Severe undernutrition in children affects tuberculin skin test performance in Southern India

Divya Reddy¹ *, Yicheng Ma², Subitha Lakshminarayanan³, Swaroop Sahu³, Laura F. White⁴, Ayiraveetil Reshma⁵, Gautam Roy⁵, Padmini Salgame⁴, Selby Knudsen⁵, Chelsie Cintron⁵, Jerrold J. Ellner⁴, C. Robert Horsburgh, Jr. ², ⁵, ⁶, Sonali Sarkar³†, Natasha S. Hochberg⁵, ⁶‡

¹ Department of Medicine, Pulmonary Division, Albert Einstein College of Medicine, Bronx, New York, United States of America, ² Department of Biostatistics, Boston University, School of Public Health, Boston, Massachusetts, United States of America, ³ Department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India, ⁴ Department of Medicine, Rutgers New Jersey Medical School, Newark, New Jersey, United States of America, ⁵ Department of Medicine, Section of Infectious Diseases, Boston University, School of Medicine, Boston, Massachusetts, United States of America, ⁶ Department of Epidemiology, Boston University, School of Public Health, Boston, Massachusetts, United States of America

† These authors are joint senior authors on this work.
* dreddy@montefiore.org

Abstract

Background

Undernutrition impairs immunity to Mycobacterium tuberculosis and is a risk factor for tuberculosis disease (TB). We aim to investigate if severe undernutrition affects the tuberculin skin test (TST) response among household contacts (HHCs) of pulmonary TB cases.

Methods

We analyzed data from HHCs (> five years) of pulmonary TB cases in Southern India. Undernutrition was defined as per World Health Organization based on body mass index (BMI) for adults (undernutrition 16–18.4 and severe undernutrition < 16 kg/m²) and BMI relative to the mean for children (undernutrition 2SD-3SD and severe undernutrition < 3SDs below mean). Univariate and multivariate models of TST positivity (> five mm) were calculated using logistic regression with generalized estimating equations.

Results

Among 1189 HHCs, 342 were children (age 5–17 years) and 847 were adults. Prevalence of TST positivity in well-nourished, undernourished and severely undernourished children was 135/251 (53.8%), 32/68 (47.1%), and 7/23 (30.4%) respectively; among adults, prevalence of TST positivity was 304/708 (42.9%), 43/112 (38.4%) and 12/26 (46.2%), respectively. Severe undernutrition in children was associated with decreased odds of TST positivity (adjusted odds ratio 0.3; 95%CI 0.1–0.9).
Conclusion
Severe undernutrition in children was associated with decreased odds of TST positivity. False-negative TSTs may result from undernutrition; caution is warranted when interpreting negative results in undernourished populations.

Introduction
Tuberculosis (TB), the leading infectious cause of death worldwide, affected 10 million people in 2017 [1]. Aerosolized droplets generated by an individual with active TB are the primary mode of spread. After inhaling such droplets, persons who become infected are largely able to control the infection and prevent progression to primary disease [2]. This *Mycobacterium tuberculosis* (*Mtb*) infection or "latent tuberculosis infection" is diagnosed by a positive tuberculin skin test (TST) or interferon gamma release assay (IGRA) in the absence of clinical signs or symptoms of disease [3]. In 2014, an estimated 1.7 billion individuals worldwide were infected with *Mtb* infection; South-East Asia, Western-Pacific, and Africa regions had the highest prevalence of infection and accounted for ~80% of those infected [4].

Several factors are linked to the progression of *Mtb* infection to disease, which occurs in 5–15% of infected individuals over their lifetime [5]. These factors include age less than five years, human immunodeficiency virus (HIV) infection, use of immunosuppressive medications such as glucocorticoids and tumor necrosis factor-α (TNF-α) inhibitors, chronic kidney disease, smoking, diabetes mellitus, and undernutrition [6]. Of these, undernutrition is of particular concern due to a high co-prevalence with *Mtb* infection in less-developed countries. According to the United Nations Food and Agricultural Organization (FAO), of the estimated 815 million people worldwide that are undernourished, the majority are from Asia (552 million, 68%) and sub-Saharan Africa (191 million, 23%) [7]. Additionally, Asia and Africa have high rates of undernutrition in children under five years of age which may compound the TB risk associated with young age [7].

The association of undernutrition with progression of *Mtb* infection to TB has been well established [8–11]. However, we recently reported in a meta-analysis that being underweight was not associated with a higher risk of a positive TST or IGRA [12]. The objective of this study, therefore, was to address whether severe undernutrition was associated with blunting of the TST and to identify other predictors of *Mtb* infection among household contacts (HHCs) of TB cases.

