Systemic Inflammation in Pregnant Women with Latent Tuberculosis Infection

Shilpa Naik¹, Mallika Alexander², ¹, Pavan Kumar³, Vandana Kulkarni², ¹, Prasad Deshpande², ¹, Su Yadana⁴, Cheng-Shiun Leu⁴, Mariana Araújo-Pereira⁵, Bruno B. Andrade⁵, Ramesh Bhosale⁴, Subash Babu³, Amita Gupta⁶, Jyoti S. Mathad⁷, Rupak Shivakoti⁸

¹B. J. Medical College & Sassoon Hospital, India, ²B. J. Medical College & Sassoon Hospital, India, ³International Centers for Excellence in Research (ICER), India, ⁴Columbia University, United States, ⁵Gonçalo Moniz Institute (IGM), Brazil, ⁶Johns Hopkins Medicine, United States, ⁷Weill Cornell Medicine, Cornell University, United States, ⁸Department of Epidemiology, Columbia University, United States

Submitted to Journal:
Frontiers in Immunology

Specialty Section:
Microbial Immunology

ISSN:
1664-3224

Article type:
Original Research Article

Received on:
26 Jul 2020

Accepted on:
09 Dec 2020

Provisional PDF published on:
09 Dec 2020

Frontiers website link:
www.frontiersin.org

Citation:
Naik S, Alexander M, Kumar P, Kulkarni V, Deshpande P, Yadana S, Leu C, Araújo-pereira M, Andrade BB, Bhosale R, Babu S, Gupta A, Mathad JS and Shivakoti R(2020) Systemic Inflammation in Pregnant Women with Latent Tuberculosis Infection. Front. Immunol. 11:3619. doi:10.3389/fimmu.2020.587617

Copyright statement:
© 2020 Naik, Alexander, Kumar, Kulkarni, Deshpande, Yadana, Leu, Araújo-pereira, Andrade, Bhosale, Babu, Gupta, Mathad and Shivakoti. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution and reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.
This Provisional PDF corresponds to the article as it appeared upon acceptance, after peer-review. Fully formatted PDF and full text (HTML) versions will be made available soon.
Systemic Inflammation in Pregnant Women with Latent Tuberculosis Infection

Authors: Shilpa Naik¹,², Mallika Alexander¹, Pavan Kumar³, Vandana Kulkarni¹, Prasad Deshpande¹, Su Yadana⁴, Cheng-Shiun Leu⁴, Mariana Araújo-Pereira⁵,⁶,⁷, Bruno B Andrade⁵,⁶,⁷,⁸,⁹,¹⁰, Ramesh Bhosale¹,², Subash Babu³, Amita Gupta¹,¹¹, Jyoti S Mathad¹², and Rupak Shivakoti⁴

Institutions:

¹Byramjee-Jeejeebhoy Government Medical College-Johns Hopkins University Clinical Research Site, Pune, India
²Byramjee Jeejeebhoy Government Medical College, Pune, India
³National Institutes of Health, National Institute for Research in Tuberculosis, International Center for Excellence in Research, Chennai, India
⁴Department of Epidemiology, Columbia University Mailman School of Public Health, New York, USA
⁵Instituto Goncalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil
⁶Multinational Organization Network Sponsoring Translational and Epidemiological Research, Fundação José Silveira, Salvador, Brazil
⁷Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Brazil
⁸Curso de Medicina, Faculdade de Tecnologia e Ciências, Salvador, Brazil
⁹Universidade Salvador (UNIFACS), Laureate Universities, Salvador, Brazil
¹⁰Escola Bahiana de Medicina e Saúde Pública (EBMSP), Salvador, Brazil
Inflammation in LTBI+ pregnant women

11Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, USA.

12Department of Medicine, Weill Cornell Medical College, New York, USA

**Keywords:** Latent TB infection, TB disease, inflammation, pregnancy, cytokines
Abstract

**Background:** Recent studies in adults have characterized differences in systemic inflammation between adults with and without latent tuberculosis infection (LTBI+ vs. LTBI-). Potential differences in systemic inflammation by LTBI status has not been assess in pregnant women.

**Methods:** We conducted a cohort study of 155 LTBI+ and 65 LTBI- pregnant women, stratified by HIV status, attending an antenatal clinic in Pune, India. LTBI status was assessed by interferon gamma release assay. Plasma was used to measure systemic inflammation markers using immunoassays: IFNβ, CRP, AGP, I-FABP, IFNγ, IL-1β, soluble CD14 (sCD14), sCD163, TNF, IL-6, IL-17a and IL-13. Linear regression models were fit to test the association of LTBI status with each inflammation marker. We also conducted an exploratory analysis using logistic regression to test the association of inflammatory markers with TB progression.

**Results:** Study population was a median age of 23 (Interquartile range: 21-27), 28% undernourished (mid-upper arm circumference (MUAC) <23 cm), 12% were vegetarian, 10% with gestational diabetes and 32% with HIV. In multivariable models, LTBI+ women had significantly lower levels of third trimester AGP, IL1β, sCD163, IL-6 and IL-17a. Interestingly, in exploratory analysis, LTBI+ TB progressors had significantly higher levels of IL1β, IL-6 and IL-13 in multivariable models compared to LTBI+ non-progressors.

**Conclusions:** Our data shows a distinct systemic immune profile in LTBI+ pregnant women compared to LTBI- women. Data from our exploratory analysis suggest that LTBI+ TB progressors do not have this immune profile, suggesting negative association of this profile with TB progression.
If other studies confirm these differences by LTBI status and show a causal relationship with TB progression, this immune profile could identify subsets of LTBI+ pregnant women at high risk for TB progression and who can be targeted for preventative therapy.
**Introduction:**

Active tuberculosis (TB) disease elicits host responses characterized by an immune profile that is clearly distinct from healthy individuals (O'Garra et al., 2013; Cliff et al., 2015). As the causative agent *Mycobacterium tuberculosis* (*Mtb*) is actively replicating during TB disease, it causes constant antigen stimulation from the bacterium that shapes the immune response. In contrast, with latent TB infection (LTBI), *Mtb* is not actively replicating in the host and antigen stimulation with *Mtb* antigens is required to generate *Mtb*-specific immune responses (O'Garra et al., 2013). While differences in immunity with *Mtb* antigen stimulation has been extensively studied for active disease or LTBI compared to healthy individuals (Tufariello et al., 2003; Mack et al., 2009; O'Garra et al., 2013; Cliff et al., 2015; de Martino et al., 2019), there are limited studies characterizing differences by LTBI status in circulating inflammatory markers, in the absence of antigen stimulation (Cowan et al., 2012; Jensen et al., 2013; LaVergne et al., 2020). This information could potentially explain why an increased risk of certain adverse outcomes (e.g. acute myocardial infarction) have been observed among LTBI+ individuals, or help identify immune profiles associated with TB progression (Andrews et al., 2012; Huaman et al., 2018b).

