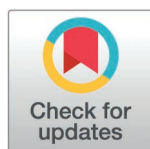RESEARCH ARTICLE

Resistance to tuberculin skin test/interferon-gamma release assay conversion among highly TB exposed, HIV infected goldminers in South Africa

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Abstract

Background

A small proportion of goldminers in South Africa resist tuberculin skin test (TST)/ interferon-gamma release assay (IGRA) conversion despite high rates of HIV and prolonged exposure to TB. We conducted a study among HIV-infected goldminers to determine the: i) proportion who resisted TST/IGRA conversion and ii) epidemiological factors associated with resistance to TST/IGRA conversion.

Methods

We enrolled HIV-infected goldminers who were on antiretroviral treatment, aged 33–60 years, with ≥15 years' service, no prior or current TB, no silicosis, and with body mass index >18.5 kg/m^2. TST/IGRA conversion was assessed at baseline, 6 months, and 12 months using TST and QuantiFERON-TB-Gold-Plus (QFT-Plus). Miners were considered resisters if they had a zero TST response and a negative QFT-Plus at all visits. Logistic regression was used to identify epidemiological factors associated with TST/IGRA conversion resistance.

Results

We enrolled 245 HIV-infected miners with median age of 48 years (interquartile-range [IQR]: 44–52 years) and median CD4 count, 506 cells/μL (IQR: 372–677 cells/μL). Overall, 98.4% (241) were males and 99.2% (243) were Black/African with a median

time of 24 years (IQR: 18–29 years) in the workforce. Of those completing all follow-ups, 24.3% (50/206) resisted TST/IGRA conversion. Miners who had a history of taking isoniazid preventive therapy (IPT) (adjusted odds ratio (aOR) 2.34; 95% confidence interval (CI): 1.14–4.80; p = 0.020) were more likely to resist TST/IGRA conversion. However, those from Mozambique (aOR 0.16; 95% CI: 0.04–0.71; p = 0.016) and those who had a CD4 count ≥500 cells/µL (aOR 0.46; 95% CI: 0.23–0.92; p = 0.028) were less likely to resist TST/IGRA conversion.

Conclusion

Similar to previous longitudinal cohort studies, we found a small proportion of HIV-infected goldminers who resisted TST/IGRA conversion. This was positively associated with prior IPT, but negatively associated with lower CD4 count and being from Mozambique. However, mechanisms underlying TST/IGRA conversion resistance are not well understood.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is a second leading cause of mortality worldwide from a single infectious agent [1]. Although the true global burden of *Mtb* infection is not well known, it was estimated around 23% in 2014 [2]. In Sub-Saharan Africa, the TB epidemic is further exacerbated by HIV infection. Goldmines are considered to be one of the major transmission hotspots for TB with an estimated incidence of TB disease of 3% [3]. In addition, the annual risk for *Mtb* infection in goldmines was estimated at 20% in a recent mathematical modeling study [3] Compared to general population, this estimate is five times higher.. TB prevalence was estimated at 2,500–3,000 cases per 100,000 within the workforce in South African mines in 2017 [4,5]. In the same period, TB incidence was estimated to be 10 times higher than the WHO threshold for a health emergency, and three times higher than the incidence in general population [4,5].

Prolonged, repeated exposure and infectiousness of TB index patient are known to increase the risk of becoming infected with *Mtb* [6]. In the absence of TB preventive therapy post infection, approximately 5–10% of the immunocompetent and ≥40% of immunocompromised persons may progress to clinical disease in their lifetime or in the first several years, respectively [7–9]. Not all persons exposed to *Mtb* become infected. This suggests that some individuals may either resist *Mtb* infection or rapidly clear it through innate immune responses [7,10–12], as evidenced by a persistently negative tuberculin skin test (TST) or interferon-gamma release assay (IGRA) despite prolonged exposure to TB in longitudinal cohorts of household contacts of persons with active pulmonary TB disease in Uganda [13–15], India [16], Indonesia [17], and South African goldmines [18–21]. In addition, a recent mathematical modelling study has estimated a 93% likelihood of having a resister phenotype in goldminers who are persistently uninfected with *Mtb* despite repeated exposure to infectious TB cases over extended period of time [6].

Earlier evidence from studies conducted in goldmines suggests that the mining population could provide a unique opportunity to identify individuals that have been exposed to TB repeatedly over decades yet remain uninfected, to gain insight into biomarkers and mechanisms of resistance to *Mtb* infection [20]. It is hypothesised that the true proportion of *Mtb* resisters among HIV-infected non anergic people may be high since HIV increases the risk of progression to active TB disease [6,20]. In addition, a monocyte transcriptional response study conducted among household contact in Uganda found that HIV-infected *Mtb* resisters had a different gene expression profile in response to *Mtb* infection compared with *Mtb* infected and HIV negative *Mtb* resisters [22]. However, it may be important to study this in different settings and populations as the effect of HIV on an individual's ability to resist developing *Mtb* infection would be particularly important given its prominence in many high-burden TB settings.

Studies previously conducted in the mines, did not determine risk factors for *Mtb* infection in HIV-infected individuals [19]. Most recently, a small proportion of HIV negative goldminers who appeared to resist *Mtb* infection were identified in South Africa, however no clear distinguishing epidemiological characteristics were identified [20]. Similarly, no epidemiological factors were associated with the resister phenotype in household contacts [16,23,24]. Therefore, studying HIV-infected individuals to determine epidemiological factors associated with remaining *Mtb* uninfected despite being highly exposed remains a priority. We conducted a study among HIV-infected goldminers to determine: i) the proportion who were resistant to *Mtb* infection and ii) epidemiological factors associated with resistance to *Mtb* infection.

Materials and methods

Study setting and population

The study was conducted in three South African goldmines: two in Orkney, North-West Province from 10th of July 2017–31st of September 2019 and one in Carletonville, Gauteng Province from 17th of October 2018–31st of September 2019. However, twelve-month follow-up visits were stopped on the 23rd of March 2020 in all sites due to COVID-19 lockdown. The target population was HIV-infected goldminers on antiretroviral treatment (ART) for ≥ 3 months, who worked for ≥ 15 years in goldmines but with no prior history of TB disease nor evidence of silicosis [20]. In addition, study participants were included in the study only if they were aged 33–60 years and had body mass index (BMI) >18.5 kg/m^2 [20]. This eligibility criteria were designed to maximise recruiting miners that had a resister phenotype.

Study design and procedures – main study

In a cohort study, herein described as the main study, we enrolled HIV-infected miners who were on ART. However, we did not collect data on ART regimen and history of default or treatment failure. To identify individuals who had high cumulative exposure to TB and yet remain resistant to *Mtb* infection, we screened potential participants for eligibility in two phases as described below. High cumulative exposure in our context was based on proxies of age, and years in the workforce; no TB disease; or not being at higher risk of TB disease. This is also based on the assumption of the high force of infection reported in Vynnycky et., al [3]. Cumulative exposure also refers to individuals who have worked in mining settings of high force of *Mtb* infection coupled with exposure to silica dust underground.

