

# The Significance of the Persistent Presence of Acid-fast Bacilli in Sputum Smears in Pulmonary Tuberculosis\*

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**Study objectives:** Identification of acid-fast bacilli (AFB) in the sputum smear at the completion of tuberculosis therapy is in some settings considered evidence of treatment failure. However, some patients with pulmonary tuberculosis (TB) will have positive smear results with negative sputum culture results at the end of therapy. The objectives of this study were to estimate the prevalence of persisting positive sputum smear results in patients with TB and to identify characteristics that distinguish patients with persistently positive sputum smear results who also had negative sputum culture results from patients identified as treatment failures.

**Design:** A population-based, historical cohort study with nested case control study.

**Setting:** British Columbia Division of Tuberculosis Control central case registry.

**Patients:** All 428 patients with culture-proven pulmonary TB in British Columbia over 7 years with sputum that was positive for AFB.

**Methods:** Review of laboratory data of all 428 patients, as well as clinical data of a subset of 30 patients with persistently positive smear results beyond 20 weeks.

**Results:** Sputum smears were positive for AFB in 205 patients (48%) at 4 weeks, in 30 patients (7%) at 20 weeks, and in 12 patients (3%) at 36 weeks. Of the patients with smear results that were persistently positive at 20 weeks, 23 (77%) had negative sputum culture results and 7 (23%) had positive sputum culture results (*ie*, they were treatment failures). Patients identified as treatment failures had more localized disease as shown on chest radiographs, had less radiographic improvement at follow-up, had a higher prevalence of drug resistance, and were less compliant with medications than patients with persistently positive smear results and negative culture results. No subject with a negative culture result relapsed over the 6- to 48-month observation period.

**Conclusion:** Sputum that is persistently positive for AFB in patients in developed countries is more likely to be associated with negative culture results than with treatment failure.

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**Key words:** acid-fast bacilli; pulmonary; sputum; tuberculosis

**Abbreviations:** AFB = acid-fast bacilli; CXR = chest radiograph; DOT = directly observed treatment; MDR-TB = multidrug-resistant TB; S+ = persistently positive sputum smear result; S+/C- = persistently positive sputum smear result and negative sputum culture result at the time of smear positivity; S+/C+ = persistently positive sputum smear result and persistently positive sputum culture result; TB = tuberculosis

In pulmonary tuberculosis (TB), positive results of sputum cultures convert to negative within 3 months following the institution of effective standard

antimycobacterial therapy in > 85% of patients.<sup>1-4</sup> By 5 to 6 months, almost all patients will have had a bacteriologic response. In addition to symptomatic and radiographic improvement, the disappearance of acid-fast bacilli (AFB) from sputum smears and the conversion to negative culture results are used to assess the response to therapy. Patients in whom sputum culture results have not converted to negative after 5 to 6 months of therapy are considered treatment failures.<sup>5</sup>

Twenty percent of patients receiving antimycobacterial therapy will have the results of their sputum cultures convert to negative before the smear results convert, a phenomenon associated with far advanced

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disease.<sup>6</sup> It also has been observed recently in Barcelona, Spain, that on completion of therapy, 2.2% of patients had sputum smears positive for AFB with negative culture results due to the presence of nonviable mycobacteria or atypical mycobacterial colonization.<sup>7</sup> This subpopulation of patients might be managed as having treatment failure and may be subjected to prolongation of therapy, to augmentation of therapy with additional drugs, or to additional investigations.

Awareness of the significance of persistently positive smear results is important in that physicians may misinterpret the positive smear results as treatment failure and may make inappropriate management decisions.<sup>6,8</sup> Further, characteristics of patients who exhibit persistently positive smear results are largely unknown. The first aim of this study was to determine the prevalence of patients with smears that were persistently positive for AFB and had negative results for cultures in a well-documented, population-based series of consecutive cases of pulmonary TB in a developed country. The second aim was to attempt to identify clinical, radiologic, and bacteriologic features of patients with a persistent presence of AFB in sputum and the impact of this on outcome.

