

Indeterminate Results of an Interferon-Gamma Release Assay Indicate an Increased Risk for Progression to AIDS Irrespective of CD4⁺ T Cell Count

Maximilian C. AICHELBERG¹, Julia TITTES¹, Florian BREITENECKER¹,
Norbert KOHRGRUBER¹, and Armin RIEGER¹

¹Department of Dermatology, Division of Immunology, Allergy and Infectious Diseases (DIAID),
Medical University of Vienna, Vienna, Austria

BACKGROUND

- *Mycobacterium tuberculosis*-specific Interferon- γ release assays are now widely used effective screening tools for the detection of tuberculosis (TB) and their implementation has been incorporated in several national TB guidelines.
- Despite the fact that their performance is dependent on the actual CD4⁺ T cell count, these tests have been demonstrated to perform well in HIV-1 infection.^{1,2}
- Indeterminate results of the QuantiFERON-TB Gold In-Tube assay (QFT-GIT) have been associated with highly advanced age and underlying disease in patients receiving immunosuppressive treatment.³
- Among HIV-1 infected individuals both men and patients aged ≥ 33 were more likely to have an indeterminate QFT-GIT result.⁴
- However, the prognostic value of indeterminate QFT-GIT results is unknown.

OBJECTIVES

- To evaluate socio-epidemiological and clinical risk factors associated with indeterminate as compared to determinate QFT-GIT results.
- To compare these results in terms of their predictive value for the subsequent development of AIDS-defining diseases and death.

MATERIAL & METHODS

- part of our prospective, longitudinal, single-center study.⁵
- the QFT-GIT result was indicated as indeterminate when either (i) unprovoked IFN- γ levels were ≥ 8.0 IU/ml in the nil plasma or (ii) the IFN- γ response was ≤ 0.5 IU/ml upon PHA stimulation with a TB antigen minus a negative control IFN- γ response of either ≤ 0.35 IU/ml or $\leq 25\%$ of the IFN- γ concentration in the nil plasma.
- clinical monitoring for TB, other AIDS-defining manifestations and death at least every 3 months for 3 years.

RESULTS

Baseline characteristics of the study participants

A total of 955 HIV-1 infected individuals were asked to participate in this study. After excluding seven patients who declined participation the remaining 948 subjects underwent QFT-GIT testing.

The QFT-GIT assay yielded indeterminate results in 5.8% (55/948) and determinate results in 94.2% (893/948) of subjects, respectively. All indeterminate QFT-GIT results were due to inadequate INF- γ response to the positive control.

There was no statistical significant difference between QFT-GIT determinate and indeterminate subjects in terms of sex, age and ethnic background. Patients with an indeterminate QFT-GIT were more likely having acquired HIV-1 infection by intravenous drug abuse than subjects with determinate QFT-GIT results ($P < 0.0125$). Among patients with indeterminate QFT-GIT results, both median actual and nadir CD4⁺ T cell counts were significantly lower (188 vs. 393 cells/mm³; $P < 0.0001$ and 107 vs. 208 cells/mm³; $P = 0.0001$) and HIV-1 RNA levels were significantly higher (3.7 vs. 1.8 log₁₀ HIV-1 RNA, $P = 0.0024$) than in subjects with interpretable results. Forty percent vs. 20.6% of subjects with an indeterminate and determinate QFT-GIT result had previously been diagnosed with an AIDS-defining disease ($P < 0.0001$). Although a higher percentage of patients with determinate as compared to indeterminate QFT-GIT results were receiving ART, this difference was not statistical significant (56.0% vs. 47.3%, $P = 0.6566$).

QFT-GIT results by CD4⁺ T cell count strata

Seventy-one patients with < 100 CD4⁺ T cells/mm³ were included in this study. 21.1% (15/71) thereof had an indeterminate result. Among patients with 100-199 CD4⁺ T cells/mm³ 15.3% (15/98) subjects had an indeterminate QFT-GIT whereas 84.7% (83/98) patients had a determinate QFT-GIT. In comparison, among patients with CD4⁺ T cell counts above 200 cells/mm³, only 8% (25/779) of subjects showed an indeterminate QFT-GIT, respectively (Table 1).