Pre-screening phase

Goldminers, attending wellness clinics within the mining hospital and/or medical stations for their routine HIV care between July 2017 and September 2019, were invited to join the study. During pre-screening, we ascertained age and years working in the mining industry while miners were waiting to see a clinician for their routine HIV care. Individuals identified as having worked for prolonged periods in the mining industry (at least 15 years) and who were at least 33 years of age were consented [20].

Full screening phase

Following an informed consenting process, a full screen was conducted among all those who met the pre-screening requirements and individuals who did not meet an earlier stage were excluded. A questionnaire was administered to collect information on medical history, including prior and current treatment for TB disease and *Mtb* infection. In addition, a symptom screen for active TB disease was performed using the WHO screening tool comprising 1) current cough of any duration; 2) fever; 3) night sweats; or 4) unintended weight loss. The most recent chest radiograph (≤3 months at a time of recruitment by study team) taken as part of routine annual examination at the Occupational Health Centre were reviewed for evidence of old or active TB and silicosis. Miners were enrolled into the study if 1) they did not report symptoms suggestive of TB; 2) no prior or current history of treatment for active TB disease was documented; 3) no evidence of silicosis documented; 4) BMI > 18.5 kg/m^2; and 5) no serious or chronic medical conditions causing severe immunodeficiency. Miners also had to be on ART for ≥ 3 months with a CD4 ≥ 200 cells/μL but not current taking treatment for cancer, no treatment with steroid tablets, inhalers, or injections. The following procedures were conducted after full screening was completed among all miners who met the inclusion criteria.

Clinical and lab procedures: Evaluation of *Mtb* infection using TST and QFT-Plus

At enrolment, participants gave blood samples for QFT-Plus and had a TST placed (RT-23 in Tween-80, Statens Serum Institute, Copenhagen, Denmark, catalogue number: XI1177KL and SP18411C) by trained professional nurses and read after 48–72 hours. The TST response was measured using a digital calliper as the maximum transverse diameter of the induration expressed in millimetres. Blood samples were collected from those who met the inclusion criteria for QFT-Plus (Qiagen, Hilden Germany) by phlebotomy trained professional nurses. All participants with a negative QFT-Plus (according to manufacturer's instruction) at enrolment, were followed up at 6 months and 12 months after baseline. In both follow-up visits QFT-Plus and TST testing were repeated.

Study design – sub-analysis and definitions

We determined the proportion of miners with *Mtb* resistance and explored epidemiological factors associated with *Mtb* resistance using measurements from baseline, 6 months, and 12 months follow-up. *Mtb* infection status was confirmed using TST and QFT-Plus, and a questionnaire was administered to collect information on factors associated with being *Mtb* resister. QFT-Plus was considered negative if both antigen tubes (TB1 and B2) were <0.35 IU·ml^{-1}. Miners were considered *Mtb* resisters if they had a zero TST (induration = 0mm) and negative QFT-Plus (response <0.35 IU·ml^{-1} on both TB1 and TB2 antigen tubes) at all three time points. Lost to follow up was defined as any miner who consented to participate or participated in this study at one point in time but was lost or could not be contacted to complete the outstanding study procedures on subsequent follow-ups.

Study outcome

The study outcome was the proportion of participants who had a zero TST (induration = 0mm) AND negative QFT-Plus (response <0.35 IU·ml^{-1} on both TB1 and TB2 antigen tubes) at baseline, 6 months, and 12 months follow-up visits, herein referred to as *Mtb* resister. Participants with a positive TST or QFT at any time point were the comparator group.

Data management

All data including participant identifiers, enrolment and follow-up questionnaires were collected using an electronic data capture system (IBM eClinical).

Statistical methods

Demographic characteristics were described using frequencies (n) and percentages (%), and Chi-square (χ^2) test or two-sided Fisher's exact test was used to compare categorical variables. For statistical comparisons, a $p \leq 0.05$ was considered significant. The univariable and multivariable logistic regression models were used to determine predictors or factors associated with a study outcome. Predictors with $p < 0.20$ in the univariable logistic regression model were introduced into the multivariable model. Manual forward and backward stepwise procedure were used to select variables for the multivariable model. A cut-off $p < 0.05$ was used to retain variables in the final multivariable model. Results were summarized using odds ratios (OR) and adjusted odds ratios (aOR) with their corresponding 95% confidence intervals (CI) and p-values. Data was analysed using Stata version 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP).

Ethics statement

The study received ethical clearance from the University of Witwatersrand Human Research Ethics Committee (WHREC Ref: 170110), University of Washington (IRB#: STUDY00001537), North West Health Research and Ethics Committee (NW_2017RP26_755), and Gauteng Health Research and Ethics Committee (GP_201804_024). Permission was also sought from internal stakeholders within the mining companies where the study was conducted. Prior to conducting study procedures, we sought informed consent from all study participants using written informed consent and information sheet available in the commonly used local languages. An impartial witness was used to witness the verbal consent for illiterate participants. A thumb print was used to document or "sign" a verbal consent.

