Value of Examining Multiple Sputum Specimens in the Diagnosis of Pulmonary Tuberculosis

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To objectively assess the value of examining multiple sputum specimens in maximizing the sensitivity of detection of *Mycobacterium tuberculosis*, we retrospectively reviewed the acid-fast bacillus smear and culture results of patients diagnosed with culture-proven pulmonary tuberculosis (TB) at Hennepin County Medical Center between 1986 and 1996. Two hundred and forty six persons were diagnosed with pulmonary TB in the time period analyzed. In 93% of these cases (229 of 246) the laboratory diagnosis was made by detection of *M. tuberculosis* in sputum specimens; however, only 52% (120 of 229) of these patients had at least three sputum specimens submitted to the laboratory at the time of diagnosis. Of the patients from whom at least three specimens were collected, 47% (56 of 120) had at least one smear-positive specimen; the third or later specimen submitted was the first smear-positive specimen for 13% (7 of 56) of these persons but was the first culture-positive specimen for only 7% (4 of 56). Of the 64 patients with smear-negative specimens, for only 5% (3 of 64) was the third or subsequent specimen submitted the first from which *M. tuberculosis* was recovered. This data indicates that, in our institution, the overwhelming majority of culture-proven pulmonary TB cases are diagnosed from the first or second sputum specimen submitted to the laboratory and that only rarely is a third specimen of diagnostic value.

The isolation and identification of *Mycobacterium tuberculosis* from respiratory specimens, most commonly expectorated or induced sputa, are required to establish a definitive diagnosis of pulmonary tuberculosis (TB). In addition, the detection of acid-fast bacilli (AFB) in smears made from concentrated sputa is of considerable clinical and epidemiological value and remains the most widely used rapid diagnostic test for TB. The resurgence of TB in the United States that occurred in the late 1980s and early 1990s resulted in a renewal of interest in improving the efficiency with which laboratories detect *M. tuberculosis* in clinical specimens (12). Much of this effort has been directed toward optimizing the methodologies used for the detection of mycobacteria by smear (8), culture (2, 7, 13), or nucleic acid amplification (1). Comparatively little attention, however, has been paid to the contribution that specimen number and quality have on the efficiency of the laboratory diagnosis of TB.

Most standard laboratory texts (6) and guidelines for mycobacteriology laboratories (3, 10) recommend that at least three sputum specimens, preferably collected on successive days, be submitted to the laboratory for AFB smear and culture for patients suspected to have TB. Unfortunately, there has been a paucity of published data analyzing the validity of this recommendation (9). In two recent clinical studies, the collection of insufficient numbers of specimens was identified as a contributing factor in the delayed diagnosis of TB (4, 5). Neither of these investigations, however, differentiated patients based on the exact numbers of specimens obtained; a division was simply made based on whether an adequate (three or greater) or inadequate (less than three) number of specimens had been obtained. In fact, many of the patients in the inadequate group had no specimens sent for AFB smear and culture.

In the present investigation, we sought to analyze what the overall contribution of each successively collected specimen was to the ultimate diagnosis of pulmonary TB for those patients from whom at least three sputa were submitted to our laboratory. Given the considerable amount of technologist labor expended in processing and examining mycobacterial sputum specimens, we felt it important to determine whether the diagnostic benefit of analyzing multiple sputum specimens compensated for the increased per-patient cost of TB testing. We retrospectively investigated the AFB smear and culture results of all patients diagnosed with culture-proven pulmonary TB at Hennepin County Medical Center (HCMC) between 1986 and 1996. The implications of the results of this analysis with respect to recommendations for the number of specimens that should be collected from patients when pulmonary TB is suspected are discussed.

MATERIALS AND METHODS

Study institution and experimental design. HCMC is a 450-bed tertiary-care, teaching facility located in Minneapolis, Minnesota. The institution averages 20,000 patient admissions and almost 400,000 outpatient clinic and emergency room visits annually. We examined the laboratory records of all patients for whom a definitive diagnosis of pulmonary TB had been made in the period from 1986 to 1996. In particular, the AFB smear and culture results of all sputum specimens received in the laboratory within 1 month of the initial diagnosis of TB were analyzed. The relative contribution of each sputum specimen collected in making an ultimate diagnosis of TB was determined. Since a record of the criteria for determining specimen quality was not available for all cultures, this variable was not addressed in the analysis.

Culture procedures. All sputum specimens were decontaminated and concentrated prior to examination by using the N-acetylcysteine–sodium hydroxide procedure recommended by the Centers for Disease Control and Prevention (3). AFB were detected microscopically in sputum concentrates, prepared by conventional centrifugation, with an auramine-rhodamine stain. Sputum sediments were inoculated both into a BACTEC 12B bottle (Becton-Dickinson Microbiology Systems, Sparks, Md.) and onto a Middlebrook 7H11 plate. Culture media were incubated at 37°C in a 5% CO₂ incubator for up to 6 weeks.
Organism identification. From 1986 through July 1992, organisms were identified as belonging to the *M. tuberculosis* complex on the basis of the ability of *p*-nitro-o-acetylamino-β-hidroypropionphenone (NAP) to inhibit organism growth in the BACTEC radiometric culture system (11). From July 1992 until the conclusion of the study, both the NAP test and the AccuProbe DNA hybridization assay (GenProbe, San Diego, Calif.) were used to identify isolates as belonging to the *M. tuberculosis* complex.

