MAJOR ARTICLE

Latent Tuberculosis Detection by Interferon γ Release Assay during Pregnancy Predicts Active Tuberculosis and Mortality in Human Immunodeficiency Virus Type 1–Infected Women and Their Children

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Background. We evaluated the prognostic usefulness of interferon γ release assays (IGRAs) for active tuberculosis and mortality in Kenyan human immunodeficiency virus type 1 (HIV-1)–infected women and their infants.

Methods. Prevalence and correlates of *Mycobacterium tuberculosis*–specific T-SPOT.TB IGRA positivity were determined during pregnancy in a historical cohort of HIV-1–infected women. Hazard ratios, adjusted for baseline maternal CD4 cell count (aHR_{CD4}), were calculated for associations between IGRA positivity and risk of active tuberculosis and mortality over 2-year postpartum follow-up among women and their infants.

Results. Of 333 women tested, 52 (15.6%) had indeterminate IGRA results. Of the remaining 281 women, 120 (42.7%) had positive IGRA results, which were associated with a 4.5-fold increased risk of active tuberculosis (aHR_{CD4}, 4.5; 95% confidence interval [CI], 1.1–18.0; $P = .030$). For immunosuppressed women (CD4 cell count, <250 cells/μL), positive IGRA results were associated with increased risk of maternal mortality (aHR_{CD4}, 3.5; 95% CI, 1.02–12.1; $P = .045$), maternal active tuberculosis or mortality (aHR_{CD4}, 5.2; 95% CI, 1.7–15.6; $P = .004$), and infant active tuberculosis or mortality overall (aHR_{CD4}, 3.0; 95% CI, 1.0–8.9; $P = .05$) and among HIV-1–exposed uninfected infants (aHR_{CD4}, 7.3; 95% CI, 1.6–33.5; $P = .01$).

Conclusions. Positive IGRA results for HIV-1–infected pregnant women were associated with postpartum active tuberculosis and mortality among mothers and their infants.

Tuberculosis and human immunodeficiency virus type 1 (HIV-1) infection significantly increase maternal and infant mortality [1–3]. In resource-limited settings, the greatest burden of tuberculosis among women occurs during childbearing age [4]. Identifying and treating latent tuberculosis infection (LTBI) during pregnancy could reduce maternal and infant morbidity and mortality. The preventive benefit of this approach is likely to be amplified in the context of HIV-1 infection but depends on accurate detection of LTBI among HIV-1–infected pregnant women.

The diagnostic usefulness of tuberculin skin test (TST) has been established in studies demonstrating that TST positivity was associated with increased risk of active tuberculosis [5–8]. However, TST has limitations with regard to specificity and sensitivity, particularly in HIV-1–infected individuals [9]. Newer T cell interferon γ release assays (IGRAs) appear to better detect LTBI after recent tuberculosis exposure than does the TST [9–11]. However, there are limited conflicting data on the predictive value of IGRAs for risk of active tuberculosis. Three studies observed significant association of IGRA positivity with subsequent active tu-

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berculosis in adults [12], children [13], and HIV-1–infected individuals [14], and 2 studies observed no predictive association [15, 16].

Screening and prophylaxis for LTBI peripartum may be a strategic approach to reduce maternal and infant mortality, particularly among HIV-1–infected women. We conducted a study to determine the association between tuberculosis IGRAs and subsequent active tuberculosis, progression of HIV-1 infection, and mortality among HIV-1–infected women and their infants.

METHODS

The study involved a historical cohort of HIV-1–infected women and their infants. We used cryopreserved peripheral blood mononuclear cells (PBMCs) archived from this previously described cohort [17, 18]. Between 1999 and 2005, 535 HIV-1 seropositive pregnant women were enrolled at 32 weeks gestation at Kenyatta National Hospital (Nairobi, Kenya) and were followed up postpartum with their infants for up to 2 years. We selected 393 women with at least 1 cryopreserved PBMC sample obtained at enrollment.

At enrollment, sociodemographic characteristics and medical history were collected, and physical examination was conducted. Women received standard antenatal care, including zidovudine, to prevent infant HIV-1 transmission. After delivery, women were seen with their infants at weeks 2 and 4, monthly thereafter to 12 months, 3 times monthly for an additional 12 months, and between scheduled visits if they had health complaints. Blood samples were collected from mothers at enrollment and serially during follow-up from mothers and infants for HIV-1 RNA level and CD4 cell count measurement and PBMC storage. HIV-1 infection in infants was confirmed by serial HIV-1 DNA [19] and RNA [20] testing.

Written informed consent for the parent study was obtained from women for their and their infants' participation. The Human Subjects Division at University of Washington (Seattle) and the Ethical Review Committee at University of Nairobi approved the parent and current studies.

