Interferon Gamma Release Assay and Tuberculin Skin Test Performance in Pregnant Women Living With and Without HIV

Samantha R. Kaplan, MD,[a] Jaclyn N. Escudero, MPH,[b] Jerphason Mecha, BS,[c] Barbra A. Richardson, PhD,[b,d] Elizabeth Maleche-Obimbo, MBChB, MMed, MPH,[b,e] Daniel Matemo, MPH,[c] John Kinuthia, MBChB, MMed, MPH,[b,c,f] Grace C. John-Stewart, MD, PhD,[b,g,h,i] and Sylvia M. LaCourse, MD, MPH[b,g]

Background: HIV and pregnancy may affect latent TB infection (LTBI) diagnostics. Tuberculin skin test (TST) and newer generation QuantiFERON-TB Gold Plus (QFT-Plus) evaluations in pregnant women living with HIV (WLHIV) and without HIV are lacking.

Methods: In this cross-sectional study, pregnant women underwent TST and QFT-Plus testing during antenatal care in Kenya. We estimated LTBI prevalence and TST and QFT-Plus performances. Diagnostic agreement was assessed with kappa statistic, participant characteristics associated with LTBI and HIV were assessed with generalized linear models, and QFT-Plus quantitative responses were assessed with Mann–Whitney *U* test.

Results: We enrolled 400 pregnant women (200 WLHIV/200 HIV-negative women) at median 28 weeks gestation (interquartile range 24–30). Among WLHIV (all on antiretroviral therapy), the median CD4 count was 464 cells/mm^3 (interquartile range 325–654); 62.5% (125) had received isoniazid preventive therapy. LTBI prevalence was 35.8% and similar among WLHIV and HIV-negative women. QFT-Plus testing identified 3-fold more women with LTBI when compared with TST (32% vs. 12%, $P < 0.0001$). QFT-Plus positivity prevalence was similar regardless of HIV status, although TB-specific antigen responses were lower in WLHIV than in HIV-negative women with LTBI (median QFT-TB1 1.05 vs. 2.65 IU/mL, $P = 0.035$; QFT-TB2 1.26 vs. 2.56 IU/mL, $P = 0.027$). TST positivity was more frequent among WLHIV than among HIV-negative women (18.5% vs 4.6%; $P < 0.0001$).

Conclusions: QFT-Plus assay had higher diagnostic yield than TST for LTBI in WLHIV and HIV-negative women despite lower TB-specific antigen responses in WLHIV. Higher TST positivity was observed in WLHIV. LTBI diagnostic performance in the context of pregnancy and HIV has implications for clinical use and prevention studies, which rely on these diagnostics for TB infection entry criteria or outcomes.

Key Words: HIV, pregnancy, latent tuberculosis, Kenya, QuantiFERON-TB Gold Plus

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INTRODUCTION

Tuberculosis (TB) during pregnancy is associated with increased risk of maternal morbidity and mortality and poor pregnancy and infant outcomes,[1,2] especially among women living with HIV (WLHIV) despite widespread antiretroviral uptake.[3,4] Although data regarding TB in pregnancy are not routinely reported, models estimate that >200,000 active TB cases occur during pregnancy annually, with the highest burden in Africa.[2] Both HIV and, potentially, pregnancy increase the risk of progression from *M. tuberculosis* infection to active TB disease,[5–7] highlighting the importance of latent TB infection (LTBI) detection in pregnancy in high HIV/TB settings, which may allow targeted prevention efforts.

There is evidence that pregnancy[8,9] and HIV[10] affect LTBI diagnostic performance.[11,12] Tuberculin skin tests (TSTs) indirectly measure *M. tuberculosis* infection by detecting a delayed-type hypersensitivity reaction in

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From the [a]Department of Medicine, University of Washington, Seattle, WA; [b]Department of Global Health, University of Washington, Seattle, WA; [c]Research and Programs, Kenyatta National Hospital, Nairobi, Kenya; [d]Department of Biostatistics, University of Washington, Seattle, WA; [e]Department of Paediatrics and Child Health, University of Nairobi, Nairobi, Kenya; [f]Department of Obstetrics and Gynaecology, Kenyatta National Hospital, Nairobi, Kenya; [g]Department of Medicine, Division of Allergy and Infectious Diseases, University of Washington, Seattle, WA; [h]Department of Epidemiology, University of Washington, Seattle, WA; and [i]Department of Pediatrics, University of Washington, Seattle, WA.

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Correspondence to: Sylvia M. LaCourse, MD, MPH, Departments of Medicine and Global Health, Division of Allergy and Infectious Diseases, University of Washington, 325 9th Avenue, Box 359931, Seattle, WA 98104 (e-mail: sylvial2@uw.edu).

individuals with cell-mediated immunity to tuberculin antigens but are subject to cross-reactivity with nontuberculous mycobacteria and Bacillus Calmette–Guérin vaccine and have poor sensitivity in immunocompromised patients.[13,14] Interferon gamma (IFN-γ) release assays (IGRAs) measure T-cell release of IFN-γ after *M. tuberculosis*–specific antigen stimulation; QuantiFERON-TB Gold (QFT-Gold) assay is an enzyme-linked immunosorbent assay (ELISA) that measures IFN-γ secreted primarily by $CD4^+$ T cells, whereas newer generation QuantiFERON-TB Gold Plus (QFT-Plus) assay detects $CD4^+$ and $CD8^+$ T-cell responses, which may increase sensitivity in populations with lower CD4 counts including people living with HIV (PLHIV).[14–16]

