Smear examination of two specimens for diagnosis of pulmonary tuberculosis in Tiruvallur District, south India

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SUMMARY
SETTINGS: A general hospital and three peripheral health institutions (PHI) in Tiruvallur District, south India.
OBJECTIVE: To validate the case detection strategy for diagnosis of pulmonary tuberculosis by smear microscopy of two sputum specimens versus three.
METHODS: In the Revised National Tuberculosis Control Programme (RNTCP), three smears from every symptomatic patient attending the PHI were examined for acid-fast bacilli (AFB) by Ziehl-Neelsen (ZN) microscopy. The data from the Tuberculosis Laboratory Registers from the above centres were analysed.
RESULTS: Of 7843 chest symptomatics who had provided three sputum specimens for examination, 895 (11.4%) were smear-positive on at least two specimens. Examination of two specimens—first spot and early morning specimens (837, 93.5%) or early morning and second spot specimens (843, 94.2%)—yielded the highest number of cases.
CONCLUSION: The examination of two sputum smears (one spot and an early morning) is as effective as examination of three smears.
KEY WORDS: DOTS; RNTCP; sputum smear examination; AFB

DIRECTLY OBSERVED TREATMENT, short-course (DOTS) chemotherapy is the global standard of care for treating tuberculosis patients. In the strategy, smear microscopy is the most efficient means of case detection among individuals presenting with symptoms suggestive of pulmonary tuberculosis (TB) (cough for 3 weeks or more, with or without other clinical symptoms). The strategy recommended by the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) for diagnosis is examination of three sputum specimens from TB suspects (one spot, an early morning and another spot) for presence of acid-fast bacilli (AFB).

This strategy may not be always be possible, due to reasons such as the workload of the technician who performs investigations for the other control programmes and routine laboratory work at the PHI, which caters for a population of 100,000. There is therefore an urgent need to explore the possibility of reducing the number of specimens from three to two, taking into consideration the convenience of the patient. This will reduce the workload of examining the third specimen so that technicians can attend to other responsibilities, and will substantially reduce the cost of sputum AFB microscopy.

Many studies have shown that the third smear does not add significantly to case finding efficiency. As early as 1970, a study from India evaluated the optimal number of sputum specimens to be examined and concluded that the first two specimens yielded the maximum number of smear-positive cases. An analysis of results of direct sputum examination from 34 laboratories in Tanzania showed that of total cases, 83.4% were detected from the first specimen and an incremental yield of respectively 12.2% and 4.4% were obtained from the second and third smear.

A comparative study in Ntcheu district in Malawi has shown that screening TB suspects using two sputum smears is as effective as screening using three sputum smears and is associated with less laboratory work and savings in costs.

A retrospective study from the Revised National Tuberculosis Control Programme (RNTCP) area of Lala Ram Sarup (LRS) Institute, New Delhi, carried out in 1998–1999, has shown that examination of two sputum smears is as effective as screening using three sputum smears and is associated with less laboratory work and savings in costs.

To evaluate the reproducibility of examining two
smears, we analysed the data available from four of the seven microscopy centres (MC) in a tuberculosis unit in Tiruvallur District, Tamil Nadu, India.

**OBJECTIVE**

To compare the potential yield of two smear-positive TB patients diagnosed by examining two sputum samples as opposed to the three conventionally recommended in the programme.

**MATERIAL AND METHODS**

**Sputum collection**

Individuals with chest symptoms attending the above selected health facilities were referred for sputum smear microscopy. Those with symptoms suggestive of tuberculosis were asked to provide three specimens—first spot, second early morning and third spot—for diagnosis of tuberculosis. When a person with chest symptoms reported at the health facility voluntarily, a good quality sputum specimen was obtained on the spot (first specimen). The patient was then provided with a disposable plastic sputum cup with a lid for collecting an early morning sputum specimen (second specimen) on the subsequent day. Another spot specimen (third specimen) was obtained on that day. The patient was given instructions on how to collect the early morning specimen at home in the cup provided. The second specimen was always the early morning collection specimen. Utmost care was taken to obtain a good quality third spot specimen. The quality of specimen was recorded on the sputum requisition form as saliva, mucopurulent or bloodstained.

