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Evaluation of the diagnostic utility of a whole blood interferon-γ assay for determining the risk of exposure to *Mycobacterium tuberculosis* in BCG-vaccinated individuals

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Abstract

We evaluated the utility of the "QuantiFERON®-TB Gold in tube" (QuantiFERON®) test that uses TB-specific antigens for the diagnosis of latent infection in such individuals. We also examined the correlation between the IFN-γ response to these antigens and the exposure risk to TB by evaluating antigen-specific IFN-γ release in comparison with IFN-γ release in response to PPD in three groups; medical students, nurses in a TB hospital, and TB patients. All nurses and TB patients responded to PPD whereas 79.2 % ($p=0.04$) and 52 % ($p<0.0001$) responded to QuantiFERON®, respectively. In the medical students, only 10.4 % responded to QuantiFERON® while 85.2 % were positive to PPD ($p < 0.0001$). There was also a significant correlation between the levels of IFN-γ production and the duration of employment in the group of nurses at the TB hospital suggesting on-going exposure in this high risk group. Thus these results demonstrate that *Mycobacterium tuberculosis*-specific IFN-γ release assay accurately discriminates low and high risk healthy subjects and might therefore be a useful diagnostic tool for the diagnosis of latent infection in BCG-vaccinated individuals.

Keywords

Tuberculosis; latent infection; IFN-γ; "QuantiFERON®-TB Gold in tube"

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Introduction

Tuberculosis (TB) remains a global public health problem with an estimated 3 million deaths and 8 million new cases yearly (Dye et al., 1999; Corbett et al., 2003). Most individuals infected with *Mycobacterium tuberculosis (M. tuberculosis)* control the bacilli and develop asymptomatic latent infection, a reservoir currently estimated to be one-third of the total human population. Latently-infected individuals face a lifetime risk of reactivation with symptomatic TB disease depending upon their immune status (American Thoracic Society, 2000; WHO, 2005). There is a dramatic increase in the risk of developing reactivation TB in HIV-coinfected individuals and in patients receiving anti-TNF antibody based therapies for, among other indications, rheumatoid arthritis (Odhiambo et al., 1999; Winthrop, 2006). As HIV infection and the use of anti-TNF antibody therapeutics have become increasingly common, there is an urgent need for more efficient ways of diagnosing latent TB (Lawn et al., 2005; Aziz et al., 2005).

The Tuberculin Skin Test (TST) has long been used as a gold standard for the diagnosis of latent tuberculosis (American Thoracic Society, 2000). The TST is a measure of a delayed-type hypersensitivity response to purified protein derivative (PPD). PPD is a mixture of mycobacterial antigens, some of which are shared between non-tuberculous mycobacteria (NTM) and *Mycobacterium bovis* BCG vaccine strains (Anderson et al., 2000; Lee et al., 2002). As a result, the TST is not adequate for the diagnosis of latent TB in populations with high BCG coverage and/or high-levels of NTM exposure (Anderson et al., 2000; Mazurek et al., 2001; Ewer et al., 2003; Pai et al., 2004). The sensitivity also may be low in individuals with decreased immune function (i.e., AIDS and other immunosuppressive conditions, advanced tuberculosis, malnutrition) (Liebeschuetz et al., 2004). To increase the specificity of such tests two *M. tuberculosis* specific antigens, Early Secretory Antigenic Target 6 (ESAT-6) and Culture Filtrate Protein 10 (CFP-10) have been employed. These proteins, encoded within the region of difference 1 (RD1) of the *M. tuberculosis* genome, are specific to *M. tuberculosis* and are not present in PPD, as they are obviously not encoded by BCG vaccine strains. In addition immunoreactive orthologs are not expressed in most NTM species (Sorensen et al., 1995; Harboe et al., 1996; Anderson et al., 2000). Several studies have reported that RD1-based (ESAT-6 and/or CFP-10) IFN-γ assays have higher specificity than TST, and are less influenced by previous BCG vaccination (Ravn et al., 1999; Arend et al., 2000; Brock et al., 2001; Mori et al., 2004; Pai et ai., 2004). Newer generation test kits using these two individual antigens have already been developed and reports of their utility are now beginning to emerge (Pai et ai., 2004; Ferrara et al., 2005; Kang et al., 2005). More recently, an enhanced and simplified QuantiFERON® assay has been developed using a mixture of overlapping peptides representing ESAT-6, CFP-10, and a portion of the TB antigen TB7.7 in order to enhance the sensitivity of the assay.

The aim of this study was to compare the usefulness of this enhanced IFN-γ based assay with PPD-stimulation for the diagnosis of latent TB infection in Korea where BCG vaccination is mandatory.

