Title: **Infectiousness of patients with smear-negative pulmonary tuberculosis, assessed by Real-time Polymerase Chain Reaction, Xpert®MTB/RIF**

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Keywords: Tuberculosis, Isolation, Polymerase-Chain Reaction, Mycobacterium tuberculosis complex, Smear-negative pulmonary tuberculosis.

This article has not been presented to any meetings.

No grants or financial support have been received for this work.

The authors declare no potential conflicts of interest.

ABSTRACT

Currently, pulmonary tuberculosis (TB) isolation recommendations are based on serial sputum smear microscopy. To assess infectiousness of smear-negative/GeneXpert-positive (Sm-/GXpert+) pulmonary TB, we evaluated 511 contacts of pulmonary TB patients attended at a teaching hospital in Spain (2010-2018). There were no statistically significant differences in rates of *Mycobacterium tuberculosis* infection (46.2% contacts of smear-positive and 34.6% contacts of Sm-/GXpert+ pulmonary TB patients, p=0.112). Sm-/GXpert+ pulmonary TB poses a substantial risk of transmission of *M. tuberculosis* infection. Our results add evidence to support including Real-time Polymerase Chain Reaction (Xpert®MTB/RIF) in the work-up diagnosis of suspected pulmonary TB cases to make decisions on air-borne isolation.

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INTRODUCTION

Besides a prompt diagnosis and an early treatment initiation, respiratory isolation of pulmonary tuberculosis (TB) patients is a key element to reduce transmission of the infection and its spread into the community (1,2). Pulmonary TB with high-bacillary loads poses a high risk of transmission. Therefore, putting in isolation all patients with newly diagnosed pulmonary TB with smear-positive sputum is recommended (1,2). Such measures can be avoided or discontinued when two to three serial smear-negative sputum samples are available (1-5). On the contrary, smear-negative pulmonary TB patients are not kept under airborne isolation, as their infectiousness is considered negligible. However, it is well established that a certain risk of transmission still exists (6,7). In this regard, direct detection of *Mycobacterium tuberculosis* by nucleic acid amplification tests (NAAT), which have higher sensitivity than smear microscopy, might help to identify those patients with a potential higher risk of transmission (8).

In this study, we aimed to assess the infectiousness of smear-negative/GeneXpert-positive (Sm-/GXpert+) pulmonary TB cases as compared to smear-positive pulmonary TB patients by determining the rates of infection among their respective contacts.

METHODS

We retrospectively identified all cases of pulmonary TB diagnosed and treated at the TB Unit of Bellvitge University Hospital, a tertiary hospital for adults in Barcelona, Spain (January 2010 - December 2018), and their contacts. As a part of the current practice of

the TB Unit, clinical and epidemiological data of patients and their contacts are prospectively collected.

Pulmonary TB cases were included if they had the diagnosis confirmed by positive culture for *M. tuberculosis* complex (MTC) from sputum samples. Patients were divided into two groups according to the sputum microscopy and NAAT results: smear-positive pulmonary TB group, defined as the presence of acid-fast bacilli (AFB) by the Ziehl-Neelsen staining, in at least one sputum sample; and smear-negative/GeneXpert positive (Sm-/GXpert+) pulmonary TB group, defined as the absence of AFB in at least two sputum samples by Ziehl-Neelsen staining and detection of MTC DNA by the real-time polymerase chain reaction (PCR) platform GeneXpert MTB/RIF® (GXpert, Cepheid, Sunnyvale, CA). Those contacts with previous TB, previous positive tuberculin skin test (TST) or previous positive interferon-gamma release assay (IGRA), whether self-reported or documented in medical reports, were excluded. During the study period, contacts of pulmonary TB cases were evaluated using TST (2010-2013) or QuantiFERON-TB Gold (QFT) (2014-2018), depending on the method in-use at each period. Close contacts were defined as those sharing an enclosed space with TB cases for at least 6 hours per day. Immunosuppressive condition was defined as having at least one of the following conditions: HIV, active malignancy, solid organ or stem cell transplantation or being under immunosuppressive therapy.

We compared rates of *M. tuberculosis* infection, defined as a positive TST (≥5 mm induration at the initial test), or positive QFT; conversions, defined as negative to positive TST with an increment of the induration of at least 5 mm, for TST, and negative to positive for QFT; and secondary pulmonary TB cases defined as active positive TB cases diagnosed in the course of contact tracing study, between contacts of smear-positive patients and those of Sm-/GXpert+ index cases. Differences between both groups were

adjusted for potential confounders. Smear-negative/GeneXpert-negative patients were not included into the analysis due to the low number of contacts identified.

Continuous variables are presented as means (± standard deviation) and categorical variables as percentages. Bivariate analyses were performed by chi-square test for categorical variables and by Student's t-test or Mann–Whitney U-test for continuous variables. Multivariate analyses were performed by using logistic regression models. All p values were two-tailed and p values < 0.05 were considered statistically significant. The statistical analysis was carried out using Stata 15 (StataCorp. 2017).