**Recording smear results**

All the smears were stained and read by laboratory technicians as per RNTCP guidelines. A specially designed Tuberculosis Laboratory Register is maintained in all MCs to record the name, age, sex, laboratory number and sputum smear results of all individuals with chest symptoms reported for sputum smear examination. The results were entered in a separate column on the same line for each patient, with the first, second and third column indicating the smear result of the first, second and third specimen, respectively.

**Quality assurance of sputum AFB microscopy**

Since the inception of the RNTCP in the study area, quality assurance methods have been followed to ensure the quality of sputum AFB microscopy. All positive slides and 10–20% of negative slides were checked by Senior Tuberculosis Laboratory Supervisor (STLS) in an unblinded fashion during the on-site evaluation, and in addition, 10% of positive and negative slides were also rechecked in a blinded fashion at Tuberculosis Research Centre (TRC). The observations made in the study area revealed that the quality of AFB sputum microscopy was good.

**Data collection and analysis**

Data were collected from the laboratory registers of four MCs, including the Tiruvallur Government Hospital from May 1999 to January 2002. The coverage for all three specimens and the yield of smear positivity for each specimen were obtained. The \( \chi^2 \) test of significance was applied to the difference in proportion of cases. A \( P \) value <0.05 was considered to be statistically significant.

**RESULTS**

During the period 9346 new patients with chest symptoms attended, of whom 7843 (84%) had three sputum specimens examined (Table 1). Of the remaining 1503, 1351 did not return after the first sputum examination, whereas 152 returned for the second sputum examination but not for the third. The age and sex distribution of the 1351 who did not turn up for subsequent sputum examinations were similar to those (7843) who attended the health facility for three sputum smear examinations (data not presented). Among the 7843 persons who had all three sputum smear examinations, 895 (11.9%) were positive on at least two specimens. Of these, 837 (93.5%) were positive on the first and second samples; 795 (88.8%) on the first and third; and 843 (94.2%) on the second and third. Of the 962 patients who were positive on at least one specimen, the first specimen detected 853 (88.7%), and the second and third specimens yielded 938 (97.5%) and 856 (89.0%) cases, respectively. The incremental yield of specimens in succession is set out in Table 2. The additional yield by the second specimen was 101 (10.5%), whereas it was only 8 (0.8%) from the third specimen.

Table 3 shows the comparison of pairs of specimens in terms of yield of cases. There were 837 cases positive on the first and second specimens: 101 cases were negative on the first specimen and positive on the other; 16 were positive on the first specimen and negative on the other; and 6889 were negative on both

| Table 1 Distribution of smear positives in successive sputum examinations |
|--------------------------------------------------|----------|----------|
| Symptomatic patients                             | 9346 (n) | —        |
| Symptomatic patients with three specimens        | 7843 (m) | 83.9 (m/n) |
| Smear-positive (on at least two specimens)       | 895 (s)  | 11.4 (s/m) |
| Smear-positive on first and second specimens     | 837 (s)  | 93.5 (s/s) |
| Smear-positive on first and third specimens      | 795 (s)  | 88.8 (s/s) |
| Smear-positive on second and third specimens     | 843 (s)  | 94.2 (s/s) |

\( m = \) maximum specimens (three); \( s = \) specimen; \( s_1 = \) first specimen; \( s_2 = \) second specimen; \( s_3 = \) third specimen.
specimens (Table 3A). Comparison of these two specimens showed that the yield of cases from the second specimen was significantly higher than that of the first specimen ($P < 0.01$). Similarly, the difference in proportion of cases yielded by the first and third specimens was not significant, showing that these two specimens were equally good in yielding cases (Table 3B). Table 3C shows that the yield of cases by the second specimen was significantly higher than that of the third specimen ($P < 0.001$). This shows that the yield of smear positivity was also greater in the early morning specimen as compared to the first and third spot specimens. The sensitivity of smear examination is of major interest because of its reliability for bacteriological confirmation of the disease. The sensitivity in our study was 94% for the two pairs of specimens (first spot and early morning, and early morning and second spot) compared to 89% for the other pair of spot specimens (first and second spot). This is very important for the RNTCP in terms of policy decisions on case finding when diagnosis is based on two specimens, of which one should be the early morning specimen.

