Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Cytokine Signatures and Clinical Characteristics Associated with Conversion and Reversion of IGRA-based TB Screening among Healthcare Workers in North-Central Nigeria: A Pilot Study

Evaezi Okpokoro
Institute of Human Virology
Nurudeen Oketade
nurudeen.oketade@colostate.edu
Colorado State University
Anzaku A. Abel
Institute of Human Virology
Monalisa Owie
Institute of Human Virology
Olawole Ayorinde
Institute of Human Virology
Sophia Osawe
Institute of Human Virology
Alash'le Abimiku
Institute of Human Virology
Novel N. Chegou
Stellenbosch University

Research Article

Keywords: Clinical Profile, Cytokine Signatures, Conversion, Reversion, Healthcare Workers, Latent Tuberculosis Infections, North-Central Nigeria

Posted Date: February 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8864362/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

The substantial burden of subclinical *Mycobacterium tuberculosis* (Mtb) infection continues to be a key reservoir and driver of the tuberculosis (TB) epidemic, particularly in countries with high TB and HIV prevalence. Fluctuations in IFN-γ release assay (IGRA) status (conversion and reversion) undermine precise estimates of the subclinical TB reservoir and hinder efforts to control transmission and progression to active disease. In this study, we assessed differences in the level of pro-inflammatory and anti-inflammatory cytokines between converters and non-converters, as well as between reverters and non-reverters.

Methods

We retrieved stored unstimulated plasma samples of 72 participants (Healthcare Workers) from selected healthcare facilities in North-central Nigeria. Samples were categorized into four groups based on two longitudinal IGRA (QFT-Plus) results at month 0 (M0) and month 18 (M18): non-converters (M0- and M18-); converters (M0- and M18+), non-reverters (M0 + and M18+); and reverters (M0 + and M18-). The samples were analyzed for the concentrations of 18 cytokines using the Luminex® technology platform. Median differences in the concentrations of cytokine between converters and non-converters, reverters and non reverters were then assessed using the Mann-Whitney test.

Results

We observed that the median level of three plasma cytokines (MIF (*p*-value = 0.067), IL2 (*p*-value = 0.055), and IL5 (*p*-value = 0.055)) were significantly different between converters and non-converters, while the median level of two plasma cytokines (IL1β (0.0547) and IL13 (0.0758)) were significantly different between reverters and non-reverters.

Conclusion

Although preliminary, this study provides foundational findings linking the potential role of cytokines as biomarkers that may be associated with IGRA conversion and reversion. Future studies will look to establish the utility of these cytokines as biomarkers for subclinical TB.

INTRODUCTION

Tuberculosis (TB) continues to be a major public health challenge globally, with approximately 10.6 million new cases and 1.6 million deaths reported in 2024, with developing countries bearing the highest burden (1). The World Health Organization (WHO) reported an estimated 1.7 billion individuals infected with Latent Tuberculosis Infection (LTBI), constituting approximately a quarter of the world's population exposed to *Mycobacterium tuberculosis* (Mtb) (2), the acid-fast bacillus that causes TB. Among those with the latent infection, about 90% will not display any clinical symptoms of TB, while 5 to 10% of this population may likely advance to the active disease stage during their lifetime if left untreated(1). Efforts to curb the global health burden caused by TB has focused majorly on the development of novel therapeutics(3, 4), vaccines(5–8) and diagnostics(9, 10). However, the successful eradication of TB requires a joint approach focusing on both developing novel biomedical tools and public health interventions targeting the subclinical TB population.

TB was recently redefined to be a continuous spectrum between active TB with lesional burden to cleared infection with little to no risk of developing disease(11). Between the extreme ends of this spectrum lies the subclinical population that are indeed infected but do not show any clinical signs(11). Recent studies have shown that this subclinical population may also contribute to disease transmission, making the diagnosis and treatment of this population a priority for the eradication of TB(12). The strategic targeting of this subclinical population for interventions required an accurate estimation of this subclinical status. However, this has been marred by inconsistencies, specifically conversion and reversion observed during serial IGRA testing for subclinical TB(13–15). This lack of consistency in IGRA testing has made the identification of subclinical TB cases difficult and the development of additional diagnostic biomarkers a priority.

Cytokines play an important role during infection by modulating immune responses. For example, IFN-γ released by activated T-cells during Mtb infection is essential to induce the control of the bacilli by macrophages(16, 17). Other pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-12 p40, IL-2 also play a protective role during Mtb infection(18). Although protective at the regulated levels, the overexpression of these cytokines may also contribute to worsened disease prognosis. For instance, IFN-γ overproduction has been linked to reactivation of latent tuberculosis infection(19). Converse to the protective role of IFN-γ, type 1 interferons, which include IFN-α2, may worsen infection and antagonize the protection rendered by IFN-γ(16). Anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 also play an important role during Mtb infection by providing a balance in the inflammatory response(18). Chemokines that guide the migration of immune cells to the site of infection also play a significant role in early innate-driven protection and the formation of granuloma(20). Given the role of cytokines and chemokines during Mtb infection, differences in their abundance in biological samples may inform infection state and risk of progression.

In a previous study from our group, we observed IGRA conversion and reversion among healthcare workers (HCWs). Similar patterns have been reported in other cohorts(14), suggesting that IGRA results can fluctuate over time. However, the biological drivers of these changes remain incompletely understood. Reversion of Mtb infection status runs counter to basic immunological principles. It is assumed that exposure to Mtb should induce a long-lasting immune memory, which can be detected using IGRA-based assays that induce a response upon re-exposure to Mtb-derived antigens (21, 22). However, this scenario does not seem to be the case as reversion of infection status is a highly common phenomenon(14, 23). Although reversion of Mtb infection has been documented since 1913, its immunological basis remains poorly defined(23). Cytokines and chemokines marshal and modulate the immune response and may influence the direction of infection upon exposure (18). We therefore conducted a pilot study among healthcare workers (HCWs) in North-central Nigeria, to examine cytokine signatures associated with conversion and reversion of IGRA-based TB screening. We measured concentrations of 18 cytokines and chemokines in unstimulated blood from converters, non-converters, reverters, and non-reverters, defined by serial IGRA screening at baseline and again 18 months after the initial test. Comparing the cytokine levels, we observed differences between converters and non-converters (IL-2, $p = 0.055$; IL-5, $p = 0.055$), and between reverters and non-reverters (IL-1β, $p = 0.0547$; IL-13, $p = 0.0758$). We also observed an association between occupational risk factors such as working in the HIV clinic and knowledge of tuberculosis infection control and IGRA conversion. Overall, our findings are consistent with a potential role for occupational exposures in shaping Mtb infection status and indicate that selected cytokines may warrant further evaluation as candidate biomarkers of IGRA conversion and reversion.

