

Cavitary Disease and Quantitative Sputum Bacillary Load in Cases of Pulmonary Tuberculosis[∇]

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Received 6 September 2007/Accepted 30 September 2007

We examined sputum bacterial loads in adults with newly diagnosed tuberculosis using quantitative culture and time-until-positive (DTP) culture in BACTEC 460. Patients with cavitary disease had higher CFU levels than those without cavities and shorter DTPs. Within radiographic strata of moderately and far advanced tuberculosis, higher CFU counts were associated with cavitary disease.

The proportion of adults with pulmonary tuberculosis (TB) who have cavitary disease at the time of diagnosis ranges from 40 to 87% (1, 9, 17). Several studies using qualitative smears and cultures have shown that patients with cavitary pulmonary TB have higher bacterial loads in their sputum (3, 9, 10, 12, 15). In 1965, Canetti and Grosset compared bacillary load in lung sections from resected lung tissue in patients with pulmonary TB. In cavity walls, the bacillary load was estimated to range from 10^7 to 10^9 in contrast with only 10^2 to 10^4 in areas of caseous necrosis (4). Few data are available using quantitative culture methods to assess the sputum bacillary load in patients with cavitary and noncavitary disease (14).

The presence of cavitary disease has been shown to be an important risk factor for treatment failure and relapse (2, 19) and possibly for development of drug resistance during treatment (11). During screening for a clinical trial enrolling newly diagnosed pulmonary TB, we examined the sputum bacillary load in patients with cavitary and noncavitary TB using quantitative culture methods.

The present study was conducted at the Universidade Federal do Espírito Santo (UFES), Vitória, Brazil, from January 2003 to January 2006. The protocol was reviewed and approved by the institutional review boards at UFES and the University Hospitals of Cleveland and by the Brazilian National Committee of Ethics in Research. Human immunodeficiency virus-uninfected adults, aged 18 to 60 years, with initial episodes of newly diagnosed pulmonary TB were screened for participation in a TB treatment trial. During the screening process, quantitative sputum cultures were performed on early-morning pretreatment sputum specimens collected from a con-

secutive temporal sample of patients with or without cavitary disease. A standard 6-foot posteroanterior (PA) chest radiograph was obtained for all patients. Cavitary disease was defined as the presence of a gas-containing lucent space at least 1 cm in diameter within the lung parenchyma surrounded by an infiltrate or fibrotic wall greater than 1 mm thick. Each X-ray film was examined independently by two experienced clinicians blinded to each other's readings (Kappa score of interobserver agreement = 0.85). The extent of radiographic disease was graded by using the U.S. National Tuberculosis and Respiratory Disease Association scheme that classifies disease into minimal, moderately advanced, and far-advanced disease (8). Note that the minimal disease classification excludes patients with cavities.

The average sputum volumes collected for cavitary and noncavitary patients were 9.7 and 9.3 ml, respectively. Aurnamine/rhodamine and/or Ziehl-Neelsen staining methods were used for sputum smear microscopy. The results were reported using the World Health Organization grading scale (18). Quantitative cultures were performed on selective Middlebrook 7H10 agar plates as described previously (5). Colonies were counted on plates with dilutions yielding 10 to 50 visible colonies. BACTEC 12B vials (Becton Dickinson, Sparks, MD) supplemented with PANTA, a lyophilized mixture of five antimicrobials (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin), were also inoculated and examined daily for growth during first 14 days, then on days 18 and 21, and weekly thereafter for up to 6 weeks. A positive culture was defined as a growth index (GI) > 30. The number of days until positive (DTP) BACTEC culture was recorded as the time after inoculation until a positive GI was obtained (7). The data were analyzed by using SAS software 9.1.

A total of 716 adults with newly diagnosed, initial episodes of pulmonary TB were screened for participation in the clinical trial. Of these, 244 patients with culture-confirmed, drug-susceptible TB were eligible for analysis, 100 with cavitary disease

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[∇] Published ahead of print on 10 October 2007.

TABLE 1. Characteristics of TB patients stratified according to the presence of cavities in the chest radiograph

Characteristic	Cavitary (n = 100)	Noncavitary (n = 144)	P ^a
Mean age in yr ± SD (range)	32.7 ± 10.8 (18–59)	32.8 ± 10.0 (18–59)	0.94*
No. of males (% total)	70 (70)	82 (57)	0.04†
Mean body mass index (kg/m ²) ± SD (range)	19.8 ± 2.7 (15–29)	20.7 ± 3.1 (15–38)	0.02*
Radiographic severity of disease: no. (%)			
Minimal	0 (0) ^b	27 (19)	<0.0001‡
Moderately advanced	29 (29)	87 (60)	
Far advanced	71 (71)	30 (21)	
Sputum AFB smear grade ^c : no. (%)			
Negative	3 (3)	55 (38)	<0.0001**
1+ to 2+	12 (12)	35 (24)	
3+	85 (85)	54 (38)	

^a *, T test for equality of means. †, X² = 4.28, df = 1; ‡, X² = 66.88, df = 2; **, X² = 58.77, df = 2.

^b There are no patients in this group because minimal disease in the U.S. National Tuberculosis and Respiratory Disease Association classification excludes patients with cavity disease.

