ORIGINAL ARTICLE

Quantitative IFN-γ and IL-2 Response Associated with Latent Tuberculosis Test Discordance in HIV-infected Pregnant Women

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Abstract

Rationale: Pregnant women with latent tuberculosis infection (LTBI) are at high risk for development of TB, especially if infected with HIV.

Objectives: To assess the performance of LTBI tests in pregnant and postpartum women infected with HIV, investigate the immunology behind discordance in pregnancy, and explore the implications for the development of postpartum TB.

Methods: We screened pregnant women in their second/third trimester and at delivery for LTBI using the tuberculin skin test (TST) and IFN-γ release assay (IGRA) (QuantiFERON Gold). A subset of antepartum women had longitudinal testing, with repeat testing at delivery and postpartum and additional cytokines measured from the IGRA supernatant. The kappa statistic and Wilcoxon rank sum test were used to determine agreement and comparison of cytokine concentrations, respectively.

Measurements and Main Results: Of 252 enrolled, 71 (28%) women had a positive IGRA but only 27 (10%) had a positive TST ($P < 0.005$). There was 75% agreement (kappa, 0.25). When stratified by pregnancy versus delivery, 20% had $IGRA^+/TST^-$ discordance at each time point. A positive IGRA was associated with known TB contact (odds ratio, 3.6; confidence interval, 1.2–11.1; $P = 0.02$). Compared with $IGRA^+/TST^+$, women with $IGRA^+/TST^-$ discordance had significantly less IFN-γ (1.85 vs. 3.48 IU/ml; $P = 0.02$) and IL-2 (46.17 vs. 84.03 pg/ml; $P = 0.01$). Five developed postpartum TB, of which three had $IGRA^+/TST^-$ discordance during pregnancy.

Conclusions: Choice of LTBI test in pregnant women infected with HIV affects results. Pregnant women with $IGRA^+/TST^-$ discordance had less IFN-γ and IL-2 than those with concordant-positive results and may represent an especially high-risk subset for the development of active TB postpartum.

Keywords: IFN-γ release assay; tuberculin skin test; pregnancy; HIV; tuberculosis

Active tuberculosis (TB) is most common in women between 15 and 45 years old, the childbearing years (1, 2). In India, the country with the highest burden of TB, 75% of cases in women are diagnosed in this age group (2). Postpartum TB incidence is also high (3–7). Because TB is a major cause of maternal mortality, especially among women infected with HIV (8, 9), understanding how pregnancy affects diagnostics and the disease course would improve TB prevention efforts in this high-risk population.

Pregnancy instigates complex changes in cell-mediated immunity (10).

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At a Glance Commentary

Scientific Knowledge on the Subject: Tuberculosis (TB) is a major killer of women of reproductive age infected with HIV. Few countries, however, follow World Health Organization recommendations to give isoniazid preventive therapy to all pregnant women infected with HIV. Instead, most treat only women who test positive for latent TB. This approach is problematic because the dynamic effects of pregnancy on latent TB tests in women infected with HIV are poorly understood.

What This Study Adds to the Field: Choice of latent TB test affects results in pregnant women infected with HIV: 20% have IFN-γ release assay–positive/tuberculin skin test–negative discordance. This discordance is associated with lower IFN-γ and IL-2 response. The magnitude of changes in IFN-γ and IL-2 response during pregnancy may impact the development of postpartum TB.

As pregnancy progresses, cell-mediated immune function and T-helper cell type 1 (Th1) response decline, increasing the risk or severity of several infections, including influenza and listeria (10, 11). Th1 response recovers 6–12 weeks postpartum (4, 5). Understanding of the details of these changes, particularly as they relate to TB, remains incomplete.

Our previous study of pregnant Indian women uninfected with HIV identified significant discordance between the two most commonly used latent TB infection (LTBI) tests, the tuberculin skin test (TST) and the IFN-γ release assay (IGRA) (12), which both rely on cell-mediated immunity. The differential effects of pregnancy on the tests provide a window into the immunology of pregnancy and the host's response to TB. Understanding discordance may provide insight into the underlying complex changes that increase the risk of TB for postpartum women.

