Tuberculin skin test conversion and primary progressive tuberculosis disease in the first 5 years of life: a birth cohort study from Cape Town, South Africa

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Introduction

Tuberculosis is a leading cause of global childhood mortality. However, the epidemiology and burden of tuberculosis in infancy is not well understood. We aimed to investigate tuberculin skin test conversion and tuberculosis in the Drakenstein Child Health study, a South African birth cohort in a community in which tuberculosis incidence is hyperendemic.

Methods

In this prospective birth cohort study, we enrolled pregnant women older than 18 years who were between 20 and 28 weeks’ gestation and who were attending antenatal care in a peri-urban, impoverished South African setting. We followed up their children for tuberculosis from birth until April 1, 2017, or age 5 years. All children received BCG vaccination at birth. Tuberculin skin tests were administered to children at 6, 12, 24, 36, 48, and 60 months of age, and at the time of a lower respiratory tract infection. An induration reaction of 10 mm or more was considered to be a tuberculin skin test conversion. To prevent boosting, we censored children with a reactive, negative tuberculin skin test.

Findings

Among 915 mother–child pairs (201 [22%] HIV-positive mothers and two [<1%] HIV-positive children), 147 (16%) children had tuberculin skin test conversion, with increasing cumulative hazard with age (0.08 at 6 months, 0.17 at 12 months, 0.22 at 24 months, and 0.37 at age 36 months). For every 100 child-years, the incidence was 11.8 (95% CI 10.0–13.8) for tuberculin skin test conversion, 2.9 (2.4–3.7) for all diagnosed tuberculosis, and 0.7 (0.4–1.0) for microbiologically confirmed tuberculosis. Isoniazid preventive therapy was effective in averting disease progression (adjusted hazard ratio 0.22, 95% CI 0.08–0.63; p<0.0001). Children with a lower respiratory tract infection were significantly more likely to also have tuberculosis than were those without one (2.27, 1.42–3.62; p<0.0001).

Interpretation

Greater focus should be placed on the first years of life as a period of high burden of transmission and clinical expression of tuberculosis infection and disease. Multifaceted interventions, such as isoniazid preventive therapy and tuberculosis screening of infants with LRTIs, beginning early in life, are needed in high-burden settings.

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We aimed to investigate tuberculin skin test conversion and tuberculosis in infants and young children from the Drakenstein Child Health study, a South African birth cohort in a community with a high tuberculosis burden.13

Methods
Study design and participants
In this prospective birth cohort study, we enrolled pregnant women who were between 20 and 28 weeks’ gestation and attending antenatal care in Paarl, a peri-urban setting outside of Cape Town, South Africa.14 In 2015, tuberculosis incidence in this area was estimated to be 880 new cases per 100 000 population.13 Participants were recruited from two clinics, TC Newman and Mbekweni, which are a few miles apart. Both clinics serve impoverished, heterogeneous communities. People attending TC Newman are of admixed ancestry, whereas the Mbekweni clinic serves mostly a black, Xhosa population. All infants were given BCG vaccination at birth, per national policy. All mothers accessed care in the public sector, which has a strong primary health-care programme, including an effective mother-to-child HIV prevention and antiretroviral therapy programme.

Women were followed up through pregnancy and childbirth, and newborn infants were followed up into early childhood, up to age 5 years. Exclusion criteria for pregnant women were being younger than 18 years and intending to leave the area within 1 year.

We also identified maternal smoking during pregnancy and lower respiratory tract infection as novel risk factors for paediatric tuberculosis, and male sex as a risk factor for tuberculin conversion.

Implications of all the available evidence
Results from this South African birth cohort study indicate that greater focus should be placed on the first years of life as a period of high burden of transmission and clinical expression of tuberculosis. The high rates of tuberculosis-related outcomes are alarming and suggest a considerable burden of unidentified transmission and undiagnosed tuberculosis disease among infants and young children in high-burden settings. There is an urgent need to review global guidelines on the management of paediatric tuberculosis in high-burden settings. Re-examination of guidelines should include consideration of integrating paediatric lower respiratory tract infection and tuberculosis control programmes. Tuberculosis infection and disease screening when infants present to primary care clinics with lower respiratory tract infections could identify a high number of undiagnosed paediatric cases. Scale-up of preventive therapy to infants with primary infection or at high risk for disease progression, such as tuberculosis-exposed or HIV-infected children, is essential to help decrease childhood mortality in sub-Saharan Africa.

Figure 1: Study flow
*Loss of pregnancy due to miscarriage, stillbirth, or intrauterine death (23 infants [including one set of twins]). †Including four pairs of twins and one set of triplets. ‡No postnatal data collected. §Participants did not have a valid tuberculin skin test result, most commonly because of national tuberculin shortages, but also because mothers and infants did not return for the induration reading.
We obtained ethics approval from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (reference numbers 401/2009 and 651/2013) and the Provincial Child Health Research Committee. Mothers provided written informed consent at enrolment and verbal assent for infants, which was renewed annually.