## MATERIALS AND METHODS

### *Patient Population*

Laboratory data from 692 consecutive culture-proven pulmonary TB cases from the period September 1988 through March 1995 were retrieved from the TB Registry of the Mycobacteriology Section, Provincial Laboratory of the British Columbia Centre for Disease Control. The laboratory is the mycobacteriology reference facility for British Columbia, the westernmost Canadian province, which has a population of approximately 3,000,000. Through its registry, the Division of TB Control maintains records of all TB cases, including details of treatment and outcome, for the entire province of British Columbia.

The study group consisted of the subset of patients whose initial sputum smears contained AFB and who had bacteriologic follow-up for at least 36 weeks or until the results of smears and cultures became negative. From this subset of patients, those with persistently positive sputum smear results (S+) beyond 20 weeks were identified and were further subclassified into those who had negative sputum culture results at that time (S+/C-) and those who had persistently positive sputum culture results (S+/C+). Sputum smears and cultures were typically obtained on a monthly basis until the results converted to negative. The medical records of the patients with persistently positive smear results after 20 weeks were reviewed to obtain data on associated medical conditions, laboratory findings, management, follow-up, compliance, and outcome, and the chest radiographs (CXR) were reviewed. Compliance with antimycobacterial therapy was defined as the use of the prescribed drugs for at least 80% of the treatment course, as documented by review of physician and nursing records.<sup>1</sup> Records of two patients were insufficient to determine compliance.

### *Mycobacteriology*

All tests were performed using standard methods that did not change over the course of the study period.<sup>9</sup> Sputum samples were decontaminated and liquefied using a 3% solution of NaOH, were neutralized with a 2.5-nanosecond exposure to HCl, and were concentrated by centrifugation for 15 min at 3,000g. Sediments were resuspended in a minimal amount of phosphate buffer; smears and cultures were made from the resuspended material. Smears were examined by the auramine acid-fast stain. Smears with positive results also were examined using the Kinyoun stain. Cultures were performed on all specimens using two media (7H11 medium; Middlebrook, Becton Dickinson; Cockeysville, MD; and Lowenstein-Jensen medium; Difco Laboratories, Detroit, MI; with added pyruvate and also by another method (BACTEC; Becton Dickinson; Mississauga, Ontario, Canada) in patients with smears positive for AFB. The first culture with a positive result was routinely tested for drug susceptibility to first-line drugs. Multidrug-resistant TB (MDR-TB) was defined as drug resistance to at least isoniazid and rifampin.

### *CXRs*

The initial and follow-up standard posteroanterior CXRs of the patients with persisting positive smears results were reviewed. The CXRs were evaluated by two physicians who were blinded to the clinical data of the patients. The disease extent on the initial CXR was based on the number of lung zones involved; each lung was considered to have three zones (upper, middle, and lower). Involvement of one or two zones was considered to be localized disease, three or four zones as moderate disease, and five or six zones as extensive disease. Follow-up CXRs were classified as showing improvement, progression, or no change.<sup>9</sup> The presence or absence of cavitation was noted.

### *Data Analysis*

The Mann-Whitney *U* test was used to compare distributions of continuous variables across two groups. Fisher's Exact Test was used to compare proportions across two groups.<sup>10</sup>

## RESULTS

### *Study Group*

Of the initial 692 patients with pulmonary TB, 162 patients (23.4%) were excluded because the result of the initial smear was negative, and an additional 102 patients were excluded by the other criteria (*ie*, there was no bacteriologic follow-up for at least 36 weeks or until the results of smears and cultures became negative). The study group, therefore, consisted of 428 cases. Almost half of the study group (48%) had smears that were positive for AFB beyond 4 weeks. Seventy-six patients (17.7%) had positive smear results beyond 12 weeks, and by 36 weeks 12 patients (2.8%) had persistently positive smear results (Fig 1). During the course of antimycobacterial therapy, the culture results converted to negative prior to the smear in 77 patients (18%). Of those with positive smear results at 4 weeks, only 20% had negative