In this study, we aimed to (*1*) determine if TST/IGRA discordance exists in pregnant women infected with HIV in a high-burden setting, (*2*) assess how the performance of these tests changes with stage of pregnancy; and (*3*) investigate immunologic contributors to $IGRA^{+}/TST^{-}$ discordance in pregnancy. We further discuss the implications for predicting postpartum TB development. Some of the results of these studies have been previously reported in the form of abstracts (13, 14).

Methods

From 2011 to 2014, we enrolled pregnant women infected with HIV into a diagnostic LTBI study at Sassoon General Hospital, a public teaching hospital affiliated with Byramjee Jeejeebhoy Government Medical College in Pune, India. Each morning, women presenting to the antenatal clinic in their late second/third trimester or to the delivery ward were approached for enrollment. The first 100 women who consented for the cross-sectional study at each site underwent TST and IGRA testing once at enrollment during pregnancy or at delivery. An additional 50 antepartum women consented to a longitudinal study to evaluate the impact of stage of pregnancy on testing. This cohort underwent repeat testing at delivery and 3 months postpartum. Women in both the cross-sectional and longitudinal studies received telephone calls every 3 months until 1 year postpartum to determine the incidence of postpartum active TB. We included women greater than or equal to 18 years old. We excluded those with a history of allergic reaction to the TST, current active TB, or an immunosuppressive condition other than HIV. The longitudinal cohort planned to deliver at Sassoon Hospital and agreed to an in-person follow-up at 3 months postpartum.

Trained counselors administered sociodemographic questionnaires, including the Household Food Insecurity Access Scale (15). Trained nurses obtained medical and obstetric histories, including HIV history, TB risk factors, and the World Health Organization (WHO)-recommended TB symptom screen (16). Women with positive screens were referred to a physician for evaluation. If active TB was excluded, the woman was eligible for enrollment.

Laboratory Testing

Trained laboratory staff obtained 3 ml of blood per enrollee for IGRA (QuantiFERON Gold In-Tube test [QGIT]; Cellestis, Valencia, CA) testing; 1 ml each for the negative control ("nil") tube, positive mitogen control tube, and *Mycobacterium tuberculosis* (MTB)-specific antigen tube. In the delivery group, blood was collected within 48 hours after delivery. The test was performed in accordance with manufacturer instructions (17) at a laboratory certified by the College of American Pathologists to perform QGIT testing. ELISA was repeated on samples with indeterminate QGIT results. If the repeat sample was indeterminate, it was recorded as such. If the sample was positive or negative, that result was recorded. Laboratory staff were blinded to the subject's clinical data, including TST results.

After phlebotomy for QGIT, trained nurses (n = 3) injected 0.1 ml (5 TU) of tuberculin purified protein derivative (Span Diagnostic Ltd., Surat, India) intradermally into the volar surface of the forearm. Outpatients were asked to return in 48–72 hours for TST interpretation by trained nurses using the ballpoint pen and ruler technique (18). Nurses were blinded to QGIT results. A compensation of INR 100 (∼$2 USD) was given to cover travel costs per local institutional review board (IRB)-recommended norms. For women enrolled at delivery, attempts were made to interpret the TST within 48–72 hours, before hospital discharge. A positive TST was defined as induration greater than or equal to 5 mm (19).

Laboratory results from both the cross-sectional and the longitudinal groups were included for antepartum analysis. Results from the tests administered to the longitudinal group at delivery were not included in the cross-sectional analysis because TSTs they received during pregnancy may have impacted subsequent results.

Remaining QGIT supernatants from nil, TB antigen, and mitogen tubes were stored at −80°C. We measured IL-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF), and IL-17a per manufacturer's instructions for the Human Th1/Th2/Th17 Cytokine BD Cytometric Bead Array (CBA) (20) on a three-color BD FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Results were processed using FCAP Array software version 3 (BD Biosciences, San Jose, CA).

This study was approved by the Johns Hopkins University IRB, the Weill Cornell Medical College IRB, the Byramjee

Jeejeebhoy Government Medical College IRB, and the Byramjee Jeejeebhoy Government Medical College Ethics Committee in India. All subjects provided written informed consent.

