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MAJOR ARTICLE

The Xpert® MTB/RIF cycle threshold value predicts *M. Tuberculosis* transmission to close contacts in a Brazilian prospective multicenter cohort

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Background: The Xpert® MTB/RIF rapid molecular test provides a quantitative measure of *Mycobacterium tuberculosis (Mtb)* DNA in the form of cycle threshold (Ct) values. This information can be translated into mycobacterial load and used as a potential risk measure of bacterial spread for tuberculosis cases, which can impact infection control. However, the role of Ct values in assessing *Mtb* transmission to close contacts has not yet been demonstrated.

Methods: A prospective study was performed to investigate the association between Xpert® MTB/RIF Ct values and *Mtb* transmission to close contacts of patients with culture-confirmed pulmonary TB in a multi-center Brazilian cohort. We evaluated clinical and laboratory data, such as age, sex, race, smoking habits, drug use, alcohol use, chest radiograph, Xpert® MTB/RIF results among pulmonary tuberculosis cases, and QuantiFERON(QFT)-Plus results at baseline and after six months for close contacts who had a negative result at baseline.

Results: A total of 1,055 close contacts of 382 pulmonary tuberculosis cases were included in the study. The median Ct values from pulmonary tuberculosis cases of QFT-Plus positive (at baseline or six months) close contacts were lower compared with those who were QFT-Plus negative. An adjusted logistic regression demonstrated that reduced Ct values from the index cases were independently associated with QFT-Plus conversion from negative to positive (OR: 1.61, 95% CI: 1.12-2.32) after adjusting for clinical characteristics.

Conclusion: Close contacts of pulmonary TB index cases exhibiting low Xpert MTB/RIF Ct values displayed higher rates of TB infection, reflecting Mtb transmission.

Keywords: *Mycobacterium tuberculosis*, tuberculosis, TB transmission, Close contacts.

INTRODUCTION

Tuberculosis (TB) is a potentially fatal infectious disease caused by *Mycobacterium tuberculosis* (Mtb). Those with active pulmonary TB who have a high bacterial load in sputum pose a significant risk of transmitting Mtb to close contacts [1]. Several studies have demonstrated an association between a higher grade of acid-fast bacilli (AFB) in sputum and Mtb transmission [2–6]. However, the several days required for growth in culture can delay TB diagnosis and treatment, which potentially results in increased Mtb spread [4].

It is estimated that 5-10% of Mtb-exposed individuals who develop TB infection (TBI) may progress to the active form of the disease during their lifetime, usually within the first 2 years after contact, if not treated with TB preventive therapy (TPT) [7]. Identification of contacts and the use of TPT in TBI patients is critical to reduce Mtb transmission. In countries where TB is endemic screening for TBI is traditionally performed using the tuberculin skin test (TST) and,

more recently, the QuantiFERON-TB Gold Plus (QFT-Plus) test, an interferon-gamma release assay (IGRA) [8,9].

Since 2010, the World Health Organization (WHO) has recommended the replacement of smear microscopy with the molecular test Xpert MTB/RIF for TB diagnosis, supported by studies that demonstrated the highest accuracy of this technique compared to smear microscopy [5]. In routine labs, Xpert MTB/RIF results are usually reported as Mtb detected or not detected. Given that this is a polymerase chain reaction (PCR) technology, it is possible to quantify the amount of bacterial DNA present in each sample through the cycle threshold (Ct) value. The Ct value is inversely related to the bacillary load; the lower the number of cycles necessary to identify Mtb DNA, the greater the bacterial load in the sample [5].

Recently, a study has demonstrated that the Xpert MTB/RIF Ct value is correlated with the AFB smear status and with time to positivity in liquid culture [10]. However, the association between Ct value and Mtb transmission to close contacts has not been directly evaluated. Thus, in the present study, we sought to investigate the Xpert MTB/RIF Ct values of persons with active pulmonary TB and to dissect its association with the risk of Mtb transmission to close contacts.

METHODS

Ethics approval

The protocol, informed consent, and study documents were approved by the institutional review boards at all study sites. Participation was voluntary and written informed consent was obtained from all participants or their legally responsible guardians.

Study and locations

We evaluated the association between Ct values of Xpert® MTB/RIF from TB index cases and QFT-Plus conversion in their respective close contacts in the Regional Prospective Observational Research in TB (RePORT-Brazil) cohort. The RePORT-Brazil consortium is an ongoing, multicenter cohort study, composed of culture-confirmed pulmonary TB cases and their close contacts, all followed for up to 24 months [26]. Participants of this study were enrolled during Phase 1 (between June 2015 and June 2019, with a follow-up through June 2021). Enrollment sites included five healthcare centers: Fundação Medicina Tropical Dr. Heitor Vieira Dourado (Manaus), Instituto Nacional de Infectologia Evandro Chagas , Clínica da Família Rinaldo Delamare, and Secretaria de Saúde de Duque de Caxias (Rio de Janeiro) and Instituto Brasileiro para Investigação da Tuberculose (Salvador). This resulted in a cohort of 1,187 cases of active TB (cohort A) and 2,700 close contacts (cohort B) of these patients.

All participants in cohort A were at least 18 years old, with new or recurrent pulmonary TB, and had culture-positive sputum. Cohort B included all close contacts irrespective of age that accepted to participate of the study.

