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Risk Factors for Smear Negative And Culture Positive Results Among Pulmonary Tuberculosis Patients in Mwanza, Tanzania

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Abstract: *Background:* Smear negative and culture positive results in pulmonary tuberculosis are not uncommon yet the underlying factors are not well established.

Objective: To determine factors associated with smear negative and culture positive results.

Methods: Pulmonary tuberculosis patients were consecutively recruited for 12 months from five health facilities in Mwanza region, Tanzania. Sputum examinations were done at the recruitment centre and at the TB reference laboratory using Auramine O technique. Culture was done at the TB reference laboratory using Lowenstein Jensen solid media. A post-hoc analysis compared patients who had a smear negative culture positive result (case) with patients who had not (controls).

Results: A total of 655 pulmonary TB patients were recruited, 18 had no culture results and were excluded from the analysis. Of the remaining 637 patients, 127 (19.9%) had three negative smears at the recruitment centre and 34 patients were a case. Current smoking was strongly associated with being a case, especially in women. Of the 127 patients who had three negative smears at the recruitment center, 104 (81.9%) also had a negative smear at the reference laboratory. Of these, 13 (12.5%) were still culture positive.

Conclusion: The frequencies of smear-negative culture-positive results differ between health facilities, indicating possible difference in quality of laboratory procedures. Strengthening of laboratory capacity is needed both for optimizing smear microscopy techniques, and for performing sputum cultures for diagnosing TB when there is a high rate of suspicion. The association between smoking and smear negative culture positive TB needs to be assessed in adequately large studies.

Key Words: Pulmonary TB, Smear, Culture, Smoking, Tanzania.

INTRODUCTION

Tuberculosis (TB) remains one of the deadliest diseases worldwide, with an estimated 8.4 million new cases and over 2 million deaths each year [1]. For decades, smear microscopy has remained the corner-stone for diagnosis of pulmonary tuberculosis (PTB). It is the most feasible microbiological method for the diagnosis of PTB in developing countries due to its rapidity, low cost, and high positive predictive value for *Mycobacterium tuberculosis* [2]. Sputum smear microscopy with Ziehl-Neelsen staining is only 60 to 70% sensitive for the diagnosis of pulmonary TB compared with sputum culture [3, 4]. However, sputum cultures need a good laboratory infrastructure; they are expensive and take up to eight weeks to get conclusive results.

Studies conducted in different settings have reported an increasing proportion of TB patients with negative smear

results but positive culture results. Known factors to influence the results of either smear microscopy or sputum culture include; HIV infection [5], tuberculin test [6], and smoking [7]. However, data on factors associated with an increased risk of smear negative and culture positive results are scarce.

In Tanzania, sputum culture is not performed for routine diagnosis but mainly for monitoring of TB treatment. With a high prevalence of HIV-infection in the general population (8%), there is the potential that a substantial number of TB-patients will be smear-negative. Considering the relatively low sensitivity of smear microscopy it is possible that a substantial number of TB patients may be missed to be initiated on treatment. Without proper treatment, they may continue suffering from the disease and others may ultimately die. Untreated TB contributes to ongoing transmission [8, 9].

We conducted post-hoc analysis to determine factors associate with smear negative and culture positive results among PTB patients at Mwanza Tanzania.

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MATERIALS AND METHODOLOGY

Study Design

The original study was a randomized, placebo-controlled trial, using a two-by-two factorial design, to assess the effects of daily supplementation with a multi-micronutrient and a zinc tablet throughout the eight months of short course TB treatment (Range TMIH 2005) [10]. The current study is post-hoc analysis using original data.

Population and Study Area

The 655 TB patients were consecutively recruited from the general out-patient department and from the in-patients from five recruitment health facilities between August 2001 and July 2002. The original randomized study included all smear-positive pulmonary TB (n=510) patients identified at the five recruitment centres in the same period. The patients were recruited based on the study inclusion criteria which included; newly diagnosed pulmonary TB (PTB) patients, aged 15 years or above, willingness to participate in the study, and residents of Mwanza city or Magu district in Mwanza, Tanzania. TB patients with the following criteria were excluded; TB patients registered as return to control or smear positive failure cases; patients with clinical evidence of renal, hepatic or cardiovascular disease; pregnant or lactating women; patients with advanced respiratory or other diseases considered unlikely to survive. For the current analysis we added the data of the TB patients who were identified at the same institutions and in the same time frame, but they did not meet the inclusion criteria of being smear-positive (n=127). The 127 local smear pulmonary cases were not randomized but rather included as controls for the different variables assessed among randomised cases for comparison of other parameters that were of interest and were assessed in the study primary objective. The variables include; HIV- infection, parasitic infections, body composition, etc. Given the passive case finding of the study subjects, and the identical participating health facilities and time of recruitment, the total population for this study can be regarded as homogeneous.