Materials and methods
Study setting and study design
This sub-study is part of an ongoing community-based observational HHC study, the Regional Prospective Observational Research for TB (RePORT) cohort, conducted by Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) in collaboration with Boston Medical Center (BMC) and Rutgers New Jersey Medical School. Detailed descriptions of the study protocol and recruitment methods have previously been published [13, 14].

Briefly, pulmonary TB cases in Puducherry and Cuddalore and Villapuram districts of Tamil Nadu were identified and recruited from the National Tuberculosis Elimination Program (NTEP) district microscopy centers and primary healthcare centers. Index case inclusion criteria were: 1. New diagnosis of smear-positive culture-confirmed pulmonary TB. 2. Intake
of less than three doses of anti-TB medication. 3. No history of TB or TB treatment. 4. Absence of multidrug resistant TB and/or contact with a multidrug resistant TB case. 5. Age greater than five years. Identification of TB cases through NTEP allowed for recruitment of a study sample representative of the TB cases in the community.

If TB cases reported at least two HHCS, these individuals were approached for participation. A HHC was defined as someone who on average slept under the same roof, shared at least one meal per day, or watched television (or the equivalent) with the TB case at least five days per week based on the groups previous work in Brazil [15–17]. Inclusion criteria for HHCs included: 1. Age > five years. 2. Had lived with the TB case ≥ three months prior to enrollment. 3. Was willing to get a TST. Exclusion criteria included: 1. Did not intend to live with the TB case in the following year or stay in the area for study duration. 2. Had a history of TB.

The main aim of the parent protocol (RePORT cohort) is to identify biomarkers of treatment failure among index TB cases and biomarkers of TB development among household contacts with the recruitment of smear positive index TB cases and their corresponding HHCs. Children less than six years of age were excluded in both the groups as the yield of smear positivity in children with TB is low and TST positive child HHCs are treated with Isoniazid preventive therapy as per standard of care. Written informed consent was obtained by all participants.

**Data collection**

For TB cases, questionnaires were used to obtain demographic, household, clinical information including co-morbidity and medication data, and HIV testing was performed. Sputum was collected for concentrated acid-fast bacilli (AFB) smear and Löwenstein–Jensen and mycobacterial growth indicator tube (MGIT) cultures.

All HHCs were screened for active TB and enrolled within eight weeks of TB case enrollment. Information was collected on demographic and clinical characteristics as well as on exposure to the TB case. Bacillus Calmette-Guérin (BCG) vaccination status was determined by the presence of a scar. Height and weight were measured to determine body mass index (BMI). A Mantoux TST (including Tubersol, Lederle, Arkray, and SPAN Diagnostics, India) was administered to HHCs as per NTEP guidelines. In brief, 5 TU of Purified Protein Derivative (Lederle, Tubersol, Arkray, and SPAN Diagnostics, India) was placed intradermally on the forearm. The diameter of induration was measured in millimeters using the “ball-point” technique by trained technicians. A pair of digital calipers was used to measure the induration, which were regularly serviced to reduce digit bias. TSTs were read between two to five days due to variant tuberculin reactivity and persistence of positive TSTs up to seven days after testing [18, 19].

Paper questionnaires were scanned and transferred to BMC with Verity TeleForm Information Capture System software V10.8 (Sunnyvale, CA, USA), and read into a Microsoft Access (Seattle, WA, USA) database. Data quality checks were performed; errors were evaluated and rectified by the study team in India. One TB patient was enrolled into the study at the age of five years. The Institutional Review Board was notified regarding this study protocol deviation and data of this patient was retained for analysis.

**Study definitions**

A TST ≥ five mm was considered positive. The modified Alcohol Use Disorders Identification Test (AUDIT-C) was used to assess alcohol use (score ≥ four in males and ≥ three in females is considered “hazardous” alcohol use) [20]. In adults (≥ 18 years of age), BMI was categorized
as severe undernutrition (< 16 kg/m²), undernutrition (16–18.4 kg/m²), and normal/overweight (≥ 18.5 kg/m²) [21]. In children (6–17 years), nutritional status was defined by standard deviations (SD) relative to the mean BMI as determined by the World Health Organization (WHO): Normal < 2SD above and below; undernutrition between 2SD to 3SD below mean; severe undernutrition as < 3SDs below mean [22]. Household location was categorized into large city (population > 100,000), small city (50,000–100,000), town (other urban area) and rural (countryside). The multidimensional poverty index (MPI), based on education, health and living standards, was calculated to categorize the households as poor and not poor [23]. Crowding was assessed based on number of individuals per room.