Study population

The population of this study was composed of 779 pulmonary TB index cases recruited in our cohort study who had Xpert Ct results available. Of these, 397 cases were excluded for not having close contacts reported and/or enrolled. In addition, close contacts with prior TB were excluded. Thus, a total of 1,055 contacts from 382 TB index cases were evaluated **(Figure 1).**

TB index case definition

Pulmonary TB patients were diagnosed with Xpert®MTB/RIF and culture positive tests. The laboratory tests (Xpert® MTB/RIF and culture) were performed according to the manufacturer's protocol [13,14].The Ct values were measured by multiple probes targeting the rpoB gene (A, B, C, D, and E), and were used to quantify the mycobacterial load, considering the maximum valid Ct as 34 for all probes [15]. Each probe indicates a different region of mutation, given that the probes hybridize to the sequence of the rpoB gene: A (codons 507–511), B (codons 512–518), C (codons 518–523), D (codons 523–529) and E (codons 529–533). Furthermore, specimens were incubated in culture with Mycobacteria Growth Indicator Tube (MGIT) at 37°C. The BD BACTEC™ MGIT™ System instrument automatically monitored for growth in MGIT.

Close contact definition and laboratory evaluation

For this study, close contacts were defined as having ≥4 hours of exposure per week with the TB index case at any time in the 6 months prior to TB diagnosis [11]. Close contacts were evaluated for Mtb infection with the QFT-Plus test at baseline (M0) and after 6 months (M6) for those who had a negative result at baseline. All contacts irrespective of age were included in the analysis. Collection, processing, and interpretation of the QFT-Plus test were performed according to the manufacturer's recommendations (QIAGEN). Briefly, venous blood was collected in four tubes (NIL, TB1, TB2, and Mitogen) and incubated at 37°C for 20h. After incubation, samples were stored at -20°C until the ELISA was performed, which was within 2 weeks. IFN-γ levels (international units [IU] per milliliter) were quantified with a 4-point standard curve. QFT-Plus analysis software was used to generate the results. The software performed a quality control assessment of the assay, generated a standard curve, and provided both quantitative (IU per milliliter) and qualitative (positive, negative, or indeterminate) results.

Variables analyzed in the study

Demographic (age, sex, self-reported race, body mass index [BMI], smoking habits, secondary smoking, alcohol use, drug use), clinical (cough, fever, night sweats, weight loss, chest radiograph), and laboratory (HIV status, CD4 count, sputum smear microscopy, MGIT culture,

and Xpert®MTB/RIF Ct value results) variables of TB index cases were collected at diagnosis. Close contact variables included age, sex, self-reported race, BMI, tobacco, alcohol use, drug use, chest radiography, HIV status, HIV-1 RNA viral load, CD4 count if HIV-seropositive, and QFT-Plus results. Moreover, QFT-Plus conversion was defined as those participants with a negative result at enrolment (around 1 month after TB index enrolment) and are QTF-positive at M6.

Statistical analysis

Statistical analyses were performed using the CompareGroups (version 4.5.1), rstatix (version 0.4.0), stats (version 3.6.2), and caret (version 6.0.86) R packages. Descriptive analyses were performed to characterize the study population. Categorical variables were compared using Pearson's chi-square test (Yate's correction) or the Fisher's two-tailed test and presented as number and frequency (%) in the tables. Continuous variables were displayed as median and interquartile range (IQR) and tested for Gaussian distribution using the D'Agostino-Pearson test. Comparisons of Xpert® MTB/RIF Ct values in TB index cases between contacts who were QFT-Plus negative *versus* positive at M0 and contacts who were QFT-Plus negative *versus* conversion to positive at M6 were performed using the Mann-Whitney U test. The Spearman rank correlation test was performed to assess relationships between Xpert® MTB/RIF Ct values in TB index cases with QFT-Plus results. Receiver Operating Characteristics (ROC) curves were performed to assess the area under the curve (AUC) and power/overall accuracy of the Xpert® MTB/RIF Ct values to discriminate QFT-Plus positive or conversion from QFT-negative controls. The best cut-off points were selected using the Youden index [12]. Furthermore, a binomial logistic regression model (ENTER method) was used to assess the independent associations between Xpert® MTB/RIF Ct values and QFT-Plus conversion. The following variables were included in multivariable-adjusted models, as follows. TB index: age, sex, race), illicit drug use , alcohol use , cavitation on chest X-ray; Contact: age, sex and smoking habits. These variables were pre-specified at the time of delineation of the analysis plan; such approach has been explored in a variety of previous studies[13–15].

RESULTS

Characteristics of TB index cases and close contacts at Baseline and Month 6 (M6)

Out of 382 TB index cases included in the study, 168 TB cases had only QFT-negative contacts (44%), 110 TB index cases had only QFT-positive contacts (29%) and 104 TB index cases had both QFT-positive and negative contacts (27%) at baseline. The overall characteristics of TB index and close contacts are detailed in **Table 1**. When comparing the characteristics of TB index cases according to QFT results from contacts, we observed that those with QFT-positive contacts presented a higher frequency of presence of cavitations on chest X-rays in contrast with those with QFT-negative contacts (TB index case of QFT-negative contact: 40.2% *versus* TB

index case of QFT-positive contact: 54.7%, p-value = 0.007). Regarding close contacts, from the identified TB index cases, 722 QFT-negative participants and 333 QFT-positive participants were enrolled. QFT-positive contacts were older, had a higher proportion of women, and had a higher BMI than those who were QFT-negative. Characteristics of TB index cases and close contacts at baseline are detailed in **Table 2**.