Predictors for indeterminate QFT-GIT results

Unadjusted analysis revealed the following risk factors of indeterminate QFT-GIT results: injection drug use (OR, 2.1; 95% CI, 1.2-3.8; $P = 0.0125$), CD4⁺ T cell count of less than 100 cells/mm³ (OR, 5.6; 95% CI, 2.9-10.8; $P < 0.0001$), HIV-1 RNA > 100 000 copies/ml (OR, 3.6; 95% CI, 2.0-6.6; $P < 0.0001$) and prior AIDS-defining manifestation (OR, 2.9; 95% CI, 1.7-5.1; $P < 0.0001$) (Table 2).

Development of AIDS-defining illnesses and/or death during follow-up

During the observational period 37 individuals (3.9%; 37/948) were diagnosed with an AIDS-defining manifestation with a median delay of 403.5 days (IQR=313.25-439.75) from inclusion. At baseline, 15% (8/55) and 3% (29/893) had been tested QFT-GIT indeterminate and determinate, respectively (OR, 5.1; 95% CI, 2.2-11.7; $P < 0.0001$).

Overall, 41 patients died during follow-up. The most frequent cause of death was bacterial and/or fungal infection followed by acute respiratory distress syndrome.

The rate of progression to AIDS or death was 22% (12/55) among QFT-GIT indeterminate subjects and 7% (58/893) among patients with positive or negative QFT-GIT results ($P < 0.0001$) (Table 3).

Progression to AIDS and/or death among individuals with a CD4⁺ T cell count < 200 cells/mm³

There was no difference between QFT-GIT determinate and indeterminate subjects in terms of median CD4⁺ T cell count, HIV-1 RNA level and ART.

Within the follow-up period however, AIDS-defining manifestations occurred in 7 QFT-GIT indeterminate compared to 11 QFT-GIT determinate individuals (23.3% vs. 8.0%; OR, 3.5; $P < 0.05$) (Table 4).

TABLE 1. STRATIFICATION OF QFT-GIT RESULTS BY CD4⁺ T CELL COUNTS

	CD4 ⁺ Strata (cells/mm ³)				Total
	< 100	100-199	200-350	> 350	
	QFT-GIT Results by CD4 ⁺ T Cell Count Strata (n = 948)				
QFT-GIT determinate	56 (79%)	83 (85%)	223 (95%)	531 (97%)	893
QFT-GIT indeterminate	15 (21%)	15 (15%)	11 (5%)	14 (3%)	55
Total	71	98	234	545	948

Definition of abbreviations: QFT-GIT = QuantiFERON-TB Gold In-Tube assay

TABLE 2. PREDICTORS FOR INDETERMINATE QFT-GIT RESULTS

	Total number of subjects with QFT-GIT results (n = 948)	Total number of subjects with indeterminate QFT-GIT results (n = 55)	OR (95% CI)	P
Male sex	664	33	0.6 (0.4-1.1)	0.0939
Age > 30 years	719	39	0.6 (0.3-1.1)	0.0841
Injection drug use	187	18	2.1 (1.2-3.8)	0.0125
CD4 ⁺ T cell count < 100 cells/mm ³	71	15	5.6 (2.9-10.8)	< 0.0001
HIV-1 RNA > 100 000 copies/ml	135	18	3.6 (2.0-6.6)	< 0.0001
On ART, at enrollment	526	26	0.9 (0.5-1.5)	0.6566
Prior AIDS manifestation	206	22	2.9 (1.7-5.1)	< 0.0001

Definition of abbreviations: CI = confidence interval; OR = odds ratio; QFT-GIT = QuantiFERON-TB Gold In-Tube assay