LTBI detection. Maternal cryopreserved PBMCs were tested for *M. tuberculosis*–specific IGRA responses with use of the T-SPOT.TB IGRA (Oxford Immunotec). Cells were incubated overnight in 5 mL AIM-V (Invitrogen) serum-free media. Cells were recounted and suspended in AIM-V at a concentration of 2.5×10^5 cells/100 μL. Mean cell viability was 73.7%. The T-SPOT.TB test procedure was performed according to the manufacturer's instructions. Duplicate wells containing 100 μL of 2.5×10^5 PBMCs per well were stimulated with 50 μL each of phytohaemagglutinin (positive control), ESAT-6, CFP-10, and AIM-V media (negative control) and were incubated overnight at 37°C in 5% carbon dioxide. Plates were washed with phosphate-buffered saline and were developed using a conjugate reagent (anti–interferon γ antibody). Dark-colored spots were visualized at addition of a colorimetric substrate solution. Developed plates were dried overnight, and spots were read using an automated ELISpot reader (CTL-Immunospot S4 Core Analyzer). Results were interpreted on the basis of criteria provided by the manufacturer. Assays were considered positive if the mean spot count in ESAT-6 or CFP-10 minus the spot count in the negative control wells was $\geqslant$6 (if the mean spot count in the negative control wells was $\leqslant$5) or if the mean spot count in ESAT-6 or CFP-10 minus the spot count in the negative control wells was >2 times the spot count in the negative control wells (if the mean spot count in the negative control wells was 6–10). Assay results were considered to be negative if the above criteria were not met, and results were considered to be indeterminate if the mean spot count was <20 in the positive control wells or >10 in negative control wells.

Clinical outcomes and progression of HIV-1 infection. Previous active tuberculosis was self-reported at enrollment, and these women were excluded from analyses (Figure 1). During follow-up, women with symptoms consistent with tuberculosis were referred for sputum testing and treatment (mothers) or Mantoux testing (infants) at the Kenyan Ministry of Health (MOH) TB Clinic (Nairobi). The Kenyan MOH protocol for suspected tuberculosis includes performance of a chest radiograph and obtainment of 3 sputum samples. Adults with at least 1 positive sputum smear result received a diagnosis of sputum-positive pulmonary tuberculosis. Adults with negative results of all 3 sputum smears but with chest radiograph images and clinical presentation suggestive of tuberculosis received a diagnosis of sputum-negative pulmonary tuberculosis. Adults with symptoms suggestive of extrapulmonary tuberculosis were tested according to the site of tuberculosis. Mantoux testing was incorporated as part of standard investigative procedures for children [21]. Information regarding tuberculosis diagnosis and treatment at MOH was obtained from mothers at the study clinic at all follow-up visits. Information regarding tuberculosis diagnostic procedures and disease classification (sputum-positive or sputum-negative) from the MOH was not collected. Information regarding symptoms near the time of death was obtained using standardized verbal autopsy forms.

Measurement of CD4 cell counts was conducted at the University of Nairobi with use of a FACScan flow cytometer (Becton Dickinson) [22]. Plasma HIV-1 RNA levels were quantified at Fred Hutchinson Cancer Research Center (Seattle, WA) with use of a transcription-mediated amplification assay developed by Gen-Probe [20].

Analysis. Women with positive and negative IGRA responses were compared with regard to baseline characteristics with use of the Student t test for means and bootstrapped standard errors to detect differences in medians for continuous variables, the χ^2 test for categorical variables, and the Fisher

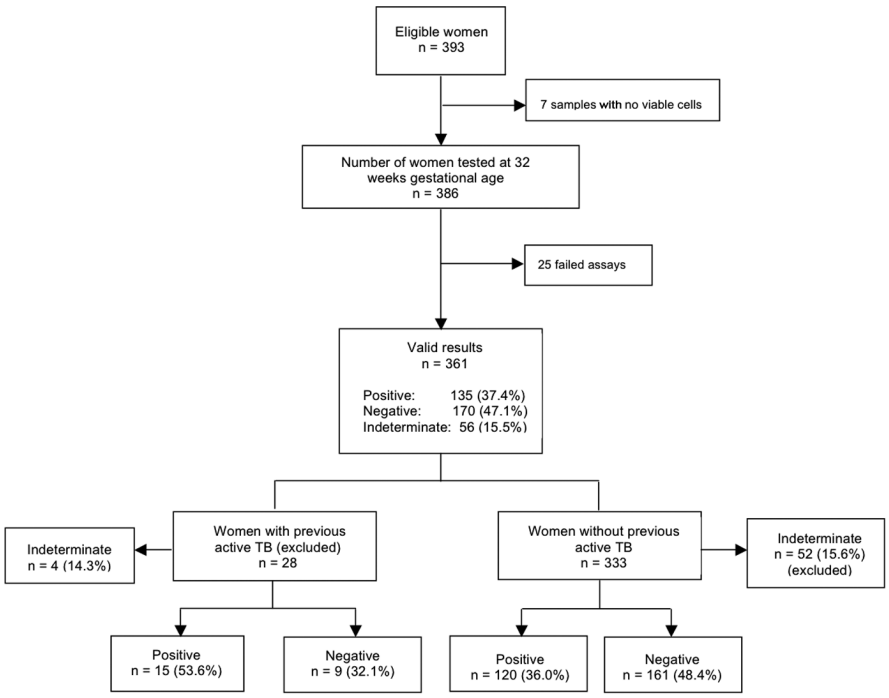

Figure 1. Flowchart of human immunodeficiency virus type 1 (HIV-1)–infected women tested for interferon γ release assay (IGRA) responses with use of T-SPOT.TB. TB, tuberculosis.

exact test for categorical variables with observed or expected cell counts of ⩽5.