Previous studies demonstrated discordance between older generation QFT-Gold assay and TST in pregnancy, with QFT-Gold assay having higher sensitivity (detecting approximately 2-fold or greater of women with LTBI) compared with TST in separate cohorts of pregnant WLHIV and HIV-negative women in India,[8,9] in Kenya,[17] and within a multinational trial of isoniazid preventive therapy (IPT) in pregnant WLHIV.[18] Pregnancy also seems to affect QFT-Gold assay as evidenced by lower mean mitogen and TB-specific antigen responses and higher rates of indeterminate results (due to lower mitogen response) during pregnancy compared with early postpartum period.[17] A cross-sectional study in Ethiopia enrolling primarily HIV-negative women using newer generation QFT-Plus assay (no TST performed) found more borderline TB-specific antigen QFT responses among pregnant WLHIV than HIV-negative women, prompting the authors to suggest consideration of lowering the QFT-positive cutoff in pregnant WLHIV.[11] A more recent Ethiopian cross-sectional study enrolling pregnant and nonpregnant women with and without HIV found that both pregnancy and HIV reduced LTBI detection by both TST and QFT-Gold assay.[12]

We designed a study to estimate LTBI prevalence and effect of HIV on diagnostic performance of the newest generation QFT-Plus assay compared with TST, including TB-specific antigen responses, in a large cross-sectional evaluation of pregnant WLHIV and without HIV in western Kenya. In addition, we investigated correlates of LTBI-diagnostic positivity and yield of varying diagnostic cutoffs.

METHODS

Study Setting and Participants

We enrolled pregnant WLHIV and HIV-negative women at 4 public antenatal clinics in Kisumu and Siaya counties in western Kenya, where HIV prevalence is 14%–21%.[19] Women were eligible if they were pregnant (between 20 and 34 weeks gestation) and aged 16 years or older. Participants were excluded if they were diagnosed with TB disease in the past year or were found to have TB on enrollment. Participants were enrolled consecutively until the predetermined sample size of 400 participants (200 WLHIV, 200 HIV-negative women) was reached.

Procedures

Enrollment

Informed consent was obtained by study staff who administered standardized questionnaires regarding sociodemographic, clinical, obstetric, and HIV-related factors; previous TB exposure and history; and maternal and household member TB symptoms using WHO symptom screen (fever, cough, weight loss, and night sweats).[20] Physical examination was performed including body mass index (BMI) calculation and middle upper arm circumference (MUAC). Medical records were used to abstract data on antiretroviral therapy (ART), IPT, maternal HIV viral load, and CD4 counts. Kenyan guidelines recommend a 6-month course of IPT for PLHIV including pregnant women; neither TST nor IGRA are used to identify LTBI prior to programmatic IPT initiation.[21] Participants continued to receive routine antenatal and HIV care. WLHIV found to have positive TST or IGRA in the study were referred for IPT evaluation if they had not already received IPT; per Kenyan guidelines at the time of the study, HIV-negative adults without known TB exposure are not routinely provided IPT.[22] Data were entered into password-protected tablets using Research Electronic Data Capture (REDCap, Vanderbilt University, Nashville, TN).

QFT-Plus and TST

QFT-Plus

Five milliliters of blood was collected into a single lithium heparinized blood collection tube, transported to Kenya Medical Research Institute Centers for Disease Control laboratory and aliquoted into QFT-Plus assay collection tubes [nil, mitogen, TB antigen 1 (ESAT-6 and CFP-10 CD4 peptides), TB antigen 2 (ESAT-6, CFP-10 CD4 and CD8 peptides)], and processed per manufacturer recommendations.[23] TB1 or TB2 antigen response of $\geq$0.35 IU/mL (minus nil, with nil $<$8 IU/mL, and positive mitogen control) was considered positive.[15]

TST

Trained study personnel injected 0.1 mL (5 international units) of tuberculin purified protein derivative intradermally to the forearm volar surface using the Mantoux method. Induration was measured at 48–96 hours after TST placement.[24,25] This reading window allowed for TST placement throughout the week and is within the allowable 7 days used in International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT).[23,26] TST of $\geq$ 5 mm was considered positive in WLHIV, and TST of $\geq$10 mm was considered positive among HIV-negative women per routine clinical cutoffs.[27]

Statistical Analysis

Baseline participant characteristics were described using proportions for categorical variables and medians and interquartile ranges (IQRs) for continuous variables. Generalized linear models with a log link and Poisson family were

used to estimate relative risk ratios (RRs) to assess differences in baseline characteristics by HIV status and correlates of LTBI in the overall cohort and stratified by HIV status. The median quantitative QFT-Plus responses were compared using Mann–Whitney *U* tests. Multinomial logistic regression was used to assess association between baseline correlates and QFT-positive or QFT-indeterminate results compared with the reference QFT-negative results. TST and QFT agreement and QFT TB1 and TB2 antigen agreement were assessed using a kappa statistic. Correlation between TST diameter and quantitative TB1 and TB 2 antigens were assessed by Spearman rho.

Sensitivity analyses were performed varying cutoffs for TST (including ≥10 and ≥5 mm for both WLHIV and HIV-negative groups) and varying QFT-Plus TB1 and TB2 antigens (minus nil) cutoffs to ≥0.20 and ≥0.70 IU/mL to represent lower and upper cutoffs described for borderline values in the literature. All estimates were reported using 95% confidence intervals: all statistical tests were 2 sided with alpha = 0.05. Analyses were performed using Stata 14 (StataCorp, College Station, TX).

Ethical Statement

This study was approved by the University of Nairobi-Kenyatta National Hospital Ethics and Research Committee and the University of Washington Institutional Review Board.

