

The Significance of *Mycobacterium tuberculosis* Antibody, Antigen 60 IgG in Patients with Abnormal Chest Radiography

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Background: The identification of acid-fast bacilli (AFB) in sputum or tissue is the definite diagnosis of tuberculosis. However, this method of diagnosis is restricted by certain limitations. The serologic diagnosis of tuberculosis has been used for a long time. The aim of this study was to determine the sensitivity and specificity of Antigen 60 (A60) immunoglobulin G (IgG) in patients with abnormal chest radiography and to assess its application in the serologic diagnosis of pulmonary tuberculosis.

Methods: Data on patients who had been diagnosed using results of culture and pathology as having active pulmonary tuberculosis (N=178), other non-tuberculosis pulmonary disease (N=34), or no pulmonary disease (N=117) was collected from January 2001 through December 2002. The data of A60 IgG using enzyme-linked immunosorbent assay (ELISA), chest radiography, tuberculosis culture and pathology were obtained retrospectively. The cutoff value of A60 IgG was chosen according to a receiver operating characteristic (ROC) analysis. The sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratio for positive and negative test were determined.

Results: The chosen cutoff value of 261.2 units defined the sensitivity (49.4%) and specificity (79.5%) of the test. The positive predictive value and likelihood ratio were 95.7% and 4.20, respectively, for patients with abnormal chest radiography and 88.2% and 2.97, respectively, for patients with abnormal chest radiography and negative AFB in sputum smear.

Conclusions: Because of the high positive predictive value and likelihood ratio, a positive A60 IgG test in the presence of an abnormal chest radiography can help make an accurate clinical diagnosis of pulmonary tuberculosis.
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Key words: antigen 60 IgG, tuberculosis, serologic test.

Early detection of pulmonary tuberculosis is important for a physician because of the infectious nature of the mycobacteria. A decision to initi-

ate combination antituberculosis chemotherapy is based mainly on clinical presentation, exposure history, laboratory test results, pathological reports, and

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radiographic findings. The most important tool currently used by physicians for tuberculosis diagnoses is microscopic examination of AFB and cultures for mycobacteria in the patient's sputum. However, the effectiveness of this approach is limited by low sputum acid-fast stain sensitivity (around 50%) when using conventional direct microscopy and the long turnaround time (more than 6 weeks) necessary for the culture results.⁽¹⁾ The use of a surrogate maker with better sensitivity and specificity may help achieve earlier diagnoses of the tuberculosis infections. The positive purified protein derivatives (PPD) tuberculin skin test can help diagnosis culture-negative pulmonary tuberculosis as well as latent tuberculosis infection, although it provides high sensitivity with low specificity.⁽²⁾

Several new techniques have been developed to improve the physicians' ability to diagnosis pulmonary tuberculosis, including radiometric methods, DNA probes, mycolic acid chromatography, polymerase chain reaction, and serologic tests. Studies conducted during the past decade have indicated that the polymerase chain reaction (PCR) test is able to detect AFB DNA with high degrees of sensitivity and specificity.⁽³⁾ However, PCR is limited by problems such as low sensitivity for smear-negative and culture-positive samples.^(3,4)

The use of serologic methods to diagnose tuberculosis have been studied since 1898⁽⁵⁾ and A60 IgG is the method most frequently used. A study comparing three different antigen antibodies showed that A60 IgG (sensitivity and specificity, 80.77 and 88.4%) was more antigenic and more effective in its determination than was 38 kda IgG (sensitivity and specificity, 64.21 and 80.74%) or Kp90 IgA (sensitivity and specificity, 62.58 and 66.3%).⁽⁶⁾ The results of other serologic test studies, including immunoglobulin antibody to diacyltrehaloses, triacyltrehaloses, cord factor, and sulfolipid I, showed relatively low sensitivity and specificity for cases of tuberculosis infection.⁽⁶⁻¹¹⁾ This study was designed to determine the diagnostic value of combining A60 IgG and chest radiography in the accurate diagnosis of pulmonary tuberculosis.