Inclusivity in global research

Additional information regarding the ethical, cultural, and scientific considerations specific to inclusivity in global research is included in the Supporting Information (S1 File).
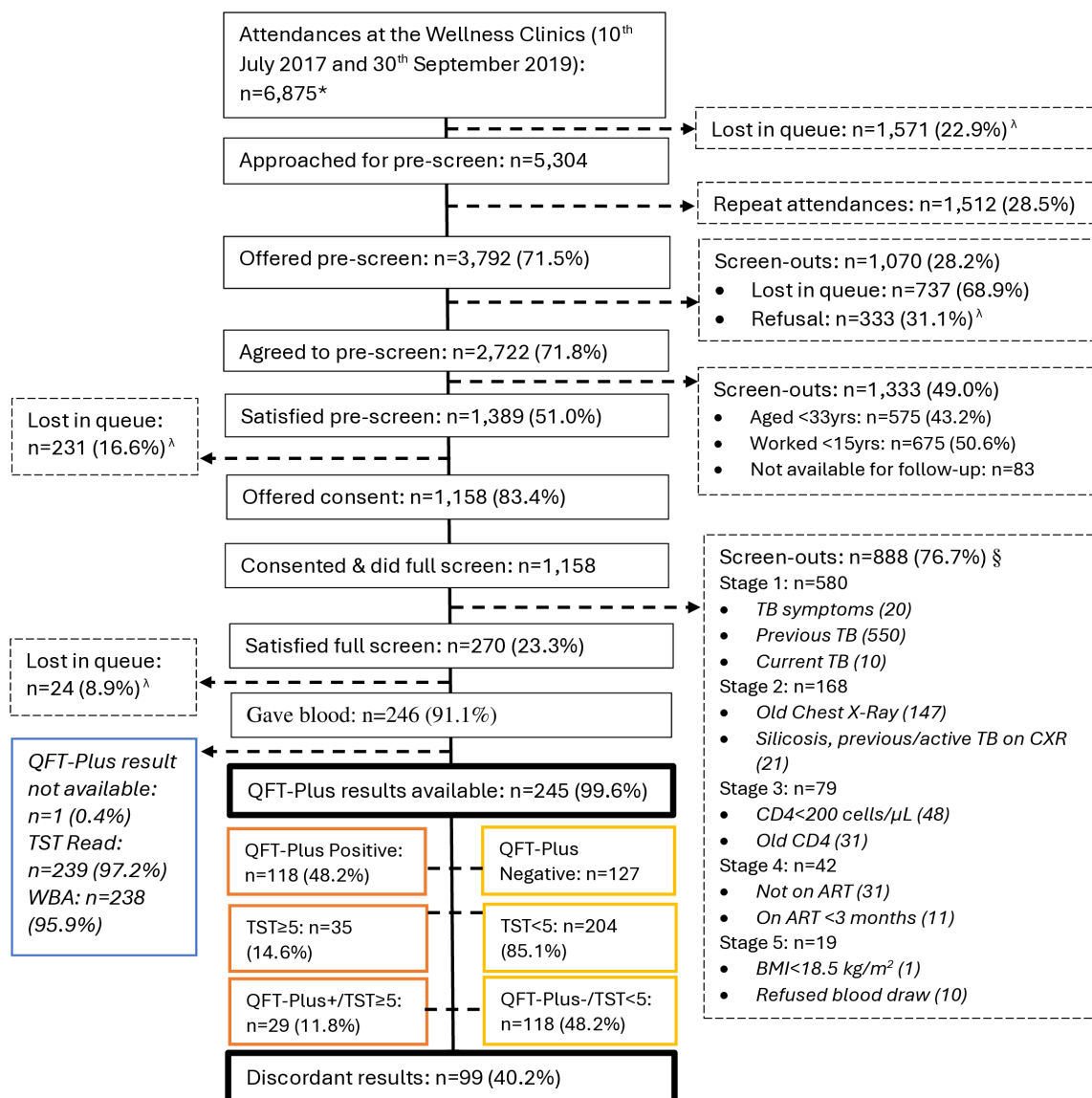
Results

Participants screening and enrolment

Between the 10th of July 2017 and 30th of September 2019, a total of 6,875 visits were recorded at the wellness clinics from a mining hospital and medical stations (Fig 1). We approached 5,304 miners (77.1%), 2,722 (71.8%) were pre-screened and remainder were either lost in queue or declined to take part in the study. Of those who were pre-screened, 1,389 (51.0%) were eligible for full screening, of whom 1,158 (83.4%) consented and completed full screening (Fig 1).

   Among the consented participants who completed full screening, 270 (23.3%) met the inclusion criteria; 888 (76.7%) were screened out. Of those meeting the inclusion criteria, 246 (91.1%) were enrolled and 24 (8.9%) were either lost in queue or lost to follow up. In total, 246 participants were enrolled, all gave blood for QFT-Plus testing, and TST was placed. Of the 246 enrolled miners, 245 (99.6%) had baseline QFT-Plus results and 239 (97.2%) had TST results (Fig 1). Fig 2 shows the interferon gamma response and TST induration by CD4 count.

Demographic characteristics of participants

In this analysis, we included 245 participants with the median age of 48 years (interquartile-range [IQR]: 44–52 years) and 98.4% (241) were male. The median time in the workforce was 24 years (IQR: 18–29 years), median CD4 count was 506 cells/µL (IQR: 372–677 cells/µL) (Table 1). Overall, 99.2% (243) were of Black/African ethnicity, 74.3% (179) had a Bacillus Calmette Guerin (BCG) vaccination scar present, 46.9% (115) were born in South Africa and half (50.6%, 124) were staying in hostels (Table 1).

Fig 1. Participants flow chart at enrolment among miners attending HIV wellness clinic in South African gold mines.

https://doi.org/10.1371/journal.pone.0318819.g001

The flow chart boxes contain:

- Attendances at the Wellness Clinics (10th July 2017 and 30th September 2019): n=6,875*
- Lost in queue: n=1,571 (22.9%)λ
- Approached for pre-screen: n=5,304
- Repeat attendances: n=1,512 (28.5%)
- Offered pre-screen: n=3,792 (71.5%)
- Screen-outs: n=1,070 (28.2%)
  - Lost in queue: n=737 (68.9%)
  - Refusal: n=333 (31.1%)λ
- Agreed to pre-screen: n=2,722 (71.8%)
- Screen-outs: n=1,333 (49.0%)
  - Aged <33yrs: n=575 (43.2%)
  - Worked <15yrs: n=675 (50.6%)
  - Not available for follow-up: n=83
- Satisfied pre-screen: n=1,389 (51.0%)
- Lost in queue: n=231 (16.6%)λ
- Offered consent: n=1,158 (83.4%)
- Consented & did full screen: n=1,158
- Screen-outs: n=888 (76.7%) §
  - Stage 1: n=580
    - TB symptoms (20)
    - Previous TB (550)
    - Current TB (10)
  - Stage 2: n=168
    - Old Chest X-Ray (147)
    - Silicosis, previous/active TB on CXR (21)
  - Stage 3: n=79
    - CD4<200 cells/µL (48)
    - Old CD4 (31)
  - Stage 4: n=42
    - Not on ART (31)
    - On ART <3 months (11)
  - Stage 5: n=19
    - BMI<18.5 kg/m² (1)
    - Refused blood draw (10)
- Satisfied full screen: n=270 (23.3%)
- Lost in queue: n=24 (8.9%)λ
- Gave blood: n=246 (91.1%)
- QFT-Plus result not available: n=1 (0.4%). TST Read: n=239 (97.2%). WBA: n=238 (95.9%)
- QFT-Plus results available: n=245 (99.6%)
- QFT-Plus Positive: n=118 (48.2%)
- QFT-Plus Negative: n=127
- TST≥5: n=35 (14.6%)
- TST<5: n=204 (85.1%)
- QFT-Plus+/TST≥5: n=29 (11.8%)
- QFT-Plus-/TST<5: n=118 (48.2%)
- Discordant results: n=99 (40.2%)

* Data from the daily attendance register. Includes repeat attendances during this time period.

λ Potentially eligible participants were not seen/missed by our study staff for various reasons and were considered as having been "lost in queue".

§ Screen out questions were asked in stages; if screened out at a stage no screening was conducted for subsequent stages. Within a stage more than one screen-out reason may apply.

