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Delayed culture conversion due to cigarette smoking in active pulmonary tuberculosis patients

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SUMMARY

Although many studies have assessed factors affecting culture conversion during tuberculosis treatment, few have looked into the effect of tobacco smoking. This study included 89 active pulmonary tuberculosis patients with positive sputum culture upon presentation and collected information regarding smoking history and culture conversion after 60 days of therapy. Current smokers had a higher risk (OR 5.6; 95%CI 1.7–18.7) of non-conversion after two months of therapy when compared to never and ex-smokers. Cavities on chest X-ray and alcohol abuse were shown to confound this association. After adjustment for cavities on the chest X-ray and alcohol abuse current smoking compared to current non-smoking remained significantly associated with culture non-conversion at 60 days of treatment (adjusted OR 6.9; 95%CI 1.8–26.7, $p = 0.002$) with a significant ($p = 0.004$) trend in adjusted OR with the number of cigarettes smoked daily to 11.6 (1.8–73.4) among those smoking more than 20 cigarettes per day. In conclusion tobacco smoking was found to delay culture conversion during treatment for pulmonary tuberculosis in a dose-dependent manner. More research is needed to elucidate the effects of smoking on tuberculosis treatment response, and of smoking cessation during tuberculosis treatment.

1. Introduction

Tuberculosis (TB) is one of the world's leading infectious diseases. In 2010 there was an estimated 8.7 million incident cases and 1.4 million deaths from TB [1]. Tobacco use is a major public health problem and an important preventable risk for premature deaths. Extensive research has shown associations between tobacco smoking and TB [2–5], and identified an increased risk of mortality among smokers due to TB [6]. Smoking causes numerous pathophysiological changes within the respiratory system, including immunological effects, decreased clearance and altered adherence of inhaled pathogens [7]. Tobacco smoking also impedes the pulmonary expression of anti-TB T-helper type 1 (Th-1) immunity via inhibiting innate immune activation and lung T-cell recruitment [8].

Adequate treatment of TB is essential to reduce morbidity and mortality, and to prevent the spread of the disease [9,10]. Smoking has been suggested to affect treatment outcome expressed as mortality or as sputum smear conversion during treatment [11,12]. Few studies have assessed the effect of smoking on culture conversion during treatment, and those that did yielded discordant results [13–18]. An effect of smoking on the time to culture conversion would be important since a delay in conversion may translate into prolonged infectiousness and increased risk of transmission to others. Furthermore persistent non-conversion after 2 months of treatment can predict relapse after successful treatment [19].

We therefore studied the effects of tobacco smoking on culture conversion in a prospective cohort of patients on first-line treatment for pulmonary tuberculosis.

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2. Methods

This study was part of a larger cohort study of TB treatment. Eligible for enrollment were all patients consecutively diagnosed with smear- and/or culture-positive pulmonary tuberculosis at two specialized hospitals in Brazil (Santa Maria Hospital and the State Institute of Chest Diseases Ary Parreiras) during the period March 2007 to September 2009. Included for the current analyses were all patients who provided informed consent and for whom sputum culture results were available before starting treatment and after 60 days of standardized first-line TB treatment consisting of isoniazid, rifampicin, pyrazinamide and ethambutol. Patients for whom the pretreatment isolate showed resistance to isoniazid and rifampicin (i.e. multidrug resistance) were excluded as these were likely to be non-responsive to first-line treatment. All were treated as in-patients until they were no longer infectious.

Clinical and socio-demographic data were collected before treatment and sputum samples were assessed at day 0 and after 60 days of TB treatment. Sputum samples were submitted to microscopic examination of Ziehl-Neelsen stained smears. Samples were then processed using the Kubica method and inoculated onto Löwenstein-Jensen medium. Smears were graded in accordance to the WHO guideline as 1+, 2+, 3+, scanty and negative [20]. Patients were classified as previously treated (i.e. with a history of use of anti-tuberculosis drugs for more than one month) or new (i.e. otherwise).

History of tobacco smoking was ascertained at entry by standardized, staff administered questionnaire as current smoking and smoking in the past. Patients were grouped in two ways: as current smokers versus current non-smokers (to reflect effects of smoking during the months preceding the TB treatment on culture conversion), and as ever smokers, i.e. current smokers or ex-smokers, versus never smokers (to reflect cumulative effects of tobacco smoke exposure on culture conversion). In addition, possible dose–response effects were examined based on the number of cigarettes currently smoked per day and the number of pack-years smoked cumulatively.

