Tuberculosis diagnosed in a rural setting in Angola. Accuracy of follow-up sputum smears to predict outcome

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Objective: To analyze treatment outcome and the accuracy of positive sputum smear at 2 months to predict treatment failure in a cohort of patients with tuberculosis (TB) in a rural setting in Angola.

Design: Observational study of patients with TB from January 2009 to August 2010 in Hospital Nossa Senhora da Paz in Angola. A multivariate analysis was performed to identify variables associated with treatment failure and death. Sensitivity, specificity, positive and negative predictive values and likelihood ratios to define the accuracy of a positive sputum smear at 2 months to predict treatment failure were calculated.

Results: One thousand four hundred and twenty-five patients were diagnosed with TB. Overall, 526 patients were cured from TB and 419 had treatment completed, so 945 (66.3%) patients achieved treatment success. The outcomes of the remaining patients were: 91 (6.4%) had treatment failure, 100 (7%) died, 49 (3.4%) interrupted treatment, and 240 (16.8%) were transferred out. Variables associated with a higher risk of treatment failure were previously treated patients (odds ratio, 2.36; 95% confidence interval, 1.32–4.2) and positive sputum smear at 2 months (odds ratio, 9.81; 95% confidence interval, 5.88–16.36). Among the group of 551 patients with sputum smear confirmed at diagnosis and specimens taken at 2 and 5 months, the positive predictive value (31%) and the positive likelihood ratio (3.21) of a positive sputum smear taken at 2 months to predict treatment failure were low.

Conclusions: Patients with positive sputum smear at 2 months have a higher risk of treatment failure. However, this by itself is a poor predictor of treatment failure.

Keywords: Tuberculosis, Predictors of treatment failure, Multidrug resistant tuberculosis, Tuberculosis in resource limited settings

Introduction

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide, especially in countries of sub-Saharan Africa.¹ In resource-limited settings, diagnosis of TB is still based on the presence of acid-fast bacilli identified in sputum smear. Treatment success in these settings is based on conversion of sputum smears to acid-fast bacilli-negative status.² A recent review suggested an inverse relationship between 2-month sputum culture conversion and relapse, nevertheless the positive predictive value to predict relapse was poor.³ In low-resource settings, where culture or other techniques for *Mycobacterium tuberculosis* detection are not performed, the control of patients with TB is based on the sputum smear examination. The ability of

a positive sputum smear at 2 months to predict treatment failure has been poorly assessed. A study from South Africa showed that patients with positive smears at 2–3 months were more likely to fail therapy than patients with negative smears.⁴

Hospital Nossa Senhora da Paz is a rural hospital in Cubal, Angola. Diagnosis of TB in this setting is based only on direct exam of clinical samples. The aim of this study is to analyze the clinical and microbiological outcome in a large cohort of patients with TB diagnosed in a rural setting in Angola and to evaluate the accuracy of positive sputum smear performed 2 months after having initiated therapy to predict treatment failure.

Patients and methods

Study population and setting

An observational study was conducted of all patients diagnosed with TB from 1 January 2009 to 31 August

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2010 in Hospital Nossa Senhora da Paz, a rural hospital located in the Benguela district, in Angola. The hospital is a reference center for the diagnosis and treatment of TB. Measures to support patient adherence include directly observed therapy and a regular supply of drugs in fixed-dose combinations and blister packs. Data from all patients with TB are routinely recorded in a written National Registry. These data were transferred to a computer with a codification system, in order to avoid identification of any patient, and were analyzed retrospectively. The study was approved by the direction board of the hospital Nossa Senhora da Paz.

Inclusion criteria

Cases of TB included both definite cases (defined by positive sputum smear) and those diagnosed clinically by a health worker (any person who presented with symptoms or signs suggestive of TB and who initiated TB treatment).⁵ A chest X-ray film was not required for diagnosis.

Data collection

The following variables were recorded: (1) socio-demographic data; (2) history of previous treatment; (3) site of TB disease (pulmonary and extrapulmonary); (4) HIV status; (5) date of initiation of TB treatment; (6) method of diagnosis of TB (clinically suspected and/or bacteriologically confirmed); (7) response to treatment; and (8) treatment outcome.

