

# Cumulative *Mycobacterium tuberculosis* Infection Incidence (Measured Primarily by Tuberculin Skin Test) Among Infants With Human Immunodeficiency Virus Exposure: Observational Follow-up of an Isoniazid Prophylaxis Trial

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Cumulative 24-month *Mycobacterium tuberculosis* infection incidence (measured primarily by tuberculin skin test [TST]) was high among human immunodeficiency virus exposed but uninfected infants (8.7 [95% confidence interval, 6.3–11.9] per 100 person-years). Trend for decreased TST positivity among infants at trial end (12 months postenrollment) randomized to isoniazid at 6 weeks of age was not sustained through observational follow-up to 24 months of age.

**Clinical Trials Registration.** NCT02613169.

**Keywords.** *M. tuberculosis* infection; HIV-exposed infant; tuberculin skin test; isoniazid.

Each year more than 5 million children aged 5 years or under are exposed to tuberculosis (TB) [1]. Recent estimates suggest that 20% of young children with evidence of *Mycobacterium tuberculosis* (*Mtb*) infection (measured by interferon- $\gamma$  release assay [IGRA] or tuberculin skin test [TST]) after household contact who do not receive preventive therapy will go on to develop TB disease [2]. Infants who are human immunodeficiency virus

(HIV) exposed but uninfected (HEU) are at particularly high risk of *Mtb* infection and TB disease [3–5].

We recently performed a randomized trial to assess whether isoniazid (INH) prevented primary *Mtb* infection among HEU infants without known TB exposure enrolled at 6 weeks of age [6, 7]. The primary trial endpoint was *Mtb* infection at 12 months following enrollment (measured by IGRA and/or TST). In the trial there was a trend for decreased TST positivity after 12 months of INH.

At trial completion, participants were invited to enroll in observational follow-up with TST placed at 24 months of age. We assessed cumulative *Mtb* infection incidence among HEU infants from trial entry through extended observational follow-up.

## METHODS

The infant TB Prevention Study (iTIPS), a randomized, non-blinded trial, evaluated efficacy of INH to prevent primary *Mtb* infection in HEU infants. Trial design and results were previously reported [6, 7]. Infants 6–10 weeks of age, born to mothers with HIV, with birth weight >2.5 kg and  $\geq 37$  weeks' gestation, were enrolled from prevention of maternal-to-child transmission of HIV clinics in western Kenya. Infants with known prior TB exposure (including maternal TB in the past year) were excluded. Participants were randomized to 12 months of daily INH (10 mg/kg dose) or no INH. At 12 months postenrollment (~14 months of age), *Mtb* infection status was assessed by IGRA (QFT-Plus) and/or TST. TST  $\geq 10$  mm induration was considered positive [8]. TST was added for a composite primary endpoint 6 months after the first participant study exit, per data safety and monitoring board recommendation due to low accrued QFT-Plus endpoints at the time, and since TST is recommended for children aged <2 years. A prespecified modified intention-to-treat analysis excluded children ( $n = 2$ ) diagnosed with HIV during the study. At trial exit, participants were invited to continue in observational follow-up with TST placement at 24 months of age (Figure 1A).

## Outcomes

The primary outcome for this analysis was cumulative *Mtb* infection by 24 months with any positive *Mtb* infection test result (TST or QFT-Plus at trial end at 12 months postenrollment, or TST at end of observational follow-up at 24 months of age) considered “ever positive.” Participants with at least 1 *Mtb* infection assessment at 12 and/or 24 months were evaluated for the primary outcome of cumulative 24-month *Mtb* infection.