One hypothesis on levels of inflammation by LTBI status is that there is no difference in circulating inflammatory markers between LTBI+ and LTBI- individuals. *Mtb* infection is mainly quiescent during LTBI and can remain in this form for a long time without harm to most individuals (Comstock et al., 1974; Vynnycky and Fine, 2000). However, recent data from studies in adults suggest that there might be differences in systemic inflammation by LTBI status (Cowan et al., 2012; Jensen et al., 2013; Huaman et al., 2016; LaVergne et al., 2020). For example, a study of Indian adults observed that after adjusting for potential confounders, LTBI+ individuals had significantly higher levels of circulating pro-inflammatory mediators IL-6 and MCP-1 but lower levels of C-
reactive protein (CRP), another pro-inflammatory marker, compared to LTBI- individuals (LaVergne et al., 2020).

While studies have started to assess potential differences in systemic inflammation by LTBI status in non-pregnant adults (Cowan et al., 2012; Jensen et al., 2013; Huaman et al., 2016; LaVergne et al., 2020), there is no data on pregnant women. Pregnant women have a distinct immune profile compared to adults and there are temporal changes in immunity during pregnancy (Mor and Cardenas, 2010). It is not currently known whether there is a difference in systemic inflammation between LTBI+ and LTBI- pregnant women, and how this might change by trimester of pregnancy. Furthermore, LTBI+ women have a higher risk of *Mtb* progression during pregnancy and post-partum but the reasons are not clear (Mathad and Gupta, 2012; Zenner et al., 2012; Jonsson et al., 2020). The immune profile during pregnancy, including the systemic inflammatory milieu, may inform on potential changes to immunity that increase susceptibility to TB disease during pregnancy. In order to address this research gap in our understanding of systemic immunity in LTBI+ pregnant women, we compared the levels of systemic inflammatory markers, at the second and third trimester, by LTBI status in a cohort of pregnant women from Pune, India, and explored the association of these immune markers with TB progression during pregnancy and post-partum.

**Methods:**

**Study Design and Population**

A cohort study of pregnant women was conducted at Byramjee Jeejeebhoy Government Medical College (BJGMC) in Pune, India from 2016-2019. Adult pregnant women, aged 18-40 years and between 13-34 weeks of gestation (confirmed by early pregnancy ultrasound), receiving antenatal care at BJGMC were enrolled for this study. Pregnant women with active TB at entry were
excluded. We enrolled four cohorts of pregnant women based on their latent tuberculosis infection (LTBI) and HIV status: 1) LTBI+HIV+ (N=35), 2) LTBI+HIV- (N=130), 3) LTBI-HIV+ (N=44) and 4) LTBI-HIV- (N=25). The sample size for this cohort was based on the primary objective of the cohort study which was to compare the concentrations of Th1 cytokines after MTB-specific antigen stimulation by stage of pregnancy. LTBI status was determined using Interferon Gamma Release Assay (IGRA Quantiferon TB-Gold) according to manufacturer’s instructions. Sampling within each cohort was based on convenience sampling of those that met eligibility criteria.

Ethics Statement

All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all participants. This study was approved by institutional review boards and ethics committees at BJGMC, Johns Hopkins University, Weill Cornell and Columbia University. We followed guidelines for human experimentation from the US Department of Health and Human Services.

Data Collection and Laboratory Procedures

Sociodemographic information and clinical data were collected from pregnant women at the enrollment visit (13-34 weeks of gestation), at the third trimester visit (for those enrolled in the second trimester), at delivery and approximately every 3 months post-partum. At each follow-up visit, women were administered a World Health Organization (WHO) TB symptom screening questionnaire. Women with a positive WHO symptom screen, unintentional weight loss since last visit or with clinical findings on examination were further investigated with sputum GeneXpert, acid-fast bacilli test, chest X-ray and abdominal ultrasound. Culture in Lowenstein Jensen (LJ) media and...
Inflammation in LTBI+ pregnant women

liquid Mycobacteria Growth Indicator Tube (MGIT) were performed for further confirmation in
those with positive findings.

Relevant to this analysis, blood was also collected at each visit in heparin tubes and plasma
samples were stored in -80°C until further use. We conducted single-plex immunoassays on second
and third trimester plasma samples according to the manufacturer’s (R&D Systems, Minneapolis,
MN) directions for soluble CD163 (sCD163), soluble CD14 (sCD14), intestinal fatty acid-binding
protein (I-FABP), C-reactive protein (CRP), alpha 1-acid glycoprotein (AGP) and interferon-β
(IFNβ). The lower and upper detection limits sensitivity of the assays were as follows: 1.6-1000.613
ng/mL for sCD163, 250-16,000125 pg/mL for sCD14, 15.6-1,0006.21 pg/mL for I-FABP, 0.8-500.02
ng/mL for CRP, 3.1-2000.54 ng/mL for AGP and 50-4,00050 pg/mL for IFNβ. Multiplex
immunoassays (Luminex assays from R&D systems) measuring IFNγ, Interleukin (IL)-1β, IL-6, IL-
13, IL-17A and TNF were also performed on these samples. The lower and upper detection
limits sensitivity of the assays were as follows: 43.9-10,6900.40 pg/mL for IFNγ, 17.5-4,2600.80
pg/mL for IL-1β, 4.7-1,1501.7 pg/mL for IL-6, 391-95,06036.6 pg/mL for IL-13, 12.9-3,1501.8
pg/mL for IL-17A, and 7.9-1,9301.2 pg/mL for TNF. These markers were chosen based on their
importance to TB, HIV and pregnancy outcomes. For Single-plex immunoassays, SpectraMax plate
readers were used with SofMax Pro 6 software. Luminex xMAP technology MAGPIX platform was
used for multiplex immunoassays with xPONENT software.