Since patients with substance abuse may be less compliant to therapy, drug and alcohol abuse were identified as potential confounders of the association between smoking and culture conversion. For the classification of alcoholism the CAGE criteria were used [21]. Other potential confounders considered in the analysis were HIV infection, sex, ethnicity, malnutrition, illiteracy, the number of pulmonary cavities on chest X-ray, and pretreatment smear microscopy results. Malnutrition was defined as a body mass index (BMI) score below 18.5 kg/m^2 [22]. Because there were only two patients with diabetes mellitus, this potential confounder was not included in the analysis.

Data were stored electronically and analyzed using Statistical Package for Social Science 13.0 (IBM Corp, Armonk NY, USA). Odds ratios (ORs) with their 95% confidence interval (95%CI) were calculated for the association with culture conversion, and *p*-values were obtained using the two-sided Fisher's exact test. We checked all covariates for potential confounding of the association between smoking history and culture conversion by bivariate logistic regression. Included in the final multivariable logistic regression model were those covariates that changed the OR for this association (i.e. displayed confounding) by more than 10%. All tests were done at the 5% significance level.

3. Results

The study enrolled 164 patients, of whom 104 (63.4%) had non-MDR TB and follow-up data at day 60 available. Pretreatment culture results were missing for another 12 (11.5%) patients, and day-60 culture results were missing for 1 (0.9%) patient. In addition, 2 (1.9%) patients were excluded because of missing smoking information (Figure 1).

The remaining 89 patients were included in the analysis (Table 1). Of these 76 (85.4%) were males; 23 (25.8%) were never-smokers, 43 (48.3%) current smokers and 23 (25.8%) ex-smokers. All current and ex-smokers smoked cigarettes.

At 60 days of treatment, 19 patients (21.3%) remained culture positive and 70 (78.7%) had culture conversion.

The odds for culture non-conversion at 60 days of treatment were significantly increased for current smokers compared to current non-smokers (odds ratio (OR) 5.63; 95% confidence interval (95%CI) 1.69–18.72): 15 of 43 (34.9%) current smokers had no culture conversion versus 4 of 46 (8.7%) current non-smokers ($p = 0.004$). Similarly, the odds for culture non-conversion at 60 days of treatment were significantly increased for current smokers compared to ex-smokers (OR 11.79, 95%CI 1.44–96.23, $p = 0.002$, Figure 1), but not for ever-smokers compared to never-smokers (OR 2.13, 95%CI 0.56–8.13, $p = 0.378$). There was a significant trend in probability of culture non-conversion with the number of cigarettes smoked daily among current smokers from 27.3% for 1–19 cigarettes (less than one package) and 35.0% for 20 cigarettes (one package) to 41.7% for >20 cigarettes (more than one package; $p = 0.003$). No clear trend was observed for the number of pack-years smoked (Figure 2).

The only other significant predictor of culture non-conversion at 60 days of treatment was the smear result at baseline (Supplement). In addition to smear test result, the number of cavities, alcohol abuse, malnutrition, and illicit drug use confounded the association between smoking and culture conversion in the bivariate analyses (Supplement). In the multivariable model, only the number of cavities and alcohol abuse remained as confounders. After adjustment for alcohol abuse and number of cavities, current smoking compared to current non-smoking remained significantly associated with culture non-conversion at 60 days of treatment (adjusted OR 6.85; 95%CI 1.76–26.70, $p = 0.002$). Compared to current non-smokers, the adjusted OR for non-conversion increased significantly with the number of cigarettes smoked daily from 4.2 for 0–19 cigarettes to 7.9 for 20 cigarettes and 11.6 for >20 cigarettes ($p = 0.015$; trend across categories $p = 0.004$; Table 2). There were no significant interactions. Adding the number of pack years (>10 years versus ≤10 years) to this model did not result in significantly improved prediction of culture conversion (adjusted OR 0.76, $p = 0.743$).