Statistical Analysis

The kappa statistic was used to quantify agreement between QGIT and TST. With our sample size, a two-sided 95% confidence interval (CI) for the kappa statistic would extend less than or equal to 0.17 from the observed value of kappa, assuming the true value of kappa is in the range 0.50–0.70 and the approximate prevalence of latent TB is 20–30%. For comparison of categorical variables, Fisher exact test was used. The Wilcoxon rank sum test was used for comparison of continuous variables. All *P* values were two-sided with statistical significance evaluated at the 0.05 alpha level. Risk factors for TST or QGIT positivity and test discordance (e.g., $TST^+/QGIT^-$ or $TST^-/QGIT^+$) were calculated using a logistic regression model. From this, odds ratios (OR) with 95% CI were determined. Variables that were statistically significant or had a trend toward significance ($P < 0.2$) in univariate analysis or were of clinical importance were included in the multivariate analysis. All data were entered into an onsite Microsoft Access database. Analyses were performed in Stata Version 12.0 (College Station, TX) and GraphPad PRISM Version 6 (La Jolla, CA).

Results

Cross-sectional Comparison

We enrolled 252 women; 149 antepartum and 103 women at delivery. Table 1 describes their baseline characteristics. Median CD4 count was over 400 cells/mm^3 and 116 (46%) were on combination three-drug antiretroviral therapy (cART), 44 (38%) of whom initiated cART during pregnancy. Median gestational age for antepartum women was 26 weeks (interquartile range [IQR], 22–31) and most (84%) had antenatal visits before enrollment. Only three (1%) women reported prior history of TB but 15 (6%) reported close contact with someone with pulmonary TB. Five (33%) reported exposure to multidrug-resistant TB. No participants had received isoniazid preventive therapy (IPT).

Table 1. Participant Characteristics and LTBI Test Results by Time Point of Screening

Characteristic	Antepartum (*n* = 149)	Delivery (*n* = 103)	*P* Value
Household sociodemographics			
Urban/periurban residence, n (%)	129 (86)	72 (69)	0.005*
House with ⩽2 rooms, n (%)	130 (87)	85 (82)	0.78
Median adults in house	2 (2–4)	2 (2–5)	0.53
Median children in house	1 (1–2)	2 (1–2)	0.01*
Personal sociodemographics, n (%)			
Employed for pay	29 (19)	18 (17)	0.31
Education ⩽fourth grade	30 (20)	39 (37)	0.03*
Biomass cooking fuel	15 (10)	25 (24)	0.002*
Moderate to severe food insecurity	14 (9)	11 (10)	0.68
Obstetric history			
Gestational age	26 (22–31)	NA	—
First prenatal visit, n (%)	24 (16)	NA	—
First pregnancy, n (%)	43 (29)	39 (38)	0.11
HIV history			
Median CD4	468 (343–606)	447 (319–622)	0.42
cART use, n (%)	68 (45)	48 (46)	0.33
cART before pregnancy	26 (18)	18 (18)	0.99
TB risk factors, n (%)			
History of TB	1 (0.6)	2 (2)	0.36
History of IPT	0 (0)	0 (0)	>0.95
Close contact with TB	12 (8)	3 (3)	0.11
Contact with MDR-TB	5 (3)	0 (0)	0.25
Positive TB symptom screen	15 (10)	11 (11)	0.79
Household contact with TB symptoms	12 (8)	5 (4)	0.31
Smoker in the house	31 (20)	23 (22)	0.38
LTBI results			
TST			0.78
Positive ⩾5 mm, n (%)	18 (12)	9 (8)	
TST^+ median induration, mm (IQR)	16 (13–21)	13 (12–15)	0.19
Negative, n (%)	125 (83)	88 (85)	
Did not return, n (%)	6 (4)	6 (6)	
QGIT			0.86
Positive, n (%)	42 (28)	29 (28)	
$QGIT^+$ median IFN-γ, IU/ml (IQR)	3.22 (1.26–9.79)	1.84 (0.84–4.63)	0.11
Negative, n (%)	103 (69)	70 (67)	
Indeterminate, n (%)	4 (2)	4 (3)	

Definition of abbreviations: cART = combination antiretroviral therapy; IPT = isoniazid preventive therapy; IQR = interquartile range; LTBI = latent tuberculosis infection; MDR-TB = multidrug-resistant tuberculosis; NA = not applicable; QGIT = QuantiFERON TB Gold In-Tube test; TB = tuberculosis; TST = tuberculin skin test.
*Statistically significant.

