Serial QuantiFERON testing and tuberculosis disease risk among young children: an observational cohort study

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Summary

Background The value of quantitative interferon-γ release assay results for predicting progression from *Mycobacterium tuberculosis* infection to active disease is unknown. We aimed to investigate the relation between QuantiFERON-TB Gold In-Tube (QFT) conversion interferon-γ values and risk of subsequent active tuberculosis disease and of QFT reversion.

Methods We analysed data from a reported vaccine efficacy trial of the tuberculosis vaccine MVA85A in South Africa. QFT negative, HIV uninfected young children aged 18–24 weeks were enrolled. We stratified participants by quantitative QFT result (interferon-γ <0·35 IU/mL, 0·35–4·00 IU/mL, and >4·00 IU/mL) at the intermediate study visit (day 336) and determined risk of progression to active tuberculosis disease over the subsequent 6–24 months. No QFT differences were observed between placebo and MVA85A groups at day 336 or end of study; therefore, both groups were included in analyses. Study clinicians were not masked to QFT values, but strict case definitions were used that excluded QFT results. We used generalised additive models to evaluate the quantitative relation between day 336 QFT value and subsequent disease risk, and we compared disease rates between QFT strata using a two-sample Poisson test.

Findings Among 2512 young children with QFT tests done at day 336, 172 (7%) were positive; 87 (7%) of 1267 in placebo group and 85 (7%) of 1245 in the MVA85A group (p=1·00). Compared with QFT non-converters (tuberculosis disease incidence 0·7 per 100 person-years [95% CI 0·4–1·1]), children with QFT conversion at interferon-γ values between 0·35–4·00 IU/mL did not have significantly increased risk of disease (2·5 per 100 person-years [95% CI 0·4–9·4]; incidence rate ratio (IRR) 3·7 (95% CI 0·4–15·8; p=0·23). However, QFT conversion at interferon-γ values higher than 4·00 IU/mL was associated with substantially increased disease incidence (28·0 per 100 person-years [95% CI 14·9–45·7]) compared with non-converters (IRR 42·5 [95% CI 17·2–99·7]; p<0·0001), and compared with children with interferon-γ values between 0·35–4·00 IU/mL (IRR 11·4 [95% CI 2·4–107·2]; p=0·00047). Among 91 QFT converters who were given a repeat test, 53 (58%) reverted from positive to negative. QFT reversion risk was inversely associated with interferon-γ value at QFT conversion and was highest with interferon-γ values less than 4·00 IU/mL (47 [77%] of 61).

Interpretation In young children, tuberculosis disease risk was not significantly increased, and QFT reversion was common, following QFT conversion at interferon-γ values up to 10 times the recommended test threshold (0·35 IU/mL). By contrast, QFT conversion at very high interferon-γ values (>4·00 IU/mL) warrants intensified diagnostic and preventive intervention because of the extremely high risk of tuberculosis disease in these young children.

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Introduction

Interferon-γ release assays (IGRAs) are diagnostic tests for *Mycobacterium tuberculosis* infection that are widely used in high-resource clinical settings to investigate contacts of tuberculosis cases and to guide isoniazid preventive therapy (IPT).[1] Among children, a positive IGRA result could improve performance of clinical algorithms for diagnosis of pauci-bacillary pulmonary tuberculosis by showing previous *M tuberculosis* exposure and infection. However, despite the modest sensitivity and specificity of IGRAs for active disease, evidence is conflicting in children.[2–8]

Young children infected with *M tuberculosis* are at very high risk of progression to tuberculosis disease and, compared with adults, are at increased risk of severe tuberculosis morbidity and mortality.[9] Major interest in the use of chemoprophylaxis among high-risk children has been shown;[10,11] however, randomised trials of untargeted IPT among children in high-transmission settings have had mixed results. IPT reduces the incidence of disease in *M tuberculosis*-infected or *M tuberculosis*-exposed children by more than 50% and is recommended in national and international guidelines.[12,13] However, implementation of IPT is poor in the resource-limited countries where effective tuberculosis prevention is most needed.

One of the key obstacles to targeted tuberculosis screening and preventive therapy is differentiating only those *M tuberculosis*-infected individuals at highest risk of disease from the majority who will remain healthy.

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Research in context

Evidence before this study

We searched PubMed for relevant articles published in English before July 26, 2016, using the search terms "(Quantiferon OR T-Spot.TB OR 'interferon gamma release assay') AND (child OR children OR pediatric OR infant) AND tuberculosis". Although several studies reported on sensitivity and specificity of interferon-γ release assays (IGRAs) for detection of active tuberculosis in children, the literature search identified no studies reporting prognostic value of IGRA conversions or predictive value of quantitative interferon-γ values for tuberculosis among young children. Among household contacts of patients with tuberculosis, positive IGRAs were associated with increased risk of tuberculosis disease, but the positive predictive value was less than 2%. Additionally, several studies have noted high rates of indeterminate IGRA results among children.