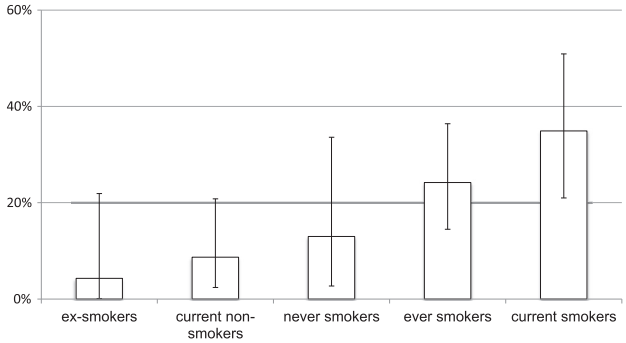

Figure 1. Proportion of patients with positive culture (non-conversion) after 2 months of treatment, by smoking status. Vertical lines denote 95% confidence intervals. Unadjusted odds ratios (95% confidence intervals): ever smokers vs. never smokers 2.13 (0.56–8.13), $p = 0.378$; current smokers vs. current non-smokers 5.63 (1.69–18.72), $p = 0.004$; current smokers vs. ex-smokers 11.79 (1.44–96.23), $p = 0.002$.

Table 1
Baseline characteristics of 89 patients with culture-positive pulmonary tuberculosis.

Characteristic		*n.* (%)
Sex	Female	13 (14.6)
	Male	76 (85.4)
Ethnicity	Non-African descent	31 (34.8)
	African descent	27 (30.3)
	Mixed African descent	25 (28.1)
	NA	6 (6.7)
Literacy	Illiterate	14 (15.4)
	Literate	75 (82.4)
Smoking	Never	23 (25.8)
	Current	43 (48.3)
	Ex-smoker	23 (25.8)
Alcohol abuse (CAGE)	No	29 (32.6)
	Yes	59 (66.3)
	NA	1 (1.1)
Illicit drug use	No	62 (69.7)
	Yes*	27 (30.3)
Diabetes mellitus	No	76 (85.4)
	Yes	2 (2.2)
	NA	11 (12.4)
Previously treated for TB	No	50 (56.2)
	Yes	36 (40.4)
	NA	3 (3.4)
HIV infection status	Negative	73 (82.0)
	Positive	13 (14.6)
	NA	3 (3.4)
Body mass index (BMI) at start of treatment	$\geq$18.5 kg/m^2	24 (27.0)
	<18.5 kg/m^2	56 (62.9)
	NA	9 (10.1)
Findings chest X-ray		
Reduced volume	Yes	36 (40.4)
	No	53 (59.6)
Increased volume	Yes	16 (18.0)
	No	73 (82.0)
Infiltrates	Yes	18 (20.2)
	No	71 (79.8)
Nodules	Yes	3 (3.4)
	No	86 (96.6)
Cavities	0	16 (18.0)
	1–2	40 (44.9)
	3–4	33 (37.1)
Culture conversion After 2 months of treatment	Yes	70 (76.9)
	No	19 (21.4)

NA: not available.
* Includes 18 (20.2%) patients who smoked illicit drugs.

