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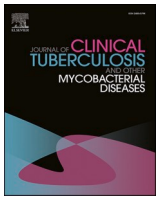

Discordance of 3rd and 4th generation QuantiFERON-TB Gold assays by pregnancy stages in India

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ABSTRACT

Background: Pregnancy and HIV affect CD4+ T lymphocytes and impact performance of QuantiFERON-TB Gold (QFT). We compared the results of QFT with QuantiFERON-TB Gold Plus (QFT-Plus), which also measures CD8+ responses to TB antigens, during pregnancy and postpartum.

Methods: We screened 516 pregnant women for TB infection (TBI) with IGRA. From 165 IGRA + pregnant women, QFT vs QFT-Plus results were compared at delivery and postpartum. Longitudinal changes in QFT-Plus were assessed in 74 pregnant women who received QFT-Plus testing at pregnancy, delivery, and postpartum.

Results: Through cross-sectional analysis of the IGRA + cohort, QFT-Plus showed higher positivity than QFT (80 % vs 65 %, p = 0.04) at delivery but no difference postpartum. Among 35 women with HIV, QFT-Plus returned more positive results than QFT at delivery and postpartum (76 % vs 47 %, p = 0.08; 90 % vs 80 %, p = 0.54), though not statistically significant. Longitudinally, QFT-Plus positivity by TB1 or TB2 was highest antepartum vs. delivery and postpartum (74 % vs. 58 % vs. 62 %; p = 0.09) and performed better than TB1 alone (100 % vs 90 %, p = 0.04) in women without HIV but not in women with HIV.

Conclusions: Performance of QFT-Plus was consistent across pregnancy, including at delivery when QFT positivity is lower. QFT-Plus may enhance antenatal TBI detection among pregnant women.

1. Introduction

The highest risk time for women to develop active tuberculosis (TB) is during and immediately after pregnancy [1,2]. If a woman develops TB during the peripartum period, it can result in multiple adverse pregnancy outcomes, including preterm birth and maternal and infant mortality [3,4]. During pregnancy, CD4+ T-cell response to *M. tuberculosis* (Mtb) decreases [5,6,7,8,9] which may explain the increased risk of TB progression postpartum, especially for women with HIV (WHIV) [10]. Pregnant women with TB infection (TBI) are also at higher risk of TB progression postpartum [11]. Therefore, detecting antenatal TBI is critical for targeted TB prevention in countries like India, where TB is endemic [12,13].

Interferon-gamma release assays (IGRA) are diagnostic tests for TBI.

Abbreviations: WHIV, Women with HIV; TBI, TB infection; QFT, QuantiFERON TB Gold in Tube; QFT-Plus, QuantiFERON TB Gold Plus; TB1, TB antigen tube 1; TB2, TB antigen tube 2.

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The 3rd generation IGRA, QuantiFERON-TB Gold in-Tube (QFT) (Cellestis Inc. CA, 91355, USA) detects TBI by measuring IFN-γ from host CD4+ T-cells after in vitro stimulation with Mtb-specific antigens (TB7.7, ESAT-6, and CFP-10). Studies from our group and others in high-burden settings have described lower TBI detection with QFT at delivery, compared to pregnancy or postpartum, and among women with HIV compared to women without HIV [6,14].

The 4th generation IGRA, QuantiFERON-TB Gold Plus (QFT-Plus) (QIAGEN GmbH, 40,724 Hilden, Germany), was designed to improve IGRA sensitivity by measuring the immune response in both CD4+ and CD8+ T-cells. The QFT-Plus consists of two TB antigen tubes (TB1 and TB2), which contain ESAT-6 and CFP-10. Like the QFT, the TB1 tube is designed to elicit CD4+ T-cell responses. The TB2 tube contains additional peptides to induce a response from CD8+ cytotoxic T-lymphocytes [15]. With the addition of the CD8+ response, we hypothesized that QFT-Plus would detect more cases of TBI compared to QFT across stages of pregnancy, especially among WHIV. Improved TBI detection would optimize TB prevention efforts and avert TB-related adverse maternal-infant outcomes.

