Cough Aerosols of *Mycobacterium tuberculosis* Predict New Infection

A Household Contact Study

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***Rationale*: Airborne transmission of *Mycobacterium tuberculosis* results from incompletely characterized host, bacterial, and environmental factors. Sputum smear microscopy is associated with considerable variability in transmission.**

***Objectives*: To evaluate the use of cough-generated aerosols of *M. tuberculosis* to predict recent transmission.**

***Methods*: Patients with pulmonary tuberculosis (TB) underwent a standard evaluation and collection of cough aerosol cultures of *M. tuberculosis*. We assessed household contacts for new *M. tuberculosis* infection. We used multivariable logistic regression analysis with cluster adjustment to analyze predictors of new infection.**

***Measurements and Main Results*: From May 2009 to January 2011, we enrolled 96 sputum culture-positive index TB cases and their 442 contacts. Only 43 (45%) patients with TB yielded *M. tuberculosis* in aerosols. Contacts of patients with TB who produced high aerosols (≥10 CFU) were more likely to have a new infection compared with contacts from low-aerosol (1–9 CFU) and aerosol-negative cases (69%, 25%, and 30%, respectively; $P = 0.009$). A high-aerosol patient with TB was the only predictor of new *M. tuberculosis* infection in unadjusted (odds ratio, 5.18; 95% confidence interval, 1.52–17.61) and adjusted analyses (odds ratio, 4.81; 95% confidence interval, 1.20–19.23). Contacts of patients with TB with no aerosols versus low and high aerosols had differential tuberculin skin test and interferon-γ release assay responses.**

***Conclusions*: Cough aerosols of *M. tuberculosis* are produced by a minority of patients with TB but predict transmission better than sputum smear microscopy or culture. Cough aerosols may help identify the most infectious patients with TB and thus improve the cost-effectiveness of TB control programs.**

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The concentration of *Mycobacterium tuberculosis* in sputum of patients with TB, usually defined by acid-fast bacilli (AFB)-positive versus AFB-negative smear microscopy, has been associated with transmission for decades. Yet, there is experimental and epidemiologic evidence of marked variability in transmission from patients with TB.

What This Study Adds to the Field

Direct measurement of cough-generated aerosols from patients with pulmonary TB seems to be a new quantitative predictor of infectiousness. Our results have several implications. First, by identifying the most infectious patients with pulmonary TB, our findings belie the current paradigm that considers all sputum AFB-positive patients as homogeneously infectious, providing a framework for more rational and cost-effective infection control decisions. Second, by reducing the number of TB contacts at highest risk of infection while increasing the yield of infected individuals, aerosols may improve the implementation of latent TB infection treatment programs by targeting contacts by virtue of their exposure. Finally, *M. tuberculosis* aerosol measurement provides a quantitative surrogate of inhaled dose in exposed contacts that reduces exposure misclassification and thus is likely to offer insights into TB immunopathogenesis and facilitate studies of vaccines, drugs, and immune responses.

Keywords: *Mycobacterium tuberculosis*; cough-generated aerosols; natural human transmission; household contact study

Tuberculosis (TB) remains one of the most significant challenges in global health (1). Exclusively transmitted by the airborne route, the TB disease cycle begins when a human host inhales infectious aerosols (airborne particles <5 μm in aerodynamic diameter) containing viable *Mycobacterium tuberculosis* (2). There is experimental (3, 4) and epidemiologic (5–10) evidence of marked variability

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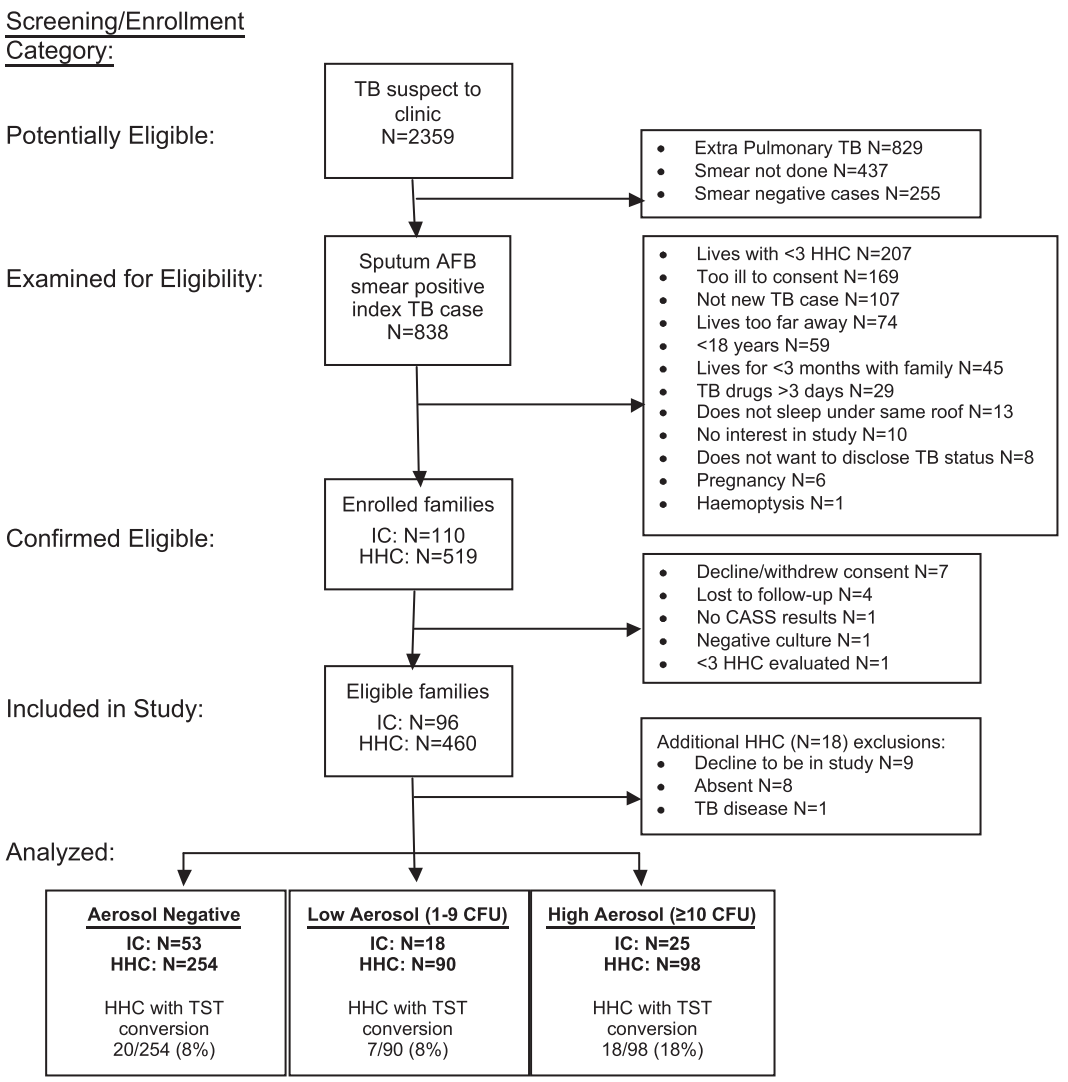