**Table 2** Incremental yield of smear positivity in successive specimens

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<th>n (%)</th>
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<tr>
<td>Smear-positive (on at least one specimen)</td>
<td>962 (100)</td>
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<tr>
<td>Smear-positive on first specimen</td>
<td>853 (88.7)</td>
</tr>
<tr>
<td>Additional smear-positive on second specimen</td>
<td>101 (10.5)</td>
</tr>
<tr>
<td>Additional smear-positive on third specimen</td>
<td>8 (0.8)</td>
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**DISCUSSION**

This retrospective analysis, undertaken using the data obtained from the laboratory registers maintained at four MCs, estimated the number of cases diagnosed from each specimen and pairs of specimens. We observed that for 7843 symptomatic patients, 962 were positive for AFB by smear on at least one specimen; 853 (88.7%) were detected by the first specimen, while the additional yield of cases was 101 (10.5%) from the second specimen and 8 (0.8%) from the third specimen. Similarly, a study of 34 laboratories in Tanzania showed that the yield of smears was 83.4% with the first, 12.2% with the second, and 4.4% with the third specimen. However, the yield of smear-positives in the first specimen was lower, and the incremental yield in the third specimen was higher in their study. In 1994, among 5988 smear-positive TB cases identified in Malawi, it was found that 1018 (17%) smear-positive cases would have gone undiagnosed if only one sputum specimen had been examined, and that 240 (4%) smear-positive cases would have gone undiagnosed if only two sputum specimens had been examined. These findings show that the majority of cases were identified by two sputum smear examinations instead of three. Another study conducted in Ntcheu district hospital, Malawi, compared the results of samples collected at two points of time, namely January–June 1998 and July–December 1998. Of 998 patients submitting three sputum samples during the first period, 160 (16%) were smear-positive, which was no different from that for the second period, where 165 (16%) of 1042 patients submitting two sputum specimens were smear-positive. In 1998, a study by the LRS Institute of TB and Allied Diseases, New Delhi, also documented that of 3738 new patients with chest symptoms registered, 719 (19.2%) were sputum-positive on at least one sputum specimen. Further analysis showed that the first spot sputum specimen detected 622 (86.5%) cases. If the second specimen was also considered, 715 (99.4%) cases were detected. The incremental yield of the third specimen was less than 1%. The National Tuberculosis Institute (NTI), Bangalore, conducted a study in the 1970s to estimate the yield of cases from additional sputum specimens examined. Eight sputum samples were collected in chronological order and examined by Ziehl-Neelsen (ZN) smear microscopy. The first and second specimens together detected 69.7% of the microscopy-positive cases and remaining patients were added after examining the other six specimens. This showed that successive examination of eight sputum specimens did not substantially increase the number of cases. Wu et al. have also established the efficiency of smear microscopy of two spontaneous sputum specimens.

In this study, a comparison of the first and second specimens showed that the proportion of smear-
positives detected on the second specimen (12.0%) was significantly higher than that detected on the first specimen (10.9%); the difference was statistically significant. The second specimen was found to be superior to the third specimen in yielding cases (12.0% vs. 10.9%). A comparison of the first and third specimens, however, showed that the proportions of positives were equal (10.9% each). These observations suggest that early morning specimens yield more positives than the spot specimens.