Patients who were suspected of having TB were confirmed through clinical examination, smear microscopy, culture and or chest x-ray. All identified patients at the recruitment centres were requested to submit an early morning sputum specimen in a universal bottle for subsequent smear microscopy and culture at the Zonal TB reference laboratory (ZTRL). The early morning sputum specimen that was sent to the TB reference laboratory was collected on the second day after the start of TB treatment. The sputum was collected before the second dose of anti-TB drugs was swallowed. Smear microscopy at the recruitment centres was done using Ziehl-Neelsen staining technique [11], while at the ZTRL, fluorochrome (Auramine O) staining technique was used. Culture was done on Lowenstein Jensen (LJ) solid media [12, 13]. Patients were given information about the study and offered inclusion after informed consent. The study was conducted within the framework of the National TB and Leprosy Programme [14], with diagnosis, classification, registration and treatment of TB patients done in accordance with recommended standard procedures [15].

All confirmed PTB patients at the recruitment centres were started on TB treatment. Prior to starting treatment, the TB Clinical Officers collected demographic and medical history data from all patients. A 10-ml sample of venous blood was collected from each patient and used to check for malarial parasitemia and HIV status, white blood cell counts, including CD4 and CD8 and viral load. Questionnaires were administered to each patient to elicit relevant information on age, marital status, tribe, religion, occupation, smoking habits and alcohol intake [16].

Ethical Consideration

Permission to conduct the study was granted from the Ethics Committee of the National Institute for Medical Research (NIMR) in Tanzania, and the Danish National Committee on Biomedical Research Ethics recommended the study. Permission was also obtained from the Regional Medical Officer and the Council Medical Officers in Mwanza Region. Informed oral consent was obtained from all study participants. Pre-test HIV counselling was given to all, and post-test counselling was given to those who were interested to know their HIV results.

Statistical Analysis

For the current post-hoc analysis we compared the characteristics of the patients who were smear negative but culture positive (case) with the patients who were not (control). A case was defined as a patient with three consecutive negative ZN microscopy smears at the recruitment center, and a positive culture at the reference laboratory. Patients who did not have a culture performed or whose culture was contaminated were excluded from the analysis.

Characteristics of the patients in the two groups were compared with Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables.

Factors independently associated with being a case were assessed through unconditional logistic regression. The different logistic models were tested for significance with the log-likelihood test. However, variables could be kept into the model despite a non-significant log-likelihood test when this improved the interpretation of the data. All logistic models included sex and age (continuous) as possible confounding variables.

Given the possibility of a differential performance of laboratory staff at the centers, we adjusted the standard errors (use of a robust standard error) around the point estimates for possible clustering of the data within a center by adding the variable 'center' as a clustering variable. The level of statistical significance was set at 5%. The analyses were performed using STATA version 9.2 (StataCorp. Texas USA).

RESULTS

The study included a total of 655 patients from five different health facilities. Of these, 18 did not have an appropriate culture result and were excluded from the analysis (Fig. 1). Of the 637 patients remaining, 127 (19.9%) had three negative ZN microscopy smear slides at the recruitment centre (Table 1). Of these, 34 (26.8%) had a positive culture in