ART, antiretroviral therapy; BMI, body mass index; CXR, Chest X-Ray; QFT-Plus, QuantiFERON-TB Gold-Plus; TST, tuberculin skin test; WBA, whole blood assay; Yrs, years

Mtb resister phenotype

Of those who had QFT-Plus results at baseline, 6 months follow-up, and 12 months follow-up, 52.8% (127/245), 85.4% (88/103), and 85.2% (75/88) were QFT-Plus negative, respectively (Fig 3). On the other hand, the proportion of those with a zero TST response and a negative QFT-Plus result was 46.5% (114/245) at baseline, 78.6% (81/103) at 6 months
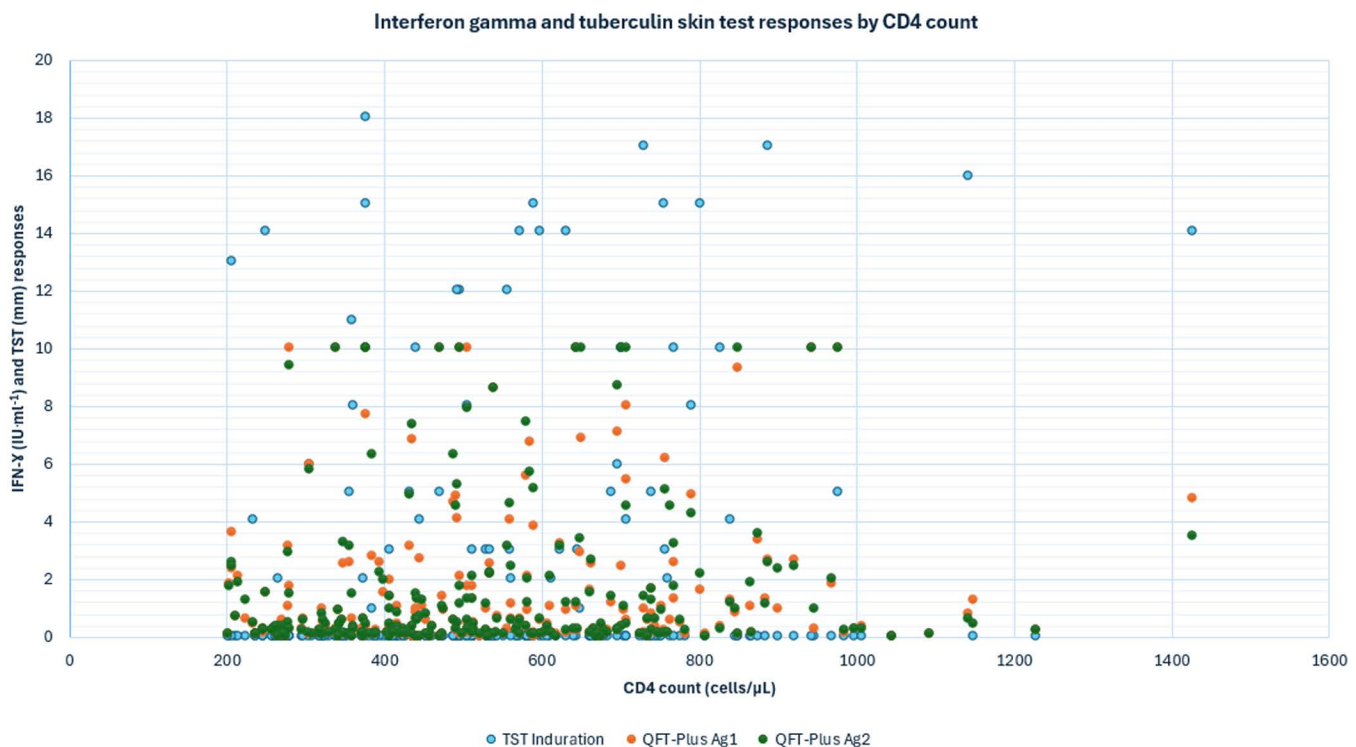
Fig 2. Interferon gamma response and tuberculin skin test responses by CD4 count among miners attending HIV wellness clinic in South African gold mines.

https://doi.org/10.1371/journal.pone.0318819.g002

follow-up, and 61.4% (54/75) at 12 months follow-up. Of the 54 participants that had a zero TST response and a negative QFT-Plus result at 12 months follow-up, 1 had TST>0mm at baseline while 3 had TST>0mm at 6 months follow-up. As a results, the 4 participants were not considered as *Mtb* resister in the final analysis. **Therefore, the overall proportion of miners who met the definition for *Mtb* resistance was 24.3% (50/206).**

Epidemiological factors associated with *Mtb* resistance

On univariable analysis, being *Mtb* resister was positively associated with a prior history of taking isoniazid preventive therapy (IPT) (OR 2.54; 95% CI: 1.33–4.83; p=0.005), but negatively associated with being from Mozambique (OR 0.23; 95% CI: 0.06–0.91; p=0.036) and having a CD4 count of ≥500 cells/µL (OR 0.43; 95% CI: 0.23–0.83; p=0.011) (Table 2). On multivariable analysis, miners who had a prior history of taking IPT (aOR 2.34; 95% CI: 1.14–4.80; p=0.020) remained two times more likely to resist *Mtb* infection. On the other hand, miners from Mozambique (aOR 0.16; 95% CI: 0.04–0.71; p=0.016) and those who had a CD4 count ≥500 cells/µL (aOR 0.46; 95% CI: 0.23–0.92; p=0.028) remained less likely to resist *Mtb* infection (Table 2).

Discussion

We found a small proportion of HIV-infected goldminers on ART who were *Mtb* resisters. Prior history of taking IPT was associated with a greater likelihood of having *Mtb* resister phenotype. In contrast, coming from Mozambique and having a CD4 count ≥500 cells/µL were associated with a lower likelihood of having a resister phenotype. Findings from this study contributes to existing scientific literature which suggests that individuals with *Mtb* resister phenotype do exist irrespective of HIV status, intensity and duration of exposure to TB [20].

Table 1. Baseline demographic characteristics of miners attending HIV wellness clinic in South African gold mines.

Variable (N = 245)		*Resister phenotype status		N (%)
		Mtb resisters – n (%)	Non-resisters – n (%)	
		50 (20.4%)	195 (79.6%)	245 (100.0%)
Employment duration				
	Median (IQR)	23.5 (17–29)	24 (19–29)	24 (18–29)
Years underground				
	Median (IQR)	21 (16–29)	24 (18–28)	23 (17–28)
Years ART				
	Median (IQR)	4 (2–9)	4 (3–8)	4 (2–8)
CD4 Count				
	Median (IQR)	418 (332–553)	530 (377–704)	506 (372–677)
Age, years				
	Median (IQR)	48 (44–51)	48 (44–52)	48 (44–52)
Age group, years				
	<45	15 (21.1%)	56 (78.9%)	71 (29.0%)
	45-49	17 (23.3%)	56 (76.7%)	73 (29.8%)
	≥50	18 (17.8%)	83 (82.2%)	101 (41.2%)
Gender				
	Female	2 (50.0%)	2 (50.0%)	4 (1.6%)
	Male	48 (19.9%)	193 (80.1%)	241 (98.4%)
Ethnicity				
	Other	0 (0.0%)	2 (100.0%)	2 (0.8%)
	Black/African	50 (20.6%)	193 (79.4%)	243 (99.2%)
Country of origin				
	Other	7 (30.4%)	16 (69.6%)	23 (9.4%)
	Mozambique	4 (9.3%)	39 (90.7%)	43 (17.6%)
	Lesotho	9 (14.1%)	55 (85.9%)	64 (26.1%)
	South Africa	30 (26.1%)	85 (73.9%)	115 (46.9%)
Marital status				
	Not married	10 (26.3%)	28 (73.7%)	38 (15.5%)
	Married	40 (19.3%)	167 (80.7%)	207 (84.5%)
Occupation				
	Skilled	9 (13.9%)	56 (86.2%)	65 (26.5%)
	Unskilled	41 (22.8%)	139 (77.2%)	180 (73.5%)
Live in hostel				
	No	29 (24.0%)	92 (76.0%)	121 (49.4%)
	Yes	21 (16.9%)	103 (83.1%)	124 (50.6%)
Sleeping arrangement				
	Alone	16 (20.5%)	62 (79.5%)	78 (31.8%)
	1 person	29 (19.7%)	118 (80.3%)	147 (60.0%)
	> 1 person	5 (25.0%)	15 (75.0%)	20 (8.2%)
BCG scar				
	No	14 (22.6%)	48 (77.4%)	62 (25.7%)
	Yes	35 (19.6%)	144 (80.4%)	179 (74.3%)
BMI				
	≤24	17 (18.5%)	75 (81.5%)	92 (37.6%)
	25-29	22 (20.8%)	84 (79.2%)	106 (43.3%)