Of the 895 cases based on at least two positive smears from three specimens, two specimens (the first and second) picked up 837 (94%) cases. The remaining 58 (6%) cases could have been diagnosed by subjecting only 125 (1.6%) of 7843 persons for third smear examination. Under routine conditions the incremental yield from a third smear examination is relatively small and hence the third specimen may not be required for routine smear examination under the RNTCP. This confirms the recommendation that examination of two consecutive sputum specimens is sufficient for case detection in the RNTCP.

The strategy of examining two smears for the diagnosis of TB is encouraging, and would reduce the workload of the technicians who can devote more time for proper sputum collection, processing and reading, and attending to other responsibilities. It is also cost-effective in the sense that the total cost towards consumables of sputum microscopy, excluding manpower, will be reduced to two-thirds. Similarly, a cost-effective analysis in a study in Zambia has shown that the incremental cost of performing a third specimen increases with only a small gain in terms of additional positives detected on the second specimen. The strategy of examining two sputum smears for the diagnosis of pulmonary TB is encouraging, and would reduce the workload of the technicians who can devote more time for proper sputum collection, processing and reading, and attending to other responsibilities. It is also cost-effective in the sense that the total cost towards consumables of sputum microscopy, excluding manpower, will be reduced to two-thirds. Similarly, a cost-effective analysis in a study in Zambia has shown that the incremental cost of performing a third specimen increases with only a small gain in terms of additional positives detected on the second specimen.

To conclude, the observations of the present study revealed that two sputum smear examinations (one spot and one early morning specimen) are sufficient for diagnosis of tuberculosis patients.

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References


CONTEXTE : L’hôpital général ainsi que trois institutions périphériques de santé (PHI) dans le district de Tiravallur en Inde du Sud.

OBJECTIF : Valider la stratégie de détection des cas pour le diagnostic de la tuberculose pulmonaire recourant à l’examen microscopique des frottis de deux ou de trois échantillons d’expectoration.

RECUEIL DES DONNEES : Dans le Programme Révisé de Lutte antituberculeuse (RNTCP), trois frottis sont examinés par l'examen microscopique et le Ziehl-Neelsen (ZN) à la recherche de bacilles acido-résistants (BAAR) chez chaque sujet symptomatique fréquentant le PHI. Les données provenant des Registres du Laboratoire de TB en provenance des centres déjà cités ont été analysées. RÉSULTATS : Sur 7.843 malades symptomatiques respiratoires qui avaient fourni trois échantillons d’expectoration pour examen, 895 (11,4%) avaient une bacilloscopie positive sur au moins deux échantillons. L’examen de deux échantillons, le premier sur place et le second tôt le matin (837 ; 93,5%) ou un échantillon tôt le matin et
CONCLUSION : L’examen de deux frottis d’expectoration (un sur place et un tôt le matin) est aussi valable que l’examen de trois frottis.

RESUMEN

CONTEXTO : Un hospital general y tres instituciones de salud periféricas (PHI) en el distrito de Tiruullur, Sur de la India.

OBJETIVO : Validar la estrategia de detección de casos para el diagnóstico de la tuberculosis pulmonar por examen microscópico de dos o tres muestras de esputo.

RECOLECCIÓN DE LOS DATOS : En el Programa Nacional Revisado de Control de la Tuberculosis (RNTCP) se examinaban tres frotis para cada enfermo sintomático que consultaba en las PHI, con el método de tinción de Ziel-Neelsen (ZN) para bacilos alcohol-ácido resistentes (BAAR). Se analizaron los datos de los Registros de Laboratorio de los centros mencionados.

RESULTADOS : De 7.843 sintomáticos respiratorios que habían proporcionado tres muestras de esputo para examen, 895 (11,4%) eran casos con baciloscopia positiva en por lo menos dos muestras. El examen de dos muestras—la primera en el momento de la consulta y temprano en la mañana (837 ; 93,5%) o temprano en la mañana y la segunda durante la consulta (843 ; 94,2%)—producía el número más elevado de casos.

CONCLUSIÓN : El rendimiento del examen de dos frotis de esputo (uno en el momento de la consulta y otro temprano en la mañana) es tan bueno como el examen de tres frotis.