Added value of this study

In this study, we present data from a large prospective cohort of young children from a tuberculosis-endemic community who were QuantiFERON (QFT)-negative at baseline and followed prospectively for QFT conversion, tuberculosis disease, or both.

We found that very high QFT interferon-γ conversion values (>4·00 IU/mL) were associated with substantially increased risk of tuberculosis disease compared with non-converters or converters with lower QFT interferon-γ values (0·35-4·00 IU/mL). Among children who did not convert QFT at 1 year but were subsequently diagnosed with disease, all whose QFT was repeated were positive at time of diagnosis. Additionally, QFT interferon-γ values above the manufacturer's threshold (0·35 IU/mL) but below 4·00 IU/mL were associated with very high rates of reversion.

Implications of all the available evidence

Guidelines from WHO, the US Centers for Disease Control and Prevention, and other advisory bodies currently recommend tuberculin skin testing over IGRAs for detection of tuberculosis infection in young children from high-burden settings, in part due to absence of evidence from this age group. These data suggest that high QFT interferon-γ conversion values could have strong prognostic or diagnostic value for tuberculosis in this population. If validated in other study populations, these findings justify revision of current international guidelines for use of IGRAs for tuberculosis screening in young children.

Individuals who test IGRA positive have only two-fold to three-fold increased risk for developing tuberculosis disease,[14–18] and the positive predictive value for disease progression among IGRA-positive household contacts, or among individuals with IGRA conversion, is less than 2%.[19,20] Further, because of the absence of natural quantitative breakpoints in the distribution of interferon-γ concentrations, the conversion threshold value of the QuantiFERON-TB Gold In-Tube test (QFT; interferon-γ ≥0·35 IU/mL), one of the most widely used IGRAs, is subject to debate.[16] Whether higher interferon-γ values represent more recent *M tuberculosis* exposure, greater aerosolised inoculum, or sustained infection—or simply reflect heterogeneity in human immune responses to *M tuberculosis*—is unclear. Importantly, evidence for whether higher QFT conversion interferon-γ values are associated with increased risk of progression to active disease is conflicting,[19,21–23] and current management algorithms do not distinguish between interferon-γ values above the QFT manufacturer's recommended test threshold of 0·35 IU/mL.

We analysed longitudinal data from a tuberculosis vaccine efficacy trial, in which no protective effect was seen on incidence of *M tuberculosis* infection or disease in a large cohort of Bacille Calmette-Guerin (BCG) vaccinated South African young children, all of whom were QFT negative at enrolment.[24] Infant BCG vaccination offers partial protection against pulmonary and disseminated tuberculosis disease in children.[25] We hypothesised that QFT conversions and quantitative QFT values predict tuberculosis disease risk among young children. We therefore investigated the relation between QFT conversion interferon-γ values and risk of subsequent active tuberculosis disease and QFT reversion.

Methods

Study design and participants

The MVA85A 020 trial was a double-blinded, randomised, placebo-controlled clinical trial undertaken in a rural region near Cape Town, South Africa, between July 15, 2009, and Oct 25, 2012. Healthy young children aged 4–6 months who had received BCG vaccination within 7 days of birth were enrolled if they were HIV ELISA negative and had no known household or other close exposure to a tuberculosis patient; QFT testing was done and those young children with a positive test were excluded.

The trial was approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee, Oxford University Tropical Research Ethics Committee, and the Medicines Control Council of South Africa. Parents or legal guardians provided written informed consent.

Procedures

Study procedures and results have been described previously.[24] In brief, young children were randomly assigned (1:1) using independently generated sequences with block sizes of four to receive one dose of the vaccine MVA85A or *Candida* spp skin test antigen (placebo control). QFT testing was done at baseline and was repeated at day 336 and at the end of the study, which

ranged from 6 months to 24 months after day 336 and was determined by development of tuberculosis disease or reaching October, 2012. Young children were actively followed every 3 months to identify signs or symptoms of disease, or history of exposure to tuberculosis. Children with persistent cough, failure to thrive, weight loss, positive tuberculin skin test (TST) or QFT conversion, or close contact with a patient with tuberculosis were admitted for standardised investigation. Investigation included QFT and TST (if not already done); chest radiography; HIV testing; and testing of two paired, consecutive induced sputa and early morning gastric lavages by auramine staining and smear microscopy, Mycobacteria growth indicator tube liquid culture and Xpert MTB/RIF. Children with positive QFT or TST results were referred to public clinics for IPT.