(Continued)

Table 1. (Continued)

Variable (N = 245)		*Resister phenotype status		N (%)
		Mtb resisters – n (%)	Non-resisters – n (%)	
		50 (20.4%)	195 (79.6%)	245 (100.0%)
	≥30	11 (23.4%)	36 (76.6%)	47 (19.2%)
Currently working underground				
	No	7 (35.0%)	13 (65.0%)	20 (8.2%)
	Yes	43 (19.1%)	182 (80.9%)	225 (91.8%)
CD4 Count				
	<500	33 (27.1%)	89 (72.9%)	122 (49.8%)
	≥500	17 (13.8%)	106 (86.2%)	123 (50.2%)
Years on ART				
	<5	28 (22.2%)	98 (77.8%)	126 (51.4%)
	≥5	22 (18.5%)	97 (81.5%)	119 (48.6%)
IPT ever				
	No	27 (15.6%)	146 (84.4%)	173 (70.6%)
	Yes	23 (31.9%)	49 (68.1%)	72 (29.4%)
Currently on IPT				
	No	44 (19.4%)	183 (80.6%)	227 (92.7%)
	Yes	6 (33.3%)	12 (66.7%)	18 (7.3%)

ART, antiretroviral therapy; BCG, Bacillus Calmette Guerin; BMI, body mass index; CD4, Clusters of differentiation 4; IPT, isoniazid preventive therapy; IQR, interquartile-range; QFT-Plus, QuantiFERON-TB Gold-Plus; TST, tuberculin skin test

*Mtb resister phenotype, defined as having a QFT-Plus Negative/TST = 0mm results at baseline, 6 & 12 months follow up.

The proportion of *Mtb* resister phenotype reported in in this study (24.3%) was within a similar range (0–35%) reported in previous study [18,20]. However, it was almost two times higher than the proportion (10.6%) reported among HIV-negative goldminers from the similar workforce [20]. The *Mtb* resister proportion reported in this study was also much higher than the *Mtb* resister proportion reported in several household contact cohorts with different force of infection profiles [13,16,24]. For instance, a multi-country study conducted among household contacts of multi-drug resistant TB cases in Botswana, Brazil, Haiti, India, Kenya, Peru, South Africa, and Thailand, reported an *Mtb* resisters proportion of 9.9% [24]. Household contact studies conducted in Uganda (11.7%) and India (6.5%), reported even lower proportion of resisters [13,16]. However, some of these studies were cross-sectional studies and did not analyse *Mtb* resister phenotypes by HIV status with some only using TST to determine *Mtb* infection status and varying TST cut-offs. It therefore remains unclear how much of a role did the HIV status, CD4 count and/or anergy play in the performance of QFT-Plus and TST in these studies. Previous studies conducted in HIV-infected cohorts found that the performance of TST and IGRA can be affected by immune status of a participant, resulting in underestimation of *Mtb* infection in this population [25–30]. Specifically, those with lower CD4 count and anergy are often unable to mount adequate immune response [31–34], with notable differences in median in CD4 count between groups [26,34]. This may result in misclassification of HIV-infected participants who are anergic as having a resister phenotype. Since we did not conduct any test for anergy, it remains unclear how much of a role HIV status and CD4 count play in the performance of QFT-Plus and TST.

In household contact studies, contacts had a well-define case source with a more precise measure of intensity and duration of exposure [3]. In contrast, age and period worked in goldmines were used as a proxy for prolonged and repeated exposure to *Mtb* in our study and we did not collect data on TB contact history. This was informed by a modelling study which suggested a greater likelihood of having a resister phenotype in miners who test negative on IGRA and TST despite working in goldmines for extended period [6]. However, it remains unclear whether the negative QFT-Plus
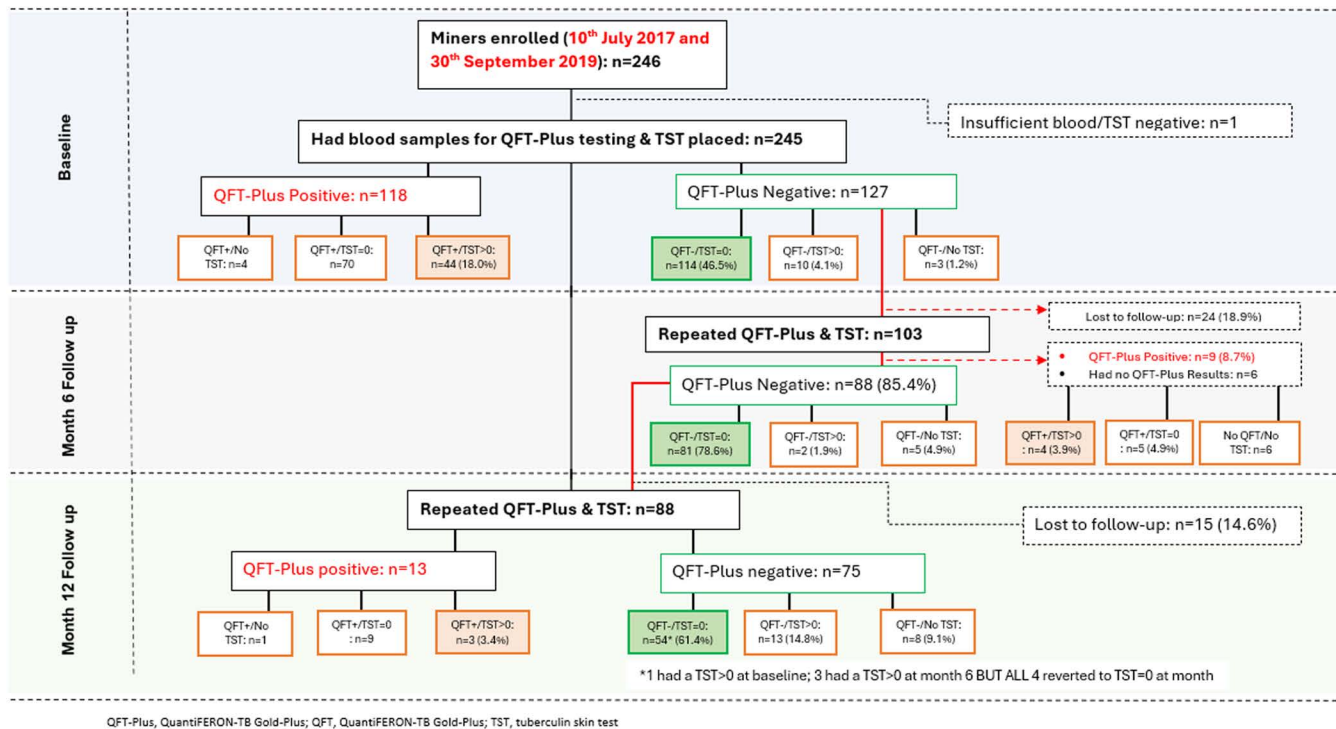
Fig 3. Participants flow chart showing phenotype stability at follow-up visit among miners attending HIV wellness clinic in South African gold mines.