RESULTS

Participant Characteristics Overall and by HIV Status

From January 2018 to December 2019, we enrolled 400 pregnant women (200 WLHIV and 200 HIV-negative women) (Fig. 1); 188 (47%) from Kisumu and 212 (53%) from Siaya counties. The median age at enrollment was 26 (IQR 22–29) years, and the median gestational age was 28 weeks (IQR 24–30) with median BMI of 24.12 kg/m^2 (IQR 22.3–27.5) (Table 1). Roughly half were employed [52.0% (209)], 46.0% (185) had running water in their home, and 22.5% (90) lived in a single-room household. The median number of years of education was 10 (IQR 8–12) years. Three women (0.8%) had a history of active pulmonary TB, and 3.3% (13) had a household member with recent TB symptoms.

For 200 WLHIV, the median CD4 count was 464 cells/mL (IQR 325–654), and most of them (87.9%; 153) were virally suppressed (HIV viral load <20 copies/mL), based on a viral load drawn at a median of 55 days (IQR 6–91) before enrollment. The median length of HIV diagnosis was 3.1 years before enrollment (IQR 0.4–6.9); 67.5% (135) were on ART before this pregnancy, and 100% were on ART at enrollment. Approximately two-thirds (62.5%; 125) of WLHIV had ever received IPT, including 30.1% (38) currently on IPT at enrollment.

WLHIV were older (median age 28 vs. 23 years), enrolled at an earlier gestational age (median 26 vs. 28 weeks), and had slightly larger MUAC (median 26.2 vs. 26.0 cm) (all $P < 0.001$) compared with HIV-negative women. WLHIV were more likely to be employed (58.0% vs. 46.5%) but less likely to have running water in their household (40.5% vs. 52.0%) or live in a single-room household (18% vs. 27%) (all $P < 0.05$).

Prevalence of LTBI including QFT-Plus and TST Results

Overall, 35.8% (143) of women were LTBI-positive (by either QFT-Plus or TST) with similar proportions of LTBI among WLHIV and HIV-negative women (Table 1 and Fig. 2). One-third (129; 32.3%) of women were QFT-positive, and 25 (6.3%) were indeterminate (most of them due to low mitogen responses: 21 of the 25; 84.0%) with similar proportions of QFT-positive and indeterminates between WLHIV and HIV-negative women, respectively (QFT-positive: 31.5% vs. 33.2%; QFT indeterminate: 6.0% vs. 6.6%). By contrast, overall TST positivity (by clinical cutoffs of ≥5 mm for WLHIV and ≥10 mm for HIV-negative women) prevalence was lower compared with QFT positivity prevalence (TST 11.6% vs. QFT-Plus 32.3%; $P < 0.0001$), although WLHIV were significantly more likely to have a positive TST than HIV-negative women (18.5% vs 4.6%; $P < 0.0001$).

Correlates of LTBI, QFT-Plus, and TST Positivity Overall and by HIV Status

Overall, women with LTBI had lower median years of education (median 9 vs. 10 years; $P = 0.006$) and were more likely to report a history of TB (2.1% vs. 0; $P < 0.001$) (Table 2). There was a trend for women with LTBI to be slightly older (median 26 vs. 35 years; $P = 0.068$). In general, QFT-positive women were similar to QFT-negative women regarding baseline characteristics. By contrast, QFT-indeterminate women were younger (median 22 vs. 25 years; $P = 0.047$) and had a lower BMI (median 23.2 vs. 24.2 kg/m^2; $P = 0.012$) (see TableE2a, Supplemental Digital Content, http://links.lww.com/QAI/B745). TST positivity defined by routine clinical cutoffs was associated with higher MUAC (median 26.5 vs. 26.0 cm), HIV (80.4% vs. 46.3%), and history of TB (4.4% vs. 0.3%) (all <0.005) (see Table E2b, Supplemental Digital Content, http://links.lww.com/QAI/B745). Correlates for LTBI positivity remained similar when adjusting for HIV status and baseline characteristics that were significantly different between LTBI-positive and LTBI-negative women (data not shown).

For both WLHIV and HIV-negative women, LTBI was associated with history of TB (both <0.001). For WLHIV, both LTBI and QFT positivity were associated with higher median CD4 counts (both <0.05) and a trend for lower likelihood of ever having received IPT (LTBI+ $P = 0.056$, QFT-Plus+ $P = 0.078$) (Table 3, see Table E2a, Supplemental Digital Content, http://links.lww.com/QAI/B745). For HIV-negative women, LTBI was associated with older age and fewer years of education (both $P < 0.02$) and TB exposure in the past 2 years ($P < 0.001$).