### Procedures

Comprehensive questionnaires about maternal health were administered at enrolment and antenatal data were collected concurrently. Detailed birth information was obtained at delivery. Obstetric care and all births took place at the regional hospital in Paarl. Follow-up visits, including clinical examinations, were done at 6, 12, 24, 36, 48, and 60 months of age. Data for environmental exposures, household characteristics, respiratory risk factors, anthropometry, and child symptoms were obtained at scheduled visits. Missed visits were rebooked with a study mobile phone network system or by study community-based fieldworkers. Mothers were counselled about respiratory symptoms at every visit and advised to attend the study site or contact study staff between scheduled study visits whenever the child developed cough or difficulty breathing. 

### Socioeconomic status

Socioeconomic status comprised a comprehensive composite of asset ownership, household income, employment, and education.14 HIV tests were given to all mothers during pregnancy. Adults were tested with Abbott Determine HIV 1/2 rapid HIV antibody test (Abbott Laboratories, North Chicago, IL, USA). If positive, a confirmatory enzyme-linked immunosorbent assay was done. Infants of HIV-positive mothers were tested with DNA PCR (Cobas Amplicrep system, Roche Molecular Systems, Branchburg, NJ, USA) at age 6 weeks, and 6 weeks after the end of breastfeeding. Children were re-tested at 18 months with the rapid antibody test.

#### Maternal characteristics

- **Age (years)**: 24·9 (21·4 to 29·7) for TC Newman (n=420), 27·2 (22·6 to 32·2) for Mbekweni (n=495), and 26·3 (22·1 to 31·0) for Total (n=915).
- **Married or cohabitating**: 380 (43%) for TC Newman, 186 (38%) for Mbekweni, and 366 (40%) for Total.
- **Tuberculosis treatment during pregnancy**: 20 (5%) for MBH, 21 (4%) for MBH, and 41 (4%) for Total.
- **Ever diagnosed with tuberculosis before pregnancy**: 13 (3%) for MBH, 25 (5%) for MBH, and 38 (4%) for Total.
- **Maternal smoking during pregnancy**: 195 (46%) for MBH, 23 (5%) for MBH, and 218 (24%) for Total.
- **Maternal education**: 102 (24%) for MBH, 112 (27%) for MBH, and 214 (23%) for Total.

#### Infant characteristics

- **Sex**: 230 (55%) for Male, 218 (48%) for Female, and 468 (51%) for Total.
- **Birthweight (kg)**: 3·0 (2·6 to 3·4) for TC Newman, 3·2 (2·8 to 3·5) for Mbekweni, and 3·1 (2·7 to 3·4) for Total.
- **Low birthweight (<2·5 kg)**: 72 (17%) for TC Newman, 63 (13%) for Mbekweni, and 135 (15%) for Total.
- **Birthweight (kg)**: 3·0 (2·6 to 3·4) for TC Newman, 3·2 (2·8 to 3·5) for Mbekweni, and 3·1 (2·7 to 3·4) for Total.
- **Low birthweight (<2·5 kg)**: 72 (17%) for TC Newman, 63 (13%) for Mbekweni, and 135 (15%) for Total.
- **Height**: 114 (27%) for TC Newman, 92 (19%) for Mbekweni, and 206 (22%) for Total.
- **Missing data**: 7 (2%) for TC Newman, 1 (<1%) for Mbekweni, and 8 (1%) for Total.

#### Household income (rand per month)

- **<1000**: 144 (34%) for TC Newman, 210 (42%) for Mbekweni, and 354 (39%) for Total.
- **1000–5000**: 210 (50%) for TC Newman, 232 (47%) for Mbekweni, and 442 (48%) for Total.
- **>5000**: 66 (16%) for TC Newman, 48 (10%) for Mbekweni, and 114 (12%) for Total.

(Table 1 continues on next page)
less than −2. Normal weight was −2 to 2, and overweight was a score greater than 2.

Tuberculin skin tests were done at the 6-month visit and then at 12, 24, 36, 48, and 60 months of age, and at the time of a lower respiratory tract infection. Tuberculin skin test conversion was defined as an induration reaction greater than or equal to 10 mm, to minimise the risk of misclassification due to BCG vaccination or exposure to environmental mycobacteria, as recommended by WHO and South Africa’s Department of Health.\textsuperscript{9,17} To prevent misinterpretation of boosted skin-test reactions due to recurrent tuberculin skin testing as tuberculosis infection, children with a reactive but negative skin test (1–9 mm) were not given another test, and were censored for the tuberculin skin test conversion analysis at that point in time. Because of the high number of censored skin tests before age 48 months, we excluded tuberculin skin tests taken after 36 months of age. Children with positive skin tests were referred to local tuberculosis clinics for isoniazid preventive therapy; however, the study investigators could not enforce that this was prescribed.