Laboratory diagnosis

Three samples of sputum were routinely collected from each patient in the early morning. Diagnosis of TB was based on the presence of acid-fast bacilli identified in sputum by Ziehl–Neelsen stain with smear microscopy. A quality control on the reading of sputum smears is routinely performed according to the guidelines of the National TB programme in Angola. During the study period, a sample of sputum specimens was sent to the reference laboratory of the National TB programme in Benguela for confirmation of the reads. Culture and drug susceptibility testing were not available. HIV testing was routinely performed in all patients using a rapid HIV test kit.⁶

Treatment regimens

The National TB programme in Angola was established according to 2003 WHO guidelines.² Treatment regimen for new cases consisted of an initial intensive phase lasting 2 months with isoniazid, rifampicin, pyrazinamide, and ethambutol and a continuation phase with isoniazid and ethambutol lasting for up to 6 months. The standard re-treatment regimen was: 2 months of streptomycin, rifampicin, isoniazid, ethambutol, and pirazinamide, four drugs (rifampicin, isoniazid, ethambutol, and pirazinamide) during the third month, and three drugs (rifampicin,

isoniazid, and ethambutol) in the continuation phase lasting for 5 additional months.

Monitoring the response to treatment

Response to treatment was assessed by sputum smear examination. Sputum smears were collected at diagnosis, at the end of the second month, during the fifth month, and in the last month of therapy. If the sputum smears remained positive at the end of the second month, smears were also checked at the end of the third month.

Definitions

WHO guidelines were used to define different patient categories as follows:^{5,7} new patients were those who had never had treatment for TB, or had taken anti-TB drugs for less than 1 month. Previously treated patients were those who had received 1 month or more of anti-TB drugs in the past. These patients were further classified by the outcome of their most recent course of treatment (relapse, failure, or default).

Definitions of treatment outcomes were: (1) cure: patients whose sputum smear was positive at diagnosis and negative in the last month of treatment and on at least one previous occasion; (2) treatment completed: patients who completed treatment but a negative sputum smear in the last month was not documented; (3) treatment success: sum of cured and completed treatment; (4) treatment failure: patients whose sputum smear remained positive at 5 months; (5) died: patients who died for any reason during the course of treatment; (6) default: patients whose treatment was interrupted for 2 consecutive months or more; and (7) transfer out: patients transferred to another unit and whose treatment outcome was unknown.

Statistical analysis

Data are presented as proportions and continuous variables are expressed as means and standard deviation. The Chi-square test was used to compare categorical variables, and the Student's *t*-test for continuous variables. Results were considered statistically significant if the two-tailed *P* value was <0.05. A multivariate analysis using a forward stepwise multiple regression model was carried out to identify variables independently associated with treatment failure and death. In this model, we included all variables that were statistically significant in the univariate analysis. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

For the group of patients with smear-positive pulmonary TB, the percentages of patients with positive and negative sputum smears at different time points and outcomes were compared between new cases and previously treated patients. Relative risks (RRs) and 95% CIs for persistence of positive sputum smear at 2 and 3 months and clinical outcome were

Table 1 Demographic and clinical characteristics of patients with tuberculosis (TB) by age group

	≤3 years	4–14 years	>14 years
Number of patients	297	131	997
Males	163 (54.9%)	64 (48.9%)	613 (61.5%)
Females	134 (45.1%)	67 (51.1%)	384 (38.5%)
Positive HIV test	7/206 (3.4%)	6/112 (5.4%)	81/963 (8.4%)
Case definition for TB			
New cases	296 (99.7%)	122 (93.1%)	841 (84.4%)
Previously treated patients	—	4 (4.1%)	133 (13.3%)
Relapse	—	3 (2.3%)	38 (3.8%)
Failure	—	—	65 (6.5%)
Default	—	1 (0.8%)	30 (3%)
Transferred	1 (0.3%)	5 (3.9%)	23 (2.3%)
Pulmonary TB	287 (96.6%)	114 (87%)	966 (96.9%)
Diagnosis of TB			
Definite case of TB (positive sputum smear)	—	25 (19.1%)	880 (88.3%)
Clinical diagnosis (negative sputum smear)	—	13 (9.9%)	99 (9.9%)
Clinical diagnosis (sputum smear not done)	297 (100%)	93 (71%)	18 (1.8%)

calculated. All statistical analyses were performed with the SPSS statistical package (version 15.0).