The ethical committee of the Bellvitge University Hospital-IDIBELL approved the study. Informed consent was waived because of the retrospective nature of the study.

RESULTS

We identified 169 patients with pulmonary TB confirmed by sputum culture. Thirty-four were excluded (17 had smear-negative sputums, but GeneXpert was not performed, 11 had negative Ziehl-Neelsen staining and negative GeneXpert sputum, and 6 had less than two smear-negative sputum). Of the remaining 135, 116 (85.9%) were smear-positive, and 19 (14.1%) were smear-negative. Eighty-eight (65.2%) were males, with a mean age of 40.3 (±17.2 SD) years, 21 (16.5%) were from countries with prevalence rates >100/100.000 cases, and 72 (53.3%) had cavitation on the X-ray. The mean time from the beginning of symptoms to diagnosis was 2.2 (±2.0 SD) months. Hospital admission was required in 53 (39.3 %) of patients, with a mean length of stay of 14.7 (±13.2 SD) days. There were no significant differences between Sm-/GXpert+ and smear-positive patients, except for their immunosuppressive condition; present in 5 (4.3%) smear-positive and 3 (15.8%) Sm-/GXpert+ (p=0.05). The proportion of close contacts among groups was 50.7% and 48.2%, respectively (p = 0.72).

Five hundred eleven contacts of the 135 pulmonary TB cases were evaluated (459 contacts of the smear-positive patients [mean per patient 3.9±2.6], and 52 contacts of the smear-negative patients [mean contacts per patient 2.8±2.3], p=0.07). Two hundred fifty-nine (50.7%) were males, with a mean age of 39.4 (±15.18 SD) years. In 260 occasions (50.9%) the contact with the index case was close. There were not significant differences between contacts of Sm-/GXpert+ and smear-positive patients, except for their origin: 69 (16.0%) of contacts of smear-positive patients were born in high endemic countries (>100 cases per 100,000 population), compared to none of contacts of smear-negative patients (p<0.01).

Mycobacterium tuberculosis infection in contacts

Mycobacterium tuberculosis infection was diagnosed in 230 (45.0%) of contacts (173 were diagnosed with TB infection without active disease due to positive TST or QFT (according to our definition of TB infection), 52 converted from negative to positive, and 5 had active TB). One hundred and thirty-two (57.4%) were diagnosed by QFT and 98 (42.6%) by TST. The overall prevalence of *M. tuberculosis* infection was not statistically different among contacts of smear-positive patients and contacts of Sm-/GXpert+ patients (46.2% and 34.6% respectively; p=0.11). Conversion from negative to positive was observed in 47 (10.2%) contacts of smear-positive patients and 5 (9.6%) contacts of smear-negative patients. Among the all 52 conversions, 29 (55.8%) converted by TST and 23 (44.2%) by QFT, with no statistically significant differences among the study groups (p = 0.61). Five contacts were diagnosed with secondary pulmonary TB; all of them belonged to the smear-positive group.

In the unadjusted logistic regression analysis, the overall risk of *M. tuberculosis* infection was not higher among contacts of the smear-positive than among Sm-/GXpert+ index TB

cases (Odds Ratio [OR] 1.62; 95%CI 0.89-2.95) (Table 1). The multivariate logistic regression analysis identified cavitation and close contact as the only variables independently associated with a higher risk of *M. tuberculosis* infection among contacts (OR 2.31; 95%CI 1.57-3.41 and 4.39; 95%CI 2.02-9.54, respectively).

DISCUSSION

Our results show that prevalence of *M. tuberculosis* infection among contacts of Sm-/GXpert+ TB patients was not significantly lower than that of contacts of smear-positive TB patients. Up to 35% of contacts of patients with negative AFB smear microscopy, detected by GeneXpert, were diagnosed with a *M. tuberculosis* infection. Previous studies reported 10-20% prevalence rates of secondary cases of smear-negative pulmonary TB patients, (6,7) having transmission being reported even at sub-clinical stages of the disease (9). Our study is the first one to assess the risk of transmission indirectly by looking at rates of infected contacts of smear-positive and smear-negative NAAT-positive pulmonary TB index cases.

While the results of the present study do not exclude a higher risk of transmission from smear-positive TB patients, as suggested by the five secondary cases and a trend towards more non-significant proportion of infected contacts from the smear-positive as compared to the Sm-/GXpert+ group, such difference would not be discriminatory enough to make clinical decisions on isolation of patients with suspicion of pulmonary TB based only in the acid fast bacilli sputum smear.