Children were followed up for tuberculosis from birth until April 1, 2017, or age 5 years. Trained study staff collected induced sputum specimens in duplicate for tuberculosis culture and mycobacterial PCR investigation (Xpert MTB/RIF; Cepheid, Sunnyvale, CA, USA) from all children with a tuberculin skin test induration of at least 10 mm, and from children who were suspected to have or had been diagnosed with tuberculosis by local health services. A chest radiograph was taken in all children with suspected pulmonary tuberculosis. Tuberculosis was diagnosed by experienced physicians and nurses in local tuberculosis clinic services. A chest radiograph was read and reported by an experienced tuberculosis clinician. We compared results using three different definitions of tuberculosis: all tuberculosis cases (clinically, radiographically suggestive, or microbiologically confirmed cases); cases that were microbiologically confirmed or radiographically suggestive only; and microbiologically confirmed cases only (positive Xpert MTB/RIF or sputum culture).

### Statistical analysis

Mother–child pairs were included in this analysis if they had at least one tuberculin skin test. For exploratory data analysis, we summarised continuous variables as medians with IQRs, and categorical variables using proportions.

For tuberculin skin test conversion, time-to-event was determined by the date on which the child was diagnosed with tuberculosis. Follow-up was censored at a reactive skin test, age 3 years. For tuberculosis, time-to-event was determined when a child was diagnosed with tuberculosis. Follow-up was censored at age 5 years.

| Tuberculin conversion events (n) | Participants (n) | Child-years of observation (n) | Incidence per 100 child-years (95% CI) | HR (95% CI, p value) |
|--------------------------------|------------------|-------------------------------|--------------------------------------|----------------------|
| All participants               | 147              | 915                           | 1248.7                               | 11.8 (10.0–13.8)     | --                   |
| Child characteristics          |                  |                               |                                      |                      |
| Sex                            |                  |                               |                                      |                      |
| Female                         | 61               | 447                           | 647.7                                | 9.4 (7.3–12.1)       | 1 (ref)              |
| Male                           | 86               | 468                           | 600.1                                | 14.3 (11.6–17.7)     | 1.53 (1.10–2.13, p=0.011) |
| Birthweight*                   |                  |                               |                                      |                      |
| Normal                         | 126              | 780                           | 1071.3                               | 11.9 (9.9–14.0)      | 1 (ref)              |
| Low                            | 21               | 135                           | 176.5                                | 11.7 (9.7–18.2)      | 1.00 (0.63–1.58, p=0.99) |
| Gestational age†               |                  |                               |                                      |                      |
| Full term                      | 124              | 768                           | 1042.3                               | 11.9 (10.0–14.2)     | 1 (ref)              |
| Preterm                        | 23               | 147                           | 205.5                                | 11.2 (7.4–16.8)      | 0.93 (0.60–1.45, p=0.75) |
| Lower respiratory tract infection |                |                               |                                      |                      |
| No                             | 74               | 475                           | 660.4                                | 11.2 (8.9–14.1)      | 1 (ref)              |
| Yes                            | 73               | 440                           | 587.4                                | 12.4 (9.9–15.6)      | 1.12 (0.81–1.54, p=0.51) |
| HIV-positive                   |                  |                               |                                      |                      |
| No                             | 147              | 913                           | 1246.0                               | 11.8 (10.0–13.9)     | --                   |
| Yes                            | 0                | 2                             | 1.8                                  | --                   | --                   |
| Weight-for-age Z score†        |                  |                               |                                      |                      |
| Underweight                    | 11               | 84                            | 101.2                                | 10.9 (6.0–19.6)      | 0.86 (0.46–1.58, p=0.62) |
| Normal weight                  | 134              | 812                           | 1111.4                               | 12.1 (10.2–14.3)     | 1 (ref)              |
| Overweight                     | 1                | 15                            | 27.0                                 | 3.7 (0.5–26.3)       | 0.31 (0.44–2.24, p=0.25) |
| Feeding choice                 |                  |                               |                                      |                      |
| Did not breastfeed             | 5                | 65                            | 85.6                                 | 5.8 (2.4–14.0)       | 1 (ref)              |
| Breastfed                      | 142              | 850                           | 1162.2                               | 12.2 (10.4–14.4)     | 1.98 (0.81–4.21, p=0.13) |