TABLE 1. Participant Characteristics and Results of LTBI Testing by HIV Status

	All Participants, n = 400	WLHIV, n = 200	HIV-Negative, n = 200		
	n (%) or Median (IQR)	n (%) or Median (IQR)	n (%) or Median (IQR)	RR* (95% CI)	*P*
Sociodemographic Characteristics					
Age, yrs	26 (22–29)	28 (24–32)	23 (21–26)	1.06 (1.05–1.08)	**<0.001**
Gestational age, wks	28 (24–30)	26 (23–30)	28 (25–32)	0.96 (0.94–0.98)	**<0.001**
BMI, kg/m^2	24.1 (22.3–27.5)	24.2 (22.2–27.5)	24.0 (22.4–27.4)	1.01 (0.99–1.03)	0.434
MUAC, cm	26.0 (25.0–28.0)	26.2 (25.1–28.8)	26.0 (24.527.2)	1.04 (1.02–1.07)	**<0.001**
Education, yrs	10 (8–12)	10 (8–12)	10 (8–12)	0.98 (0.95–1.00)	0.082
Employed	209 (52.0)	116 (58.0)	93 (46.5)	1.26 (1.03–1.54)	**0.023**
Residential characteristics					
Running water in home	185 (46.0)	81 (40.5)	104 (52.0)	0.79 (0.65–0.97)	**0.024**
Electricity in home	255 (64.0)	131 (65.5)	124 (62.0)	1.08 (0.88–1.33)	0.472
Pit latrine use	377 (94.3)	187 (93.5)	190 (95.0)	0.88 (0.61–1.27)	0.493
Persons in household	4 (3–5)	4 (3–5)	3 (2–5)	1.05 (0.99–1.12)	0.077
Single-room household	90 (22.5)	36 (18.0)	54 (27.0)	0.76 (0.57–0.99)	**0.046**
TB history, symptoms, and exposures					
Previous TB	3 (0.8)	2 (1.0)	1 (0.5)	1.34 (0.60–3.00)	0.481
WHO TB symptom in the past mo †	16 (4.0)	10 (5.0)	6 (3.0)	1.26 (0.85–1.87)	0.244
Household WHO TB symptom	13 (3.3)	7 (3.5)	6 (3.0)	1.08 (0.65–1.80)	0.770
TB exposure in the past 2 yrs	4 (1.0)	3 (1.5)	1 (0.5)	1.51 (0.85–2.68)	0.162
HIV					
CD4, cells/mm^3 (n = 90) §		464 (325–654)			
HIV viral load, copies/mL (n = 174) §		0 (0–54)			
HIV viral load undetectable (n = 174)‖		153 (87.9)			
Time since HIV diagnosis, yrs		3.1 (0.4–6.9)			
ART before pregnancy		135 (67.5)			
ART on enrolment		200 (100.0)			
Any IPT		125 (62.5)			
IPT on enrollment (n = 125)		38 (30.1)			
LTBI test results					
LTBI+ (TST+ or QFT-Plus+)	143 (35.8)	74 (37.0)	69 (34.5)	1.06 (0.86–1.29)	0.600
TST+ (n = 398) ‡	46 (11.6)	37 (18.5)	9 (4.6)	1.74 (1.45–2.08)	**<0.001**
QFT-Plus (n = 399)					
Positive	129 (32.3)	63 (31.5)	66 (33.2)	0.96 (0.77–1.19)	0.691
Negative	245 (61.4)	125 (62.5)	120 (60.3)	Ref	
Indeterminate	25 (6.3)	12 (6.0)	13 (6.6)	0.94 (0.61–1.44)	0.779

*Relative risk (RR) estimated using a generalized linear model (GLM) with log link and Poisson family.
†WHO TB symptoms: fever, cough, weight loss, and night sweats.
‡TST-positive defined as ≥5 mm induration if HIV-positive and ≥10 mm induration if HIV-negative; the median time of TST read was 47 hours (IQR 45–70).
§CD4 and viral load data were collected from routine programmatic data; viral load was collected at a median of 55 (6–91) days before enrollment.
‖Undetectable viral load ≤20 copies/mL

QFT-Plus Quantitative Results by HIV Status

The median QFT mitogen (mitogen-nil) response was similar among women with and without HIV (6.68 vs. 8.19 IU/mL; $P = 0.332$), whereas median nil response was higher among HIV-negative women (WLHIV 0.08 vs. HIV-negative 0.12 IU/mL; $P < 0.001$) (Fig. 3A; see Table E3, Supplemental Digital Content, http://links.lww.com/QAI/B745). Among women with LTBI (either QFT-positive or TST-positive, n = 142), WLHIV had significantly lower median TB1 (1.05 vs. 2.65 IU/mL; $P = 0.035$) and TB2 (1.26 vs. 2.56 IU/mL; $P = 0.027$) antigen responses (Fig. 3B; see Table E3, Supplemental Digital Content, http://links.lww.com/QAI/B745). TB1 and TB2 antigen responses remained lower in WLHIV than in HIV-negative women in analyses limited to TST-positive or QFT-positive women but not significantly so (see Table E3, Supplemental Digital Content, http://links.lww.com/QAI/B745).

QFT-Plus and TST Concordance

Among 397 participants with QFT-plus assay and TST (using clinical cutoffs for TST) results, agreement was 66.0% (kappa 0.19; $P < 0.001$) (see Table E4, Supplemental Digital Content, http://links.lww.com/QAI/B745). Stratified by HIV status, WLHIV had 70.0% agreement (kappa 0.31; $P < 0.001$) and HIV-negative women had 61.9% agreement

