Comparison of Plasma Interferon-Gamma and Antigen 60 Immunoglobulin G in Diagnosing Pulmonary *Mycobacterium Tuberculosis* Infection

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Background: An elevated interferon-gamma (IFN-γ) level has been reported in the plasma of patients with pulmonary tuberculosis. Serologic diagnosis of tuberculosis using Antigen 60 immunoglobulin G (A60 IgG) is a well-known diagnostic approach. This study evaluated plasma IFN-γ compared to A60 IgG in diagnosing *Mycobacterium tuberculosis.*

Methods: This study recruited 65 patients with tuberculosis and 59 controls. The plasma levels of A60 IgG and IFN-γ were measured using enzyme-linked immunosorbent assay kits.

Results: The cutoff values of IFN-γ and A60 IgG tests were set at 0.137 pg/ml and 261.2 units. The sensitivity and specificity of the IFN-γ test were 27.7% and 91.5%. The positive and negative predictive values of the IFN-γ test were 53.4% and 78.3%. Moreover, the sensitivity and specificity of the A60 IgG test were 53.8% and 67.8%. The positive and negative predictive values of the A60 IgG test were 37.0% and 80.7%. Finally, the sensitivity and specificity of the combined A60 IgG and IFN-γ tests were 64.6% and 62.7%. The positive and negative predictive values of the combined A60 IgG and IFN-γ tests were 37.8% and 83.4%.

Conclusions: The combined tests did not achieve significant improvement in disease prediction. The A60 IgG test and IFN-γ test had similar diagnostic value in pulmonary *Mycobacterium tuberculosis* infection.

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Key words: interferon-gamma, Antigen 60 immunoglobulin G, tuberculosis, serologic test.

The main tool currently used by physicians for diagnosing pulmonary tuberculosis in the absence of a sputum sample is the tuberculin skin test (TST) using purified protein derivative (PPD).[1] However, the effectiveness of this approach is limited due to the need for patients to return for test reading, as well as the variability and subjectivity of the test reading. Furthermore, the PPD test has low specificity, since the antigen used for TST is a mixture of mycobacterial antigens that are also present in nontuberculous mycobacteria (NTM) and the Bacille Calmette Guerin (BCG) vaccine strains.[2] The use of a surrogate marker with better diagnostic value than the PPD test could help in diagnosing *Mycobacterium tuberculosis* infection.

Several new techniques have been designed to

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diagnose *Mycobacterium tuberculosis* infection, including radiometric methods, DNA probes, mycolic acid chromatography, polymerase chain reaction, and serologic tests. Antigen 60 immunoglobulin G (A60 IgG) is the most frequently used method and has good sensitivity and specificity.[3] A combination of A60 IgG with other serologic tests may increase the accuracy of diagnosis. Many studies have observed an elevated concentration of interferon-gamma (IFN-γ) in tuberculosis patients.[4-6] One serologic test that has recently received considerable attention is the IFN-γ level in the culture supernatant of peripheral blood mononuclear cells (PBMCs).[7-9] However, this assay may be unsuitable for clinical application because it requires numerous reagents and needs overnight incubation with subsequent measurement of IFN-γ by enzyme-linked immunosorbent assay (ELISA). Perhaps, the measurement of plasma IFN-γ level alone may be sufficient to diagnose *Mycobacterium tuberculosis* infection. This report determined the diagnostic value of the IFN-γ test and compared the accuracy of A60 IgG and IFN-γ tests in diagnosing pulmonary tuberculosis.

METHODS

Tuberculosis patients

To estimate sensitivity, this study recruited 65 consecutive tuberculosis patients who were admitted to our hospital between October 2003 and October 2004. The patients had open pulmonary tuberculosis with an initial positive acid-fast bacilli (AFB) in sputum and a final positive culture for *Mycobacterium tuberculosis*.

Healthy volunteers

Twenty-eight healthy volunteers from our staff were included. The healthy volunteers had normal chest radiography and no respiratory symptoms or signs.

Pneumonia patients

Thirty-one pneumonia patients were consecutively recruited as controls in this study in the same study period. Pneumonia patients had initial negative AFB in sputum and final negative cultures for *Mycobacterium tuberculosis*. Pneumonia was defined as infiltration on chest radiography and typical symptoms and signs, such as fever, dyspnea, and cough with sputum.

We excluded patients who had received corticosteroids and prior anti-tuberculosis chemotherapy. Informed consent was obtained from all subjects and the study methods were approved by the Institutional Review Board/Chang Gung Memorial Hospital (IRB/CGMH). Plasma was obtained after centrifugation of 2 ml of whole blood from the subjects. Plasma samples from tuberculosis patients were obtained within two weeks of beginning anti-tuberculosis chemotherapy and plasma samples of pneumonia patients were obtained within one week of beginning antibiotic therapy. All samples were stored at -80°C until use.