MATERIALS AND METHODS

Study design and location

The samples used for this study is a subset from a previous perspective cohort study (24, 25). The parent study was conducted in selected healthcare facilities in North-central Nigeria between December 2019 and April 2021. The main goal of the parent study was to determine the prevalence and risk factors for LTBI among HCWs in this region. HCWs from the selected facilities were screened for LTBI at baseline (M0), month 6 (M6), month 12 (M12) and month (M24), using the IGRA based QFT-Plus kit. Information on sociodemographic, clinical history, occupational and non-occupational exposure were collected for each participant. In the parent study, 45% of the 1043 participants reported a positive IGRA at baseline (24). For this pilot study, participants were selected based on the result of the serial QFT-Plus kit. We examined the cytokine and chemokine profile of four groups of individuals at two timepoints, 18 months apart: converters (negative to positive), reverters (positive to negative), non-converters (negative at both time points) and non-reverters (positive at both time points). For profiling, we measured the abundance of 18 cytokines and chemokines using the Luminex® technology. Conversion was defined as the development of new immunological reactivity following a previous negative IGRA and increase in IFN-γ responses while reversion was defined as a previously positive IGRA becoming negative upon repeat testing and decrease in IFN-γ responses over an 18-month period. The cutoff for positive and negative IGRA results were determined according to QFT-Plus kit manufacturers instruction with positive QFT-Plus being an IFN-γ ≥ 0.35 IU/mL and negative QFT-Plus being an IFN-γ < 0.35 IU/mL.

Eligibility criteria

For the parent study, HCWs according to the WHO criteria employed at the selected study sites were eligible for the study. Only HCWs that were 18 years or older and that provided informed consent to participate were included. HCWs with a known diagnosis of HIV infection, TB infection or having a household member with TB, and those on TB preventative therapy were excluded from the parent study.

Sample Size

Given that this was a pilot study, stored samples of study participants were itemized if they met the selection criteria for the sub study described earlier. Thus, of the 215 participants who met our eligibility criteria for the sub study, stored samples of 72 participants were randomly selected and categorized as non-reverters (M0 + and M18+), reverters (M0 + and M18-), non-converters (M0- and M18-) and converters (M0- and M18+) based on the IGRA results.

Sample collection procedure

At enrollment and follow-up visits, venipuncture whole blood was collected into two separate 5 mL lithium heparin tubes for QFT-Plus ELISA and Luminex analysis. For QFT-Plus ELISA, 1mL each of the heparinized whole blood from tube-1 was sub-aliquoted into each of the four QFT-Plus collection tubes (Nil, TB1, TB2, Mitogen), and subsequently mixed 10 times by inversion for antigen stimulation. Tubes were incubated upright at 37°C for 16–24 hours, aiming at 18 hours of incubation within 3 hours of collection. After incubation, samples were centrifuged at 3000rpm for 15 minutes, and plasma samples were harvested from each tube into separate labelled cryovial tubes and were subsequently stored at 2–8°C until ELISA was performed. For Luminex assay, heparinized blood from tube-2 was centrifuged and separated into plasma aliquots and stored at -80°C freezer at I-HAB until Luminex assay was performed.

Immunoassay

QFT-Plus ELISA

For ELISA, all reagents and plasma samples were brought to room temperature, and 50 µl of standards, controls, and each stimulated plasma sample was added to designated wells of the microplate pre-coated with IFN-γ antibody. Then, 50 µl of enzyme conjugate was added to each well, the plate was covered and incubated at room temperature for 2 hours. Wells were washed six times with the provided wash buffer, 100 µl of enzyme substrate solution was added, shaken, and incubated in the dark for 30 minutes, after which 50 µl of stop solution was added to each well and was read at 450 nm wavelength within 5 minutes. IFN-γ concentrations (IU/ml) were calculated using QFT software as provided by Qiagen.

Luminex procedure

The stored unstimulated plasma samples were retrieved for four groups of HCWs categorized based on their serial screening for LTBI at baseline (M0) and month 18 (M18) using QFT-plus test kits as previously described in the eligibility criteria. Plasma cytokine at M18 was tested for the following 18 cytokines and chemokines: Interferon (IFN)-α2, IFNγ, interleukin (IL)-1β, IL-2, IL-3, IL-5, IL-8, IL-12 (p40), IL-13, IFNγ-inducible protein (IP-10), Monokine induced by gamma (MIG/CXCL9), Tumor Necrosis Factor (TNF)-α, TNFβ, Stem cell factor (SCF), Leukemia Inhibitory Factor (LIF), TNF-related apoptosis-inducing ligand (TRAIL), Nerve Growth Factor (NGF), Macrophage Migration Inhibitory Factor (MIF) (26). Stored plasma samples were retrieved from − 80°C and allowed to thaw at room temperature, and further centrifuged at 10,000rpm for 10 minutes at 4°C to remove any residual debris. Multiplex bead assays were performed using Luminex® technology with four panels: Human Cytokine/Chemokine/Growth Factor Panel A, Human Cytokine/Chemokine Panel II, Adipokine Magnetic Bead II Assay, and Human Sepsis Panel I, following the manufacturer's protocol.