https://doi.org/10.1371/journal.pone.0318819.g003

and/or TST in this study was due to non-exposure to *Mtb* bacilli or having *Mtb* resister phenotype. It also remains unclear whether the observed QFT-Plus and/or TST conversions, at 6- and 12-month follow-ups, may be attributed to most recent exposure rather than a prolonged exposure. In addition, a validated exposure risk score capturing cumulative exposure through indicators of exposure duration in mines does not exist. Further work is needed to determine and validate exposure risk scores for *Mtb* infection in mines.

Miners from Mozambique were less likely to have *Mtb* resister phenotype and this association was statistically significant. The association between *Mtb* resister phenotype and geographic location such as country of origin has never been reported in previous studies. However, it may suggest that settings with higher force of infection profiles, as demonstrated in previous studies [13,16,24], may have a lower proportion of *Mtb* resister phenotype.

Lastly, this study found that miners with a prior history of IPT were two times more likely to have *Mtb* resister phenotype. IPT is widely used as a prophylaxis for *Mtb* infection [35–37]. However, studies in diverse settings have reported TST or QFT-Plus reversion after IPT, particularly in persons with recent conversion and small reaction diameters [38,39]. Therefore, the observed association between *Mtb* resistance and taking IPT may be indicative of treatment efficacy rather than true resistance to *Mtb* infection.

Similar to previous studies, we did not find plausible epidemiological characteristics associated with *Mtb* resister phenotype. We hypothesize that *Mtb* resister phenotype can be explained by immunological and cellular mechanisms underlying the clinical phenomenon of resistance to *Mtb* infection. Therefore, further studies comparing cellular responses, innate immune responses and gene expression profiles are needed to provide some insight.

Table 2. Factors associated with Mtb resistance, defined as having a zero TST response and a negative QFT-Plus result at the end of follow-up period, among miners attending HIV wellness clinic in South African gold mines.

Variable (N = 245)		Mtb resisters		Univariable analysis			Multivariable analysis		
		N	%	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Age group, years									
	<45	15	21.1	1	Reference	Reference	1	Reference	Reference
	45-49	17	23.3	1.13	0.52 - 2.49	0.755	0.92	0.39 - 2.16	0.842
	≥50	18	17.8	0.81	0.38 - 1.74	0.588	0.68	0.29 - 1.57	0.362
Gender									
	Female	2	50.0	1	Reference	Reference	1	Reference	Reference
	Male	48	19.9	0.25	0.03 - 1.81	0.170	0.41	0.04 - 3.79	0.432
Country									
	Other	7	30.4	1	Reference	Reference	1	Reference	Reference
	Mozambique	4	9.3	0.23	0.06 - 0.91	0.036	0.16	0.04 - 0.71	0.016
	Lesotho	9	14.1	0.37	0.12 - 1.16	0.089	0.36	0.11 - 1.20	0.097
	South Africa	30	26.1	0.81	0.30 - 2.15	0.668	0.56	0.19 - 1.65	0.296
Marital status									
	Not married	10	26.3	1	Reference	Reference	–	–	–
	Married	40	19.3	0.67	0.30 - 1.49	0.328	–	–	–
Occupation									
	Skilled	9	13.9	1	Reference	Reference	1	Reference	Reference
	Unskilled	41	22.8	1.84	0.84 - 4.03	0.130	1.64	0.71 - 3.82	0.249
Live in hostel									
	No	29	24.0	1	Reference	Reference	1	Reference	Reference
	Yes	21	16.9	0.65	0.35 - 1.21	0.174	0.74	0.36 - 1.52	0.414
Sleeping arrangement									
	Alone	16	20.5	1	Reference	Reference	–	–	–
	1 person	29	19.7	0.95	0.48 - 1.89	0.889	–	–	–
	> 1 person	5	25.0	1.29	0.41 - 4.09	0.663	–	–	–
BCG scar									
	No	14	22.6	1	Reference	Reference	–	–	–
	Yes	35	19.6	0.83	0.41 - 1.68	0.610	–	–	–
BMI									
	≤24	17	18.5	1	Reference	Reference	–	–	–
	25-29	22	20.8	1.16	0.57 - 2.34	0.688	–	–	–
	≥30	11	23.4	1.35	0.57 - 3.17	0.494	–	–	–
Currently working underground									
	No	7	35.0	1	Reference	Reference	1	Reference	Reference
	Yes	43	19.1	0.44	0.17 - 1.17	0.098	0.41	0.13 - 1.27	0.121
CD4 Count									
	<500	33	27.1	1	Reference	Reference	1	Reference	Reference
	≥500	17	13.8	0.43	0.23 - 0.83	0.011	0.46	0.23 - 0.92	0.028
Years on ART									
	<5	28	22.2	1	Reference	Reference	–	–	–
	≥5	22	18.5	0.79	0.42 - 1.48	0.469	–	–	–
IPT ever									
	No	27	15.6	1	Reference	Reference	1	Reference	Reference

(Continued)

Table 2. (Continued)

Variable (N = 245)		*Mtb* resisters		Univariable analysis			Multivariable analysis		
		N	%	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
	Yes	23	31.9	2.54	1.33 - 4.83	0.005	2.34	1.14 - 4.80	0.020
Currently on IPT									
	No	44	19.4	1	Reference	Reference	1	Reference	Reference
	Yes	6	33.3	2.08	0.74 - 5.85	0.165	1.86	0.59 - 5.86	0.292

ART, antiretroviral therapy; BCG, Bacillus Calmette Guerin; BMI, body mass index; CD4, Clusters of differentiation 4; IPT, isoniazid preventive therapy; IQR, interquartile-range; QFT-Plus, QuantiFERON-TB Gold-Plus; TST, tuberculin skin test.

https://doi.org/10.1371/journal.pone.0318819.t002

Study limitations

Our study had some limitations. For instance, while it was mandatory for mines to conduct baseline and annual TB and silicosis surveillance through radiography screening, miners could still seek care outside the mining healthcare system. It is therefore possible that a very small proportion of miners who accessed care elsewhere (private and public clinics) were missed by our study team during recruitment and enrolment process. In addition, *Mtb* infection and *Mtb* resister phenotype were defined based on indirect measure of adaptive immune response to *Mtb* infection, similar to HIV negative cohort [20]. A negative TST, IGRA or non-conversion of these test may be due to inadequate exposure, involvement of innate immunity or other adaptive immune mechanisms other than interferon-gamma response [20].