Materials and Methods

Study subjects

There were three cohorts of study participants. Group A included 48 healthy students of a medical school in Busan and was considered to be a “low-risk group”. They consisted of 32 males and 16 females (mean age 23.7 ± 0.2 years). All of them had a scar on the shoulder indicating that they had been BCG-vaccinated. Group B included 25 nurses who have been working for the past 1 to 27 years (mean 12.6 ± 1.6 years) at the National Masan TB Hospital (NMTH) in Masan, South Korea. All were female (mean age 36.5 ± 1.5 years) and one of them

did not have a BCG scar. These nurses have regular daily contact with TB patients in the hospital (ca 5 hours a day). We therefore considered this a “high-risk group”. The total length of their employment at the hospital was distributed as follows; less than 1 year : 2 persons, 1 – 5 years : 4 persons, 6 – 10 years : 3 persons, 11 – 15 years : 6 persons, 16 – 20 years : 7 persons, more than 20 years : 3 persons. Group C included 25 active pulmonary TB patients (23 males and 2 females, mean age 43.6 ± 2.5 years) who showed positive results in sputum AFB smear and culture and had TB lesions by radiological examination. They include 7 newly diagnosed, 11 relapsed, and 7 incurable cases. These patients had been admitted to NMTH and had received anti-tuberculosis drugs for less than 1 week at the time of blood collection. This study was approved by a local IRB committee at NMTH and informed written consent was obtained from each participant.

IFN-γ assays

Two methods for measuring antigen-specific IFN-γ release were used in this study. First, diluted whole blood (1/10) was incubated with PPD (Statensserum institute; Copenhagen, DK) and IFN-γ was measured from the supernatants. This in vitro whole blood IFN-γ response to PPD correlates well with TST (Black et al., 2001). Briefly, diluted whole blood was incubated with PPD at the final concentration of 10 μg/ml in a 5 % CO_2 atmosphere at 37 °C for 5 days. The supernatants were harvested and stored at − 70 °C until use for the measurement of IFN-γ by ELISA with OptEIA mAb set (BD Pharmingen, LA, California). The detection limit of the assay was 31 pg/ml and a positive response was defined as ≥ 62 pg/ml, twice the detection limit. Second, the “QuantiFERON®-TB Gold in tube” test kit (Cellestis Ltd, Victoria, Australia) was performed according to the manufacturer’s instructions. Blood was directly collected in two 1 ml heparin containing tubes. One tube contained only heparin as negative control and the second tube contained overlapping peptides representing the entire sequences of CFP-10 and ESAT-6, and another peptide representing a portion of TB7.7. The tubes were incubated for 20 – 24 hrs according to the manufacturer’s recommendation. After incubation, plasma was removed and frozen until used for ELISA. The IFN-γ values were calculated by subtracting the value of negative control and the cut-off value was 0.35 IU/ml according to the manufacturer’s instruction.

Statistical analysis

The comparison of response rates between the two assay methods in each group was analyzed using the two-sided Fisher’s exact test (GraphPad PRISM, version 4.0). The differences of IFN-γ response levels among groups were analyzed using one way analysis of variance (ANOVA) and a nonparametric Mann-Whitney tests. Statistical significance was accepted when the P value was less than 0.05.

Results

Whole blood response to PPD in BCG-vaccinated healthy subjects and TB patients

Whole blood IFN-γ production in response to PPD was measured in three cohorts of volunteers with very different risk of having latent or active tuberculosis. Group A was composed of healthy normal medical students. Both because of their age and their lack of clinical experience we considered this group to be at relatively “low-risk” for TB exposure. All of these volunteers had a BCG scar and, as expected, stimulation of their whole blood with PPD identified 85.2 % of Group A as producing significant amounts of IFN-γ in response to PPD (≥ 62 pg/ml) (Figure 1 and Table 1).

Group B was composed of 25 nurses who had been working at the National Masan TB Hospital (NMTH) in Masan, South Korea. NMTH is the largest public tertiary care facility in South Korea specifically for TB patients. These nurses have had daily contact with tuberculosis

patients for the duration of their employment (mean 12.6 ± 1.6 years, range 1 to 27) we therefore considered them to have been at high risk for exposure and latent TB. Group C consisted of 25 active pulmonary TB patients (23 males and 2 females, mean age 43.6 ± 2.5 years) who were both smear and culture positive and had radiologic and clinical evidence of disease. Stimulation of whole blood from volunteers in either of these two high-risk groups with PPD showed that all of them produced significant amounts of IFN-γ in response to PPD (≥62 pg/ml) (Figure 1 and Table 1).