In order to analyze the value of a positive sputum smear at 2 months to predict treatment failure, we calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value. To ask whether or not sputum smear after the intensive phase of treatment predicts treatment failure, we have analyzed patients who meet all the following conditions: (1) initially diagnosed with a positive sputum smear; (2) had a sputum smear taken at the end of the intensive phase of treatment; and (3) had a sputum smear taken at 5 months of treatment. Sensitivity is defined as the proportion of TB patients with treatment failure with a positive sputum smear or culture at 2 months. Specificity refers to the proportion of patients not experiencing treatment failure with negative sputum at 2 months. PPV is the proportion of those with positive sputum and treatment failure. Negative predictive value is the proportion of those with negative sputum who did not experience treatment failure. These definitions have been used elsewhere.⁸ We also calculated positive and negative likelihood ratios (LRs). Low negative LR (<0.1) and high positive LR (>10) are considered useful for ruling out and ruling in decisions, respectively.⁹

Results

Study population

From 1 January 2009 to 31 August 2010, 1425 patients with a mean age of 24.1 ± 17.1 years were

diagnosed with TB. There were 585 (41.1%) females and 840 (58.9%) males. HIV rapid test was performed in 1281 (89.9%) patients and it was positive in 94 (7.3%) of them. Table 1 shows the main demographic and clinical characteristics of patients by age group.

Evaluation of clinical outcome

Overall, 526 (36.9%) patients were cured of TB and 419 (29.4%) had treatment completed, so 945 (66.3%) patients achieved treatment success. The outcomes of the remaining patients were: 91 (6.4%) had treatment failure, 100 (7%) died during the course of treatment, 49 (3.4%) interrupted treatment for ≥ 2 months; and 240 (16.8%) were transferred out. The outcomes of patients by age group are shown in Table 2.

In Table 3, we present treatment success, death, and treatment failure rates for different groups of patients. In the multivariate analysis (Table 4), the variables independently associated with a higher risk of treatment failure were previously treated patients at presentation (OR, 2.36; 95% CI, 1.32–4.2) and positive sputum smear at 2 months (OR, 9.81; 95% CI, 5.88–16.36). Variables associated with a higher risk of death were female sex (OR, 1.76; 95% CI, 1.06–2.92), previously treated patients at presentation (OR, 3.35; 95% CI, 1.81–6.18), and HIV infection (OR, 6.93; 95% CI, 3.87–12.40).

Assessing treatment response in new and previously treated pulmonary TB patients with microbiologically confirmed diagnosis

A total of 905 patients had a positive sputum smear at diagnosis. At presentation 754 (83.3%) were new cases, 137 (15.1%) were previously treated patients, and the remaining 14 patients could not be classified.

At the end of the second month, a sputum sample was collected in 802 (88.6%) patients. In 225 of these 802 (28.1%) patients with a sputum sample taken at 2 months, it remained positive. Of patients with a positive smear at 2 months, a subsequent sample was collected at 3 months in 205 cases and it was still

Table 2 Outcome of patients diagnosed with tuberculosis (TB) by age group

	≤3 years	4–14 years	>14 years
Treatment success	212 (71.4%)	101 (77.1%)	632 (63.4%)
Cured	—	13 (9.9%)	513 (51.5%)
Treatment completed	212 (71.4%)	88 (67.2%)	119 (11.9%)
Treatment failure	—	2 (1.5%)	89 (8.9%)
Died	24 (8.1%)	6 (4.6%)	70 (7%)
Default	13 (4.4%)	4 (3.1%)	32 (3.2%)
Transfer out	48 (16.2%)	18 (13.7%)	174 (17.5%)

Table 3 Univariate analysis of treatment success, treatment failure, and death rates for patients with tuberculosis (TB)