Figure 1. Study profile. AFB = acid-fast bacilli; CASS = cough aerosol sampling system; HHC = household contacts; IC = index tuberculosis cases; IGRA = interferon-γ release assay; TB = tuberculosis; TST = tuberculin skin test.

in transmission from patients with TB. Riley and coworkers (11) demonstrated this variability of infectiousness in pulmonary TB more than 50 years ago but this knowledge has never been translated into practice or policy (4, 11), and the mechanisms underlying this variability remain poorly understood.

Successful TB transmission results from a variety of incompletely characterized determinants. These factors relate to the contagiousness of the source case; environmental conditions, such as ambient air humidity, ventilation, and exposure to ultraviolet light; and the pulmonary ventilation rate in exposed human hosts (12). Risk factors for transmission include the sputum bacillary concentration and cough frequency in the source case, and the proximity or duration of the exposure (13). The concentration of *M. tuberculosis* in sputum of patients with TB (14), usually defined by acid-fast bacilli (AFB)–positive versus AFB-negative smear microscopy, has been associated with transmission for decades. As a result, the sputum AFB smear is widely considered to define TB infectiousness. However, it is estimated that less than 30% of sputum AFB-positive source cases transmit infection to their contacts (15). Conversely, sputum AFB-negative sources have accounted for up to 13–17% of *M. tuberculosis* transmission in several settings (16–18). Thus, there is a need for an improved laboratory marker of TB transmission. We conducted this study among patients with pulmonary TB with sputum AFB greater than or equal to 1+ at screening to determine if colony-forming units (CFU) of *M. tuberculosis* in cough-generated aerosols predict recent infection in household contacts and to compare its predictive ability with that of sputum AFB smear microscopy grade.

METHODS

Study Population

We enrolled consecutive patients with pulmonary TB attending the Mulago Hospital National Tuberculosis and Leprosy Program (NTLP) clinic in Kampala, Uganda, and their household contacts. Patients with TB were eligible if they (*1*) were 18 years or older, (*2*) had a new TB episode with at least one sputum specimen that was initially AFB greater than or equal to 1+ with subsequent growth of *M. tuberculosis* in culture, (*3*) were untreated or had received 5 days or less of anti-TB treatment, and (*4*) lived with three or more contacts. We excluded patients with medical conditions that could be worsened by cough (19) or if they were too ill to consent, or unable to understand or to comply with the protocol. A contact was defined as an individual of any age sleeping under the same roof with the index TB case for 3 or more months before enrollment and no prior history of TB disease. We obtained informed consent and assent in accordance with age-specific ethical guidelines from participating institutions.

Measurements

TB cases. Evaluation of TB cases included three sputa specimens for AFB smear microscopy (auramine O fluorescent stain) (20) and cultures performed on 7H10 Middlebrook agar and liquid media (BACTEC MGIT 960, Becton Dickinson, Franklin Lakes, NJ). The radiologic extent of disease was graded on a four-category ordinal scale. We evaluated cough using a self-reported visual analog scale (21) and three cough peak

TABLE 1. CHARACTERISTICS OF 96 PATIENTS WITH PULMONARY TUBERCULOSIS AND THEIR 442 HOUSEHOLD CONTACTS ACCORDING TO COUGH AEROSOL RESULTS