In this study, QFT was done according to manufacturer's instructions (Qiagen, Venlo, The Netherlands). Briefly, QFT is used to detect in-vitro *M tuberculosis*-specific immune responses by measuring interferon-γ concentration in plasma harvested from whole blood incubated with *M tuberculosis*-specific antigens (TB Ag) minus interferon-γ detected in unstimulated control (nil). The amount of interferon-γ is quantified by ELISA using interferon-γ standards between 0 IU/mL and 4 IU/mL (outside the USA where standards go up to 8 IU/mL). To assess the detectable range of interferon-γ concentration beyond the standard curve, blood samples from 42 healthy adult volunteers were recruited from the same high tuberculosis burden community and were stimulated in the TB Ag tube. Undiluted and diluted (1:5, 1:10, and 1:25) plasma samples were analysed by QFT interferon-γ ELISA. Linearly extrapolated results were compared with each of the diluted results to assess reliability of extrapolation beyond the highest standard, which was assessed by Pearson's product-moment correlation coefficient.

Outcomes

We defined a positive QFT as TB antigen minus nil interferon-γ value greater than or equal to 0·35 IU/mL, as per the manufacturer's instructions. QFT conversion was defined as a positive test that followed a negative test, and QFT reversion as a negative test that followed a positive test. For the original trial, the primary disease endpoint (endpoint 1) required either microbiological confirmation (defined as at least one positive liquid culture for *M tuberculosis* or Xpert MTB/RIF from any clinical specimen) or, in the absence of microbiological confirmation, specific chest radiographic and clinical findings indicative of tuberculosis disease in addition to positive TST or QFT conversion. Study clinicians were not masked to QFT results. Because QFT was a component of the latter composite definition, we used a revised endpoint 1 for this analysis that removed QFT conversion from the diagnostic criteria to avoid bias towards association with QFT status. Additionally, after a tuberculosis disease diagnosis, most children did not have QFT repeated, because they had met the main study endpoint.

Statistical analysis

Consistent with the primary trial finding that QFT conversion and tuberculosis disease incidence did not differ by study group,[24] we analysed QFT dynamics and tuberculosis risk in control and MVA85A vaccine participants together. We analysed QFT conversions at day 336 and at the end of the study (determined by development of tuberculosis disease or October, 2012). We compared QFT converters and non-converters at the day 336 study visit and analysed subsequent incidence of tuberculosis disease through to the end of study. We also included tuberculosis cases that were diagnosed within 60 days of the end of the study, as they probably had tuberculosis at the end of the study visit, which in most cases prompted the tuberculosis diagnostic process that resulted in their diagnosis. We further stratified QFT-positive results by interferon-γ value at the day 336 study visit, first as continuous values, and then using ordinal categories based on inspection of model results of disease risk, reversion risk, and positive predictive value of various thresholds. For our main results, we selected thresholds of 0·35 IU/mL (manufacturer's recommended value) and 4·00 IU/mL, because the latter is the maximum standard provided in international kits. The relation
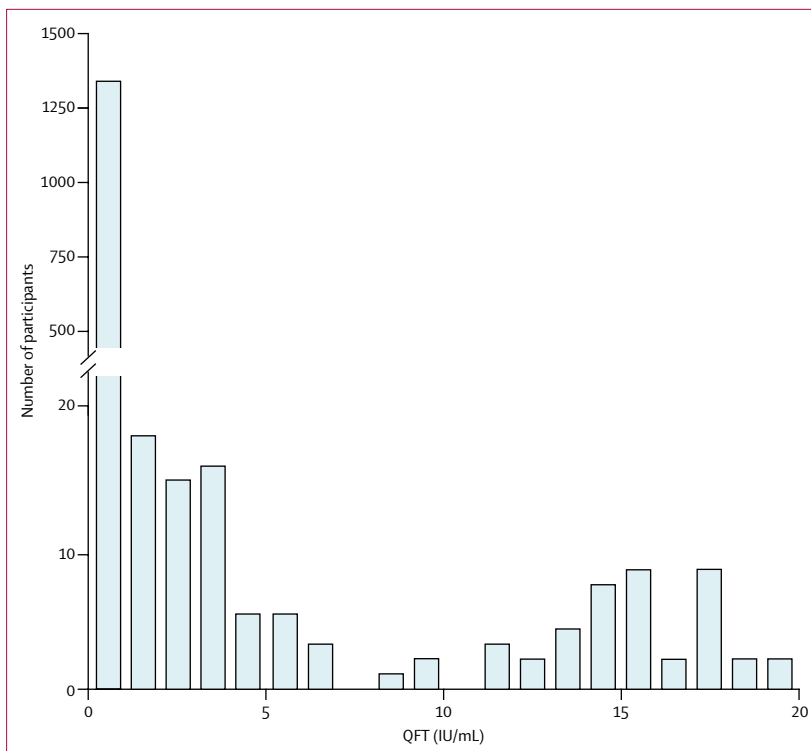