Statistical analyses
We analyzed data from patients recruited into the RePORT cohort from May 2014—March 2018. Descriptive statistics were calculated using chi-square or Fischer’s exact tests for categorical and t-tests for continuous variables. Both univariate and multivariable analyses, with TST status as the outcome, were performed using logistic regression fit with generalized estimating equations (GEE) to account for household-level clustering effects. Separate models were constructed for adults and children. Undernutrition status and variables with p-values ≤ 0.2 in univariate analyses were included in each multivariable model. Diabetes mellitus (in adults) and history of BCG vaccination were retained in the models due to known associations with TST positivity [24, 25]. As nutritional status is a component of the MPI calculation, MPI was excluded from the multivariable model evaluating the impact of undernutrition on TST. Similarly, as undernutrition status correlated with the number of meals shared per day with the TB case, the latter was not included in the multivariable models. All data analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

This sub-study protocol was reviewed and approved by the BMC, Albert Einstein College of Medicine and Rutgers Institutional Review Boards and the JIPMER Ethics and Scientific Advisory Committees.

Results
Demographic characteristics of study population
Of the 1395 HHCs screened for study enrollment, 27 were ineligible and 76 did not consent. 103 HHCs of the remaining 1292 HHCs were excluded due to missing information. Our study therefore analyzed data from 1189 HHCs of 401 TB cases. Only 4/1189 (0.34%) HHCs were found to have active TB disease when screened at the time of study enrollment. The median age of all HHCs was 26 years (range: 5–93); 847 (71.2%) were adults and 342 (28.8%) children (Tables 1 and 2). Females accounted for 538/847 (63.5%) adults and 164/342 (48.0%) children. The largest proportion (520/1185 [43.9%]) of HHCs were children of TB cases, followed by siblings (n = 281 [23.7%]) of TB cases. Of adults, 112/847 (13.2%) were undernourished and 26/847 (3.1%) were severely undernourished. Among children, 68/342 (19.9%) and 23/342 (6.7%) were undernourished and severely undernourished, respectively. Tables 1 and 2 provide further details on demographic, household and index case characteristics in addition to details of time spent with index case by adult and child HHCs respectively.

Predictors of TST positivity among adult HHCs
Of 1189 HHC, 661 (55.6%) had a positive TST. Of the adults, 487/847 (57.5%) were TST positive. In univariate analyses, TST positive adults were more likely to be older (median 37 vs 32.5 years; p = 0.03), female (OR 1.6, 95%CI 1.24–2.10), the spouse of the TB case (OR 1.5, 95% CI
Table 1. Univariate and multivariate models of predictors of tuberculin skin test positivity in adult (≥18 years of age) household contacts of pulmonary TB cases in India, n = 847.