Characteristic	Cough Aerosol Negative	Cough Aerosol Positive	
		Low Aerosol (1–9 CFU)	High Aerosol (≥10 CFU)
Index TB cases			
N	53	18	25
Age, yr	29.3 (23.1–36.5)	28.4 (20.8–40.3)	28.5 (24–40)
Sex			
Male	26 (49)	8 (44)	15 (60)
Female	27 (51)	10 (56)	10 (40)
Body mass index, kg/m^2	19.0 (17.8–20.7)	18.9 (18.1–20.7)	18.7 (17.2–20.7)
Karnofsky performance score			
70	2 (4)	0 (0)	0 (0)
80	2 (4)	0 (0)	2 (8)
90	49 (92)	18 (100)	23 (92)
HIV status			
Uninfected	44 (83)	10 (56)	19 (76)
Infected	8 (15)	8 (44)	5 (20)
CD4 cell count, cells/ml	315 (226–351)	172 (116–264)	288 (217–360)
Weeks sick before enrollment	12 (8–20)	12 (8–12)	12 (8–15)
Chest radiograph findings			
Extent of lung disease			
Normal	0 (0)	1 (6)	0 (0)
Minimal	4 (8)	2 (13)	1 (4)
Moderate	15 (29)	6 (37)	9 (38)
Far advanced	32 (63)	7 (44)	14 (58)
Cavitations			
Absent	14 (27)	8 (50)	8 (33)
Present	37 (73)	8 (50)	16 (67)
Sputum characteristics			
Volume, ml	5 (4–10)	5 (3–7.5)	5 (5–15)
Acid-fast bacilli smear			
Negative	1 (2)	0 (0)	0 (0)
Scanty	1 (2)	0 (0)	0 (0)
1+	8 (15)	3 (17)	3 (12)
2+	11 (21)	2 (11)	1 (4)
3+	32 (60)	13 (72)	21 (84)
MGIT 960 culture (DTP)	6 (4–8)	5 (4–8)	6 (4–10)
CASS characteristics			
Cough during aerosol collection			
Weak	21 (40)	8 (44)	7 (28)
Strong	32 (60)	10 (56)	18 (72)
Days on TB treatment before aerosol collection	1 (1–2)	1 (1–2)	1 (0–1)
Aerosol CFU	0 (0)	2 (1–5)	26 (17–55)
Household contacts			
N	254	90	98
Age	12 (7–24)	15 (8–24)	13 (6–26)
BCG scar			
Present	188 (74)	68 (76)	74 (76)
Absent or uncertain	66 (26)	22 (24)	24 (24)
HIV status			
Infected	4 (2)	3 (4)	3 (3)
Uninfected	232 (91)	67 (74)	78 (80)
Unknown	18 (7)	20 (22)	17 (17)
TST induration diameter, mm			
Baseline	18 (0–24)	20 (0–25)	20 (4–25)
Six weeks (in contacts with first TST <5 mm only)	0 (0–14)	0 (0–18)	20 (0–24)
IGRA positive, n/N (%)			
Baseline	163/239 (68)	56/83 (68)	67/96 (70)
Six weeks (in contacts with first IGRA negative only)	28/77 (36)	16/34 (47)	21/27 (78)

Definition of abbreviations: BCG = bacille Calmette-Guérin vaccine; CASS = cough aerosol sampling system; DTP = days to detection (MGIT 960); IGRA = interferon-γ release assay; IQR = interquartile range; TB = tuberculosis; TST = tuberculin skin test.

Values are median (IQR) or n (%), unless otherwise specified.

Missing data for index TB cases as follows: body mass index (2); HIV (2); CD4 cell count (11); weeks sick before enrollment (1); cough visual analog scale at 6 weeks (2); extent of lung disease/cavitations (5); and MGIT 960 culture (1).

Missing data for household contacts as follows: TST result at baseline (4) and TST at 6 weeks (10). IGRA result at baseline (24) and IGRA at 6 weeks (34).

flow rates (MicroDirect, Inc., Lewiston, ME). We cultured *M. tuberculosis* from cough aerosols using a cough aerosol sampling system (CASS), a simple, safe, and reproducible method initially developed in the United States (22) and subsequently evaluated at this site with minor modifications (19). A detailed description of the aerosol sampling method is provided in the online supplement. Briefly, patients were instructed to voluntarily cough into the CASS as frequently and as strongly as they could for 5 minutes. They then rested for 5 minutes before a second 5-minute period of coughing. After aerosol collection, the CASS agar plates were read at 1 week to detect contaminants, and then at 3 and 6 weeks to

count CFU of *M. tuberculosis*, which were confirmed by polymerase chain reaction; the 6-week CFU counts were taken as the primary outcome measure of the cough aerosol collection. Before aerosol collection, we recorded room temperature and humidity. The NTLP program provided standard TB treatment and offered routine HIV testing. HIV-infected patients had a CD4 performed and were referred for HIV care.

Household contacts. We used modified US Centers for Disease Control and Prevention recommendations for household contact investigations (23). Before enrollment, we trained staff in tuberculin skin test (TST) (Tubersol; Sanofi Pasteur, Swiftwater, PA) placement and reading and completed interreader and intrareader evaluations (kappa agreement >90%). We obtained blood for an interferon-γ release assay (IGRA) (QuantiFERON Gold-In-Tube; Cellestis, Chadstone, Australia) before TST placement. Household contacts with a negative TST or IGRA at baseline were retested after 6 weeks. To minimize misclassification of TST converters (24), we used two different definitions of TST conversion (*see* the online supplement). Contacts were not tested for HIV and thus, HIV infection was not taken into account in determining TST and IGRA results. We visited the participants' dwellings to assess individual contact time, crowding, and ventilation conditions. Following NTLP recommendations (25), infected contacts at highest risk of developing active TB disease and secondary TB suspects were referred for evaluation and treatment.