Overall, 71 (28%) women had a positive QGIT but only 27 (10%) had a positive TST ($P < 0.005$). Of 232 (92%) women with both a valid QGIT and TST result, the proportion positive by each test was significantly different in both the antepartum (28% $QGIT^+$ vs. 12% TST^+; $P = 0.002$) and delivery (26% $QGIT^+$ vs. 9% TST^+; $P = 0.002$) groups (Figure 1A). For those with a positive TST, the median induration was smaller in the delivery group than antepartum (13 vs. 16 mm), although this did not reach statistical significance ($P = 0.19$). Similarly, IFN-γ concentration of those with positive QGIT was lower at delivery than antepartum (1.80 IU/ml, IQR 0.8–5.2 vs. 3.20 IU/ml, IQR 1.2–9.6; $P = 0.07$) (Figure 2).

Among the 12 (4%) women who did not return for TST reading, seven (58%) were QGIT positive (three antepartum, four delivery). All eight (3%) women who had indeterminate QGIT results had low IFN-γ mitogen responses and were TST negative.

In multivariate analysis controlling for CD4 and cART use, a positive QGIT was significantly associated with contact with someone with active TB (OR, 3.6; CI, 1.2–11.1; $P = 0.02$). Controlling for the same factors, a positive TST was not associated with any known TB risk factors.

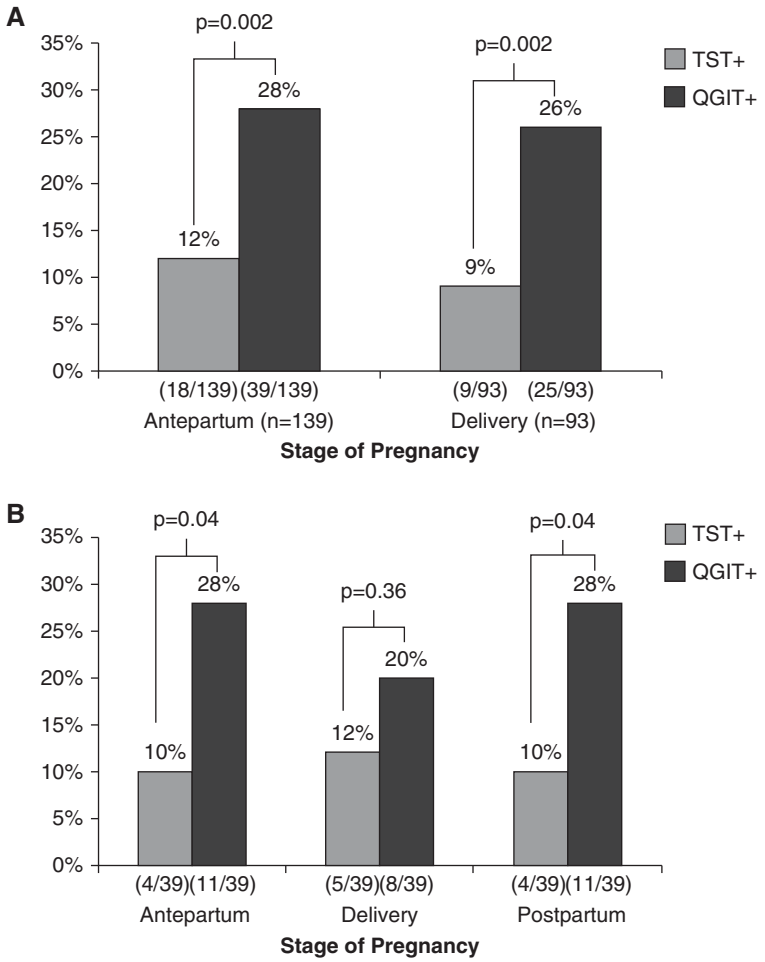

Figure 1. Prevalence of latent tuberculosis infection by stage of pregnancy in women infected with HIV. (*A*) Cross-sectionally, there was insignificantly lower percent positivity of TST and QGIT at delivery versus during pregnancy but significant discordance between TST and QGIT. (*B*) In the longitudinal cohort, there was a decrease in QGIT-positive results at delivery with rebound postpartum, although these were not significant changes. The TST, however, mirrored the changes in the QGIT with increased positivity at delivery and a decrease postpartum, suggesting that decreased TST performance is more related to HIV than pregnancy. QGIT = QuantiFERON TB Gold In-Tube test; TST = tuberculin skin test.

