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The Value of Submitting Multiple Sputum Specimens for Accurate Diagnosis of Pulmonary Tuberculosis

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Is a multiple number of sputum specimens necessary for the diagnosis of pulmonary tuberculosis? To answer this question, 6844 respiratory specimens obtained from previously untreated patients suspected of having pulmonary tuberculosis between 1998 and 2001 were evaluated retrospectively. All of the specimens were evaluated by acid fast bacilli smear and BACTEC 460 TB culture system. A total of 785 (11%) specimens from 353 patients were positive for *Mycobacterium tuberculosis* complex. For 76% (270/353) of these patients the organism was detected from sputum specimens collected sequentially for daily basis. *Mycobacterium tuberculosis* was isolated in the first, second and third samples of the majority (98%, 195/199) of patients who had three or more sputum samples sent to the laboratory. Our results indicate that, we could carry out *Mycobacterium tuberculosis* isolation in the first, second and third sputum samples of the overwhelming majority of the patients and the diagnostic value of four or more sputum specimens submitted to the laboratory was very low (2%). We recommend that, for definitive and cost-effective diagnosis of pulmonary tuberculosis at least three sequential sputum specimens be collected for all patients suspected pulmonary tuberculosis.

Key words: sputum, laboratory diagnosis, pulmonary tuberculosis, TB culture system

Pulmonary tuberculosis remains a major health problem in the developing countries. The basis for effective treatment and cure of a patient is rapid diagnosis of the disease and detection of its causative agent. Probably, the most important factor is taking suitable measures to minimize the contagion. Despite the availability of effective preventive measures and chemotherapy, the prevalence of tuberculosis is increasing in the developing world and in much of the industrialized world as well. The presumptive diagnosis of active disease depends upon the demonstration of acid fast bacilli (AFB) by microscopy, and definitive diagnosis by subsequent culture of *M.tuberculosis* Tenover *et al.*, 1993; (Nelson *et al.*, 1998; Harvell *et al.*, 2000). Most of standard laboratory texts and guidelines for mycobacteriology laboratories recommend that at least three sputum specimens should be collected for three successive days for AFB smear and culture (Levy *et al.*, 1989; Shinnick and Good, 1995 Kevin, 1996; Nolte and Metchock, 1999). Recently, the number of sputum specimens contributed has become a matter of debate to assess the benefit and to reduce hospital expenses in the diagnosis of pulmonary tuberculosis (Nelson *et al.*, 1998; Finch and Beaty, 1997 Cascina *et al.*, 2000; Craft *et al.*, 2000; Harvell *et al.*, 2000).

To answer these questions, we retrospectively investigated the result of AFB smear and cultures with BACTEC 460 TB culture system (Siddigi, 1996) and the efficiency of sequential sputum specimens to aid the rapid and accurate diagnosis of pulmonary tuberculosis in our center, which is a tertiary referral hospital in pulmonary tuberculosis endemic region.

Materials and Methods

All of the AFB smear and culture results of sputum specimens sent to Mycobacteriology Laboratory of the Department of Microbiology and Clinical Microbiology of Gulhane Medical Academy between January 1998 and May 2001 were retrospectively evaluated. The specimens were decontaminated by N-acetly-L-cysteine procedure recommended by the Centers for Disease Control (Kent and Kubica, 1985). Each specimen was concentrated by refrigerated centrifugation at 3000×g for 15-20 min after adding phosphate buffer (pH 6.8). Following centrifugation, the sediment was diluted with phosphate buffer and inoculated into Middle-brook 7H12 medium (BACTEC 12B vials) and incubated at 37°C. BACTEC

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12B vials were screened three times per week for the first three weeks, and weekly for the next three weeks using the BACTEC 460 instrument. Any vial with a growth index (GI) 210 was accepted as positive. At the end of the six weeks, vials with GI<10 were considered as negative. Smears from both specimens and growth positive 12B vials were stained by Ehrlich-Ziehl-Neelsen method in order to confirm presence of AFB. Differentiation of the *M. tuberculosis* complex and non-tuberculous mycobacteria was achieved by selective inhibition of the *M. tuberculosis* complex in the presence of 5 μl/ml of p-nitro-α-acetyl-amino-β-hydroxypropiophenone (NAP) according to BACTEC TB manual (Siddigi, 1996).