Statistical Methods

Our primary exposure variable was the cough aerosol status of the index TB patient in the household. To evaluate a relationship between number of CFU in aerosol and TST conversion, we divided aerosol status into three groups after first looking at the trend in risk of TST conversion by finely divided categories of CFU. Our final groupings were (*1*) aerosol negative; (*2*) low aerosol (1–9 CFU); and (*3*) high aerosol (≥10 CFU). We chose a 10-CFU cut-off because this was the point at which we noted an increase in TST conversion risk. We also performed a sensitivity analysis using different CFU cut-points. Our primary outcome was TST conversion in contacts (using Criterion 1). To examine the possibility of TST or IGRA boosting in contacts, we performed a quantitative analysis of interferon-γ (international units per milliliter) values for each group of contacts. We use standard box plots to represent normalized (QuantiFERON Gold-In-Tube: TB antigen–nil) interferon-γ levels after calculating a delta value for each individual from paired blood samples (6-wk level–baseline level). We calculated descriptive statistics to identify clinical and demographic characteristics and differences between households of aerosol-positive (CFU >0) and aerosol-negative index patients with TB. To determine factors associated with TST conversion in contacts, we performed unadjusted analyses using our primary definition of conversion. Predictor variables included clinical and epidemiologic characteristics of the index case and household contacts, and environmental factors. Variables associated with TST conversion in contacts in the unadjusted analysis ($P < 0.15$) were retained in the final multivariable analysis. To account for clustering within a household, we fit logistic regression models of conversion using generalized estimating equations (SAS version 9.1, SAS Institute, Inc., Cary, NC) (26) using an independent working correlation matrix. Because we identified no significant interactions, we present only main effects. We present our results as unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (CI).

Ethical Approvals

The study was approved by the Makerere University Faculty of Medicine Research and Ethics Committee, the Uganda National Council for Science and Technology, and the Institutional Review Boards of the University of Medicine and Dentistry of New Jersey and Boston University Medical Campus.

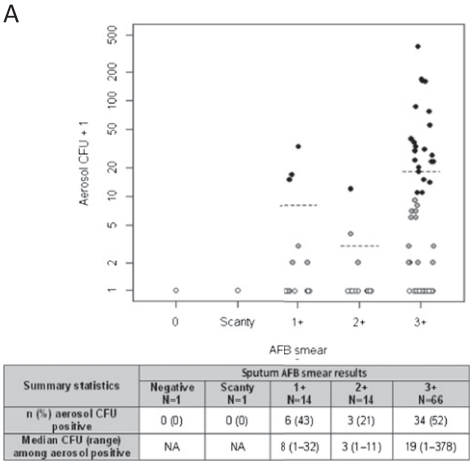
RESULTS

Between May 2009 and January 2011, we screened 838 patients with TB (Figure 1). The 728 patients excluded were either ineligible (n = 710) or refused (n = 18). Of 110 enrolled families, 14 were withdrawn because they declined participation, were lost to follow-up, or had missing results. Eighteen (4%) eligible contacts were excluded. Thus, this analysis includes 96 index TB cases and their 442 household contacts.

TB Cases and Cough Aerosols of *M. tuberculosis*

Index patients with TB were a median age of 28.9 years with advanced pulmonary TB disease as measured by duration of cough (median, 12 wk); sputum smear microscopy; solid and liquid culture results; and, chest radiograph (Table 1). The 21 (22%) HIV-infected patients had a median $CD4^+$ cell count of 264 cells/μl and 12 (57%) were on antiretroviral therapy at enrollment.

Fifty-five percent of patients with TB produced no aerosols with culturable *M. tuberculosis*; 19% produced low aerosols (1–9 CFU); and 26% high aerosols (≥10 CFU). Cough aerosol positivity varied across sputum AFB smear microscopy grades (Figure 2A); the proportion of patients with high aerosols in each of the sputum-positive AFB smear grades (1+, 2+, and 3+) was 21%, 7%, and 32%, respectively. There was considerable variation in CFU (median, 14 CFU; range, 1–378 CFU) among aerosol-positive patients (Figure 2B). Patients with high aerosols had median cough peak flow rates that were 60–80 L/min higher than patients with negative or low aerosols (*see* Table E1 in the online supplement).



Summary statistics	Sputum AFB smear results				
	Negative N=1	Scanty N=1	1+ N=14	2+ N=14	3+ N=66
n (%) aerosol CFU positive	0 (0)	0 (0)	6 (43)	3 (21)	34 (52)
Median CFU (range) among aerosol positive	NA	NA	8 (1–32)	3 (1–11)	19 (1–378)