TST and QGIT Concordance and Discordance

Overall, the TST and QGIT had 75% agreement ($\kappa = 0.25$, fair agreement). Seventeen (7%) women had concordant positive tests, 158 (68%) had concordant negative tests, and 47 (20%) had $QGIT^+/TST^-$ discordance. Only 10 (4%) had $QGIT^-/TST^+$ discordance.

In the antepartum group, agreement between QGIT and TST was 74% for antepartum women ($\kappa = 0.25$, fair agreement). Eleven (8%) had concordant positive tests. Similarly, in the delivery group, there was 76% agreement ($\kappa = 0.24$, fair agreement). Only six (6%) had concordant positive tests. In each group, 20% had $QGIT^+/TST^-$ discordance. Changing the cutoff for a positive test for either the TST or QGIT did not significantly affect concordance (data not shown).

Compared with the antepartum group, the delivery group had lower median IFN-γ concentrations (measured by QGIT) from the mitogen tubes (5.20 IU/ml, IQR 1.9–9.8 at delivery vs. 10.0 IU/ml, IQR 3.9–10.0 antepartum; $P = 0.0001$). In MTB-antigen stimulated tubes, women with $QGIT^+/TST^-$ discordance also had significantly lower IFN-γ than women who had concordant positive results (1.85 IU/ml, IQR 0.8–4.5 vs. 3.48 IU/ml, IQR 1.6–10.0; $P = 0.02$) (Figure 3A). When stratified by pregnancy stage, IFN-γ remained significantly lower in antepartum women with $QGIT^+/TST^-$ versus $QGIT^+/TST^+$ (2.10 IU/ml, IQR 1.1–4.4 vs. 6.0 IU/ml, IQR 3.3–10.0; $P = 0.04$).

Similar to IFN-γ, IL-2 (measured by CBA) was only produced after MTB-antigen stimulation. Overall, those with $QGIT^+/TST^-$ discordance had lower IL-2 concentrations compared with those with $QGIT^+/TST^+$ (46.17 pg/ml, IQR 14.2–121.8 vs. 84.03 pg/ml, IQR 20.8–212.2; $P = 0.01$) (Figure 3B). When stratified by pregnancy stage, a similar decrease in IL-2 was seen in discordant $QGIT^+/TST^-$ compared with concordant positive in pregnancy (28.15 pg/ml, IQR 3.6–59.9 vs. 143.33 pg/ml, IQR 50.4–219.1; $P = 0.15$) with a marginal difference at delivery (90.10 pg/ml, IQR 22.6–190.0 in $QGIT^+/TST^-$ vs.170.20 pg/ml, IQR 58.1–398.1 in $QGIT^+/TST^+$; $P = 0.63$).

In multivariate analysis adjusting for CD4 count, cART use, and history of TB contact, having a higher IFN-γ concentration had an inverse association with $QGIT^+/TST^-$ discordance (OR, 0.86; CI, 0.67–0.96; $P = 0.01$).

Longitudinal Comparison

Fifty women were enrolled into the longitudinal cohort; 39 (83%) completed all three visits at antepartum, delivery, and 3 months postpartum. Figure 1B shows that women in this cohort also displayed $QGIT^+/TST^-$ discordance. Similar to the cross-sectional groups, the median concentration of IFN-γ in $QGIT^+$ decreased between antepartum and delivery but increased by 3 months postpartum (2.50 IU/ml, IQR 1.1–6.0 to 1.06 IU/ml, IQR 0.7–1.9, to 2.13 IU/ml, IQR 1.1–3.1; $P = 0.35$). Unlike the cross-sectional analysis, the median TST induration among TST^+ increased marginally between antepartum and delivery and continued to increase postpartum (17 to 19.5 to 28 mm; $P = 0.41$).