METHODS

Subjects

Clinical data on 329 subjects who had under-

gone tests for A60 IgG during the most recent 2-year period were reviewed. Of this group, 178 were diagnosed with active pulmonary tuberculosis and 34 with non-tuberculosis pulmonary disease. The remainder (117) were diagnosed as healthy. A diagnosis of active pulmonary tuberculosis was made when a subject had an abnormal chest radiography and positive sputum culture with negative or positive AFB in smear. A non-tuberculosis pulmonary disease diagnosis was made when a subject had (1) an abnormal chest radiography, (2) negative sputum smear and culture for tuberculosis, and (3) pathologic proof of non-tuberculosis process from lung tissue. A healthy diagnosis was made when a subject had a normal chest radiography, with no symptoms or signs of respiratory problems. Most of the subjects in the healthy category underwent the test due to prior contact with one or more pulmonary tuberculosis patients.

Antigen 60 IgG Measurement

Two ml of whole blood was collected and centrifuged, with the supernatant stored at -20°C for a period, but not exceeding 4 weeks. The sample was removed from storage on the day in which it was subjected to an ELISA test. The A60 IgG level was measured using an ELISA kit (Anda Biologicals, Strasbourg Cedex, France).⁽⁶⁾

Statistical Analysis

Sensitivity and specificity were defined as the proportion of patients correctly identified by the test as abnormal and the proportion of healthy subjects correctly identified, respectively. Positive predictive value was the proportion of patients with active tuberculosis among those with positive test results. The negative predictive value was the proportion of subjects without tuberculosis among those with negative test results. For positive test results, the likelihood ratio was the odds of positive test results in patients with tuberculosis, versus positive test results in patients without tuberculosis. For negative test results, the likelihood ratio was the odds of negative test results in patients without tuberculosis, versus negative test results in patients with tuberculosis. Because of the limitation of our ELISA reader, titers of A60 IgG less than 200 units were treated as 200 ELISA units and titers of A60 IgG greater than 1600 units were treated as 1600 ELISA units. A reason-

able cutoff value was chosen according to one ROC analysis, which also produced areas under the curve (95% confidence intervals). The Kruskal-Wallis test and the Mann-Whitney rank sum test were used to compare the differences of A60 IgG among and between the groups, respectively. The Mann-Whitney rank sum test was also used to compare A60 IgG between the sexes. The Chi-Square test was used to compare the distribution of gender among the different groups. The One-Way ANOVA test was used to compare the differences of ages among the different groups using the Bonferroni method for post hoc multiple comparisons. The Spearman rank correlation was used to measure the correlation between A60 IgG and age. For the A60 IgG test, the Fisher's exact test was used to compare the differences between the groups after 261.2 units was set as a cutoff value. A *p* value less than 0.05 was considered statistically significant.

RESULTS

The demographic data for the subjects included in our survey are shown in Table 1. A60 IgG, gender and age were significantly different among groups. Age was a variable that showed significantly different both between groups with tuberculosis and groups that were healthy and between groups with tuberculosis and groups suffering from other non-tuberculosis pulmonary diseases. The healthy patient group and tuberculosis patient group differed significantly in terms of gender distribution, while the tuberculosis and non-tuberculosis pulmonary disease patient groups did not.