| Household Contact Characteristics | Total n = 847 | Tuberculin skin test status | Univariate OR (95% CI) | p-value | Multivariate OR (95% CI) |
|----------------------------------|--------------|-----------------------------|------------------------|---------|-------------------------|
|                                  |              | Positive n = 487 (n %)      | Negative n = 360 n %   |         |                         |
| Demographic characteristics      |              |                             |                        |         |                         |
| Median age, years (range)        | 35 (18–93)   | 37 (18–80)                  | 32.5 (18–93)           | 1.01 (1.00, 1.02) | 0.03 | 1.0 (0.99–1.02)         |
| Sex                              |              |                             |                        |         |                         |
| Male                             | 309 (36.5%)  | 156 (32.0%)                 | 153 (42.5%)            | reference | 0.0004 | reference                |
| Female                           | 538 (63.5%)  | 331 (68.0%)                 | 207 (57.5%)            | 1.6 (1.24–2.10) | 0.03 | 1.0 (0.97–1.04)         |
| Median years of education (range)| 8 (0–19)     | 8 (0–18)                    | 9 (0–19)               | 0.97 (0.95, 1.00) | 0.03 | 1.0 (0.97–1.04)         |
| Relationship to index case       |              |                             |                        |         |                         |
| Spouse                           | 263 (31.1%)  | 175 (35.9%)                 | 91 (25.3%)             | 1.5 (1.05, 2.23) | 0.03 | 1.3 (0.83–2.06)         |
| Parent                           | 121 (14.3%)  | 69 (14.2%)                  | 52 (14.4%)             | 1.2 (0.72, 1.88) | 0.04 | 1.1 (0.62–1.90)         |
| Child                            | 266 (31.4%)  | 134 (27.5%)                 | 129 (35.8%)            | 0.8 (0.55, 1.21) | 0.28 | 0.9 (0.58–1.40)         |
| Sibling/other                    | 197 (23.3%)  | 109 (22.4%)                 | 88 (24.4%)             | reference | 0.005 | reference               |
| Tobacco smoking status           |              |                             |                        |         |                         |
| Current/former                   | 89 (10.5%)   | 48 (9.9%)                   | 41 (11.4%)             | 0.9 (0.62–1.44) | 0.77 |                         |
| Never                            | 758 (89.5%)  | 439 (90.1%)                 | 319 (88.6%)            | reference | 0.005 | reference               |
| Hazardous Alcohol use            |              |                             |                        |         |                         |
| Yes                              | 53 (6.3%)    | 25 (5.1%)                   | 28 (7.8%)              | 0.7 (0.39, 1.18) | 0.17 | 0.9 (0.46–1.69)         |
| No                               | 794 (93.7%)  | 462 (94.9%)                 | 332 (92.2%)            | reference | 0.005 | reference               |
| BCG vaccination status           |              |                             |                        |         |                         |
| Yes                              | 721 (85.1%)  | 407 (83.6%)                 | 314 (87.2%)            | 0.8 (0.51, 1.22) | 0.28 | 0.7 (0.42–1.08)         |
| No/Don’t know                    | 126 (14.9%)  | 80 (16.4%)                  | 46 (12.8%)             | reference | 0.005 | reference               |
| Comorbidities                    |              |                             |                        |         |                         |
| History of Diabetes Mellitus     |              |                             |                        |         |                         |
| Yes                              | 50 (5.9%)    | 31 (6.4%)                   | 19 (5.3%)              | 1.4 (0.80, 2.46) | 0.23 | 1.6 (0.68–3.95)         |
| No/Don’t know                    | 797 (94.1%)  | 456 (93.6%)                 | 341 (94.7%)            | reference | 0.005 | reference               |
| Nutritional status               |              |                             |                        |         |                         |
| Severe malnourished              | 26 (3.1%)    | 14 (2.9%)                   | 12 (3.3%)              | 0.9 (0.42, 1.98) | 0.04 | 1.0 (0.46–2.37)         |
| Malnourished                     | 112 (13.2%)  | 69 (14.2%)                  | 43 (12.0%)             | 1.2 (0.81, 1.81) | 0.31 | 1.3 (0.86–2.08)         |
| Well-nourished                   | 708 (83.7%)  | 404 (83.0%)                 | 304 (84.7%)            | reference | 0.005 | reference               |
| Variables related to time spent with TB case | | | | | | |
| Meals shared with TB case per day|              |                             |                        |         |                         |
| None                             | 166 (19.6%)  | 95 (19.5%)                  | 71 (19.7%)             | reference | 0.005 | reference               |
| 1                                | 385 (45.5%)  | 219 (45.0%)                 | 166 (46.1%)            | 1.1 (0.71, 1.56) | 0.28 |                         |
| 2                                | 171 (20.2%)  | 94 (19.3%)                  | 77 (21.4%)             | 0.98 (0.62,1.53) | 0.005 |                         |
| 3 or more                       | 125 (14.8%)  | 79 (16.2%)                  | 46 (12.8%)             | 1.3 (0.82, 2.21) | 0.04 |                         |
| Sleeping Proximity relative to TB Case | | | | | | |
| Same room, same bed              | 63 (7.4%)    | 40 (8.2%)                   | 23 (6.4%)              | 1.5 (0.90, 2.63) | 1.3 (0.74–2.42) | |
| Same room, different bed         | 316 (37.3%)  | 194 (39.8%)                 | 122 (33.9%)            | 1.4 (1.03, 1.83) | 1.3 (0.97–1.79) | |
| Other T                         | 468 (55.3%)  | 253 (52.0%)                 | 215 (59.7%)            | reference | 0.005 |                         |
| Hours spent caring for TB case   |              |                             |                        |         |                         |
| 0                                | 145 (17.1%)  | 87 (17.9%)                  | 58 (16.1%)             | reference | 0.008 | reference               |
| <1 hour per day                 | 316 (37.3%)  | 160 (32.9%)                 | 156 (43.3%)            | 0.8 (0.51, 1.19) | 0.7 | 0.46–1.12               |
| 1–6 hours per day               | 347 (41.0%)  | 212 (43.3%)                 | 135 (37.5%)            | 1.2 (0.78, 1.76) | 0.8 | 0.48–1.22               |
| >6 hours per day                | 39 (4.6%)    | 28 (5.8%)                   | 11 (3.1%)              | 2.0 (0.92, 4.54) | 1.3 (0.56–3.15) |
| Household characteristics        |              |                             |                        |         |                         |
| (Continued)                      |              |                             |                        |         |                         |
1.05–2.23), and have less education (median 8 vs 9 years; p = 0.03) compared to TST negative adult HHCs (Table 1). Sleeping in the same room but in a different bed was also associated with an increased odds of TST positivity (OR 1.4, 95% CI 1.03–1.83) compared to sleeping in a different room and/or different building, and TST positive adult HHCs were more likely (OR 2.7, 95% CI 1.13–6.60) to live in towns than large cities. As shown in Fig 1A, undernutrition status did not significantly differ (p = 0.58) between TST positive and TST negative adult HHCs. In a multivariable model, after adjusting for age, sex, years of education, relationship to TB case, BCG vaccination, hazardous alcohol use, diabetes mellitus, location of household, sleeping proximity to TB case and hours spent taking care of TB case, undernutrition (aOR 1.3, 95% CI 0.86–2.08) and severe undernutrition (aOR 1.0, 95% CI 0.46–2.37) were not
Table 2. Univariate and multivariate models of predictors of tuberculin skin test positivity in child (5–17 years of age) household contacts of pulmonary TB cases in India, n = 342.