A more stringent cut-off for discriminating these groups did not improve the situation as similar results were obtained using a cut-off of 300 pg/ml (100 % of both Group B and Group C responded along with 81.5 % of Group A) Although no difference was observed between Group A and Group B, the level of IFN γ detected following stimulation of the TB patient group (Group C) was significantly higher than that in medical students (Group A)($p = 0.0001$) and in nurses (Group B)($p = 0.0001$) (Figure 1).

IFN-γ assay by "QuantiFERON®" test kit in BCG-vaccinated healthy subjects and TB patients

To assess whether the *M. tuberculosis* specific antigens would discriminate between these groups more accurately we performed whole blood stimulation using the same samples and the QuantiFERON® test kit. The results of the antigen-specific IFN-γ values assessed by the QuantiFERON® test kit are shown in Figure 2 and Table 1. About 80 % of Group C were positive (≥ 0.35 IU/ml) in this assay, significantly less ($p = 0.04$) than the response rate to PPD stimulation. In nurses who have been at high risk of *M. tuberculosis* infection (Group B), the positivity rate was 52 % when tested by the in tube method compared to PPD response which was 100 % (Figure 2 and Table 1). In the low-risk group (Group A), only 10.4 % were positive. The IFN-γ levels were highest in Group C (15.5 ± 4.5 IU/ml), followed by Group B (11.7 ± 5.5 IU/ml) and Group A (1.3 ± 0.9 IU/ml). Highly significant differences were found between Group C and Group A ($p = 0.0002$) and Group B and Group A ($p = 0.01$) but no difference was observed between Group B and Group C (Figure 2).

Correlation of the duration of exposure to M. tuberculosis and the level of IFN-γ responses in high-risk group

Because the magnitude of the IFN-γ response in the NMTH nursing staff had a large range of values that overlapped Group A and Group C, we re-evaluated the magnitude of the response in terms of the length of time that each nurse had worked at the hospital. The duration of their employment at the hospital should reflect the period of their exposure to bacilli, and may be related to the degree of IFN-γ response. A significant positive correlation ($r = 0.424$, $p = 0.035$) was found using the data from the QuantiFERON® test but not the data from PPD stimulation (Figure 3).

Discussion

In this study, we were successful in discriminating latent infection from BCG vaccination in three cohorts at varying risk for TB exposure using an IFN-γ assay kit based on the *M. tuberculosis* specific antigens. TST is also likely to be a good indicator of latent infection in a population of BCG-unvaccinated subjects but is confounded by BCG vaccination (Brock et al., 2004). In the present study, we demonstrated that QuantiFERON® test kit clearly differentiated the BCG-vaccinated healthy individuals at low risk of exposure from TB patients in contrast to the results obtained using only PPD. The most recent generation of this test kit, known as "QuantiFERON®-TB Gold in tube" used in this study is the third-generation of QuantiFERON®-TB assay kit. The first-generation (QuantiFERON®-TB) is a whole-blood assay measuring IFN-γ response to PPD as the stimulating antigen, while the second generation (QuantiFERON®-TB Gold) uses *M. tuberculosis*–specific antigens such as ESAT-6 and

CFP-10 in separate tubes. The "QuantiFERON®-TB Gold in tube" assay contains a mixture of overlapping peptides representing ESAT-6, CFP-10, and a portion of TB antigen TB7.7 in one tube and has consequently been proposed to have enhanced sensitivity and specificity. Therefore, the first aim of this study was to evaluate this new test kit for the diagnosis of latent TB infection in Korea where TB is endemic and BCG vaccination is mandatory. Since there is no gold standard for the diagnosis of latent TB, we compared the results of QuantiFERON® with those in PPD response.

