Clinical Study

Factors Influencing Sputum Conversion among Smear-Positive Pulmonary Tuberculosis Patients in Morocco

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Background. Sputum smear-positive pulmonary tuberculosis patients expel infectious viable bacilli for a period following the commencement of treatment. **Objective.** To determine the time to sputum smear conversion and study the factors influencing it. **Design.** A prospective study was undertaken at our hospital in Rabat over a six-month period on a cohort of 119 sputum smear positive patients. Patients were followed up fortnightly. At each followup, specimens were collected and processed for microscopy using standard protocol. **Results.** 96.6% of our patients completed the study (4 deaths). Sputum conversion rate was 42% after two weeks, 73% after one month, and 95% after two months. Univariate and stepwise regression analysis showed that patients who had high smear grading, miliary, and bilateral radiologic lesions were more likely to undergo delayed sputum conversion ($P < 0.05$). Other factors were thought to influence sputum conversion but were not statistically proven in our study. **Conclusion.** Since viable bacilli continue to be expelled for up to two months, infection control measures should be maintained for such a time. Patients with high smear grading, miliary, and bilateral radiologic lesions need to be monitored more closely.

1. Introduction

The World Health Organization (WHO) estimates that there are almost 13.7 million people living with tuberculosis and that the disease kills more young people and adults than any other infectious disease in the world.

In Morocco, the new cases of tuberculosis in 2008 were 27000, and the incidence was 82/100000 people. Patients in our health system receive intermittent therapy with multidrug regimen based on directly observed treatment, short-course (DOTS).

Tuberculosis control aims to reduce the spread of the infection, and the most efficient method for preventing transmission is the identification and cure of infectious pulmonary tuberculosis patients [1].

Sputum smear-positive (SSP) pulmonary tuberculosis patients are the most significant source of infection for tuberculosis because, when they cough or sneeze, they expel droplet nuclei which carry infectious bacilli [2]. One untreated infectious tuberculosis patient is likely to infect 10 to 15 persons annually [3].

When SSP patients are initiated on multidrug antituberculosis treatment, there is a multifold reduction in bacillary load expelled in sputum [4]. Patients, who respond, are likely to become smear and culture negative during the course of treatment. However, viable bacilli continue to be expelled for a period of time, during which they may continue to spread infection.

It is expected that 80 to 90% of patients will undergo smear conversion within two to three months of treatment [5]. Several factors have been identified that may delay the time to smear conversion. These include high initial sputum smear acid fast bacilli (AFB) grade, cavitary lesion, uncontrolled hyperglycaemia/diabetes mellitus, old age, certain ethnic initial treatment with less than four antitubercular drugs, and nonrifamycin-based treatment regimens [6].

Infection control measures are recommended for all sputum smear-positive patients to minimize the spread of infection.

Measures are to be maintained until noninfectiousness has been demonstrated. Demonstration of noninfectiousness is best done by demonstration of culture conversion. The

time required to report culture results and the availability of resources are limitations to the use of culture for the purpose of infection control. On the other hand, sputum smear microscopy, thought less sensitive than culture, can be reported much earlier. Current recommendations include serial negative sputum smears for AFB before removal of infection control measures [7, 8].

In this background, it is essential to evaluate risk factors such as age, smear grading, weight, and associated comorbid conditions like HIV infection and diabetes mellitus among TB patients that are likely to influence smear conversion. Although risk factors which influence smear conversion have been studied widely [9], there are no prospective studies on smear conversion in Moroccan patients.

The present study was undertaken to determine the time to smear conversion in Category I DOTS patients receiving uninterrupted therapy and to determine factors that prolong smear conversion.

Smear conversion is defined as new smear-positive PTB cases who became smear negative after a period of anti-TB treatment and are therefore no longer infectious (confirmed by at least two consecutive negative sputum acid fast bacillus (AFB)).

2. Study Population and Methods

This is a six-month prospective study. It was undertaken at Moulay Youssef University Hospital, Rabat, Morocco. In this study, all new smear-positive pulmonary TB inpatients of our tertiary care hospital were enrolled from 1 January 2010 to 30 June 2010. All the patients were consecutive ones.