| Household Contact Characteristics | Total n = 342 | Tuberculin skin test status | Univariate OR (95% CI) | p-value | Multivariate OR (95% CI) |
|----------------------------------|--------------|-----------------------------|------------------------|---------|------------------------|
|                                  |              | Positive n = 174 (50.9%)    | Negative n = 168 (49.1%) |         |                        |
| Demographic characteristics      |              |                             |                        |         |                        |
| Median age, years (range)        | 13 (5–17)    | 13 (5–17)                   | 13 (6–17)              | 1.1 (0.98–1.14) | 0.16 | 1.1 (0.85–1.41) |
| Sex                              |              |                             |                        |         |                        |
| Male                             | 178 (52.1%)  | 98 (56.3%)                  | 80 (47.6%)             | reference | 0.13 | 1.4 (0.94–2.11) |
| Female                           | 164 (48.0%)  | 76 (43.7%)                  | 88 (52.4%)             | 0.7 (0.50–1.10) | 0.18 | reference |
| Median years of education (range)| 8 (1–13)     | 9 (1–13)                    | 8 (1–13)               | 1.1 (0.98, 1.14) | 0.18 | 1.0 (0.75–1.25) |
| Relationship to index case (missing = 4) |          |                             |                        |         |                        |
| Child                            | 254 (75.1%)  | 128 (74.4%)                 | 126 (75.9%)            | 0.95 (0.55, 1.62) | 0.84 |                     |
| Sibling/other                   | 84 (24.9%)   | 44 (25.6%)                  | 40 (24.1%)             | reference |       |                     |
| Tobacco smoking status           |              |                             |                        |         |                        |
| Current/Former                   | 2 (0.6%)     | 1 (0.6%)                    | 1 (0.06%)              | NA      | NA                     |
| Never                            | 340 (99.4%)  | 173 (99.4%)                 | 167 (99.4%)            | NA      |                        |
| Hazardous alcohol use            |              |                             |                        |         |                        |
| No                               | 342 (100%)   | 174 (100%)                  | 168 (100%)             | NA      | NA                     |
| BCG vaccination status           |              |                             |                        |         |                        |
| Yes                              | 286 (83.6%)  | 149 (85.6%)                 | 137 (81.6%)            | 1.5 (0.85, 2.71) | 0.16 | 1.6 (0.87–2.93) |
| No/Don’t know                    | 56 (16.4%)   | 25 (14.4%)                  | 31 (18.5%)             | reference |       | reference |
| Comorbidities                    |              |                             |                        |         |                        |
| History of Diabetes Mellitus     |              |                             |                        |         |                        |
| Yes                              | 1 (0.3%)     | 1 (0.6%)                    | 0 (0.0%)               | NA      | NA                     |
| No/Don’t know                    | 341 (99.7%)  | 173 (99.4%)                 | 168 (100%)             | NA      |                        |
| Nutritional status               |              |                             |                        | 0.07    |                       |
| Severely malnourished            | 23 (6.7%)    | 7 (4.0%)                    | 16 (9.5%)              | 0.7 (0.40, 1.17) | 0.3 | (0.12–0.85) |
| Malnourished                     | 68 (19.9%)   | 32 (18.4%)                  | 36 (21.4%)             | 0.7 (0.37, 1.22) | 0.8 | (0.45–1.34) |
| Well-nourished                   | 251 (73.4%)  | 135 (77.6%)                 | 116 (69.1%)            | reference |       |                     |
| Hours spent with the TB case in the same house everyday |             |                             |                        | 1.00    |                       |
| < 6 hours per day                | 40 (11.7%)   | 19 (10.9%)                  | 21 (12.5%)             | reference |       |                     |
| 6–12 hours per day               | 266 (77.8%)  | 136 (78.2%)                 | 130 (77.4%)            | 0.99 (0.49, 1.98) |       |                     |
| >12 hours per day                | 36 (10.5%)   | 19 (10.9%)                  | 17 (10.1%)             | 0.99 (0.39, 2.57) |       |                     |
| Meals shared with TB case per day every day^2 |              |                             |                        | 0.007   |                       |
| None                             | 43 (12.6%)   | 28 (16.1%)                  | 15 (8.9%)              | reference |       |                     |
| 1                                | 175 (51.2%)  | 99 (56.9%)                  | 76 (45.2%)             | 0.7 (0.34, 1.59) |       |                     |
| 2                                | 109 (31.9%)  | 40 (23.0%)                  | 69 (41.1%)             | 0.3 (0.16, 0.75) |       |                     |
| 3 or more                        | 15 (4.4%)    | 7 (4.0%)                    | 8 (4.8%)               | 0.5 (0.14, 1.72) |       |                     |
| Sleeping Proximity relative to TB Case |              |                             |                        | 0.53    |                       |
| Same room, same bed             | 35 (10.2%)   | 18 (10.3%)                  | 17 (10.1%)             | 1.2 (0.57, 2.65) |       |                     |
| Same room, different bed        | 158 (46.2%)  | 86 (49.4%)                  | 72 (42.9%)             | 1.3 (0.81, 2.18) |       |                     |
| Other ^1                         | 149 (43.6%)  | 70 (40.2%)                  | 79 (47.0%)             | reference |       |                     |
| Household characteristics        |              |                             |                        | 0.42    |                       |
| Location of household (missing = 3) |              |                             |                        |         |                       |
| Large city                       | 8 (2.4%)     | 1 (0.6%)                    | 7 (4.2%)               | reference |       |                     |
| Small city                       | 17 (5.0%)    | 10 (5.8%)                   | 7 (4.2%)               | 8.3 (0.75–91.22) |       |                     |
| Town                             | 144 (42.5%)  | 76 (43.7%)                  | 68 (41.2%)             | 6.6 (0.72–61.32) |       |                     |
| Rural                            | 170 (50.1%)  | 87 (50.0%)                  | 83 (50.3%)             | 6.3 (0.68–58.28) |       |                     |