2. Methods

Between May 2016 and December 2019, we conducted a prospective observational cohort study of pregnant women with and without HIV (PREgnancy Associated CHanges In Tuberculosis immunology (PRACHITi)). The PRACHITi study was conducted at Byramjee Jeejeebhoy Government Medical College and Sassoon General Hospitals in Pune, India. The primary objective of the study was to examine the longitudinal effects of pregnancy and HIV infection on the Mtb immune response in women with and without HIV. We consecutively screened women presenting to the antenatal clinic and followed them through 12 months postpartum. We purposely enrolled a disproportionate number of women with HIV and women with TBI to have adequate power to study the effect of HIV and pregnancy on immune responses to Mtb.

2.1. Study population and Sub-cohorts

Pregnant women with and without HIV, over 18 years of age who presented to the antenatal clinic with gestational age between 14–34 weeks were screened for the study. All participants were screened for TBI with IGRA (QFT from June 2016–2017; QFT-Plus from September 2017–2020). If IGRA positive, shielded chest radiograph and sputum Gene Xpert (Cepheid, Sunnyvale, CA, USA) were done to rule out active TB. Women with active TB or a history of active TB in the past two years, those on immunosuppressants or antibiotics continuously for 15 days before screening, or those with hemoglobin < 7.5 g/dL were excluded. Further details on enrolment criteria are previously published (8).

Pregnant women who met inclusion criteria completed a WHO TB symptom screen at each visit, and TBI testing repeated within 5 days of delivery, and at 6 months postpartum. All participants provided written informed consent prior to enrolment. The study was approved by the institutional review boards at Byramjee Jeejeebhoy Government Medical College, Johns Hopkins University, and Weill Cornell Medicine.

In the parent PRACHITi study, we screened 516 pregnant women for TBI with either QFT or QFT-Plus; their results were used for TBI prevalence (**Group A of** Fig. 1). From these 516 pregnant women, we enrolled 234 women with and without HIV based on the inclusion criteria detailed above. Of the 234, 165 IGRA-positive women (35 with HIV and 130 without HIV) were included for cross-sectional comparisons of QFT vs QFT-Plus at delivery and postpartum (**Group B of** Fig. 1). Of the 234 enrolled women, 74 received QFT-Plus testing at antepartum, delivery, and postpartum and constituted the longitudinal cohort (**Group C of** Fig. 1).