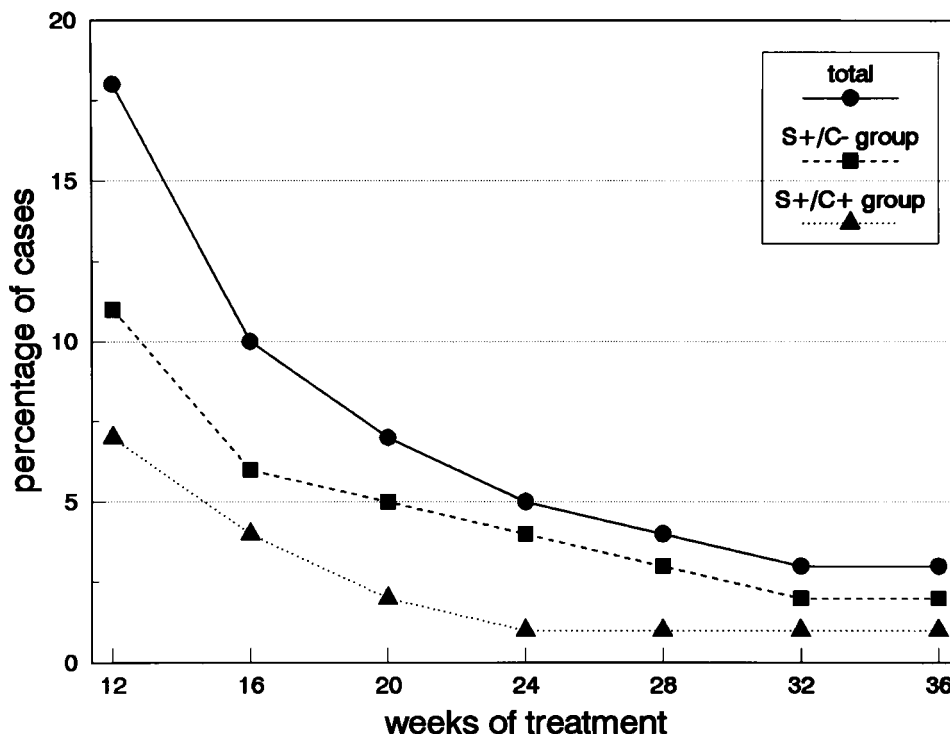


FIGURE 1. Surveillance of sputum smears positive for AFB in 428 patients with pulmonary TB after an initial 12 weeks of antimycobacterial chemotherapy. The majority of patients with smears persistently positive for AFB after 12 weeks had negative culture results (S+/C-). Fewer had positive culture results (S+/C+).

culture results. Beyond 12 weeks of therapy, approximately two-thirds of the patients with positive smear results had negative culture results, and only a minority had persistently positive culture results (Table 1). After 20 weeks, when the subclassified patient group was identified, 30 patients (7.1%) had persistently positive smear results, of whom 21 patients (76.7%) converted to negative culture results before negative smear results and 7 patients (23.3%)

**Table 1—Surveillance of Patients With Sputum Smears Positive for AFB With and Without Positive Culture Results Beyond 4 Weeks\***

Surveillance	S+/C- Group	S+/C+ Group	Total†
Beyond 4 weeks	40 (19.5)	165 (80.5)	205 (48.0)
Beyond 8 weeks	56 (45.5)	67 (54.5)	123 (28.7)
Beyond 12 weeks	47 (62.8)	29 (38.2)	76 (17.8)
Beyond 16 weeks	28 (63.6)	16 (36.4)	44 (10.3)
Beyond 20 weeks	21 (70.0)	9 (30.0)	30 (7.1)
Beyond 24 weeks	16 (72.8)	6 (27.3)	22 (5.1)
Beyond 28 weeks	13 (72.2)	5 (27.8)	18 (4.2)
Beyond 32 weeks	10 (71.4)	4 (28.6)	14 (3.2)
Beyond 36 weeks	8 (66.7)	4 (33.3)	12 (2.8)

\*Values given as No. (%). The percentages in the first two columns are those of the row total.