Comparison between tuberculosis and healthy subjects groups

The A60 IgG levels in the tuberculosis, non-tuberculosis pulmonary disease, and healthy groups are shown in Table 2. The A60 IgG levels were significantly different between the tuberculosis and healthy subject groups. In the active pulmonary tuberculosis group, the A60 IgG serum levels in patients with positive AFB were significantly higher than that those in patients with negative AFB. There were no differences between A60 IgG and gender in the healthy subjects and tuberculosis patients. As a whole, A60 IgG had no significant correlation to age (correlation coefficient (*r*) of -0.076). However, a significant correlation was found in the tuberculosis group (*r* = -0.250 (*p* = 0.01)), which was not replicated in the healthy group (*r* = -0.017), non-tuberculosis pulmonary disease (*r* = -0.077), or combined group

Table 2. A60 IgG Levels in Tuberculosis, Non-tuberculosis Pulmonary Disease and Healthy Groups

Study Group	A60 IgG ELISA units	
	Median	Range
Active pulmonary tuberculosis	253.95*†	200.00 - 1600.00
Positive AFB	371.20‡	200.00 - 1600.00
Negative AFB	200.00	200.00 - 1600.00
Healthy	200.00	200.00 - 1535.20
Non-Tuberculosis pulmonary disease	200.00	200.00 - 738.40

Abbreviations: A60 IgG: Antigen 60 immunoglobulin G; ELISA: enzyme-linked immunosorbent assay; AFB: acid-fast bacilli

**p* < 0.001 compared with healthy subjects

†*p* < 0.001 compared with non-tuberculosis pulmonary disease

‡*p* < 0.001 compared with negative AFB tuberculosis

Table 1. Subject Demographic Data

Study Group	Subjects (N)	Age (years)		Gender (M/F)
		Mean	Range	
Active pulmonary tuberculosis	178	60.11*†	14 - 93	131/47†
Positive AFB	92	60.05	15 - 91	64/28
Negative AFB	86	60.16	14 - 93	67/19
Non-Tuberculosis pulmonary disease	34	68.26‡	47 - 88	26/ 8
Healthy	117	44.32	10 - 86	52/65

Abbreviations: M: male; F: female; AFB: acid-fast bacilli

**p* < 0.001 compared with non-tuberculosis pulmonary disease group

†*p* < 0.001 compared with healthy subjects

‡*p* < 0.001 compared with healthy subjects

of both tuberculosis patients and healthy subjects ($r = -0.036$). We did one ROC analysis of data on the two groups of pulmonary tuberculosis patients and healthy subjects. The value of the area under the curve was 0.676 (0.615-0.736). With a cutoff value set at 261.2 ELISA units (Fig. 1), the sensitivity and specificity for these groups were 49.4% and 79.5%, respectively.

Comparison of tuberculosis and non-tuberculosis pulmonary disease

The data of the A60 IgG test showed statistically significant differences between the patients in the tuberculosis and non-tuberculosis pulmonary disease groups (Table 2). There were no differences

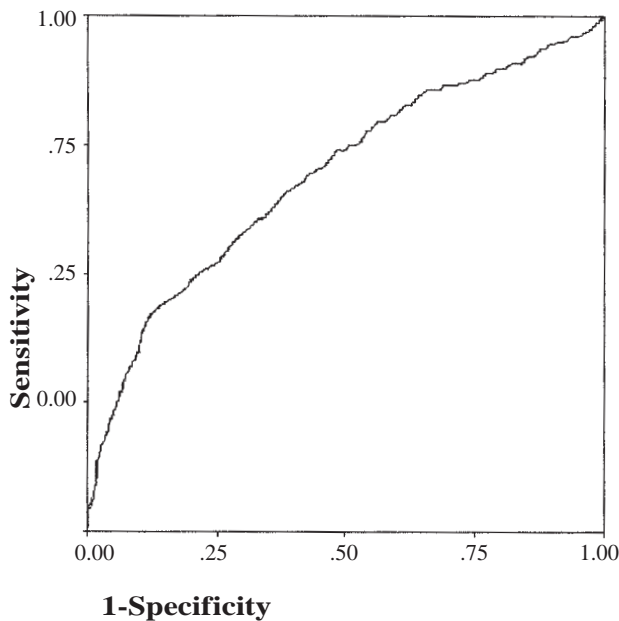


Fig. 1 ROC curve plotting A60 IgG in pulmonary tuberculosis and healthy patient group.

between A60 IgG and sex distribution in groups of non-tuberculosis pulmonary disease. The A60 IgG test results in groups with abnormal chest radiography are showed in Table 3 and the reliability of A60 IgG test in patients with abnormal chest radiographs are showed in Table 4. The reliability of the A60 IgG test was found to be inferior in patients with negative AFB, with the exception of a slightly better negative predictive value.