Statistical Analysis

We combined the LTBI+ cohorts (HIV+ and HIV-) and LTBI- cohorts (HIV+ and HIV-) to
study the relationship of LTBI status with second or third trimester inflammatory markers among 220
women with available inflammatory data. Differences in study population characteristics by LTBI
status were determined using Fisher’s exact test for categorical variables and Wilcoxon rank-sum test
for continuous variables. A p-value less than 0.05 was considered statistically significant and a p-value of less than 0.004 (0.05/12) was considered statistically significant after Bonferroni correction for multiple comparisons. We also compared median levels of each inflammatory marker, during the second and third trimester, between LTBI+ and LTBI- pregnant women using the Wilcoxon rank-sum test. Inflammatory markers were log$_2$-transformed for the data to approximate normality.

We conducted univariable and multivariable linear regression to determine the change in log$_2$ concentrations of each inflammatory marker (outcome variable) by change in LTBI status (exposure variable), with separate cross-sectional analyses for markers measured in second trimester or third trimester. Multivariable models adjusted for age, mid-upper arm circumference (MUAC), HIV status, vegetarian diet and gestational diabetes status. We also tested models that further adjusted for smoking, education or preeclampsia. MUAC at the time of plasma sample collection (i.e. second or third trimester) was used in multivariable models as it is a more reliable indicator of nutritional status during pregnancy compared to body mass index. Sub-set analysis was performed using Wilcoxon rank-sum test to determine whether similar relationships between LTBI status and inflammatory markers were observed for only HIV-negative populations.

We also conducted an exploratory analysis, using univariable and multivariable logistic regression analyses, to determine whether third trimester inflammation levels (exposure variable) was associated with TB progression during pregnancy or post-partum (outcome variable). Progressors were defined as those who prospectively developed active TB after sample collection in third trimester and within study follow-up of one-year post-partum. We used STATA software version 15.0 for the data analysis.
Inflammation in LTBI+ pregnant women

Study Population Characteristics

Our study population of pregnant Indian women (N=220) had a median age of 23 years (interquartile range (IQR): 21-27) (Table 1). Only 25% had an education of less than secondary education and 34% had an income below India’s poverty line (monthly income <10,255 Indian rupees). Around 28% of the women had a mid-upper arm circumference (MUAC) less than 23 cm (an indicator of undernutrition in pregnancy(Ververs et al., 2013)) and 7% had an MUAC>30.5 cm, indicative of overweight (Table 1). Most of the women (88%) did not smoke and 12% were vegetarians. Ten percent had gestational diabetes and 11% had preeclampsia. As this cohort was stratified by HIV status, 32% of the pregnant women were HIV+ (all on antiretroviral therapy). Study population characteristics did not differ by LTBI status except for lower proportion of HIV (p-value <0.001) in LTBI+ women; as mentioned above, this was due to the stratified design of the study. LTBI+ women also had a lower proportion of gestational diabetes (p=0.08) and less post-high school education (p=0.09) but these differences were not statistically significant (Table 1).

Levels of Inflammatory markers by LTBI status

We compared the median log2-transformed levels of third trimester inflammatory markers by LTBI status using Wilcoxon-rank sum tests (Figure 1). IL-1β (3.64 vs. 2.25 pg/mL; p=0.0002), TNF (1.76 vs. 1.54 pg/mL; p=0.004), IL-6 (4.08 vs. 1.25 pg/mL; p<0.0001) and IL-17a (2.48 vs. 2.16 pg/mL; p=0.0001) were significantly higher in LTBI- women compared to LTBI+ women (Figure 1). IFNγ production upon Mtb antigen stimulation is used to define LTBI positivity; of note, IFNγ was lower (3.63 vs. 3.73 pg/mL; p=0.15) in plasma (i.e. unstimulated samples) of LTBI- women compared to LTBI+ women, but this association was not statistically significant (Figure 1). Similar
results were also observed when using log2 concentrations of markers measured in plasma samples from the second trimester (Supplementary Figure 1). LTBI- women had significantly higher levels of second trimester AGP, I-FABP, IL-1β, TNF, IL-6 and IL-17a compared to LTBI+ women (Supplementary Figure 1). LTBI- women also had lower levels of IFNγ compared to LTBI+ women, although this was not statistically significant (p=0.08) (Supplementary Figure 1).

Association of inflammation by LTBI status

Next, we assessed the relationship of third trimester inflammation with LTBI status using univariable and multivariable linear regression models. LTBI+ women had significantly lower levels of I-FABP (mean log2 change: -0.41, 95% confidence intervals (CI): -0.78 to -0.04; p=0.03), IL1β (mean log2 change: -1.03, 95% CI: -1.53 to -0.54; p<0.001), IL-6 (mean log2 change: -1.36, 95% CI: -1.93 to -0.80; p<0.001) and IL-17a (mean log2 change: -0.34, 95% CI: -0.50 to -0.17; p<0.001) compared to LTBI- women in univariable models (Figure 2). AGP (mean log2 change: -0.20, 95% CI: -0.42 to 0.02; p<0.08) and sCD163 (mean log2 change: -0.18, 95% CI: -0.39 to 0.03; p<0.10) was also lower in LTBI+ women but this relationship was not statistically significant (Figure 2).