	Treatment success (n=945)	P	Treatment failure (n=91)	P	Died (n=100)	P
Males	539/840 (64.2%)	0.040	63/840 (7.5%)	0.047	50/840 (6%)	0.073
Females	406/585 (69.4%)		28/585 (4.8%)		50/585 (8.5%)	
Age <14 years	313/428 (73.1%)	<0.0001	2/428 (0.5%)	<0.0001	30/428 (7.0%)	1
Age >14 years	632/997 (63.4%)		89/997 (8.9%)		70/927 (7.0%)	
New cases	863/1259 (68.5%)	<0.0001	64/1259 (5.1%)	<0.0001	75/1259 (6%)	<0.0001
Previously treated patients	63/137 (46%)		25/137 (18.2%)		21/116 (15.3%)	
Microbiologically confirmed diagnosis (definite cases)	577/905 (63.8%)	0.004	88/905 (9.7%)	<0.0001	59/905 (6.5%)	0.334
Clinical diagnosis	368/520 (70.8%)		3/520 (0.6%)		41/520 (7.9%)	
Positive HIV test	44/94 (46.8%)	<0.0001	7/94 (7.4%)	0.834	21/94 (22.3%)	<0.0001
Negative HIV test	817/1187 (68.8%)		83/1187 (7.0%)		50/1187 (4.2%)	
Positive sputum smear at 2 months	125/229 (54.6%)	<0.0001	64/229 (27.9%)	<0.0001	7/229 (3.1%)	0.310
Negative sputum smear at 2 months	511/667 (76.6%)		24/667 (3.6%)		13/667 (1.9%)	
Positive sputum smear at 3 months	39/106 (36.8%)	<0.0001	54/106 (50.9%)	<0.0001	1/106 (0.9%)	1
Negative sputum smear at 3 months	85/102 (83.3%)		3/102 (2.9%)		1/102 (1.0%)	

positive in 103 (50.2%) cases. At the end of the fifth month, a sputum sample could be collected in 559 cases and 68 (12.2%) of them had a positive sputum smear.

Table 5 shows the proportion of patients with positive sputum smear at 2 and 3 months and the outcome in new cases and previously treated patients. At the second month, previously treated patients had significantly higher risk of having a positive sputum smear than new cases (RR, 1.92; 95% CI, 1.26–2.94). At the third month, previously treated patients with a positive sputum smear at 2 months had also a significantly higher risk of having a positive sputum smear than new cases (RR, 3.37; 95% CI, 1.49–7.64). The risks of treatment failure (RR, 2.53; 95% CI, 1.52–4.21) and death (RR, 3.71; 95% CI, 2.09–6.61) were significantly higher in previously treated patients.

Accuracy of a positive sputum smear at 2 months to predict treatment failure

Table 6 shows the accuracy of a positive sputum smear at 2 months to predict treatment failure. This analysis has been made in the group of 551 patients initially diagnosed with a positive sputum smear and

who had sputum samples collected at 2 and 5 months. Sensitivity to predict treatment failure was 74% when the sputum remained positive at 2 months. However, both the PPV (31%) and the positive LR (3.21) were low.

Discussion

In our cohort of patients with TB in a rural setting in Angola, the overall rate of treatment success was 66.3%. However, it should be taken into account that 16.8% of patients were transferred out which explains partly this low rate of treatment success. If we discard these patients, the rate of treatment success rises to 80%. Primary resistance to antituberculous drugs, which is unknown in this setting, may contribute to our understanding of this rate of treatment success.

We have also observed that patients in whom the sputum smears remain positive at 2 months have a higher risk of treatment failure. This risk is even higher if the sputum is positive at 3 months. In our study, we have used the WHO definitions for TB outcomes in which treatment failure is defined as positive sputum smear at the fifth month or later during TB treatment.⁵ These results are similar to

Table 4 Multivariate analysis of variables associated with treatment failure and death

	Odds ratio (95% confidence interval)	P
Variables associated with treatment failure		
Previously treated patients at presentation	2.36 (1.32–4.2)	0.004
Positive sputum smear at 2 months	9.81 (5.88–16.36)	<0.0001
Variables associated with death		
Female sex	1.76 (1.06–2.92)	0.029
Previously treated patients at presentation	3.35 (1.81–6.18)	<0.0001
HIV infection	6.93 (3.87–12.40)	<0.0001

Table 5 Microbiological and clinical outcome of patients with positive sputum smear tuberculosis (TB)

	Previously treated patients (n=137)	New cases (n=754)	P
Positive sputum smear at 2 months	43/105 (41.0%)	182/686 (26.5%)	0.003
Positive sputum smear at 3 months	26/35 (74.3%)	77/167 (46.1%)	0.003
Treatment outcomes			
Treatment success	63/137 (46.0%)	508/754 (67.4%)	<0.0001
Treatment failure	25/137 (18.2%)	61/754 (8.1%)	0.001
Died	21/137 (15.3%)	35/754 (4.6%)	<0.0001
Default	6/137 (4.4%)	21/754 (2.8%)	0.28
Transferred out	22/137 (16.1%)	129/754 (17.1%)	0.901

those reported in previous studies. In South Africa, an analysis of four cohorts that included 562 adults with culture positive pulmonary TB found that, although patients with positive smears at 2–3 months had a 4.5 times higher risk to fail therapy than patients with negative smears, PPV for treatment failure was only 12.5%.⁴ In another study among patients with MDR-TB, outcomes were significantly worse for patients with positive sputum culture within 2 months after starting second-line therapy.¹⁰

