Incidence and Significance of Acid-fast Bacilli in Sputum Smears at the End of Antituberculous Treatment*

Rafael Vidal, MD, PhD; Nuria Martín-Casabona, MD, PhD; Arturo Juan, MD; Teresa Falgueras, MD; and Marc Miravitlles, MD

**Objective:** A group of patients with pulmonary tuberculosis (PT) who received and correctly completed antituberculous therapy were studied to determine the incidence and significance of positive acid-fast bacilli (AFB) in sputum smears at the end of this treatment.

**Design:** Retrospective chart review of persons with bacteriologically proved PT.

**Patients:** Of 1,052 patients diagnosed as having PT between 1988 and 1992, 453 who fulfilled the following criteria were included in the study: (1) diagnosis established by positive AFB and positive culture in sputum smears; (2) no previous antituberculous treatment had been received; (3) HIV serologic test results were negative; (4) treatment was correctly completed; (5) they were followed up throughout the period of treatment; and (6) expectoration was still present at the end of treatment and at least two spontaneous sputum samples could be obtained.

**Results:** Positive AFB of sputum smears were found at the end of treatment in 10 (2.2%) of the 453 patients studied. Five patients had only one positive smear, and the other five had more than one. Of these ten cases, sputum culture was negative in eight, which were considered to be unviable bacilli, and positive for nontuberculous mycobacteria in two. Clinical symptoms or worsening on chest radiograph were observed only in one patient with unviable bacilli, but they were caused by a concomitant nonspecific respiratory tract infection.

**Conclusions:** Positive AFB smear results at the end of completed treatment regimens analyzed in this study have occurred because of unviable bacilli and nontuberculous mycobacteria colonization. The presence of more than one positive smear seems not to increase the probability of treatment failure and is more frequently due to nontuberculous mycobacteria. Results of culture can thus be awaited without the need to prolong or modify antituberculous therapy.


**Key words:** antituberculous treatment; nontuberculous mycobacteria; pulmonary tuberculosis; relapse; treatment failure; unviable bacilli

**Abbreviations:** AFB=acid-fast bacilli; E=ethambutol; H=isoniazid; LJ= Löwenstein-Jensen culture; NTM=nontuberculous mycobacteria; PT=pulmonary tuberculosis; R=rifampin; UVB=unviable bacilli; Z=pyrazinamide

Short therapeutic regimens (6 or 9 months) for treatment of pulmonary tuberculosis (PT) with rifampin (R), isoniazid (H), and pyrazinamide (Z), with the addition in some cases of ethambutol (E), have proved to be effective.1,2 After the start of treatment, the number of bacilli in sputum decreases rapidly and at 3 months, acid-fast bacilli (AFB) in sputum smears are negative in more than 90% of cases and should not become positive again either during or after treatment.3,4 The reappearance of positive smears at the end of treatment may be due to relapse of the tuberculous disease, failure of treatment, the presence of unviable bacilli (UVB), or colonization or disease by nontuberculous mycobacteria (NTM).4,6 In any event, the fact that a smear proves positive at the end of treatment places in doubt whether treatment has been effective and whether retreatment with three new drugs should be started.5 International guidelines provide no concrete answer to this question.7-9

The aim of this study was to determine the incidence and significance of the appearance of AFB in sputum smears at the end of some most-often-used-correctly completed treatment regimens and the appropriate therapeutic approach.

**Materials and Methods**

**Population**

A total of 1,051 patients with bacteriologic diagnosis of PT were treated and followed up between 1988 and 1992. The study group...
was composed of 453 patients with PT who fulfilled the following criteria: (1) diagnosis was established by positive AFB and positive culture in sputum smears; (2) they had not received previous antituberculous treatment; (3) HIV serologic test results were negative; (4) treatment was correctly completed; (5) they were followed up throughout the period of treatment; and (6) expectoration was still present at the end of treatment and at least two spontaneous sputum samples could be obtained.

**Therapeutic Regimens**

All patients were prescribed an antituberculous regimen of 9 months’ duration with R, H, and E for the first 2 months, followed by R and H for 7 months (2RHE/7RH); or 6 months with R, H and Z with or without E for the first 2 months, followed by R and H for 4 months (2RHEZ/4RH or 2RHZ/4RH) at the doses previously described.1,3

**Follow-up of Patients**

All patients were followed up on an outpatient basis and seen at 15 days, 1 month, 2 months, and 3 months, and then every 2 or 3 months until completion of treatment.

Chest radiograph, AFB in sputum smears, and culture in Löwenstein-Jensen (LJ) medium of spontaneous sputum were performed in all cases at the end of treatment. At least two samples were processed for every patient.

All patients were followed up for at least 1 year after completion of treatment, and samples of sputum were collected at 2 or 3 months when spontaneous expectoration was present.

**Bacteriologic Study of Mycobacteria**

The samples obtained were centrifuged at 3,500 rpm for 20 min. The supernatant was digested and decontaminated with sodium lauryl sulfate, neutralized with orthophosphoric acid, and recentrifuged at 3,500 rpm for 20 min. The sediment was screened for AFB with both auramine and Ziehl-Neelsen techniques and cultured into LJ tubes that were incubated at 37°C and checked weekly for a period of 8 weeks. Positive LJ cultures were confirmed by using Ziehl-Neelsen techniques. After isolation, differentiation of NTM was carried out according to the guidelines of the Centers for Disease Control and by hybridization probe technique (Gen-Probe or Accuprobe Inc; San Diego, Calif) in cases of *Mycobacterium tuberculosis*, *Mycobacterium avium complex*, *Mycobacterium kansasii*, and *Mycobacterium gordonae*.