Kaplan Meier survival analysis was conducted for the following outcomes, by maternal IGRA response, within 24 months after delivery: first diagnosis of active tuberculosis, mortality, and composite outcome of first diagnosis of active tuberculosis or mortality among women and their infants. Cox proportion hazards regression models were fit to estimate unadjusted and adjusted hazard ratios (aHRs). Among infants, HRs of the outcomes were estimated using maternal IGRA and were further stratified by infant HIV-1 infection status; HIV-1 infection status was treated as a time-varying covariate. Rate of change in HIV-1 progression markers (CD4 cell count and HIV-1 load), by IGRA response, were compared using linear mixed effects models, allowing for random intercepts and random slopes. In all multivariate models, we adjusted for baseline CD4 cell count, because it is a primary risk factor for tuberculosis and mortality and was associated with IGRA positivity. We conducted additional multivariate analyses, adjusting for potential tuberculosis exposure correlates and those associated with IGRA positivity in univariate analyses. Women were censored at initiation of antiretroviral therapy, death, last seen, or 24 months postpartum. Some women reported a history of tuberculosis between 2 clinic visits but never received a diagnosis of tuberculosis, using the standard study procedure. These women were censored at the last visit that they were known to be free of tuberculosis. Analyses were performed using Stata software (version 10.0; Stata).

RESULTS

Prevalence of LTBI. Of 333 HIV-1–infected women tested for *Mycobacterium tuberculosis*–specific IGRA responses at 32 weeks gestation, 120 (36.0%) had positive responses, 161 (48.4%) had negative responses, and 52 (15.6%) had indeterminate responses (Figure 1). The mean age of women was 24.9 years (range, 18–40 years). Most women were married (92.5%), had less than primary education (57%), were unemployed (67%), lived in a single room (78%), and shared a toilet (91%). The median CD4 cell count was 440 cells/μL (interquartile range [IQR], 306–644), and the median $\log_{10}$ plasma viral load was 4.8 copies/mL (IQR, 4.2–5.3).

Excluding women with indeterminate responses, the prevalence of positive IGRA responses was 42.7%. Women with positive IGRA responses had a higher median CD4 cell count (477 vs 396 cells/μL; $P = .03$) and were more likely to use a flush toilet (60.8% vs 44.7%; $P < .008$), compared with women with negative IGRA responses (Table 1).

Of the 52 indeterminate responses, 44 (84.6%) were indeterminate on the basis of >10 spot counts in the negative control well and 8 (15.4%) were indeterminate on the basis of <20 spot counts in the positive control well. Women with indeter-

Table 1. Baseline Sociodemographic and Clinical Characteristics of Pregnant Human Immunodeficiency Virus Type 1 (HIV-1)–Infected Women, by Interferon γ Release Assay (IGRA) Response at 32 Weeks Gestation

Variable	IGRA response		
	Positive (n = 120)	Negative (n = 161)	P
Sociodemographic characteristic			
Mean age, years (95% CI)	24.9 (24.1–25.7)	24.9 (24.2–25.6)	.98
Ever married			
Yes	113 (94.2)	148 (91.9)	.47
No	7 (5.8)	13 (8.1)	
More than primary education			
Yes	49 (41.5)	71 (44.4)	.60
No	69 (58.5)	88 (55.0)	
Employed			
Yes	43 (35.8)	50 (31.1)	.4
No	77 (64.2)	111 (68.9)	
Residential conditions			
Living in 1 room			
>1 room	27 (22.7)	27 (17.1)	.24
1 room	92 (77.3)	131 (82.9)	
Mean no. of rooms (95% CI)	1.3 (1.2–1.4)	1.3 (1.2–1.4)	.61
Use of toilet			
Flush toilet	73 (60.8)	72 (44.7)	.008
Pit toilet	47 (39.2)	89 (55.3)	
Use of shared toilet			
Yes	107 (89.2)	148 (91.9)	.43
No	13 (10.8)	13 (8.1)	
No. of persons per room (95% CI)	3.0 (2.8–3.2)	3.0 (2.8–3.2)	.95
History of HIV-1–related illness			
Yes	10 (8.3)	14 (8.7)	.91
No	110 (91.7)	147 (91.3)	
HIV-1 markers			
Mean CD4 cell count, cells/μL (95% CI)	530.1 (480.1–580.1)	464.2 (421.5–506.9)	.05
Median CD4 cell count, cells/μL (IQR)	477.5 (345–669)	396.0 (299–606)	.03
CD4 cell count, cells/μL			
<250	17 (14.7)	28 (17.8)	.48
⩾250	99 (85.3)	129 (82.2)	
Mean CD8 cell count, cells/μL (95% CI)	979.7 (892.4–1067.1)	959.8 (901.2–1018.4)	.70
Median CD8 cell count, cells/μL (IQR)	873.5 (653.0–1184.5)	874.0 (689.0–1183)	.99
Mean HIV-1 load, $\log_{10}$ copies/mL (95% CI)	4.5 (4.4–4.7)	4.7 (4.6–4.8)	.11
Median HIV-1 load, $\log_{10}$ copies/mL (IQR)	4.7 (4.2–5.2)	4.9 (4.2–5.3)	.13
HIV-1 load, copies/mL			
>50,000	57 (47.9)	87 (54.0)	.31
⩽50,000	62 (52.1)	74 (46.0)	

NOTE. Data are no. (%) of women, unless otherwise indicated. CI, confidence interval; IQR, interquartile range.

minate responses did not differ from women with valid assays with regard to CD4 cell count and viral load (data not shown).