RESULTS

The Clinical Microbiology Laboratory at HCMC received 17,723 respiratory specimens for AFB smear and culture from 1986 to 1996, with 2,545 being positive for mycobacteria. Seven hundred and six (28%) of these positive respiratory cultures contained *M. tuberculosis*. A total of 246 persons were diagnosed with culture-proven pulmonary TB during this 10-year interval. For 93% (229 of 246) of these individuals, the organism was recovered from one or more sputum specimens. The remaining 17 cases were diagnosed by recovering the organism from bronchoalveolar lavage (11 cases), pleural fluid (4 cases), a bronchial wash (1 case), and a lung biopsy (1 case).

Of the *M. tuberculosis* culture-positive patients with sputum specimens submitted to the laboratory, 25% (58 of 229) had only a single specimen sent for AFB smear and culture, 22% (51 of 229) had two sputa sent for examination for mycobacteria, and 53% (120 of 229) had three or more sputum specimens collected. The AFB smear was positive for 46% (28 of 58) of persons for whom only a single sputum specimen was examined. AFB were detected by direct microscopic examination at least once for 60% (31 of 51) of patients who had two sputum specimens obtained and for 45% (56 of 120) of patients who had three or more sputa sent to the laboratory.

More extensive analysis was performed on the data from patients who had at least the currently recommended number of three sputum specimens submitted at the time of their initial diagnosis. The total number of culture-positive specimens received for each patient in this group is shown in Table 1. For 21% (25 of 120) of these patients only a single sputum specimen of the three or more collected was positive, and for 96% (24 of 25) of these persons the sole culture-positive specimen was smear negative. At least three specimens were culture positive for 62% (74 of 120) of patients from whom three or more sputa were collected. One or more smear-positive specimens were obtained from 69% (51 of 74) of the individuals in this group.

Of more significance, however, is the relative frequency with which the first, second, third, or a subsequent specimen proved to be the first one positive by smear or culture. This data is shown in Table 2. For 9% (113 of 120) of the patients, either the first or second specimen collected proved to be diagnostic. For only 5% (7 of 120) of the patients was a third specimen required to make a definitive diagnosis of TB. In the smear-positive group of patients, 13% (7 of 56) required more than two specimens to be collected before a smear-positive specimen was obtained. Interestingly, there was no difference between the smear-positive and smear-negative groups in terms of the diagnostic value of a third specimen. For 7% (4 of 56) of the smear-positive patients and 5% (3 of 64) of the smear-negative patients, the first culture-positive specimen was the third one obtained.

DISCUSSION

This 10-year retrospective review of data collected on patients with culture-proven pulmonary TB at HCMC enabled us to objectively assess the value of examining multiple sputum specimens in diagnosing this disease.

The first significant observation was that for only a slim majority (52%) of positive patients were the minimum recommended number of sputum specimens submitted to the laboratory. Furthermore, for a significant minority (25%) of *M. tuberculosis* culture-positive individuals only a single specimen was obtained, in spite of the presence of institutional guidelines recommending that multiple specimens be sent for the evaluation of patients suspected to have TB. Interestingly, the rate of smear positivity for the patient group from which only one specimen per person was collected did not differ significantly from that observed for the first specimens received from the group of patients from whom three or more specimens were collected (46 versus 45%). This suggests that the reporting of a positive smear result by the laboratory was not a primary factor in determining whether more than one specimen would be collected. The frequency with which single sputum specimens were submitted for mycobacterial culture noted in this study is in general agreement with the findings of a Q-Probe study conducted by the College of American Pathologists (CAP) in 1994 (9). In that investigation, approximately 65% of TB-positive patients had three or more specimens submitted for examination for AFB and 17.5% of patients who were *M. tuberculosis* culture positive had only one specimen submitted. Primarily because positive cultures were detected disproportionately more often among patients from whom multiple specimens were obtained, the authors of the CAP survey concluded that the recommendation that three or more sputum specimens be examined for patients suspected to have TB was justified. No clinical data was analyzed in the CAP study; thus, it was not possible to determine how many individuals in the *M. tuberculosis* culture-negative group, from

### Table 1. Total number of *M. tuberculosis* culture-positive specimens for patients from whom three or more sputum specimens were obtained for AFB smear and culture

<table>
<thead>
<tr>
<th>No. of culture-positive specimens</th>
<th>No. of patients (%) that were:</th>
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<tbody>
<tr>
<td></td>
<td>Either&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>25 (21)</td>
</tr>
<tr>
<td>2</td>
<td>21 (17)</td>
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<tr>
<td>3</td>
<td>49 (41)</td>
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<tr>
<td>4 or more</td>
<td>25 (21)</td>
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<sup>a</sup> Either smear positive or smear negative.<br><sup>b</sup> At least one specimen obtained from each member of this group of patients was smear positive.