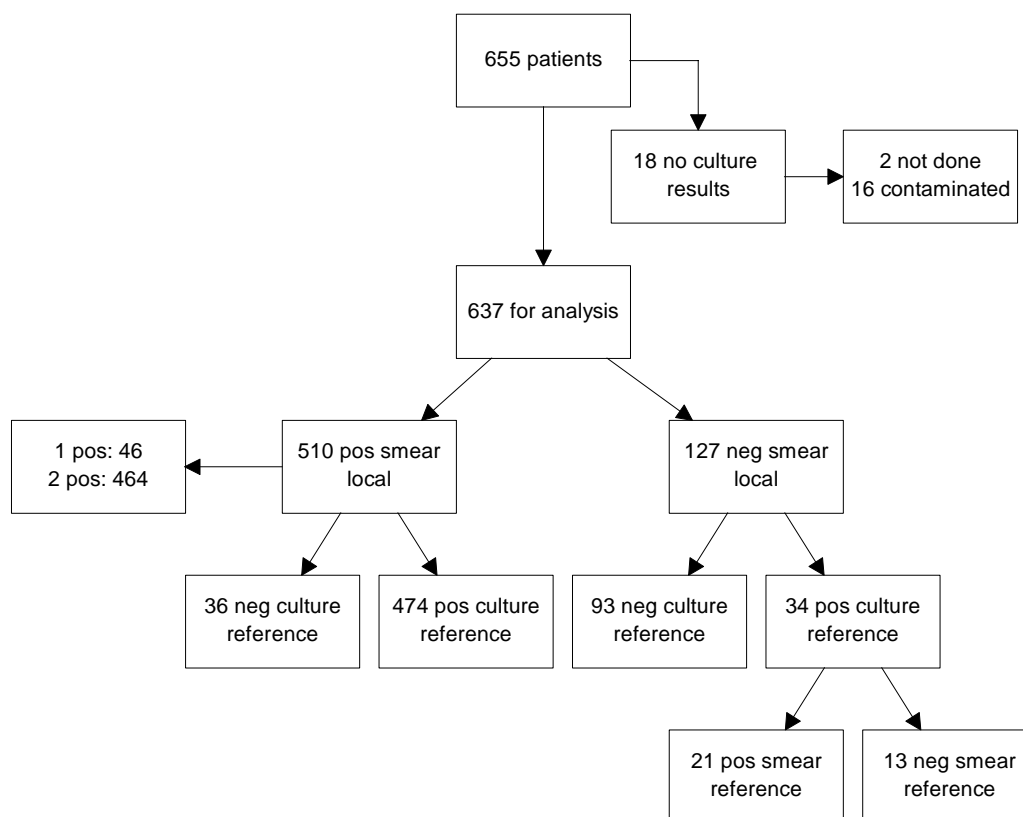


Fig. (1). Patient deposition.

the reference laboratory and were considered a case for this study, all other patients were considered a control.

Table 1. Distribution of Smear-Negative Patients and Cases by Diagnostic Centers

	Patients	Smear-Negative (%)	Cases (%)
Sekou Toure	290	36 (12.4)	21 (7.2)
Bugando	136	54 (39.7)	8 (5.9)
Magu	109	19 (17.4)	3 (2.8)
Buzuruga	63	14 (22.2)	0 (0.0)
Butimba	39	4 (10.3)	2 (5.1)

The characteristics of the two groups of patients are summarized in Table 2. The number of cases differed per diagnostic center with relatively more cases in Sekou Toure, and relatively less cases in Magu and Buzuruga (no cases at all). The HIV-status of the patients did not differ statistically significant between the two groups but was numerically higher in the cases. The severity of the immunosuppression of HIV-positive patients was comparable between cases and controls based on CD4+ cell counts. Nor did the body weight or body mass index differ across the group. The only statistically significant difference between the two groups was the percentage of the patients who were current smokers. This was 26.5% for the cases and 11.9% for the controls ($p=0.029$).

From the variables reported in Table 2 we included current smoking, current alcohol use, and HIV-status in subsequent logistic regression model in addition to sex and age (Table 3). Only current smoking was identified as being associated with being a case (OR: 3.61; 95% Confidence interval [CI]: 1.57 – 8.33). Given the differential smoking habits in the general population between men and women, we tested whether there was an interaction between sex and current smoking. The interaction term was indeed strong (OR: 0.16; 95% CI: 0.28 – 1.00), indicating a stronger association for smoking women to be a case as compared to smoking men. Compared to non-smoking patients, the OR for smoking men of being a case was 2.50 (95% CI: 1.38 – 4.51), while it was 17.75 for women (95% CI: 3.04 – 103.47). The reason for these wide confidence intervals is that there were only 5 women who smoked, out of them 2 (40%) were a case, compared to 76 men who smoked out of them 7 (9%) were a case.

There was no association between past smoking and being a case (OR 1.10; 95% CI: 0.48 – 2.54). None of the 8 women who used to smoke were a case, compared to 6 of the 108 (5.5%) men.

In the reference laboratory, fluorescence microscopy was performed before the culture was initiated. Of the 127 patients who had three negative smears at the recruitment center, 104 (81.9%) had also a negative smear at the reference laboratory. Of these, 13 (12.5%) were culture positive. Twenty three patients who had three negative smears at the recruitment center had a positive smear at the reference laboratory and of these, 21 (91%) were culture positive.