Of 333 women who had samples tested using IGRA, 321 were seen after delivery. Excluding women with indeterminate IGRA responses, 269 women remained in longitudinal analyses, contributing 345.2 person-years of follow-up (PYFU).

Maternal postpartum active tuberculosis. Nine women developed active tuberculosis during follow-up (2.7 cases per 100 PYFU; 95% confidence interval [CI], 1.2–5.1 cases per 100 PYFU). Six cases occurred among 110 women with positive IGRA responses (4.2 cases per 100 PYFU; 95% CI, 1.5–9.1 cases per 100 PYFU), and 3 occurred among 148 women with neg-

ative IGRA responses (1.6 cases per 100 PYFU; 95% CI, 0.3–4.7 cases per 100 PYFU; $P = .2$). In univariate analysis, a positive IGRA result was associated with a 2.6-fold increased risk of active tuberculosis, but the association was not statistically significant (HR, 2.6; 95% CI, 0.7–10.3; $P = .17$) (Table 2). Number of persons in the household (HR, 1.5; 95% CI, 1.0–2.4; $P = .05$), baseline CD4 cell count of <250 cells/μL (HR, 6.8; 95% CI, 1.9–24.4; $P = .003$), and mean HIV-1 load (HR, 2.8; 95% CI, 1.2–6.6; $P = .02$) were significantly associated with incident active tuberculosis.

In multivariate analysis, adjusting for baseline CD4 count, IGRA positivity was associated with a 4.5-fold increased risk of active tuberculosis (aHR, 4.5; 95% CI, 1.1–18.0; $P = .03$). Baseline CD4 cell count of <250 cells/μL was associated with a 9.3-fold increased risk of incident active tuberculosis (aHR, 9.3; 95% CI, 2.4–36.1; $P = .001$), adjusting for IGRA response.

Maternal mortality. The postpartum maternal mortality in this cohort was 3.8 deaths per 100 PYFU (95% CI, 2.0–6.4 deaths per 100 PYFU). In both unadjusted and CD4 cell count–adjusted analyses, IGRA positivity was not significantly associated with mortality (Table 2). Baseline CD4 cell count of <250 cells/μL was associated with a 19-fold increased risk of mortality (aHR, 19.5; 95% CI, 4.8–78.5; $P < .001$). Stratifying by baseline CD4 cell count (<250 and ⩾250 cells/μL), a positive IGRA result was associated with a 3.5-fold increased risk of mortality among women with a baseline CD4 cell count of <250 cells/μL (aHR, 3.5; 95% CI, 1.02–12.1; $P = .045$) (Table 2 and Figure 2*B*).

Maternal active tuberculosis or mortality. Because undiagnosed tuberculosis may contribute to mortality, a combined outcome of active tuberculosis or mortality was assessed. IGRA positivity was associated with a 3.3-fold increased risk of either active tuberculosis or mortality (aHR, 3.3; 95% CI, 1.3–8.4; $P = .01$). Among women with baseline CD4 cell counts of <250 cells/μL, IGRA positivity was associated with a 5.2-fold greater risk of active tuberculosis or death (aHR, 5.2; 95% CI, 1.7–15.6; $P = .004$) (Table 2).

Adjusting for potential LTBI or tuberculosis cofactors (ie, age, employment, and flush toilet), the associations between IGRA response and CD4 cell count and tuberculosis, mortality, and tuberculosis or mortality remained statistically significant (Table 2).

Change in HIV-1 RNA level and CD4 cell count. CD4 cell counts decreased and HIV-1 loads increased significantly during follow-up, as expected, because of HIV-1 disease progression in women (Table 3 and Figure 3). However, the rate of change in the 2 HIV-1 progression markers did not differ significantly between IGRA-positive and IGRA-negative women.

Active tuberculosis and mortality among infants. Eight cases of active tuberculosis were diagnosed among infants (3.5

Table 2. Crude and Adjusted Hazard Ratios (HRs) for Clinical Outcomes in Human Immunodeficiency Virus Type 1 (HIV-1)–Infected Women with Positive Tuberculosis Interferon γ Release Assay (IGRA) Results, Compared with HIV-1–Infected Women with Negative Tuberculosis IGRA Responses