In this study, instead of TST, *in vitro* whole blood IFN-γ assay to PPD was performed in all subjects. It has been previously reported that in vitro whole blood IFN-γ response to PPD correlates well with TST (Black et al., 2001). We found poor agreement between QuantiFERON® and PPD tests in all groups, which is consistent with other reports (Brock et al., 2001 & 2004; Mori et al., 2004; Kang et al., 2005) and confirms that QuantiFERON® test is substantially more specific than TST in BCG vaccinated persons. Brock and co-workers (2004) showed that, in BCG-vaccinated persons, 50 % of high-exposure and 6 % of low-exposure group responded to QuantiFERON®, which gives the similar overall prevalence to those of the unvaccinated subjects, 53 % and 5 %, respectively. A more recent study by Kang et al. (2005) was performed in Korea and showed 4 %, 44 % and 81 % of positive responses to QuantiFERON® test in low risk, high risk subjects and TB patients, respectively whereas ours are 10.4 %, 52 %, and 79.2 %. The sensitivity of the assay in TB patients was similar in both studies. However, the results in healthy subjects differed. The subjects comprising the low risk group were medical students in both studies. Kang et al. (2005) mentioned that the 4 % of IFN-γ response in low risk individuals was underestimated probably because the IFN-γ assay reflects recent rather than remote TB infection. In our study, however, we obtained 10.4 % of positive rate which is twice higher but this is still low considering the predicted prevalence of TB infection (33 %) in the Korean population (Korean National Tuberculosis Association, 2005). Although the group size in that study was twice as large as ours, the higher response rate in our study suggests that the higher sensitivity of the test kit in tube type containing the mixture of 3 antigens compared to individual antigen plate used in their study. Another plausible explanation for this observation is the difference in regional prevalence rates because the medical students were recruited from different regions in the two studies. In the high risk group, we also obtained a higher response rate than that of Kang et al (2005). This may be because of the different characteristics of the cohort in our study. Although many reports indicate that BCG vaccination affects TST in diagnosis of TB infection (Anderson et al., 2000; Mazurek et al., 2001; Ewer et al., 2003; Pai et al., 2004), others report controversial data. Pai et al. (2005) and Dogra et al. (2007) showed a high concordance between TST and QuantiFERON® either in health care workers or in children in India, a highly TB endemic country, suggesting there is little influence of BCG vaccination on TST. However, the participants in their study received BCG vaccine once only at birth perhaps reflecting a waning effect of BCG vaccination (Chadha, 2001; Wang et al., 2002). Repeat vaccinations may have a more discernible and persistent effect on TST responses (Menzies et al., 2000; Floyd et al., 2002). On the contrary, in Nigeria, another high endemic country, TST responsiveness in high risk grouped children was lower than that of QuantiFERON® (49 % vs 74 %), suggesting TST could underestimate risk for infection with TB in children in areas with high incidence of infectious diseases (Nakaoka et al., 2006).

In the present study, the members of the high risk group were composed of nurses at TB hospital. They have contact with TB patients in the hospital for more than 5 h daily and their working durations were widely distributed between 1 to 27 years with an average of 12 years indicating long-term duration of exposure to *M. tuberculosis.* Therefore, we examined if there was any correlation between the time of exposure to *M. tuberculosis* and IFN-γ secretion levels in response to PPD or *M. tuberculosis*–specific antigens in the nurse group. No correlation was found when the analysis was performed with the IFN-γ responses to PPD. However, a

significant correlation was observed between two factors when using QuantiFERON®, suggesting that the frequency and intensity of exposure to *M. tuberculosis* reflect the risk factor for *M. tuberculosis* infection. This correlation could have also been due to the change of age not to exposure time. Therefore, we analyzed the correlation between the age and IFN-γ secretion levels but no correlation was found (data not shown). Our results indicate that the new "QuantiFERON®-TB Gold in tube" based on a mixture of *M. tuberculosis*–specific antigens might become a useful diagnostic tool for detecting latent TB infections particularly in BCG-vaccinated individuals in TB endemic countries.

References

American Thoracic Society. Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Pespir Crit Care Med 2000;161:S221–S247.

Anderson P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet 2000;356:1099–1104. [PubMed: 11009160]

Arend SM, Anderson P, van Meijgaarden KE, Skjot RLV, Subronto YW, van Dissel JT, Ottenhoff THM. Detection of active tuberculosis infection by T cell responses to early-secreted antigenic target 6-kDa protein and culture filtrate protein 10. J Infect Dis 2000;181:1850–1854. [PubMed: 10823800]

Aziz MA, Wright A. The World Health Organization/International Union Against Tuberculosis and Lung Disease Global Project on Surveillance for Anti-Tuberculosis Drug Resistance: a model for other infectious diseases. Clin Infect Dis 2005;15:S258–S262. [PubMed: 16032561]

Black GF, Fine PEM, Warndorff DK, Floyd S, Weir RE, Blackwell JM, Bliss L, Sichali L, Mwaungulu L, Chaguluka S, Jarman E, Ngwira B, Dockrell HM. Relationship between IFN-γ and skin test responsiveness to Mycobacterium tuberculosis PPD in healthy, non-BCG-vaccinated young adults in Northern Malawi. Inter J Tuberc Lung Dis 2001;5:664–672.

Brock I, Munk ME, Kok-Jensen A, Andersen P. Performance of whole blood IFN-γ Test for tuberculosis diagnosis based on PPD or the specific antigens ESAT-6 and CFP-10. Inter J Tuberc Lung Dis 2001;5:462–467.