(Continued)
| Household Contact Characteristics | Total n = 342 | Tuberculin skin test status | Univariate OR (95% CI) | p-value | Multivariate OR (95% CI) |
|-----------------------------------|--------------|----------------------------|------------------------|---------|-------------------------|
|                                   | Positive n = 174 | Negative n = 168          |                        |         |                         |
| Multidimensional poverty index    |              |                            |                        |         |                         |
| Poor                              | 219 (64.0%) | 109 (62.6%) | 110 (65.5%) | 0.9 (0.54–1.43) | 0.61 |
| Not poor                          | 123 (36.0%) | 65 (37.4%)  | 58 (34.5%)  | reference  |         |
| Wood fuel use                     |              |                            |                        |         |                         |
| Yes                               | 188 (55.0%) | 95 (54.6%)   | 93 (55.4%)   | 0.95 (0.60, 1.52) | 0.84 |
| No                                | 154 (45.0%) | 79 (45.4%)   | 75 (44.6%)   | reference  |         |
| Number of persons per room (median, range) | 1.3 (0.33–6.33) | 1.3 (0.40–6.33) | 1.0 (0.33–6.33) | 1.1 (0.85–1.34) | 0.59 |
| Index case characteristics        |              |                            |                        |         |                         |
| Median age, years (range)         | 42.0 (14.0–75.0) | 42.0 (15.0–75.0) | 42.0 (14.0–73.0) | 0.99 (0.969–1.006) | 0.18 |
| Sex (missing = 1)                 |              |                            |                        |         |                         |
| Male                              | 259 (76.0%) | 130 (75.1%) | 129 (76.8%) | reference  | 0.83 |
| Female                            | 82 (24.1%)  | 43 (24.9%)  | 39 (23.2%)  | 0.9 (0.54–1.64) |         |
| Tobacco smoking status            |              |                            |                        |         |                         |
| Current/Former                    | 177 (51.8%) | 82 (47.1%)  | 95 (56.6%)  | 0.7 (0.43–1.10) | 0.12 |
| Never                             | 165 (48.3%) | 92 (52.9%)  | 73 (43.5%)  | reference  |         |
| Hazardous Alcohol use             |              |                            |                        |         |                         |
| Yes                               | 157 (45.9%) | 73 (42.0%)  | 84 (50.0%)  | 0.7 (0.44–1.14) | 0.15 |
| No                                | 185 (54.1%) | 101 (58.1%) | 84 (50.0%)  | reference  |         |
| HIV infection                      |              |                            |                        |         |                         |
| Yes                               | 1 (0.3%)    | 0 (0.0%)    | 1 (0.6%)    | NA        | NA          |
| No                                | 341 (99.7%) | 174 (100%) | 167 (99.4%) |             |            |
| Median duration of illness, weeks (range), missing = 3 | 4.0 (1.0–16.0) | 4.0 (1.0–12.0) | 4.0 (1.0–16.0) | 0.9 (0.81–1.02) | 0.16 |
| Median TTP-MGIT†, hours (range), missing = 6 | 800.0 (221.0–2814.0) | 801.0 (221.0–2814.0) | 722.0 (300.0–1920.0) | 1.0 (0.99–1.00) | 0.41 |