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| Tuberculin conversion events (n) | Participants (n) | Child-years of observation (n) | Incidence per 100 child-years (95% CI) | HR (95% CI, p value) |
|--------------------------------|------------------|-------------------------------|--------------------------------------|----------------------|
| TC Newman (n=420) Mbekweni (n=495) Total (n=915) |
| Housing                        |                  |                               |                                      |                      |
| Shack or hokkie                | 124 (30%)        | 202 (41%)                     | 326 (36%)                            |                      |
| House or flat                  | 296 (70%)        | 293 (59%)                     | 589 (64%)                            |                      |
| Crowding (people per household) | 5 (4 to 7)       | 4 (3 to 6)                    | 4 (3 to 6)                           |                      |
| ≥3                             | 92 (22%)         | 205 (41%)                     | 297 (32%)                            |                      |
| 4–5                            | 169 (40%)        | 144 (29%)                     | 313 (34%)                            |                      |
| ≥7                             | 157 (37%)        | 146 (29%)                     | 303 (33%)                            |                      |
| Missing data                   | 2 (1%)           | 0                             | 2 (<1%)                              |                      |
| Children younger than 5 years per household |                  |                               |                                      |                      |
| 0                              | 252 (60%)        | 311 (63%)                     | 563 (62%)                            |                      |
| 1                              | 139 (33%)        | 152 (31%)                     | 291 (32%)                            |                      |
| >1                             | 29 (7%)          | 32 (6%)                       | 61 (7%)                              |                      |
| Household exposure to tuberculosis in the year before first study visit |                  |                               |                                      |                      |
| 0                              | 40 (10%)         | 88 (18%)                      | 128 (14%)                            |                      |

Data are n (%) or median (IQR). Column totals vary across different characteristics because of missing values for some participants. *Self-reported smoking status at the baseline study visit; self-reported smoking was highly correlated with maternal cotinine levels.

Table 1: Sociodemographic and clinical characteristics of included mother-child pairs, by clinic
### Household characteristics

| Socioeconomic status (quartile) | 
|---------------------------------|---|
| Highest                         | 35 206 284.9 12 3 (8-17.4) 1 (ref) |
| Low                             | 45 233 333.7 14 3 (10-19.2) 1.14 (0.73-1.77, p=0.56) |
| Moderate low                    | 32 242 331.9 9 6 (6-13.6) 0.82 (0.48-1.25, p=0.30) |
| Moderate high                   | 35 226 303.8 11 5 (8-16.0) 0.93 (0.58-1.48, p=0.75) |
| Clinic                          | 
| Mbekweni                        | 72 495 681.6 10 6 (8-13.3) 1 (ref) |
| TC Newman                       | 75 420 566.2 13 2 (10-16.6) 1.24 (0.90-1.71, p=0.19) |
| Household income (rand per month) | 
| >5000                           | 16 114 166.3 9 6 (5-15.7) 1 (ref) |
| 1000-5000                       | 69 442 627.1 11 0 (8-13.9) 1.67 (0.62-1.85, p=0.80) |
| <1000                           | 62 354 454.4 13 6 (10-17.5) 1.34 (0.77-2.32, p=0.30) |
| Crowding (people per household) | 
| ≤3                              | 44 297 412.4 10 7 (7-14.3) 1 (ref) |
| 4-5                             | 41 313 438.3 9 4 (6-12.7) 0.86 (0.56-1.32, p=0.49) |
| >5                              | 62 303 396.1 15 7 (12-20.1) 1.43 (0.97-2.11, p=0.067) |
| Children younger than 5 years per household | 
| 0                               | 80 563 776.6 10 3 (8-12.8) 1 (ref) |
| 1                               | 54 291 406.0 13 3 (10-17.4) 1.31 (0.93-1.85, p=0.13) |
| ≥1                              | 13 61 65.3 19 9 (11-34.3) 1.81 (1.00-3.25, p=0.048) |
| Patient with tuberculosis in household in past year | 
| No                              | 131 779 1075.5 12 2 (10-14.5) 1 (ref) |
| Yes                             | 13 128 162.6 8 0 (4-13.8) 0.65 (0.37-1.15, p=0.14) |

Missing data are detailed in table 1. HR=hazard ratio. *Normal birthweight is 2·5 kg or more and low birthweight is less than 2·5 kg. †Full term is 37 weeks or more and preterm is less than 37 weeks. ‡Some data are missing for this variable. §Self-reported smoking status at the baseline study visit; self-reported smoking was highly correlated with maternal cotinine levels.

Table 2: Tuberculin skin test conversion in children, stratified by child, maternal, and household characteristics

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We investigated the timing of lower respiratory tract infections and tuberculosis diagnoses to determine whether case ascertainment bias could possibly explain an association seen between the two diseases. We assumed case ascertainment was likely if diagnosis of both diseases occurred simultaneously in children with multiple diagnoses. To investigate this, among children diagnosed with both diseases during the study period, we calculated the proportion diagnosed with a lower respiratory tract infection and tuberculosis less than 2 weeks apart.

We used two-sided p values and 95% CIs to assess statistical significance in all models. The likelihood ratio test was used to derive all p values from Cox regression models. We did all analyses with Stata (version 14.1).

