

Clinical application, evaluation and analysis of influencing factors of on tuberculosis γ -Interferon enzyme-linked immunosorbent assay

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Abstract

Objective: To assess sensitivity and specificity of the interferon gamma release assay test, and to pinpoint the influencing factors that should be taken care of in clinical application.

Methods: This study was conducted at the First People's Hospital in the Yunnan Province of China from October 2018 to March 2019, and comprised samples collected from outpatient and inpatients. To detect mycobacterium tuberculosis, acid-fast staining on sputum smear was performed on relevant tissues suspected of extrapulmonary tuberculosis. Tuberculosis interferon gamma release assay test and pathology samples were examined. Tuberculosis-specific cell reaction assay kit was used for sampling. SysmexXN-2000 haematology analyser, VACUETTE SRS100/II and Beckman Coulter AU5800 were used to perform various analyses. Data was grouped and analysed using R statistical software.

Results: Of the 960 samples, 516(53.75%) cases tested positive for tuberculosis infection and 444(46.25%) tested negative. The sensitivity of the pathological results was 86% and the specificity was 96%. The sensitivity of the interferon gamma release assay test was 95% and specificity 82%. Interferon release test, pathological results and final diagnosis showed significant comparisons ($p < 0.05$). Significant relationships were also established for factors, such as age, interferon release quantity, lymphocyte, C-reactive protein and counts of mono-nuclear cell ($p < 0.05$).

Conclusions: Interferon gamma release assay test was found to have high consistency with pathological results and final diagnosis and can be used as a subsidiary to traditional clinical imaging and pathological judgment.

Keywords: Tuberculosis, Interferon release, CRP, Hormone use. (JPMA 71: 2543; 2021)

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Introduction

Rapid diagnosis and treatment are key factors in tuberculosis (TB) control. To date, there is no accurate, rapid and reliable method for detecting mycobacterium TB (MTB) infection. Commonly used bacteriological detection methods include sputum smear microscopy and culture methods, as well as TB clinical gene amplification method and serological detection methods. However, these methods also have issues, like low sensitivity, poor specificity, high operational complexity, and long testing period. The World Health Organisation's 2014 report showed that the diagnostic rate of TB was only 37%¹ and even in developed countries, only 50% of TB cases were confirmed by bacteriological methods.² Therefore, the development of rapid and specific diagnosis of TB has become an urgent task to control its spread. TB can induce a strong cellular immune response, and, therefore, detection of TB-specific T lymphocytes can be used as a new path to detect infection.³ The current study was planned to assess sensitivity and specificity of the interferon gamma release assay test, and to pinpoint the influencing factors that should be taken care of in clinical application.

Materials and Methods

This study was conducted at the First People's Hospital in the Yunnan Province of China from October 2018 to March 2019. Fixed-point sampling method was used to select suspected TB patients who were grouped as suspect positive group A and suspect negative group B. The diagnostic criteria for active tuberculosis (ATB) required MTB culture to be positive in sputum, bronchoalveolar lavage fluid, tissue or other specimens in addition to typical clinical symptoms and imaging findings. The diagnostic criteria for latent tuberculosis infection-1 (LTBI) dictated TB-interferon test positive in routine screening, but no clinical symptoms and imaging findings related to TB⁴ with any of the following elements present; age <18 years; immunocompromised people having diabetes, malignant tumours, chronic renal failure; or anti-TB treatment >2 weeks.

In case of hospitalised patients, 4ml of fresh heparin lithium anticoagulated whole blood was sampled on the morning after the hospital admission and was stored at room temperature. Patients' sputum smear was examined, and conditional bronchoscopy brush was checked for acid-fast bacilli, with extrapulmonary TB checked by taking smears of relevant areas. Interferon gamma release assay (IGRA) test and pathology samples were sent for examination. QIAGEN M. TB-specific cell reaction enzyme-linked