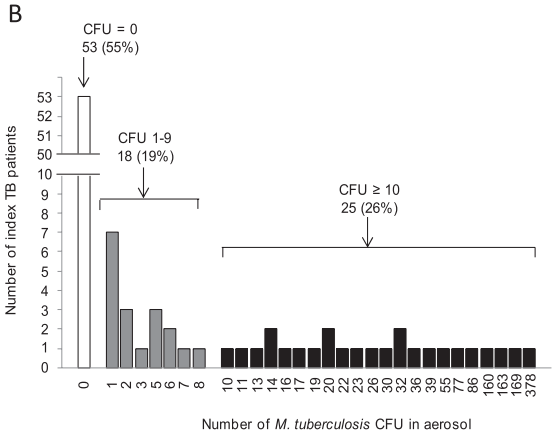


Figure 2. Distribution of CFU of *Mycobacterium tuberculosis* in aerosols from 96 patients with pulmonary tuberculosis. (*A*) Distribution of cough aerosol results according to sputum acid-fast bacilli (AFB) smear microscopy grade. Patients with 0 CFU *M. tuberculosis* in aerosols (*white circles*). Patients with 1–9 CFU of *M. tuberculosis* in (low) aerosol (*gray circles*). Patients with greater than or equal to 10 CFU of *M. tuberculosis* in (high) aerosol (*black circles*). *Dashed horizontal lines* indicate the median for subjects who produced aerosol (CFU ≥1). Below the graph, the median CFU and range are given for subjects who produced aerosol (CFU ≥1). Because aerosol CFU data are presented on a log scale, 1 was added to all CFU for plotting purposes. (*B*) Distribution of CFU counts in cough aerosols. TB = tuberculosis.

Household Contacts and Prevalent TB Infection

The number of contacts exposed to aerosol-negative, low- and high-aerosol index TB cases was 254, 90, and 98, respectively (Table 1). Enrolled contacts were 52% female, had a median age of 13 years, and 74% had a bacille Calmette-Guérin vaccination scar. Nearly all were biologically related to the TB index case. Participant dwellings were small and crowded; 52% of contacts slept in the same bed or room as the index TB case. At baseline, the prevalence of TB infection in contacts was comparable in all three cough-aerosol groups by either TST greater than or equal to 10 mm or IGRA criteria (*see* Figure E1A). The use of different TST cut-off values (5, 10, and 15 mm of induration) had a limited impact on TST sensitivity (variation between 5% and 8%).

Incident TB Infection

After excluding contacts that were infected at baseline, the number of contacts "at-risk" of TST conversion in each of the three groups was 66 (aerosol negative), 28 (low aerosol), and 26 (high aerosol). Compared with contacts of aerosol-negative patients with TB (30%), the odds of TST conversion in contacts of low-aerosol cases (25%) was similar (OR, 0.77; 95% CI, 0.27–2.17; $P = 0.62$) but over fivefold greater (OR, 5.18; 95% CI, 1.52–17.61; $P = 0.009$) in contacts of high-aerosol patients (69%) (Figure 3). Although not reaching statistical significance, a similar pattern was seen when we only included contacts (data not shown) of patients with TB with sputum AFB greater than or equal to 3+ (OR, 3.14; 95% CI, 0.75–13.12; $P = 0.12$). When we used IGRA alone (OR, 9.53; 95% CI, 2.51–36.24; $P = 0.0009$) or TST and IGRA conversion together (OR, 20.9; 95% CI, 3.94–110.8; $P = 0.0004$) to define new TB infection, we observed a stronger positive association with cough aerosols (Figure 3A; *see* Figure E2A). By comparison, the same analysis using sputum AFB smear grade to classify exposure groups did not show clear or consistent risk stratification (Figure 3A).

To examine the possibility of boosting, we analyzed TST and IGRA results as a continuous variable. The TST induration size