For each patient enrolled, at least one culture was done to confirm the diagnosis and to exclude a drug resistant tuberculosis. All patients received a four drug regimen (isoniazid, rifampicin, pyrazinamide, and ethambutol).

When a patient was enrolled in the cohort, details of demographic, clinical and radiological findings, past history of tuberculosis, tobacco, alcohol, and drugs consumption, BCG status, diabetes mellitus, renal diseases, and HIV coinfection were noted.

Patients were followed up fortnightly for up to 6 months or until they underwent smear conversion whichever was earlier. At least two smear specimens were collected in each evaluation.

All expectorated and induced sputum specimens obtained by the microbiology laboratory for AFB smear were decontaminated with sodium hydroxide in combination with N-acetyl-L-cysteine and processed in a standard manner. The Ziehl-Neelsen stain was used throughout the study.

The smear grading was based on this classification: negative (smear contains no AFB in 100 fields), 1+ (10–99 AFB in 100 fields), 2+ (1–9 AFB/field in at least 50 fields), and 3+ (>10 AFB/field in at least 20 fields).

Statistical analysis to determine factors that prolong time to conversion was done by univariate analysis and stepwise regression analysis using SPSS 17.0 software. *P* value of <0.05 was considered as significant. Any patient unable to complete the required followup was excluded from data analysis.

TABLE 1: Sociodemographic characteristics and comorbidities of baseline population.

	Number	Percent	<i>P</i> value
Sex			
Male	77	65%	0.02
Female	42	35%	
Age Group			
10–20	10	8.4%	0.14
20–30	37	31.1%	
30–40	24	20.2%	
40–50	19	16%	
50–60	16	13.4%	
60–70	9	7.6%	
>70	4	3.3%	
Smoking			
Yes	43	36.1%	0.01
No	61	51.3%	
Weaned	15	12.6%	
Comorbidities			
Diabetes	9	7.6%	0.77
Respiratory diseases	4	3.4%	
HIV	1	0.8%	
Others	10	8.4%	

The goal and benefits of the study were explained to the study participants, and oral informed consents were obtained from the participants prior to enrollment.

3. Results

From 1 January 2010 through 30 June 2010, 119 cases of smear-positive tuberculosis were diagnosed, which included 77 men and 42 women. Patients were aged between 17 to 79 years. The mean age for both men and women was 39 years. 96.6% of our patients completed the study, four of our patients died during the study. They died of acute respiratory distress (2 cases), septic shock (1 case), and hemoptysis lightning (1 case).

The characteristics of the patients are given in Table 1.

Of these patients, 88 (74%) had pulmonary disease alone, 8 (7%) had pulmonary and pleural disease, 15 (13%) had pulmonary and lymph node tuberculosis, and the 7 remaining cases had pulmonary and another extrapulmonary location. All cases were confirmed by culture, and no drug resistance was detected.

The rate of sputum conversion at the end of one month of treatment was 73.1% ($P < 0.01$) while it was 95% ($P < 0.05$) at the end of the second month.

Table 2 shows other characteristics of TB in the enrolled patients, while Table 3 shows patients undergoing smear conversion.

Smear grading (44.5% negatization in the 1st fortnight in 1+/2+ group versus 12.1% in the 3+/4+ group; $P = 0.02$), miliary (7.1% negatization in the 1st fortnight versus 57.1% in the 4th fortnight or later; $P = 0.01$), bilateral radiologic lesions (26.9% negatization in the 1st fortnight versus 40.4%

TABLE 2: TB features.