Figure 1: Distribution of QuantiFERON-TB Gold In-Tube (QFT) interferon-γ values at the intermediate study visit (day 336)
Bars represent numbers of individuals with QFT values between numbers (eg, 0·01–1·00, 1·01–2·00).

between QFT conversion value, tuberculosis disease incidence, and probability of QFT reversion at end of study were analysed with logistic regression models and generalised additive models with penalised splines. We evaluated the effect mediation of sex, age, vaccine assignment, and IPT on the relationship between QFT value and tuberculosis risk in generalised linear models. We calculated exact 95% Poisson CIs and compared incidence of tuberculosis disease in groups, expressed in incidence rate ratios, using a two-sample Poisson test. Data were analysed with R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

Role of the funding source
No additional funding was obtained for this analysis of trial data. Aeras was the funder of the MVA85A 020 Trial. BSL and JBM are employees of Aeras who participated in writing the report. JRA is supported by the National Institutes of Health (K01 AI104411). JRA, EN, TJS, and MH had complete access to the data. All authors reviewed and interpreted analyses and contributed to the writing of the report. MH had final responsibility for the decision to submit for publication.

Results
Among the 2797 young children enrolled between July 15, 2009, and May 4, 2011, in the MVA85A 020 trial at median age 20·4 weeks (IQR 19·3–22·0), 2772 (99%) young children had a negative QFT at enrolment, five (<1%) had no quantitative results available, and 20 (1%) had an indeterminate result. 1399 young children were allocated to MVA85A and 1398 were allocated to placebo. Among those 2772 young children with a negative QFT at baseline, 2512 (91%) had a QFT done at the day 336 visit. In this group, 172 (7%) were positive and 13 (1%) were indeterminate. Among the 172 QFT converters with a positive test at day 336, median QFT conversion interferon-γ value was 3·41 IU/mL (IQR 0·82–13·72). A bimodal distribution of interferon-γ values was noted, with a nadir of about 7·0 IU/mL (figure 1). QFT conversion rate did not differ among young children receiving the MVA85A vaccine (85 [7%] of 1245 children assigned to MVA85A who had QFTs available at day 336) and those receiving placebo (87 [7%] of 1267 children assigned to placebo who had QFTs at day 336; p=1·00). QFT conversion values similarly did not differ for the two groups (median 3·46 IU/mL [IQR 0·86–13·19] in the MVA85A vaccine group vs median 3·41 IU/mL [0·72–13·80] in the placebo group; p=0·83). At the end of study visit, 2045 of those children who were QFT negative at day 336 had a repeat QFT done and 116 (6%) had converted. A non-significant trend towards higher QFT conversion among those who were negative at day 336 was noted in the MVA85A vaccine group compared with the placebo group at the end of the study (p=0·090).

Because of the bimodal distribution of interferon-γ values, we investigated whether very high QFT conversion

interferon-γ values, which exceed the maximum value of the standard curve and were therefore estimated through linear extrapolation, were a reliable estimate of true interferon-γ concentrations. In 42 samples from healthy adult volunteers with varying interferon-γ values that were diluted 5-fold, 10-fold, and 25-fold, we found high correlation between extrapolated measurements in
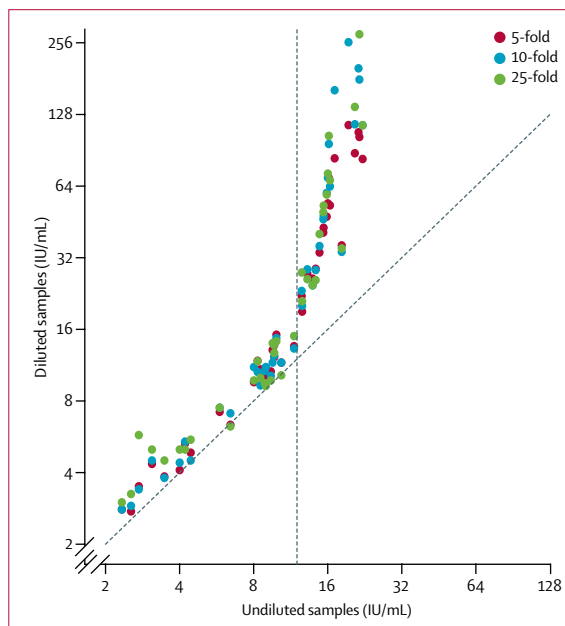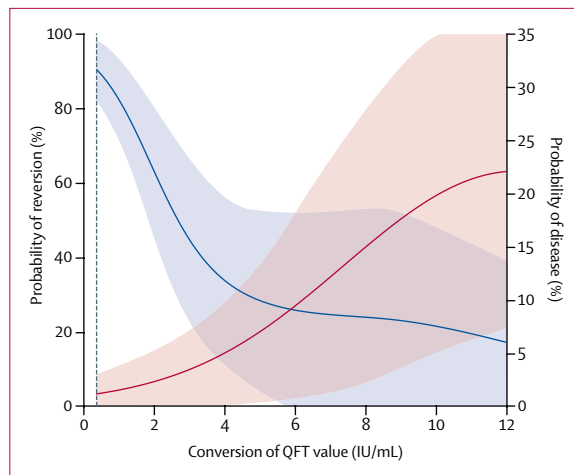
Figure 2: Comparison of QuantiFERON interferon-γ values estimated by dilutions with QuantiFERON interferon-γ estimates from undiluted values, with linear extrapolation above 4 IU/mL
To estimate whether interferon-γ values extrapolated above the assay higher standard (4 IU/mL) were reliable, measurements obtained from undiluted samples were correlated with those obtained by diluting the same samples 5-fold (red marker), 10-fold (blue marker), and 25-fold (green marker). Measurements obtained from diluted samples were multiplied by the dilution factor to estimate the pre-dilution interferon-γ concentration and correlated with values measured in the corresponding undiluted samples. The diagonal dashed line represents perfect correlation. The vertical dashed line depicts an undiluted interferon-γ value of 12, above which extrapolated results became less reliable.