†Percentage of a total of 428 cases.

had persistently positive culture results. Two of the 30 patients had negative culture results only after 25 weeks (see "Mycobacteriology" section below).

#### *Patients With Persistently Positive Smear Results Beyond 20 Weeks*

**Demographic Data:** The demographic characteristics of the 30 patients with persistently positive smear results are shown in Table 2. Those in the S+/C- group tended to be older ( $p = 0.09$ ) than those in the S+/C+ group (60.8 years [SD, 16.9] vs 48.4 years [SD, 11.2], respectively). Only two patients (29%) in the S+/C+ group were caucasian compared with 14 patients (61%) in the S+/C- group ( $p > 0.10$ ). There was little difference between the two groups in regard to the country of origin, however (Table 2).

**Associated Medical Conditions:** All but eight patients (35%) in the S+/C- group had associated medical problems. Ten patients (43%) had diseases that caused impaired immunity: 2 patients had AIDS; 2 were receiving prolonged systemic corticosteroid therapy for steroid-dependent asthma and Addison's disease, respectively; 1 had rectal adenocarcinoma; 4 had longstanding diabetes mellitus; and

**Table 2—Demographic Characteristics of 30 Patients With Pulmonary TB Who Had Persistently Positive Sputum Smear Results Beyond 20 Weeks\***

Patient Characteristics	S+/C- Group	S+/C+ Group
No. of patients	23	7
Age, yr		
Mean $\pm$ SD	60.8 $\pm$ 16.9	48.4 $\pm$ 11.2
Range	26–81	37–67
Sex		
Male	16 (70)	5 (71)
Female	7 (30)	2 (29)
Race or ethnic group		
Caucasian	14 (61)	2 (29)
Aboriginal	3 (13)	3 (43)
Oriental	6 (26)	2 (29)
Region of origin		
North America	14 (61)	4 (57)
Europe	2 (9)	0 (0)
China or Southeast Asia	5 (26)	2 (28)
India	2 (9)	0 (0)
Latin America	0 (0)	1 (14)

\*Values given as No. (%), unless otherwise indicated.

I was receiving long-term hemodialysis. Eight patients in this group had chronic airways obstruction: five patients had COPD, two had bronchial asthma, and one had bronchiectasis. Nine patients had a substance abuse history, with seven being heavy alcohol abusers and two being illicit drug users. The number of patients in the S+/C+ group was too small to determine whether the distribution of associated medical problems in this group was different from that in the S+/C- group.

**Mycobacteriology:** In the S+/C- group, smear results remained positive up to 112 weeks, with a median duration of 31 weeks; smear results were positive in 76.2% of patients after 24 weeks, 61.9% after 28 weeks, 47.6% after 32 weeks, and 38.1% after 36 weeks. In the S+/C+ group, the median period of persistently positive smear results was 39 weeks. Patients in the S+/C- group generally had negative culture results before the end of 20th week; two patients in this group had positive culture results up to 25 weeks but had consistently negative culture results thereafter. The smear results of these latter two patients continued to be positive up to 97 and 35 weeks, respectively. The median duration of positive culture results in the S+/C- group was 10 weeks. In the S+/C+ group, three patients never converted either their smear or culture results to negative, one patient had active disease at 65 weeks when study follow-up ended, and two patients died still having positive results of smears and cultures. An atypical mycobacterial organism was isolated from one patient in both the S+/C- and S+/C+ groups after 20

weeks (both *Mycobacterium avium-intracellulare* complex).

Nineteen patients in the S+/C- group (83%) had a fully sensitive *Mycobacterium TB* organism. Four patients in the S+/C- group (17%) had organisms initially resistant to isoniazid; none had MDR-TB. In the S+/C+ group, two patients had MDR-TB ( $p < 0.05$  vs the S+/C- group). One additional patient in this group had initial resistance to isoniazid and, because of rifampin intolerance, required prolonged administration of second-line drugs.