**Role of the funding source**

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Results**

Between March 5, 2012, and March 31, 2015, 1225 pregnant women were recruited and enrolled in the birth cohort (figure 1). Of 1143 livebirths, 68 (6%) were excluded because of perinatal death or study termination, and 160 infants (14%) were enrolled but did not have a valid tuberculin skin test, predominantly because of national and global shortages of tuberculin. Thus,
915 infants with tuberculin skin test results and follow-up for active disease were included in our analysis. The characteristics of excluded infants were largely similar to those of included infants, except that mothers of excluded infants were more likely to be young, have a high income, and were less likely to have ever been previously diagnosed with tuberculosis (appendix).

Although 201 (22%) mothers were HIV-seropositive, only two (<1%) infants tested were HIV-positive, because of an effective antenatal antiretroviral therapy programme (table 1). These HIV-positive infants were breastfed but did not have household tuberculosis exposure (appendix). 850 (93%) women breastfed their newborn infants. Household tuberculosis exposure in the year before the primary study visit occurred in 128 (14%) of all surveyed households, and was almost twice as frequent at Mbekweni than at TC Newman (18% vs 10%, p<0.003). 440 (48%) of 915 infants had at least one lower respiratory tract infection; of 440 cases, 100 (23%) led to hospital admission and 101 (23%) were classified as severe.

For the tuberculin skin test conversion analysis, 915 children accrued 1248.7 years of follow-up (table 2). In total, 147 children (16%) had a positive result and most of these conversions occurred before age 1 year (appendix). All of these children were referred to local clinics for isoniazid preventive therapy, but only 33 (22%) were medically registered as receiving the treatment. The incidence of tuberculin skin test conversion was 11.8 (95% CI 10.0–13.8) per 100 child-years. The cumulative hazard of conversion was high 6 months after birth (0.08) and consistently increased with age to 0.17 after 12 months, 0.22 after 24 months, and 0.37 after 36 months (figure 2; appendix).

Univariable Cox regression of tuberculin conversion and tuberculosis results is shown in tables 2 and 3. In univariable analyses, boys and children in households with more than one child younger than 5 years had significantly increased risk of tuberculin skin test conversion (table 2). A larger proportion of infants exposed to maternal smoking during pregnancy had tuberculosis conversion (table 2). A larger proportion of infants with more than five people in the household had increased risk of tuberculin skin test conversion. The HR was 1.6 (95% CI 1.2–2.2), and incidence of microbiologically confirmed tuberculosis was 2.9 (95% CI 2.4–3.7), incidence of microbiologically confirmed or radiographically suggestive tuberculosis was 1.6 (95% CI 1.2–2.2), incidence of microbiologically confirmed tuberculosis was 0.7 (95% CI 0.4–1.0). Cumulative hazard at the end of follow-up was 0.10 for all diagnosed disease and 0.02 for microbiologically confirmed disease, and increased most in the first 3 years of life (figure 2; appendix). Among participants who had a tuberculin skin test conversion, the hazard of diagnosed paediatric tuberculosis was approximately 0.4 in infancy (<1 year old) and decreased substantially to less than 0.1 by age 2 years.

In the univariable analysis, risk factors for diagnosed tuberculosis included male sex, maternal smoking during pregnancy, TC Newman clinic, households with more than five people, and a lower respiratory tract infection (table 3). Tuberculosis risk increased with the number of lower respiratory tract infections, regardless of the diagnostic definition used for tuberculosis (p<0.0001; figure 3; appendix). The Cox model was identifiable after applying the random effects, and the proportional hazards assumption was met for all Cox regression analyses of both outcomes, except for socioeconomic status (both outcomes) and crowding for tuberculosis (appendix).

In multivariable analysis, risk factors for diagnosed tuberculosis that remained significant were tuberculin...
### Table 3