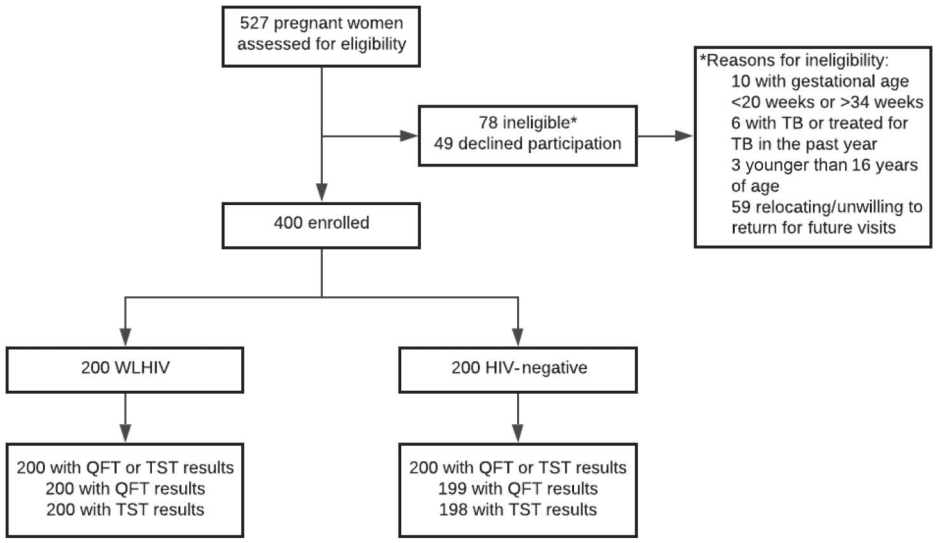

FIGURE 1. Study flow evaluating QFT-Plus and TST among women with and without HIV.

(kappa 0.07; $P = 0.022$). For QFT-Plus results, overall, there was 95.5% agreement between TB1 and TB2 antigen positive results (kappa 0.90; $P < 0.001$), which was similar when stratified by HIV status (see Table E5a, Supplemental Digital Content, http://links.lww.com/QAI/B745).

For WLHIV, there was a positive significant correlation between TST induration (mm) and both TB1 (Spearman rho 0.293; $P < 0.001$) and TB2 antigen responses (Spearman rho 0.387; $P < 0.001$), but not for HIV-negative women (TST/TB1: 0.00, $P = 0.980$; TST/TB2: 0.019, $P = 0.787$) (see Table E5b, Supplemental Digital Content, http://links.lww.com/QAI/B745 and Fig. E1, Supplemental Digital Content, http://links.lww.com/QAI/B745).

Sensitivity Analyses Varying QFT-Plus and TST Cutoffs

By increasing TST positivity cutoff to ≥10 mm for WLHIV, 17 (8.5%) fewer WLHIV would have been considered TST-positive, but differences between WLHIV and HIV-negative women remained statistically significant (WLHIV 10.0% vs. HIV-negative 4.6%; $P = 0.011$) (see Supplemental Digital Content Table E1,http://links.lww.com/QAI/B745), without improvement of agreement (see Table E4, Supplemental Digital Content, http://links.lww.com/QAI/B745). Lowering the TST positivity threshold to ≥5 mm for HIV-negative women would identify an additional 17 (8.5%) HIV-negative women as TST-positive, with differences between groups no longer statistically significant (WLHIV 18.5% vs. HIV-negative 13.1%; $P = 0.116$).

Lowering the QFT positivity threshold to 0.20 IU/mL from 0.35 IU/mL increased the overall proportion of QFT positivity to 38.1% (from 35.8%) by identifying 23 additional women as QFT-positive (10 WLHIV; 13 HIV-negative); but similar to the conventional cutoff, there were no significant differences in QFT-Plus positivity between WLHIV and HIV-negative women (see Table E1, Supplemental Digital Content, http://links.lww.com/QAI/B745). Data regarding proportion and correlates of LTBI positivity of WLHIV and HIV-negative women at various LTBI, TST, and QFT-Plus cutoffs are summarized in Tables E1, E2a, E2b, Supplemental Digital Content, http://links.lww.com/QAI/B745.

DISCUSSION

In this large cross-sectional analysis of pregnant women evaluated for LTBI in a high HIV/TB burden setting in western Kenya, newer generation IGRA assay, QFT-Plus, identified 3 times as many pregnant women overall with LTBI compared with TST (32% vs. 12%), with similar prevalence of QFT-Plus positivity in WLHIV and HIV-negative women. The finding of higher prevalence of QFT-Plus positivity compared with TST was further amplified among HIV-negative women, even when increasing TST cutoffs to ≥10 mm for both WLHIV and HIV-negative women. Among women with LTBI, quantitative TB1 and TB2 antigen responses were significantly lower in WLHIV compared with HIV-negative women despite similar prevalence of QFT positivity between groups.

Our finding of 30% QFT positivity among pregnant women is similar to previous studies in Kenya[17,28] and other high HIV/TB burden settings including India and Ethiopia.[8,9,11] We found TST had lower rates of positivity in detecting LTBI among pregnant women irrespective of HIV status, which has been reported in separate cohorts of WLHIV and HIV-negative women with the older generation QFT-Gold assay.[8,9,17,18] To our knowledge, this is one of the first studies to directly compare TST and QFT positivities between pregnant women with and without HIV infection concurrently with next generation QFT-Plus. Although overall TST positivity was lower compared with QFT-Plus positivity, our study found a larger proportion of TST positivity in WLHIV compared with HIV-negative pregnant women when using recommended clinical TST cutoffs based on HIV status. This higher TST positivity prevalence among WLHIV remained even when we increased the cutoff to ≥10 mm for all women in

