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Statistical Analysis of Interferon-γ for the Effectiveness of an Anti-Tuberculous Treatment

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***Abstract*—**Tuberculosis (TB) is a potentially serious infectious disease that remains a health concern. The Interferon Gamma Release Assay (IGRA) is a blood test to find out if an individual is tuberculous positive or negative. This study applies statistical analysis to the clinical data of interferon-gamma levels of seventy-three subjects who diagnosed pulmonary TB in an anti-tuberculous treatment. Data analysis is performed to determine if there is a significant decline in interferon-gamma levels for the subjects during a period of six months, and to infer if the anti-tuberculous treatment is effective.

***Keywords*—**Data analysis, interferon gamma release assay, statistical methods, tuberculosis infection.

I. Introduction

TB is a major human health concern. The World Health Organization (WHO) estimated that in 2014, approximately 9.6 million people fell ill with TB, and 1.5 million people died as a result of this disease [8]. The progresses made in the diagnosis and treatment of TB has saved an estimated 43 million lives between 2000 and 2014. However, TB remains a leading cause of death worldwide. WHO adopted the End TB Strategy in May 2014 that serves as a blueprint for countries to reduce the number of TB death by 90% by 2030 (compared with 2015 levels), and to cut new cases by 80% [8]. Continuing research and development of effective TB diagnosis and treatment are needed to achieve the goal set by WHO.

TB is caused by *Mycobacterium tuberculosis* (*M. tb*), first discovered in 1882 by Dr. Robert Koch [4]. *M. tb* attacks lungs to get oxygen for its survival, and hence causes pulmonary TB. *M. tb* also attacks other organs like stomach, kidney, and brain to cause extrapulmonary TB. However, in most people, *M. tb* is contained by the host immune response and remains latent. During the lifetime of about 10% of these latently infected individuals, *M. tb* is able to overcome the immune response and lead to highly transmissible and often debilitating active disease. A widely used test to diagnose latent TB is IGRA. In contrast to the older skin test, where *M. tb* proteins are injected directly into the person to stimulate a measurable immune response, IGRA involves stimulating and measuring an immune response from a blood sample using more specific *M. tb* antigen [1]. IGRA is also used to monitor the treatment response of TB subjects by measuring the level of interferon-gamma (IFN-γ) level (IU/mL) released by blood cells after the stimulation [3], [7]. One of the two commercially available IGRA, QuantiFERON-TB Gold In-Tube assay (QFT-GIT), detects the level of IFN-γ produced in response to the *M. tb* antigens. Based on the interpretation criteria for QFT-GIT recommended by manufacturers a subject is considered TB positive if the IFN-γ ≥ 0.35 IU/ml and negative if IFN-γ < 0.35 [2].

In this study, clinical data was collected from seventy-three subjects diagnosed with pulmonary TB, who were treated with multidrug regimens for a six month period. Their IFN-γ levels were measured at the beginning of treatment (baseline), and also at the end of one month, two months, four months and six months. This paper describes how statistical methods are applied to the data, and to investigate if there is a significant decline in IFN-γ level based on the data analysis.

II. Summary of Data

Seventy-three subjects, diagnosed pulmonary TB by IGRA or other means like X-ray, underwent a six-month anti-TB treatment. The data of their baseline IFN-γ levels and those of one, two, four and six months after treatment are listed in an Excel file. A sample of the first four rows is shown in Fig. 1. As mentioned before IFN-γ level of 0.35 IU/ml is the cutoff to separate TB positive and TB negative.

Subject ID	baseline	1 month	2 months	4 months	6 months
A-03	1.525	1.365	0.335	1.6	0.43
A-05	2.07	2.275	1.95	1.48	2.735
A-06	1.255	0.255	0.435	0.085	0.16
⋮					

Fig. 1 The first 4 rows of the clinical data

In this section descriptive statistics of the clinical data are summarized. Table I shows the means and standard errors of the IFN-γ levels of the 73 pulmonary TB subjects at each of the five measurements over six months.