Measurement of A60 IgG and IFN-γ levels

A diagnostic kit for A60 IgG was obtained from Anda Biologicals (Strasbourg Cedex, France). A60 IgG was measured in duplicate according to the instructions of the manufacturer and expressed in ELISA units as the average of the two readings. IFN-γ was measured in duplicate using a human IFN-γ ELISA Kit (Pierce Biotechnology, Illinois, U.S.A) according to the instructions of the manufacturer. Data were expressed in pg/ml as as the average of the two readings.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 11.0.1 for Windows (SPSS Inc., Chicago, IL, USA). The Mann-Whitney test was used to compare differences in age, A60 IgG levels and IFN-γ levels between tuberculosis patients and controls. The Pearson Chi-square test was used to compare differences in gender and serologic tests between tuberculosis patients and controls. The cutoff values of the A60 IgG and IFN-γ tests were chosen according to receiver operating characteristic (ROC) analysis. The area under the curve (AUC) was also calculated. The probability of the diagnostic procedure was presented as the sensitivity, specificity, positive predictive value, and negative predictive value. Multivariate logistic regression was used to obtain adjusted parameter estimates for clinical characteristics and serologic tests. *Mycobacterium tuberculosis* infection served as the response variable. The level of significance was set at $p < 0.05$.

RESULTS

Table 1 lists subject and AUC data. The chosen cutoff values for the A60 IgG and IFN γ tests were 261.2 units and 0.137 pg/ml, respectively. The AUC of the A60 IgG and IFN-γ tests was 0.605 and 0.596. Table 2 shows a statistically significant difference in IFN-γ, A60 IgG, and the combined test between tuberculosis patients and controls. Furthermore, Table 3 reveals that the combined A60 IgG and IFN-γ test was the most sensitive for diagnosing mycobacterium tuberculosis infection. Notably, the IFN-γ test had excellent specificity. When adjusted for prevalence of tuberculosis, serologic tests displayed poor positive predictive values but good negative predictive values (Table 4). There was a statistically significant difference in the IFN-γ test between subjects with tuberculosis and controls after adjustment for age and gender (Table 5). The IFN-γ test was statistically more significant than the A60 IgG test after including the A60 IgG and IFN-γ tests in the statistical analysis (Table 6). No test was statistically significant after including the A60 IgG, IFN-γ and combined A60 IgG and IFN-γ tests in the statistical analysis.

Table 2. Tuberculosis Test Evaluation in All Subjects

IFN-γ test	Tuberculosis	Control (Pneumonia + Healthy)
Positive	18*	5
Negative	47	54
A60 IgG test		
Positive	35*	19
Negative	30	40
Combined IFN-γ and A60 IgG test		
Positive	42*	22
Negative	23	37

Abbreviations: A60 IgG: Antigen 60 immunoglobulin G; IFN-γ: Interferon-gamma.

* $p < 0.05$ compared with control group by Pearson Chi-Square test.

Table 3. Values of Tests for Tuberculosis

	A60 IgG (%)	IFN-γ (%)	Combined A60 IgG and IFN-γ (%)
Sensitivity	53.8	27.7	64.6
Specificity	67.8	91.5	62.7

Abbreviations: A60 IgG: Antigen 60 immunoglobulin G; IFN-γ: Interferon-gamma.

DISCUSSION

Sensitivity and specificity are the most frequently used tools in evaluating the quality of diagnostic procedures. However, in daily practice clinicians are more concerned with predictive values, which vary markedly with the prevalence of a disease in a particular community. The probability of tuberculosis in Taiwanese hospitals has been estimated to be approximately 0.26.[10] With this tuberculosis prevalence, the positive predictive values for the A60 IgG test, IFN-γ test and combined A60 IgG and IFN-γ test were 37.0%, 53.4% and 37.8%, respectively (Table 4). Plasma IFN-γ had a better positive predictive value than A60 IgG and even the combined test. In this study, the A60 IgG test, IFN-γ test and even the combined A60 IgG and IFN-γ test were not good diagnostic methods to predict pulmonary *Mycobacterium tuberculosis* infection. However, the negative predictive values of the A60 IgG test, IFN-γ test and combined A60 IgG and IFN-γ test were 80.7%, 78.3%, and 83.4%, respectively. Plasma IFN-γ had less disease exclusion ability compared with the combined A60 IgG and IFN-γ test. The difference in the nega-