After adjusting for age, third trimester MUAC, HIV status, vegetarian diet, and gestational diabetes in multivariable models, levels of IL-1β (mean log2 change: -1.15, 95% CI: -1.70 to -0.60; p<0.001), IL-6 (mean log2 change: -1.22, 95% CI: -1.87 to -0.58; p<0.001) and IL-17a (mean log2 change: -0.39, 95% CI: -0.57 to -0.21; p<0.001), but not I-FABP (mean log2 change: -0.25, 95% CI: -0.67 to 0.15; p=0.22), remained significantly lower in LTBI+ women compared to LTBI- women (Figure 2). In addition, AGP was also significantly lower in LTBI+ women (mean log2 change: -0.29, 95% CI: -0.54 to -0.04; p=0.02) (Figure 2). After Bonferroni correction to adjust for multiple comparisons, third trimester IL1β, IL-6 and IL-17a were significantly lower in LTBI+ women in multivariable models.
Further adjusting for smoking, education or preeclampsia in multivariable models did not change the direction or significance of the results. Finally, we also conducted sensitivity analysis to show that when we limited the analysis only to HIV- subjects, the levels of these inflammatory markers were still lower in LTBI+ pregnant women compared to LTBI- women (Supplementary Figure 2), suggesting that HIV was not driving the observed relationships.

Results using second trimester inflammatory markers instead of third trimester showed similar associations with LTBI status (Figure 3). In univariable models, LTBI+ pregnant women had significantly lower levels of AGP, I-FABP, IL1β, TNF, IL-6 and IL-17a compared to LTBI- pregnant women (Figure 3). In multivariable models, we observed similar results observed in univariable models with significantly lower levels of the AGP, I-FABP, IL-1β, IL-6, and IL-17a, but not TNF in LTBI+ compared to LTBI- women (Figure 3). In addition, sCD163 levels were significantly lower and IFNγ was significantly higher in LTBI+ women compared to LTBI- women (Figure 3). After Bonferroni correction to adjust for multiple comparisons, second trimester AGP, IL1β, IL-6 and IL-17a were significantly lower in LTBI+ women in multivariable models.

Inflammatory markers during pregnancy and progression of TB

We also conducted an exploratory analysis to test whether the systemic immune profile observed in LTBI+ pregnant women was associated with progression to active TB during pregnancy or post-partum. In our study, there were nine women, all LTBI+ at study baseline, who progressed to active TB either during the third trimester of pregnancy (n=1) or post-partum (i.e. within one year of delivery) (n=8). Given that all of the progressors were LTBI+ women, we present data comparing progressors and non-progressors only among LTBI+ women. Interestingly, levels of these markers in LTBI+ progressors, while higher than non-progressor LTBI+ pregnant women, were similar to LTBI- women (data not shown), suggesting that lower levels of these markers might be protective against
TB progression in LTBI+ pregnant women. There was a significantly increased odds of progression per log₂ increase in third trimester plasma levels of IL-1β (adjusted odds ratio (aOR): 1.64, 95% CI: 1.05-2.57), IL-6 (aOR: 1.58, 95% CI: 1.05-2.39), and IL-13 (aOR: 2.43, 95% CI: 1.12-5.27) after adjusting for age, MUAC and HIV status (Figure 4). There was also an increased odds for IL-17a (aOR: 5.49, 95% CI: 0.84-35.97), but this association was not statistically significant (Figure 4).

Similar results were observed when we limited the analysis only to post-partum progressors (data not shown).

**Discussion:**

In our study of LTBI+ and LTBI- pregnant women from India, LTBI+ women had lower levels of various pro-inflammatory cytokines such as IL-1β, IL-6 and IL-17a compared to LTBI- women. In contrast, the levels of IFNγ were higher (significant in second trimester) in LTBI+ women. While increased levels of IFNγ might be related to the use of this cytokine to define IGRA-based LTBI (Pai et al., 2004), the results with the other cytokines were surprising. These findings suggest that LTBI in pregnancy is characterized by a distinct immune profile with higher levels of IFNγ but lower levels of other immune markers with known roles in TB disease. Interestingly, LTBI+ women who progressed to active TB during pregnancy and post-partum did not have this profile in our exploratory analysis, suggesting the distinct immune profile in LTBI+ pregnant women might have a protective role against TB progression. Future larger studies will need to confirm these findings and determine whether these markers play a causal role and could be used to identify LTBI+ pregnant women at increased risk for TB progression and a target for preventative therapy.

LTBI+ pregnant women had significantly increased levels of IFNγ in the second trimester compared to LTBI- women. While the association was not statistically significant, the IFNγ levels were also higher for LTBI+ women in the third trimester. In our study, we used the IGRA test, which
Inflammation in LTBI+ pregnant women

is dependent on IFNγ production (Pai et al., 2004), to define LTBI status; thus it might be expected

IFNγ is higher in LTBI+ women. On the other hand, it should be noted that we measured IFNγ in
plasma samples and it is not obvious that IFNγ levels in circulation should also be higher for LTBI+
individuals. Our results here do indicate that higher levels of IFNγ are observed in circulation for

LTBI+ pregnant women even without Mtb antigen stimulation. Similar results for IFNγ have also
been observed from plasma samples of non-pregnant LTBI+ adults (Huaman et al., 2016; Huaman et
al., 2018a). While the reasons are not clear, it is possible that despite being a latent infection, there
could be periodic activity of some component (e.g. mRNA, protein) or low-level replication of Mtb
that induces IFNγ production (Huaman et al., 2016). Furthermore, LTBI is thought to be a spectrum
of host-pathogen interactions, with ongoing replication and metabolic activity in certain subsets
while quiescence in other Mtb subsets (Barry et al., 2009; Huaman et al., 2018b).

Our data showed lower levels of immune markers, especially IL-1β, IL-6, IL-17a and AGP, in
both trimesters, in LTBI+ women compared to LTBI- women. Higher levels of IFNγ can partly
explain the lower levels of these other markers, as studies of Mtb have shown that IFNγ can have
counteractive roles with IL-1β, IL-6 and IL-17a in certain instances (Nandi and Behar, 2011; Dutta et
al., 2012; Eigenbrod et al., 2013). Pregnancy-specific changes in immune profile could also in part
help explain these observations (Mor and Cardenas, 2010). For example, during pregnancy there is
an increase in neutrophil levels (Sacks et al., 1998; Luppi et al., 2002), which have been linked to
lower levels of IL-6 and IL-17 in Mtb infection (Zhang et al., 2009; O'Garra et al., 2013).