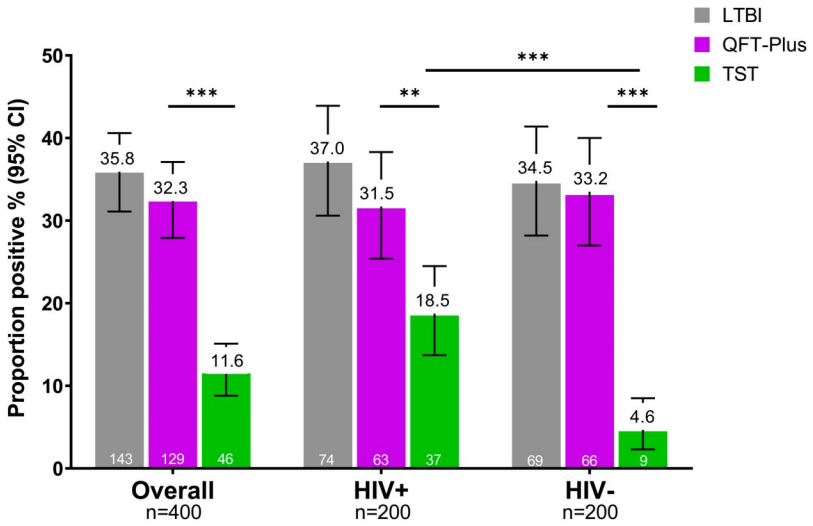

FIGURE 2. Prevalence of latent tuberculosis infection by TST and QFT-Plus among pregnant women by HIV status. TST-positive defined as ≥5 mm induration for women living with HIV (HIV+) and ≥10 mm induration if HIV-negative (HIV−). ***$P < 0.0001$, **$P < 0.005$. full color online

sensitivity analyses, suggesting that this difference was not due to an artifact of differing routine clinical cutoffs by HIV status. Although TST identified a lower proportion of pregnant women with LTBI overall, TST identified a different population as having LTBI than QFT-Plus, as shown in the concordance analysis. WLHIV are potentially at higher risk of *M. tuberculosis* infection, which the TST may have identified through broader or different antigen selection.

Our findings differ from an Ethiopian study that found a higher proportion of QFT and TST positivities among HIV-negative women than WLHIV.[12] The authors suggested that these differences were due to test performance rather than differences in true LTBI positivity between groups because the study was conducted in an area with more than 50% LTBI prevalence.[29,30] A study in Ugandan postpartum mothers found similar QFT-Gold positivity but higher TST positivity in HIV-negative women compared with that in WLHIV.[31] We

TABLE 2. Participant Characteristics by LTBI Status

	All Participants (n = 400)			
	LTBI-Positive,* n = 143	LTBI-Negative, n = 257		
	n (%) or median (IQR)	n (%) or median (IQR)	RR† (95% CI)	*P*
Sociodemographic characteristics				
Age, yrs	26 (22–30)	25 (21–28)	1.02 (1.00–1.04)	0.068
Gestational age, wks	28 (24–30)	28 (24–30)	1.00 (0.97–1.03)	0.892
BMI, kg/m^2	24.1 (22.2–27.5)	24 (22.3–27.3)	1.00 (0.96–1.03)	0.721
MUAC, cm	26 (25–28)	26 (24.8–28)	1.00 (0.96–1.04)	0.979
Education, yrs	9 (8–11)	10 (8–12)	0.95 (0.92–0.99)	**0.006**§
Employed	78 (54.6)	131 (51.0)	1.10 (0.84–1.43)	0.494
Residential characteristics				
Running water in home	63 (44.1)	122 (47.5)	0.92 (0.70–1.19)	0.513
Electricity in home	93 (65.0)	162 (63.0)	1.06 (0.80–1.40)	0.692
Pit latrine use	136 (95.1)	241 (93.8)	1.19 (0.63–2.23)	0.599
Persons in household	3 (3–5)	4 (3–5)	1.03 (0.95–1.12)	0.475
Single-room household	28 (19.6)	62 (24.1)	0.84 (0.60–1.18)	0.311
TB history, symptoms, and exposures				
Previous TB	3 (2.1)	0	2.84 (2.48–3.24)	<**0.001**§
WHO TB symptom in the past mo ‡	6 (4.2)	10 (3.9)	1.05 (0.55–2.01)	0.880
Household WHO TB symptom	4 (2.8)	9 (3.5)	0.86 (0.37–1.96)	0.714
TB exposure in the past 2 yrs	2 (1.4)	2 (0.8)	1.40 (0.52–3.78)	0.502
HIV (n = 200)	74 (51.8)	126 (49.0)	1.07 (0.82–1.40)	0.603

*LTBI defined as TST-positive or QFT-positive, where TST-positive ≥5 mm for WLHIV and ≥10 mm for HIV negative women.
†Relative risk (RR) estimated using a generalized linear model (GLM) with log link and Poisson family.
‡WHO TB symptoms: fever, cough, weight loss, and night sweats.
§Remained statistically significant in multivariable models adjusting for education, previous TB, and HIV status (data not shown).