Briefly, 25 µl of each plasma sample was added per well in a 96-well microplate containing magnetic beads that were re-coated with capture antibodies specific to each analyte, followed by incubation with shaking at room temperature for 2 hours. Plates were then washed using a magnetic plate washer, and 25 µl of biotinylated detection antibodies were added to each well and incubated with shaking for 1 hour. Subsequently, 25 µl of streptavidin-phycoerythrin (SA-PE) was added and incubated for 30 minutes to allow signal detection (27). After final washes and resuspension in 150 µl of assay buffer, plates were read on the MAGPIX® system, and data were analyzed using a five-parameter logistic (5-PL) standard curve generated for each analyte on MyAssays.com to determine cytokine concentrations in pg/ml (28).

Statistical analysis

A non-parametric test was employed to compare the median differences in cytokine concentrations between the different groups using the Mann-Whitney U test. All statistical analyses were performed using Stata version 18.0 (StataCorp LLC, College Station, TX, USA). Hypothesis tests were two-tailed and performed at a 5% alpha level (29).

Ethical consideration

The study was carried out per the tenets of the Declaration of Helsinki as cited by the General Assembly of the World Medical Association (30). Upon ethics approval from the National Health Research Ethics Committee (NHREC), an Informed Consent (IC) was obtained from consenting participants for enrolment, and each participant was assigned

a unique de-identified code to ensure privacy and confidentiality. All study data were encrypted to maintain the research data protection policy (31). Participation in the study was completely voluntary and at no cost to the consenting participants.

RESULTS

Sociodemographic attributes of participants

Table 1 provides the sociodemographic data of selected participants broken down based on conversion and reversion between M0 and M18 for 72 participants selected from the parent study. 33.33% and 11.11% of the 72 participants were non-converters and converters respectively, while 20.83% and 34.72% were non-reverters and reverters respectively. Majority of the participants were above 35 years (63.9%) with the overall mean age of 38 years. In terms of educational status, majority (72.2%) had tertiary education, 16.7% had a form of secondary/post-secondary education while 9.7% attained only primary education. Participants predominantly resided in densely populated areas (45.8%), while 38.9% and 15.3% resided in moderately populated and sparsely populated areas respectively. Interestingly, none of the socioeconomic factors captured in the sociodemographic data was significantly associated with conversion or reversion.

Table 1
Socioeconomic risk factors stratified by QFT serial test results

	Conversion group			**Reversion group**			
Variables	Non converters n(%)	Converters n(%)	*p*-value	Non reverters n(%)	Reverters n(%)	*p*-value	Overall n(%)
	24(33.33)	**8(11.11)**		**15(20.83)**	**25(34.72)**		
Age [media(IQR)]	40(16.50)	38(15.00)	0.10	37(18.00)	38(11.00)	0.36	38(16.00)
Age_group			0.10			0.19	
0–34	14(87.50%)	2(12.50%)		2(20.00%)	8(80.00%)		26(36.10%)
35+	10(62.50%)	6(37.50%)		13(43.30%)	17(56.70%)		46(63.90%)
Sex			0.22			0.67	
Female	12(66.70%)	6(33.30%)		10(40.00%)	15(60.00%)		43(59.70%)
Male	12(85.70%)	2(14.30%)		5(33.30%)	10(66.70%)		29(40.30%)
Education			0.24			0.33	
No education	0(0.00%)	0(0.00%)		0(0.00%)	1(100.00%)		1(1.40%)
Primary	2(100.00%)	0(0.00%)		1(20.00%)	4(80.00%)		7(9.70%)
Secondary/post-secondary school	3(50.00%)	3(50.00%)		4(66.70%)	2(33.30%)		12(16.70%)
Tertiary/postgraduate	19(79.20%)	5(20.80%)		10(35.70%)	18(64.30%)		52(72.20%)
Religion			0.22			0.28	
Christian	20(71.40%)	8(28.60%)		13(41.90%)	18(58.10%)		59(81.90%)
Islam	4(100.00%)	0(0.00%)		2(22.20%)	7(77.80%)		13(18.10%)
Marital			0.82			0.29	
Married	17(73.90%)	6(26.10%)		13(43.30%)	17(56.70%)		53(73.60%)
Single	7(77.80%)	2(22.20%)		2(28.60%)	5(71.40%)		16(22.20%)
Widowed	0(0.00%)	0(0.00%)		0(0.00%)	3(100.00%)		3(4.20%)
Residence			0.46			0.11	
Densely populated	9(64.30%)	5(35.70%)		9(47.40%)	10(52.60%)		33(45.80%)
moderately populated	11(84.60%)	2(15.40%)		6(40.00%)	9(60.00%)		28(38.90%)
sparsely populated	4(80.00%)	1(20.00%)		0(0.00%)	6(100.00%)		11(15.30%)
Biomass cooking fuel			0.29			0.8	
No	13(68.40%)	6(31.60%)		9(36.00%)	16(64.00%)		44(61.10%)
Yes	11(84.60%)	2(15.40%)		6(40.00%)	9(60.00%)		28(38.90%)

	Conversion group			Reversion group		
toilet			0.39			0.38
Aqua privy	0(0.00%)	0(0.00%)		0(0.00%)	3(100.00%)	5(6.90%)
Pit toilet	1(50.00%)	1(50.00%)		2(40.00%)	3(60.00%)	5(6.90%)
water cistern	23(76.70%)	7(23.30%)		13(40.60%)	19(59.40%)	62(86.10%)

Clinical history and risk factors

We assessed the occupational and behavioral factors associated with IGRA conversion and reversion (Table 2). For the professional roles of selected participants, 51.4% were clinical staff, indicating a balance between clinical and non-clinical staff selected for the study. Most participants (72.2%) had previously worked in an HIV clinic, and 33.3% were working at an HIV clinic at the time of enrollment and follow-up. 63.9% of participants reported to have received the BCG vaccine. In terms of behavioral risks factors, most of the selected participants reported non-consumption of alcohol (80.6%) and cigarette (97.2%).