Interestingly, in our exploratory analyses, LTBI+ TB progressors had a profile more similar
to LTBI- women, with higher levels of IL-1β, IL-6, IL-13 and IL17a and generally lower levels of
IFNγ compared to LTBI+ non-progressors. These inflammatory markers have been recognized for
their complex role in TB disease where while a deficiency is linked to reduced control of Mtb
infection, excessive levels can result in tissue damage and immunopathology (Martinez Cordero et
al., 2008; Tadokera et al., 2011; Martinez et al., 2013; O'Garra et al., 2013; Barber et al., 2014; Zhang et al., 2014; Shen and Chen, 2018) as well as progression to active TB disease in non-pregnant adults (Manabe et al., 2019). Given the small number of progressors in this study, these findings will need to be confirmed in other studies with a larger sample size. If these findings are confirmed, this profile could be used to identify subsets of LTBI+ pregnant women (i.e. those without this profile) at an increased risk of TB progression and would further support the idea of LTBI as a spectrum where subgroups of LTBI+ are protected from progression while others are not (Andrews et al., 2012; Huaman et al., 2018b). In addition, future studies would also need to determine whether this relationship of the systemic immune profile with TB progression is causal as it could partly explain the increased risk of *Mtb* progression during pregnancy and post-partum (Mathad and Gupta, 2012; Zenner et al., 2012; Jonsson et al., 2020).

Our study has some limitations. We did not have data on inflammation markers from pregnant women during the first trimester or non-pregnant women. This data would be informative to understand whether the relationship of these markers with LTBI status was also similar in early pregnancy compared to later pregnancy, or in pregnant women compared to non-pregnant women. Regardless, our study did have longitudinal data on inflammatory markers in the second and third trimester of pregnancy, and showed consistent results with LTBI status in both trimesters that was robust to adjustments for multiple comparisons. Another limitation of this study is that we only assessed soluble markers of inflammation. The next steps for this study is to better understand the cellular sources of these differences by assessing potential differences in immune cell phenotype and function by LTBI status. The sample size for the analysis of TB progression was limited; while we were able to detect significant differences in multiple markers, this was an exploratory analysis that will need to be confirmed in larger studies. Future large studies should also address whether the
In summary, we characterize the systemic immune profile in LTBI+ pregnant women showing higher levels of IFNγ but lower levels of other immune markers compared to LTBI- pregnant women. These findings describe a circulating cytokine and immune milieu indicating a distinct immune profile in LTBI+ women. Exploratory analysis suggests that this profile is negatively associated with TB progression. Future studies should confirm these findings in diverse settings in order to test the potential causal role along with the utility of this profile to identify women at high risk for TB progression and who may benefit from preventative therapy.
Acknowledgments: The authors thank the study participants for their time and contributions as well as the study staff who meticulously collected detailed data.

Financial Support: This work was supported primarily by the United States National Institutes of Health, NIH, Bethesda, MD, USA (R00HD089753 to RS and R01HD081929 to AG). JSM received support from NIAID (K23AI129854). Additional support for this work was the NIH-funded Johns Hopkins Baltimore-Washington-India Clinical Trials Unit for NIAID Networks (U01AI069465 to AG). BBA is a senior investigator from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil. MAP received a research fellowship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; finance code 001). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Conflict of Interest: None declared

Author Contributions: SN contributed to study design, implementation and interpretation. MA contributed to study design and interpretation and led the data collection. PK and SB conducted the laboratory assessments and contributed to interpretation of findings. VK and PD contributed to laboratory data collection and writing of this manuscript. SY and CSL contributed to data analysis. MAP and BBA created the statistical scripts used to plot the analyses and graphs, and helped with the interpretation of findings. RB, AG and JM led the parent study and also contributed to the design, implementation and interpretation of this study. RS led the conceptual design, analysis and wrote the primary version of the manuscript. All authors have approved the final manuscript and agreed to publication.
**References:**

Andrews, J.R., Noubary, F., Walensky, R.P., Cerda, R., Losina, E., and Horsburgh, C.R. (2012). Risk of progression to active tuberculosis following reinfection with Mycobacterium tuberculosis. *Clin Infect Dis* 54(6), 784-791. doi: 10.1093/cid/cir951.

Barber, D.L., Andrade, B.B., McBerry, C., Sereti, I., and Sher, A. (2014). Role of IL-6 in Mycobacterium avium--associated immune reconstitution inflammatory syndrome. *J Immunol* 192(2), 676-682. doi: 10.4049/jimmunol.1301004.

Barry, C.E., 3rd, Boshoff, H.I., Dartois, V., Dick, T., Ehrt, S., Flynn, J., et al. (2009). The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol* 7(12), 845-855. doi: 10.1038/nrmicro2236.

Cliff, J.M., Kaufmann, S.H., McShane, H., van Helden, P., and O'Garra, A. (2014). Role of IL-6 in *Mycobacterium avium*--associated immune reconstitution inflammatory syndrome. *J Immunol* 192(2), 676-682. doi: 10.4049/jimmunol.1301004.

Barber, D.L., Andrade, B.B., McBerry, C., Sereti, I., and Sher, A. (2014). Role of IL-6 in Mycobacterium avium--associated immune reconstitution inflammatory syndrome. *J Immunol* 192(2), 676-682. doi: 10.4049/jimmunol.1301004.

Barry, C.E., 3rd, Boshoff, H.I., Dartois, V., Dick, T., Ehrt, S., Flynn, J., et al. (2009). The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol* 7(12), 845-855. doi: 10.1038/nrmicro2236.

Cliff, J.M., Kaufmann, S.H., McShane, H., van Helden, P., and O'Garra, A. (2015). The human immune response to tuberculosis and its treatment: a view from the blood. *Immunol Rev* 264(1), 88-102. doi: 10.1111/imr.12269.

Comstock, G.W., Livesay, V.T., and Woolpert, S.F. (1974). The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 99(2), 131-138. doi: 10.1093/oxfordjournals.aje.a121593.