Guidelines have based recommendations on isolating patients with pulmonary TB on serial sputum smear microscopy (1,2). These recommendations are questioned by previous experience and our own findings, which putting together definitively supports the current tendency of including NAAT in the work-up of patients with suspicion of

pulmonary TB when making decisions on air-borne isolation (10-13). While two consecutive negative NAAT of sputum have a 100% negative predictive value for TB, a positive test implies a substantial risk of transmission of TB, and advocate for isolation of the patient (13). Although the optimal moment to perform NAAT to guide isolation is not clear, given the low incidence of Sm-/GXpert+ cases over the study period (14.1% of cases included), taking into account their costs and according to previous consensus, we would support the use of NAAT in patients with clinical suspicion of TB and no alternative diagnosis after 2 negative smear samples, who require admission to hospital in countries with low TB incidence (13).

Our study has some limitations that deserve comment. First, the retrospective design of the study might prevent from some potential confounders not being controlled. However, as stated above, these potential small differences would not be enough relevant to guide decisions on isolation of patients. Second, the low statistical power, due to the small number of patients with Sm-/GXpert+ pulmonary TB and the respective contacts might have not allowed to detect existing differences in infectiousness between the different study groups.

In conclusion, Sm-/GXpert+ pulmonary TB poses a substantial risk of transmission of *M. tuberculosis* infection. Our results add evidence to support including Real-time Polymerase Chain Reaction (Xpert®MTB/RIF) in the work-up diagnosis of suspected pulmonary TB cases to make decisions on air-borne isolation. They should be considered when issuing recommendations at local and programmatic levels in TB low-incidence countries.

Table 1. Descriptive, bivariate and multivariate analysis of factors associated with *M. tuberculosis* infection in contacts of pulmonary tuberculosis patients

	Descriptive analysis		**Bivariate analysis**		**Adjusted analysis**	
	MTB infection (N = 230)	No MTB infection (N = 281)	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Characteristics of contacts						
Male gender, n (%)	124 (53.91)	135 (48.04)	1.27 (0.89-1.79)	0.187		
Age (years), mean ± SD	40.23 ±15.38	38.73 ± 15.00	1.01 (0.99-1.02)	0.267		
Endemic country*[2], n/N (%)	32/219 (14.61)	37/256 (14.45)	1.37 (0.82-2.30)	0.235		
Close contact, n (%)	147 (63.91)	113 (40.21)	**2.63 (1.84-3.77)**	**<0.001 ***	**4.39 (2.02-9.54)**	**< 0.001***
Cohabitants, n (%)	145 (63.04)	127 (45.20)	**2.07 (1.45-2.95)**	**<0.001 ***	0.64 (0.30-1.38)	0.235
Case classification - Sm-/GXpert+, n (%) - Smear-positive, n (%)	 18 (7.83) 212 (92.17)	 34 (12.10) 247 (87.90)	1.62 (0.89-2.95)	0.112		
Characteristics of the index case						
	(N = 72)	(N = 63)				

Male gender, n (%)	44 (61.11)	44 (69.84)	0.70 (0.33-1.39)	0.288		
Age (years), mean ± SD	37.89 ± 15.11	43.27 ± 19.16	0.98 (0.96-1.01)	0.076		
Endemic country*[2], n/N (%)	16/69 (23.19)	5/58 (8.62)	**3.20 (1.09-9.37)**	**0.028***	0.81 (0.49-1.32)	0.388
Time of symptoms (months), mean ± SD	2.05 ± 1.75	2.34 ± 2.23	0.93 (0.78-1.11)	0.417		
Cavitation, n (%)	46/72 (63.89)	26 (41.27)	**2.52 (1.26-5.04)**	**0.009***	**2.31 (1.57-3.41)**	**<0.001***
Immunosupression*[3], n (%)	2/72 (2.78)	6 (9.52)	0.27 (0.05-1.40)	0.145		
Resistance to anti-tuberculosis agents*[4], n (%)	10/72 (13.89)	5 (7.94)	1.87 (0.60-5.80)	0.272		
Admission to hospital, n (%)	27/72 (37.50)	26 (41.27)	0.85 (0.43-1.71)	0.655		
Days of admission, mean ± SD	17.15 ± 14.36	12.24 ± 11.73	0.97 (0.93-1.02)	0.188		

CI: Confidence interval. n: number of patients. MTB: *Mycobacterium tuberculosis*. N: study group sample size. OR: Odds ratio. Sm-/GXpert+: Smear-negative/GeneXpert-positive.

*: statistically significant.

*[2]: Endemic country defined as having a TB incidence > 100 cases per 100,000 population.

*[3]: Immunosuppressive condition was defined as having at least one of the following conditions: HIV, active malignancy, solid organ or stem cell transplantation or being under immunosuppressive therapy.

*[4] Resistance to any anti-tuberculous drug confirmed by phenotypic susceptibility tests or GeneXpert®MTB/RIF.

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