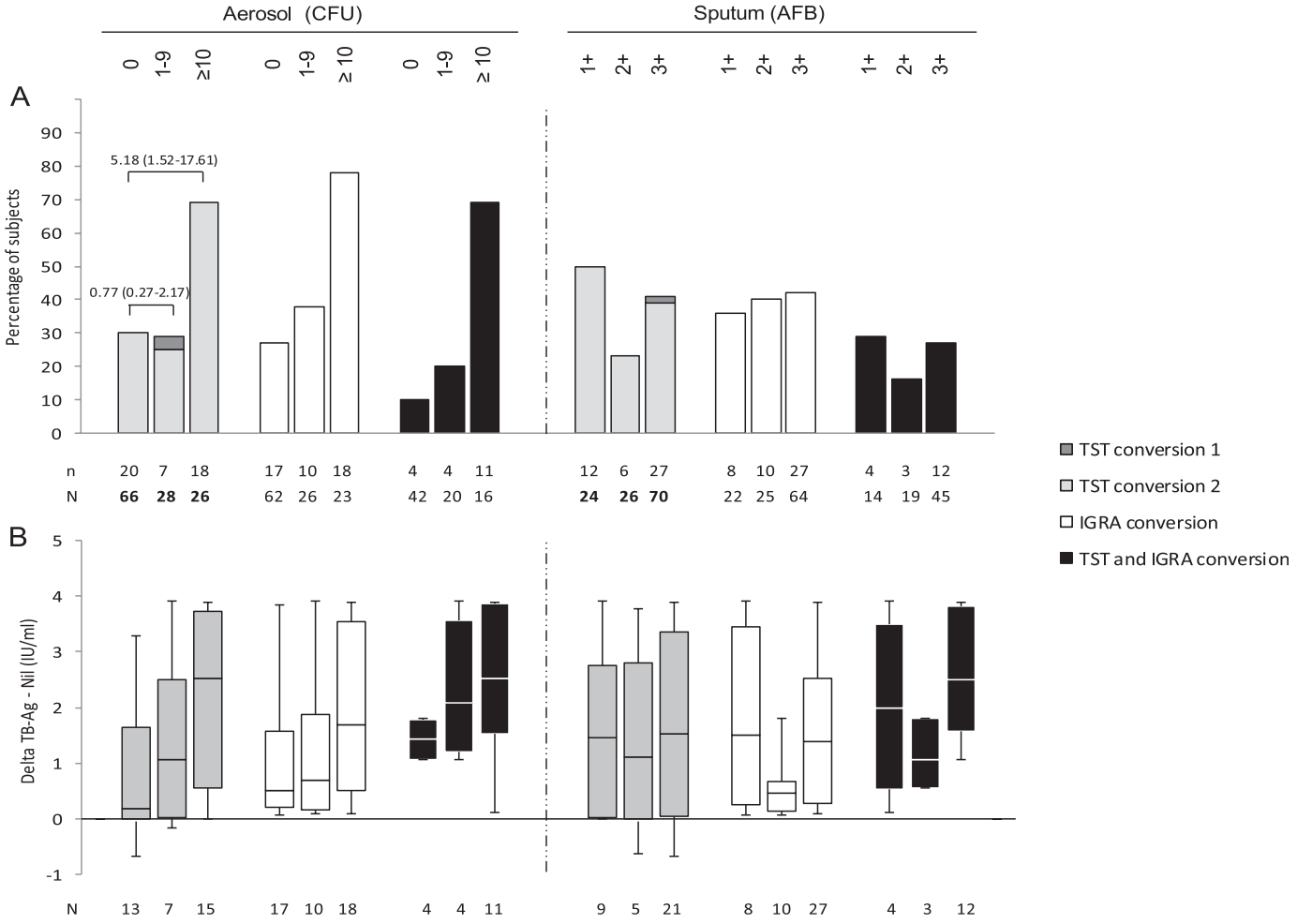


Figure 3. Tuberculin skin test (TST) and interferon-γ release assay (IGRA) results in household contacts uninfected at baseline, according to aerosol or sputum results in index tuberculosis (TB) cases. (*A*) Proportion of contacts with TST, IGRA, or TST and IGRA (both together) conversion at 6 weeks. We present data using two different criteria to define TST conversion. Criterion 1: first TST less than 10 mm, second TST greater than or equal to 10 mm, and difference between first and second TST greater than or equal to 10 mm. Criterion 2: first TST less than 5 mm, second TST greater than or equal to 10 mm, and difference between first and second TST greater than or equal to 6 mm. Comparisons (odds ratios adjusted for clustering and 95% confidence intervals) are based on Criterion 1. The n under each column denotes the number of contacts fulfilling each outcome criterion. The N under each column denotes the number of household contacts "at risk" of conversion in each exposure category. We highlight the N to emphasize a change in exposure categories when using aerosol versus sputum. (*B*) Quantitative IGRA analysis. Standard box plots of interferon-γ values for each group of contacts. Values are normalized interferon-γ (TB antigen − Nil) results after calculating a delta for each individual from paired blood samples (6-wk level − baseline level).

TABLE 2. UNADJUSTED* AND ADJUSTED† ODDS RATIOS FOR INDEX TUBERCULOSIS CASE, HOUSEHOLD CONTACT, AND ENVIRONMENTAL CHARACTERISTICS ASSOCIATED WITH TUBERCULIN SKIN TEST CONVERSION IN EXPOSED HOUSEHOLD CONTACTS