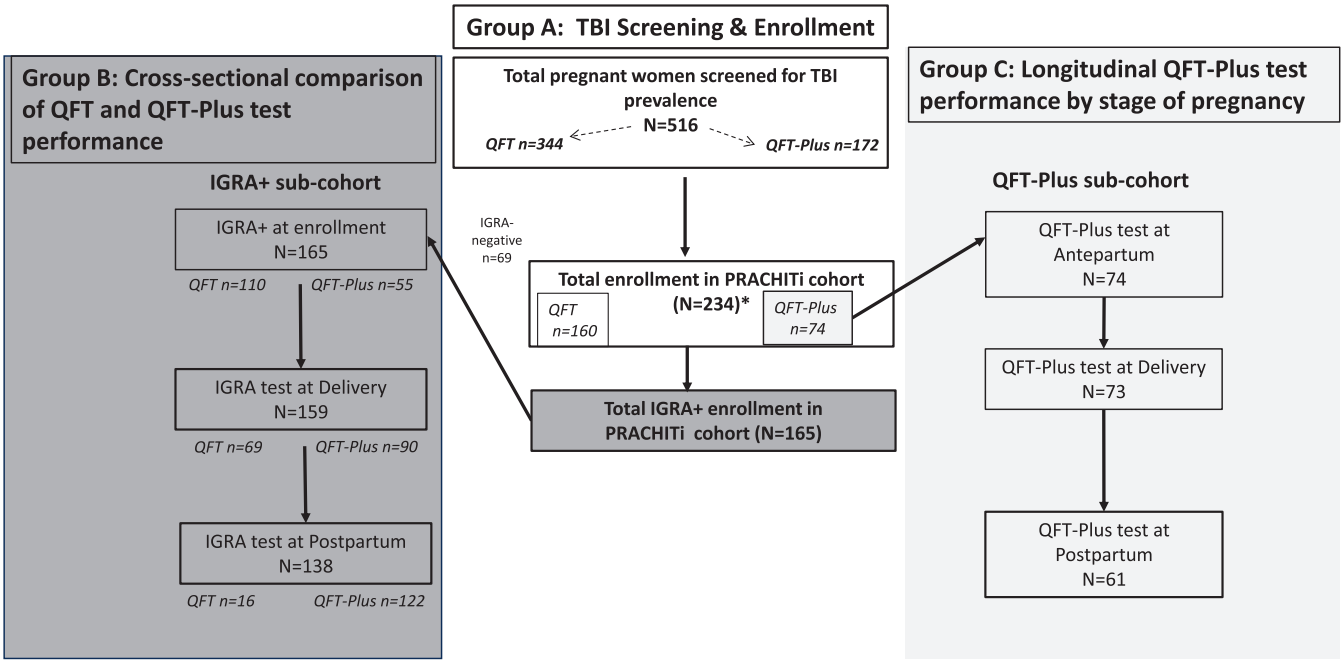

Fig. 1. Study Flow Diagram. A total of 516 pregnant women were screened for TBI using QFT and QFT-Plus and results were used for TBI prevalence. (Group A, TBI Screening and Enrollment) [3]. A total of 234 women were enrolled in the PRACHITi study based on enrolment criteria (see Methods). Of the 234, 165 had a positive IGRA during pregnancy. We compared positivity rates cross-sectionally at antepartum, delivery, and postpartum in these women (Group B, Cross-sectional). Of the 234, 74 women were tested with the QFT-Plus at all time points. We compared positivity rates longitudinally in these women (Group C, Longitudinal). [3] Bhosale R, Alexander M, Deshpande P, Kulkarni V, Gupte N, Gupta A, et al. Stages of pregnancy and HIV affect diagnosis of tuberculosis infection and Mycobacterium tuberculosis (MTB)-induced immune response: Findings from PRACHITi, a cohort study in Pune, India. International Journal of Infectious Diseases. 2021 Nov;112:205–11.

2.2. Study procedures

Trained study staff collected baseline sociodemographic, clinical data, and laboratory specimens. The women with HIV underwent CD4 testing using flow cytometry (FACS Count, Becton-Dickinson, CA, USA) and HIV viral load using Realtime HIV-1 viral load assay (Abbott Laboratories, Illinois, USA). All participants were screened for active TB symptoms at each visit. IGRA was done at screening (during pregnancy) in all women and was repeated at delivery and 6 months postpartum in enrolled women.

2.3. Interferon-gamma release assay

From June 2016 to 2017, the QuantiFERON-TB Gold In-Tube (Cellestis Inc. CA, 91355, USA,) was performed per the manufacturer's instructions. After the manufacturer replaced QFT with QuantiFERON-TB Gold Plus (QIAGEN GmbH, 40,724 Hilden, Germany) in September 2017, QFT-Plus was used exclusively. For QFT-Plus, whole blood was collected in two different TB antigen tubes (TB1 and TB2). Plasma was separated and stored at −80 °C from all tubes. ELISA was performed as per the manufacturer's instructions. The results were interpreted as positive, negative, or indeterminate.

2.4. Outcomes and Definitions

The primary outcomes were the prevalence of TBI at screening (defined as a positive QFT or QFT-Plus) and the positivity of QFT and QFT-Plus at delivery and postpartum. The secondary outcome was the longitudinal change in the positivity of QFT-Plus over time.

2.5. Statistical analysis

Baseline characteristics were summarized as proportions and medians with interquartile range (IQR). Categorical and continuous variables were compared using Fisher's exact test and Wilcoxon rank-sum test respectively. The analysis was restricted to factors that were statistically different between women with and without TBI or considered relevant to TB antigen reactivity, including HIV status and pregnancy stage.

The primary prevalence outcome was assessed in the total number of women screened for the study (n = 516). For the cross-sectional comparison of QFT and QFT-Plus at delivery and postpartum, we only included women enrolled in the PRACHITi study with a positive IGRA at the study entry (n = 165). The secondary outcome was assessed in a subset who received QFT-Plus testing at all three time points using the two-sample z-test (n = 74) (Fig. 1). This analysis was stratified by HIV status. Given our sample size, we had 89 % power to detect a significant difference between QFT and QFT-Plus at each time point. Statistical analyses were performed using Stata 14.2 (StataCorp 2015. Stata Statistical Software: Release 14.2 College Station, TX: StataCorp LP).