Further work is needed to determine cellular immune functions associated with apparent resistance to *Mtb* infection. Potential cellular mechanisms underlying the clinical phenomenon of resistance to *Mtb* infection despite high exposure, were identified in household contacts in Uganda and among HIV negative gold miners. Having *Mtb* resister phenotype was associated with novel chromosomal loci in a genome scan study among household contacts in Uganda [15]. Further work is needed to determine genetic factors that may potentially play a key role in *Mtb* infection in this population group. Furthermore, individuals with *Mtb* resister phenotype had a distinct gene expression profiles in monocytes in a gene expression study from distinct Ugandan and South African cohorts [40,41]. Similar analysis on gene expression profiles in monocytes is planned in this population group to provide further insight.

Conclusions

We found a small proportion of HIV-infected goldminers who had a resister phenotype. However, we did not find plausible epidemiological characteristics associated with *Mtb* resister phenotype. Our results are consistent with the study among HIV negative miners but not similar to other *Mtb* resister studies conducted in household contacts.

Supporting information

S1 File. Inclusivity in global research.
(DOCX)

Acknowledgments

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References

1. WHO. Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO [Internet]. 2023 [cited 2024 Jan 7]. Available from: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023

2. Houben RMGJ, Dodd PJ. The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling. PLoS Med. 2016;13(10):e1002152. https://doi.org/10.1371/journal.pmed.1002152 PMID: 27780211

3. Vynnycky E, Sumner T, Fielding KL, Lewis JJ, Cox AP, Hayes RJ, et al. Tuberculosis control in South African gold mines: mathematical modeling of a trial of community-wide isoniazid preventive therapy. Am J Epidemiol. 2015;181(8):619–32. https://doi.org/10.1093/aje/kwu320 PMID: 25792607

4. World Bank. The Southern Africa TB in the Mining Sector Initiative. 2017 [cited 2023 Jan 25]. Available from: https://www.worldbank.org/en/programs/the-southern-africa-tb-in-the-mining-sector-initiative

5. WHO. Global tuberculosis report 2022. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO. [Internet]. 2022 [cited 2023 Jan 24]. Available from: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022

6. Wallis R. Mathematical Models of Tuberculosis Reactivation and Relapse. Front Microbiol [Internet]. 2016 May 17 [cited 2023 Jan 25];7. Available from: https://pubmed.ncbi.nlm.nih.gov/27242697/.

7. Barry CE, Boshoff H, Dartois V, Dick T, Ehrt S, Flynn J, et al. The spectrum of latent tuberculosis: rethinking the goals of prophylaxis. Nat Rev Microbiol. 2009 Dec;7(12):845–55.

8. Horsburgh CR Jr. Priorities for the treatment of latent tuberculosis infection in the United States. N Engl J Med. 2004;350(20):2060–7. https://doi.org/10.1056/NEJMsa031667 PMID: 15141044

9. Getahun H, Matteelli A, Chaisson RE, Raviglione M. Latent Mycobacterium tuberculosis Infection. N Engl J Med. 2015 May 28;372(22):2127–35.

10. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. Eur Respir J. 2013;41(1):140–56. https://doi.org/10.1183/09031936.00070812 PMID: 22936710

11. Emery JC, Richards AS, Dale KD, McQuaid CF, White RG, Denholm JT, et al. Self-clearance of Mycobacterium tuberculosis infection: implications for lifetime risk and population at-risk of tuberculosis disease. Proc R Soc B Biol Sci [Internet]. 2021 Jan 1 [cited 2023 Jan 24];288(1943). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7893269/.

12. Behr MA, Edelstein PH, Ramakrishnan L. Is Mycobacterium tuberculosis infection life long? BMJ. 2019 Oct 24;367:l5770.

13. Ma N, Zalwango S, Malone LL, Nsereko M, Wampande EM, Thiel BA, et al. Clinical and epidemiological characteristics of individuals resistant to M. tuberculosis infection in a longitudinal TB household contact study in Kampala, Uganda. BMC Infect Dis. 2014 Jun 27;14:352.

14. Stein CM, Nsereko M, Malone LL, Okware B, Kisingo H, Nalukwago S, et al. Long-term stability of resistance to latent M. tuberculosis infection in highly exposed TB household contacts in Kampala, Uganda. Clin Infect Dis Off Publ Infect Dis Soc Am. 2018 Aug 28.

15. Stein CM, Zalwango S, Malone LL, Won S, Mayanja-Kizza H, Mugerwa RD, et al. Genome scan of M. tuberculosis infection and disease in Ugandans. PLoS One. 2008;3(12):e4094. https://doi.org/10.1371/journal.pone.0004094 PMID: 19116662

16. Mave V, Chandrasekaran P, Chavan A, Shivakumar SVBY, Danasekaran K, Paradkar M, et al. Infection free "resisters" among household contacts of adult pulmonary tuberculosis. PLoS One. 2019;14(7):e0218034. https://doi.org/10.1371/journal.pone.0218034 PMID: 31318864

17. Verrall AJ, Alisjahbana B, Apriani L, Novianty N, Nurani AC, van Laarhoven A, et al. Early Clearance of Mycobacterium tuberculosis: The INFECT Case Contact Cohort Study in Indonesia. J Infect Dis. 2020 Mar 28;221(8):1351–60.