| HR (95% CI, p value) | Disease events* (n) | Participants (n) | Child-years of observation | Incidence per 100 child-years (95% CI) |
|---------------------|---------------------|------------------|----------------------------|-------------------------------------|
| **All participants** | 81                  | 915              | 2736.8                     | 2.9 (2.4–3.7)                       |
| **Child characteristics** |                  |                  |                            |                                    |
| **Sex**             |                     |                  |                            |                                    |
| Male                | 49                  | 468              | 1381.5                     | 3.5 (2.7–4.7)                       |
| Female              | 32                  | 447              | 1355.3                     | 2.4 (1.7–3.3)                       |
| **Birthweight†**    |                     |                  |                            |                                    |
| Normal              | 64                  | 780              | 2331.1                     | 2.7 (2.1–3.5)                       |
| Low                 | 17                  | 135              | 405.8                      | 4.2 (2.6–6.7)                       |
| **Gestational age‡**|                     |                  |                            |                                    |
| Full term           | 65                  | 768              | 2298.0                     | 2.8 (2.2–3.6)                       |
| Preterm             | 16                  | 147              | 438.8                      | 3.6 (2.2–6.0)                       |
| **Lower respiratory tract infection** |             |                  |                            |                                    |
| No                  | 27                  | 475              | 1502.0                     | 1.8 (1.2–2.6)                       |
| Yes                 | 54                  | 440              | 1234.9                     | 4.4 (3.3–5.7)                       |
| **HIV status**      |                     |                  |                            |                                    |
| Negative            | 80                  | 913              | 2731.8                     | 2.9 (2.4–3.6)                       |
| Positive            | 1                   | 2                | 5.1                        | 198 (28–1000)                      |
| **Weight-for-age Z score§** |                |                  |                            |                                    |
| Underweight         | 7                   | 84               | 244.6                      | 2.9 (1.4–6.0)                       |
| Normal weight       | 73                  | 812              | 2437.9                     | 3.0 (2.4–3.8)                       |
| Overweight          | 1                   | 15               | 41.1                       | 2.4 (0.3–17.3)                      |
| **Breastfeeding**   |                     |                  |                            |                                    |
| Did not breastfeed  | 2                   | 65               | 202.4                      | 1.0 (0.2–4.0)                       |
| Breastfed           | 79                  | 850              | 2534.4                     | 3.1 (2.5–3.9)                       |
| **Isoniazid preventive therapy** |              |                  |                            |                                    |
| Among converters    | 43                  | 117              | 261.6                      | 16.4 (12.2–22.2)                    |
| No                  | 2                   | 33               | 110.1                      | 1.8 (0.0–7.3)                       |
| Yes                 | 33                  | 737              | 2721.1                     | 1.5 (1.0–2.0)                       |
| Among non-converters |                    |                  |                            |                                    |
| No                  | 3                   | 28               | 941                        | 3.2 (1.0–9.9)                       |
| Yes                 | 3                   | 28               | 941                        | 3.2 (1.0–9.9)                       |
| **Maternal characteristics** |               |                  |                            |                                    |
| Age (years)         | --                  | --               |                            | 0.98 (0.94–1.02, p=0.37)            |
| Tuberculosis treatment in pregnancy |                 |                  |                            |                                    |
| No                  | 75                  | 874              | 2627.0                     | 3.0 (2.3–3.6)                       |
| Ever diagnosed with tuberculosis § |             |                  |                            | 1 (ref)                            |
| No                  | 79                  | 874              | 2619.3                     | 3.0 (2.4–3.8)                       |
| Yes                 | 2                   | 38               | 109.0                      | 1.8 (0.4–7.3)                       |
| Maternal smoking during pregnancy § |             |                  |                            | 0.59 (0.14–2.39, p=0.46)            |
| No                  | 46                  | 692              | 2097.4                     | 2.2 (1.6–2.9)                       |
| Yes                 | 34                  | 218              | 622.6                      | 5.5 (3.9–7.6)                       |
| Maternal education § |                     |                  |                            |                                    |
| Primary school only | 10                  | 73               | 218.4                      | 4.6 (2.5–8.5)                       |
| Some secondary school | 45              | 500              | 1486.4                     | 3.0 (2.3–4.1)                       |
| Finished secondary school | 26             | 342              | 1032.0                     | 2.5 (1.7–3.7)                       |

(Table 3 continues on next page)

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skin test conversion, maternal smoking during pregnancy, and a lower respiratory tract infection. Isoniazid preventive therapy was also effective in averting disease progression (table 4). When we confined tuberculosis definition to radiographically suggestive or microbiologically confirmed disease, tuberculosis skin test conversion and a lower respiratory tract infection remained significant predictive factors (table 4). The protective effect of isoniazid preventive therapy had a similar point estimate to that noted for all diagnosed tuberculosis cases, although the confidence intervals were wider (table 4). When including only microbiologically confirmed tuberculosis, the only factor that remained associated with tuberculosis was a lower respiratory tract infection (table 4). When restricting the analysis to only the TC Newman clinic, infants exposed to maternal smoking during pregnancy were at a higher risk of diagnosed tuberculosis, but this did not reach significance (adjusted HR 1.66, 0.96–2.86; appendix).

To explore the potential role of case ascertainment in determining the relation between tuberculosis and lower respiratory tract infections, we investigated the timing of a lower respiratory tract infection in relation to tuberculosis for each child with both diagnoses (n=55; appendix). In most cases, both diagnoses occurred at least 2 weeks apart, regardless of the method used to ascertain tuberculosis (41 [75%] of 55 with diagnosed tuberculosis, 26 [76%] of 34 with radiographically suggestive or microbiologically confirmed diagnoses, and 11 [65%] of 17 with microbiologically confirmed disease). Moreover, the diagnosis of tuberculosis occurred before that of the lower respiratory tract infection in 23 (42%) of 55 diagnosed tuberculosis cases, 16 (47%) of 34 radiographically suggestive or microbiologically confirmed diagnoses, and six (35%) of 17 microbiologically confirmed disease cases. It is thus unlikely that the association is due only to case ascertainment.