3. Results

3.1. Prevalence estimates in screening group

We screened 516 women for TBI (Fig. 1**, Group A)**; 344 (67 %) were tested by QFT, and 172 (33 %) were tested by QFT-Plus. The median age of screened women was 23 years (IQR 20–26) and the median gestational age at screening was 21.6 weeks (IQR 18–26.5); 116 (23 %) had HIV. Overall TBI prevalence was 35 %; 125 (36 %) were diagnosed by QFT and 60 (35 %) by QFT-Plus (p = 0.76). There were no significant demographic differences between people tested with QFT vs QFT-Plus. (Supplementary Table 1**)**.

3.2. Higher QFT-Plus positivity in cross-sectional comparisons of QFT and QFT-Plus

We included 165 women enrolled in PRACHITi with a positive IGRA during pregnancy for the cross-sectional analysis at delivery and postpartum stages **(**Fig. 1**, Group B)**. The demographics of these women were similar to the screening group described above (Table 1**)**. Five (3 %) had a known TB contact. All 35 (21 %) WHIV were on antiretroviral therapy (ART). The median CD4 count was 476 cells/mm^3 (IQR, 399–586) and 11 (31 %) WHIV had detectable HIV-1 viral load (>40 copies). The median viral load (VL) was 109 copies/mL (IQR, 40–266).

In our study, QFT-Plus returned a significantly higher number of positive results than QFT at delivery (80 % vs 65 % p = 0.04) but not at postpartum (89 % vs 88 % p = 0.90) **(**Fig. 2A**)**. In the 35 WHIV, QFT-Plus had higher positivity than QFT at delivery (76 % vs 47 %, p = 0.08) and at postpartum (90 % vs 80 %, p = 0.54) **(**Fig. 2B**)**, but the differences were not statistically significant.

3.3. Longitudinal QFT-Plus results across stages of pregnancy

For the longitudinal sub-cohort, we included 74 women who underwent QFT-Plus testing at entry, delivery, and postpartum. This group included women with a positive or negative IGRA at the study entry (Fig. 1**, Group C**). The median age was 24 years (IQR 20–27), and the median gestational age at entry was 19.8 weeks (IQR 16–25). Of the 74 women, 34 (46 %) had HIV with a median CD4/mm^3 of 490 (IQR 363–550); 16 (47 %) with a detectable viral load (>40 copies), the median viral load (VL) was 420 copies/mL (IQR 161—908) (Supplementary Table 2). Of the 74 women included in the longitudinal analysis, 55 (74 %) had a positive QFT-Plus during pregnancy (Fig. 3A).

QFT-Plus positivity (TB1 or TB2 minus NIL ≥ 0.35 IU/mL) was highest in pregnancy but did not differ statistically across pregnancy, delivery, and postpartum (74 % vs. 58 % vs. 62 %; p = 0.09) (Fig. 3A). Among women without HIV, there was discordance in test positivity based on TB1 results alone vs TB1 or TB2 during pregnancy (90 % vs 100 %, p = 0.04) but not at delivery (73 % vs 78 %, p = 0.61) or postpartum (79 % vs 79 %) (Fig. 3B**)**. Among WHIV, no discordance was

Table 1
Overall IGRA positive demographic characteristic table by QFT and QFT-Plus.