The QFT-negative contacts at baseline were retested after six months, and 91/717 (12.7%), presented a positive result (QFT-conversion) at M6. Thus, we evaluated the same characteristics of TB index cases and their contacts according to the QFT-Plus result at M6. Similar to our primary result, we found that only the frequency of chest X-ray of cavitation in TB index cases had a statistically significant difference when comparing the two groups (QFT-negative in both timepoints: 40.0%; and QFT-conversion: 53.5%, p=0.007) (**Supplementary Table 1**).

Comparison of Xpert® MTB/RIF CT values from TB index cases with QFT-Plus results of close contacts at both timepoints

After comparing the characteristics of TB index cases and their close contacts according to the QFT-Plus result of the contacts at baseline and M6, we investigated the Ct values of Xpert® MTB/RIF in each of these groups at both timepoints. Comparing the Ct values from TB index cases, the Ct values of those with QFT-positive contacts were lower than the Ct values of those with QFT-negative contacts at baseline, except probe E**.** A similar result was observed when comparing the Ct values related to QFT-plus results at M6. That is, the Ct values from TB index cases of QFT-conversion contacts were significantly lower than those who remained negative on the second test, considering the probe mean, probes A, C, and E. The Ct value from TB index cases at baseline and M6 are detailed in **Supplementary Table 2 and 3.**

Independent association of decreased Xpert MTB/RIF CT values with QFT-Plus conversion at M6

After identifying that lower Ct values from TB index cases were related to contacts who were QFT-Plus positive (baseline or M6), we performed a binomial logistic regression analysis to assess whether the decrease in these values was associated with QFT- conversion independent of other factors (**Figure 2**). The results demonstrated that the lower Xpert® MTB/RIF Ct values were independently associated with QFT-conversion of close contacts, regardless of the probe, as well, considering the mean value (-CT mean, aOR: 1.61, IQR: 1.12-2.32, p<0.001). Each model was adjusted for the following parameters, which were pre-specified: age (TB index), sex (TB index), ethnicity (TB index), drug use (TB index), alcohol use (TB index), cavitation on chest Xray (TB index) age (contact), sex (contact) and smoking habits (contact). The details of the variables used for adjustment of the full logistic regression models are described in **Table 3** , whereas the specific odds ratio values for the Xpert CT values are described in **Figure 2**.

Correlation between Xpert® MTB/RIF CT values and quantitative QFT-Plus results

A Spearman correlation analysis was carried out to evaluate the relationship between the Xpert® MTB/RIF Ct values and QFT-Plus results at M6. **Supplementary Figure 1** summarizes the significant negative correlation between Xpert® MTB/RIF Ct values of index cases with quantitative QFT-Plus values of contacts, regardless of whether isolated values from the Xpert® MTB/RIF probes or their mean were used. This analysis has shown that low Ct values were significantly correlated with high quantitative QTF values, demonstrating that there is an association between the TB index parameters (CT value) and the close contacts parameters (QFT value), which can be related to TB transmission.

Xpert® MTB/RIF CT values from TB cases predict QFT-Plus conversion in close contacts

In order to test the performance of Xpert® MTB/RIF Ct values in classifying close contacts according to QFT-Plus results (negative or positive) at M0 and QFT-Plus results (negative or conversion) at M6, we used a ROC curve analysis (**Figure 3**). We found that the mean probes of Xpert® MTB/RIF Ct values had better power to discriminate close contacts with QFT-Plus conversion in relation to the QFT-Plus negative (area under the curve [AUC] of 0.999 (CI 95%: 0.997-1.000)) in comparison with each probe individually, with 47% specificity and 78% sensitivity (**Figure 3**). In addition, similar data, although with a lower power of discrimination (lower AUC), were observed in mean probes of Xpert® MTB/RIF Ct values at M0 or any timepoint of study (M0+M6 data) (**Figure 3**).

DISCUSSION

In our study, Xpert® MTB/RIF Ct values demonstrated the potential to be used to identify TB index cases at higher risk of Mtb transmission. We observed that close contacts with a QFT-positive result (at either M0 or M6) were more likely to be close contacts of TB index cases with cavitation on chest X-ray, and with lower Ct values than those TB index cases who had a QFT-negative contacts. In addition, a decrease of 1 unit in any of the Xpert® MTB/RIF Ct values from the probes was associated with a risk of QFT-conversion at month 6. In our study, the consistent result of the association between the decreased Ct values and QFT conversion across all probes underscores the robustness and validity of our findings. Such consistency, irrespective of the probe used, reduces potential false-positive results, avoids artifacts tied to any specific probe, and bolsters confidence in the observed association. This uniformity suggests that the phenomenon is not restricted to a particular genetic region but represents a more general feature of the presence of the pathogen. Thus, it is noted that Xpert® MTB/RIF Ct values evaluation of TB patients is essential to the diagnosis, as well as to identify those at high risk of Mtb transmission, being critical to reduce the incidence of TB and to control Mtb dissemination [16–18].

The spread of Mtb is strongly affected by the characteristics of the environment, microorganisms, and host response, with some TB index cases generating considerably more new infections than others [3]. There is an increased risk of Mtb transmission to close contacts when the TB index case has a high bacillary load on smear sputum microscopy at the time of screening [18]. The identification and follow-up of close contacts at higher risk of Mtb transmission can help to prioritize investigations and preventive treatment strategies [18].