### Discussion

Few prospective studies have measured community-based incidence and prenatal and early-life risk factors for tuberculosis transmission and disease in high-burden areas. As a result, pragmatic approaches to guide prevention and management strategies for young children in these settings are absent. In this longitudinal birth cohort study of 915 infants and young children from South Africa, we noted a high burden of *Mycobacterium tuberculosis* infection and subsequent disease. The incidence for tuberculosis skin test conversion and primary progressive disease found in our study—equating to 2900 cases per 100 000 population per year—is alarming and suggests a considerable burden of unidentified transmission and undiagnosed tuberculosis among young children in this community. We also identified modifiable risk factors for these outcomes that have important practical implications, and which could
be used to reduce infections and prevent incident tuberculosis in children.

Incidences of both tuberculosis infection and disease in our cohort are among the highest reported in young children and are slightly higher than those reported in a study of South African infants enrolled in a vaccine trial.16 In that study, Quantiferon Gold In-Tube conversion after approximately 1 year was 7%, and incident tuberculosis was 2% for diagnosed disease (2% for microbiologically confirmed tuberculosis) measured over the subsequent 6–24 months. Although clinical overdiagnosis might have occurred in our study, we believe this is unlikely for two reasons. First, despite the low sensitivity of Xpert and sputum culture testing in young children, the incidence of microbiologically confirmed tuberculosis was 0·7 per 100 child-years (or 700 per 100000 population per year) and cumulative hazard was 0·02 over 3 years, both of which are high for this age group. Second, the proportion of children with a positive tuberculin conversion was also high, suggesting that many children in this setting acquire infection in the first 2–3 years of life, embodying a large pool at high risk for progressive disease. We found that the hazard of disease progression after primary infection was approximately 0·40 in the first year of life, reducing to 0·04 thereafter, resulting in an estimated risk of 0·7 per 1000 child-years in young children, the incidence of tuberculosis in children during follow-up, stratified by child, maternal, and household characteristics.

Table 2: Diagnosed tuberculosis in children during follow-up, stratified by child, maternal, and household characteristics

| Household characteristics | Disease events (n) | Participants (n) | Child-years of observation | Incidence per 100 child-years (95% CI) | HR (95% CI, p value) |
|---------------------------|-------------------|-----------------|---------------------------|---------------------------------------|---------------------|
| Socioeconomic status (quartile) |  |  |  |  |  |
| Lowest | 24 | 233 | 726 6 | 3 3 (2.2–4.9) | 1 (ref) |
| Moderate low | 23 | 242 | 712 2 | 3 2 (2.1–4.9) | 0·94 (0.53–2.42, p=0.83) |
| Moderate high | 18 | 226 | 656 6 | 2 7 (1.7–4.4) | 0·79 (0.43–1.46, p=0.46) |
| Highest | 15 | 206 | 614 9 | 2 4 (1.5–4.8) | 0·72 (0.38–1.37, p=0.32) |
| Clinic |  |  |  |  |  |
| Mbekweni | 26 | 495 | 1472 4 | 1 8 (1.2–2.6) | 1 (ref) |
| TC Newman | 55 | 420 | 1264 4 | 4 3 (3.3–1.7) | 2·55 (1·60–4.07, p<0.0001) |
| Household income (rand per month) |  |  |  |  |  |
| <2000 | 38 | 354 | 1114 1 | 3 4 (2.5–4.7) | 1 (ref) |
| 1000–5000 | 38 | 442 | 1261 2 | 3 0 (2.2–4.1) | 0·84 (0.53–1.31, p=0.43) |
| >5000 | 5 | 114 | 361 5 | 1·4 (0.6–3.3) | 0·41 (0.16–1.03, p=0.059) |
| Crowding (people per household) |  |  |  |  |  |
| ≤3 | 18 | 297 | 886 3 | 2 0 (1.3–3.7) | 1 (ref) |
| 4–5 | 28 | 313 | 940 6 | 3 0 (2.1–4.3) | 1·50 (0.83–2.70, p=0.18) |
| >5 | 35 | 303 | 904 2 | 3 9 (2.8–5.4) | 1·94 (1·10–3.42, p=0.022) |
| Children younger than 5 years per household |  |  |  |  |  |
| 0 | 42 | 563 | 1705 4 | 2·5 (1.8–3.3) | 1 (ref) |
| 1 | 31 | 291 | 851 5 | 3·6 (2.6–5.2) | 1·45 (0.91–2.13, p=0.12) |
| >1 | 8 | 61 | 179 9 | 4·4 (2.2–8.0) | 1·75 (0.82–3.73, p=0.15) |
| Patient with tuberculosis in household in past year |  |  |  |  |  |
| No | 64 | 779 | 2386 1 | 2·7 (2.1–3.4) | 1 (ref) |
| Yes | 15 | 128 | 329 5 | 4·6 (2·7–7·6) | 1·52 (0·86–2·67, p=0·15) |