Baseline socio-demo & clinical profile	Total N (%) (165)	QFT (3rd generation) N = 110	QFT-Plus (4th generation) N = 55
Median Age, years (IQR)	23 (21–27)	23 (21–26)	25 (20–27)
Median GA at entry, wks (IQR)	21.5 (18.2–27.1)	20.8 (18.1–28.3)	22.2 (18.4–26.6)
Median GA at delivery, wks (IQR)	38.5 (37.5–39.4)	38.4 (37.4–39.4)	38.6 (38–39.6)
HIV positive n (%)	35 (21)	20 (18)	15 (27)
ART status, n (%)	35 (100)	20 (100)	15 (100)
Median CD4/mm^3 (IQR)	476 (399–586)	457 (403–702)	490 (363–544)
Detectable viral load (>40 copies)	11 (31)	6 (30)	5 (33)
Viral Load median copies/mL (IQR)	109 (40–266)	135 (104–1269)	302 (156–802)
HH income: n (%)			
<10255 ($126)	123 (75)	88 (80)	35 (64)
One room tenement n (%)	63 (38)	40 (36)	23 (42)
Live with family members (%)			
>3	80 (48)	58 (53)	22 (40)
≤3	85 (52)	52 (47)	33 (60)
Education, n (%)			
≤ to 4th grade	40 (24)	32 (29)	8 (15)
>4th grade	125 (76)	78 (71)	47 (85)
TB contact	5 (3)	2 (2)	3 (6)