The relationship between smear sputum microscopy status and bacillary load as a marker of Mtb transmission is used to guide public health and treatment decisions. Nevertheless, smear status provides an inaccurate estimate of bacillary load [4]. Another bacillary load-related variable is the presence of cavitations on the chest X-ray of patients with TB [19]. In our study, this variable showed significance related to TB transmission, both in univariate and multivariate analyses, demonstrating its role as a potential predictor of TB transmission to close contacts. However, chest X-ray evaluation is not available in all settings and depends on operator and assessor training.

Our results, corroborate with other studies that estimated the risk of transmission [2–5]. In addition, Xpert® MTB/RIF Ct values correlate well with smear grade and with time to positivity in liquid culture. Given the current global recommendation to substitute smear with Xpert® MTB/RIF as the initial diagnostic test for TB as culture results take longer, the results of Xpert® MTB/RIF Ct values may provide the only means to assess bacillary load [22]. This assertion extends to the Xpert MTB/RIF Ultra version [10]. Therefore, our study results hold relevance and should be extrapolated to encompass this technology as well.

Although Xpert® MTB/RIF Ct values do not ensure the viability of bacilli, this information can be used as a measure for infection control and contact tracking, as suggested previously [2,3,16]. Variables of TB index cases such as age, sex, ethnicity, drug use, alcohol use and cavitations on chest X-ray, as well variables of close contacts such as age, sex, and smoking habits were used to adjust our regression analyses and did not influence the results shown in our study. Thus, Xpert® MTB/RIF Ct values were associated with Mtb transmission regardless of these variables, including chest X-ray cavitations. Although it has been demonstrated that the Xpert® MTB/RIF Ct values are influenced by factors related to the TB index case [6], its association with Mtb bacillary burden is clear. Thus, the CT values are important indicators of transmission [20].

This study has certain limitations. We did not evaluate all the cohort of TB index cases, given that 397 participants were excluded because they did not have reported contacts. Finally, the contacts included in the study were drawn from the RePORT-Brazil cohort, which encompasses individuals linked to culture-confirmed TB cases. While the RePORT-Brazil TB index cases cohort is believed to be representative of all TB patients in Brazil [21], it is unclear whether close contacts are representative of all close contacts in this population. Moreover, it is essential to expand these investigations to settings where it is feasible to assess delays in diagnosis, symptoms among close contacts, and the prevalence of HIV infection at a higher frequency, and

to explore their potential impact on QTF conversion results. Additionally, validating these findings with molecular epidemiology tools would be of paramount importance.

Regardless of such limitations, this study raises the possibility that Xpert® MTB/RIF Ct values could be used to identify patients at higher risk of transmission and can be useful in prioritizing contact investigations.

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TABLES

Table 1: Characteristics of close contacts and TB index cases.

	TB index cases (n=382)	Close contacts (n=1055)
Age (years), median (IQR):	35 (25-47)	31 (15-46)
Sex (Female), n (%):	130 (34)	622 (58.9)
Ethnicity (admixed), n (%):	215 (56.3)	655 (62.1)
BMI, median (IQR):	24.9 (20.6-29.7)	20.5 (18.2-23.1)
Smoking habits, n (%):	202 (52.9)	90 (8.5)
Alcohol use, n (%):	156 (46.8)	352 (33.3)
Illicit drug use, n (%):	34 (8.90)	25 (2.4)
HIV, n (%):	8 (2.00)	2 (0.61)

Table note: The results are presented as median and interquartile range (IQR) or frequency absolute and percentage. abbreviations: BMI: Body Mass Index; HIV: Human Immunodeficiency Virus; IQR: Interquartile Range; QFT: QuantiFERON-TB Gold Plus

Table 2: Characteristics of close contacts and TB index cases according to the Baseline (M0) QFT-Plus results.

Close contacts	QFT negative (n=722)	QFT positive (n=333)	p value
Age (years), median (IQR):	29.4 (13.9-43.7)	35.4 (16.9-50.1)	**<0.001**
Sex (Female), n (%):	401 (55.5)	221 (66.4)	**0.001**
Ethnicity (admixed), n (%):	466 (64.5)	189 (56.8)	**0.002**
BMI, median (IQR):	23.7 (19.3-28.0)	24.9 (20.6-29.7)	**0.001**
Smoking habits, n (%):	52 (7.20)	38 (11.4)	0.071
Alcohol use, n (%):	233 (32.3)	118 (35.4)	0.587
Substance use, n (%):	16 (2.22)	9 (2.70)	0.884
HIV, n (%):	7 (1.00)	2 (0.61)	0.336
CD4 count, median (IQR):	400 (218-596)	362 (259-708)	0.896
Quantitative QTF, median (IQR)[1]:	0.06 (0.02-0.15)	1.56 (1.03-3.24)	**0.069**
TB index cases	**(n=228)**	**(n=149)**	**p value**
Age (years), median (IQR):	35.0 (25.0-48.0)	35.0 (25.0-47.0)	**0.674**
Sex (Male), n (%):	152 (65.2)	100 (67.1)	0.789
Ethnicity (admixed), n (%):	137 (58.8)	77 (51.7)	0.207
BMI, median (IQR):	20.3 (18.1-22.5)	20.9 (18.4-23.7)	0.134
Smoking habits, n (%):	107 (45.7)	95 (64.2)	0.107
Secondary smoking, n (%):	83 (35.5)	44 (29.7)	0.294
Alcohol use, n (%):	89 (45.4)	67 (45.3)	0.239
Substance use, n (%):	26 (33.3)	18 (34.6)	0.999
Cough, n (%):	219 (93.6)	141 (95.3)	0.644
Fever, n (%):	186 (79.5)	121 (81.8)	0.680
Weight loss, n (%):	213 (91.0)	135 (92.5)	0.763
Night sweats, n (%):	153 (65.4)	102 (69.4)	0.486
Cavitations, n (%):	94 (40.2)	81 (54.7)	**0.007**
HIV, n (%):	8 (4.67)	6 (4.72)	0.997
CD4 count, median (IQR):	132 (55.0-234.0)	156 (40.8-373.0)	0.691
Smear positive, n (%):	185 (79.1)	120 (81.1)	0.727
Solid culture positive, n (%):	221 (98.4)	140 (94.6)	0.286