Outcome	No. of events	Incidence, cases per 100 PYFU[a]	P[b]	HR (95% CI)	P	Adjusted HR[c] (95% CI)	P	Adjusted HR[d] (95% CI)	P
Active tuberculosis									
Whole cohort	9	2.7	…	2.6 (0.7–10.3)	.17	4.5 (1.1–18.0)	.03	4.8 (1.2–19.7)	.03
Baseline CD4 cell count, cells/μL									
⩾250[e]	4	1.5	…	3.3 (0.4–31.1)	.29	3.9 (0.4–38.9)	.24	2.8 (0.1–62.9)	.52
<250[e]	5	9.7	<.001	3.5 (0.7–18.4)	.14	6.2 (1.1–36.0)	.04	8.0 (0.3–210.5)	.22
Mortality									
Whole cohort	13	3.8	…	1.1 (0.4–3.3)	.86	2.5 (0.7–8.4)	.15	2.7 (0.7–10.1)	.15
Baseline CD4 cell count, cells/μL									
⩾250[e]	3	1.1	…	0.5 (0.05–4.9)	.56	0.7 (0.1–8.1)	.74	0.5 (0.1–2.3)	.36
<250[e]	10	18.2	<.001	2.3 (0.7–7.7)	.18	3.5 (1.0–12.1)	.05	4.6 (1.1–19.3)	.04
Active tuberculosis or mortality									
Whole cohort	20	6.0	…	1.6 (0.7–3.8)	.30	3.3 (1.3–8.4)	.01	3.7 (1.3–10.6)	.01
Baseline CD4 cell count, cells/μL									
⩾250[e]	7	2.5	…	1.4 (0.3–6.2)	.63	1.8 (0.4–8.0)	.5	1.4 (0.2–7.9)	.72
<250[e]	13	25.2	<.001	4.0 (1.3–12.2)	.01	5.2 (1.7–15.6)	.004	10.2 (1.5–69.0)	.02

[a] Incidence rate among women with CD4 cell counts of ⩾250 and <250 cells/μL, not accounting for IGRA status. PYFU, person-years of follow-up.
[b] Log-rank P value comparing incidence rates among women with CD4 cells counts of ⩾250 cells/μL with those among women with CD4 cells counts of <250 cells/μL.
[c] Adjusted for maternal baseline CD4 cell count.
[d] Adjusted for maternal baseline CD4 cell count, age, employment, and use of shared flush toilet.
[e] Baseline CD4 cell count was adjusted within strata of CD4 cell counts (<250 and ⩾250 cells/μL).

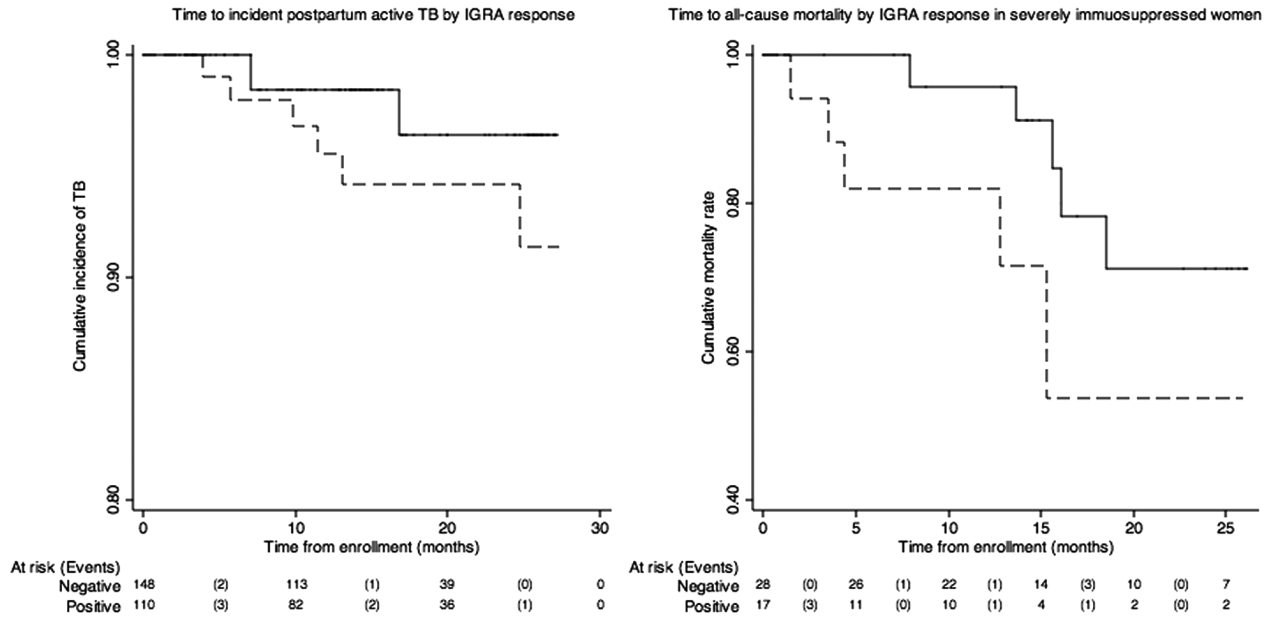

Figure 2. Kaplan-Meier survival curves for time to incident postpartum active tuberculosis (TB) (*left*) and time to mortality (*right*) in severely immunosuppressed women (CD4 cell count, <250 cells/μL), by interferon γ release assay (IGRA) response, during pregnancy. Positive IGRA responses are indicated by the dashed line, and negative IGRA responses are indicated by the solid line. $P = .16$ for equality of survivor functions (incidence of postpartum tuberculosis); $P = .09$ for mortality among severely immunosuppressed women.

cases per 100 PYFU; 95% CI, 1.5–6.8 cases per 100 PYFU); 5 infants with active tuberculosis had mothers with positive IGRA responses (4.8 cases per 100 PYFU; 95% CI, 1.6–11.2 cases per 100 PYFU), and 3 had mothers with negative IGRA responses (2.4 cases per 100 PYFU; 95% CI, 0.5–6.9 cases per 100 PYFU; $P = .36$).