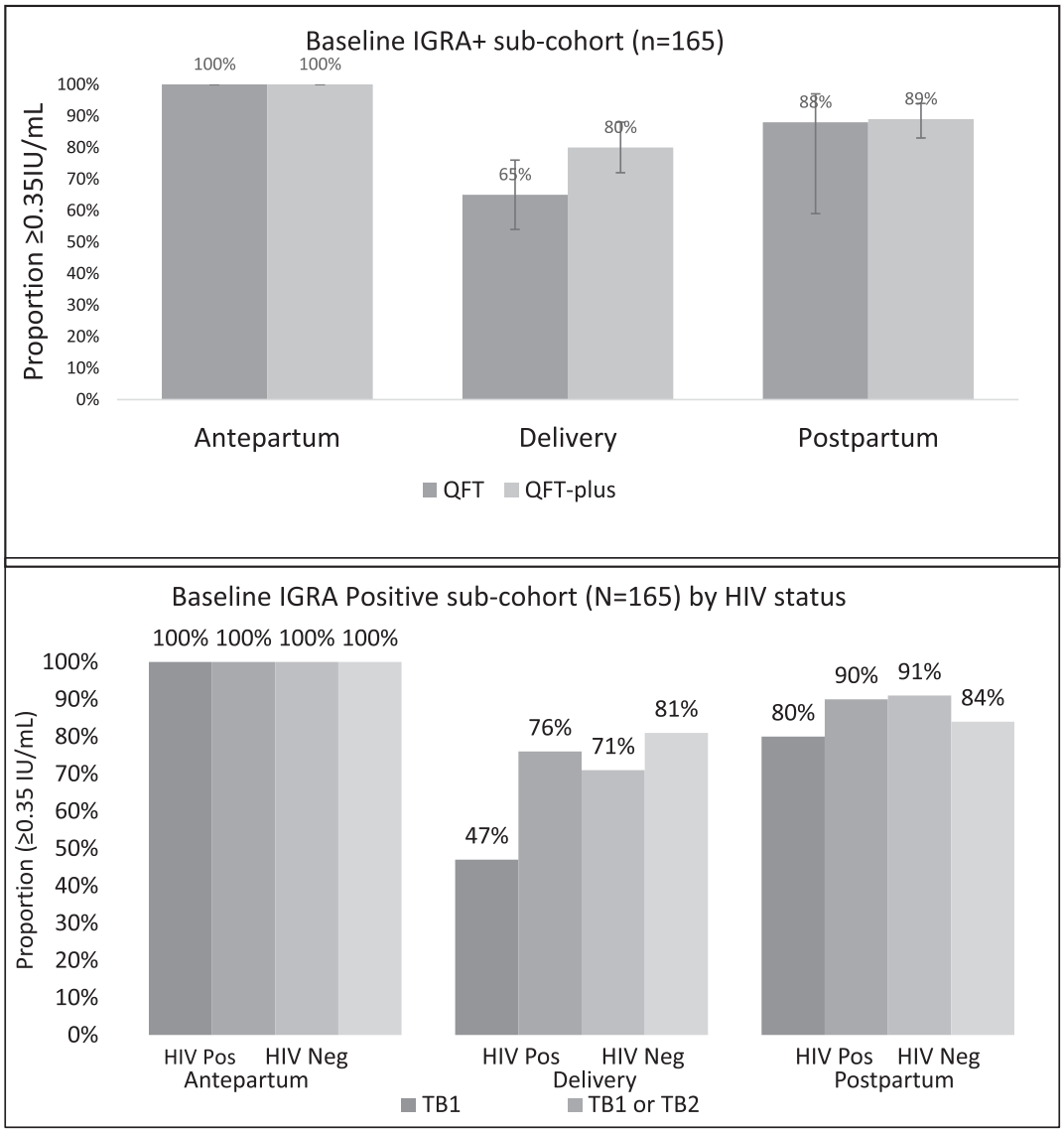

Fig. 2. A and B. Cross-sectional IGRA + Sub-cohort. Comparison between 3rd generation-QuantiFERON-TB Gold In-Tube (QFT) and 4th generation-QuantiFERON-TB Gold Plus (QFT-Plus) positivity at each pregnancy stage (Cross-sectional) (N=165) A) Baseline IGRA+ sub-cohort B) Baseline IGRA+ sub-cohort stratified by HIV status. This illustrates overall positivity stratified by HIV status using QFT/ QFT-Plus performance.

noted between TB1 alone and TB1 or TB2 at all stages of pregnancy (44 % vs 44 % at antepartum, 33 % vs 33 % at delivery, and 41 % vs 41 % at postpartum) (Fig. 3C).

When analyzing TB1 and TB2 tubes separately, the addition of TB2 resulted in four additional cases detected during pregnancy and two at delivery in women without HIV (Fig. 3B **&** Supplementary Table 3). The addition of TB1 added four cases at pregnancy and one postpartum with none at delivery. In WHIV, the addition of TB2 did not result in the detection of any additional cases of TB infection (Fig. 3C **&** Supplementary Table 3), whereas TB1 added two additional cases at antepartum in these women.

A sub-analysis of the QFT and the QFT-Plus, with a lowered cut-off for TB antigen 1-nil $\geq$ 0.2 and/or TB antigen 2-nil $\geq$ 0.2, was performed to determine if changing the cutoff would identify more cases. The QFT-Plus with the lowered cut-off detected more TBI cases at delivery (80 out of 111) compared to the standard cut-off (72 out of 111), this difference was statistically significant ($p = 0.01$) **(**Supplementary Table 4**).**

4. Discussion

In our study, we found that QFT-Plus had significantly more positive results at delivery than QFT, suggesting that the addition of CD8+ T-cell stimulation improves the sensitivity of TBI diagnosis in pregnant women, especially at delivery. This time point is important because it may be the only time a woman interacts with the health care system in countries, like India, where many women do not access antenatal care [13,16,17]. Interestingly, TB2 results compared to TB1 did not seem to improve the sensitivity of QFT-Plus vs QFT in peripartum WHIV (Fig. 3C).

Nonetheless, the broader use of QFT-Plus in antenatal clinics at any stage of pregnancy is likely to improve the prevention of TB for mothers and infants in TB-endemic countries like India.

The prevalence of TBI during pregnancy as assessed by QFT and QFT-Plus was 35 %-36 %, which is consistent with TBI estimates in non-pregnant populations in India. We expected that the QFT-Plus, with TB antigens for CD4+ and CD8+ T-cell stimulation, would detect TBI in significantly more women than QFT, particularly in WHIV, as HIV directly infects and destroys CD4+ T-cells but not CD8+ T cells [18,19].