## Statistical Analysis

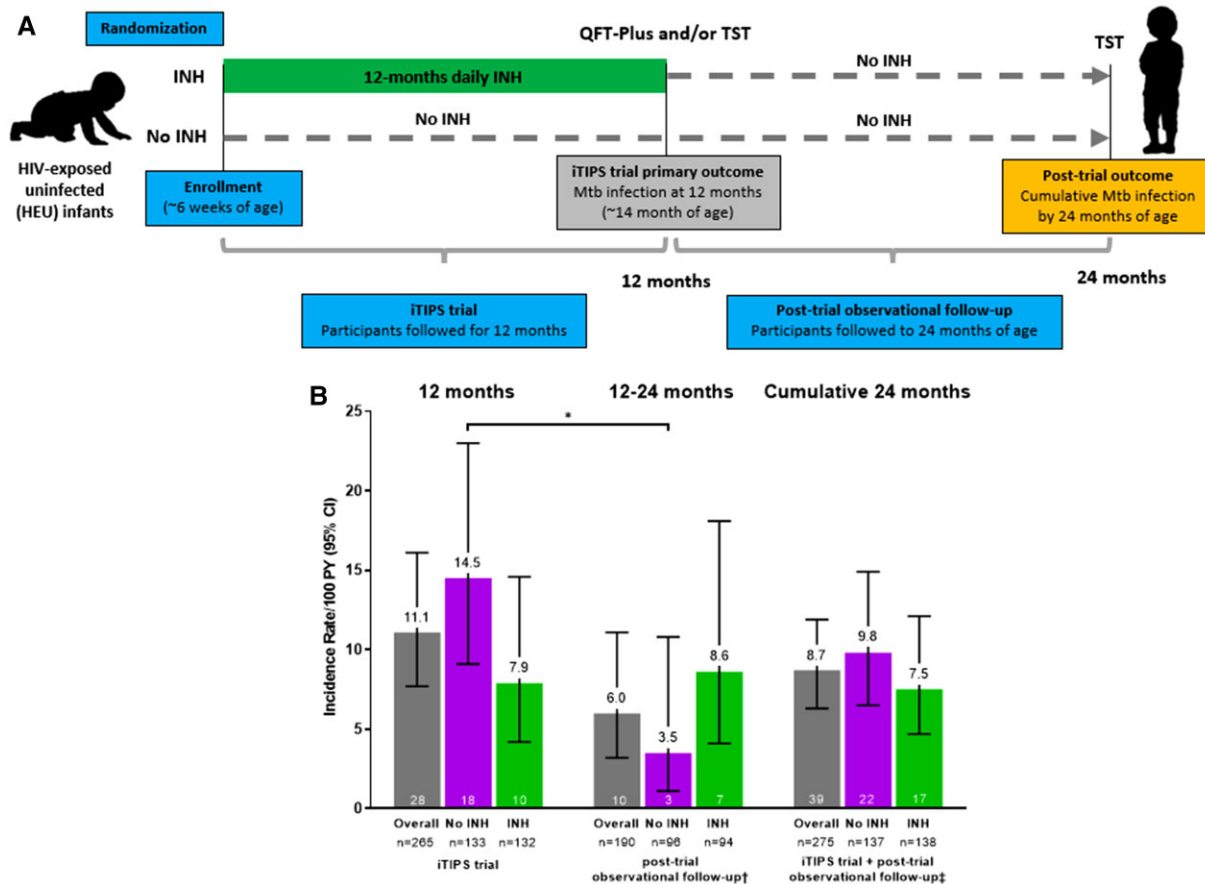
We compared *Mtb* infection incidence between trial arms and between trial and posttrial observational periods using Cox proportional hazards regression among children with at least 1 *Mtb*

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**Figure 1.** Study design of the infant TB Prevention Study and posttrial observational follow-up and incidence of *Mycobacterium tuberculosis* (*Mtb*) infection among human immunodeficiency virus–exposed but uninfected children. *A*, Study design. *B*, *Mtb* infection incidence at trial end, posttrial observational follow-up, and cumulative 24 months. Incidence is among children with at least 1 test for *Mtb* infection. \* $P < .02$ . †Among children with negative 12-month *Mtb* infection results. ‡Includes 10 children with 24-month-only *Mtb* infection testing. Abbreviations: CI, confidence interval; HEU, human immunodeficiency virus exposed but uninfected; HIV, human immunodeficiency virus; INH, isoniazid; iTIPS, infant TB Prevention Study; *Mtb*, *Mycobacterium tuberculosis*; PY, person-years; QFT-Plus, Quantiferon Plus; TST, tuberculin skin test.

infection assay result. Timing of infection was estimated to have occurred at 6 months for children with a positive test at 12 months postenrollment, 18 months for children with a negative test at 12 months postenrollment but positive at 24 months, and 12 months for children missing 12-month *Mtb* infection assessment but positive at 24 months. Infants without a positive test were censored at their last negative test.