	N	Cases	Incidence (95% CI)	IRR (95% CI)	p value
Revised case definition 1					
<0·35 IU/mL	2232	16	0·7 (0·4–1·1)	Ref	Ref
0·35–4·00 IU/mL	79	2	2·5 (0·4–9·4)	3·7 (0·4–15·8)	0·23
>4·00 IU/mL	63	10	28·0 (14·9–45·7)	42·5* (17·2–99·7)	<0·0001
Culture or Xpert positive					
<0·35 IU/mL	2232	11	0·5 (0·2–0·8)	Ref	Ref
0·35–4·00 IU/mL	79	2	2·5 (0·4–9·4)	5·4 (0·6–24·8)	0·13
>4·00 IU/mL	63	7	19·6 (8·9–36·8)	43·3† (14·2–122·3)	<0·0001

Incidence reported in cases per 100 person-years. IRR=incidence rate ratio. Ref=reference. *IRR of higher than 4·00 vs 0·35–4·00 for revised case definition 1: 11·4 (95% CI 2·4–107·2), p<0·000047. †IRR of higher than 4·00 vs 0·35–4·00 for culture or Xpert positive: 8·0 (95% CI 1·5–78·8); p=0·0094.

Table 1: Incidence of tuberculosis (cases per 100 person-years) according to day 336 QuantiFERON interferon-γ value by case definition

undiluted samples and actual measurements in diluted samples up to about 12·00 IU/mL (Pearson's r 0·94–0·97). Measurements in undiluted samples underestimated interferon-γ concentrations higher than 12·00 IU/mL (Pearson's r 0·66–0·88; figure 2).


Figure 3: **Probability of QuantiFERON-TB Gold In-Tube (QFT) reversion (blue line, left axis) between the intermediate and final study visit and prospective risk of disease (red line, right axis), as a function of the QFT conversion interferon-γ value**
The shaded areas reflect 95% CIs from a generalised additive model. Vertical dashed line reflects the current recommended cutoff (0·35 IU/mL).

Figure 4: **Tuberculosis-free survival from day 336 study visit, stratified by quantitative QuantiFERON-TB Gold In-Tube (QFT) result**

Overall, 58 (2%) of 2797 children met diagnostic criteria for active tuberculosis disease, including 42 (2%) who had microbiologically confirmed tuberculosis. 37 cases were diagnosed between day 336 and the end of the study, but nine of them were missing day 0 or day 336 QFT results. An additional 107 children were treated for tuberculosis disease, without meeting the study definition, before day 336 and were excluded from further analysis. Among the 172 converters at day 336, 30 were diagnosed and treated for tuberculosis before day 336, leaving 142 converters available for analysis. Among the 2232 children with negative QFT at day 336, 16 were diagnosed with tuberculosis disease by the end of the study (incidence 0·7 per 100 person-years), and 11 of them had microbiologically confirmed tuberculosis disease (incidence 0·5 per 100 person-years; table 1). Risk of tuberculosis disease increased substantially at a conversion QFT value of above 4·00 IU/mL (figure 3). Among 79 children with a QFT conversion interferon-γ value between 0·35 IU/mL and 4·00 IU/mL, two were subsequently diagnosed with tuberculosis (incidence 2·5 per 100 person-years) and both cases were microbiologically confirmed (table 1). Among 63 children with a QFT conversion interferon-γ value higher than 4·00 IU/mL, ten were diagnosed with tuberculosis (28·0 cases per 100 person-years) and seven were microbiologically confirmed (19·6 cases per 100 person-years; table 1).