	Household Contacts					
Characteristic	Number At Risk	Number (%) with TST Conversion	Unadjusted* Odds Ratio (95% Confidence Interval)	*P* Value‡	Adjusted Odds Ratio (95% Confidence Interval)	*P* Value
Index cases						
Age (per 10-yr increment)			0.93 (0.56–1.55)	0.79		
Sex						
Male	53	18 (34)	1			
Female	67	27 (40)	1.31 (0.68–2.52)	0.41		
Body mass index, kg/m^2						
<15.0	2	1 (50)	1			
15.0–18.5	33	13 (39)	0.65 (0.29–1.46)			
>18.5	78	30 (38)	0.63 (0.32–1.22)	0.68		
HIV status						
Infected	19	6 (32)	1			
Uninfected	100	39 (39)	1.39 (0.49–3.91)	0.55		
Family size						
2–3 contacts	27	7 (26)	1			
4–5 contacts	42	17 (40)	1.94 (0.56–6.72)			
⩾6 contacts	51	21 (41)	1.99 (0.51–7.83)	0.49		
Cough measurements at baseline						
Visual analog scale (per unit increase)			1.08 (0.85–1.37)	0.51	1.00 (0.93–1.06)	0.12
Cough peak flow§ (per 10 L/min increase)			1.00 (0.99–1.01)	0.91		
Cough during aerosol collection						
Weak	46	15 (33)	1			
Strong	74	30 (41)	1.41 (0.56–3.56)	0.48		
Chest radiograph						
Normal/minimal	14	7 (50)	1			
Moderate	49	18 (37)	0.58 (0.16–2.17)			
Far advanced	49	19 (39)	0.63 (0.19–2.11)	0.73		
Cavitations						
Absent	43	15 (35)	1			
Present	69	29 (42)	1.35 (0.50–3.66)	0.55		
Sputum appearance						
Mucoid	35	14 (40)	1			
Mucopurulent	27	6 (22)	0.43 (0.12–1.52)			
Mucosalivary	32	12 (38)	0.90 (0.24–3.37)			
Salivary/watery	24	12 (50)	1.50 (0.29–7.68)	0.45		
Sputum acid-fast bacilli smear						
Negative/scanty/ 1+	24	12 (50)	1			
2+	26	6 (23)	0.30 (0.05–1.68)			
3+	70	27 (39)	0.63 (0.16–2.48)	0.42		
7H11 culture, CFU						
<200	16	4 (25)	1			
⩾200	104	41 (39)	1.95 (0.64–6.00)	0.30		
MGIT 960 culture (DTP)						
⩾7	44	14 (32)	1		1	
<7	71	26 (37)	1.24 (0.42–3.69)	0.70	1.14 (0.38–3.41)	0.81
Cough aerosol, CFU						
Negative	66	20 (30)	1		1	
Low (1–9 CFU)	28	7 (25)	0.77 (0.27–2.17)		0.83 (0.29–2.42)	0.74
High (⩾10 CFU)	26	18 (69)	5.17 (1.52–17.61)	0.08	4.81 (1.20–19.23)	0.03
Household contacts						
Age, yrs						
⩾18	32	10 (31)	1		1	
5–17	63	24 (38)	1.35 (0.60–3.07)		1.14 (0.44–2.97)	0.97
<5	23	11 (48)	2.02 (0.74–5.52)	0.40	1.29 (0.40–4.19)	0.66
Sex						
Male	59	20 (34)	1			
Female	61	25 (41)	1.35 (0.49–3.77)	0.56		
BCG scar						
Absent/uncertain	31	11 (35)	1			
Present	89	34 (38)	1.12 (0.48–2.61)	0.78		
Dwellings						
Number of habitable rooms		36 (40)				
⩾2	90	8 (36)	1			
<2	22		0.86 (0.23–3.15)	0.81		

(Continued)

TABLE 2. (CONTINUED)

Characteristic	Household Contacts					
	Number At Risk	Number (%) with TST Conversion	Unadjusted* Odds Ratio (95% Confidence Interval)	*P* Value‡	Adjusted Odds Ratio (95% Confidence Interval)	*P* Value
Sleeping arrangement with index						
Different room	71	23 (32)	1		1	
Same room, different bed	39	19 (49)	1.98 (0.79–4.98)		1.06 (0.38–2.94)	0.91
Same room, same bed	10	3 (30)	0.89 (0.18–4.37)	0.33	0.64 (0.10–4.08)	0.64

Definition of abbreviations: BCG = bacille Calmette-Guérin vaccine; CASS = cough aerosol sampling system; DTP = days to detection (MGIT 960); TB = tuberculosis; TST = tuberculin skin test.

* Adjusted for clustering using generalized estimating equations with an independent working correlation matrix.

† Adjusted for clustering and for all other covariates listed.

‡ Global *P* value.

§ The highest of three cough peak flow measurements was used for each individual.

in contacts that developed a TST greater than or equal to 5 mm after the baseline visit was larger in contacts from high-aerosol households (Table 1); similarly, we observed a strong dose–response correlation between interferon-γ levels and aerosol CFU (Figure 3B). Furthermore, a sensitivity analysis varying CFU cut-points showed similar results but additionally suggests that higher cut-points to define high aerosol production improves the predictive value of cough aerosols and reveals a consistently increasing trend with higher aerosol CFU (*see* Figure E2B).

By 6 weeks (*see* Figure E1B), the proportion of contacts with TST greater than or equal to 10 mm was similar in contacts from aerosol-negative (78%) and low-aerosol (76%) households (OR, 0.85; 95% CI, 0.41–1.79; $P = 0.67$) but significantly higher (92%) in contacts from high-aerosol households (OR, 3.10; 95% CI, 1.13–8.48; $P = 0.03$). This result did not change when we used a greater than or equal to 5 mm ($P = 0.03$) or greater than or equal to 15 mm ($P = 0.04$) TST cut-off level or IGRA positivity ($P = 0.03$) to define TB infection (*see* Figure E1B).

Factors Associated with TST Conversion in Contacts

The median age (range) of contacts with TST conversion in each of the three aerosol groups (negative, low, and high) was 9 (0.6–76), 11 (2–26), and 8 (1–43) years, respectively. In analyzing index TB patients, household contact, and environmental factors associated with TST conversion in contacts (Table 2), the only significant factor in an unadjusted analysis was the TB case producing high aerosols (OR, 5.17; 95% CI, 1.52–17.61). In a multivariable model adjusted for clustering and other covariates, high aerosol status remained the only variable associated with TST conversion (OR, 4.81; 95% CI, 1.20–19.23; $P = 0.03$). This result did not significantly change using other multivariable models.

DISCUSSION

This is the first study to demonstrate an association between cough-generated aerosols of *M. tuberculosis* and transmission among humans. Our study design and use of TST and IGRA to measure incident TB infection provide robust evidence that exposure to high aerosols increases the risk of infection in contacts, despite high background infection rates in this setting. The results from this study suggest that a minority of patients with pulmonary TB transmit more than others (27, 28), and that in future studies, cough aerosols may serve as a more refined predictor of infectiousness and a more precise surrogate of exposure on inhaled dose in recently infected individuals. The findings from this study have generated several important questions that need to be investigated in future work.