Table note: The results are presented as median and interquartile range (IQR) or frequency absolute and percentage. *Quantitative QFT-Plus was obtained as following, using data from month 6: AgTB1+AgTB2-Nil. Statistical analysis was performed using the Mann-Whitney U test or (continuous variables, two by two) or the Pearson's Chi-Square ($\chi 2$) test (for data on frequency). For both analyses, p was considered significant when <0.05. Bold-type font indicates statistical significance. Abbreviations: BMI: Body Mass Index; HIV: Human Immunodeficiency Virus; IQR: Interquartile Range; QFT: QuantiFERON-TB Gold Plus

Table 3: Logistic regression models, with all variables included.

	Model 1		
	crude OR (95%CI)	adj. OR (95%CI)	Pvalue
Age (continuous) [Contact]	1.01 (0.99-1.02)	1.01 (0.99 - 1.02)	0.264
Sex (female x male) [Contact]	0.89 (0.54-1.47)	0.86 (0.5-1.47)	0.579
Smoking habits (yes x no) [Contact]	1.46 (0.62-3.41)	1.53 (0.62-3.78)	0.374
Age (continuous) [TB index]	0.99 (0.98-1.01)	0.99 (0.97-1.02)	0.423
Sex (female x male) [TB index]	1.42 (0.85-2.36)	1.54 (0.90-2.64)	0.115
Race (non-white x white) [TB index]	0.67 (0.41-1.11)	0.79 (0.45-1.38)	0.4
Substance use (yes x no) [TB index]	2.07 (1.01-4.24)	1.6 (0.73-3.54)	0.254
Alcohol use (yes x no) [TB index]	1.43 (0.86-2.37)	1.2 (0.66-2.16)	0.55
X-ray cavitation (yes x no) [TB index]	2.77 (1.6-4.79)	2.31 (1.29-4.12)	**0.003**
Xpert (-CT mean)	1.60 (1.10-2.30)	1.61 (1.12-2.32)	**0.001**
	Model 2		
	crude OR (95%CI)	adj. OR (95%CI)	Pvalue
Age (continuous) [Contact]	1.01 (0.99-1.02)	1.01 (0.99-1.02)	0.272
Sex (female x male) [Contact]	0.89 (0.54-1.47)	0.87 (0.51-1.49)	0.612
Smoking habits (yes x no) [Contact]	1.46 (0.62-3.41)	1.45 (0.58-3.6)	0.438
Age (continuous) [TB index]	0.99 (0.98-1.01)	0.99 (0.97-1.01)	0.422
Sex (female x male) [TB index]	1.42 (0.85-2.36)	1.59 (0.93-2.71)	0.093
Race (non-white x white) [TB index]	0.67 (0.41-1.11)	0.79 (0.45-1.38)	0.414
Substance use (yes x no) [TB index]	2.07 (1.01-4.24)	1.64 (0.75-3.61)	0.227
Alcohol use (yes x no) [TB index]	1.43 (0.86-2.37)	1.19 (0.66-2.15)	0.554
X-ray cavitation (yes x no) [TB index]	2.77 (1.6-4.79)	2.26 (1.25-4.06)	**0.005**
Xpert (-CT Probe A)	1.60 (1.10-2.30)	1.61 (1.11-2.32)	**0.001**
	Model 3		

