stronger the decay of IFN-γ responses were during follow-up. Previous reports noticed that the increase in IFN-γ response at baseline and early conversion of the IGRA response were an important predictive marker for recurrence.[10,11] It was postulated that inhibitory immune factors that induce T cell energy, including Treg cells, may have played a role in regulating IFN-γ release. Besides, it has been accepted that immunosuppression plays a role in the pathogenesis of tuberculosis. Previous studies confirmed that CD4+CD25highFoxp3+ T cells were significantly increased in TB patients.[19,20] We observed that the change of the proportion of CD4+CD25highFoxp3+T cells was in accordance with the change of IFN-γ response, providing indirect evidence that quantitative IFN-γ response is influenced by the immunosuppressive factors.

There are several limitations of this study, including the small number of patients and the fact that QFT-GIT was not the most suitable test for measuring IFN-γ. However, there was still sufficient data to show interesting information on the effects of anti-TB treatment, and data related to its impact on serial IGRA responses were provided. This study further sheds light on the impact of the immunosuppressive factors on IFN-γ responses.

5. Conclusion

This study showed that the patterns of IGRA responses during treatment in tuberculous pleurisy. Quantitative IFN-γ response was correlated with patient's age and symptom duration. Fluctuation of IFN-γ response during follow up was influenced by baseline IFN-γ responses. This dynamic tendency was similar between the proportion of CD4+CD25highFoxp3+ T cells and the IFN-γ responses at each time-point, indicating that quantitative IFN-γ response was influenced by host immune status rather than disease burden and anti-tuberculosis treatment. Nevertheless, a variety of factors that could potentially suppress the IGRA response should be investigated further and the test performance of the IGRA during treatment should be improved in future studies.

Acknowledgment

We would like to thank all the patients that were involved in this study and the staff of Huashan Hospital, Fudan University and Wuxi Infectious Diseases Hospital.

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References

[1] Diel R, Loddenkemper R, Nienhaus A. Evidence-based comparison of commercial interferon-gamma release assays for detecting active TB: a metaanalysis. Chest 2010;137:952–68.
[2] Lalvani A, Pathan AA, McShane H, et al. Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. Am J Respir Crit Care Med 2001;163:824–8.
[3] Pathan AA, Wilkinson KA, Klenerman P, et al. Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in Mycobacterium tuberculosis-infected individuals: associations with clinical disease state and effect of treatment. J Immunol 2001;167:5217–25.
[4] Clifford V, He Y, Zufferey C, et al. Interferon gamma release assays for monitoring the response to treatment for tuberculosis: a systematic review. Tuberculosis (Edinb) 2015;95:639–50.
[5] Udwadia ZF, Sen T. Pleural tuberculosis: an update. Curr Opin Pulm Med 2010;16:399–406.
[6] Ates G, Yildiz T, Ortakoylu MG, et al. Adapted T cell interferon-gamma release assay for the diagnosis of pleural tuberculosis. Respiration 2011;82:351–7.
[7] Gao Y, Zhang S, Ou Q, et al. Characterization of CD4/CD8+ alphabeta and Vgamma2Vdelta2+ T cells in HIV-negative individuals with different Mycobacterium tuberculosis infection statuses. Hum Immunol 2015;76:801–7.
[8] Clifford V, Zufferey C, Germano S, et al. The impact of anti-tuberculous antibiotics and corticosteroids on cytokine production in QuantiFERON-TB Gold In Tube assays. Tuberculosis (Edinb) 2015;95:343–9.
[9] Denkinger CM, Pai M, Patel M, et al. Gamma interferon release assay for monitoring of treatment response for active tuberculosis: an explosion in the spaghetti factory. J Clin Microbiol 2013;51:607–10.
[10] Hang NT, Matsushita I, Shimbo T, et al. Association between tuberculosis recurrence and interferon-gamma response during treatment. J Infect 2014;69:616–26.
[11] Kaneko Y, Nakayama K, Kinoshita A, et al. Relation between recurrence of tuberculosis and transitional changes in IFN-gamma release assays. Am J Respir Crit Care Med 2015;191:480–3.
[12] Adetifa IM, Ota MO, Walther B, et al. Decay kinetics of an interferon gamma release assay with anti-tuberculosis therapy in newly diagnosed tuberculosis cases. PLoS One 2010;9:
[13] Bugiani M, Bonora S, Carosso A, et al. The effect of antituberculosis treatment on interferon-gamma release assay results. Monaldi Arch Chest Dis 2011;75:215–9.
[14] Gao L, Lu W, Bai L, et al. Latent tuberculosis infection in rural China: baseline results of a population-based, multicentre, prospective cohort study. Lancet Infect Dis 2015;15:310–9.
[15] Li T, Bao L, Diao N, et al. Influencial factors of the performance of interferon-gamma release assays in the diagnosis of childhood tuberculosis. Clin Exp Med 2015;15:303–9.
[16] Bao L, Li T, Diao N, et al. Fluctuating behavior and influential factors in the performance of the QuantiFERON-TB gold in-tube assay in the diagnosis of tuberculosis. PLoS One 2015;10:e0103763.
[17] Adetifa IM, Ota MO, Jeffries DJ, et al. Interferon-gamma ELISPOT as a biomarker of treatment efficacy in latent tuberculosis infection: a clinical trial. Am J Respir Crit Care Med 2013;187:439–45.
[18] Park JS, Lee JS, Kim MY, et al. Monthly follow-ups of interferon-gamma release assays among health-care workers in contact with patients with TB. Chest 2012;142:1461–8.
[19] Li L, Lao SH, Wu CY. Increased frequency of CD4(+)CD25(high) Treg cells inhibit BCG-specific induction of IFN-gamma by CD4(+) T cells from TB patients. Tuberculosis (Edinb) 2007;87:526–34.
[20] Roberts T, Beyers N, Aguirre A, et al. Immunosuppression during active tuberculosis is characterized by decreased interferon- gamma production and CD25 expression with elevated forkhead box P3, transforming growth factor- beta, and interleukin-4 mRNA levels. J Infect Dis 2007;195:870–8.