We have now performed aerosol studies in more than 200 patients with TB and have found that only a minority of patients with pulmonary TB generates aerosols during coughing. Although unexplained at this time, we doubt that this observation is spurious or is related to an insensitive aerosol collection method. Whereas in our first two studies less than 30% of TB cases produced aerosols (19, 22), the greater frequency (45%) of aerosol-positive patients in this study is explained by our recruitment strategy. We collected aerosols earlier and enrolled mostly ambulatory patients who had stronger coughs compared with the hospitalized patients recruited in our previous study at this site (19). Our aerosol production results have been consistent across two different settings (United States and Uganda) and our reproducibility studies have shown reliable results (19). The Andersen cascade impactor was chosen for our sampling method because it has been the reference standard for viable bioaerosol measurement for decades, supported by a considerable body of literature. It provides validated particle size distribution data (29) and it has been used previously to collect mycobacterial aerosols (30, 31). Furthermore, the same aerosol collection method yielded positive cough aerosol cultures of *Pseudomonas aeruginosa* from 96% of patients with cystic fibrosis who were chronically infected (32).

An important roadblock to developing a reliable predictor of natural TB transmission has been the long reliance on studies of sputum, despite established evidence that TB is transmitted by aerosols. Although aerosol production was correlated with sputum AFB smear grade in this and our previous studies, most subjects with high-grade smears (i.e., AFB $\geq$3+) were aerosol negative, suggesting great variability in infectiousness independent of sputum AFB smear microscopy. This observation runs in opposition to the current paradigm of TB transmission, which considers all smear-positive cases as equal transmitters. Similarly, aerosol generation (and therefore rate of conversion in contacts) was not associated with semiquantitative sputum culture results or MGIT DTP, two markers that are commonly assumed to be indicative of bacillary burden and presumably infectiousness. Without the aerosol measurement, however, the identification of the most infectious patients with TB is not straightforward because all patients had similar characteristics at baseline. If cough aerosol collection can be modified to incorporate a real-time read out in a point-of-care test (33, 34), the identification of the most infectious patients could allow for more cost-effective use of resources for infection control in hospitals (e.g., isolation rooms) and for targeting preventive treatment in individuals at highest risk of TB infection by virtue of their exposure.

In addition to providing a more precise marker of source infectiousness, cough aerosols may help determine the individual risk of *M. tuberculosis* infection after exposure, which can be variable and is poorly understood (35). Previous efforts to explain this variability have focused on identifying environmental and host immunogenetic factors in close contacts (36). More recently, there

has been great interest in identifying strain virulence factors (37–40). The results from this study suggest that differential ability of source cases to transmit *M. tuberculosis* may be an underappreciated source of variability. A related issue has been the lack of appreciation of the importance of the inhaled dose in humans. First demonstrated by Robert Koch himself (41), the immunopathology of TB in animal models is clearly dependent on the size of the infecting inoculum (41–43). In contrast, most of the epidemiologic evidence supporting the importance of inoculum size on TB outcomes in humans is based on inference, mostly from household contact studies. Interestingly, whereas the incorporation of IGRA substantially increased the discriminatory capacity of aerosols, we observed differential TST-IGRA responses between contacts exposed to aerosol-negative and aerosol-positive index TB cases. In particular, contacts of aerosol-negative patients with TB who underwent TST conversion had very low interferon-γ levels, suggesting the possibility their TST results may be explained by boosting. Further work is needed to determine if differences in inhaled inoculum may lead to a qualitatively different infection in humans (44). If our results are reproduced in future studies, cough aerosols may serve as an exposure biomarker to study natural *M. tuberculosis* transmission in humans.

Limitations

The predictive value of cough aerosols over time is unknown. Like the sputum smear and culture, cough aerosols measured shortly after TB diagnosis is a surrogate of past exposure. As a consequence, contacts that were already infected at enrollment may have been infected by the TB index case at an earlier time point when infectiousness (and aerosol positivity) may have differed, or in the community. Also, it is possible that the high background rate of *M. tuberculosis* infection in contacts, although consistent with our previous finding at this same site (36), may have obscured weaker predictors of TST conversion in this study. The generalizability of our findings is unknown for several reasons. First, we restricted study participation to sputum AFB-positive patients at screening, which in this setting biased the sample of patients toward having more advanced disease. Second, we only included two index cases that were AFB-negative/scanty in this sample and are therefore unable to evaluate aerosol production in that group. Finally, because we only enrolled households with three or more contacts, our results may not apply to less crowded dwellings. We measured cough, an important predictor of TB transmission, in several ways but none of these provided an objective, continuous measurement of cough strength.

Our results have several implications. First, by identifying the most infectious patients with pulmonary TB, our findings belie the current paradigm that considers all sputum AFB-positive patients as homogeneously infectious, providing a new framework for more rational and cost-effective infection control decisions. Second, by reducing the number of TB contacts at highest risk of infection while increasing the yield of infected individuals, aerosols may improve the implementation of latent TB infection treatment programs by targeting contacts by virtue of their exposure. Last, and perhaps most importantly, *M. tuberculosis* aerosol measurement provides a quantitative surrogate of inhaled dose in exposed contacts that reduces exposure misclassification and thus, is likely to offer insights into natural TB transmission and immunopathogenesis, and facilitate studies of vaccines, drugs, and immune responses.

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