Table 1. Subject Data and AUC of A60 IgG and IFN-γ

	Tuberculosis	Control	*p* value	AUC (95% CI)
Number	65	59		
Age (mean ± SD years)	64.71 ± 17.06	58.02 ± 20.75	0.084*	
Gender (Male / Female)	49 / 16	42 / 17	0.597†	
A60 IgG (mean ± SD units)	432.1 ± 391.4	285.4 ± 242.0	0.044*	0.605 (0.505-0.705)
IFN-γ (mean ± SD pg/ml)	0.940 ± 2.658	0.214 ± 0.987	0.007*	0.596 (0.497-0.696)

Abbreviations: A60 IgG: Antigen 60 immunoglobulin G; IFN-γ: Interferon-gamma; SD: Standard deviation; AUC: Area under the curve; CI: Confidence interval.

* Mann-Whitney test.

† Pearson Chi-square test.

Table 4. Positive and Negative Predictive Values of Tests in a Population with Different Prevalences of Tuberculosis

Prevalence	Positive predictive value (%)			Negative predictive value (%)		
	A60 IgG	IFN-γ	Combined A60 IgG and IFN-γ	A60 IgG	IFN γ	Combined A60 IgG and IFN-γ
0.5	62.6	76.5	63.4	59.5	55.9	63.9
0.4	52.7	68.5	53.6	68.8	65.5	72.7
0.26	37.0	53.4	37.8	80.7	78.3	83.4
0.1	15.7	26.6	16.1	93.0	91.9	94.1

Abbreviations: A60 IgG: Antigen 60 immunoglobulin G; IFN-γ: Interferon-gamma.

Table 5. Logistic Regression Predicting *Mycobacterium tuberculosis* Infection After Adjusting for Subject Characteristics and IFN-γ Test

Independent variable	*p* value	Odds	95% CI for odds	
			Lower	Upper
Age	0.099	1.017	0.997	1.037
Gender	0.867	1.075	0.465	2.485
IFN-γ test	0.014	3.867	1.320	11.323

Abbreviations: IFN-γ: Interferon-gamma; CI: Confidence interval.

Table 6. Logistic Regression Predicting *Mycobacterium tuberculosis* Infection After Adjusting for Subject Characteristics, A60 IgG, and IFN γ Tests

Independent variable	*p* value	Odds	95% CI for odds	
			Lower	Upper
Age	0.153	1.015	0.995	1.035
Gender	0.966	1.019	0.435	2.384
A60 IgG test	0.050	2.147	1.001	4.605
IFN-γ test	0.020	3.637	1.222	10.824

Abbreviations: A60 IgG: Antigen 60 immunoglobulin G; IFN-γ: Interferon-gamma; CI: Confidence interval.

tive predictive value of the A60 IgG test, IFN-γ test and combined A60 IgG and IFN-γ test was around 5%. The combined test is twice as expensive as a single test. Therefore, testing plasma A60 IgG and IFN-γ concurrently is not recommended, because the negative predictive value of the combined test is only 6% higher than the IFN-γ test alone. In this study, the negative predictive values for the IFN-γ and A60 IgG tests were around 80%. We might correctly exclude 80% of patients with suspected pulmonary *Mycobacterium tuberculosis* infection using the A60 IgG or IFN-γ test.

To our knowledge, this is the first investigation to compare plasma IFN-γ and A60 IgG in terms of their ability to diagnose *Mycobacterium tuberculosis* infection. Elevated concentrations of IFN-γ in tuberculosis patients have been found in numerous studies.[4-6,11] The elevated IFN-γ levels in tuberculosis patients may result from leakage from tissue into the circulation, because T cells and macrophages at the infection site can produce IFN-γ.[12,13] This study identified a statistically significant difference in the IFN-γ level between tuberculosis patients and controls. The specificity of using 0.137 pg/ml as the cutoff value was extremely high, reaching 91.5%, but the sensitivity was low. The IFN-γ test was statistically significant for prediting *Mycobacterium tuberculosis* infection after adjusting for age and gender with an odds ratio of 3.867 (Table 5). Thus, a patient with a positive IFN-γ test had almost a 4 times greater risk of having *Mycobacterium tuberculosis* infection than a patient with a negative IFN-γ test. Moreover, after including the A60 IgG test in the statistical analysis, the statistical significance of the IFN-γ test persisted and the odds ratio of the IFN-γ test was greater than that of the A60 IgG test (3.637 vs. 2.147) (Table 6). This suggested that the IFN-γ test might be more useful than the A60 IgG test for diagnosing *Mycobacterium tuberculosis* infection. However, after including the combined IFN-γ and

A60 IgG test in the statistical analysis, the significance of all tests disappeared. This suggested that the combined IFN-γ and A60 IgG test might not be more useful than either the IFN-γ or A60 IgG tests alone.