Median IL-2 decreased marginally between antepartum and delivery and 3 months postpartum (79.66 pg/ml, IQR 24.9–171.5 to 65.79 pg/ml, IQR 21.2–274.6, to 30.99 pg/ml, IQR 11.7–93.7; $P = 0.48$). There were no significant differences in levels of IL-4, IL-10, TNF, or IL-17a between unstimulated and stimulated samples or based on LTBI status.

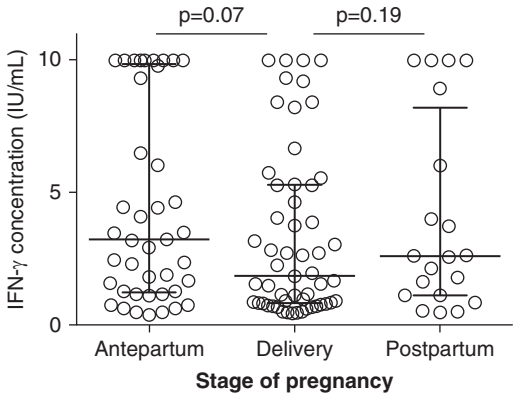

Figure 2. Although percent of QuantiFERON TB Gold In-Tube test positive remained relatively constant, there was a trend for decreased median concentration of IFN-γ between antenatal and delivery ($P = 0.07$), and between delivery and postpartum ($P = 0.19$).

Women Who Developed Active TB

Five (2%) of 252 enrolled women developed active TB (three from longitudinal, two from cross-sectional), all within 1 year postpartum. The median time to development of TB was 97 days postpartum (IQR, 51–270). This yielded an incidence estimate of 2 cases per 100 person years. The median CD4 count at enrollment for these five women was 683 cells/mm^3 and four (80%) had initiated cART during pregnancy. All five women had a positive QGIT at enrollment and one had a positive TST. Based on this, the sensitivity of the QGIT for development of postpartum TB was 100%, but the specificity was 27% and the positive predictive value was only 7%.

Women who developed TB had larger decreases in IFN-γ and IL-2 between antepartum and delivery compared with those who did not. Of the three women who developed active TB from the longitudinal study, the mean IFN-γ in mitogen-stimulated samples decreased 1.6-fold (10–6.06 IU/ml) between antepartum and delivery, and 2.9-fold (8.4–2.9 IU/ml) in MTB antigen-stimulated samples. In comparison, the eight women in the longitudinal study with a positive QGIT that did not develop active TB (three who initiated cART in pregnancy) had mean IFN-γ from mitogen samples increase 1.2-fold (6.17–7.34 IU/ml) between pregnancy and delivery but decrease 2.4-fold in MTB-stimulated samples (3.95–1.64 IU/ml). Similarly, MTB antigen-stimulated samples from women who developed active TB experienced an eightfold drop in mean IL-2 (189.79–23.14 pg/ml), but women who did not develop active TB had only a 1.9-fold drop (149.0–75.60 pg/ml) between pregnancy and delivery.