Cowan, J., Pandey, S., Filion, L.G., Angel, J.B., Kumar, A., and Cameron, D.W. (2012). Comparison of interferon-gamma-, interleukin (IL)-17- and IL-22-expressing CD4 T cells, IL-22-expressing granulocytes and proinflammatory cytokines during latent and active tuberculosis infection. *Clin Exp Immunol* 167(2), 317-329. doi: 10.1111/j.1365-2249.2011.04520.x.

Eigenbrod, T., Bode, K.A., and Dalpke, A.H. (2013). Early inhibition of IL-1beta expression by IFN-gamma is mediated by impaired binding of NF-kappaB to the IL-1beta promoter but is independent of nitric oxide. *J Immunol* 190(12), 6533-6541. doi: 10.4049/jimmunol.1300324.

De Martino, M., Lodi, L., Galli, L., and Chiappini, E. (2019). Immune Response to *Mycobacterium tuberculosis*: A Narrative Review. *Front Pediatr* 7, 350. doi: 10.3389/fped.2019.00350.

Dutta, R.K., Kathania, M., Raje, M., and Majumdar, S. (2012). IL-6 inhibits IFN-gamma induced autophagy in *Mycobacterium tuberculosis* H37Rv infected macrophages. *Int J Biochem Cell Biol* 44(6), 942-954. doi: 10.1016/j.biocel.2012.02.021.

Eigenbrod, T., Bode, K.A., and Dalpke, A.H. (2013). Early inhibition of IL-1beta expression by IFN-gamma is mediated by impaired binding of NF-kappaB to the IL-1beta promoter but is independent of nitric oxide. *J Immunol* 190(12), 6533-6541. doi: 10.4049/jimmunol.1300324.

Huanan, M.A., Deepe, G.S., Jr., and Fichtenbaum, C.J. (2016). Elevated Circulating Concentrations of Interferon-Gamma in Latent Tuberculosis Infection. *Pathog Immun* 1(2), 291-303. doi: 10.20411/pai.v1i2.149.

Huanan, M.A., Henson, D., Rondon, P.L., Ticona, E., Miranda, G., Kryscio, R.J., et al. (2018a). Latent tuberculosis infection is associated with increased unstimulated levels of interferon-gamma in Lima, Peru. *PLoS One* 13(9), e0202191. doi: 10.1371/journal.pone.0202191.

Huanan, M.A., Ticona, E., Miranda, G., Kryscio, R.J., Mugruza, R., Aranda, E., et al. (2018b). The Relationship Between Latent Tuberculosis Infection and Acute Myocardial Infarction. *Clin Infect Dis* 66(6), 886-892. doi: 10.1093/cid/cix910.

Jensen, A.V., Jensen, L., Faurholt-Jepsen, D., Aabye, M.G., Praygod, G., Kidola, J., et al. (2013). The prevalence of latent *Mycobacterium tuberculosis* infection based on an interferon-gamma release assay: a cross-sectional survey among urban adults in Mwanza, Tanzania. *PLoS One* 8(5), e64008. doi: 10.1371/journal.pone.0064008.

Jonsson, J., Kuhlmann-Berenzon, S., Berggren, I., and Bruchfeld, J. (2020). Increased risk of active tuberculosis during pregnancy and postpartum: a register-based cohort study in Sweden. *Eur Respir J* 55(3). doi: 10.1183/13993003.01886-2019.

LaVergne, S., Umlauf, A., McCutchan, A., Heaton, R., Benson, C., Kumarasamy, N., et al. (2020). Impact of Latent Tuberculosis Infection on Neurocognitive Functioning and Inflammation in HIV-Infected and Uninfected South Indians. *J Acquir Immune Defic Syndr*. doi: 10.1097/QAI.0000000000002368.
Luppi, P., Haluszczyk, C., Trucco, M., and Deloia, J.A. (2002). Normal pregnancy is associated with peripheral leukocyte activation. *Am J Reprod Immunol* 47(2), 72-81. doi: 10.1034/j.1600-0897.2002.10041.x.

Mack, U., Migliori, G.B., Sester, M., Rieder, H.L., Ehlers, S., Goletti, D., et al. (2009). LTBI: latent tuberculosis infection or lasting immune responses to M. tuberculosis? A TBNET consensus statement. *Eur Respir J* 33(5), 956-973. doi: 10.1183/09031936.00120908.

Manabe, Y.C., Andrade, B.B., Gupte, N., Leong, S., Kintali, M., Matoga, M., et al. (2019). A Parsimonious Host Inflammatory Biomarker Signature Predicts Incident TB and Mortality in Advanced HIV. *Clin Infect Dis*. doi: 10.1093/cid/ciz1147.

Martinez, A.N., Mehra, S., and Kaushal, D. (2013). Role of interleukin 6 in innate immunity to Mycobacterium tuberculosis infection. *J Infect Dis* 207(8), 1253-1261. doi: 10.1093/infdis/jit037.

Martinez Cordero, E., Gonzalez, M.M., Aguilar, L.D., Orozco, E.H., and Hernandez Pando, R. (2008). Alpha-1-acid glycoprotein, its local production and immunopathological participation in experimental pulmonary tuberculosis. *Tuberculosis (Edinb)* 88(3), 203-211. doi: 10.1016/j.tube.2007.10.004.

Mathad, J.S., and Gupta, A. (2012). Tuberculosis in pregnant and postpartum women: epidemiology, management, and research gaps. *Clin Infect Dis* 55(11), 1532-1549. doi: 10.1093/cid/cis732.

Mor, G., and Cardenas, I. (2010). The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol* 63(6), 425-433. doi: 10.1111/j.1600-0897.2010.00836.x.

Nandi, B., and Behar, S.M. (2011). Regulation of neutrophils by interferon-gamma limits lung inflammation during tuberculosis infection. *J Exp Med* 208(11), 2251-2262. doi: 10.1084/jem.20110919.

O'Garra, A., Redford, P.S., McNab, F.W., Bloom, C.I., Wilkinson, R.J., and Berry, M.P. (2013). The immune response in tuberculosis. *Annu Rev Immunol* 31, 475-527. doi: 10.1146/annurev-immunol-032712-095939.

Pai, M., Riley, L.W., and Colford, J.M., Jr. (2004). Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis* 4(12), 761-776. doi: 10.1016/S1473-3099(04)01206-X.