Table I indicates that the means of IFN-γ level decreases from 1.739 IU/ml at the beginning of treatment to 1.157 IU/mL at the end of 6 months with the lowest level 1.043 IU/mL detected at the end of 2 months. Although IFN-γ remains constantly above the 0.35 IU/ml threshold the treatment seems working in reducing the mean IFN-γ level but not in a very fast rate. Fig. 2 also shows the declination of the means of IFN-γ level over six months.

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TABLE I
MEANS AND STANDARD ERRORS OF IFN-γ LEVEL

	Baseline	1 Month	2 Months	4 Months	6 Months
Average	1.739	1.444	1.043	1.336	1.157
Standard error	0.156	0.153	0.128	0.142	0.126

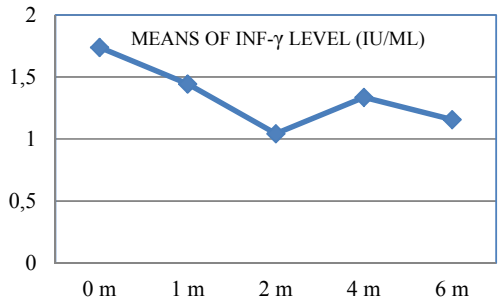

Fig. 2 Means IFN-γ level over six month treatment

Other important statistics of the data set, such as maximum, minimum, median, percentage of INF-γ level < 0.35, are listed in Table II.

TABLE II
MAXIMUM, MINIMUM, MEDIAN, AND PERCENTAGE

	Baseline	1 month	2 months	4 months	6 months
Max	4.10	3.86	4.03	3.99	3.91
Min	0	0	0	0	0
Median	1.57	0.93	0.63	0.95	0.79
INF-γ ≥ 0.35	57 (78%)	53 (73%)	48 (66%)	53 (73%)	55 (75%)
INF-γ < 0.35	16 (22%)	20 (27%)	25 (34%)	20 (27%)	18 (25%)

The percentages of INF-γ level < 0.35 for baseline and one, two, four and six months after treatment are also presented in Fig. 2.

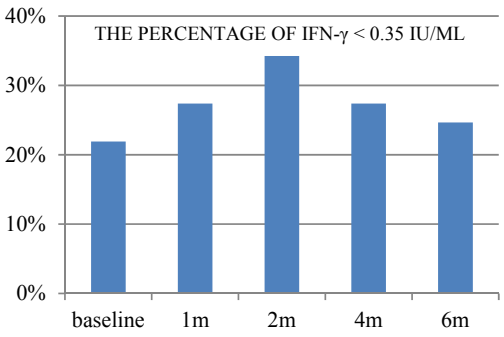

Fig. 3 The percent of IFN-γ < 0.35 IU/mL

Fig. 3 indicates that the percentage of subjects with IFN-γ level < 0.35 IU/mL increases from 22% at the beginning of the treatment to 25% after 6 months of treatment. It is also noticed that the biggest improvement in the percentage occurs after 2 months of treatment when a remarkable 34% of subjects tested IFN-γ level < 0.35 IU/mL. Furthermore, the percentage of subjects with IFN-γ level < 0.35 IU/mL increases for the first two months of treatment but gradually decreases after it reaches the peak at the end of two month. Still the percentages after 4 months and 6 months of treatment are higher than baseline percentage.

III. STATISTICAL ANALYSIS AND INFERENCE OF THE DATA

In this section, several statistical analysis methods are applied to the clinical data to investigate if there is a significant difference among the five measurements of IFN-γ levels over the six month period, and also quantitatively determine the mean IFN-γ level that is reduced by the treatment. The statistical calculations are carried out using SAS or Excel [6].

A. Analysis of Variance (ANOVA)

First, a single-factor ANOVA with a significance level of α = 0.05 is used to compare the means of the five measurements of IFN-γ levels. The objective is to test the null hypothesis that all the five means are the same against the alternative hypothesis that at least two of them are different. It follows from the test statistics $F = 3.650$ and the degrees of freedoms that P-value is 0.006. Since P-value is less than α, the null hypothesis is rejected. Therefore, the means of IFN-γ levels is not the same for all the five measurements over six months. It is thus concluded that the treatment does make a difference on the IFN-γ level over a six month periods.