Results

The mycobacteriology laboratory collected 6844 respiratory specimens from previously untreated patients suspected of having pulmonary tuberculosis for AFB smear and culture between January 1998 and May 2001. A total of 785 (11%) respiratory specimens submitted from the 353 patients were positive for *M. tuberculosis* complex. For 76% (270/353) of these patients the organism was detected from sputum specimens.

Of the patients with sputum specimens submitted to the laboratory sequentially for daily basis, 16% (42/270) had only a single specimen sent for examination, 11% (29/270) had two specimens collected, and 74% (199/270) had at least three specimens sent to the laboratory. The AFB smear was found as positive for 55% (23/42) of patients of whom only a single sputum specimen was sent whereas the AFB smear was detected at least once for 55% (16/29) of persons who had two specimens sent to the laboratory. AFB smear was positive for 69% (137/199) of the patients who had three or more sputum specimens collected.

Of 199 patients having three or more sputum specimens sent to the laboratory 24% (48/199) had just one of the specimens which was culture positive. AFB smear was positive for 9% (13/137) of these persons (Table 1). At least four cultures of 38% (75/199) of the same persons were positive and AFB smear was positive at least once in 50% (68/137) of these patients.

Table 1. AFB smear results with respect to the number of positive specimens from *M.tuberculosis* culture positive patients having three or more sputum specimens collected

No. of culture positive specimens	Number of patients (%) that were:		
	Either[a] (n=199)	Smear positive[b] (n=137)	Smear negative (n=62)
1	48 (24)	13 (9)	35 (56)
2	39 (20)	23 (17)	16 (26)
3	37 (19)	33 (24)	4 (6)
4 or more	75 (38)	68 (50)	7 (11)

[a]Total number of AFB smear positive and negative specimens.
[b]At least one sequential specimen of the patients was AFB smear positive

M. tuberculosis was isolated in the first, second and third samples of the overwhelming majority (98%, 195/199) of the patients having three or more sputum samples sent to the laboratory. The diagnostic value of four or more specimens submitted to the laboratory was very low (2%, 4/199). The first, the second and the third specimens of the patients having three or more sputa collected were AFB smear positive (99%, 198/199) and the percentage of AFB smear positive specimens of the patients from whom four or more sputa submitted to the laboratory was observed quite low (1%, 1/199) (Table 2).

Discussion

Tuberculosis can be controlled successfully by early and adequate detection and effective therapy of the infectious patients in a community. Rapid identification of *M. tuberculosis* facilitates the selection of an appropriate drug therapy and helps patient isolation to minimize the spread. It has been estimated that ten secondary infections result from each untreated AFB smear-positive patient of pulmonary tuberculosis (Kramer *et al.*, 1990).

Interestingly, we detected the rate of AFB smear posi-

Table 2. AFB smear results with respect to the collection order of culture positive specimens from *M. tuberculosis* culture positive patients having three or more sputum specimens collected.

Collection order of culture positive specimens	Number of patients (%) that were:						
	Total[a] (n=199)	Smear positive[b] (n=137)	Smear positive[c]				Smear negative (n=62)
			1.	2.	3.	4.	
First	155 (78)	120 (88)	96	19	3	2	35 (56)
Second	27 (14)	12 (9)	2	6	3	1	15 (24)
Third	13 (7)	4 (3)	2	-	1	-	9 (15)
Fourth or later	4 (2)	1 (1)	-	-	1	-	3 (5)

[a]Total number of both AFB smear positive and negative specimens with respect to collection order of culture positive specimens.
[b]At least one sequential specimen collected from the patients was smear positive.
[c]Which AFB smear was positive with respect to culture positive specimens.