Table 4. Values Used to Evaluate A60 IgG in Patients with Abnormal Chest Radiography

Reliability	Patients with negative AFB	All patients
Sensitivity	34.9%	49.4%
Specificity	88.2%	88.2%
Positive predictive value	88.2%	95.7%
Negative predictive value	34.9%	25.0%
LR for a positive test	2.97	4.20
LR for a negative test	1.36	1.75

Abbreviations: A60 IgG: Antigen 60 immunoglobulin G; AFB: acid-fast bacilli; LR: likelihood ratio

DISCUSSION

Early serologic tests used PPD or other crude antigens with results showing relatively poor sensitivity and specificity due to the fact that many antibody responses are shared with common antigens.⁽¹²⁻¹⁴⁾ Our results indicated that accurate diagnosis of pulmonary tuberculosis using A60 IgG serum data alone was unsatisfactory. However, the likelihood ratio for positive test results for A60 IgG in patients with abnormal chest radiography was as high as 4.20. Therefore, it is of some use in diagnosing pulmonary tuberculosis.

It is important to determine the cutoff value for

Table 3. 2 × 2 Table Evaluating Tuberculosis in Patients with Abnormal Chest Radiography

A60 IgG	Non-tuberculosis pulmonary disease	Tuberculosis		
		Total	AFB(+)	AFB(-)
Positive	4	88*	58*	30†
Negative	30	90	34	56

Abbreviations: A60 IgG: Antigen 60 immunoglobulin G; AFB: acid-fast bacilli

* $p < 0.001$ compared to non-tuberculosis pulmonary disease

† $p < 0.05$ compared to non-tuberculosis pulmonary disease

this serologic test. This value may differ in areas with different prevalence of pulmonary tuberculosis and BCG vaccination policies. In a study by Chiang, A60 IgG levels in Taiwanese patients had the cutoff value of 340 ELISA units, which defined the sensitivity and specificity for his tests of 80.77% and 88.40%, respectively.⁽⁶⁾ Although our study population was similar to that studied by Chiang, our results were different. The most likely reason is that the inclusion criteria between the two studies differed. The tuberculosis group in the previous study included patients diagnosed clinically and those with positive AFB but negative results on sputum cultures.⁽⁶⁾ That may have increased the sensitivity. It may be difficult to distinguish pulmonary tuberculosis from other pulmonary diseases through chest radiography alone.^(15,16) Although it is reasonable to begin anti-tuberculosis chemotherapy using results of chest radiography if no other diagnosis is made,⁽¹⁷⁾ in the study by Chiang, the true percentage of correctly diagnosed tuberculosis by chest radiography alone was uncertain. The percentage of patients with positive AFB but negative results on sputum culture in Chiang's study was greater than 4%,⁽⁶⁾ however, the percentage of specimens shown to be positive by smear but negative on culture was reportedly around 2%.⁽¹⁸⁻²⁰⁾ Our results may be more prudent and similar to the results of others.^(21,22) In our data, the A60 IgG level was higher in patients with positive AFB in the sputum smear than in those with negative AFB. The results were similar to those of other investigators,^(23,24) as greater exposure to antigens should induce a greater antibody response.^(25,26)