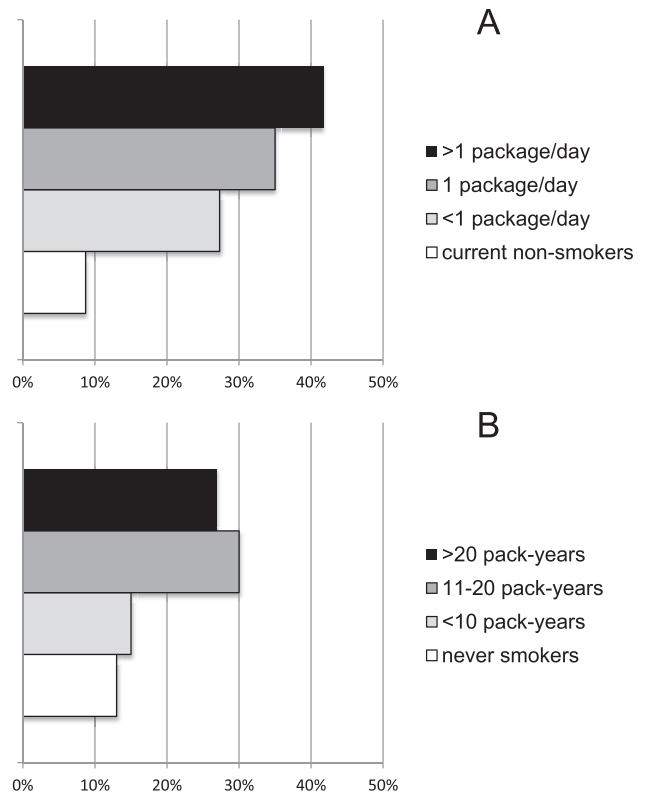

Figure 2. Proportion of patients with positive culture (non-conversion) after 2 months of treatment. A. By average number of packages of cigarettes currently smoked par day. Cuzick's non-parametric test for trend: $p = 0.003$. B. By number of pack-years of cigarette smoking. Cuzick's non-parametric test for trend: $p = 0.147$.

4. Discussion

This prospective cohort study of pulmonary TB patients found a strong and independent association between current smoking and a positive *Mycobacterium tuberculosis* culture at 60 days of standard first-line treatment. There was a significant dose-response relationship with the number of cigarettes smoked daily. No such association was found for ever smoking compared to never smoking, nor for the number of pack-years as an indicator of cumulative exposure to tobacco smoke.

Two previous studies showed an association between a history of ever smoking and delayed culture conversion during treatment [13,14], while three others gave ambiguous results [15–17]. Ever smokers compared to never smokers had significantly longer time to conversion in a randomized-controlled therapeutic vaccination trial and in a cohort study, both conducted in South Africa (adjusted hazard ratio for conversion 0.58 and 0.45, respectively) [13,14]. However, neither routine data from Hong Kong nor a cohort study from Turkey showed delayed culture conversion for ever smokers [15,16], and in a multi-country, randomized-controlled drug trial culture conversion at two months was associated with ever smoking in univariate, but not in multivariate analysis [17]. Only one study specifically assessed the effect of current smoking: a case-control study from Brazil found the risk of culture positivity at two months of therapy to be 3-fold increased for current smokers compared to non-smokers, and in addition 2-fold increased for smoking >20 cigarettes per day [18].

Taken together with our results this suggests that it is in fact current smoking that defines the effect of smoking on culture conversion, that different proportions of current smokers among the ever smokers may explain the contradicting results from previous studies, and that cumulative lung damage due to smoking seems to be less relevant. This would support the hypothesis that immunological changes caused by smoking interfere with anti-tuberculosis treatment.

Several studies found that higher pretreatment sputum smear grades, cavitary disease and/or prior history of TB predict delayed culture conversion [12,14,16,18]. We found similar associations with 60-day culture conversion, although this was statistically significant only for smear grade, probably due to the relatively small size

Table 2
Multivariable logistic regression model for the association between current smoking and culture conversion at 60 days of anti-tuberculosis treatment.

Variable	Category	Adjusted odds ratio (95% confidence interval)	*p*-value
Current tobacco smoking*: daily number of cigarettes	0	1	0.015
	1–19	4.22 (0.72–24.94)	
	20	7.89 (1.59–39.08)	
	$\geq$20	11.57 (1.82–73.42)	
Alcohol abuse	No abuse	1	0.027
	Abuse	0.22 (0.05–0.90)	
Cavities	Number†	2.30 (0.96–5.54)	0.050

* *p*-value for trend (fitted as linear variable) 0.004. Current smoking versus no current smoking: adjusted odds ratio 6.85 (95%CI 1.76–26.70), $p = 0.002$.
† Average odds ratio for each additional cavity.

of our study population. Of these potential risk factors, only the number of cavities confounded the association between smoking and culture conversion, which may reflect that smokers with TB more frequently have cavities [23]. The effect of current smoking on 60-day culture conversion remained after multivariable adjustment, indicating that this is independent of the extent of disease and residual lung damage due to earlier TB episodes. A previous study reported delayed smear conversion in association with smoking only among patients with extensive TB disease defined by radiological criteria and initial smear grade [12]. We found no such interactions for culture conversion but the power of our study to detect these as statistically significant was small.