* Not included in the multivariable model due to its association with nutritional status
† Others: same building, different room, different building that is part of the same household, other.

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**Fig 1.** a. Distribution of tuberculin skin test positivity in adult household contacts of pulmonary TB cases, stratified by household contact nutritional status. b. Distribution of tuberculin skin test positivity in child (<18 years) household contacts of pulmonary TB cases, stratified by household contact nutritional status.

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significantly associated with TST positivity among adult HHCs. The median TST induration results didn’t significantly differ by nutrition status (p = 0.37) among adult HHCs (Fig 2A). We found that compared to large cities, living in a town was associated (aOR 2.5, 95% CI 0.97–6.22) with higher odds of TST positivity among adult HHCs; these results reached borderline significance.

Predictors of TST positivity among child HHCs
Among child HHCs, 174/342 (50.9%) had positive TSTs. The prevalence of TST positivity in well-nourished, undernourished, and severely undernourished children decreased from 135/251 (53.8%), 32/68 (47.1%) to 7/23 (30.4%) respectively (Fig 1B). There was a significant decreasing trend (p = 0.02) in the proportion TST positive among child HHCs as nutritional status decreased from well nourished to severely undernourished. Among children, comorbidities and time spent with TB cases were not significantly associated with TST positivity in univariate analyses (Table 2). However, in a multivariable model after adjusting for age, sex, years of education and BCG vaccination, severe undernutrition in children was associated with a significantly lower odds (aOR 0.3, 95%CI 0.12–0.85) of TST positivity compared to well-nourished children, while undernutrition was not (aOR 0.8, 95% CI 0.45–1.34). The median ages of the child HHCs within the well nourished (13 years), undernourished (13 years) and severely undernourished (14 years) groups were not significantly different (p = 0.33). The median TST size in severely undernourished child HHCs (2 mm) was significantly smaller compared to the undernourished HHCs (4 mm; p = 0.03) and the well-nourished child HHCs (5 mm; p = 0.05; Fig 2B).

Discussion
In a large cohort of household contacts (HHCs) of index pulmonary tuberculosis cases in South India, we found that ~55% of the HHCs had a positive TST; prevalence was high among
adults (56%) and children (51%). Nearly one fifth of the studied HHCs were undernourished; among children, rates of undernutrition and severe undernutrition were high (20% and 7% respectively). We also found that severe undernutrition in child HHCs of pulmonary TB cases in India was associated with decreased odds of TST positivity.

Our finding of a lower odds of TST positivity in severely undernourished children suggests that the TST may not be a reliable measure of *Mtb* infection in severely undernourished children. Although there is no gold standard for diagnosis, we suspect that undernutrition led to a blunted immune response and under-diagnosis, rather than the true prevalence of infection being lower. Previous studies have been conflicting. In a study of 212 adults in Peru, protein energy undernutrition was associated with a lower likelihood of TST positivity [26], and among 6,608 adolescents in India, recent weight loss was associated with TST non-response [27]. Similarly, TST positivity post BCG vaccination increased with improved nutritional status and weight for age [28]. Other studies have failed to demonstrate an association [12, 29, 30]; however, all of these studies failed to account for the severity of undernutrition and/or rates of severe undernutrition were not documented. This is important as severe protein undernutrition in children (kwashiorkor) affected TST conversion six months post BCG vaccination while milder forms of undernutrition did not [31]. Furthermore, a retrospective evaluation of baseline TST results in patients who later developed active TB also showed that negative TSTs in malnourished individuals are false negatives [32].