In our study of patients with abnormal chest radiography, the positive predictive value and likelihood ratio for positive test results was as high as 95.7% and 4.20, respectively. This indicates that the positive test results increased by at least fourfold compared with the pretest odds of tuberculosis in patients with abnormal chest radiography and that at least 90% of patients with abnormal chest radiographies and positive A60 IgG test results may actually have tuberculosis. The diagnostic value of a test depends on its positive and negative predictive values, and these values vary markedly with the prevalence of the disease in a community.⁽⁶⁾ In a sub-group study of patients with negative AFB tuberculosis and non-tuberculosis pulmonary disease, the likelihood ratio for positive test results was 2.97 and the posi-

tive predictive value was 88.2%. As we expected, the reliability of the A60 IgG test was inferior in tuberculosis patients with negative AFB because of lower A60 IgG levels. In the study by Luh, the positive predictive value of A60 IgG in tuberculosis patients with negative AFB in the sputum smear was 67.9% using a study population of 26% active tuberculous infection rate in Taiwan.⁽²⁷⁾ Thus, A60 IgG still has good diagnostic value in patients with abnormal chest radiography and negative AFB sputum smears. Although the number of non-tuberculosis pulmonary disease patient was small, the results of the analysis were statistically significant. As the number of patients in our study was small, a large scale study should be performed.

The A60 IgG level was not statistically correlated to age as expected in the healthy group. In patients with tuberculosis, A60 IgG had minor, but significant, negative correlation to age with an $r = -0.250$. This may indicate that antibody levels are low in older patients with pulmonary tuberculosis. We found no available literature discussing the correlation of A60 IgG and aging through a Medline search. However, there are many articles discussing the relationship between immunity and aging. There is a consensus that the immune system decreases with age.^(28,29) Reduced proliferation and response of T lymphocytes to stimulation are associated with diminished production of interleukin-2 and reduced expression of the high affinity interleukin-2 receptor.^(30,31) Decreased number of B cells, which are responsible for T cell-independent antibody production,⁽³²⁾ and lack of T cell effectiveness are primary causes of decreased antibody production. Thus, it seems reasonable to speculate that aging may reduce the production of A60 IgG during the course of tuberculosis infection.

Although with low sensitivity and specificity, A60 IgG in combination with chest radiography could help us to diagnose tuberculosis. Positive results could potentially aid in clinical decisions regarding the recommendation of anti-tuberculosis chemotherapy for patients with abnormal chest radiography and negative sputum smear for AFB.

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肺結核抗體及抗原60免疫球蛋白在不正常胸部X光片病患的重要性

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背景：肺結核病的確定診斷是經由在痰液或組織中找到嗜酸性結核桿菌。但是此方法卻有些限制。目前利用血液學試驗診斷肺結核已經有一段時間。此實驗的目的在探討抗原60免疫球蛋白，在不正常胸部X光片病患用來診斷肺結核病的敏感度及特異性；評估抗原60免疫球蛋白在肺結核病血液學診斷的應用。

方法：我們收集西元2001年1月到西元2002年12月中診斷為肺結核病(N=178)、其他非肺結核肺部疾病(N=34)、無肺部疾病的健康人(N=117)的資料，包含抗原60免疫球蛋白濃度、胸部X光片判讀、細菌培養及病理組織報告。抗原60免疫球蛋白的截止數值是根據ROC統計方法來決定。我們依此抗原60免疫球蛋白的截止數值來計算敏感度、特異性、陽性預估值、陰性預估值、陽性及陰性反應可能比率。

結果：抗原60免疫球蛋白的截止數值為261.2單位，敏感度為49.4%，特異度為79.5%。在不正常胸部X光片病患中，陽性預估值為95.7%，陽性反應可能比率為4.20。在不正常胸部X光片及痰液鏡檢陰性嗜酸性結核桿菌病患中，陽性預估值為88.2%，陽性反應可能比率為2.97。

結論：因為此試驗有高陽性預估值及可能比率，在一個不正常胸部X光片病患，有陽性抗原60免疫球蛋白試驗可以幫助我們下一個正確的肺結核病臨床診斷。
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關鍵字：抗原60免疫球蛋白，肺結核病，血液學試驗。

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