TABLE III
ANOVA TABLE FOR THE CLINICAL DATA

Source of Variation	*SS*	*df*	*MS*	*F*
Treatments	21.297	4	5.324	3.650
Error	525.073	360	1.459	
Total	546.369	364		

B. Paired t Test

Next a paired t test is performed to compare the baseline IFN-γ levels with those at the end of six months. Let μ_1 = mean of baseline IFN-γ level (before the treatment), μ_2 = mean of IFN-γ level after 6 month treatment, and $\mu_d = \mu_1 - \mu_2$ = the mean difference between the IFN-γ levels before and 6 months after treatment. The paired t-test with a significance level of α = 0.05 is carried out for the null hypothesis H_0: $\mu_d = 0$ (no difference) versus alternative hypothesis H_a: $\mu_d > 0$ (IFN-γ level being lower after 6 month treatment).

TABLE IV
PAIRED T TEST FOR H_0: $M_D = 0$ VS H_A: $M_D > 0$

Pearson correlation	0.506
hypothesized mean difference	0
degree of freedoms	72
t Statistics	4.090
P (T <= t) one-tail	0.00006

Because P-value < α, the null hypothesis is rejected. It is concluded that the clinical data does provide the evidence that mean IFN-γ level is lower after 6 month treatment. Furthermore, paired t tests are used to quantitatively determine how much lower the IFN-γ level changes after 6 month of treatment. With a significance level of α = 0.05 a series of

paired *t*-tests are carried out for the null hypothesis H_0: $\mu_d = \mu_0$ versus alternative hypothesis H_a: $\mu_d > \mu_0$, with μ_0 being the hypothesized mean difference with values equal to 0.32, 0.33, 0.34 and 0.35 IU/mL, respectively.

TABLE V
PAIRED *t* TESTS FOR H_0: $\mu_d = \mu_0$ VS H_a: $\mu_d > \mu_0$

	H_0: $\mu_d = \mu_0$ vs H_a: $\mu_d > \mu_0$			
μ_0	0.32	0.33	0.34	0.35
t Statistics	1.841	1.771	1.701	1.630
P-value	0.035	0.040	0.047	0.053

Table V shows *P*-values of paired *t* tests for hypothesized mean differences μ_0 = 0.32, 0.33, 0.34 and 0.35, respectively. The *P*-values for μ_0 = 0.32, 0.33, and 0.34 IU/ml are less than the significant level α = 0.05, while *P*-value for μ_0 = 0.35 IU/ml is greater than α. The comparisons with significance level α = 0.05 leads to the conclusion that six-month treatment with multidrug regimens reduces IFN-γ level by about 0.34 IU/ml. The same technique is applied to compare the baseline mean IFN-γ level to those of one month, two months and four months treatment and it is found that the largest reduction occurs after two month treatment.

TABLE VI
MEAN IFN-γ LEVEL REDUCTION AFTER TREATMENT

	Mean IFN-γ level is reduced by
After 1 month	0.11 IU/ml
After 2 months	0.47 IU/ml
After 4 months	0.14 IU/ml
After 6 months	0.34 IU.ml

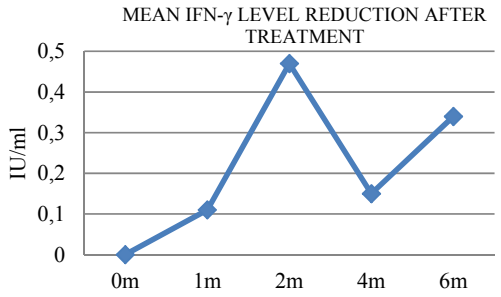

Fig. 4 Mean IFN-γ level reduction after treatment

Table VI and Fig. 4 indicate that the anti-TB treatment effectively reduces the mean IFN-γ level by 0.34 IU/mL after six months of treatment. Even more importantly, the best result of the treatment again occurs after two months of treatment when the mean IFN-γ level is reduced by 0.47 IU/mL.

C. McNemar's Test

To further confirm that the treatment has the best effect after two months McNemar's test is performed. Table VII is constructed based on the data set: 43 subjects tested positive (IFN-γ ≥ 0.35) in baseline remained positive after two months of treatment and 14 subjects tested positive in baseline changed to negative (IFN-γ < 0.35) after two months; meanwhile, 5 subjects tested negative in baseline changed to positive after 2 months and 11 remained negative after two months.