Sacks, G.P., Studena, K., Sargent, K., and Redman, C.W. (1998). Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol* 179(1), 80-86. doi: 10.1016/s0002-9378(98)70254-6.

Shen, H., and Chen, Z.W. (2018). The crucial roles of Th17-related cytokines/signal pathways in M. tuberculosis infection. *Cell Mol Immunol* 15(3), 216-225. doi: 10.1038/cmi.2017.128.

Tadokera, R., Meintjes, G., Skolimowska, K.H., Wilkinson, K.A., Matthews, K., Seldon, R., et al. (2011). Hypercytokinaemia accompanies HIV-tuberculosis immune reconstitution inflammatory syndrome. *Eur Respir J* 37(5), 1248-1259. doi: 10.1183/09031936.0091010.

Tufariello, J.M., Chan, J., and Flynn, J.L. (2003). Latent tuberculosis: mechanisms of host and bacillus that contribute to persistent infection. *Lancet Infect Dis* 3(9), 578-590. doi: 10.1016/s1473-3099(03)00741-2.

Ververs, M.T., Antierens, A., Sackl, A., Staderini, N., and Captier, V. (2013). Which anthropometric indicators identify a pregnant woman as acutely malnourished and predict adverse birth outcomes in the humanitarian context? *PLoS Curr* 5. doi: 10.1371/ currents.dis.54a8b618c1bc031ea140e3f2934599c8.

Vynnycky, E., and Fine, P.E. (2000). Lifetime risks, incubation period, and serial interval of tuberculosis. *Am J Epidemiol* 152(3), 247-263. doi: 10.1093/aje/152.3.247.

Zenner, D., Kruijshaar, M.E., Andrews, N., and Abubakar, I. (2012). Risk of tuberculosis in pregnancy: a national, primary care-based cohort and self-controlled case series study. *Am J Respir Crit Care Med* 185(7), 779-784. doi: 10.1164/rccm.201106-1083OC.
Zhang, G., Zhou, B., Li, S., Yue, J., Yang, H., Wen, Y., et al. (2014). Allele-specific induction of IL-1beta expression by C/EBPbeta and PU.1 contributes to increased tuberculosis susceptibility. *PLoS Pathog* 10(10), e1004426. doi: 10.1371/journal.ppat.1004426.

Zhang, X., Majlessi, L., Deriaud, E., Leclerc, C., and Lo-Man, R. (2009). Coactivation of Syk kinase and MyD88 adaptor protein pathways by bacteria promotes regulatory properties of neutrophils. *Immunity* 31(5), 761-771. doi: 10.1016/j.immuni.2009.09.016.
Table 1. Characteristics of the study population (N = 220)

| Characteristic                      | Overall (N=220) | LTBI+ (N=155) | LTBI- (N=65) | P-value  |
|------------------------------------|-----------------|---------------|--------------|----------|
| Age median (IQR)                   | 23 (21-27)      | 23 (21-27)    | 24 (21-27)   | 0.51     |
| Monthly Income                     |                 |               |              |          |
| ≤ Rs. 10,255                       | 75 (34)         | 51 (33)       | 24 (38)      | 0.54     |
| > Rs. 10,255                       | 143 (66)        | 103 (67)      | 40 (62)      |          |
| Education                          |                 |               |              |          |
| None to primary                    | 54 (25)         | 40 (26)       | 14 (22)      | 0.09     |
| Middle school to high school       | 139 (63)        | 101 (65)      | 38 (58)      |          |
| Post-high school                   | 27 (12)         | 14 (9)        | 13 (20)      |          |
| Mid-upper arm circumference        |                 |               |              |          |
| < 23 cm                            | 62 (28)         | 48 (31)       | 14 (21)      | 0.37     |
| 23 – 30.5 cm                       | 143 (65)        | 97 (63)       | 46 (71)      |          |
| >30.5 cm                           | 15 (7)          | 10 (6)        | 5 (8)        |          |
| Smoking status                     |                 |               |              |          |
| Yes                                | 26 (12)         | 20 (13)       | 6 (9)        | 0.50     |
| No                                 | 194 (88)        | 135 (87)      | 59 (91)      |          |
| Preeclampsia                       |                 |               |              |          |
| Yes                                | 25 (11)         | 18 (12)       | 7 (11)       | 0.99     |
| No                                 | 195 (89)        | 137 (88)      | 58 (89)      |          |
| Gestational Diabetes status        |                 |               |              |          |
| Yes                                | 21 (10)         | 11 (7)        | 10 (16)      | 0.08     |
| No                                 | 195 (90)        | 141 (93)      | 54 (84)      |          |
| HIV                                |                 |               |              |          |
| Yes                                | 70 (32)         | 31 (20)       | 39 (60)      | <0.001   |
| No                                 | 150 (68)        | 124 (80)      | 26 (40)      |          |

Legend: Data are presented as number (%) of subjects unless otherwise stated. P-values were calculated using Fisher’s exact test for categorical variables and Wilcoxon rank-sum for continuous variables to determine the difference between LTBI+ and LTBI- pregnant women.
**Figure 1: Levels of third trimester inflammation by LTBI status (N=220)**

Legend: A) Median and interquartile range (IQR) Log₂ levels of markers, measured in the 3rd trimester is shown for LTBI+ (n=155) and LTBI- (n=65) pregnant women. Wilcoxon rank-sum test was used to calculate p-values. *p < 0.05, **p < 0.01 and ***p < 0.001. B) Relative fold-change is shown for each marker by LTBI status. Red bars indicate p-value < 0.05.

**Figure 2: Association of LTBI status with third trimester inflammation (N=220)**

Legend: Using linear regression, the mean change in Log₂ concentrations of each inflammation marker and 95% confidence intervals (95% CI) among LTBI+ individuals compared to LTBI- individuals is shown in the forest plot. Inflammation markers were measured in samples collected at the third trimester of pregnancy. Multivariate models adjusted for age, mid-upper arm circumference, HIV status, diet and gestational diabetes status. Only immune markers with a p-value <0.2 in the univariate model are shown.