Table 2
Occupational and behavioral risk factors stratified by QFT serial test results

	Conversion Group			Reversion Group			
	Non converters n(%)	Converters n(%)	*p*-value	Non-reverters n(%)	Reverters n(%)	*p*-value	Overall n(%)
Employment category			0.10			0.46	
Clinical	14(87.50%)	2(12.50%)		9(42.90%)	12(57.10%)		37(51.40%)
Non-clinical	10(62.50%)	6(37.50%)		6(31.60%)	13(68.40%)		35(48.60%)
Previously worked in the HIV Centre.			0.19			0.57	
No	15(68.20%)	7(31.80%)		12(40.00%)	18(60.00%)		52(72.20%)
Yes	9(90.00%)	1(10.00%)		3(30.00%)	7(70.00%)		20(27.80%)
Currently working in the HIV Centre.			**0.03**			0.06	
No	14(63.60%)	8(36.40%)		7(26.90%)	19(73.10%)		48(66.70%)
Yes	10(100.00%)	0(0.00%)		8(57.10%)	6(42.90%)		24(33.30%)
BCG_Vaccine			0.27			0.54	
No	5(55.50%)	4(45.50%)		5(29.4%)	12(70.60%)		26(36.10%)
Yes	19(82.60%)	4(17.40%)		10(43.50%)	13(56.50%)		46(63.90%)
TST_test			0.73			0.71	
No	22(75.90%)	7(24.10%)		14(36.80%)	24(63.20%)		67(93.10%)
Yes	2(66.70%)	1(33.30%)		1(50.00%)	1(50.00%)		5(6.90%)
The facility actively screens for TB.			0.40			0.75	
No	22(73.30%)	8(26.70%)		12(36.40%)	21(63.60%)		63(87.50%)
Yes	2(100.00%)	0(0.00%)		3(42.90%)	4(57.10%)		9(12.50%)
***Facemask, n = 47**			0.85			0.99	
No	11(78.60%)	3(21.40%)		8(36.40%)	14(63.60%)		36(50.00%)
Yes	4(66.70%)	2(33.30%)		2(40.00%)	3(60.00%)		11(15.30%)
Handwash			0.38			0.42	
Always	12(80.00%)	3(20.00%)		7(36.80%)	12(63.20%)		34(47.20%)
Never	0(0.00%)	0(0.00%)		1(100.00%)	0(0.00%)		1(1.40%)

	Conversion Group			Reversion Group			
	Non converters n(%)	**Converters n(%)**	***p*-value**	**Non-reverters n(%)**	**Reverters n(%)**	***p*-value**	**Overall n(%)**
Often	3(100.00%)	0(0.00%)		2(66.70%)	1(33.30%)		6(8.30%)
Rarely	4(80.00%)	1(20.00%)		1(16.70%)	5(83.30%)		11(15.30%)
Sometimes	5(55.60%)	4(44.40%)		4(36.40%)	7(63.60%)		20(27.80%)
Alcohol			0.08			0.24	
No	17(68.00%)	8(32.00%)		11(33.30%)	22(66.70%)		58(80.60%)
Yes	7(100.00%)	0(0.00%)		4(57.10%)	3(42.90%)		14(19.40%)
Smoking			0.56			0.28	
Ex-smoker of cigarettes	0 (0.00%)	0 (0.00%)		2 (66.70%)	1 (33.30%)		3(4.20%)
Non-smoker of cigarettes	23 (74.20%)	8 (25.80%)		24 (64.9%)	13 (35.10%)		68(94.40%)
Occasional smoker	1(100.00%)	0(0.00%)		0 (0.00%)	0 (0.00%)		1(1.40%)
Facility TB control program			**0.04**			0.33	
No	13(65.00%)	7(35.00%)		8(50.00%)	8(50.00%)		36(50.00%)
Yes	11(91.70%)	1(8.30%)		7(29.20%)	17(70.80%)		36(50.00%)
Cigarette frequent smoking			0.26			0.13	
No	24(75.00%)	8(25.00%)		13(34.20%)	25(65.80%)		70(97.20%)
Yes	0(0.00%)	0(0.00%)		2(100.00%)	0(0.00%)		2(2.78%)

We observed evidence of an association between currently working in HIV clinic and being in the conversion or non-conversion group (*p*-value = 0.028). Hence, higher proportions of converter (36.4%) were not currently working in the HIV clinics compared to those in the HIV clinics (0%). We also observed an association between conversion status (conversion vs non-conversion) and the awareness of a TB infection control program at the participants' source facility. (*p*-value = 0.040). Thus, higher proportions of converters (35%) were unaware of TB infection control program in their health facility compared those who were aware (8.3%)

Cytokine profiling of HCWs across conversion and reversion categories

To assess the relationship between the levels of selected cytokines and chemokines and IGRA conversion and reversion, we compared the median cytokine concentration between converters and non-converters as well as reverters and non-reverters at month 18 of the study (Table 3). A total of 72 samples (month 18) were retrieved for HCWs across the 14 health facilities from the parent study. Among the 18 cytokines profiled, we observed a

statistically significant difference in the median concentration (p-value < 0.1) for MIF, IL1β, IL2, IL5, and IL13. MIF, IL2 and IL5 were significantly different between converters and non-converters with MIF and IL5 being higher in non-converters and IL2 being higher in the converters. For the comparison between reverters and non-reverters, IL1β was significantly higher in the non-reverters while IL13 was significantly higher in the reverters. Although we expected a difference in IFNα2 and IFNγ between the groups, their levels were similar across all the groups due to their concentration being below the limit of detection for our assay.