Maternal IGRA positivity was not significantly associated with increased risk of active tuberculosis or mortality among infants, overall or by HIV-1 infection status. However, maternal IGRA positivity was associated with increased risk of mortality among infants born to mothers with baseline CD4 cell counts of <250 cells/μL (aHR, 3.0; 95% CI, 1.0–9.1; $P = .05$) (Table 4). Maternal IGRA positivity was not associated with the combined outcome of either active tuberculosis or mortality among infants, overall or by HIV-1 infection status. However, among infants born to mothers with baseline CD4 cell counts of <250 cells/μL, maternal IGRA positivity was associated with an increased risk of active tuberculosis or mortality among all infants (aHR, 3.0; 95% CI, 1.0–8.9; $P = .05$) and among HIV-1–exposed uninfected infants (aHR, 7.3; 95% CI, 1.6–33.5; $P = .01$).

DISCUSSION

In this longitudinal assessment of HIV-1–infected women and their infants, we observed that 36% of HIV-1–infected women

Table 3. Rate of Change in CD4 Cell Counts and Log_{10} Plasma Human Immunodeficiency Virus Type 1 (HIV-1) RNA Levels during Follow-Up in Women with Positive and Negative Tuberculosis Interferon γ Release Assay Responses during Pregnancy

Variable	Change per month in CD4 cell count, cells/μL (95% CI)[a]	*P*	Change per month in log_{10} plasma HIV-1 RNA level, copies/mL[b] (95% CI)	*P*
Negative response	−7.2 (−10.0 to −4.5)	<.001	0.02 (0.01–0.03)	<.001
Positive response	−7.9 (−11.0 to −4.8)	<.001	0.02 (0.01–0.03)	<.001
Difference	−0.65 (−4.8 to 3.5)	.76	0.003 (−0.008 to 0.014)	.61

NOTE. CI, confidence interval.

[a] Estimates adjusted for baseline CD4 cell count.

[b] Estimates adjusted for baseline log_{10} plasma viral load.

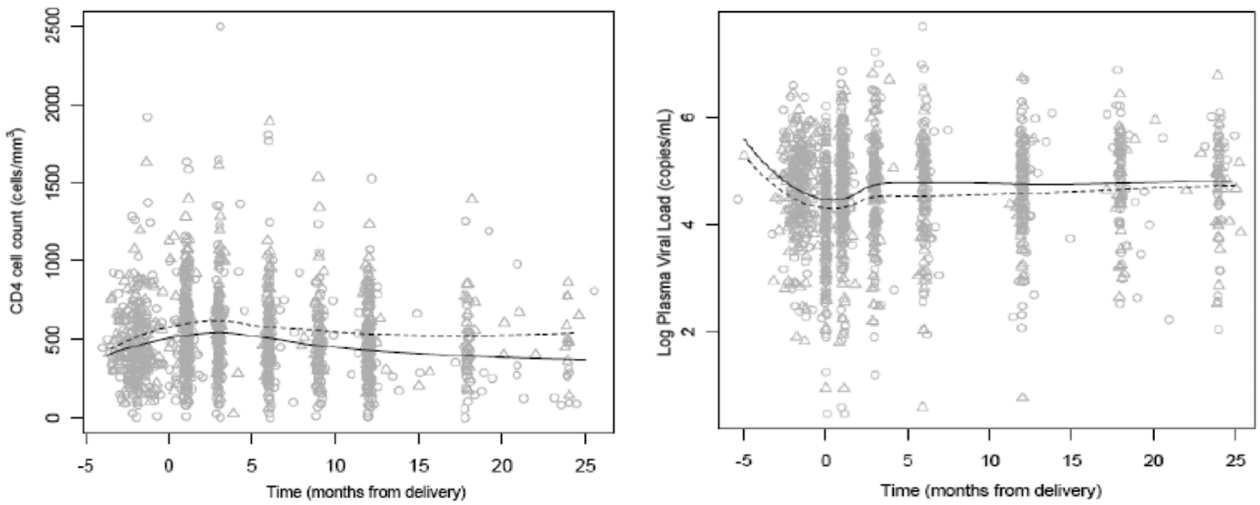

Figure 3. Change in CD4 cell count (*left*) and $\log_{10}$ plasma human immunodeficiency virus type 1 (HIV-1) RNA level (*right*) from enrollment to end of follow-up in women with positive (*dotted line, triangles*) and negative (*solid line, circles*) interferon γ release assay (IGRA) responses during pregnancy.

were positive for *Mycobacterium tuberculosis* by IGRA during pregnancy, which may reflect LTBI. Among women with determinate assays, 42.7% had positive IGRA responses. IGRA positivity was associated with a significant 4-fold increased risk of active tuberculosis, compared IGRA negativity. Among immunosuppressed women, a positive IGRA response was associated with a 3-fold increased risk of mortality and a 5-fold risk of either active tuberculosis or mortality. In addition, we observed a significant 3-fold increased risk of either active tuberculosis or mortality among infants born to immunosuppressed women with a positive IGRA result. Together, these data suggest that IGRAs performed for HIV-1–infected mothers during pregnancy identify a critical target group for tuberculosis prophylaxis that may prevent maternal active tuberculosis and enhance survival and, potentially, confer similar benefits in their infants.