	crude OR (95%CI)	adj. OR (95%CI)	Pvalue
Age (continuous) [Contact]	1.01 (0.99-1.02)	1.01 (0.99-1.02)	0.293
Sex (female x male) [Contact]	0.89 (0.54-1.47)	0.86 (0.5-1.47)	0.579
Smoking habits (yes x no) [Contact]	1.46 (0.62-3.41)	1.54 (0.62-3.83)	0.364
Age (continuous) [TB index]	1 (0.98-1.01)	0.99 (0.97-1.01)	0.311
Sex (female x male) [TB index]	1.42 (0.85-2.36)	1.57 (0.92-2.69)	0.102
Race (non-white x white) [TB index]	0.67 (0.41-1.11)	0.79 (0.45-1.38)	0.401
Substance use (yes x no) [TB index]	2.07 (1.01-4.24)	1.56 (0.71-3.47)	0.283
Alcohol use (yes x no) [TB index]	1.43 (0.86-2.37)	1.26 (0.7-2.28)	0.444
X-ray cavitation (yes x no) [TB index]	2.77 (1.6-4.79)	2.32 (1.3-4.13)	**0.003**
Xpert (-CT Probe B)	1.62 (1.10-2.50)	1.65 (1.12-2.44)	**0.001**
	Model 4		
	crude OR (95%CI)	adj. OR (95%CI)	Pvalue
Age (continuous) [Contact]	1.01 (0.99-1.02)	1.01 (0.99-1.02)	0.257
Sex (female x male) [Contact]	0.89 (0.54-1.47)	0.85 (0.5-1.46)	0.564
Smoking habits (yes x no) [Contact]	1.46 (0.62-3.41)	1.5 (0.61-3.72)	0.394
Age (continuous) [TB index]	0.99 (0.98-1.01)	0.99 (0.97-1.01)	0.404
Sex (female x male) [TB index]	1.42 (0.85-2.36)	1.53 (0.89-2.62)	0.124
Race (non-white x white) [TB index]	0.67 (0.41-1.11)	0.79 (0.45-1.39)	0.416
Substance use (yes x no) [TB index]	2.07 (1.01-4.24)	1.57 (0.71-3.47)	0.278
Alcohol use (yes x no) [TB index]	1.43 (0.86-2.37)	1.23 (0.68-2.22)	0.49
X-ray cavitation (yes x no) [TB index]	2.77 (1.6-4.79)	2.31 (1.29-4.13)	**0.003**
Xpert (-CT Probe C)	1.57 (1.10-2.30)	1.58 (1.11-2.25)	**0.002**
	Model 5		
	crude OR (95%CI)	adj. OR (95%CI)	Pvalue
Age (continuous) [Contact]	1.01 (0.99-1.02)	1.01 (0.99-1.02)	0.244

Sex (female x male) [Contact]	0.89 (0.54-1.47)	0.85 (0.5-1.46)	0.555
Smoking habits (yes x no) [Contact]	1.46 (0.62-3.41)	1.51 (0.61-3.74)	0.386
Age (continuous) [TB index]	0.99 (0.98-1.01)	0.99 (0.97-1.01)	0.387
Sex (female x male) [TB index]	1.42 (0.85-2.36)	1.52 (0.89-2.61)	0.126
Race (non-white x white) [TB index]	0.67 (0.41-1.11)	0.8 (0.46-1.4)	0.433
Substance use (yes x no) [TB index]	2.07 (1.01-4.24)	1.54 (0.7-3.4)	0.299
Alcohol use (yes x no) [TB index]	1.43 (0.86-2.37)	1.24 (0.69-2.23)	0.483
X-ray cavitation (yes x no) [TB index]	2.77 (1.6-4.79)	2.33 (1.3-4.15)	**0.003**
Xpert (-CT Probe D)	1.58 (1.10-2.32)	1.59 (1.10-2.30)	**0.002**
	Model 6		
	crude OR (95%CI)	adj. OR (95%CI)	Pvalue
Age (continuous) [Contact]	1.01 (0.99-1.02)	1.01 (0.99-1.03)	0.222
Sex (female x male) [Contact]	0.89 (0.54-1.47)	0.86 (0.5-1.47)	0.576
Smoking habits (yes x no) [Contact]	1.46 (0.62-3.41)	1.61 (0.65-3.96)	0.318
Age (continuous) [TB index]	0.99 (0.98-1.01)	0.99 (0.98-1.01)	0.615
Sex (female x male) [TB index]	1.42 (0.85-2.36)	1.55 (0.91-2.65)	0.11
Race (non-white x white) [TB index]	0.67 (0.41-1.11)	0.78 (0.44-1.36)	0.378
Substance use (yes x no) [TB index]	2.07 (1.01-4.24)	1.76 (0.81-3.82)	0.167
Alcohol use (yes x no) [TB index]	1.43 (0.86-2.37)	1.13 (0.63-2.03)	0.685
X-ray cavitation (yes x no) [TB index]	2.77 (1.6-4.79)	2.62 (1.48-4.62)	**<0.001**
Xpert (-CT Probe E)	1.60 (1.10-2.31)	1.61 (1.12-2.31)	**<0.001**

Table note: Bold-type font indicates statistical significance. OR: Odds Ratio; CI: Confidence Interval.

FIGURE LEGENDS

Figure 1. Study flow chart. Of the 779 index TB cases recruited and with Xpert results available, 382 were included because they had close contacts identified and recruited. From this, 1094 close contacts were identified, of which 1055 were included in this study.