TABLE VII
TEST RESULTS CHANGE FROM BASELINE TO 2 MONTHS

Baseline	2 months after treatment		
	N (IFN-γ < 0.35)	P (IFN-γ ≥ 0.35)	Row total
N (IFN-γ < 0.35)	11	5	16
P (IFN-γ ≥ 0.35)	14	43	57
Column total	25	48	73

The null hypothesis of McNemar's test is "marginal homogeneity", that is, the 2 months of treatment has no effect. With a significance level α = 0.05 McNemar's test is applied to the data in Table VII and it is found that the test statistic is 4.263 and *P*-value is 0.0389, which is < α. Therefore, the null hypothesis is rejected and it is concluded that 2 months of the anti-TB treatment does have an effect.

IV. SUMMARY AND DISCUSSION

TB has been a critical health concern, and the development of effective diagnosis and treatment of TB has been the continuing efforts of researchers. IGRA is a test that assesses human immune response to tuberculous to determine if an individual is tuberculous positive or negative. Clinical data was collected from seventy-three subjects diagnosed with pulmonary TB, whose IFN-γ levels were monitored during six months of anti-TB treatment.

In this paper, statistical analysis is applied to the clinical data and verifies that there is a decline in IFN-γ levels between the beginning of the treatment and at the end of one, two, four and six months. Descriptive statistics indicates that the mean IFN-γ level declined and the percentage of subjects with TB negative increased. ANOVA and paired *t*-test confirmed that there is a difference in the IFN-γ level over a six month periods. A series of paired *t*-test also quantitatively determines that the IFN-γ level is reduced by 0.34 IU/mL after a 6 months treatment. All these results point to the fact that this anti-tuberculous treatment is effective although not at a fast pace as we would desire.

The statistical analysis also reveals a remarkable phenomenon that the best result of the treatment occurs not at the end of whole six months of treatment but at the end of the second month. Mean IFN-γ level is at the lowest, the percentage of subjects with TB negative is at its highest, and the IFN-γ level reduction is also at the highest after two month of treatment. This phenomenon is worth the attention of scientists, medical doctors and pharmaceutical researchers. One possible explanation is that *M. tb* starts to have drug resistance after two months so the treatment is more effective during the first two months. Another possible explanation is that an immune response develops to mycobacterial antigens after two months of particular drug dosages [5]. It is important to find a way to modify the treatment so that the significant

improvement during the first two months can continue to the end of treatment.

REFERENCES

[1] Centers for Disease Control & Prevention, *Tuberculosis (TB) Fact Sheets: Tuberculin Skin Testing*, Atlanta, GA, U.S. Retrieved Sept. 29, 2015, http://www.cdc.gov/tb/publications/factsheets/testing/skintesting.htm.

[2] ECDC (Europe Center for Disease Prevention and Control) GUIDANCE: *Use of interferon-gamma release assays in support of TB diagnosis*, Stockholm, March 2011. ISBN 978-92-9193-240-5.

[3] Kobashi, Y., Mouri, K., Yagi, S., Obase, Y., Miyashita, N., and Oka, M., "Transitional changes in T-cell responses to Mycobacterium tuberculosis-specific antigens during treatment", *Journal of Infection*, vol. 58, no. 3, pp. 197–204, 2009.

[4] Madigan, Michael T., et al. *Brock Biology of Microorganisms: Thirteenth edition.* Benjamin Cummings: Boston, 2012.

[5] Menzies, D., "Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion", *Am J Respir Crit Care Med*, 1999. 159(1): p. 15-21.

[6] Ronald P. Cody and Jefferey K. Smith, *Applied Statiistics and the SAS Programming Language*, 5th ed. Pearson Prentice Hall; 2006.

[7] Sauzullo, I., Mengoni, F., Lichtner, M. et al., "In vivo and in vitro effects of antituberculosis treatment on mycobacterial interferon-γ T cell response," *PLoS ONE*, vol. 4, no. 4, Article ID e5187, 2009.

[8] World Health Organization Global Tuberculosis Report 2015, Executive Summary pp 1 – 3. 2015.