Compared with QFT non-converters, children with day 336 QFT conversion interferon-γ values greater than 4·00 IU/mL had substantially increased incidence of active tuberculosis disease by both case definitions (incidence rate ratio [IRR] for revised endpoint 1: 42·5 [95% CI 17·2–99·7]; IRR for microbiologically confirmed cases: 43·3 [14·2–122·3]; both p<0·0001). Using the threshold of 4·00 IU/mL, the sensitivity and specificity for subsequent disease were 36% and 99% and the positive and negative predictive values were 16% and 99%, respectively. The positive and negative predictive values using the manufacturer's cut-off of 0·35 IU/mL were 8% and 99%, respectively. Median time to diagnosis from the time of QFT testing among QFT non-converters was 210 days (IQR 134–373) and among QFT converters was 44 days (30–72; p=0·0020), respectively (figure 4). Among the 16 individuals with a negative QFT at day 336 who were subsequently diagnosed with tuberculosis, 14 had QFT repeated at diagnosis, and all (14 [100%] of 14) were positive. We found no effect mediation of vaccine assignment (p=0·93), age (p=0·96), sex (p=0·16), or receipt of IPT (p=0·79) on the relation between QFT values and risk of tuberculosis.

Among the 172 QFT converters at day 336, 91 (53%) children had a repeat QFT at the end of the study, and 53 (58%) reverted from positive to negative QFT. QFT reversion rates did not differ by vaccine or placebo assignment (29 [56%] of 52 in the MVA85A vaccine group vs 24 [62%] of 39 in the control group; p=0·74) or among
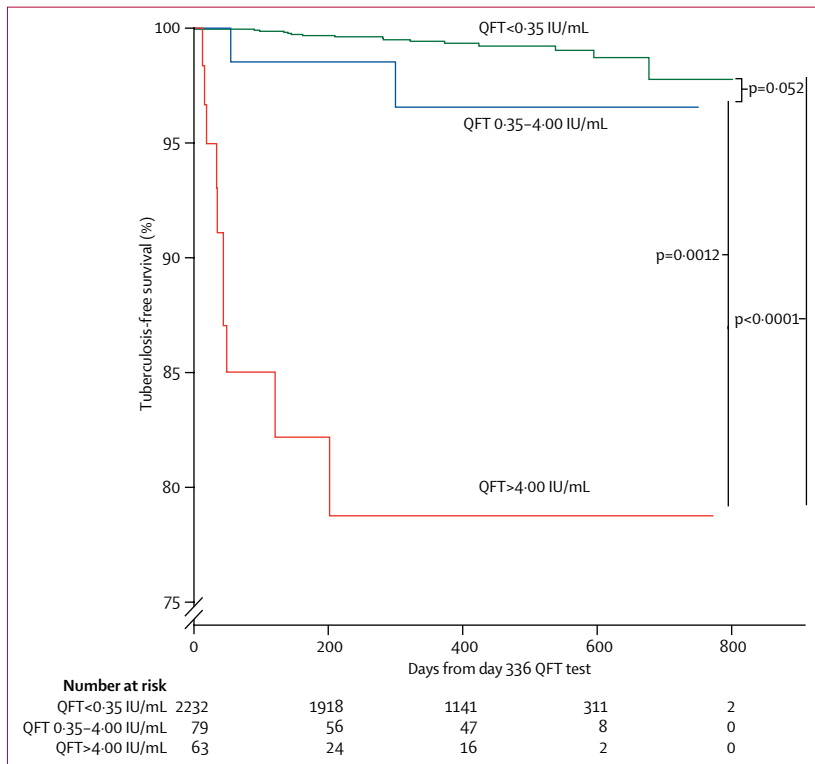
those who subsequently received IPT or four-drug curative therapy (29 [53%] of 55 who received *vs* 24 [67%] of 36 who did not receive; p=0·27). We analysed risk of QFT reversion at the end of the study as a function of the QFT conversion interferon-γ value at day 336 (table 2; figure 3). At QFT conversion interferon-γ values between 0·35–4·00 IU/mL, subsequent reversion rates were high (47 [77%] of 61). Very high QFT conversion interferon-γ values were associated with substantially lower risk of QFT reversion (odds ratio 0·07 [95% CI 0·02–0·21] for QFT conversion interferon-γ value >4 IU/mL *vs* ≤4 IU/mL; figure 3), although probability of QFT reversion for QFT conversion interferon-γ values even above 4 IU/mL remained moderately high (six [20%] of 30; table 2).