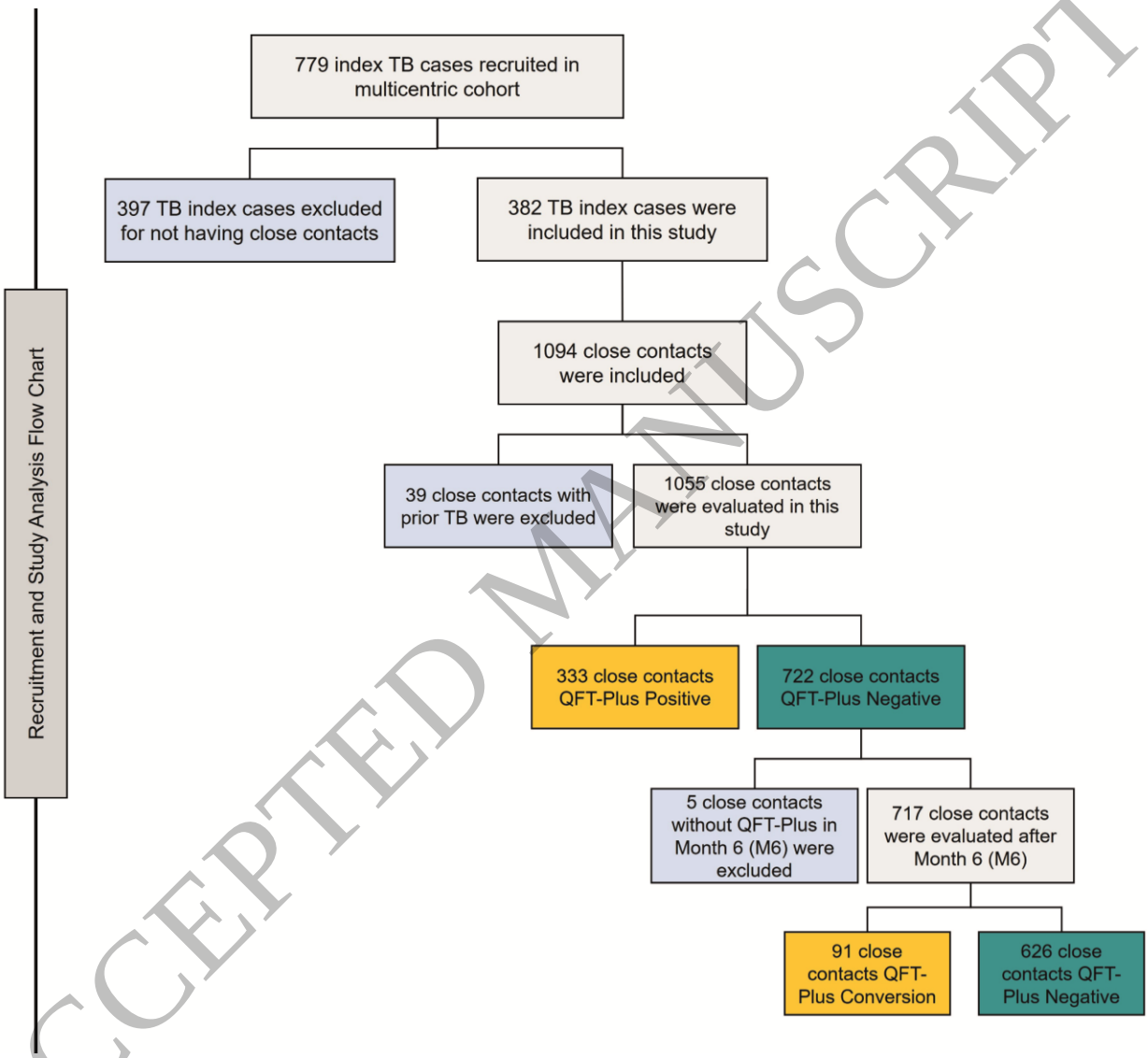


Figure 2. Binomial logistic regression model to evaluate independent associations between Xpert® MTB/RIF Ct values of the TB index cases and the QFT-Plus conversion results in close contacts. We performed a logistic regression model (method "enter") for each probe using the inverse values to assess whether decreased Ct values were associated with QFT-Plus results conversion. Statistical analyses were performed using Binomial logistic regression model. The variables included in the model were: age (TB index), sex (TB index), race (TB index), illicit drug use (TB index), alcohol use (TB index), cavitation on chest X-ray (TB index), age (contact), sex (contact) and smoking habits (contact). The odds ratio values for each combination of variables used to adjusted the multivariable models are described in Table 3.