Correlates of cumulative 24-month *Mtb* infection were evaluated using Cox proportional hazards regression. For infants with TST results at both timepoints, TST conversions (negative [ $<10$  mm] to positive [ $\geq 10$  mm]) and reversions (positive to negative) were calculated and compared between trial arms with generalized linear models. All hypothesis testing was 2-sided. Analyses were conducted in Stata (version 15) and GraphPad Prism (version 9.3.1) software.

#### Ethical Considerations

Caregivers provided written informed consent for the parent trial and extended observational follow-up. Study procedures

were approved by the University of Washington Institutional Review Board, Kenyatta National Hospital/University of Nairobi, Jaramogi Oginga Odinga Teaching and Referral Hospital Ethics and Research Committees, and Kenya Pharmacy and Poisons Board. The trial is registered at ClinicalTrials.gov (NCT02613169).

#### RESULTS

Between August 2016 through June 2018, 300 infants 6–10 weeks of age were enrolled and randomized to 12 months of INH or no INH (150 per arm); 275 (92%, 138 INH, 137 no INH) infants had at least 1 *Mtb* infection assessment by 24 months (12 and/or 24 months) (Supplementary Figure 1). Baseline characteristics of participants with at least 1 *Mtb* infection assessment were similar between arms (Supplementary Table 1).

*Mtb* infection incidence at trial end among 265 children with QFT and/or TST results was 11.1 per 100 person-years (PY)

(95% confidence interval [CI], 7.7–16.1) (INH vs no INH, 7.9 vs 14.5/100 PY; hazard ratio [HR], 0.55 [95% CI, .25–1.20];  $P = .13$ ) (Supplementary Table 2A) and driven by TST positivity (25 were TST positive and 3 were QFT-Plus positive with no overlap in positive results). Among 187 children with TST results at trial end, there was a trend for decreased TST positivity among children randomized to INH (INH: 8.6% [8/93] vs no INH: 18.1% [17/94]; 9.0 vs 19.8/100 PY; HR, 0.47 [95% CI, .20–1.08];  $P = .08$ ) (Supplementary Table 3A).

Two-hundred twenty-eight children completed observational follow-up; 8.3% (19) were TST positive at 24 months of age: 6.8% (8/110) in the INH and 10.0% (11/118) in the no INH arm (relative risk [RR], 1.48 [95% CI, .61–3.54];  $P = .38$ ).

Of 275 children with at least 1 *Mtb* infection assessment at 12 and/or 24 months, 14.2% (39) were positive (QFT-Plus positive, 3/39; TST positive, 36/39). Cumulative *Mtb* infection incidence up to 24 months of age was 8.7/100 PY and similar between arms (INH vs no INH: 7.5 vs 9.8/100 PY; HR, 0.75 [95% CI, .40–1.42];  $P = .382$ ) (Figure 1B and Supplementary Table 2A). In the posttrial observational period, *Mtb* infection incidence was 6.0/100 PY and lower in the no INH arm, though not significantly (INH vs no INH: 8.6 vs 3.5/100 PY; HR, 2.44 [95% CI, .63–9.46];  $P = .196$ ) (Supplementary Table 2B). There was a trend for increased *Mtb* infection incidence during the trial (12 months) vs posttrial period (12–24 months) (11.1 vs 6.0/100 PY; HR, 2.00 [95% CI, .97–4.12];  $P = .06$ ). Children in the no INH arm had 4.5-fold increased *Mtb* infection incidence in the trial vs posttrial period (14.5 vs 3.5/100 PY; HR, 4.45 [95% CI, 1.31–15.12];  $P = .017$ ). Analyses restricted to TST had similar results, with statistically significant increases in TST positivity in the trial vs posttrial period overall and among children randomized to no INH (Supplementary Table 3A and 3B). Cumulative *Mtb* infection was associated with TB exposure during the study and lack of indoor plumbing (a proxy for socioeconomic status) (Supplementary Table 4).