^c The World Health Organization scale consists of five groups: negative; exact figure (1 to 9 AFB)/100 fields; 1+, 10 to 99 AFB/100 fields; 2+, 1 to 10 AFB/50 fields; and 3+, >10 AFB/20 fields.

and 144 with noncavitary disease. Demographic and clinical characteristics of the patients are summarized in Table 1. Eighty-nine percent of the patients had moderately advanced or far-advanced radiographic disease. Eighty-five percent of the cavitary patients had “3+” (i.e., >10 acid-fast bacilli [AFB] per field in at least 20 fields) sputum smears, whereas only 38% of the noncavitary patients had 3+ smears.

The sputum bacillary loads measured by CFU and DTP BACTEC culture were determined. The bacillary loads in 244 TB patients stratified according to the presence of cavity lesions on their initial posteroanterior chest radiographs were as follows. For cavitary (n = 100) and noncavitary (n = 144) patients, the mean log₁₀ CFU/ml of sputum values were 5.2 ± 1.4 (range, 1.1 to 7.3) and 4.0 ± 1.6 (range, 0.9 to 7.4), respectively (P = <0.0001 [T test for equality of means]). For cavitary (n = 93) and noncavitary (n = 136) patients, the BACTEC mean DTP values were 3.5 ± 2.8 (range, 1 to 15) and 9.0 ± 5.8 (range, 1 to 38), respectively (P = <0.0001 [T test for equality of means]). In combined cavitary and noncavitary groups the BACTEC DTP was inversely proportional to the CFU (r² = 0.63). Cavitary patients had significantly higher CFU counts than patients with noncavitary disease (5.2 log₁₀ CFU/ml versus 4.0 log₁₀ CFU/ml) and a lower BACTEC DTP (3.5 days versus 9.0 days). The radiographic extent of disease and the presence of cavity disease on initial chest radiographs were also compared to bacillary load (Table 2). The bacterial burden increased with the radiographic severity of disease in both cavitary and noncavitary groups (P < 0.05). Sputum specimens from cavitary patients had higher CFU counts regardless of the extent of disease category (P < 0.05).

Cavitary disease is an important independent risk factor for treatment failure and relapse in TB (2, 13, 19). In an earlier histopathological study, the internal necrotic zone and softened caseous material of the tuberculous cavity were shown by semiquantitative readings on Lowenstein-Jensen slants to contain higher numbers of tubercle bacilli, greatly exceeding those in closed lesions (3). Eighty percent of the closed cavities had >200 colonies per slant, whereas only 22% of the closed le-

sions had an equivalent number. Although not directly comparable because of methodological differences, our results confirmed earlier histologic and bacteriologic studies showing that the number of tubercle bacilli is much greater in patients with cavitary lesions than in noncavitary patients (5.2 versus 4.0 log CFU/ml).

Previously, Wallis et al. demonstrated that DTP in BACTEC can serve as a surrogate for CFU measurement (16). The number of days to reach a positive GI was inversely proportional to the log₁₀ CFU of the inoculum (r² = 0.99). Recently, Perrin et al. investigated the relationship of DTP and cavitary disease by using the BacT/ALERT culture system (bioMérieux) (14). The DTP was significantly lower in patients with cavities (8.4 days) than in those without cavitation (16.2 days). In patients with four to five cavities, the DTP was lower (5.3 days) than those with one to three cavities (13.4 days) and without cavities (17.5 days) (14). We observed a high inverse correlation between BACTEC DTP and CFU in the combined cavitary and noncavitary groups (r² = 0.63), confirming that DTP is a reliable surrogate of sputum bacillary load. Also, there was a relationship between DTP and the presence or absence of cavities in the chest radiograph. A low DTP (3.5 days) was associated with cavitary disease and a higher DTP (9.0 days) was associated with noncavitary disease. These DTP values are lower than those reported with the BacT/ALERT

TABLE 2. Comparison of sputum bacillary load and disease severity in patients with cavitary and noncavitary tuberculosis

Patient group	CFU/ml of sputum (SE) at various disease severities			P ^a
	Minimal	Moderately advanced	Far advanced	
Cavitary		4.79 (0.22)	5.35 (0.17)	<0.05
Noncavitary	3.05 (0.28)	4.11 (0.17) ^b	4.64 (0.25) ^b	<0.05

^a Wilcoxon Mann-Whitney test.

^b P < 0.05 (cavitary versus noncavitary).

system, suggesting that growth in the BACTEC 460 is more rapid than in the BacT/ALERT and/or the overall mycobacterial loads of our patients were much higher.

In an assessment of early bactericidal activity of isoniazid, Donald et al. observed that the CFU counts of baseline sputum specimens were associated with the patient's radiographic extent of disease ($P < 0.001$) and cavity size ($P < 0.001$) (6). We observed a correlation between severity of disease in chest X-ray and both the presence of cavities and CFU counts, thus confirming this finding. The bacillary load in cavitary and non-cavitary patients increased according to the progression of disease based on the amount of lung involved, the density of lesions, and the presence and extent of cavitary lesions.

A limitation of our study was the presence of cavitary disease was made using standard PA chest radiographs. Small cavitary lesions may have been found in some of the noncavitary patients if computed tomography of the chest had been used. However, PA chest radiographs are the most common radiographic examination for TB suspects in TB control programs worldwide.

Using the DTP in liquid culture as an indicator of bacterial load will be useful in assessing the efficacy of new treatment regimens and in quantifying mycobacteria in patients who are nonresponsive to treatment.

This study was supported by contract NO1-AI95383 (Tuberculosis Prevention and Research Control Unit) of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and, in part, by funding for CNPq and Rede Brasileira de Pesquisa em Tuberculose (REDE-TB).

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