Table 3
Cytokines expression level following conversion and reversion for healthcare workers

	Conversion group			Reversion group		
Variable	**Non-converters n(%)**	**Converters n (%)**	**P-value**	**Non-reverters n (%)**	**Reverters n (%)**	**P-value**
	24 (33.33)	**8 (11.11)**		**15 (20.83)**	**25 (34.72)**	
IFNα2, median (IQR)	13.03 (13.03–13.03)	13.03 (13.03–13.03)	1.00	13.03 (13.03–13.03)	13.03 (13.03–13.03)	1.00
MIF, median (IQR)	159.79 (98.11–1302.46)	98.11(98.11–401.51)	**0.07**	116.32 (98.11–393.48)	146.6 (98.11–881.43)	
IFN-γ, median (IQR)	1.7 (1.70–6.67)	1.7 (1.70–4.70)	0.98	1.7 (1.70–2.83)	1.7 (1.70–7.03)	0.37
IL1β. median (IQR)	2.67 (2.13–121.6)	9.04 (2.13–2272.20)	0.14	4.41 (2.13–49.85)	2.13(2.13–12.60)	**0.05**
IL2, median (IQR)	0.65 (0.65–2.56)	0.97 (0.65–1.31)	**0.06**	0.65 (0.65–2.56)	0.65 (0.65–6.55)	0.83
IL3, median (IQR)	1.7 (1.70–1.70)	1.7 (1.70–1.70)	1.00	1.7 (1.70–1.70)	1.7 (1.70–3.66)	0.44
IL5, median (IQR)	1.09 (0.42–5.70)	0.90 (0.47–12.99)	**0.06**	1.39 (0.42–7.15)	0.90 (0.42–3.60)	0.53
IL8, median (IQR)	5.44 (5.44–5831.09)	32.17 (5.44–12229.94)	0.22	5.44 (5.44–11166.00)	5.44 (5.44–4873.00)	0.53
IL12p40, median (IQR)	9.24 (5.38–40.11)	21.25 (3.60–166.21)	0.41	18.47 (5.50–524.71)	10.28 (5.49–106.31)	0.10
IL13, median (IQR)	8.75 (8.75–91.90)	19.24 (8.75–66.09)	0.51	8.75 (8.75–524.00)	8.75 (8.75–698.67)	**0.08**
MIGCXCL9, Median (IQR)	730.1(50.02–17026.24)	630.63 (366.11–13772.00)	0.93	1060.32 (402.99–2465.61)	988.88 (382.46–5287.04)	0.94
TNFα, median (IQR)	15.12 (5.94–6991.04)	36.07 (5.94–15951.04)	0.11	26.8 (5.94–3846.08)	10.99 (5.94–902.15)	0.19
TNFβ, median (IQR)	2.08 (1.34–26.88)	2.19 (1.34–31.36)	0.75	1.34 (1.34–76.80)	1.34 (1.34–23.68)	0.18
SCF, median (IQR)	14.46 3.11 3.11 3.11 3.11–57.24)	13.55 (3.11–57.78)	0.57	13.11 (3.11–29.45)	8.85 (3.11–217.00)	0.96
LIF, median (IQR)	1.22 (1.22–5.55)	1.22 (1.22–2.61)	0.75	1.22 (1.22–8.10)	1.22 (1.22–18.54)	0.47
TRAIL, median (IQR)	59.4 (34.16–197.54)	73.58 (43.82–95.36)	0.14	55.65 (20.86–120.45)	57.0 (38.64–95.04)	0.97
NGF, median (IQR)	0.44 (0.44–0.53)	0.44 (0.44–0.44)	0.57	0.44 (0.44–0.69)	0.44 (0.44–0.65)	0.68

DISCUSSION

The eradication of TB requires a multipronged strategy that couples biomedical innovation and implementation of strengthened public health measures. Recently, the disease burden posed by subclinical TB has been recognized as a severe limiting factor towards achieving the goals of the WHO's End TB Strategy(32). Currently, the diagnosis of subclinical TB relies on TST-based and IGRA-based tests(21, 22). Unfortunately, both tests have been recognized to have significant drawbacks. In the case of Tuberculin Skin Test (TST)-based tests, its cross reactivity with BCG vaccination makes it undeployable in highly endemic regions where BCG vaccine are part of the routine vaccine regimen(22, 33). For IGRA-based, there has been reports of reversion from positive to negative which impacts our ability to accurately determine the prevalence of subclinical TB(14, 23). This instability in TB infection status also impacts the effective implementation of public health strategies to reduce the burden of subclinical TB(12). Optimal implementation of public health strategies also requires an in-depth understanding of the risk factors associated with subclinical TB. One of such risk factors includes occupational exposure. HCWs have an increased risk of becoming infected with TB(34). However, public policies and strategies aimed at reducing TB risk among HCWs remain limited(35, 36).

In this study, we examined the effect of socio-economic, behavioral and occupational risk factors on the stability of IGRA-defined TB infection status using retrospectively collected sociodemographic, clinical and occupational data from a prior cohort(25). Previous studies have shown strong links between factors such as income, education status, alcohol intake, smoking and residency and increased risk of TB infection, poor treatment outcome and poor health seeking behavior(24, 34, 37, 38). Importantly, occupational risk specifically for HCWs has been shown to increase the likelihood of infection two to three times that of the general population(25, 34). In this population, we sought to identify if similar risk factors also contribute to the stability of IGRA-defined TB infection status. We observed significant association between working in a HIV clinic (p-value = 0.03) and the presence of infection control strategies (p-value = 0.04) to conversion over an 18-month duration. Interestingly, all the converters in this study worked outside the HIV clinic. It is possible that workers in the HIV clinic are more cautious with the use of personal protective equipment which reduces the risk of TB infection. For facility-wide TB infection control, most of the converters were in facilities without a control program indicating the importance of such strategies to reduce the likelihood of TB infection. Converse to what we observed for these two occupational risks, we did not observe any significant association between change in infection status and well-known risk factors such as residence, level of education, BCG vaccine status, use of personal protective equipment, smoking and alcohol drinking habits. This result may reflect selection-related skewing of our study population, as we primarily drew participants from a subset of HCWs in the parent study. This is particularly relevant because, in the parent cohort, we observed significant associations between QFT positivity and age as well as residency(24, 25).