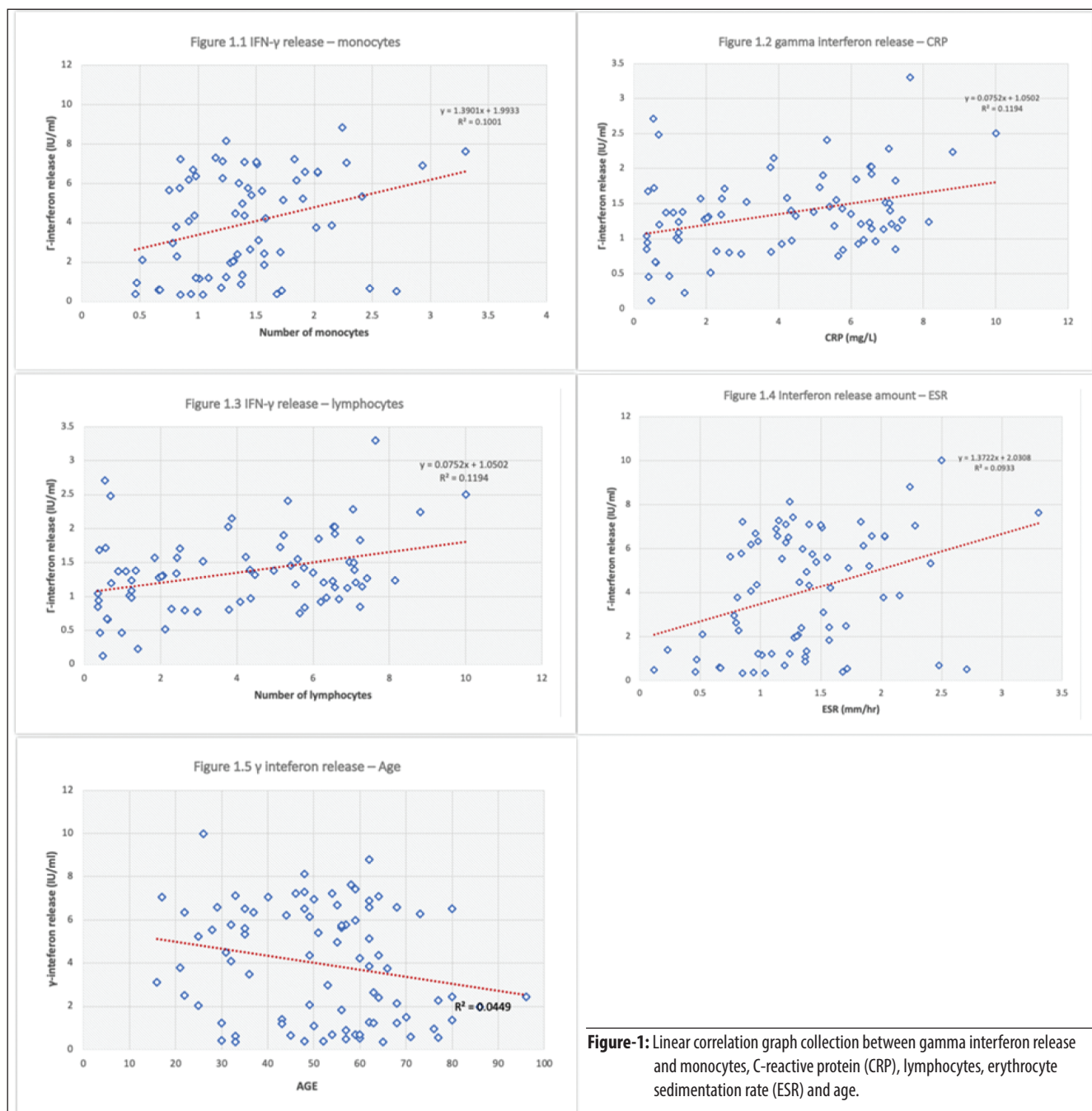
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immunosorbent assay (ELISA) kit was used during sampling. SysmexXN-2000 haematology analyser, VACUETTE SRS100/II and Beckman Coulter AU5800 were used to obtain mononuclear cells, lymphocytes, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and factors which may be related to the serological indicators of TB infection. All testing systems used matching reagents controlling for quality.

Group A was isolated by pathological and bacteriological examinations and clinical testing, while group B had

patients negative for TB by means of the detection of MTB by culture. IGRA results were compared with pathological results and its sensitivity, specificity, accuracy and consistency was tested for correlation. IGRA and pathological results were compared for final diagnosis using paired chi-squared tests. After the raters were confirmed, Cohen's Kappa index, a statistic used to measure inter-rater reliability, was calculated. Linear regression was performed on each individual explanatory and potentially confounding variable. In order to eliminate confounding



factors, analysis was performed on selected factors with statistically significant single factors. Binary logistic regression was chosen because the dependent variable, correct diagnosis, was dichotomous. Gamma release value, ESR, lymphocyte, CRP, and number of monocytes were continuous variables, while age was a discrete variable. R statistical software was used to produce correlation plots to guide final data fitting and modelling. The following generalised linear model regression function was used:

Glm (formula = data\$GAMMA ~ data\$GAMMA.VALUE + data\$AGE + data\$BLOOD + data\$Lymphyte + data\$CELL, family = binomial (link = "logit"), data = data).