Brock I, Weldingh K, Lillebaek T, Fillmann F, Andersen P. Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. Am J Respir Crit Care Med 2004;170:65–69. [PubMed: 15087297]

Chadha VK. Tuberculin test. Indian J Pediatr 2001;68:53–58. [PubMed: 11237237]

Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, Dye C. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med 2003;163:1009–1021. [PubMed: 12742798]

Dogra S, Narang P, Mendiratta DK, Chaturvedi P, Reingold AL, Colford JM, Roley LW, Pai M. Comparison of a whole blood interferon-γ assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. J Infec 2007;54:267–276. [PubMed: 16733068]

Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement: global burden of tuberculosis: estimated incidence, prevalence, and mortality by country: WHO Global Surveillance and Monitoring Project. JAMA 1999;282:677–686. [PubMed: 10517722]

Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Anderson P, Monk P, Lalvani A. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. Lancet 2003;361:1168–1173. [PubMed: 12686038]

Floyd S, Ponnighaus JM, Bliss L, Nkhosa P, Sichali L, Msiska G, Fine PE. Kinetics of delayed-type hypersensitivity to tuberculin induced by bacille Calmette-Guerin vaccination in northern Malawi. J Infect Dis 2002;186:807–814. [PubMed: 12198615]

Harboe M, Oettinger T, Wiker HG, Rosenkrands I, Anderson P. Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis* BCG. Infect Immun 1996;64:16–22. [PubMed: 8557334]

Kang YA, Lee HW, Yoon HI, Cho B, Han SK, Shim YS, Lim JJ. Discrepancy between the tuberculin skin test and the whole-blood interferon γ assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA 2005;293:2756–2761. [PubMed: 15941805]

Korean National Tuberculosis Association. Tuberculosis. [Accessibility verified May 4, 2005]. Available at http//www.knta.or.kr/korea/knowledge/knowledge09.asp

Lawn SD, Badri M, Wood R. Tuberculosis among HIV-infected patients receiving HAART: long term incidence and risk factors in a South African cohort. AIDS 2005;19:2109–2116. [PubMed: 16284460]

Lee E, Holzman RS. Evolution and current use of the tuberculin test. Clin Infec Dis 2002;34:365–370. [PubMed: 11774084]

Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet 2004;364:2196–2203. [PubMed: 15610806]

Mazurek GH, Lobuer PA, Daley CL, Bernardo J, Lardizabal AA, Bishai WR, Iademarco MF, Rothel JS. Comparison of a whole-blood interferon-γ assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection. JAMA 2001;286:1740–1747. [PubMed: 11594899]

Menzies D. What does tuberculin reactivity after bacilli Calmette-Guerin vaccination tell us ? Clin Inf Dis 2000;31:S71–S74.

Mori T, Sakatani M, Yamagishi F, Takashima T, Kawabe Y, Nagao K, Shigeto E, Harada N, Mitarai S, Okada M, Suzuki K, Inoue Y, Tsuyuguchi K, Sasaki Y, Mazurek GH, Tsuyuguchi I. Specific detection of tuberculosis infection; An interferon-γ-based assay using new antigens. Am J Respir Crit Care Med 2004;170:59–64. [PubMed: 15059788]

Nakaoka H, Lawson L, Squire SB, Coulter B, Ravn P, Brock I, Hart CA, Cuevas LE. Risk for tuberculosis among children. Emerg Infect Dis 2006;12:1383–1388. [PubMed: 17073087]

Odhiambo JA, Borgdorff MW, Kiambih FM, Kibuga DK, Kwamanga DO, Nganga L, Agwanda R, Kalisvaart NA, Misljenovic O, Nagelkerke NJ, Bosman M. Tuberculosis and the HIV epidemic: Increasing annual risk of tuberculosis infection in Kenya, 1986–1996. Am J Public Health 1999;87:1078–1082. [PubMed: 10394319]

Pai M, Gokhale K, Joshi R, Dogra S, Kalantri S, Mendiratta DK, Narang P, Daley CL, Granichi RM, Mazurek GH, Reingold AL, Riley LW, Coldford JM Jr. Mycobacterium tuberculosis infection in health care workers in rural India; comparison of a whole-blood interferon r assay with tuberculin skin testing. JAMA 2005;293:2746–2755. [PubMed: 15941804]

Pai M, Riley LW, Colford JM Jr. Interferon-r assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis 2004;4:761–776. [PubMed: 15567126]