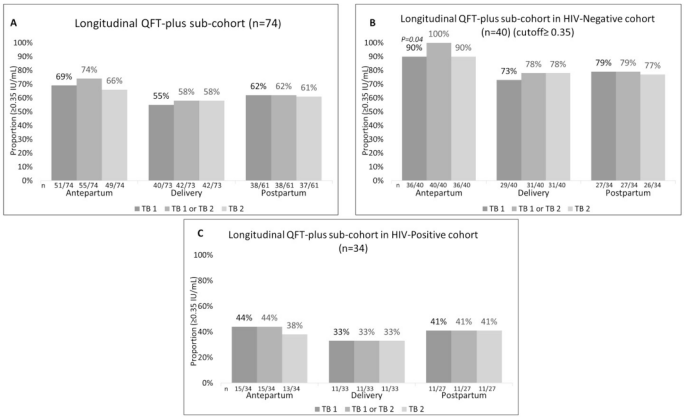

Fig. 3. A, B, and C. Longitudinal QFT-PLUS Sub-cohort. Comparison between 4th generation-QuantiFERON-TB Gold Plus (QFT- Plus) (Longitudinal) (N=74) and the TB1, TB1 or TB2 and TB2 positivity at each pregnancy stage using cut-off ≥0.35IU/mL. (A) Longitudinal QFT-Plus sub-cohort considering TB1 as a surrogate for QFT and TB1 or TB2 for QFT-Plus showing no discordance performance at delivery and postpartum. (B) Longitudinal QFT-Plus sub-cohort in the HIV-Negative cohort (N=40), QFT-plus showed significantly higher performance in HIV-negative. (C) Longitudinal QFT-Plus sub-cohort in HIV-Positive cohort (N=34), showed no discordance in performance across peripartum.

The lack of improvement with the addition of the TB2 tube in WHIV, however, supports data suggesting that CD8+ T-cells are also indirectly affected by chronic HIV infection [20]. Even when HIV infection is well controlled with ART, chronic inflammation persists, leading to immune activation, dysfunction, and exhaustion of the CD8+ cells despite treatment with ART [8]. In our cohort, the majority of WHIV were virally suppressed and adherent to their ART. ART improves reconstitution of CD4+ and CD8+ T cell counts and activation levels but may not optimally improve function [19]. It is also possible that ART is so effective at restoring sufficient CD4+ T-cell function that the benefit of measuring the CD8+ response is no longer additive [20]. The median gestational age at entry was the mid-second trimester, when immune changes of pregnancy may be modest [21,22]. Our data are similar to studies conducted in Ethiopia and South Korea where no difference was noted in the performance of QFT versus QFT-Plus between pregnant and non-pregnant populations with HIV [23,24]. A Zambian study on adults with pulmonary TB with and without HIV also noted that the sensitivity of QFT-Plus was similar to QFT except for people living with HIV (PLHIV) with severe immunosuppression, in whom QFT sensitivity was lower [25].

However, in pregnant women without HIV, TB1 and TB2 were found complementary to each other, showing maximum positivity when considering responses to TB1 or TB2 compared to TB1 response only. This finding is distinct from other studies showing strong agreement between TB1 and TB2 tubes, including those in an adult population with TBI in Italy, children without HIV in Eswatini, and diverse adult and health care worker populations with TBI and TB disease in the United States [26,27]. A Spanish study on children and adolescents with a risk of TBI showed no added value of TB2 tube [28] similar to a study in Iran on adults [29]. However, most of the studies assessed sensitivity between QFT and QFT-Plus in response to TB1 or TB2 antigens in a non-pregnant, immunocompetent population from low to medium TB prevalence settings [30]. Conversely, our study suggests there is a benefit of adding responses to the TB2 antigens during pregnancy and especially at delivery in a high-endemic setting. A study of the performance of QFT plus versus other IGRA tests from the US showed 97 % agreement, with 1 % TB1+/TB2- and 2 % TB1-/TB2 + discordance [31]. Our longitudinal analysis of QFT plus data revealed a higher TB1+/TB2- discrepancy during pregnancy, likely due to our study's focus on pregnant women with a higher proportion of HIV-positive individuals in whom immune suppression could have contributed to TB1/TB2 test discordance.

To determine if the discordance was related to borderline cases, we also analyzed the extended cut-offs of our population of pregnant women with and without HIV and found that lowering the cutoff of TB1 or TB2 minus nil to ≥ 0.2 IU/ml did not significantly increase the diagnosis of TBI by either QFT or QFT-Plus except at delivery. At delivery, QFT-Plus diagnosed significantly more TBIs with the lowered cutoff ($p = 0.01$). This is likely due to greater variability in QFT-Plus results, including more conversions, reversions, and indeterminate results, which could be due to lower IFN- γ level at delivery (8).