IGRA positivity was common in this cohort of HIV-1–infected pregnant women. Unlike most tuberculosis studies that

Table 4. Adjusted Hazard Ratios (HRs) for Clinical Outcomes in Infants Born to Mothers with Positive Tuberculosis Interferon γ Release Assay (IGRA) Responses, Compared with Women with Negative Tuberculosis IGRA Responses, Overall and Stratified by Infant Human Immunodeficiency Virus Type 1 (HIV-1) Status

	All infants			HIV-1–infected infants			HIV-1–uninfected infants		
Outcome	No. of events	Adjusted HR[a,b] (95% CI)	*P*	No. of events	Adjusted HR[a,b] (95% CI)	*P*	No. of events	Adjusted HR[a,b] (95% CI)	*P*
Active tuberculosis	8	2.1 (0.5–8.8)	.34	3	2.7 (0.5–14.7)	.25	5	1.9 (0.3–11.8)	.50
Mortality									
Whole cohort	47	0.8 (0.4–1.4)	.44	19	0.4 (0.1–1.3)	.12	28	1.0 (0.5–2.2)	.91
CD4 cell count, cells/μL									
⩾250[c]	34[d]	0.6 (0.3–1.2)	.16	11	0.2 (0.01–1.4)	.09	23[d]	0.9 (0.4–2.1)	.81
<250[c]	11[d]	3.0 (1.0–9.1)	.05	8	1.7 (0.5–5.5)	.37	3[d]	7.3 (1.6–33.5)	.01
Active tuberculosis or mortality									
Whole cohort	52	0.9 (0.5–1.5)	.58	20	0.5 (0.2–1.5)	.23	32	1.0 (0.5–2.1)	.91
CD4 cell count, cells/μL									
⩾250[c]	41	0.7 (0.4–1.3)	.21	12	0.3 (0.1–1.6)	.16	29	0.8 (0.4–1.7)	.61
<250[c]	11	3.0 (1.0–8.9)	.05	8	2.1 (0.6–7.0)	.24	3	7.3 (1.6–33.5)	.01

[a] Adjusted HR for incidence of outcome among infants born to women with positive IGRA responses, compared with infants born to women with negative IGRA responses. CI, confidence interval.
[b] Adjusted for maternal baseline CD4 cell count.
[c] Maternal baseline CD4 cell count was adjusted within strata of maternal baseline CD4 cell count (<250 and ⩾250 cells/μL).
[d] Numbers do not add up to the total because of missing maternal CD4 cell count data.

involve contacts of active tuberculosis cases, women in this study were recruited from an antenatal clinic in Kenya. Therefore, we expect that the prevalence of IGRA positivity is representative of HIV-1–infected adults in Kenya. We excluded women with self-reported history of active tuberculosis. Thus, a positive IGRA result likely represented latent tuberculosis rather than relapsed or incompletely treated active tuberculosis. The earliest case of tuberculosis in our cohort of women occurred ~4 months after IGRA testing, suggesting that all of the women with positive IGRA results had latent rather than undiagnosed active tuberculosis. Our estimate of 36% IGRA positivity lies within the range of TST positivity reported by other studies from areas where tuberculosis is endemic among HIV-1–infected women during pregnancy and the postpartum period [23, 24]. To our knowledge, there are no studies specifically involving tuberculosis IGRAs for HIV-1–infected women in general or during pregnancy. The prevalence of IGRA positivity in other studies of tuberculosis-exposed individuals has been 36%–68% and has varied by study population, IGRA used (T-SPOT.TB or QuantiFERON-TB [Cellestis]), and presence of HIV-1 [14, 15, 25].

IGRA positivity was associated with higher CD4 cell counts, which suggests that the test may be compromised by immunosuppression, as would be expected if CD4 cells contribute to *M. tuberculosis* response or sustain a CD8 cell and *M. tuberculosis* response. Conflicting data exist on the extent to which immunosuppression modifies IGRA responses. Some studies suggest that tuberculosis IGRA responses are independent of CD4 cell count [25–27], whereas others have shown an association between positive IGRA responses and higher CD4 cell counts, consistent with our observation [25, 27]. Studies of adapting T-SPOT.TB criteria to include a lower spot count as indicating positivity in immunosuppressed individuals may be required, analogous to a smaller dermal TST reaction being considered as indicating positivity in HIV-1–infected individuals.