Table 2. Characteristics of Cases and Controls

	Controls	Cases	P-value
Number of patients	603	34	
Male, n (%)	357 (59.2)	19 (55.9)	0.723
Age, median (iqr)	34 (28-43)	35 (27-40)	0.537
Centre			0.186
Sekou Toure	269 (44.6)	21 (61.8)	
Bugando	128 (21.2)	8 (23.5)	
Magu	106 (17.6)	3 (8.8)	
Buzuruga	63 (10.5)	0 (0.0)	
Butimba	37 (6.1)	3 (5.9)	
Cough, n (%)	593 (98.3)	33 (97.1)	0.456
Fever, n (%)	420 (69.7)	25 (73.5)	0.705
Current smoking, n (%)	72 (11.9)	9 (26.5)	0.029
Current alcohol use, n (%)	211 (35.0)	15 (44.1)	0.276
Any co-infection	298 (49.4)	17 (50.0)	1.00
HIV-positive	279 (46.3)	21 (61.8)	0.111
CD4+ cells , mm ³ (iqr)	346 (166-625)	253 (160-365)	0.240
Low CD4 cell count*	86 (30.8)	6 (28.6)	1.00
PvL, log ₁₀ (iqr)	4.2 (3.6-4.8)	4.5 (3.9-5.0)	0.157
High PvL [#]	52 (18.6)	6 (28.6)	0.259

Iqr = inter-quantile range; Any co-infection = Malaria, *S. mansoni*, *S. hematobium*, *A. lumbricoides*, *T. trichiura* and *S. stercoralis*, HIV = Human Immunodeficiency virus; PvL = Plasma viral Load; * Low CD4 cell count = < 200 cells mm³; [#]High PvL = > 5 log₁₀.

Table 3. Risk Factors for Being a Case

	Univariable*			Multivariable		
	OR	95% CI [#]	p	OR	95% CI [#]	p
Male	0.934	0.57 - 1.56	0.808			
Age, per year	0.98	0.96 - 1.00	0.184	0.97	0.95 - 1.00	0.068
Smoking	3.61	1.57 - 8.33	0.003			
Alcohol use	0.10	0.40 - 6.67	0.488			
HIV-positive	1.89	0.88 - 4.04	0.101			
Smoking men [§]				2.50	1.38 - 4.51	0.002
Smoking women [§]				17.75	3.04 - 103.47	0.001

* In addition to sex and age; [#] based on robust standard error; [§] in comparison to non-smoking patients.

DISCUSSION

We found numerical differences in the percentage of TB patients with smear negative and culture positive results by diagnostic centers. This could possibly be due to laboratory quality services issues in terms of personnel qualifications

and workload. A centre like Sekou Toure hospital and Bugando Medical Centre (BMC) had more patients recruited for the study and had more cases (smear negative but culture positive results). For program operational improvement it may be necessary to conduct AFB microscopy refresher

training to the laboratory personnel to minimize the differences on quality of sputum smear microscopy. In addition, recruitment of additional staff to cope with a high workload could probably improve the quality of laboratory services. Laboratory monitoring and routine quality assurance should be reinforced. High workload and inadequate training of laboratory personnel in Tanzania have recently been described as factors related to a high proportion of false-negative smears [17]. A similar finding was described in a study performed in Nairobi Kenya [18].

The study also showed that replacing routine culture by a negative confirmation smear seems not a viable option, as the 13 patients who were confirmed smear negative at the reference TB laboratory but still had a positive culture, would have been missed based on smear negative confirmation. With the possibility of increasing numbers of smear negative TB cases due to the HIV/AIDS epidemic, complementing smear negative results with culture seems to be a better option with respect to case detection.

Our study showed that current cigarette smoking was strongly associated with being a case (OR 3.61 95% CI; 1.57- 8.33), and that this association was stronger in women than in men. According to our knowledge, this is the first time that this association has been described. However, due to the very wide confidence intervals, the data can only serve as a first indication of a possible relationship between smoking and sputum smear results, and the possible modifying effect of sex on this relationship. Further studies that have an adequate number of patients must assess this relationship. If this relationship is indeed confirmed, modified diagnostic procedures for current smokers might be needed.

Smoking has been reported to be associated with an increased risk of developing TB disease [19], a slow healing process, delayed sputum conversion [7], and a high death rate [20].

The main limitation of our study is its post-hoc design. Result from such an analysis should be interpreted with care, since adequate measuring of confounding factors might not have been optimal. This design is also prone to selection bias. We have included all TB patients from the diagnostic centers who were confirmed cases through clinical suspicion, sputum examination and X-ray. Sputum culture was not part of the diagnostic work-up. Any misclassification of TB-patients is therefore non-differential with respect to culture result and would not have influenced the results of this study.

CONCLUSION

The frequencies of smear-negative culture-positive results differ between health facilities, indicating possible difference in quality of laboratory procedures. Strengthening of laboratory capacity is needed both for optimizing smear microscopy techniques, and for performing sputum cultures for diagnosing TB when there is a high rate of suspicion.

The TB program and other stakeholders should have collaborative efforts to control smoking. Messages on TB control to create public awareness should include strategies on the control of cigarette smoking to minimize its effect on TB. The association between smoking and smear negative

culture positive TB needs to be assessed in adequately large studies.

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