Missing data are detailed in table 1. HR=hazard ratio. *Any tuberculosis diagnosis made in the clinic (clinically, radiographically, or microbiologically). Normal birthweight is ≥2·5 kg and low birthweight is less than 2·5 kg. †Normal smoking was highly correlated with maternal cotinine levels. ‡Full term is 37 weeks or more and preterm is less than 37 weeks. §Some data are missing for this variable. ¶Self-reported smoking status at the baseline study visit; self-reported smoking was highly correlated with maternal cotinine levels.
The increased risk for tuberculin skin test conversion in boys in our study might be explained by immunological or test-specific differences between sexes. 19-22 Infants of mothers who smoked during pregnancy had 69% greater risk of tuberculosis during follow-up than did unexposed infants. An association between passive smoking and tuberculosis-related outcomes has been shown in adults 23 and young children. 24 However, an association between childhood tuberculin skin test conversion was associated with familial smoking. 

Pulmonary tuberculosis is a severe, acute disease in infants younger than 3 years, and we have shown that a large proportion of children who had tuberculin conversion rapidly progressed to disease. 20,21 Isoniazid preventive therapy was effective in preventing tuberculosis. This finding supports previous findings 22,23 that preventive therapy is one of the most effective interventions to avert progressive disease in hyper-endemic settings. Follow-up of these children into the school years will allow us to assess whether preventive therapy provides long-term protection in young children living in such a high-burden setting. It is concerning that of the 147 children with a positive tuberculin skin test who were referred to local clinics for isoniazid preventive therapy, more than 70% were not prescribed isoniazid. This represents a missed opportunity for tuberculosis prevention. Strengthening of current prevention programmes to promote adherence to prophylaxis and development of new strategies for prevention are urgently needed.

Limitations of our study include challenges in the interpretation of longitudinal tuberculin skin test results in a high tuberculosis incidence area. Boosting through BCG vaccination or repeated skin tests could have led to false-positive conversion results. To address this issue, any infant with a positive skin test reaction of any size did not have a repeat skin test, and we acknowledge that dealing with boosting in this manner might have resulted in overestimating or underestimating overall conversion rates. Additionally, tuberculin skin tests are one of several criteria used in clinical diagnosis, potentially inflating disease rates due to tuberculin skin test conversion. Infants with positive skin tests might have been more likely to receive microbiological testing, potentially leading to ascertainment bias. However, in our study sample, most microbiologically confirmed cases were symptomatic and therefore this is doubtful. Additionally, in our tuberculin skin test conversion analysis, informative censoring might have been possible for children diagnosed with recurrent lower respiratory tract infection since they are more likely to receive multiple tuberculin skin tests and thus be censored because of boosting. For all other subgroups and for our disease outcome, censoring in the survival analysis was non-informative. The results might not be generalisable to communities in settings of low tuberculosis prevalence. However, tuberculosis prevalence is high in many African and low-income countries; furthermore, the inclusion of two heterogeneous communities in our study, with risk factors such as poor nutrition or poverty, which are common in many communities in Africa, make these results generalisable to many areas of high tuberculosis prevalence. Lastly, we used self-reported smoking status from mothers. This can be subject to social desirability biases, which, if present, would probably bias the association between maternal smoking and tuberculosis towards the null. Furthermore, maternal self-reported smoking during pregnancy in this cohort was strongly correlated with high urine cotinine levels in both mothers and newborn infants, 25 suggesting that this bias is unlikely.

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In conclusion, we found high incidences of *M tuberculosis* infection and disease in this South African birth cohort, indicating that much greater focus should be placed on the first years of life as a period of high transmission burden and clinical tuberculosis expression. These results support the need for multifaceted interventions, such as wide-scale preventive therapy and integration of control programmes for lower respiratory tract infections and tuberculosis, beginning early in life with the goal of interrupting transmission and preventing progressive disease in paediatric populations living in endemic settings such as South Africa.

**Contributors**

LM analysed the data and was the main investigator responsible for interpretation of results and drafting of the manuscript. HJZ is the principal investigator, obtained funding, conceived and designed the study, and assisted with drafting of the manuscript. MPN is the lead microbiologist. DMR assisted with epidemiological analysis, study coordination, operational oversight, and drafting of the manuscript. AS is the study clinician and coordinator. WB is the project manager and provided operational oversight. All authors reviewed, contributed to, and approved the final manuscript.

**Declaration of interests**

HJZ and MPN report grants from the Bill & Melinda Gates Foundation. HJZ also reports grants from Medical Research Council South Africa, the National Research Foundation South Africa, and the National Institutes of Health during completion of the study. All other authors declare no competing interests.

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