There is limited and conflicting evidence on the association between IGRAs and the risk of active tuberculosis. To our knowledge, there is only 1 study that has evaluated IGRAs for prediction of active tuberculosis in HIV-1–infected adults. In this study involving immigrants to Austria from countries with a high prevalence of tuberculosis, all 3 active tuberculosis cases occurred in persons with positive IGRA (QuantiFERON-TB) results, with an incidence of 8.1% [14]. In another study involving HIV-1–uninfected household contacts of persons with tuberculosis in Germany, tuberculosis incidence was 14.6% and all tuberculosis cases occurred in persons with positive QuantiFERON-TB results who refused isoniazid prophylaxis [12]. Among child contacts of persons with active tuberculosis in Turkey, positive ELISpot predicted a 3–4-fold increased risk of active tuberculosis, despite most children having received isoniazid; those who had not received isoniazid had an 11-fold increased risk [13]. In contrast, 2 studies in the Gambia and the Netherlands that involved mostly HIV-1–uninfected contacts of persons with active tuberculosis reported that neither TST nor IGRA responses were significantly associated with active tuberculosis [15, 16]. One of the reasons suggested for lack of prognostic value of ELISpot or TST in African settings is the endemicity of tuberculosis; community transmission may dilute prognostic usefulness [13]. We observed an association consistent with that observed in studies involving HIV-1–uninfected individuals in regions where tuberculosis is not endemic. Despite the high exposure to *M. tuberculosis* because of the high incidence of tuberculosis in Kenya (330 cases per 100,000 population per year [28]), we found that a positive IGRA response was significantly associated with active tuberculosis and is likely to be an underestimate of the true prognostic value of the test.

Our findings are consistent with those in the study by Gupta et al [23] in which a positive TST result at delivery was associated with a 3-fold increased risk of postpartum tuberculosis in HIV-1–infected women in India. We also observed that immunosuppressed women with positive IGRA responses had an increased risk of mortality. In Africa, tuberculosis is often the first manifestation of HIV-1 infection, is often misdiagnosed, and contributes substantially to mortality [29]. Our results reveal that a group of women with positive IGRA responses and CD4 cell counts of <250 cells/μL had the greatest of tuberculosis and mortality.

We found that IGRA positivity did not adversely influence CD4 cell count or viral load changes, consistent with LTBI being a contained infection that may not have systemic impact on HIV-1 until progression to active tuberculosis [30]. Maternal immune status and maternal mortality have been shown to independently predict mortality among infants born to HIV-1–infected mothers [31]. Gupta et al [23] observed a 3.4-fold increased risk of mortality among infants born to mothers with active tuberculosis. We found that IGRA positivity in women with immunosuppression was subsequently associated with either active tuberculosis or mortality in infants.

Our study had important strengths and limitations. The study involved a large cohort with carefully characterized longitudinal outcomes, enabling us to evaluate the associations between positive IGRA responses and a variety of important outcomes, including maternal active tuberculosis and mortality, progression of maternal HIV-1 infection (viral load and CD4 cell count), and infant active tuberculosis and/or mortality. This constellation of outcomes is of value for maternal-child health and for consideration of HIV-1 and tuberculosis programmatic links. Limitations of the study include the use of cryopreserved specimens rather than fresh specimens, which is recommended by the test manufacturer. This may have contributed to a higher prevalence of indeterminate assay responses (15%) than has

been reported in previous studies (0.6%–14%) [25–27, 32–34]. However, a new, prospective design would be difficult to envision, because positive IGRA responses would inform management and, thus, preclude evaluation of outcomes. Moreover, other studies have used frozen PBMCs for T-SPOT.TB and other ELISpot assays with favorable results. A study in Germany observed similar results with use of fresh and frozen PBMCs but also noted a decrease in assay sensitivity with frozen cells [35]. In our study, decreased sensitivity would have led to underestimation of prevalence of positive assay results and bias of potential predictive associations to the null. Other studies comparing ELISpots of fresh samples with ELISpots of frozen samples for varicella zoster or HIV-1 cellular responses observed no significant compromise of assays of frozen specimens [36, 37].

We lack maternal TST results, which would have been useful to compare with the IGRA results. Finally, the study was not primarily designed to evaluate tuberculosis; thus, confirmatory evidence of tuberculosis was limited. However, we assume that the MOH tuberculosis clinics used consistent tuberculosis diagnostic criteria for women and infants referred from the study and that these criteria were established before maternal IGRAs were performed. Thus, limitations in tuberculosis diagnosis were nondifferential, which would bias the HRs to null.

In summary, our study suggests that IGRA positivity during the peripartum period may have maternal and child benefits for HIV-1–infected women in areas with a high prevalence of HIV-1 infection and tuberculosis. Although isoniazid prophylaxis in HIV-1–infected individuals with positive TST results is recommended [38], there are limited data on IGRAs. Randomized clinical trials involving different durations and forms of tuberculosis preventive therapy are needed to determine optimal approaches for treating individuals with positive IGRA results, to reduce their risk of tuberculosis and mortality. In an era during which the burden of the twin HIV-1–tuberculosis epidemics is increasingly apparent and with scale-up of programs to prevent mother-to-child transmission of HIV-1 infection, there is an ideal opportunity to better integrate tuberculosis prevention and enhance maternal-child outcomes with use of systematic detection and treatment of LTBI.

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