Among 162 infants with TST results at both 12 and 24 months, 4.9% (8) remained persistently positive, 4.3% (7) converted from negative to positive, and 10.5% (17) reverted from positive to negative (Supplementary Figure 2). While rates of posttrial TST conversions were similar between arms (no INH: 2/83 [2.4%] vs INH: 5/79 [6.3%]; RR, 0.4 [95% CI, .08–2.2];  $P = .30$ ), children randomized to no INH were more likely to have TST reversions (no INH: 13/83 [15.7%] vs INH: 4/79 [5.1%]; RR, 3.4 [95% CI, 1.04–10.8];  $P = .04$ ), partially reflecting more positive TST results at trial end in the no INH arm. Restricting analysis to TST-positive participants at trial end with subsequent 24-month assessment, participants in the no INH arm still had more frequent reversions than the INH arm (13/17 [76.5%] vs 4/8 [50.0%]; RR, 1.53 [95% CI, .72–3.26];  $P = .27$ ), but this difference was not statistically significant.

## DISCUSSION

In this extended observational follow-up of an infant *Mtb* infection prevention trial among HEU children, 24-month cumulative *Mtb* infection incidence (measured primarily by TST) was substantial (8.6/100 PY) despite high uptake of maternal programmatic isoniazid preventive therapy (IPT) and few reports of TB exposure. The trend for decreased TST positivity among infants randomized to INH at trial end was not sustained during observational follow-up to 24 months. *Mtb* infection incidence was higher in the first vs second year of life, most clearly demonstrated among children not receiving INH who had a 4.5-fold higher risk of *Mtb* infection at 12 compared to 24 months. Cumulative 24-month infection was associated with report of TB exposure and lack of indoor plumbing.

This study provides one of the few estimates of *Mtb* infection among HEU infants with longitudinal follow-up. *Mtb* infection incidence (measured by TST) was comparable in a South African birth cohort (11.8/100 PY), in which, in contrast, only 22% of mothers had HIV, maternal IPT was not reported, and TB exposure was not an exclusion criterion [9]. Most TST positivity occurred before 1 year of age, with a similar decline in incidence in the second year of life (16.5/100 PY at 12 months vs 5.1/100 PY between 12 and 24 months). Higher incidence in the first year of life is likely due to increased susceptibility in young children to *Mtb* infection after exposure and is in alignment with natural history studies in the prechemotherapy era which found that children <1 year of age were at highest risk of developing TB after exposure, with risk dropping in subsequent years [10]. Conversely, our estimates are lower than reported among HEU infants evaluated cross-sectionally by TST at 12–24 months of age in Uganda (26%), in which 70% of mothers were on antiretroviral therapy without reported maternal IPT use [5].

Our cumulative 24-month estimates may have underestimated or overestimated *Mtb* infection incidence as half of the children enrolled in the parent trial received INH throughout the first year of life and we relied primarily on TST, an imperfect measure of *Mtb* infection with potential BCG cross-reactivity [11]. The observed correlation between TB exposure and TST positivity suggests that TST (with its limitations) is a reliable measure of *Mtb* infection outcome in this young infant population. An alternate interpretation for our findings is INH reduced BCG-induced TST positivity during the trial, with waning effect in the posttrial observational period. In recent evaluation, infant mycobacteria-specific T-cell responses at 6 weeks correlated with 12-month TST induration among those in the no-IPT arm which was attenuated in those in the INH arm. Delayed trial TST implementation may have led to underestimation of *Mtb* infection at 12 months. However, we found higher incidence of *Mtb* infection at 12 months than at 24 months when there was wider TST implementation,

suggesting that this did not compromise our estimates. Future assessment of T-cell responses at the 24-month timepoint may enable us to disentangle BCG- vs TB-specific responses [12].

In summary, risk of *Mtb* infection is substantial among HEU infant in high-TB-burden areas, particularly during the first year of life. Durable interventions targeted specifically for HEU children at greatest risk of progression to TB disease during early life will remain important, even with availability of community-level HIV treatment and TB prevention.

### Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

**Author contributions.** G. J.-S., B. A. R., J. K., and S. M. L. designed the parent randomized clinical trial. G. J.-S., B. A. R., and S. M. L. were responsible for the statistical design of the trial and this analysis. G. J.-S. is the principal investigator and protocol chair and T. R. H. is the immunology principal investigator. J. K. is the protocol co-chair and site principal investigator. S. M. L. and J. N. E. performed the data analysis. J. M. managed study data and J. M. and N. C. conducted interim analyses. S. M. L. wrote the initial draft of the manuscript. S. M. L., D. M., A. W., J. K., J. M., and G. J.-S. participated in clinical cohort implementation. All authors read and approved the manuscript.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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