We also assessed the level of pro-inflammatory and anti-inflammatory cytokines and chemokines upon reversion and conversion. Several studies have shown that cytokine and chemokine profiles can recapitulate the stratification of individuals according to TB infection status, including distinctions between uninfected, latent TB infection, and active TB(18, 33, 39–41). One study showed a higher level of TNF-α, IFN-γ, IL-6, IP-10, and MIP-1β in the plasma samples of TB patients in comparison to infected individuals(39). Another study showed that a combination of six to seven cytokines and chemokines which included MCP-1, LIF, IL-2, IL12-p40, IFN-γ, MIF, TRAIL, IL-2Ra and PDGF could be used to accurately predict the infection status of individuals(42). These findings provided a basis for the potential use of cytokines as biomarkers. In our study, we observed a decrease in MIF and IL5 and an increase in IL2 upon conversion to IGRA-positive. MIF, a proinflammatory chemokine has been shown to be involved in modulating the innate immune response(43). MIF has also been shown to have therapeutic and prognostic potential for active TB cases(43–45). MIF was shown to be higher in patients with advanced and drug-resistant disease in comparison to

mild-to-moderate and drug sensitive disease(45). However, a comparison to uninfected individuals were not made in this study. Given the pro-inflammatory role of MIF, we may hypothesize that the reduced level observed in IGRA conversion may indicate a loss of innate control of Mtb. IL5, a Th2 cytokine, is involved in humoral immunity(46). Previous studies have linked an increase in Th2 response to reduced protection from tuberculosis(47). Converse to this finding, exposure of cells to Mtb led to a decrease in IL5(48). Although the reduced level of IL5 opposes what we should observe for Th2 cytokines upon conversion, this change may be a part of a more complex immune response to TB infection and cannot be used to determine the complete profile of Th2 cytokines. Unlike IL5, IL2 is part of the Th1 cytokines which are deemed important for the control of TB infection(46). Similar to IFN-γ, the production of IL2 increases during active TB(49). In fact, IL2 has been shown to possess compelling diagnostic potential improving the ability to diagnose both latent and active TB cases when used in combination with IGRA(49, 50). Our observation of an increase in IL2 upon conversion concurs with previous studies and builds a strong case for its use as a diagnostic biomarker(49, 50). Comparing the cytokine profile between reverters and non-reverters, we observed a decrease in IL1β and an increase in IL13 upon reversion. IL1β plays an important protective role during TB infection by recruiting antimicrobial effector molecules and by inducing a reprogramming of immune cells towards an antimycobacterial profile(51). The inhibition of IL1β production severely impacts the ability to control Mtb infection(52). In the context of our study, the reduction in IL1β level upon reversion may portend the resolution of TB infection and relaxation of the immune response towards Mtb. Similar to IL5, IL13 is also a Th2 cytokine and considered an anti-inflammatory cytokine that polarizes macrophages towards the unprotective M2 macrophage phenotype(46, 53). Counterintuitive to the anti-inflammatory role of IL13, it has been shown to play a protective role during Mtb infection likely through providing a counterbalance to pro-inflammatory signals(54). Increase in IL13 upon reversion may indicate the assumption of an anti-inflammatory response post TB clearance.

The immune response is highly complex and a causal role of the highlighted cytokines and chemokines in this study for conversion and reversion cannot be made. However, these findings serve as a starting point for further studies to understand their role in the stability of TB status. Further evaluation of their immunomodulatory and biomarker roles will be important to determine the clinical relevance of our findings.

Limitations of the study

One limitation of this study is the relatively small sample size of 72 participants. Larger and well-powered cohorts are needed to confirm the associations observed. Another point worth mentioning was our inability to detect any significant difference in the levels of IFNα2 and IFN-γ between groups. It is possible that our collection and storage conditions may have depleted these important cytokines below the limit of detection of the assay employed. A further limitation of the study is the potential temporal variability in cytokine levels. Given the cross-sectional design of the study, the observed differences or absence of differences may be influenced by variability in the timing of cytokine peaks and decay across participants. Future studies will look to adopt longitudinal design to address this limitation.

Conclusion

In conclusion, we identified cytokines and chemokines associated with conversion (MIF, IL5 and IL2) and reversion (IL1β and IL13). Our eventual goal will be to validate these findings using a more balanced and larger cohort.

Abbreviations

TB

Tuberculosis
Mtb
Mycobacterium tuberculosis
IGRA
Interferon-Gamma Release Assays
QFT
QuantiFERON plus
TST
Tuberculin Skin Test
HCWs
Healthcare Workers

Declarations

Ethics approval and consent to participate

Ethical approval for the parent study was obtained from the National Health Research Ethics Committee (NHREC/01/01/2007–09/04/2021). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki, and all personal information was kept confidential.

Consent for publication

Not Applicable

Competing interests

The authors declare no competing interests.

Funding

This study was funded by the European Developing Countries Clinical Trial Partnership (EDCTP) under the Career Development Fellowship (CDF) Trading Mobility Action (TMA) number (TMA2016CDF-1583).