Discussion

We found high rates of QFT conversion and high incidence of tuberculosis disease, using a rigorous endpoint definition, in a cohort of young children living in a rural community in South Africa. Consistent with our previous studies in older populations, we found an increased risk of incident tuberculosis disease following QFT conversion.[18] However, contrary to traditional interpretation of the QFT result, we additionally found that incidence of tuberculosis disease was significantly higher among children with very high QFT conversion interferon-γ values (>4·00 IU/mL), with tuberculosis disease incidence more than 40-fold that of QFT non-converters and 11-fold that of QFT converters with interferon-γ value between 0·35–4·00 IU/mL. By contrast, children with QFT conversion interferon-γ values less than 4·00 IU/mL (more than 10 times higher than the assay threshold value) had a high risk of QFT reversion and did not have significantly increased risk of progression to active disease, compared with QFT non-converters. Together, these results indicate that very high QFT conversion interferon-γ values, much higher than previously considered clinically useful, are a powerful indicator of substantially increased risk for progression from *M tuberculosis* infection to active tuberculosis disease in this vulnerable population.

Despite the increased incidence and mortality from tuberculosis among children in high-burden countries, few large cohort studies have characterised the role of IGRAs to predict progression from infection to disease in this population. The existing literature consists predominantly of cross-sectional studies showing the accuracy of IGRAs in the diagnosis of active or latent tuberculosis, frequently in comparison with TST. Results have been conflicting at times, with some studies showing comparable sensitivity and specificity to TST, whereas others found higher or lower sensitivity.[3,26–32] Rates of indeterminate QFT tests have been higher in young children than in adults;[26,33–36] although in this study, we noted a low rate of indeterminate tests (0·4% of all tests).

It is possible that very high QFT conversion interferon-γ values among children are a marker of very early,

	n	Reverted (n [%; 95% CI])
0·35–4·00 IU/mL	61	47 (77%; 64·2–86·5)
>4·00 IU/mL	30	6 (20%; 8·4–39·1)
Total (>0·35) IU/mL	91	53 (58%; 47·4–68·3)

Table 2: Risk of QuantiFERON reversion at end of study, among children who converted at the intermediate study visit, according to the day 336 quantitative QuantiFERON conversion interferon-γ value (IU/mL)

incipient disease, or subclinical active disease that was present at the time of QFT testing. This hypothesis is supported by the relatively short interval between QFT testing and diagnosis of tuberculosis disease in very high QFT converters in this study. These findings also echo a recent study in adolescents that identified increased expression of interferon-regulated genes as a predictor of subsequent tuberculosis risk.[37] The crucial question is whether identification of those *M tuberculosis*-infected children at highest risk of having or developing the disease offers an opportunity for both early case detection of subclinical tuberculosis, and in those children in whom aggressive diagnostic measures do not reveal tuberculosis, the use of preventive therapy to interrupt progression to active disease. Untargeted IPT did not prevent tuberculosis among HIV-exposed young children in a recent randomised trial;[11] whether QFT-targeted preventive therapy—with one or more drugs—could reduce tuberculosis morbidity and disease in this very high-risk population merits further study. Such strategies would need to be balanced against the risk of under-treating undiagnosed active disease, causing selective pressure for antibiotic resistance.

Several studies have addressed the question of whether QFT interferon-γ values have additional value for prediction of incident tuberculosis, and results have been conflicting. Diel and colleagues[21] found that 16 (84%) of 19 contacts who subsequently progressed to tuberculosis disease had interferon-γ values higher than 3·5 IU/mL at initial investigation. In a German study, Geis and colleagues[22] reported that five (83%) of six contacts with disease had interferon-γ values higher than 10 IU/mL, and suggested that raising the QFT test threshold could improve the positive predictive value and reduce the number needed to treat. By contrast, Haldar and colleagues[23] did not find an association between QFT conversion interferon-γ value and progression to tuberculosis disease among adult contacts in the UK, and Zellweger and colleagues[19] found minimal variability in risk by interferon-γ value in a cohort of contacts in a large European network. Notably, these studies measured interferon-γ values in primarily adult tuberculosis contacts in low endemicity areas, whereas our study enrolled young children in a high transmission area who were known to be QFT negative and were then followed prospectively for QFT conversion and subsequent disease.

The absence of an inflection point in the distribution of interferon-γ values in previous studies has called into

question the validity of the 0·35 IU/mL test threshold.[19] For the first time, we have shown in young children that the distribution of interferon-γ values is clearly bimodal, as has similarly been shown previously for the distribution of TST in older age groups.[19] In the absence of a gold standard test, determination of how this distribution relates to *M tuberculosis* infection is impossible, but we suggest that based on the nadir of measured QFT conversion interferon-γ values at 7·0–8·0 IU/mL, it is unlikely that the bimodal distribution differentiates *M tuberculosis*-uninfected children from *M tuberculosis*-infected children. We note that this bimodal distribution differs from the unimodal distribution of QFT values reported in adolescent and adult studies, including one from the same laboratory and community, suggesting that the naive infant immune response to *M tuberculosis* might differ from that of adults.[20]

For our main analyses, we selected a threshold of 4·00 IU/mL based on disease and reversion risks above this inflection point (figure 3) and availability of interferon-γ standards up to 4·00 IU/mL in the international kits. We note that high rates of disease (2·5 per 100 person-years) were found even among those with QFT values 0·35–4·00 IU/mL, and the optimal threshold from a perspective of targeting interventions might be lower. Because interferon-γ values greater than 4·00 IU/mL lie beyond the maximum value within the standard curve of the assay, we did several dilution experiments using plasma from healthy volunteers and noted that observed interferon-γ values estimated through linear extrapolation were a reliable estimate of true interferon-γ concentrations up to about 12·00 IU/mL, suggesting that the observed distribution nadir is not an analytical artefact. We infer that the bimodal distribution of QFT conversion interferon-γ values might be related to other factors such as recent or transient *M tuberculosis* infection, size of inoculum, or bacillary load.