**Figure 3: Association of LTBI status with second trimester inflammation (N=187)**

Legend: Using linear regression, the mean change in Log₂ concentrations of each inflammation marker and 95% confidence intervals (95% CI) among LTBI+ individuals compared to LTBI- individuals is shown in the forest plot. Inflammation markers were measured in samples collected at the second trimester of pregnancy. Multivariate models adjusted for age, mid-upper arm circumference, HIV status, diet and gestational diabetes status. Only immune markers with a p-value <0.2 in the univariate model are shown.
Figure 4: Association of third trimester inflammation markers with TB progression (N=155; 9 progressors)

Legend: Using logistic regression, the odds ratio and 95% confidence intervals (95% CI) of TB progression per log₂ increase in each inflammation marker among LTBI+ pregnant women is shown in the forest plot. Progressors were defined as those who developed TB either during the third trimester of pregnancy (n=1) or up to one year post-partum (n=8). Inflammation markers were measured in samples collected at the third trimester of pregnancy. Multivariable models adjusted for age, mid-upper arm circumference and HIV status. Only immune markers with a p-value <0.2 in the univariate model are shown.
Supplementary Material

1 Supplementary Figure Title and Legends

Supplementary Figure 1: Levels of second trimester inflammation by LTBI status (N=187)

Legend: A) Median and interquartile range (IQR) Log₂ levels of markers, measured in the 2ⁿᵈ trimester is shown for LTBI+ (n=124) and LTBI- (n=58) pregnant women. Wilcoxon rank-sum test was used to calculate p-values. *p < 0.05, **p < 0.01 and ***p < 0.001. B) Relative fold-change is shown for each marker by LTBI status. Red bars indicate p-value < 0.05.
Supplementary Figure 2: Levels of Inflammation by LTBI status in HIV- women in 3rd trimester (N=139)

Legend: A) Median and interquartile range (IQR) Log2 levels of markers, measured in the 3rd trimester is shown for HIV- pregnant women with (n=124) and without (n=58) LTBI. Wilcoxon rank-sum test was used to calculate p-values. *p < 0.05, **p < 0.01 and ***p < 0.001. B) Relative fold-change is shown for each marker by LTBI status. Red bars indicate p-value < 0.05.
| biomarker | model      | Mean change (95% CI)   | p-value |
|-----------|------------|------------------------|---------|
| AGP       | univariate | -0.20 (-0.42 to 0.02) | 0.08    |
|           | multivariate | -0.29 (-0.54 to -0.04) | 0.02    |
| I-FABP    | univariate | -0.41 (-0.78 to -0.04) | 0.03    |
|           | multivariate | -0.25 (-0.67 to 0.15) | 0.22    |
| IL-1β     | univariate | -1.03 (-1.53 to -0.54) | <0.001  |
|           | multivariate | -1.15 (-1.70 to -0.60) | <0.001  |
| sCD163    | univariate | -0.18 (-0.39 to 0.03) | 0.10    |
|           | multivariate | -0.28 (-0.51 to -0.05) | 0.02    |
| TNF       | univariate | -0.19 (-0.49 to 0.10) | 0.20    |
|           | multivariate | -0.24 (-0.57 to 0.10) | 0.17    |
| IL-6      | univariate | -1.36 (-1.93 to -0.80) | <0.001  |
|           | multivariate | -1.22 (-1.87 to -0.58) | <0.001  |
| IL-17a    | univariate | -0.34 (-0.50 to -0.17) | <0.001  |
|           | multivariate | -0.39 (-0.57 to -0.21) | <0.001  |
| biomarker | model     | Mean change (95% CI)        | p-value |
|-----------|-----------|----------------------------|---------|
| AGP       | univariate| -0.32 (-0.56 to -0.08)     | 0.01    |
|           | multivariate| -0.52 (-0.79 to -0.22)    | 0.001   |
| I-FABP    | univariate| -0.60 (-1.15 to -0.04)     | 0.04    |
|           | multivariate| -0.71 (-1.36 to -0.06)    | 0.03    |
| IFNγ      | univariate| 0.32 (-0.007 to 0.65)      | 0.06    |
|           | multivariate| 0.45 (0.07 to 0.82)       | 0.02    |
| IL-1β     | univariate| -0.91 (-1.45 to -0.37)     | 0.001   |
|           | multivariate| -1.08 (-1.7 to -0.46)     | 0.001   |
| sCD163    | univariate| -0.23 (-0.50 to 0.04)      | 0.09    |
|           | multivariate| -0.36 (-0.67 to -0.05)    | 0.03    |
| TNF       | univariate| -0.31 (-0.58 to -0.05)     | 0.02    |
|           | multivariate| -0.23 (-0.54 to 0.08)     | 0.14    |
| IL-6      | univariate| -1.47 (-2.05 to -0.89)     | <0.001  |
|           | multivariate| -1.28 (-1.96 to -0.59)    | <0.001  |
| IL-17a    | univariate| -0.46 (-0.75 to -0.16)     | 0.002   |
|           | multivariate| -0.51 (-0.86 to -0.16)    | 0.004   |
| biomarker | model     | Odds ratio (95% CI) | value |
|-----------|-----------|---------------------|-------|
| I-FABP    | univariate| 1.63 (1.01 to 2.61) | 0.04  |
|           | multivariate | 1.50 (0.88 to 2.54) | 0.13  |
| IL-1β     | univariate| 1.52 (0.99 to 2.30) | 0.05  |
|           | multivariate | 1.64 (1.05 to 2.57) | 0.03  |
| sCD163    | univariate| 2.02 (0.78 to 5.23) | 0.15  |
|           | multivariate | 2.10 (0.47 to 9.37) | 0.33  |
| IL-6      | univariate| 1.66 (1.15 to 2.40) | 0.007 |
|           | multivariate | 1.58 (1.05 to 2.39) | 0.03  |
| IL-17a    | univariate| 4.72 (1.06 to 21.06) | 0.04  |
|           | multivariate | 5.49 (0.84 to 35.97) | 0.08  |
| IL-13     | univariate| 1.96 (0.99 to 3.84) | 0.05  |
|           | multivariate | 2.43 (1.12 to 5.27) | 0.02  |

Association with TB