Author Contribution

EO and NO conceptualized the study; EO, NO, AAA, MO, SO implemented the study; EO, NO, AAA, OA conducted the analysis and interpretation of these data; all authors contributed to the drafting of the manuscript; EO, AA, NC critically revised and supervised the manuscript. All authors read and approved the submitted version of the manuscript.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Global Tuberculosis Report 2024 [Internet]. [cited 2025 May 26]. Available from: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024
2. Houben RMGJ, Dodd PJ. The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling. PLOS Med. 2016;13(10):e1002152.
3. Dartois VA, Mizrahi V, Savic RM, Silverman JA, Hermann D, Barry CE. Strategies for shortening tuberculosis therapy. Nat Med. 2025;31(6):1765–75.
4. Aldridge BB, Barros-Aguirre D, Barry CE, Bates RH, Berthel SJ, Boshoff HI, et al. The Tuberculosis Drug Accelerator at year 10: what have we learned? Nat Med. 2021;27(8):1333–7.
5. Sable SB, Posey JE, Scriba TJ. Tuberculosis Vaccine Development: Progress in Clinical Evaluation. Clin Microbiol Rev. 2019;33(1):10.1128/cmr.00100 – 19.
6. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet Lond Engl. 2013;381(9871):1021–8.
7. Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, Bilek N, et al. Prevention of M. tuberculosis Infection with H4:IC31 Vaccine or BCG Revaccination. N Engl J Med. 2018;379(2):138–49.
8. Tait DR, Hatherill M, Meeren OVD, Ginsberg AM, Brakel EV, Salaun B, et al. Final Analysis of a Trial of M72/AS01E Vaccine to Prevent Tuberculosis. N Engl J Med. 2019;381(25):2429–39.
9. Ockhuisen T, Nooy A de, Jenkins HE, Han A, Russell CA, Khan S, et al. Cost-effectiveness of diagnostic tools and strategies for the screening and diagnosis of tuberculosis disease and infection: a scoping review. BMJ Public Health [Internet]. 2024 Apr 19 [cited 2025 Aug 26];2(1). Available from: https://bmjpublichealth.bmj.com/content/2/1/e000276
10. Mahato RP, Kumar S. The future in diagnostic tools for TB outbreaks: A review of the approaches with focus on LAMP and RPA diagnostics tests. J Microbiol Methods. 2024;227:107064.
11. Dheda K, Migliori GB. New framework to define the spectrum of tuberculosis. Lancet Respir Med. 2024;12(6):426–8.
12. Kendall EA, Shrestha S, Dowdy DW. The Epidemiological Importance of Subclinical Tuberculosis. A Critical Reappraisal. Am J Respir Crit Care Med. 2021;203(2):168–74.
13. Auld SC, Kasmar AG, Dowdy DW, Mathema B, Gandhi NR, Churchyard GJ, et al. Research Roadmap for Tuberculosis Transmission Science: Where Do We Go From Here and How Will We Know When We're There? J Infect Dis. 2017;216(suppl_6):S662–8.
14. Wang MS, Li-Hunnam J, Chen YL, Gilmour B, Alene KA, Zhang YA, et al. Conversion or Reversion of Interferon γ Release Assays for Mycobacterium tuberculosis Infection: A Systematic Review and Meta-analysis. Clin Infect Dis. 2025;80(1):168–79.
15. Yamasue M, Komiya K, Usagawa Y, Umeki K, Nureki S ichi, Ando M, et al. Factors associated with false negative interferon-γ release assay results in patients with tuberculosis: A systematic review with meta-analysis. Sci Rep. 2020;10(1):1607.
16. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. Nat Rev Immunol. 2015;15(2):87–103.

17. Lu LL, Smith MT, Yu KKQ, Luedemann C, Suscovich TJ, Grace PS, et al. IFN-γ-independent immune markers of Mycobacterium tuberculosis exposure. Nat Med. 2019;25(6):977–87.
18. Domingo-Gonzalez R, Prince O, Cooper A, Khader S. Cytokines and Chemokines in Mycobacterium tuberculosis infection. Microbiol Spectr. 2016;4(5):10.1128/microbiolspec.TBTB2-0018-2016.
19. Sakai S, Kauffman KD, Sallin MA, Sharpe AH, Young HA, Ganusov VV, et al. CD4 T Cell-Derived IFN-γ Plays a Minimal Role in Control of Pulmonary Mycobacterium tuberculosis Infection and Must Be Actively Repressed by PD-1 to Prevent Lethal Disease. PLOS Pathog. 2016;12(5):e1005667.
20. Lyu J, Narum DE, Baldwin SL, Larsen SE, Bai X, Griffith DE, et al. Understanding the development of tuberculous granulomas: insights into host protection and pathogenesis, a review in humans and animals. Front Immunol [Internet]. 2024 Dec 9 [cited 2025 Aug 26];15. Available from: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2024.1427559/full
21. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. The Lancet. 2000;356(9235):1099–104.
22. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, et al. Gamma Interferon Release Assays for Detection of Mycobacterium tuberculosis Infection. Clin Microbiol Rev. 2014;27(1):3–20.
23. Dale KD, Schwalb A, Coussens AK, Gibney KB, Abboud AJ, Watts K, et al. Overlooked, dismissed, and downplayed: reversion of Mycobacterium tuberculosis immunoreactivity. Eur Respir Rev [Internet]. 2024 Jul 24 [cited 2025 Aug 26];33(173). Available from: https://publications.ersnet.org/content/errev/33/173/240007
24. Okpokoro E, Lawal T, Oyedele O, Etuk V, Ijioma S, Adetiba T, et al. Prevalence, perception of risk and mycobacterium tuberculosis control practices among healthcare workers in HIV care and treatment centres in North Central Nigeria. BMC Infect Dis. 2025;25(1):220.
25. Okpokoro E, Lesosky M, Osa-Afiana C, Bada F, Okwor U, Odonye G, et al. Prevalence and Risk Factors for Mycobacterium tuberculosis Infection among Health Workers in HIV Treatment Centers in North Central, Nigeria. 2023 Jul 5 [cited 2025 Aug 26]; Available from: https://www.ajtmh.org/view/journals/tpmd/109/1/article-p60.xml
26. Obeng-Aboagye E, Frimpong A, Amponsah JA, Danso SE, Owusu EDA, Ofori MF. Inflammatory cytokines as potential biomarkers for early diagnosis of severe malaria in children in Ghana. Malar J. 2023;22(1):220.
27. Numis AL, Fox CH, Lowenstein DJ, Norris PJ, Di Germanio C. Comparison of multiplex cytokine assays in a pediatric cohort with epilepsy. Heliyon. 2021;7(3):e06445.
28. MyAssays - Data Analysis Tools and Services for Bioassays [Internet]. [cited 2025 Aug 26]. Available from: https://www.myassays.com/
29. Statistical software for data science | Stata [Internet]. [cited 2025 Aug 26]. Available from: https://www.stata.com/
30. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013;310(20):2191–4.
31. Karasneh RA, Al-Azzam SI, Alzoubi KH, Hawamdeh SS, Muflih SM. Patient Data Sharing and Confidentiality Practices of Researchers in Jordan. Risk Manag Healthc Policy. 2019;12:255–63.
32. The End TB Strategy [Internet]. [cited 2025 May 26]. Available from: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/the-end-tb-strategy
33. Leo S, Narasimhan M, Rathinam S, Banerjee A. Biomarkers in diagnosing and therapeutic monitoring of tuberculosis: a review. Ann Med. 2024;56(1):2386030.