Several recent studies have reported high rates of IGRA reversion (>60%) among health-care workers in low-burden countries.[38–41] In a study of adolescents in the same high-burden community, we previously reported a reversion rate of 23·7% following QFT conversion.[20] Very few studies of QFT reversion risk among young children have been published. Shah and colleagues[42] found low rates of reversion (15%) among QFT-positive household contacts of tuberculosis cases. In this study, we found high overall rates of QFT reversion among young children (58%) and, as seen in our adolescent study,[20] we found an inverse relation between QFT conversion interferon-γ value and reversion risk; however, reversion rates remained fairly high (20%) even at very high interferon-γ values (>4·00 IU/mL).

The traditional approach to early detection of childhood tuberculosis has been the investigation of household contacts of adult tuberculosis cases. However, recent studies from South Africa have shown that half or more of tuberculosis cases in children are not linked to an adult case in the household or are diagnosed before the adult.[43] The use of serial IGRA testing represents a potential instrument for early identification of children with subclinical or incipient tuberculosis; whether this would be cost-effective as a testing strategy, either standalone or in conjunction with contact tracing, remains to be determined.

The results of this analysis should be interpreted within the context of the limitations of the study design and available data. First, although we excluded QFT from the rigorous case definition for tuberculosis used in this analysis, clinicians were not masked to QFT results and it is possible that decision to investigate on the basis of a positive QFT led to increased diagnosis of tuberculosis disease; however, it is unlikely that this would have explained the increased disease incidence associated with greater quantitative interferon-γ values. Additionally, our findings were consistent when using a microbiologically confirmed endpoint. To avoid potential for boosting of QFT responses, TSTs were not routinely done in this study, precluding direct comparison. Children who lived in a household with a smear-positive tuberculosis case were screened for active tuberculosis; several of such children were identified as having active tuberculosis before day 336 and therefore not included in the prospective analysis. We are unable to assess effect mediation between exposures and QFT interferon-γ values on tuberculosis risk. Participants with a positive QFT who did not have tuberculosis were referred for IPT, but IPT did not seem to confound the relation between interferon-γ values and risk of disease. We used undiluted samples with QFT values extrapolated from the standard curve above 4·00 IU/mL in our main analyses, because we did not have samples available from this cohort to do measurements on diluted samples; our analysis from healthy adult controls suggests that this extrapolation would be reliable up to 12·00 IU/mL. Several technical factors can affect quantitative results of QFT testing, as recently outlined by Banaei and colleagues.[44] To minimise risk of variation, all assays were done according to a trial protocol in a single laboratory accredited with the South African National Accreditation System (SANAS), by well trained technicians with tested competency, under a rigorous quality assurance programme, and with external monitoring by SANAS and the trial funder, Aeras. Finally, we did this study in a community with extremely high rates of tuberculosis; whether these findings will hold true in lower burden settings should be investigated.

Current WHO guidelines recommend that IGRAs not be used to replace TSTs in high-burden, low-income, and middle-income countries, and several national and regional guidelines recommend TSTs over IGRAs in young children.[12,45] However, many of the same guidelines recommend IGRAs preferentially over TSTs in BCG-vaccinated individuals, which leaves an evidence gap for BCG-vaccinated children. We found that QFT

testing in a high-burden, BCG-vaccinated population can identify children at highest risk of subclinical disease or imminent progression to tuberculosis disease, who might benefit from more intensive diagnostic and chemotherapeutic interventions. Very high QFT conversion interferon-γ values (>4·00 IU/mL) in particular were associated with lower probability of QFT reversion and substantially increased risk of tuberculosis disease. By contrast, conversion values in the range of 0·35–4·00 IU/mL had low predictive value for tuberculosis disease and were associated with high rates (77%) of reversion. The current recommended QFT threshold interferon-γ value of 0·35 IU/mL might therefore be too low in this population, and a higher test threshold might be indicated for risk-targeted intervention. Similarly, infant QFT conversion values in the range of 0·35–4·00 IU/mL might warrant repeat testing if preventive therapy is considered. If validated in other study populations, these findings justify revision of current international guidelines for use of IGRAs in young children.[12,45]

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