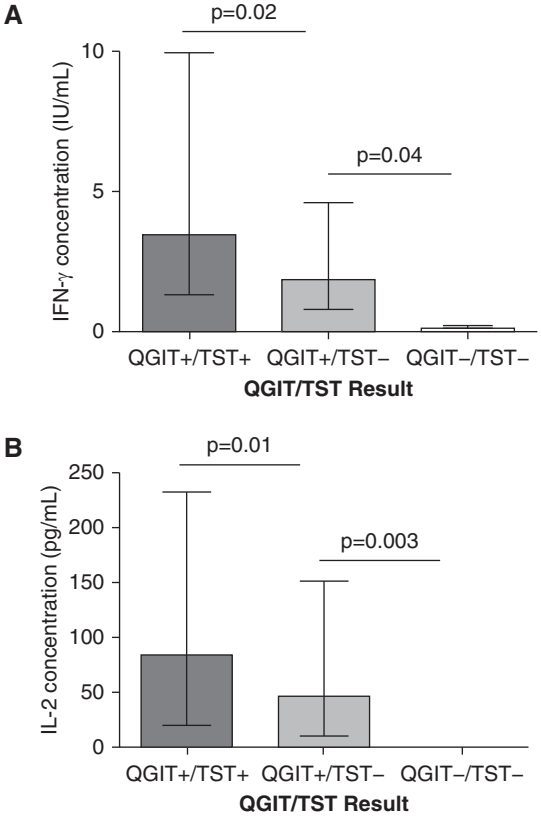

Figure 3. Women with discordant QGIT$^+$/TST$^-$ had lower IFN-γ (*A*) and IL-2 (*B*) production as compared with concordant positive QGIT$^+$/TST$^+$, suggesting a difference in population and not just a difference in false-positive and -negative latent tuberculosis infection test results. QGIT = QuantiFERON TB Gold In-Tube test; TST = tuberculin skin test.

Discussion

Our study has several key findings. We demonstrate that choice of LTBI test for pregnant and postpartum women infected with HIV matters. To our knowledge, this is the first study comparing the performance of these two tests in pregnant women infected with HIV. We found that QGIT positivity was almost three times higher than the more widely used TST at every time point tested. Although there is no gold standard for the diagnosis of LTBI, the prevalence of LTBI as defined by a positive QGIT in our study was more consistent than TST with the prevalence of LTBI in the general population uninfected with HIV in India (~35–40%) (21). Moreover, we show that a positive QGIT is associated with having contact with someone with pulmonary TB, similar to our prior observations among pregnant women uninfected with HIV in India and that of others (12, 22–24). Although other studies in pregnant women uninfected with HIV in the United States (22, 25) did not show the same QGIT$^+$/TST$^-$ discordance, the

patients were substantially less likely to have been exposed to TB. Only 5–13% of women in these studies were $IGRA^+$ (vs. 10–23% TST^+) and the main discordance seen was $IGRA^-/TST^+$ (7–16%). Testing was also conducted earlier in pregnancy, when immune suppression is milder (25). A recent yet unpublished study of pregnant Kenyan women infected with HIV in their second/third trimester, by contrast, reported 25% $QGIT^+/TST^-$ discordance (26).

Our findings suggest that TST underestimates the burden of LTBI in pregnancy, and that QGIT has high sensitivity for predicting postpartum TB, similar to a report in Kenyan women (6). Countries that already perform targeted screening or are considering integrating LTBI screening into antenatal programs may benefit from using the IGRA in addition to or instead of TST. Although the TST is less expensive, it presents operational challenges, which result in unread TST results in 15% or more of pregnant and postpartum women (27).

By measuring IFN-γ concentrations longitudinally, we also discovered that stage of pregnancy seems to specifically affect the IFN-γ response to MTB antigens. Although there was no difference in the number of $QGIT^+$ women by stage, the IFN-γ concentration among $QGIT^+$ trended down between pregnancy and delivery in both the mitogen and the MTB antigen tubes. This finding was also seen in a study from the United States (22) and is consistent with the known immune changes of pregnancy that favor suppression of the proinflammatory Th1 immune response to allow a successful pregnancy (28). This suppression reaches a nadir late in pregnancy (29, 30), near delivery. We believe the decrease in IFN-γ response to MTB antigens between pregnancy and delivery is a novel finding and suggests that pregnant women have an impaired immune response to TB. Larger cohorts are needed to validate this finding, but reduced IFN-γ response to MTB antigens has also been seen in patients infected with HIV before cART initiation who developed post-cART TB immune reconstitution inflammatory syndrome (31). Rapid increase in immune recovery has also been associated with increased risk of TB immune reconstitution inflammatory syndrome (31, 32). Similarly, we hypothesize that immune control of LTBI weakens during pregnancy, allowing progression from latent to active TB, which only becomes symptomatic during rapid postpartum immune recovery. We only measured response to MTB antigens at one postpartum time point, but future studies are underway to intensively investigate immune changes in the immediate postpartum period.

Another key finding of our study is the 20% prevalence of $QGIT^+/TST^-$ discordance among pregnant women infected with HIV in a TB endemic area. $IGRA^+/TST^-$ discordance has been documented in other high-risk populations, including recent household TB contacts (33); the elderly (34); recent immigrants from TB-endemic countries (34, 35); and immunocompromised populations (35, 36), including those with rheumatoid arthritis (37) and HIV (38). The immunologic etiology behind this type of discordance, however, has not been thoroughly investigated.