Results

Of the 960 cases, 528(55%) were males and 432(45%) were females. The overall mean age was 49.14 ± 17.42 years (range: 40-67 years). Of them 516(53.75%) tested positive for TB infection and were placed in group A; 288(56%) males and 228(44%) females with an overall mean age of 49.14 ± 17.81 years (range: 49-67 years). The remaining 444(46.25%) cases tested negative and were placed in group B; 240(54%) males and 204(46%) females, with an overall mean age of 40.86 ± 17.04 years (40-58 years).

Table-1: Sensitivity, specificity, and accuracy of pathological results and IGRA test against traditional screening.

	Sensitivity	Specificity	Accuracy
Pathological Results	86%	96%	82%
Interferon Release Test	95%	82%	77%

IGRA: Interferon gamma release assay.

Table-2: Comparison of interferon release test results and pathological results in 960 patients with final diagnosis.

		Final Diagnosis		Pathological Results	
		+ve	-ve	+ve	-ve
IGRA Test	+ve	422 (95%)	94 (18%)	460 (96%)	70 (14%)
	-ve	20 (5%)	424 (82%)	18 (4%)	436 (86%)
Pathological Results	+ve	500 (85%)	16 (4%)		
	-ve	82 (15%)	362 (96%)		

IGRA: Interferon gamma release assay.

Table-3: Report of correlation statistics of influencing factors on interferon release test results.

Category/Statistics	+ve (n=516)	-ve (n=444)	Kappa/t	p-value
Sex				
Male	288 (55.8%)	240 (54.1%)	0.299	0.585
Female	228 (44.2%)	204 (45.9%)		
Age (years)	49.14 ± 17.81	40.86 ± 17.04	7.347	0.47
IG Release (IU/ml)	3.69 ± 3.57	0.02 ± 0.02	23.351	0.001
ESR (mm/hr)	30.33 ± 33.22	28.58 ± 28.53	0.868	0.386
Lymphocyte/ μ L	1.66 ± 0.91	0.75 ± 0.59	18.577	0.000
CRP (mg/L)	20.08 ± 33.93	29.33 ± 50.93	3.258	0.001
Hormone (mg/ml)	0.50 ± 0.19	0.62 ± 1.11	2.248	0.025

IG: interferon gamma; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein.

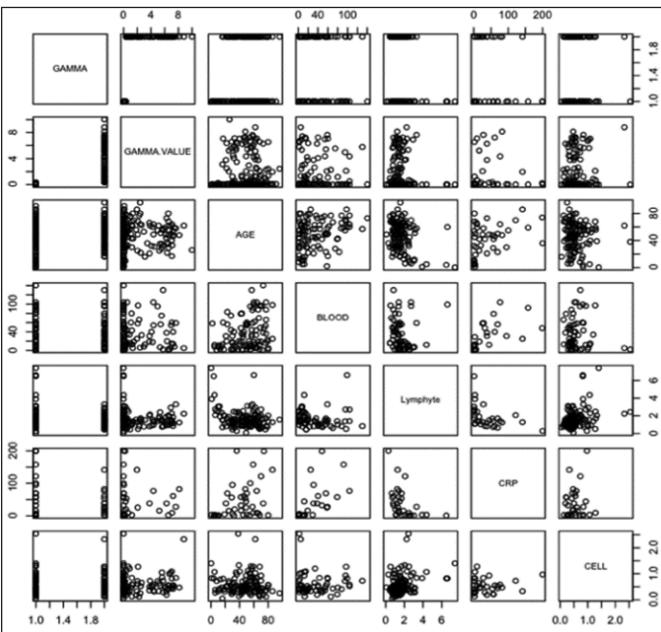


Figure-2: Overview of correlation scatterplot matrix.

The sensitivity of pathological results was 86% and specificity was 96%. The sensitivity of the IGRA test was 95% and specificity was 82% (Table 1). IGRA, pathological results and final diagnosis were significantly associated with each other (Table 2). Kappa value showed high degree of consistency between the two testing methods (Table 3). The amount of interferon-gamma released was positively correlated with the number of monocytes, CRP, number of lymphocytes and ESR ($p < 0.05$), and it was negatively correlated with age (Figure 1)

In the negative group B, there were significant differences in interferon release, lymphocytes, CRP and monocytes. ($p < 0.05$), and non- significant differences in terms of gender, age and EDR ($p > 0.05$) (Figure 2).