Ravn P, Demissie A, Eguale T, Wondwosson H, Lein D, Amoudy HA, Mustafa AS, Jensen AK, Holm A, Rosenkrands I, Oftung F, Olobo J, von Reyn F, Anderson P. Human T cell responses to the ESAT-6 antigen from *Mycobacterium tuberculosis*. J Infect Dis 1999;179:637–645. [PubMed: 9952370]

Sorenson AL, Nagai S, Houen G, Anderson P, Anderson AB. Purification characterization of a low-molecular-mass T-cell antigen secreted by Mycobacterium tuberculosis. Infect Immun 1995;63:1710–1717. [PubMed: 7729876]

Wang L, Turner MO, Elwood RK, Schulzer M, FitzGerald JM. A meta-analysis of the effect of Bacille Calmette Guerin vaccination on tuberculin skin test measurements. Thorax 2002;57:804–809. [PubMed: 12200526]

World Health Organization. Global tuberculosis control: Surveillance, planning, financing WHO report 2005. Geneva, Switzerland: World Health Organization; 2005.

Winthrop KL. Risk and prevention of tuberculosis and other serious opportunistic infections associated with the inhibition of tumor necrosis factor. Nat Clin Pract Rheumatol 2006;2:602–610. [PubMed: 17075599]

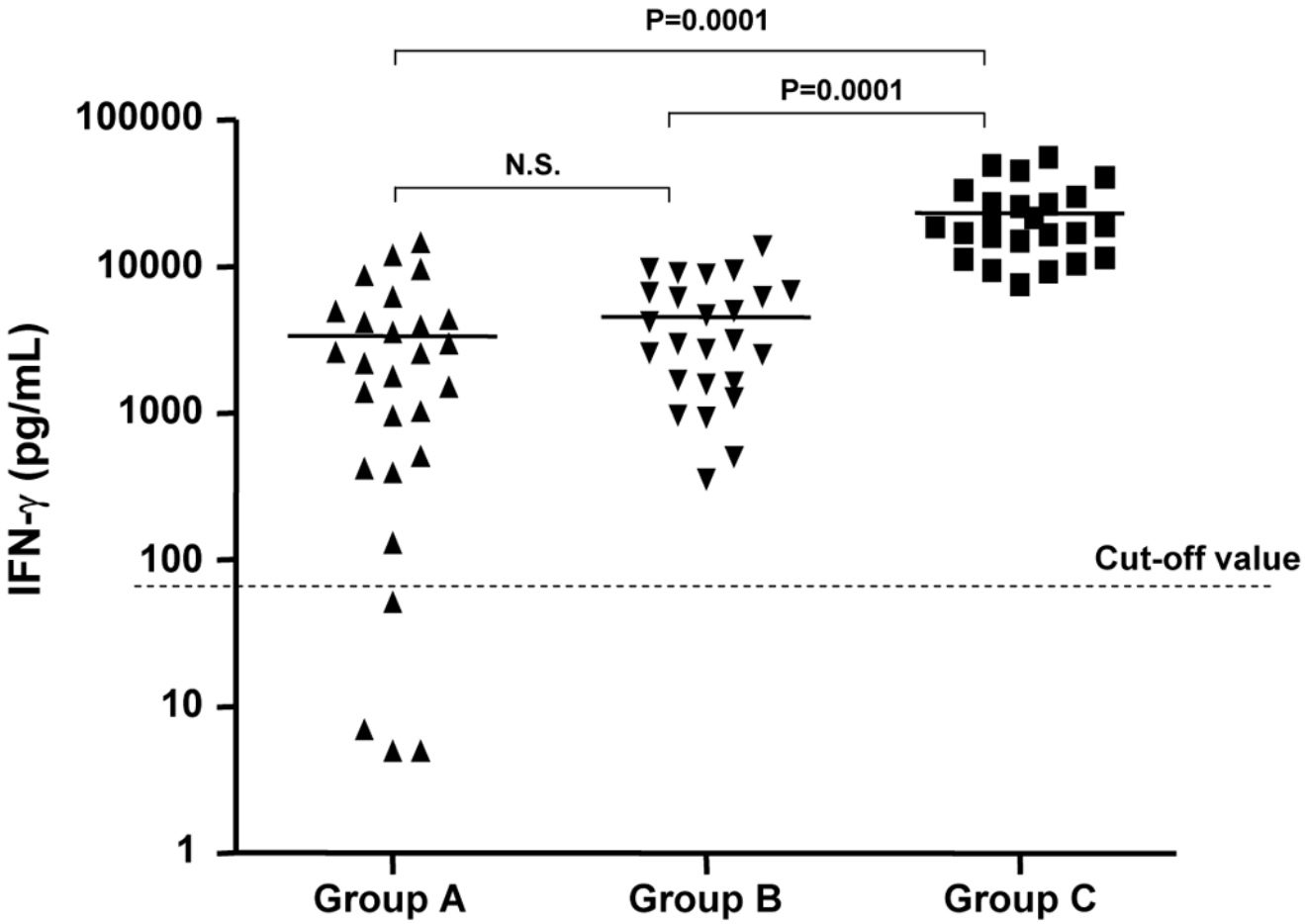

Figure 1.
Whole blood response to PPD in different groups. Group A : Medical students (low risk); Group B : nurses at TB hospital (high risk); Group C : TB patients. Whole blood was stimulated with PPD for 5 days. IFN-γ release was determined by ELISA. Broken line shows the cut-off value for positive responders (62 pg/ml). Horizontal bars represent mean IFN-γ value. The significant difference of IFN-γ production levels was analyzed between two groups and P value less than 0.05 was considered significant. N.S. : not significant.

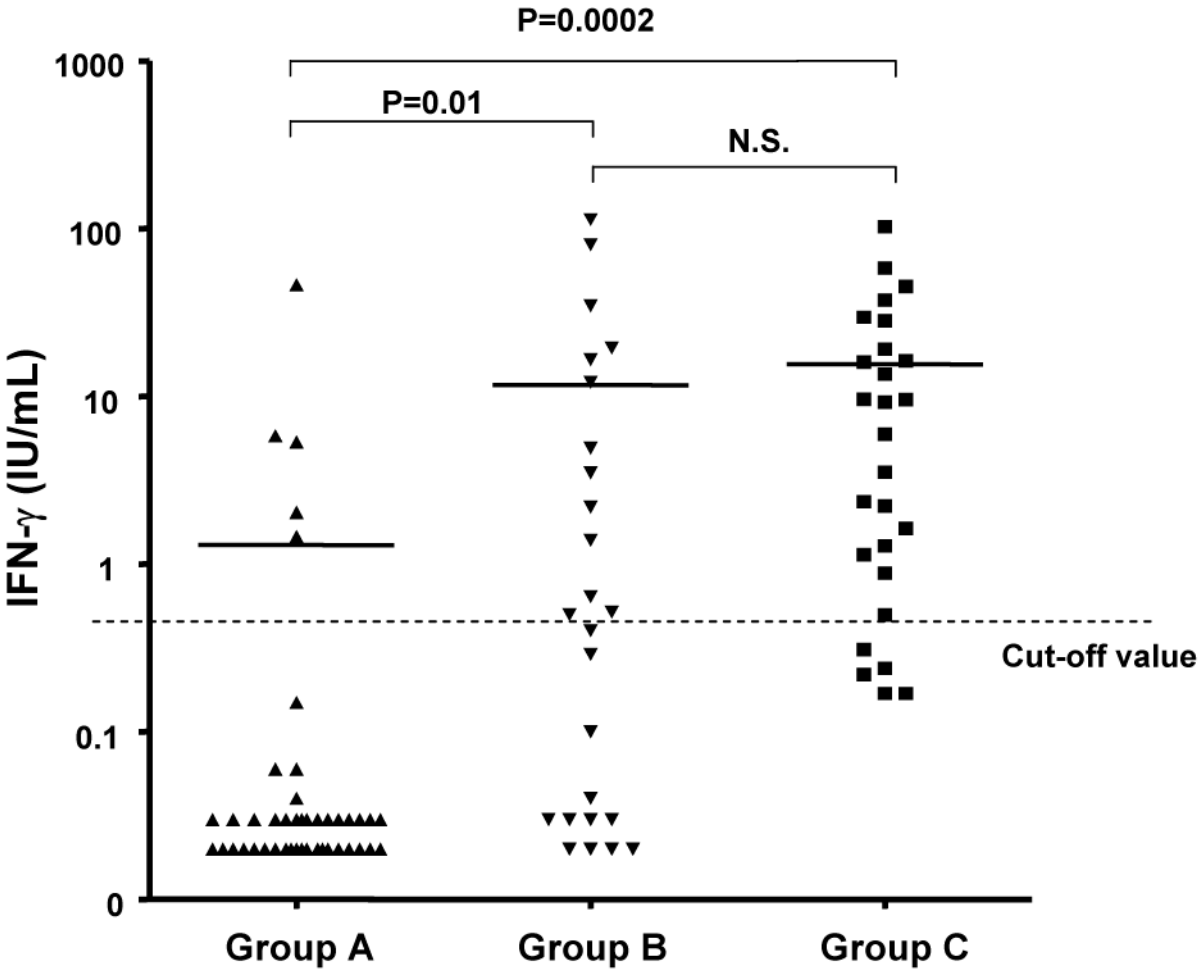

Figure 2.