Table 3. Comparison of previously published studies with the results of AFB smear of from *M.tuberculosis* culture positive patients having three or more sputum specimens sent according to collection order.

Collection order of the culture positive specimens	The number of the culture positive patients (%)							
	Cascina *et al.*[a] (1989-1998)[c] (n=84)		Nelson *et al.*[b] (1986-1996)[c] (n=120)		Harvell *et al.*[b] (1994-1996)[c] (n=143)		Present study[b] (1998-2001)[c] (n=199)	
	Culture	Smear	Culture	Smear	Culture	Smear	Culture	Smear
First	64 (76)	46 (82)	80 (67)	41 (73)	117 (82)	44 (96)	155 (78)	120 (88)
Second	13 (16)	5 (9)	33 (28)	8 (14)	14 (10)	2 (4)	27 (14)	12 (9)
Third	7 (8)	5 (9)	7 (5)	4 (7)	12 (9)		13 (7)	4 (3)
Fourth				3 (6)			4 (2)	1 (1)

[a]*M. tuberculosis* culture was performed with Löwenstein-Jensen medium.
[b]*M. tuberculosis* culture was performed with BACTEC 460 TB culture system.
[c]The time interval of the studies were performed.

tivity for the culture positive patients from which three or more sputum specimens were collected was slihtly higher than the patients from whom only one specimen was collected (55%, 69%) in this present study. The positivity rates were 46% and 45% in the study of Nelson *et al.* (1998) and were 43% and 67% in the study of Cascina *et al.* (2000), respectively. However, these findings were not primary factors in determining the optimal number of sputum specimens to be collected. Obtaining at least three specimens was a positive factor and did somewhat increase the sensitivity of the AFB smear.

According to the observations of several investigators, *in vitro* detection of *M. tuberculosis* is directly proportional to the total number of specimens that were sent to the laboratory (Levy *et al.*, 1989; Harvell *et al.*, 2000). Some previous studies indicate that insufficient specimen collection is an important contributing factor to delayed diagnosis and treatment of tuberculosis (Kramer *et al.*, 1990; Mathur *et al.*, 1994). In our study, the first positive culture was obtained from the third specimen for only 7% of the patients having three or more sputum samples submitted to the laboratory. These findings are in agreement with those previously reported (Table 3) (Nelson *et al.*, 1998; Cascina *et al.*, 2000 Harvell *et al.*, 2000). These results suggest that two sputum samples are sufficient for the diagnosis of pulmonary tuberculosis. In our study the first positive culture was obtained from the fourth specimen for only 2% of the patients having four sputum specimens collected. Hence, the minimal increase of the positivity rate in recovery from the fourth or more sequential specimen has led to the recommendation that only three sputum specimens are necessary for the definitive diagnosis of pulmonary tuberculosis. Delays in diagnosis and treatment can result in significant patient morbidity and mortality, and, in addition, undiagnosed cases of tuberculosis can be an important reservoir for the transmission of multidrug-resistant tuberculosis and related outbreaks (Tenover *et al.*, 1993; Craft *et al.*, 2000). Since, BACTEC liquid medium used in our laboratory for the detection of *M. tuberculosis* is expensive and dependent foreign currency, the cost of diagnosis per patient is quite high. For only one sputum specimen, the total cost of AFB smear, culture with Löwenstein-Jensen medium and BACTEC 460 TB culture system are approximately $40 US. So, it appears that the recommendation that more than three sputum specimens be collected for all of the patients suspected pulmonary tuberculosis could be excessive and that examination of most of these additional specimens could also be an inefficient use of laboratory resources. However, obtaining more than one specimen to increase the sensitivity of AFB smear and *M. tuberculosis* culture is definitely recommended.