Characteristics	All patients (<i>n</i> = 119)	<i>P</i> value
New cases, <i>n</i> (%) yes/no	100 (84)/19 (16)	0.005
Radiological involvement, <i>n</i> (%) unilateral/bilateral	50 (42)/68 (57.1)	0.56
Cavitation, <i>n</i> (%) yes/no	42 (35.3)/76 (63.9)	0.06
Nodules and micronodules, <i>n</i> (%) yes/no	101 (84.9)/17 (14.3)	0.01
Infiltrate, <i>n</i> (%) yes/no	103 (86.5)/15 (12.6)	0.03
Smear grading, <i>n</i> (%)		
1–9 AFB/100 fields (1+)	8 (6.7)	0.02
1–9 AFB/10 fields (2+)	19 (16)	
1–9 AFB/field (3+)	37 (31.1)	
>9 AFB/field (4+)	55 (46.2)	

TABLE 3: Cumulative patients undergoing smear conversion.

Time	Smear conversion patients			<i>P</i> value
	Number	%	Cumulative %	
1st fortnight	51	42,86	42,86	<0.001
2nd fortnight	36	30,25	73,11	<0.01
3rd fortnight	16	13,45	86,56	0.02
4th fortnight	10	8,4	94,96	0.03
8th fortnight	2	1,68	96,64	>0.05

in the 4th fortnight or later; $P < 0.01$) were associated with longer smear conversion time.

There were no statistically significant differences in other evaluated variables, such as age, sex, weight, smoking, alcoholism, addictions, respiratory diseases, diabetes mellitus, HIV infection, cavitations, TB contagion, previous TB disease, alternative anti-TB treatment, and related toxicity.

Multivariate logistic regression analysis indicated that all 3 significant variables from the univariate analysis were independently associated with delayed smear conversion (smear grading 3+: OR 7.1, 95% CI 2.5–11.2; military: OR 8.8, 95% CI 2.3–19.4; bilateral radiologic lesions: OR 13.4, 95% CI 1.8–55.6).

4. Discussion

The World Health Organization (WHO) recommends that patients with previously untreated pulmonary TB receive a four-drug regimen during the two-month initial phase of treatment that includes rifampicin. The overall rate of failure or relapse (poor outcome) in patients receiving directly observed treatment, short-course (DOTS) with a rifampicin-containing regimen, is low [10].

This study is a prospective assessment on TB cases registered and treated in a tertiary care Moroccan hospital. The sample size (119) is relatively small for definitive biostatistical conclusions but is close to some other studies.

TB was suspected on the basis of symptoms, clinical signs, and chest X-ray and confirmed by sputum smear examination. No case was confirmed by sputum culture.

The search for tools to estimate the duration of respiratory isolation and to monitor the treatment of tuberculosis

patients continues. Studies have shown that nonconversion of positive smears at the end of the two months of treatment is one of the strongest predictors for treatment failure [11–13], although it is not a very reliable indicator because of its low positive predictive value [14].

Indeed a positive sputum smear for AFB does not permit one to know whether these are still viable bacteria or not after two months of antituberculosis treatment. To assess sputum sterilization, therefore, it is ideal to study cultures for mycobacteria at the end of the two months. However under programmed conditions, cultures for mycobacteria are not available under field conditions. Furthermore, conventional results of culture on media would be available far too late (after more than two months) to be useful [14].

Currently the duration of infectiousness after the initiation of effective treatment is still a subject of discussion. From our results it is shown that after two weeks of treatment about 57.14% of the patients were still potentially infectious. This is in contrary to the belief that patients become noninfectious after two weeks of standard treatment regimen. This finding is in line with other results which have also shown that the conversion to a negative test and hence the loss of infectiousness of pulmonary tuberculosis patients during therapy does not occur rapidly in all patients [15–17]. This finding has implications to those countries which practise patients' isolation during the infectious period and are using two weeks as a time which usually a patient is considered to become noninfectious. For example, in France and UK, guidelines indicate that the isolation of smear-positive tuberculosis patients without risk factors for multidrug resistant tuberculosis is generally only required for 2 weeks [18, 19]. Factors such as higher pretreatment smear grading, military, and bilateral radiographic involvement were associated with

the delay of smear conversion. This applies to the univariate analysis and the multivariable analysis. Similar findings have been reported earlier [15, 20–22].