TABLE 3. Participant Characteristics by LTBI Status Stratified by HIV Status

	WLHIV (n = 200)				HIV-Negative (n = 200)			
	LTBI*-Positive, n = 74	LTBI-Negative, n = 126			LTBI*-Positive, n = 69	LTBI-Negative, n = 131		
	n (%) or Median (IQR)	n (%) or Median (IQR)	RR† (95% CI)	*P*	n (%) or Median (IQR)	n (%) or Median (IQR)	RR† (95% CI)	*P*
Sociodemographic characteristics								
Age, yrs	28 (23–32)	28 (24–31)	1.00 (0.97–1.04)	0.700	24 (22–27)	23 (20–26)	1.04 (1.01–1.08)	**0.017**
Gestational age, wks	28 (24–30)	26 (22–30)	1.02 (0.98–1.07)	0.240	28 (24–30)	28 (25–32)	0.97 (0.93–1.01)	0.190
BMI, kg/m^2	24.1 (22.1–27.4)	24.2 (22.3–27.6)	0.99 (0.94–1.04)	0.658	24.1 (22.4–27.6)	24.0 (22.3–27.2)	1.00 (0.95–1.05)	0.935
MUAC, cm	26.0 (25.5–28.8)	26.5 (25.0–29.0)	0.99 (0.94–1.05)	0.760	26 (25–27.4)	25.6 (24.5–27.0)	1.01 (0.95–1.07)	0.821
Education, yrs	8.5 (8.0–11.0)	10 (8–12)	0.97 (0.92–1.02)	0.212	9 (8–12)	10 (8–12)	0.94 (0.90–0.99)	**0.014**
Employed	40 (54.1)	76 (60.3)	0.85 (0.59–1.22)	0.385	38 (55.1)	55 (42.0)	1.41 (0.96–2.07)	0.080
Residential characteristics								
Running water in home	25 (33.8)	56 (44.4)	0.75 (0.51–1.11)	0.149	38 (55.1)	66 (50.4)	1.13 (0.77–1.66)	0.530
Electricity in home	46 (62.2)	85 (67.5)	0.87 (0.60–1.25)	0.443	47 (68.1)	77 (58.8)	1.31 (0.86–1.99)	0.208
Pit latrine use	71 (96.0)	116 (92.1)	1.65 (0.60–4.53)	0.335	65 (94.2)	125 (95.4)	0.86 (0.39–1.88)	0.697
Persons in household	4 (3–5)	4 (3–5)	1.08 (0.97–1.20)	0.183	3 (2–5)	3 (2–5)	0.98 (0.86–1.11)	0.748
Single-room household	11 (14.9)	25 (19.8)	0.80 (0.47–1.35)	0.398	17 (24.6)	37 (28.2)	0.88 (0.56–1.39)	0.592
TB history, symptoms, and exposures								
Previous TB	2 (2.7)	0 (0.0)	2.75 (2.29–3.31)	**<0.001**	1 (1.5)	0 (0.0)	2.93 (2.41–3.55)	**<0.001**
WHO TB symptom in the past mo ‡	4 (5.4)	6 (4.8)	1.09 (0.50–2.38)	0.837	2 (2.9)	4 (3.1)	0.97 (0.31–3.05)	0.952
Household WHO TB symptom	2 (2.7)	5 (4.0)	0.77 (0.23–2.51)	0.660	2 (2.9)	4 (3.1)	0.97 (0.31–3.05)	0.952
TB exposure in the past 2 yrs	1 (1.4)	2 (1.6)	0.90 (0.18–4.52)	0.898	1 (1.5)	0 (0.0)	2.93 (2.41–3.55)	**<0.001**
HIV								
CD4, cells/mm^3 (N = 90) §	546 (391–725)	448 (287–597)	1.00 (1.00–1.00)	**0.039**				
HIV viral load, copies/mL (n = 174) §	0 (0–40)	0 (0–65)	1.00 (1.00–1.00)	0.114				
HIV viral load undetectable (n = 174)‖	58 (90.6)	95 (86.4)	1.32 (0.65–2.69)	0.434				
Time since HIV diagnosis, yrs	2.8 (0.4–7.6)	3.3 (0.5–6.3)	1.00 (0.96–1.05)	0.847				
ART before pregnancy	50 (67.6)	85 (67.5)	1.00 (0.68–1.48)	0.988				
Any IPT	40 (54.1)	85 (67.5)	0.71 (0.49–1.00)	**0.056**				
IPT on enrollment (n = 125)	12 (30.0)	26 (30.2)	0.99 (0.57–1.74)	0.979				

*LTBI defined as TST-positive or QFT-positive, where TST-positive ≥5 mm for WLHIV and ≥10 mm for HIV-negative women.
†Relative risk (RR) estimated using a generalized linear model (GLM) with log link and Poisson family.
‡WHO TB symptoms: fever, cough, weight loss, and night sweats.
§CD4 and viral load data were collected from routine programmatic data.
‖Undetectable viral load ≤20 copies/mL

postulate that pregnant WLHIV in our study were relatively immunocompetent because they were all on ART with relatively high CD4 counts, which may have allowed them to mount a TST response. Our results indicate that HIV-negative women had slightly lower MUAC than WLHIV, which indicates that nutritional status could have contributed to false negatives. When decreasing the TST cutoff to ≥5 mm for all women, the difference in TST positivity between WLHIV and HIV-negative women was no longer significant, suggesting that perhaps these 2 groups are more similar regarding immune status than other studies with WLHIV who were more immunosuppressed. In addition, there may be some other explanations contributed to negative results among HIV-negative women. TST false negativity, or anergy, has been associated with not only HIV but also immunosuppression, malnutrition, chronic kidney disease, and alcohol use.[32,33]

Although the prevalence of QFT positivity was similar between pregnant women with and without HIV in our study, QFT-Plus IFN-γ levels in response to TB1 and TB2 antigens were lower in WLHIV compared with HIV-negative women with LTBI. This is consistent with studies of pregnant women in Ethiopia,[11] adults in Italy,[34] and recent unpublished data from western Kenya, all of which showed lower TB1 and TB2 responses in PLHIV compared with that in HIV-negative