### Table 2. Frequency distribution of the first positive specimen for patients from whom three or more specimens were collected for AFB smear and culture

<table>
<thead>
<tr>
<th>Collection order of specimen</th>
<th>No. of patients (%) that were:</th>
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<tbody>
<tr>
<td></td>
<td>Either&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>First</td>
<td>80 (67)</td>
</tr>
<tr>
<td>Second</td>
<td>33 (28)</td>
</tr>
<tr>
<td>Third</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Fourth or later</td>
<td>0 (0)</td>
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</table>

<sup>a</sup> Numbers are for the first culture-positive specimens for all patients, both smear positive and smear negative.<br><sup>b</sup> At least one specimen obtained from each member of this group of patients was smear positive.
whom less than three specimens were obtained, were deemed clinically to have TB.

In the present study, we concentrated on assessing the contribution of each specimen collected to the ultimate diagnosis of TB for patients with culture-proven disease and from whom at least the minimum recommended number of sputum specimens had been collected. For 25 of the 120 patients in our study who fit these criteria, M. tuberculosis was recovered from only a single specimen. This figure (21%) is somewhat higher than the 9.1% reported in the CAP Q-Probe study (9). As might be expected given that smear positivity is presumably reflective of increased organism burden, in 24 of these cases the single culture-positive specimen was smear negative. In contrast, the majority of patients (69%) who had M. tuberculosis recovered from three or more specimens had at least one smear-positive result. The relatively high frequency with which M. tuberculosis was recovered from only one specimen, even for patients from whom multiple specimens were obtained, appears to lend credence to the idea that diagnostic efficiency would be greatly compromised if less than three specimens were examined. Further analysis of this data, however, supports a somewhat different conclusion. When the relative diagnostic significance of each specimen collected is assessed, it becomes clear that for the overwhelming majority (95%) of patients from whom three or more specimens were collected, the first or second specimen proved to be diagnostic (Table 2).

In addition, the third specimen was of no higher value for smear-negative patients (for 3 of 64 of these individuals the third specimen collected was the first from which M. tuberculosis was cultured) than for smear-positive patients (4 of 56), despite the presumed lower organism burden of the former. This is especially interesting given the propensity of smear-negative patients to have fewer total positive specimens than smear-positive patients. Obtaining three or more specimens, perhaps not surprisingly, did somewhat increase the sensitivity of the AFB smear. For 7 of the 56 smear-positive patients (13%), AFB were detected microscopically only in the third or later specimen provided.

Clearly, the examination of more than one sputum specimen is necessary to maximize the sensitivity of culture for M. tuberculosis. Somewhat disturbingly, 25% of patients with culture-proven TB had only a single specimen submitted to the laboratory. The frequency of smear positivity for this group was no higher than for patients from whom multiple specimens were obtained; thus, one cannot hypothesize that a positive AFB smear result dissuaded clinicians from collecting additional specimens. The frequency with which single specimens were obtained in both this study and the prior CAP investigation (9) suggests that insufficent specimen collection is a contributing factor to delayed diagnosis and treatment of TB. This finding notwithstanding, our data strongly indicates that the collection of two sputum specimens is almost always adequate to make a diagnosis, irrespective of the quality of the specimens obtained (this variable was not addressed in our study). Only rarely did the examination of three or more specimens increase the overall sensitivity of M. tuberculosis culture. It appears, therefore, that the recommendation that at least three sputum specimens be collected for all patients with suspected TB is excessive and that examination of most of these additional specimens is an inefficient use of laboratory resources.

It seems clear that efforts to improve the efficiency of laboratory diagnosis should be focused on two areas. First, there should be an effort to ensure that more than one specimen is collected from patients suspected to have TB. The diagnostic value of the second specimen collected is considerable, improving the overall sensitivity of M. tuberculosis culture by nearly 30% in our study. Second, there should be an effort to improve the sensitivity of rapid diagnostic testing. For only 47% of patients with three or more sputa submitted to the laboratory was at least one specimen smear positive, even though 79% of these same individuals had multiple culture-positive specimens. In the CAP Q-Probe study, the inevitable delay between specimen receipt and the reporting of a positive result for smear-negative individuals had a significant impact on the time to initiation of therapy (9). It seems, therefore, that one of the most valuable potential uses of new rapid-testing methodologies would be to bridge the gap between smear and culture sensitivity, thus minimizing treatment delays. The education of care-givers so that they send an adequate but not excessive number of specimens and the judicious use by the clinical laboratory of sensitive rapid-diagnostic-testing methods appear to be necessary to achieve an acceptable sensitivity of M. tuberculosis diagnosis while efficiently utilizing laboratory resources.

REFERENCES