The potential biologic mechanism for blunting of the TST in severe undernutrition is suppression of innate and adaptive immune responses [11, 33]. The TST relies on an effective delayed type hypersensitivity response; upon intradermal injection of *Mtb* antigens, pro-inflammatory cytokines are released that stimulate adhesion molecules to attract monocytes and T cells [34]. Protein-deprived *Mtb* H37Rv vaccinated mice and guinea pigs have shown diminished lymphoproliferation and defective T cell interaction with macrophages after purified protein derivative (PPD) stimulation [35]. In these models, *Mtb* antigen stimulation was associated with reduced interleukin-2, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) and increased regulatory cytokines such as transforming growth factor-β (TGF-β) [36, 37].

Interferon gamma release assays (IGRAs) such as Quantiferon Gold In-Tube (QGIT) measure T cell IFN-γ release following stimulation with *Mtb* antigens and phytohemagglutinin-P (a non-specific T-cell stimulator) and rely on an adequate cellular response. Reports suggest that IGRA may also not be a reliable test in the setting of undernutrition, with an increase in indeterminate results and more negative tests compared with normal weight persons [12, 38].

The strength of the study lay in collection of detailed information using validated questionnaires and clinical measures for factors associated with *Mtb* infection risk (e.g., AUDIT-C, BMI). These data allowed us to perform multivariable analyses to control for potential confounders of the effect. One limitation was our inability to assess the effect on children less than six years old; these were excluded from the RePORT cohort. It is likely that severe undernutrition affects TST sensitivity in that group as well. The HIV status of our HHCs was unknown, but as Puducherry and Tamil Nadu have low HIV prevalence and rates are <1% in RePORT TB cases [14, 39], this is unlikely to be a significant risk factor for *Mtb* infection. The different formulations of purified protein derivative used for TSTs may have affected diagnosis of *Mtb* infection; however, as the prevalence of *Mtb* infection (55.6%) is comparable to other published studies, marked under-diagnosis is unlikely [40]. Median years of education among child HHCs likely is collinear with age and not a potential marker of community TB exposure like in the adults. We did not collect additional details on risk of community TB exposure in children as part of our study protocol. We analyzed the data to assess differences in time spent with the index TB case to explain the TST status. However, due to our small sample size of
undernourished children [91/342 (26.6%)] we were unable to further explore differences in
time spent with the index TB case by nutritional status. Lastly because we relied on reported
history of diabetes mellitus, under-diagnosis is possible.

Conclusion
As the number of TB cases decline, targeted screening and treatment of Mtb infected persons
in high burden countries will be of increasing importance for TB eradication. As part of the
stop TB strategy, the WHO currently recommends isoniazid preventive therapy (IPT) for
recent TST converters, TST or IGRA positive children with close contact with a TB disease
patient and TST positive persons living with HIV after TB disease has been excluded [41]. Our
study found that severe undernutrition blunts the TST response in children and suggests that
calor is warranted when interpreting TST results in this population. Due to the high preva-
lence of undernutrition in India and other TB-endemic countries [7], this study has public
health relevance. TB programs might consider a lower cut-off point for severely undernour-
ished young HHCs or presumptive treatment of Mtb infection regardless of TST status.

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Author Contributions
Conceptualization: Divya Reddy, Sonali Sarkar, Natasha S. Hochberg.

Formal analysis: Yicheng Ma, Laura F. White, C. Robert Horsburgh, Jr.

Funding acquisition: Gautam Roy, Padmini Salgame, Jerrold J. Ellner, Sonali Sarkar.

Investigation: Subitha Lakshminarayanan, Sonali Sarkar, Natasha S. Hochberg.

Methodology: C. Robert Horsburgh, Jr., Natasha S. Hochberg.

Project administration: Subitha Lakshminarayanan, Swaroop Sahu, Ayiraveetil Reshma,
Selby Knudsen.

Supervision: Subitha Lakshminarayanan, Swaroop Sahu, Gautam Roy, Padmini Salgame, Jer-
rold J. Ellner, Sonali Sarkar, Natasha S. Hochberg.

Writing – original draft: Divya Reddy.

Writing – review & editing: Subitha Lakshminarayanan, Swaroop Sahu, Gautam Roy, Pad-
mini Salgame, Chelsie Cintron, Jerrold J. Ellner, C. Robert Horsburgh, Jr., Sonali Sarkar,
Natasha S. Hochberg.

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