Also alcohol abuse confounded the association between smoking and culture conversion, but contrary to previous studies patients who abused alcohol had a tendency towards higher probability of culture conversion by day 60. The available literature suggests that alcohol abuse predisposes for a poor response to antituberculosis treatment [24], is linked to poor compliance to therapy [25,26], and therefore, by taking fewer than prescribed drug doses, results in a higher risk of non-conversion [4]. Possibly the patients with alcohol abuse in the present study were monitored more strictly for taking their medication. This can only be speculated, as we did not collect information about treatment adherence. However the whole study population was treated as in-patients, which would make non-compliance less plausible. As the results may be contradictory to the literature, the possibility of uncontrolled confounding has to be considered. Alcohol abuse was defined using the CAGE criteria at the start of therapy and no additional information was available concerning the duration and degree of alcohol dependency as identified by other instruments such as the Michigan Alcohol Screening Test [27]. and therefore this possibly important distinction could not be made. Instead there might be a biological explanation to the association between alcoholism and non-conversion, which smoking possibly emphasizes.

Two partially related hypotheses may explain the delayed culture conversion among current smokers. One could be the altered activity of nitric oxide (NO). NO is an important effector molecule in the defense against intracellular organisms [28]. Activated macrophages that are capable of suppressing multiplication of, or killing, *M. tuberculosis* express nitric oxide synthase (NOS) which is essential for the production of NO; its anti-microbial activity was lost when NOS inhibitors were present [28]. Cigarette smoking is a NOS inhibitor and its irreversible inhibitory effect has been shown in pulmonary endothelial cells [29]. Another hypothesis is altered iron content of alveolar macrophages. In addition to its anti-microbial properties, NO is an important regulator of iron metabolism [30]. When iron levels become excessive, NO interacts with iron–sulfur clusters in enzymes to generate toxic radicals that may damage intracellular proteins leading to a loss of function of activated macrophages including growth control of *M. tuberculosis* [30,31]. The iron content of bronchoalveolar macrophages of smokers was shown to be at least twice that of non-smokers [31], with the higher iron load likely to come from cigarettes. As reported by Thompson et al., smoking one pack per day presents 1.12 μg of iron [32]. If cigarette smoking hinders host-defense by inhibition of NO production and the production of toxic radicals, then it is plausible that smoking delays clearance of multiplying bacilli and thereby culture conversion.

Our study had a number of strengths. It was prospective, all patients were treated as in-patients and we collected detailed information on clinical and behavioral factors that might underlie associations between smoking and culture conversion.

It also had limitations. The sample size was small, and only 19 patients had delayed conversion. Although we found strong and significant associations, this small sample size might have limited the statistical power of finding relevant interactions with e.g. extent of disease. Smoking histories are subject to recall bias and we did not collect details about the type of cigarettes smoked and possible cessation periods. Patients who smoked very little might have considered themselves as non-smokers, others might have cited wrongly the number of cigarettes smoked daily in the past. As it is known to cause preventable health problems, smoking might be subject to stigmatization, leading some patients to answer untruthfully about their smoking behavior. Our smoking data were collected before treatment and we did not have information about smoking cessation during treatment. Additionally, no information was collected on the persistent use of solid fuel sources in the homes.

Finally, ours was a rather selected study population of mainly disadvantaged TB patients with high levels of alcoholism, drug abuse and malnutrition. Even though we made adjustments in our analyses for possible confounding effects caused by these characteristics, this study population may not be fully representative of other TB patients with regard to the effect of smoking on treatment response.

In conclusion, pulmonary TB patients who smoked at the start of standard first-line treatment were found to have delayed response to therapy based on *M. tuberculosis* culture results after 2 months compared to non-smokers or ex-smokers. Therefore, smokers likely have prolonged infectiousness, and together with the higher tendency for cough, may give rise to increased risks of transmission during the initial phase of treatment. Further research is needed to understand the mechanisms that underlie the effect of smoking on the response to anti-TB treatment as well as the impact of smoking cessation during tuberculosis treatment.

Ethical approval: The study protocol was approved by the Ethic Committee of University Hospital, Federal University of Rio de Janeiro.

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Contribution of authors: Design of the study: AK, OMM; collection and analysis: DdPR, PFCM, KdL, EM, HO, RNdB, JBdOeSF, FC, OMM; development of article: all authors.

Competing interests: None of the authors has any conflict of interest to disclose.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tube.2013.10.005.

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