Use of the QuantiFERON®-TB Gold Assay in Pregnant Patients

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Abstract

Background. The QuantiFERON®-TB Gold assay is a new test for latent tuberculosis infection. It is thought to be more reliable and have fewer false positives than the tuberculin skin test (TST). Both tests are dependent on a normal immune system for diagnostic accuracy. However, no comparisons of the two tests have assessed the accuracy in pregnant women. This investigation assessed the consistency of results between the two tests in both pregnant and non-pregnant women.

Methods. The study included 152 women presenting for care at the Sedgwick County Health Department. They were divided into two groups of pregnant and non-pregnant women. Both groups were assessed with the Quantiferon assay and the TST. Subjects were adults between the ages of 18 and 45. All had a pregnancy test and a negative HIV test. None had existing morbidities that would influence the test results.

Results. Concordant results between the tests were shown in 131 subjects (86.2%). Of the pregnant women, 91.2% had concordant results. Of the non-pregnant women, 76% had concordant results. Significantly more discordant results occurred in non-pregnant women ($p<.022$).

Conclusion. Current guidelines favor using either test in healthy individuals. Although more discordant results occurred in the non-pregnant women, both tests were effective in pregnant women. Thus, TST and Quantiferon are accurate to use in pregnant women. The decision to use either test in pregnant women should be based mainly on the compliance of the patient to return to have the TST read. *KJM 2010; 3(2):24-30.*

Introduction

Tuberculosis is a communicable disease caused by infection with *M. tuberculosis* complex organisms, which typically spreads to new hosts via airborne droplet nuclei from patients with respiratory tuberculosis disease.[1] A newly-infected individual can become ill from tuberculosis within weeks to months, but most infected individuals remain well. Latent tuberculosis infection (LTBI), a non-communicable asymptomatic condition, persists in some who might develop tuberculosis disease months or years later.[1,2]

Until recently, the tuberculin skin test (TST) was the only available method for diagnosing LTBI. Cutaneous sensitivity to tuberculin develops from 2 to 10 weeks after infection. This test uses a small amount of purified protein derivative (PPD) prepared from *M. tuberculosis,* placed intradermally

and measured 48 to 72 hours later to identify the delayed hypersensitivity response, indicating previous infection.[2] However, some infected individuals, including those with a wide range of conditions hindering immune functions, but also others without these conditions, do not respond to tuberculin. Conversely, some individuals who are unlikely to have *M. tuberculosis* infection exhibit sensitivity to tuberculin and have positive tuberculin skin test (TST) results after vaccination with bacilli Calmette-Guerin (BCG) infection with mycobacteria other than *M. tuberculosis* complex or undetermined other factors.[3-5]

LTBI must be distinguished from tuberculosis disease, a reportable condition which usually involves the lungs and lower respiratory tract, although other organ systems also may be affected. Tuberculosis disease is diagnosed from historical, physical, radiological, histological, and mycobacteriological findings.[1,5,6]

The QuantiFERON®-TB Gold test is a new test for cell mediated immune (CMI) responses to peptide antigens that simulate mycobacterial proteins.[4] These proteins, ESAT-6 and CFP-10, are absent from all BCG strains and from most non-tuberculosis mycobacteria with the exception of *M. kansasii*, *M. szulgai*, and *M. marinum.* It is thought to be more reliable and has fewer false positives than the TST. Individuals infected with *M. tuberculosis* complex organisms usually have lymphocytes in their blood that recognize these and other mycobacterial antigens. This recognition process involves the generation and secretion of the cytokine, IFN-γ. The detection and subsequent quantification of IFN-γ forms the basis of this test.[4,7-9]

Since the Quantiferon test is dependant on immune mediators and factors, like TST, it also is affected by changes in the immune system of the tested subjects.[10,11] Both tests are dependent on a normal immune system for diagnostic accuracy. Therefore, any condition that alters the immune system, especially that will depress this system, theoretically can cause false-negative results with this test.[12-16]

In the US Centers for Disease Control and Protection (CDC) guidelines of 2003, pregnancy was one of the conditions in which use of this test was not recommended, because pregnancy has the potential to decrease the immune response.[7] In the CDC guidelines of 2005, the spectrum to use QuantiFERON®-TB Gold was broadened and pregnancy no longer showed as one of the conditions for which it is not recommended.[17] However, no serious investigations have been done concerning the correlation of pregnancy with the accuracy of the Quantiferon assay, and the CDC arbitrarily included, then excluded, pregnancy in the contraindication category for that assay.