In our study, women with $QGIT^+/TST^-$ discordance had significantly decreased IFN-γ and IL-2 production in response to MTB antigens compared with those with concordant positive results, providing a possible immunologic explanation for discordance. The TST is an indirect measurement of the Th1 immune response; it requires IFN-γ, TNF-α, IL-2, and other Th1 cytokines to stimulate a delayed-type hypersensitivity reaction, which results in skin induration (31, 39). The IGRAs, however, only measure IFN-γ produced in the blood. Our results suggest that lower IFN-γ and IL-2 may prevent the immune system from triggering the delayed-type hypersensitivity reaction, resulting in a falsely negative TST. It is possible that $QGIT^+/TST^-$ discordance could be related to operator-dependent errors with the TST. We do not think this is likely because our nurses were formally trained to perform TST. Any potential operational errors that are inherent to the TST, however, simply emphasize the risks of TST reliance. If discordance was caused by the different antigens or duration of antigen stimulation, we would expect to observe $QGIT^+/TST^-$ more often. Studies in other populations with discordance are needed to confirm if these findings are generalizable or unique to pregnancy.

Finally, decreased IL-2 and IFN-γ may also be associated with increased risk of active TB. IL-2 is produced primarily by memory T cells, indicating remote exposure to TB; IFN-γ is secreted by effector cells and represents a more acute exposure (31, 40). Studies have shown patients with active TB have a lower IL-2/IFN-γ ratio than those with LTBI (41). No longitudinal studies of predictive value of low IL-2 and IFN-γ exist. In our study, five women developed active TB postpartum. Of the four women who had recorded TST and QGIT results during pregnancy, three of them had discordant results. Moreover, the three women with samples from pregnancy and delivery showed a 2.9-fold decrease in IFN-γ and eightfold decrease in IL-2 between antepartum and delivery. These findings are hypothesis generating, suggesting that reduced IL-2 and IFN-γ associated with discordance and the magnitude of decline in these cytokines during pregnancy may be predictors of progression to active TB. Longitudinal studies with larger sample sizes are needed to validate this finding.

Our study had the following limitations. Because the additional cytokine analyses were a secondary outcome, the blood samples were processed per the manufacturer's instructions to measure IFN-γ. Longer incubation times may have been needed to measure maximal concentrations of other cytokines, including IL-2 (42). We were also limited in the number of additional cytokines tested and samples we could run with the CBA. We plan on more extensive studies, which will include other TB-relevant cytokines/chemokines (e.g., IP-10, type 1 IFN). We also did not collect information regarding comorbidities that could affect the Th1 immune response, such as helminth infections, hepatitis B, or diabetes. Given our sample size and the less than 5% prevalence of gestational diabetes and hepatitis B in this population (3, 43), we were unable to adjust for such conditions. Because these women had CD4 counts above 400, were pregnant, and did not have any opportunistic infections, we do not think our findings are a result of unadjusted concomitant comorbidities. By screening women for active TB using the WHO TB symptom screen and physical examination, it is possible that we missed women with subclinical or extrapulmonary TB, although all women had follow-up through 1 year postpartum.

Pregnancy offers a unique opportunity to screen for and treat TB, because it is one of the few times young women in

high-burden TB countries voluntarily access health care (44). Best practices, however, remain unknown, largely because of the poorly understood impact of the immune changes associated with pregnancy on TB screening and pathogenesis. The WHO recommends IPT for all people infected with HIV (45) but few countries have adopted this policy for pregnant women. Where targeted treatment is preferred, our study suggests that the QGIT may be superior to the TST in identifying pregnant populations infected with HIV that would benefit from IPT in TB-endemic countries, such as India. The results also suggest that delivery is not the optimal time for LTBI screening. Importantly, our results also provide a starting point for further investigation into the immune changes of pregnancy that specifically impact TB pathogenesis. Pregnancy also provides a useful model to examine the interaction between host immune changes and response to pathogens more generally, giving our findings potential significance beyond pregnant women. Dedicated immunologic studies in pregnancy are urgently needed to improve TB prevention and management strategies in pregnant and postpartum women and their children. ■

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