**Radiographic Findings:** CXRs of 20 patients in the S+/C- group were available for review (Table 3). Follow-up radiographs were available for the period from 4 to 28 months after patients began treatment for TB, with a mean CXR follow-up of 19 months. The seven patients in the S+/C+ group had radiographic follow-up from 4 to 32 months after they began treatment, with a mean follow-up of 18 months. None of the patients in the latter group had extensive disease by CXR, whereas 9 of the 20 patients in the S+/C- group had extensive radiographic disease ( $p = 0.06$ ).

Twelve patients (60%) in the S+/C- group had cavitory disease. The persistence of positive smear results in these patients occurred for a longer time (median, 33 weeks), than in those without cavitory disease (median, 22 weeks) ( $p < 0.05$ ). The extent of disease did not significantly affect the persistence of AFB on smear; the median duration of positive smear results was 35 weeks in patients with extensive disease compared to 27 weeks in those without ( $p > 0.10$ ). However, within the subgroup of those with cavitory disease, extensive disease was associated with the prolongation of AFB on smear, with a median duration of positive smear results of 51 week compared to 28 weeks in those with cavitory, but nonextensive, disease ( $p < 0.10$ ). The duration of

**Table 3—Radiographic Characteristics of 27 Patients With Persistently Positive Sputum Smear Results Beyond 20 Weeks\***

Characteristics	S+/C-	S+/C+
No. of patients	20	7
Extent of disease		
1–2 zones (localized)	8 (40)	4 (57)
3–4 zones (moderate)	3 (15)	3 (43)
5–6 zones (extensive)	9 (45)	0 (0)
Cavitory disease	12 (60)	5 (71)
Noncavitory disease	8 (40)	2 (29)
Follow-up		
No change or progressed	2 (10)	5 (71)
Improvement	18 (90)	2 (29)

\*Values given as No. (%).



positive culture results was not significantly affected by the extent of the disease.

All but two patients in the S+/C- group (90%) showed radiologic improvement with treatment, whereas only two patients in the S+/C+ group (29%) had showed radiologic improvement ( $p < 0.01$ ).

*Treatment:* The majority of patients (78%) in the S+/C- group received first-line therapy with isoniazid and rifampin for a mean period of 12.7 months and with pyrazinamide for the first 2 to 3 months. Because of hepatic toxicity, pyrazinamide was discontinued in two patients and rifampin in one of those patients. Nine of these 23 patients received directly observed treatment (DOT). The duration of therapy in patients that had either DOT or nonstandard first-line treatment did not differ significantly from those on standard therapy. None of the patients in this group had changes in treatment based on the persistently positive smears, apart from a prolongation in the course of treatment.

In the S+/C+ group, four of the seven patients began receiving standard first-line medications and continued for a mean period of 13.3 months. Prolonged treatment with second-line drugs for 32 to 36 weeks was required for two patients with MDR-TB and one patient with isoniazid resistance and rifampin intolerance. The two patients with MDR-TB underwent limited surgical resection of the diseased lung; one patient died after 33 months, with TB considered to be a contributory factor, while the other patient continued to have positive smear and culture results after 65 weeks of follow-up. Five of the seven patients received DOT.

*Compliance and Posttreatment Follow-up:* Eighteen patients in the S+/C- group (78.3%) and 4 patients in the S+/C+ group (57.1%) were compliant with treatment ( $p > 0.10$ ). In the 18 of 23 patients in the S+/C- group who had documented completion of treatment, follow-up ranged from 6 to 48 months (median, 17 months). None of these patients relapsed during the period of follow-up. Of the remaining five patients in this group, two were still on treatment at the time of data analysis, two were lost to follow-up, and one patient with rectal carcinoma died for reasons unrelated to TB. One patient in the S+/C+ group had follow-up for 55 months after completing treatment without disease relapse. Of the remaining six patients, one was still on treatment at the time of data analysis, three did not appear for follow-up after completing treatment, and two were dead, with TB considered to be contributory factors in the deaths.