This investigation assessed the consistency of results between the the QuantiFERON®-TB Gold assay and the TST in both pregnant and non-pregnant women.

Methods

Study population. Subjects were women presenting for care at the Sedgwick County Health Department. Eligibility criteria included:

- Female patients
- Age between 18 and 45 years old
- HIV negative status
- No history of corticosteroids use, taking the equivalent of greater than 15 mg/day of prednisone for one month or more
- Non-diabetic patients
- Non-transplant patients
- No current treatment with immuno-suppressive drugs
- No renal failure

- No pre-existing hematologic problems (e.g., myeloproliferative disorders, leukemias, and lymphomas)
- No pre-existing malignancies (e.g., carcinoma of the head, neck, or lung)

Exclusion criteria included:

- Abortion prior to study completion
- Conversion to HIV positive during study participation
- Any diversion from the eligibility criteria
- Withdrawal of informed consent by the subject

Procedures. Approval of the Institutional Review Board at the University of Kansas School of Medicine-Wichita was obtained. Subjects were recruited by clinical personnel at the Sedgwick County Health Department. All pregnant subjects were recruited during visits to the prenatal clinic. Non-pregnant subjects were recruited from other Health Department clinics.

Each woman signed an informed consent in either Spanish or English, as appropriate. Each subject was given four clinical tests. Two tests determined eligibility for participation: a pregnancy test and an HIV test. Then, the two TB tests (Quantiferon and TST) were given when eligibility was established. Women who were pregnant received the TST as part of their usual care regardless of study participation.

Blood was drawn by clinical personnel at the Health Department to determine pregnancy (for the non-pregnant control group) and HIV status. Pregnant subjects did not receive an additional pregnancy test because they entered the study with a known pregnancy status from the prenatal clinic. Subjects were required to return to the Health Department two to three days after the TST to have the test results read by a trained person. Laboratory testing was performed according to standard Health Department policy and procedures.

Tuberculin Skin Testing. The TST was administered by the Mantoux method using 0.1 ml (5TU) of Tubersol (Connaught Laboratories Inc., Toronto, Ontario) and interpreted by trained clinical personnel according to American Thoracic Society (ATS)/CDC guidelines.[18] Transverse induration at the TST site was measured 48 to 78 hours after injection of purified protein derivative (PPD). TST results were interpreted using the risk-stratified interpretation of induration as recommended by the ATS/CDC guidelines.

QuantiFERON®-TB Gold Assay. The assay was performed and interpreted according to the manufacturer's instructions using previously described cut-points to identify infected persons.

Statistical measures. Statistical measures of agreement (*kappa*) were performed using MedCalc for Windows, version 10.1.3 (MedCalc Software, Mariakerke, Belgium). All other tests were conducted in StatXact, version 8.0.0, Cytel Studio.

Results

A total of 152 women (102 pregnant subjects and 50 non-pregnant controls) participated in the study. All met study criteria to participate. Group demographics according to pregnancy status are shown in Table 1. Pregnant subjects were younger, with greater representation of Caucasians and Hispanics. Non-pregnant subjects were older and more racially diverse, but with fewer Hispanics.

Table 2 shows concordant results between the two TB tests in 131 subjects (86.2%). A *kappa* statistic revealed fair agreement between the two tests (K=0.288).[19]

Table 3 shows the TB test results of the 102 pregnant women. A total of 93 (91.2%) had concordant results between both tests and nine had discordant results (8.8%). A *kappa* statistic revealed fair agreement

Table 1. Composition of the pregnant and non-pregnant subject groups.