TABLE 3. DEVELOPMENT OF AIDS-DEFINING ILLNESSES AND/OR DEATH DURING FOLLOW-UP

	QFT-GIT determinate (n = 893)	QFT-GIT indeterminate (n = 55)	OR (95% CI)	P
Active TB	6 (1%)	0 (0%)	1.2 (0.1-22.1)	---
AIDS-defining manifestations including TB	29 (3%)	8 (15%)	5.1 (2.2-11.7)	< 0.0001
Other AIDS-defining manifestations	23 (3%)	8 (15%)	6.4 (2.7-15.2)	< 0.0001
Death	37 (4%)	4 (7%)	1.8 (0.6-5.3)	0.2682
AIDS or Death	58 (7%)	12 (22%)	4.0 (2.0-8.0)	< 0.0001

Definition of abbreviations: OR = odds ratio; QFT-GIT = QuantiFERON-TB Gold In-Tube assay; TB = tuberculosis

TABLE 4. DEVELOPMENT OF AIDS-DEFINING ILLNESSES AND/OR DEATH DURING FOLLOW-UP IN INDIVIDUALS WITH CD4⁺ COUNTS < 200 CELLS/MM³

	QFT-GIT determinate (n = 138)	QFT-GIT indeterminate (n = 30)	OR (95% CI)	P
Median CD4 ⁺ T cell count, at enrollment [IQR]	116 [65-160.8]	94 [24.75-140.3]	...	0.1616
Median log ₁₀ HIV-1 RNA [IQR]	4.385 [1.7-5.275]	5 [2.83-5.23]	...	0.1183
On ART, at enrollment	66 (48%)	10 (33%)	0.6 (0.2-1.3)	0.1624
Active TB	0 (0%)	0 (0%)	...	---
Other AIDS-defining manifestations	11 (8%)	7 (23%)	3.5 (1.2-10.0)	0.0137
Death	16 (12%)	3 (10%)	0.8 (0.2-3.1)	0.8027
AIDS or Death	26 (20%)	10 (33%)	2.1 (0.9-4.9)	0.1422

Definition of abbreviations: ART = antiretroviral therapy; IQR = interquartile range; OR = odds ratio; QFT-GIT = QuantiFERON-TB Gold In-Tube assay; TB = tuberculosis

SUMMARY & CONCLUSION

- The QFT-GIT yielded indeterminate results in 5.8% of the 948 HIV-1 infected individuals tested in this study.
- In patients with indeterminate QFT-GIT results, median CD4⁺ T cell counts were significantly lower as compared to subjects with a determinate QFT-GIT result.
- The rate of progression to AIDS-defining illnesses was significantly higher among individuals with an indeterminate QFT-GIT result as compared to patients with a determinate QFT-GIT result.
- In patients with a CD4⁺ T cell count of less than 200 cells/mm³, an indeterminate QFT-GIT result indicated a significantly increased risk for the development of AIDS-defining diseases.

⇒ Among patients with advanced immunodeficiency, those with an indeterminate QFT-GIT result are at increased risk of serious clinical events other than TB.

⇒ Thus, in HIV-1-infected patients with advanced quantitative CD4⁺ T cell depletion, an indeterminate QFT-GIT might indicate an additional loss of global T cell function.

References

1. Rangaka MX, Wilkinson KA, Seldon R, et al. (2007). Effect of HIV-1 infection on T-cell-based and skin test detection of tuberculosis infection. *Am J Respir Crit Care Med*, 175(5): 514-20.
2. Aichelberg MC, Rieger A, Breitenecker F, et al. (2009). Detection and prediction of active tuberculosis disease by a whole-blood interferon- γ release assay in HIV-1 infected individuals. *Clin Infect Dis*, 48(7): 954-962.
3. Kobashi Y, Sugita T, Mouri K, Ohase Y, Miyashita N, Oka M. (2009). Indeterminate results of QuantiFERON TB-2G test performed in routine clinical practice. *Eur Respir J*, 33(4): 812-5.
4. Aabye MG, Ravin P, Pray-God G, et al. (2009). The impact of HIV infection and CD4 cell count on the performance of an interferon gamma release assay in patients with pulmonary tuberculosis. *PLoS ONE*, 4:e4220.