34. Zein RA, Wulandari RD, Ridlo IA, Hendriani W, Suhariadi F, Rianto A. The characteristics of occupational tuberculosis risk in healthcare workers. Int J Health Plann Manage. 2022;37(5):2669–83.
35. Joshi R, Reingold AL, Menzies D, Pai M. Tuberculosis among Health-Care Workers in Low- and Middle-Income Countries: A Systematic Review. PLOS Med. 2006;3(12):e494.
36. Westhuizen HM van der, Dorward J, Roberts N, Greenhalgh T, Ehrlich R, Butler CC, et al. Health worker experiences of implementing TB infection prevention and control: A qualitative evidence synthesis to inform implementation recommendations. PLOS Glob Public Health. 2022;2(7):e0000292.
37. Kapwata T, Breetzke G, Wright CY, Marcus TS, Eales O. Demographic and socio-economic risk factors associated with self-reported TB. Int J Tuberc Lung Dis. 2022;26(1):33–7.
38. Uden L, Barber E, Ford N, Cooke GS. Risk of Tuberculosis Infection and Disease for Health Care Workers: An Updated Meta-Analysis. Open Forum Infect Dis. 2017;4(3):ofx137.
39. Xiong W, Dong H, Wang J, Zou X, Wen Q, Luo W, et al. Analysis of Plasma Cytokine and Chemokine Profiles in Patients with and without Tuberculosis by Liquid Array-Based Multiplexed Immunoassays. PloS One. 2016;11(2):e0148885.
40. Mensah GI, Amponsah JA, Alahaman NB, Anim-Baidoo I, Tetteh JKA, Addo KK, et al. Performance of eight serum cytokine/chemokine biomarkers in discriminating between active and latent tuberculosis infection in Ghana. Front Immunol [Internet]. 2025 Jun 6 [cited 2026 Feb 12];16. Available from: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2025.1600712/full
41. Sampath P, Rajamanickam A, Thiruvengadam K, Natarajan AP, Hissar S, Dhanapal M, et al. Cytokine upsurge among drug-resistant tuberculosis endorse the signatures of hyper inflammation and disease severity. Sci Rep. 2023;13(1):785.
42. La Manna MP, Orlando V, Li Donni P, Sireci G, Di Carlo P, Cascio A, et al. Identification of plasma biomarkers for discrimination between tuberculosis infection/disease and pulmonary non tuberculosis disease. PloS One. 2018;13(3):e0192664.
43. Le Hiress M, Tu L, Ricard N, Phan C, Thuillet R, Fadel E, et al. Proinflammatory Signature of the Dysfunctional Endothelium in Pulmonary Hypertension. Role of the Macrophage Migration Inhibitory Factor/CD74 Complex. Am J Respir Crit Care Med. 2015;192(8):983–97.
44. Ngobeni R, Abhyankar MM, Jiang NM, Farr LA, Samie A, Haque R, et al. Entamoeba histolytica-Encoded Homolog of Macrophage Migration Inhibitory Factor Contributes to Mucosal Inflammation during Amebic Colitis. J Infect Dis. 2017;215(8):1294–302.
45. Wang Q, Han W, Niu J, Sun B, Dong W, Li G. Prognostic value of serum macrophage migration inhibitory factor levels in pulmonary tuberculosis. Respir Res. 2019;20(1):50.
46. Kelsey A, Chirch LM, Payette MJ. Tuberculosis and interleukin blocking monoclonal antibodies: Is there risk? Dermatol Online J [Internet]. 2018 [cited 2025 Aug 26];24(9). Available from: https://escholarship.org/uc/item/58j4n38m
47. Abebe F. Synergy between Th1 and Th2 responses during Mycobacterium tuberculosis infection: A review of current understanding. Int Rev Immunol. 2019;38(4):172–9.
48. Calvo S, Rodrigo-Muñoz JM, Tarancón R, Uranga S, Martín C, Pozo V del, et al. Correlation between systemic allergen desensitisation and long-term asthma protection in mice following intravenous administration of the live tuberculosis vaccine MTBVAC. eBioMedicine. 2024;107:105272.
49. Tan Y, Tan Y, Li J, Hu P, Guan P, Kuang H, et al. Combined IFN-γ and IL-2 release assay for detect active pulmonary tuberculosis: a prospective multicentre diagnostic study in China. J Transl Med. 2021;19(1):289.

50. Wang S, Diao N, Lu C, Wu J, Gao Y, Chen J, et al. Evaluation of the Diagnostic Potential of IP-10 and IL-2 as Biomarkers for the Diagnosis of Active and Latent Tuberculosis in a BCG-Vaccinated Population. PLOS ONE. 2012;7(12):e51338.
51. Jayaraman P, Sada-Ovalle I, Nishimura T, Anderson AC, Kuchroo VK, Remold HG, et al. IL-1β Promotes Antimicrobial Immunity in Macrophages by Regulating TNFR Signaling and caspase-3 activation. J Immunol Baltim Md 1950. 2013;190(8):4196–204.
52. Mayer-Barber KD, Andrade BB, Barber DL, Hieny S, Feng CG, Caspar P, et al. Innate and Adaptive Interferons Suppress IL-1α and IL-1β Production by Distinct Pulmonary Myeloid Subsets during Mycobacterium tuberculosis Infection. Immunity. 2011;35(6):1023–34.
53. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25(12):677–86.
54. Lundahl ML, Mitermite M, Ryan DG, Case S, Williams NC, Yang M, et al. Macrophage innate training induced by IL-4 and IL-13 activation enhances OXPHOS driven anti-mycobacterial responses. Yona S, Rath S, editors. eLife. 2022;11:e74690.