Another cause of the low sensitivity in the IFN-γ test may be BCG vaccination. Data from the Center for Disease Control, Taiwan, R.O.C., indicates that Taiwan has had a policy of BCG vaccinations for all children since 1951. Thus Taiwanese over 55 years old may not have received BCG vaccinations. In a guinea pig model with BCG vaccination, expression of IFN-γ mRNA in peripheral blood mononuclear cells, spleen cells and lymph nodes was increased.[14,15] Other studies also demonstrated that subjects who did not receive BCG vaccination had poor IFN-γ responses to *Mycobacterium tuberculosis* antigen.[16,17] To our knowledge, no previous study has examined the difference in plasma IFN-γ in tuberculosis patients with and without BCG vaccination, but BCG vaccination should influence the baseline IFN-γ level. In this study, the absence of BCG vaccination could have affected the plasma IFN-γ level in the tuberculosis group and decreased the difference in the IFN-γ level between tuberculosis patients and controls. Because the plasma IFN-γ level decreases with anti-tuberculosis chemotherapy,[4,11] the IFN-γ test should be limited in patients who received minimal or no treatment at the time of testing, as in this study.

The treatment regimen for active and latent tuberculosis is different. Physicians should distinguish between patients with latent tuberculosis and those with active tuberculosis. Many studies have demonstrated that the whole blood IFN-γ assay is a good test to detect latent tuberculosis.[7,17-19] Only Mori reported excellent sensitivity and specificity for diagnosis of active pulmonary *Mycobacterium tuberculosis* infection using the whole blood IFN-γ assay.[8] However, there was a significant difference in age between tuberculosis and control subjects in Mori's study. IFN-γ production from type 1 T cells is impaired with aging.[20] Study of a whole blood IFN-γ assay for diagnosing active pulmonary tuberculosis should exclude the age effect. It is still unclear whether a whole blood IFN-γ assay can be used for diagnosing active pulmonary tuberculosis.

It is important to accurately detect and treat people with *Mycobacterium tuberculosis* infection in Taiwan. The IFN-γ test had better specificity and positive predictive value than the A60 IgG test, but the sensitivity was lower. Although the combined IFN-γ and A60 IgG tests could increase the sensitivity for diagnosing *Mycobacterium tuberculosis* infection, this effect could easily be achieved by adjusting the cutoff value. However the total costs increase. This study also showed that the IFN-γ test might be more useful to exclude pulmonary tuberculosis. The number of patients in this investigation was small. A large scale study should be conducted.

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比較丙種干擾素與抗原 60 免疫球蛋白在診斷肺部結核分支桿菌感染

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背 景： 結核菌素皮膚測試使用純化蛋白衍生物，來診斷結核分支桿菌感染有很大的限制。在肺結核病患，已報導過有上升的血漿丙種干擾素濃度。抗原 60 免疫球蛋白是廣被所知的血液學診斷方法。這個研究嘗試比較丙種干擾素與抗原 60 免疫球蛋白對結核分支桿菌的診斷價值。

方 法： 此研究囊括 65 名結核病患與 59 名對照組。血漿中的丙種干擾素與抗原 60 免疫球蛋白濃度是經由蛋白酶免疫螢光法試劑來測出。

結 果： 丙種干擾素與抗原 60 免疫球蛋白的分隔數值分別爲 0.137 pg/ml 與 261.2 units。丙種干擾素測試的敏感度與特異度爲 27.7% 與 91.5%。丙種干擾素測試的陽性預估值與陰性預估值爲 53.4% 與 78.3%。抗原 60 免疫球蛋白測試的敏感度與特異度爲 53.8% 與 67.8%。抗原 60 免疫球蛋白測試的陽性預估值與陰性預估值爲 37.0% 與 80.7%。合併丙種干擾素與抗原 60 免疫球蛋白兩種測試的敏感度與特異度爲 64.6% 與 62.7%。合併丙種干擾素與抗原 60 免疫球蛋白兩種測試的陽性預估值與陰性預估值爲 37.8% 與 83.4%。

結 論： 合併測試對疾病預估並沒有明顯進步。與抗原 60 免疫球蛋白測試比起來，丙種干擾素測試對於結核分枝感菌感染擁有相似的診斷價值。

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關鍵字： 丙種干擾素，抗原 60 免疫球蛋白，結核病，血液學測試。

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