Scatterplot matrix showed all the interrelationships between explanatory variables. Analysis using logistic regression revealed a low Akaike Information Criterion index (AIC: 14) indicating a better fit model, and a reduced possibility of data overfitting. The analysis used gamma release amount as the dependent variable (IU/ml), while using age, EDR, lymphocyte, CRP and number of monocytes as the independent/explanatory variables. The median deviance residual was $-2.110e-08$ with the number of Fisher Scoring iterations at 25. After statistical reference, all five factors were found to be significant influencing factors ($p < 0.05$).

Discussion

The findings further established γ -interferon as a powerful complement to clinical traditional imaging and

pathological judgment of TB. Specifically, this indicates that the interferon test results were not independent of the pathological results and the final diagnosis, strengthening the fact that IGRAs test results and pathological results were also highly consistent between themselves. The IGRAs tests detects gamma interferon produced by T cells at a specific antigen of MTB; its purpose is to determine whether there is an infection of MTB. IGRAs can make up for the deficiency of purified protein derivative (PPD) test and, furthermore, it has been used in many countries to diagnose LTBI, a special state after host infection of MTB. The MTB in the infected person is in a state of retention and cannot be diagnosed as ATB, but has the risk of developing into ATB. Early diagnosis and appropriate intervention for high-risk groups of LTBI have significant impact in TB control.⁵ The results suggest that the specificity of IGRAs for the diagnosis of MTB infection was highly consistent with the pathological results and final diagnosis, which is consistent with related studies.⁶ As a result of Yunnan's status as a human immunodeficiency virus (HIV)-prone area, it is unsurprising that TB is the leading cause of death for HIV-infected patients. The reason, while IGRAs (especially cell detection) is less affected by the number of CD4+ T cells is that the number of individual nuclear cells has been normalised during the IGRAs test, i.e. the number of lymphocytes is reduced in the detection procedure, with its impact being increased sensitivity. The IGRAs diagnosis uses the genomic region of difference 1 (RD1) of MTB to encode an antigenic polypeptide as a specific antigen, mainly having an early secretory antigen target (ESAT-6) with a relative molecular mass of 6000 and a culture filtrate protein (CFP-10) antigen with a relative molecular mass of 10,000. On this basis, the TB7.7 antigen polypeptide was added.⁷ While ESAT-6 and CFP-10 are mainly present in the MTB complex, but are absent in bacille Calmette-Guerin (BCG) and most non-TB mycobacteria, the specificity of IGRAs is better. However, there are certain limitations in sensitivity to gamma interferon, especially in patients with impaired immune function, such as rheumatoid arthritis, organ transplants, and other special patients with diabetes, or for glucocorticoids.

Immunosuppressive drugs, such as tumour necrosis factor-alpha (TNF- α) antagonists, lead to defects in the immune function, and with the patient's immune system impaired, the result is the inability of T cells to produce γ -interferon. This could have caused the lower sensitivity of IGRAs to that of PPD.⁸ However, this type of patient is a high-risk group of LTBI who develops TB and henceforth, the interpretation of IGRAs test results in clinical work must be analysed based on patient's comprehensive factors in the best interest of the patient.

The current study focussed on age, gamma interferon release, lymphocyte count, CRP, monocytes and other influencing factors. According to relevant studies, non-steroidal anti-inflammatory drugs (NSAIDs)⁹, T cell activation inhibition agent¹⁰ and the application of glucocorticoids led to an increase in the total number of white blood cells, decreasing lymphocytes, resulting in a decrease in gamma interferon.¹¹ The current study also confirmed that γ -interferon release, hormone use, lymphocyte count and CRP were non-significant, thus reaffirming the belief that these factors need to be further reviewed when the results of the gamma interferon test are uncertain.

Conclusion

The specificity of IGRAs diagnosis of MTB was highly consistent with the final pathological results, but there were certain limitations in sensitivity. In patients with impaired immune system and immunodeficiency, clinical evidence should also be considered. At the same time, age, beta-interferon release, hormone use, lymphocyte number and CRP were found to be factors that need to be reviewed when the results of the interferon test are uncertain.

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Conflict of Interest: None.

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