	Pregnant Subjects n=102[#]	Non-Pregnant Subjects N=50[#]	*p*
Mean Age (sd)[*]	25.94 (5.25)	29.34 (7.25)	0.004
Race [§]			
Caucasian (%)	97 (.97)	37 (.79)	<0.001
African-American (%)	3 (.03)	6 (.13)	
Asian (%)	0 (.00)	4 (.08)	
Ethnicity[§]			
Hispanic (%)	87 (.87)	12 (.26)	<0.001
Non-Hispanic (%)	13 (.13)	35 (.74)	

[#] Race and ethnicity data were available for 47 of the 50 non-pregnant and 100 of 102 pregnant subjects.
[*] Two sample t-test with unequal variance.
[§] Pearson Chi-Square Test.

Table 2. Comparisons between the QuantiFERON®-TB Gold assay and TST results for all subjects.

		Quantiferon			
		Positive	Negative	Indeterminate	*kappa*
TST	Positive	5 (3.3%)	15 (9.9%)	1 (.7%)	0.288
	Negative	2 (1.3%)	126 (82.9%)	3 (2.0%)	

Table 3. Comparisons between the QuantiFERON®-TB Gold assay and TST results for pregnant subjects.

		Quantiferon			
		Positive	Negative	Indeterminate	*kappa*
TST	Positive	3 (3%)	7 (7%)	0 (0%)	0.358
	Negative	2 (2%)	90 (88%)	0 (0%)	

Table 4. Comparisons between the QuantiFERON®-TB Gold assay and TST results for all non-pregnant subjects.

		Quantiferon			
		Positive	Negative	Indeterminate	*kappa*
TST	Positive	2 (4%)	8 (16%)	1 (2%)	0.213
	Negative	0 (0%)	36 (72%)	3 (6%)	

Table 5. Level of concordance between the two TB tests in pregnant and non-pregnant subjects.

	Concordant	Discordant	*p*[*]
Pregnant	93 (91%)	9 (9%)	0.022
Non-Pregnant	38 (76%)	12 (24%)	

[*] Fisher's Exact Test.

between the tests for pregnant women (*K*=0.358).

Table 4 shows the TB test results of the 50 non-pregnant women. A total of 38 (76%) had concordant results between both tests and 12 (24%) had discordant results. A *kappa* statistic also revealed fair agreement between the tests for non-pregnant women (*K*=0.213).

Fisher's exact testing revealed that significantly more discordant results occurred in non-pregnant than pregnant women ($X^2 = 6.159$; $p<.022$; see Table 5).

Discussion

The increase in prevalence of tuberculosis and the emergence of multidrug-resistant strains have created a public health urgency for early identification of *M. tuberculosis*-infected individuals.[1,2] The gold standard for detecting exposure remains the TST and is one of the oldest tests still in clinical use. Despite the long history of clinical application, limitations and controversy with regard to placement and interpretation remain.[2-4]

The TST has several drawbacks including false-positive reactivity due to non-tuberculin strains, such as BCG, inter-observer variability in reading, false-negative results due to underlying immunosuppression, and variability with repeat testing. Due to changes in disease prevalence and demographics, a heightened need for early detection and better testing methodologies has emerged.[2-5]

The Quantiferon test and TST are dependant on immune mediators and factors, thus, affected by changes in the immune system of the tested subjects. Pregnancy certainly falls within that category. However, current guidelines favor using either test in healthy individuals.[9,20]

In this study, results between the two TB tests in all subjects (86.2%) showed fair agreement between the two tests with 91.2% of pregnant women and 76% of non-pregnant women having concordant results. Interestingly, concordance between test results in a population of university international students was only 59%.[21]

Although the groups in this study differed by age and race/ethnicity, it is doubtful whether these differences were clinically significant. No evidence is available that indicates that delayed type hypersensitivity reacts differently by race/ethnicity or in the relatively narrow age range of the reported subjects.

Even though more discordant results occurred in the non-pregnant women, both tests were effective in pregnant women. To our knowledge, this is the first study that compared both tests in pregnant individuals. In conclusion, the TST and Quantiferon tests can be used with pregnant women. The decision to use either test in pregnant women should be based mainly on the compliance of the patient to return to have the TST read.

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