## DISCUSSION

In this population, 7.1% and 2.8% of treated pulmonary TB patients had persistent sputum smear positivity beyond 20 and 36 weeks, respectively. The presence of AFB on smear after conversion to negative culture results was observed at some point during treatment in 18% of the patients. This phenomenon occurred as early as after 4 weeks of therapy. Beyond 12 weeks of therapy, 63% to 73% of patients with persistently positive smear results had negative culture results. Similar findings have been observed by other investigators<sup>6,11</sup> who have reported that approximately two-thirds of patients had positive smear results and negative culture results at 14 weeks. It is important that this group of patients not be confused with those identified as treatment failures, who are defined as having persistently positive culture results. Whereas treatment failure requires a change in the treatment regimen, our study shows that a patient with persistently positive smear results alone has a good prognosis and does not require treatment modification. Mistakenly identifying those patients with persistently positive smear results only as treatment failures may also potentially result in unnecessary prolongation of antimycobacterial treatment.<sup>12</sup>

Only 30% of the subset of 30 patients with persistently positive smear results beyond 20 weeks represented treatment failure in this population. Demographic features that tended to characterize those with treatment failure rather than persistently positive smear results alone were younger age and non-Caucasian ethnicity. Somewhat paradoxically, the radiographic feature that distinguished the S+/C- group from the S+/C+ group was more extensive lung involvement in the former. Lack of improvement or worsening of the patient's condition on CXR with treatment were useful in identifying the treatment failure group, with 71% of the treatment failures showing no radiographic improvement, but only 10% of the S+/C- group showing no improvement. Kim and colleagues<sup>6</sup> observed that persistently positive smear results with negative culture results occurred between 4 to 20 weeks and was more common in patients with far advanced cavitary disease.<sup>6</sup> Radiographic improvement on treatment with persistently positive smear results should suggest that the positive smear result is due to the presence of nonviable organisms. It is not clear why cavitary disease should predispose to persistently positive smear results due to nonviable organisms. Possibly, the vastly increased bacillary load results in such an overwhelming number of dead bacilli that

more time is required to clear the large load. Also, it is possible that clearance from cavities is relatively delayed.

All of the AFB organisms in the sputum of patients in the S+/C- group appeared to be nonviable *Mycobacterium TB* organisms rather than atypical mycobacteria. The one patient who also grew an atypical mycobacterial organism only grew the organism from two of multiple specimens that had positive smear results.

It is not known with certainty whether patients in the S+/C- group require prolongation of antimycobacterial therapy in order to avoid an increased risk of eventual relapse. In all patients in this population, treatment was extended beyond the 6 to 9 months that is currently recommended.<sup>5</sup> We do not know whether treatment for only the standard duration would have resulted in relapse. With a mean treatment duration of 13 months, no relapses were observed over the brief period of follow-up, which ranged from 6 to 48 months. Whereas the need for a longer treatment course is not known, it does not appear that a change in drug regimen is required in patients with S+/C-. In none of our patients were the drugs that were used in the treatment regimen altered because of persistently positive smear results, yet the results of treatment were good.

Management of S+/C- patients, therefore, is quite different from management of S+/C+ patients, who would be considered treatment failures. The therapy for this latter group of patients requires the addition of at least two new antimycobacterial drugs to which the patient's organism is sensitive. The CXR is helpful in distinguishing the two groups; continued resolution by CXR despite the persistently positive smear results strongly suggests that the persistently positive smear results are due to nonviable mycobacteria and do not require a change in the regimen.

It should be stressed that these recommendations apply to a modern TB program in a developed country. Where program conditions and resources differ, the implications of persistently positive smear results might well indicate treatment failure in the majority of patients. Poor compliance and high rates of drug resistance will clearly influence the frequency of this phenomenon.

In summary, in the absence of MDR-TB, our findings indicate that the persistence of AFB in smears at the end of therapy is not necessarily a treatment failure. If the patient demonstrates clinical and radiographic improvement, a change of therapy may not be required based on positive smear results alone. The results of cultures and drug susceptibility tests are essential for the physician to have prior to any decision regarding prolongation or change in therapy.

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