In this study, the presence of numerous bacilli on initial pretreatment sputum smears was also an independent predictor of a delay of conversion of positive sputum smears. The direct influence of initial bacillary load on the absence of sputum conversion at the two months of therapy has been reported by several authors [11, 13, 20, 23]. Rieder et al. observed that sputum conversion at the end of the two months of directly observed therapy among patients with initial weakly positive sputum was 90.9% while it was, respectively, 77.9% and 61.7% among patients with initial moderately positive and strongly positive sputum [11]. Singla et al. reported that patients with numerous bacilli on pretreatment sputum smear examination had an almost six times greater risk of persistent sputum positivity than patients with few bacilli [20]. Meanwhile, Lienhardt et al. [13] reported sputum conversion at the end of two months in patients with initial sputum smear 1+, 2+, and 3+ to be 96.2%, 85.8%, and 81.8%, respectively [13].

Our results indicated also that bilateral radiological involvement and miliary lesions were independent risk factors for delayed smear conversion, due to the high baseline bacillary burden of those patients.

In contrast with previous studies [20, 24, 25], our results indicated no relationship of cavitation with delayed smear conversion, although its presence has been significantly associated with a longer time to smear conversion. This discrepancy could be due to differences in methodologies and populations.

In our cohort, it is difficult to speculate on the influence of HIV status in smear conversion, as long as there is only one patient.

The relationship between factors like age, sex, weight, diabetes mellitus, smoking, alcoholism, addictions, respiratory diseases, previous TB contagion, previous TB disease, alternative anti-TB treatment, and related toxicity and delays of conversion of positive sputum smears after two months of treatment in our study had no statistically significant association.

There has been a concern that HIV-infected patients with tuberculosis are more infectious and are infectious for a longer period of time than patients with tuberculosis who are not HIV-infected. In our study we had only one coinfection HIV-TB which negated in the 2nd fortnight. Some studies, by investigating contacts, have also concluded that HIV-associated pulmonary tuberculosis is not more infectious than tuberculosis alone [26]. Otherwise, Telzak et al. and Domínguez-Castellano et al. showed that it was more likely that the sputum smears and cultures of HIV-infected patients would convert more rapidly than the smears and cultures of HIV-uninfected patients [21, 27].

The role of diabetes in sputum conversion had been studied. A study in India suggested that, although patients with combined pathologies of pulmonary tuberculosis and diabetes mellitus had higher sputum conversion rate compared to the rate in nondiabetic patients [28], in our study we did not find any significant association of these two factors.

Supervised control of diabetes for two months in hospitalized patients might have reduced the difference between nondiabetics and well-controlled diabetic patients in our study.

No age groups had any significant association with a delay in negativation. Kuaban et al. showed that age above or equal to 40 years was an independent predictor of nonconversion of sputum smears [14]. Singla et al. observed in a similar study that patients aged over 60 years had an almost six times greater risk of remaining sputum positive after two months of treatment than patients aged 21–40 years, while patients aged 41–60 years were twice as likely to remain sputum positive [28]. Liu et al. also reported that the elderly were the least likely to have documented sputum conversion after two months of treatment. The reason why old age should predispose to delayed sputum smear conversion is not exactly known [29].

There is insufficient evidence to support an association of smoking and delayed smear conversion [30]. Three researchers examined this association; one study showed significant effects, but two others did not [31–33]. New studies should use cohort and case-control designs to examine the association of passive and active smoking with slower smear conversion.

Our study has several strengths, its prospectivity, sample size, standard protocols of treatment and supervising (DOTS), and studying environmental conditions.

It has also some limitations; the information was based on selected inpatients of our tertiary care hospital, at which only complicated cases are admitted. It may not reflect the patterns in the community in general.

5. Conclusion

In conclusion, our analysis showed delayed smear conversion in more than half of the patients. Smear grading, miliary, and bilateral radiologic lesions were independently associated with delayed smear conversion. We suggest that intensified treatment and precautions against transmission should be especially considered for TB patients with these risk factors, allowing the optimization of national TB control measures.

More prospective studies about sputum conversion and also culture conversion are needed to consolidate our findings.

Conflict of Interests

The authors have no conflict of interests to declare.

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