A strength of our study was the ability to compare the two generations of IGRAs, which reflect different immune pathways, at pregnancy, delivery, and postpartum in a large cohort of pregnant women living in a TB-endemic country. One weakness of our study is that our longitudinal analysis of QFT-Plus performance over time was limited to a smaller cohort ($n = 74$), as the QFT-Plus assay only became available during the latter part of the parent cohort study. Furthermore, the manufacturer's decision to withdraw QFT test kits when QFT-Plus was released precluded us from testing participants with both test kits at each pregnancy stage which is a limitation. However, a subanalysis of TB1 antigen alone vs TB1 or TB2 antigens could be interpreted as a comparison between QFT vs QFT using TB antigen (TB1) from QFT-Plus as a surrogate marker for QFT results. We had sufficient power to detect a significant difference in performance between QFT and QFT-Plus during pregnancy and at delivery and to explore the performance of the test in pregnant WHIV. It's intriguing that IGRA positivity rates for QFT and QFT-Plus showed no overall discordance and indicated similar TBI prevalence. This suggests that both tests are detecting similar levels of TBI, despite QFT-Plus including additional peptides to stimulate CD8+ cytotoxic T-lymphocytes. This similarity may be attributed to the comparable sensitivity and specificity of the QFT and QFT-Plus tests.

Because of the excellent retention of our cohort (96 % at delivery, 90 % at 6 months postpartum, and 93 % overall), we were able to evaluate the relative performance of QFT and QFT-Plus at different stages of pregnancy.

5. Conclusion

Pregnancy presents an ideal opportunity to implement strategies to prevent TB and avoid adverse pregnancy outcomes in high-burden settings. Without a gold standard diagnostic, integrating QFT-Plus into routine antenatal and postpartum care may enhance TBI detection for targeted TB prevention in this high-risk population.

Ethical approvals

All participants provided written informed consent prior to enrolment in the study. The approvals for the study were requested and

obtained from the institutional review boards at Byramjee Jeejeebhoy Government Medical College # NA, dated 20 March 2015, Johns Hopkins University # 00068973/CIR00015742 dated 21 February 2016, and Weill Cornell Medicine #1503016041 dated 23 November 2015.

Ethical statement

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Author contributions statement

We acknowledge the Department of Obstetrics and Gynecology at Byramjee Jeejeebhoy Government Medical College for providing clinical support to our study participants; the study team of clinicians, counselors, research nurses, and laboratory and data management staff for their contributions to the quality of the study data and the excellent retention rate; Katherine N McIntire MD for providing editorial and data preparation assistance; and Persistent Systems (Pune, India) for supporting the study's electronic data management system. VK wrote the original draft and JM, MA, AG, and NN contributed to the review and editing of the manuscript. JM, AG, MA, VK, and PD contributed to conceptualization and methodology. NG, DJ, EG, AV, and AC contributed to data curation. DJ and NG contributed to the formal analysis.JM, AG, MA, RB, SN, and VK contributed to the investigation. MA, EG, AV, AC, and PD contributed to project management and supervision. RB and SN provided resources. JM and AG contributed to the fund acquisition. All authors performed a critical review and editing of the manuscript. All authors had full access to the underlying data. All authors contributed to important content during manuscript drafting and revisions. All authors accept accountability for overall work.

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CRediT authorship contribution statement

Vandana Kulkarni: Conceptualization, Methodology, Investigation, Writing – original draft. **Mallika Alexander:** Conceptualization, Project administration, Supervision, Data curation, Writing – review & editing. **Ramesh Bhosale:** Investigation, Resources. **Divyashri Jain:** Data curation, Formal analysis. **Prasad Deshpande:** Methodology, Supervision, Data curation. **Emily Shira Gitlin:** Project administration, Data curation. **Arthi Vaidyanathan:** Project administration, Data curation. **Andrea Chalem:** Project administration, Data curation. **Shilpa Naik:** Investigation, Resources. **Nikhil Gupte:** Supervision, Formal analysis. **Neelu Nawani:** Methodology, Writing – review & editing. **Amita Gupta:** Conceptualization, Funding acquisition, Investigation, Writing – review & editing. **Jyoti Mathad:** Conceptualization, Funding acquisition, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jctube.2024.100504.

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