**Therapeutic Approach**

Prolongation of antituberculous treatment was considered only when AFB in sputum smears were obtained in at least two sputum samples at completion of treatment and while awaiting LJ culture results.

**Definitions**

We considered as UVB the AFB in sputum smear obtained during or at the end of treatment and that do not grow in LJ culture.4,11 Colonization by NTM was established when NTM were detected in sputum in the absence of diagnostic criteria of NTM-induced disease.11,12

**Results**

Of the 453 patients studied, 10 (2.2%) had AFB in sputum smears at the end of treatment. Eight of them had less than ten AFB per sputum smear; only two of these had positive culture for NTM. The sputum samples obtained from one patient displayed more than one AFB per microscopic field, but cultures were negative. Finally, the last patient presented 50 to 100 AFB per smear in different sputum samples, but no growth was observed in cultures (Table 1).

Five of the ten patients had AFB in sputum smears in a single specimen at the end of treatment (patients 2 to 6). All five patients had good clinical and radiologic evolution and antituberculous treatment was prolonged in only two patients until the results of LJ culture were obtained. In the remaining five patients, acid-fast smears in sputum were positive in more than one sample and antituberculous treatment was continued until LJ culture results were available. Nine patients were asymptomatic, although residual radiologic lesions persisted in four. LJ cultures were negative in seven of these nine patients who were asymptomatic; these patients were considered to have UVB; the other two were isolated NTM and considered to be NTM colonized, since NTM were not identified on subsequent controls (patients 9 and 10).

Clinical manifestations and chest radiographic findings suggestive of progression of the disease were identified in one patient (patient 7). Moreover, AFB in sputum smears were obtained immediately after the end of treatment. Consequently, the patient was diagnosed as having bacteriologic failure and a new treatment was started with new drugs (Table 1). A broad-spectrum antibiotic was added owing to persistence of symptoms, and the condition resolved after a few days. Posterior LJ cultures were negative. The patient was therefore considered to have UVB and the accompanying symptoms were attributed to a coinciding nonspecific respiratory tract infection. The new antituberculous treatment was discontinued.

**Discussion**

Our results show that AFB in sputum smears at the end of correct treatment were observed with a 2.2% incidence in our selected population of previously untreated patients diagnosed as having PT. In most cases, the AFB in sputum smears corresponded to UVB (8/10, 80%), and less frequently to colonization by NTM (2/10, 20%). Treatment failure was not observed in any of the cases.

Although the diagnosis of active PT is usually suspected by AFB in sputum smears in respiratory samples, it is essential to show mycobacterial growth in culture sample for confirmation.5,6 Therefore, the basic criterion indicating therapeutic effectiveness and recovery from PT is microbiologic and requires definitive negativity of the initially positive culture.

The reappearance of positive cultures, during or after correct treatment, usually indicates therapeutic failure or relapse, respectively. In the first case, a therapeutic regimen with antituberculous drugs different from those used up to that point must be established, whereas in the case of relapse, when the
The incidence of lesions.

extensive tuberculous disease may be due to a coinciding nonspecific respiratory tract infection.

Criteria for treatment failure require at least two positive sputum smears followed by positive cultures. Five of our patients initially fulfilled these criteria and consequently treatment was prolonged. Nevertheless,
results of cultures were negative for *M tuberculosis* in all five, as they were in the patients with only one positive sputum smear. If other studies confirm these findings, the suspicion of treatment failure based on the number of positive samples should be revised. The results of our study suggest that the only valid criterion for failure should be positive culture, and even cases with more than one positive sputum smear will probably be due to either UVB or NTM. Thus, the decision to prolong treatment in such patients should be based on clinical and radiographic data instead of the number of positive smears, while awaiting the cultures.

A further cause of AFB in sputum smears during or at the end of treatment is NTM due to colonization of residual tuberculous lesions. Neither signifies the presence of disease or requires treatment. In contrast, lung disease by NTM in non-HIV carriers is mainly observed in patients with previous lung tuberculosis, chronic lung disease, or both, and in all cases it produces clinical symptoms and abnormal chest radiographic findings. In the present study, AFB in sputum smears and LJ culture for NTM were detected in two patients (0.4%) who were asymptomatic with no changes on chest radiograph and corresponded to colonization in both cases, since criteria for disease by NTM could not be demonstrated.

In conclusion, from the results obtained, it can be stated that the presence of AFB in sputum smears at the end of correct antituberculous treatment is mainly due to UVB or NTM colonization, neither of which is accompanied by clinical symptoms or chest radiographic changes and has no clinical significance. Thus, the presence of AFB in sputum smears at the end of treatment in itself is no indication for prolonging or reintiating treatment while awaiting the results of culture, unless this phenomenon is accompanied by clinical symptoms or abnormal findings on chest radiograph.

**REFERENCES**