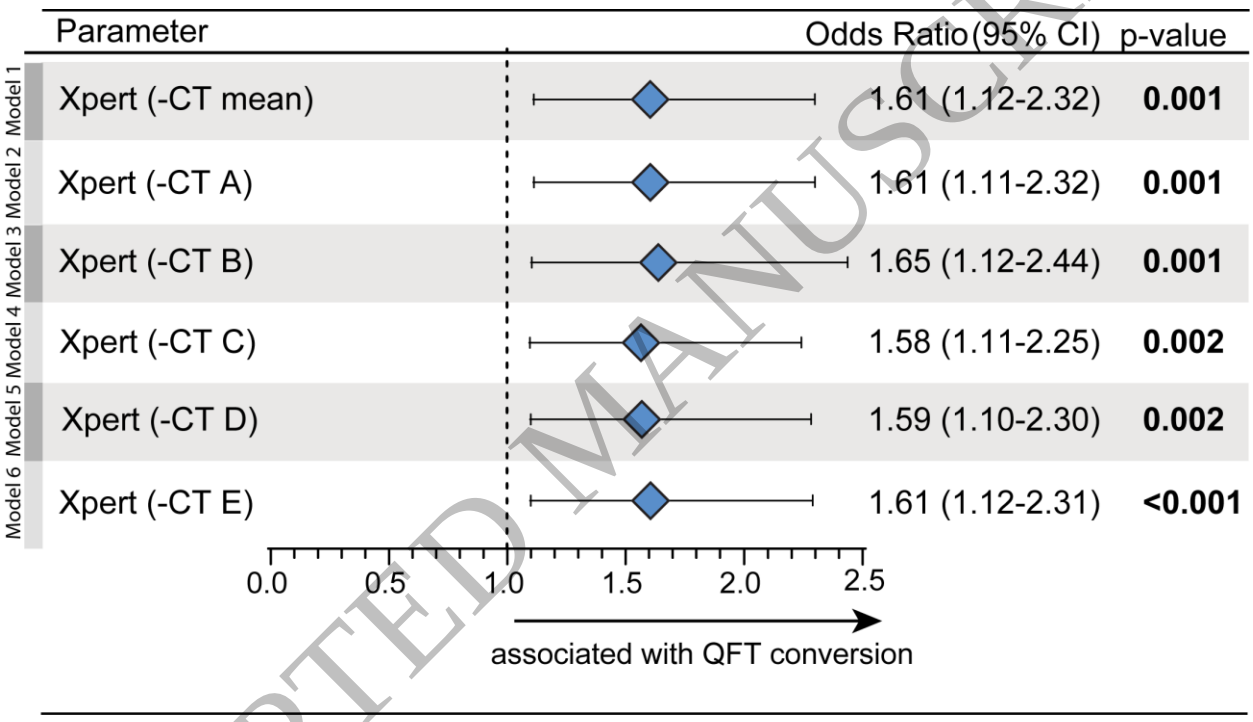
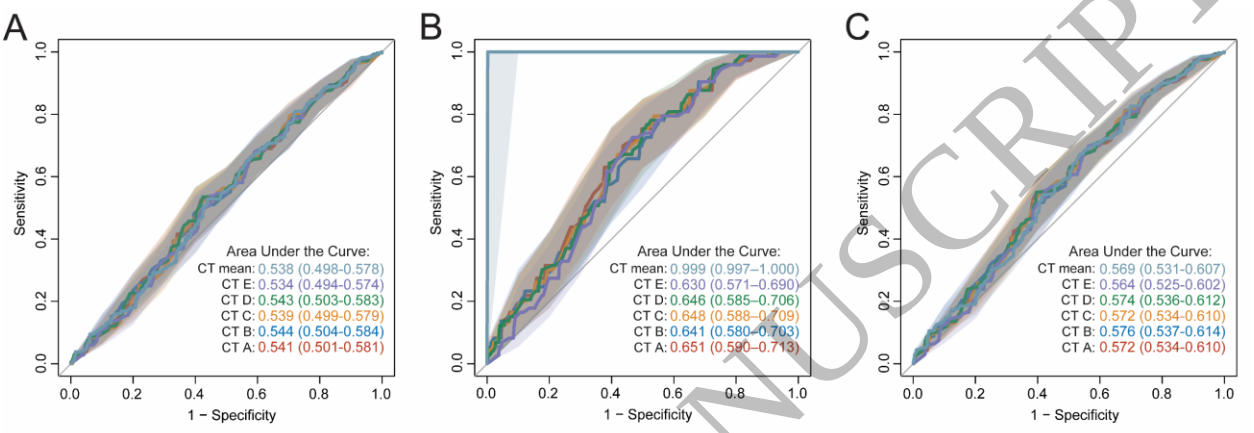


Figure 3. Receiving Operated Curve (ROC) analysis to evaluate the power of Xpert CT parameters (from TB index cases) to discriminate contact QFT positive. The evaluation was performed at baseline (A), conversion at month 6 (B) and QFT positive in any timepoint (baseline+month 6 – C). The thresholds were identified using the Youden index. The area under the curve, sensibility and specificity are displayed with 95% confidence intervals. Abbreviatures: CT: cycle threshold.



	Threshold	Specificity	Sensitivity
CT A	18.4	0.58 (0.55-0.63)	0.53 (0.47-0.58)
CT B	19.5	0.6 (0.56-0.64)	0.49 (0.43-0.54)
CT C	18.6	0.58 (0.54-0.62)	0.52 (0.47-0.58)
CT D	19.6	0.58 (0.54-0.61)	0.54 (0.48-0.60)
CT E	22.1	0.41 (0.37-0.45)	0.68 (0.63-0.73)
CT mean	19.3	0.56 (0.52-0.60)	0.53 (0.47-0.60)

	Threshold	Specificity	Sensitivity
CT A	18.7	0.56 (0.52-0.61)	0.70 (0.59-0.79)
CT B	21.7	0.40 (0.45-0.49)	0.78 (0.68-0.86)
CT C	20.3	0.46 (0.42-0.51)	0.79 (0.70-0.88)
CT D	20.9	0.49 (0.44-0.53)	0.77 (0.67-0.86)
CT E	20.5	0.54 (0.49-0.58)	0.73 (0.63-0.82)
CT mean	20.8	0.47 (0.42-0.51)	0.78 (0.68-0.88)

	Threshold	Specificity	Sensitivity
CT A	18.4	0.61 (0.57-0.65)	0.54 (0.48-0.59)
CT B	21.7	0.45 (0.40-0.30)	0.68 (0.63-0.73)
CT C	18.6	0.60 (0.56-0.65)	0.53 (0.48-0.59)
CT D	19.5	0.60 (0.56-0.65)	0.55 (0.49-0.60)
CT E	22.1	0.41 (0.37-0.45)	0.71 (0.66-0.76)
CT mean	20.9	0.46 (0.42-0.51)	0.67 (0.62-0.72)