Despite the higher risk of treatment failure, the accuracy of a positive sputum smear at 2 months to predict treatment failure is only modest. Although we have found a sensitivity and specificity of 74 and 77%, respectively, the PPV and the positive LR to predict treatment failure are too low to rely on this parameter to predict treatment failure. Our observation is in accordance with WHO guidelines which state that smear status at the end of the intensive phase is a poor predictor of relapse and treatment failure.¹¹ In a meta-analysis that included controlled trials, cohort and case-control studies of previously untreated patients with pulmonary TB, the pooled sensitivity for the 2-month smear to predict failure was 57% with a poor positive predictive value.⁸ Nevertheless, the authors of this meta-analysis recognize that the studies analyzed were heterogeneous, particularly concerning the type of sputum specimen (smear or culture), month of specimen examination, and outcomes, so the results of this meta-analysis should be taken with caution.

Although a positive sputum smear at the end of the intensive phase may suggest that the patient may have drug-resistant TB, it also may indicate other situations such as poor adherence, slow resolution because of extensive cavitations or even non-viable bacteria that remain visible by microscopy.

The risk of failure is even higher if the sputum remains positive at 3 months. A small retrospective study found that only 21 of 35 (60%) patients whose sputum was still positive at the end of 3 months of treatment were ultimately cured.¹²

Although patients with a positive sputum smear at 2 months had a significantly lower chance of cure from TB, they do not seem to have a higher risk of death. In our study variables independently associated with death were female sex, HIV infection, and previously treated patients at presentation. Different studies have also shown the delay in diagnosis of TB and the higher mortality that occur in women, probably because of difficulties in access to care.^{13–15}

In our study, previously treated patients had a significantly higher percentage of positive sputum smears at 2 and 3 months than new cases. They also had a significant higher risk of treatment failure and death. Previously treated patients are difficult to retreat and represent a serious threat to TB control worldwide because of their higher rate of drug resistance.^{16–22} The high percentage of patients with treatment failure, especially in the cohort of previously treated patients, indicates that the prevalence of drug resistance should be high in this setting.

Table 6 Accuracy of a positive sputum smear at 2 months to predict treatment failure*

	Treatment failure	
	YES (positive sputum smear at 5 months)	NO (negative sputum smear at 5 months)
Positive sputum smear at 2 months	50	111
Negative sputum smear at 2 months	17	373
Accuracy of a positive sputum smear at 2 months to predict treatment failure		
Sensitivity	74%	
Specificity	77%	
Positive predictive value	31%	
Negative predictive value	96%	
Positive likelihood ratio	3.21	
Negative likelihood ratio	0.34	

Note: *The analysis has been made in the group of 551 patients initially diagnosed with a positive sputum smear and who have sputum samples collected at 2 and 5 months.

We are aware that our study has several limitations, mainly because it has been performed in a resource-limited setting. The most important limitations that should be acknowledged include the lack of culture confirmation of diagnoses or treatment outcomes and the lack of drug resistance testing. These limitations reflect the reality of TB diagnosis and management not only in this area of Angola but also in many other poor resource settings. Another aspect that should be taken into account is that nearly 17% of patients were transferred out so their final outcome could not be evaluated. Finally, because an active follow-up after the end of the treatment was not systematically performed, our study cannot tell if sputum smears taken at 2 months have any predictive value for prediction of relapse after treatment was completed. For this reason, the objective of our study was restricted to analyze the predictive value of sputum smears at 2 months to predict treatment failure. Despite these limitations, in our opinion, the main strength of our study is that it reflects the actual problem of TB treatment in a large cohort of patients in a setting with very few known data. These data may allow the identification of problems, so that the national TB programme can institute appropriate actions to improve programme performance.

In summary, we have observed that patients with positive sputum smear at 2 months have a higher risk of treatment failure; however, by itself this is a poor predictor of treatment failure. The implementation of rapid tests to detect TB resistance and new markers to monitor TB in resource-limited settings, are urgently needed in order to improve therapeutic results in patients with TB especially in those previously treated.

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