IFN-γ assay by "QuantiFERON®-TB Gold in tube" test in different groups. Group A : Medical students (low risk); Group B : nurses at TB hospital (high risk); Group C : TB patients. Whole blood were stimulated in a tube containing a mixture of ESAT-6, CFP-10, and a portion of TB antigen TB 7.7 for 20 h. IFN-γ release was determined by ELISA. Broken line shows the cut-off value for positive responders (0.35 IU/ml). Horizontal bars represent mean IFN-γ value. The significant differences of IFN-γ production levels were analyzed between two groups and P value less than 0.05 was considered significant. N.S. : not significant.

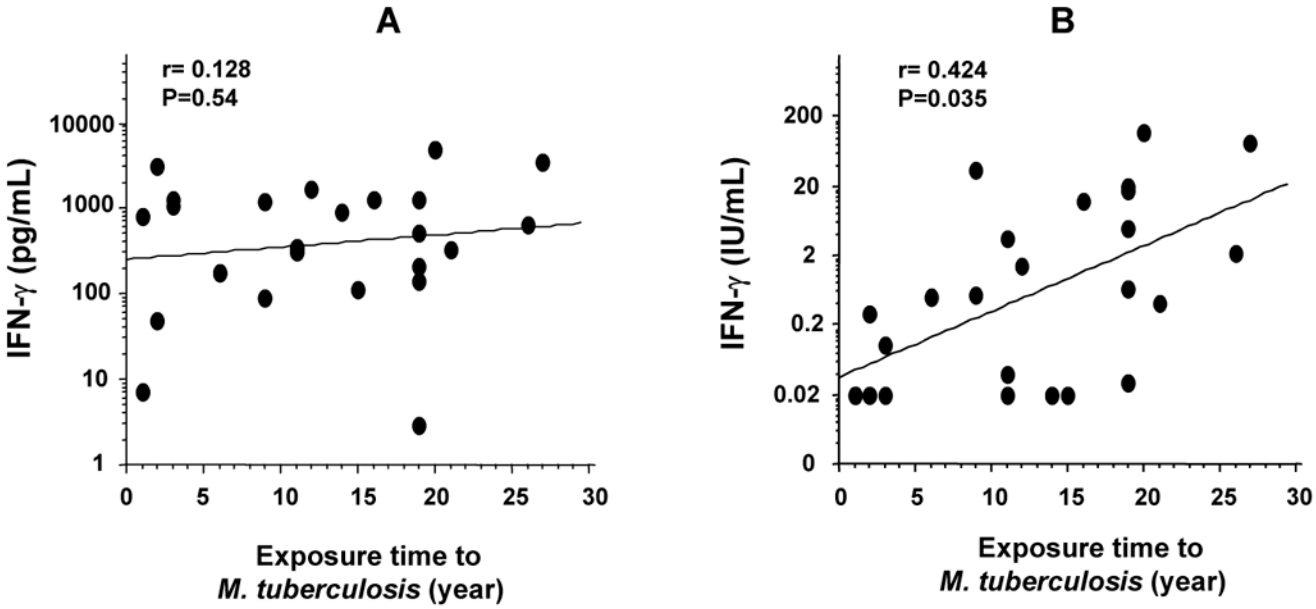

Figure 3.

The correlation analysis in high risk individuals (Group B) between the exposure period to *M. tuberculosis* and the secretion levels of IFN-γ in response to PPD (A) or to a mixture of *M. tuberculosis*-specific antigens (B). Each point represents each individual value. A significant correlation (r = 0.424, p = 0.035) was found by *M. tuberculosis*-specific antigens. N.S. : not significant.

Table 1

IFN-γ assay results in response to PPD or *M. tuberculosis*-specific antigens

IFN-γ assay in response to	Group A (Low risk group)	Group B (High risk group)	Group C (TB patients)
PPD (≥ 62 pg/ml) ESAT-6/CFP-10/TB7.7 (≥ 0.35 IU/ml)	85.2 % (23/27) 10.4 % (5/48)	100 % (25/25) 52 % (13/25)	100 % (24/24) 79.2 % (19/24)
Fisher's test	p < 0.0001	p < 0.0001	p = 0.04
Correlation coefficient	r = 0.157 p = 0.45	r = 0.113 p = 0.59	r = 0.308 p = 0.14

The frequency of positive responses to PPD and *M. tuberculosis*-specific antigens were analyzed by Fisher's test.
Correlation analysis was performed for IFN-γ responses between PPD and *M. tuberculosis*-specific antigens in each group.