In conclusion, for the laboratories having have improved rapid diagnostic test systems, the collection of two sputum specimens per patient may be sufficient for accurate diagnosis of pulmonary tuberculosis. When we take into consideration the importance of the pulmonary tuberculosis for the health care and the reality of the underdeveloped and developing countries without rapid diagnostic test facilities, the cost of the third specimen and loss of time should not be a matter of debate. The recommendation of at least three sequential sputum samples is sufficient for a definitive and cost-effective laboratory diagnosis of pulmonary tuberculosis in developed countries should be explained to clinicians who sent the specimens and for the laboratories there should be an effort to improve themselves and the sensitivity of rapid diagnostic testing.

References

Siddigi, S.H. 1996. Procedure for primary isolation of mycobacteria from clinical specimens. In BACTEC TB System Product and Procedure Manual. Revision, Becton, Dickinson and Company, Sparks, Maryland, p II.1-II.13.

Cascina, A., A. Fietta, and L. Casali. 2000. Is a large number of sputum specimens necessary for the bacteriological diagnosis of tuberculosis? *J. Clin. Microbiol.* 38, 466.

Craft, D.W., M.C. Jones, C.N. Blanchet, and R.L. Hopfer. 2000. Value of examining three acid-fast bacillus sputum smears for removal of patients suspected of having tuberculosis from the "airborne precautions" category. *J. Clin. Microbiol.* 38, 4285-

4287.

Finch, D. and C.D. Beaty. 1997. The utility of a single sputum specimens in the diagnosis tuberculosis. *Chest* 111, 1174-1179.

Harvell, J.D., W.K. Hadley, and L. Valeria. 2000. Increased sensitivity of the BACTEC 460 Mycobacterial Radiometric Broth Culture System does not decrease the number of respiratory specimens required for a definitive diagnosis of pulmonary tuberculosis. *J. Clin. Microbiol.* 38, 3608-3611.

Kent, B.D. and G.P. Kubica. 1985. Public health mycobacteriology: A guide for the level III laboratory. U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta, p.1-207.

Kevin, M. 1996. Tuberculosis control in resource-poor settings with high rates of HIV Infection. *Am. J. Public. Health.* 86, 1071-1073.

Kramer, F., T. Modilevsky, A.R. Waliany, J.M. Leedom, and P.F. Barnes. 1990. Delayed diagnosis of tuberculosis in patients with *Human Immunodeficiency Virus* infection. *Am. J. Med.* 89, 451-456.

Levy, H.C., C. Fedman, H. Sacho, H. van der Meulen, J. Kallenbach, and H.A. Koornhof. 1989. A reevaluation of sputum microscopy and culture in the diagnosis of pulmonary tuberculosis. *Chest* 95, 1193-1197.

Mathur, P., L. Sacks, G. Auten, R. Sall, C. Levy, and F. Gordin. 1994. Delayed diagnosis of pulmonary tuberculosis in city hospitals. *Arch. Intern. Med.* 154, 306-310.

Nelson, S.M., M.A. Deike, and C.P. Cartwright. 1998. Value of examining multiple sputum specimens in the diagnosis of pulmonary tuberculosis. *J. Clin. Microbiol.* 36, 467-469.

Nolte, F.S. and B. Metchock. 1999. Mycobacterium, p. 399-428. *In* P.R. Murray, E.J. Baron, M. A. Pfaller, F. C. Tenover, R.H. Yolken (eds.), Manuel of Clinical Microbiology. 8th ed. American Society for Microbiology, Washington, D.C.

Shinnick, T.M. and R.C. Good. 1995. Diagnostic mycobacteriology laboratory practies. *Clin. Infect. Dis.* 21, 291-299.

Tenover, F.C., J.T. Crawford, R.E. Huebner, L.J. Geiter, C.R. Horsburgh, and R.C. Good. 1993. The resurgence of tuberculosis: Is your laboratory ready? *J. Clin. Microbiol.* 31, 767-770.