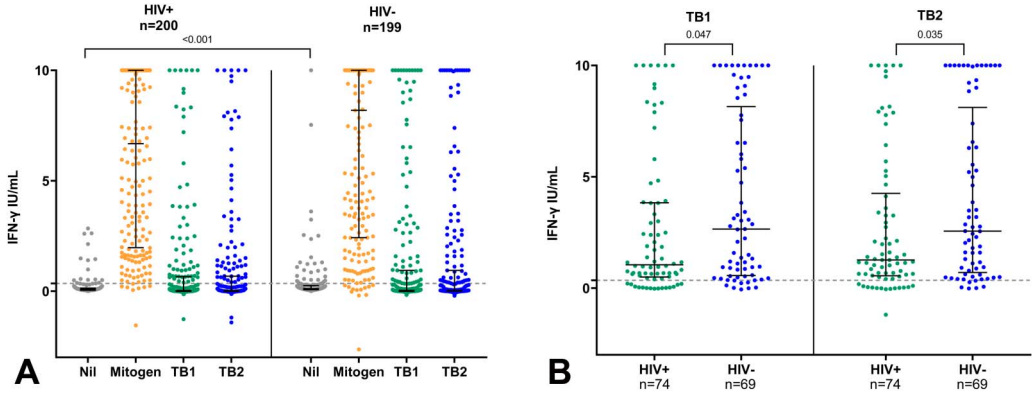

FIGURE 3. Quantitative QFT-Plus responses by HIV status among pregnant women. A, Quantitative QFT-Plus responses compared between women with and without HIV, and (B) TB-specific antigen responses compared between women with and without HIV among LTBI+ (QFT+ or TST+) women. Mitogen = mitogen–nil, TB1 = TB1–nil, TB2 = TB2–nil. full color online

persons.[35] CD4 cells from PLHIV have impaired IFN-γ–secreting capacity,[36] and IFN-γ concentration in response to TB-specific antigens (using QFT-Gold assay) has been correlated to CD4 cell count.[37] In our study, WLHIV with LTBI had higher median CD4 counts than those with negative LTBI results, consistent with this mechanism. Higher IPT usage among LTBI-negative WLHIV may have also influenced results; although IPT decreases risk of active TB disease, it may have reduced risk of LTBI and has been associated with a loss of QFT positivity,[18] TST reversion,[38] and decrease in IFN-γ concentration.[39]

Previous studies have suggested lowering QFT-plus cutoffs for pregnant WLHIV.[11] However, in our study, QFT positivity prevalence between WLHIV and HIV-negative women remained similar after adjusting the QFT positivity threshold to ≥0.20 IU/mL or ≥0.70 IU/mL instead of the manufacturer threshold of ≥0.35 IU/mL. Similarly, changing the QFT threshold did not have an impact on differential LTBI prevalence in our cohort, suggesting that WLHIV in our study (with relatively high median CD4 count and universal ART coverage) may have had robust immune responses to TB antigens used in QFT-Plus, in contrast to more immunosuppressed cohorts.[40]

We found higher TST/QFT agreement in WLHIV compared with that in HIV-negative women (70% vs 62%, respectively), which is lower agreement but showing a similar trend to the study conducted by Birku et al (98% vs. 90%).[12] Other studies found similar, lower TST/QFT agreement in pregnant WLHIV in Kenya (kappa 0.20)[17] and India (kappa 0.25)[9] and at delivery in a multinational study (kappa 0.40)[18] because of a similar pattern of higher QFT positivity compared with TST. It is postulated that because the TST response requires IFN-γ, TNF-α, IL-2, and other cytokines to stimulate a delayed-type hypersensitivity reaction, there may be more falsely negative TSTs in pregnant women or immunocompromised populations, which could explain the lower TST positivity compared with QFT.[9] This would not, however, explain why our study showed a higher proportion of TST positivity in WLHIV than in HIV-negative women, which remained even after using the higher cutoff for both groups in sensitivity analyses.

There were limitations and strengths to our study. Baseline characteristics differed between WLHIV and HIV-negative women, which may have influenced LTBI diagnostic performance. To address this, we assessed cofactors of LTBI in the entire cohort and in analyses stratified by HIV status. We relied on participant report of IPT initiation, which may have affected our estimates of IPT use. Although IPT rollout is high in western Kenya and estimates of IPT use overall among pregnant WLHIV were similar to those reported in the same setting in our previously published estimates, notably, only 54% of WLHIV with evidence of LTBI in this study had received IPT.[41] Given the cross-sectional nature of our study and limitations, in general, for LTBI diagnosis, we are unable to ascertain the timing of *M. tuberculosis* infection acquisition and previous IPT use. Of note, only 54% of WLHIV with evidence of LTBI had received IPT. There are some data to suggest that LTBI diagnostics could be potentially most affected by changes occurring in the late third trimester, and the median gestational age in our cohort was 28 weeks.[42] Our study has significant strengths in that it is one of the largest studies to directly compare LTBI diagnostics, including the newer generation QFT-Plus, between pregnant WLHIV and HIV-negative pregnant women and in the contemporary setting of universal ART coverage and high IPT uptake among WLHIV.

In conclusion, this study adds to our knowledge regarding performance of TST and QFT-Plus diagnostics in detecting LTBI in pregnant women with and without HIV. Our data showed a pattern of overall lower TST positivity compared with QFT in pregnancy, and although QFT positivity was similar between WLHIV and HIV-negative women, HIV infection was associated with lower TB-specific antigen responses among women with LTBI. Although the current Kenyan guidelines recommend IPT for all PLHIV including among those who are pregnant, our data identified WLHIV with LTBI but had not yet received IPT. Given the results of a recent large-scale, randomized, noninferiority study demonstrating the association of higher risk for adverse pregnancy outcomes with initiation of IPT during pregnancy, understanding how pregnancy affects the performance of LTBI diagnostics will be crucial to safely target groups with the highest risk for TB prevention, including pregnant

mothers with particular risk factors.[43] Furthermore, the ability to detect LTBI accurately with diagnostics influences how policy makers and researchers measure LTBI prevalence and risk factors of infection, which has implications for larger public health interventions and design of vaccine and other prevention studies that rely on these diagnostics for TB infection entry criteria or outcomes.

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