18. Simmons JD, Stein CM, Seshadri C, Campo M, Alter G, Fortune S, et al. Immunological mechanisms of human resistance to persistent Mycobacterium tuberculosis infection. Nat Rev Immunol. 2018;18(9):575–89. https://doi.org/10.1038/s41577-018-0025-3 PMID: 29895826

19. Hanifa Y, Grant AD, Lewis J, Corbett EL, Fielding K, Churchyard G. Prevalence of latent tuberculosis infection among gold miners in South Africa. Int J Tuberc Lung Dis. 2009;13(1):39–46. PMID: 19105877

20. Chihota VN, Ntshiqa T, Maenetje P, Mansukhani R, Velen K, Hawn TR, et al. Resistance to Mycobacterium tuberculosis infection among highly TB exposed South African gold miners. PLoS One. 2022;17(3):e0265036. https://doi.org/10.1371/journal.pone.0265036 PMID: 35302992

21. Ntshiqa T, Chihota V, Mansukhani R, Nhlangulela L, Velen K, Charalambous S, et al. Comparing QuantiFERON-TB Gold Plus with QuantiFERON-TB Gold in-tube for diagnosis of latent tuberculosis infection among highly TB exposed gold miners in South Africa. Gates Open Res. 2022;5:66. https://doi.org/10.12688/gatesopenres.13191.3 PMID: 37560544

22. Hong H, Dill-Mcfarland KA, Benson B, Simmons JD, Peterson GJ, Benchek P, et al. Mycobacterium tuberculosis-induced monocyte transcriptional responses associated with resistance to tuberculin skin test/interferon-γ release assay conversion in people with HIV. AIDS. 2023 Dec 1;37(15):2287.

23. Stein C, Z S, LI M, B T, E M, N M, et al. Resistance and Susceptibility to Mycobacterium tuberculosis Infection and Disease in Tuberculosis Households in Kampala, Uganda. Am J Epidemiol [Internet]. 2018 Jan 7 [cited 2023 Jan 25];187(7). Available from: https://pubmed.ncbi.nlm.nih.gov/29304247/.

24. Baliashvili D, Gandhi NR, Kim S, Hughes M, Mave V, Mendoza-Ticona A, et al. Resistance to Mycobacterium tuberculosis Infection Among Household Contacts: A Multinational Study. Clin Infect Dis Off Publ Infect Dis Soc Am. 2021 Sep 15;73(6):1037–45.

25. NIH. Mycobacterium tuberculosis Infection and Disease | NIH [Internet]. 2022 [cited 2023 Jan 28]. Available from: https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-opportunistic-infections/mycobacterium-0

26. König Walles J, Tesfaye F, Jansson M, Tolera Balcha T, Winqvist N, Kefeni M, et al. Performance of QuantiFERON-TB Gold Plus for detection of latent tuberculosis infection in pregnant women living in a tuberculosis- and HIV-endemic setting. PLoS One. 2018;13(4):e0193589. https://doi.org/10.1371/journal.pone.0193589 PMID: 29617458

27. Stephan C, Wolf T, Goetsch U, Bellinger O, Nisius G, Oremek G, et al. Comparing QuantiFERON-tuberculosis gold, T-SPOT tuberculosis and tuberculin skin test in HIV-infected individuals from a low prevalence tuberculosis country. AIDS. 2008 Nov 30;22(18):2471.

28. Aabye MG, Ravn P, PrayGod G, Jeremiah K, Mugomela A, Jepsen M, et al. The impact of HIV infection and CD4 cell count on the performance of an interferon gamma release assay in patients with pulmonary tuberculosis. PLoS One. 2009;4(1):e4220. https://doi.org/10.1371/journal.pone.0004220 PMID: 19156218

29. Balcells ME, Pérez CM, Chanqueo L, Lasso M, Villanueva M, Espinoza M, et al. A comparative study of two different methods for the detection of latent tuberculosis in HIV-positive individuals in Chile. Int J Infect Dis. 2008;12(6):645–52. https://doi.org/10.1016/j.ijid.2008.03.005 PMID: 18534887

30. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med. 2007;146(5):340–54. https://doi.org/10.7326/0003-4819-146-5-200703060-00006 PMID: 17339619

31. Cattamanchi A, R S, Kr S, Jz M, A D, C C, et al. Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals: a systematic review and meta-analysis. J Acquir Immune Defic Syndr 1999 [Internet]. 2011 Jan 3 [cited 2023 Jan 28];56(3). Available from: https://pubmed.ncbi.nlm.nih.gov/21239993/.

32. Telisinghe L, Amofa-Sekyi M, Maluzi K, Kaluba-Milimo D, Cheeba-Lengwe M, Chiwele K, et al. The sensitivity of the QuantiFERON®-TB Gold Plus assay in Zambian adults with active tuberculosis. Int J Tuberc Lung Dis. 2017;21(6):690–6. https://doi.org/10.5588/ijtld.16.0764 PMID: 28482964

33. N M, H Ni, W Tc, H Pc, G J, K Pa, et al. Tuberculin and anergy testing in HIV-seropositive and HIV-seronegative persons. Pulmonary Complications of HIV Infection Study Group. Ann Intern Med [Internet]. 1993 Jan 8 [cited 2023 Jan 28];119(3). Available from: https://pubmed.ncbi.nlm.nih.gov/8100692/.

34. Brock I, Ruhwald M, Lundgren B, Westh H, Mathiesen LR, Ravn P. Latent tuberculosis in HIV positive, diagnosed by the M. tuberculosis specific interferon-gamma test. Respir Res. 2006;7(1):56. https://doi.org/10.1186/1465-9921-7-56 PMID: 16579856

35. Churchyard GJ, Fielding KL, Lewis JJ, Coetzee L, Corbett EL, Godfrey-Faussett P, et al. A trial of mass isoniazid preventive therapy for tuberculosis control. N Engl J Med. 2014;370(4):301–10. https://doi.org/10.1056/NEJMoa1214289 PMID: 24450889

36. Mølhave M, Wejse C. Historical review of studies on the effect of treating latent tuberculosis. Int J Infect Dis. 2020 Mar 1;92:S31–6.

37. Smieja MJ, Marchetti CA, Cook DJ, Smaill FM. Isoniazid for preventing tuberculosis in non-HIV infected persons. Cochrane Database Syst Rev. 2000;1999(2):CD001363. https://doi.org/10.1002/14651858.CD001363 PMID: 10796642

38. Johnson DF, Malone LL, Zalwango S, Mukisa Oketcho J, Chervenak KA, Thiel B, et al. Tuberculin skin test reversion following isoniazid preventive therapy reflects diversity of immune response to primary Mycobacterium tuberculosis infection. PLoS One. 2014;9(5):e96613. https://doi.org/10.1371/journal.pone.0096613 PMID: 24796677

39. Daniel TM, Bowerfind ES Jr. Reversion of recently acquired tuberculin reactivity during isoniazid prophylaxis. Am Rev Respir Dis. 1967;95(3):500–2. https://doi.org/10.1164/arrd.1967.95.3.500 PMID: 6018709

40. Simmons JD, Pt V, Cm S, V C, T N, P M, et al. Monocyte metabolic transcriptional programs associate with resistance to tuberculin skin test/interferon-γ release assay conversion. J Clin Invest [Internet]. 2021 Jul 15 [cited 2023 Jan 28];131(14). Available from: https://pubmed.ncbi.nlm.nih.gov/34111032/.

41. Simmons JD, Dill-McFarland KA, Stein CM, Van PT, Chihota V, Ntshiqa T, et al. Monocyte Transcriptional Responses to Mycobacterium tuberculosis Associate with Resistance to Tuberculin Skin Test and Interferon Gamma Release Assay Conversion. mSphere. 2022 Jun 13;7(3):e00159–22.