What a difference a day makes: same-day vs. 2-day sputum smear microscopy for diagnosing tuberculosis

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<http://dx.doi.org/10.5588/pha.16.0062>

Setting: Nine district-level microscopy centres in Assam and Tripura, India.

Objective: Same-day sputum microscopy is now recommended for tuberculosis (TB) diagnosis. We compared this method against the conventional 2-day approach in routine programmatic settings.

Methods: During October–December 2012, all adult presumptive TB patients were requested to provide three sputum samples (one at the initial visit, the second 1 h after the first sample, and the third the next morning) for examination by Ziehl-Neelsen smear microscopy. Detection of acid-fast bacilli with any sample was diagnostic. The first and second spot sample comprised the same-day approach, and the first spot sample and next-day sample comprised the 2-day approach.

Results: Of 2168 presumptive TB patients, 403 (18.6%) were smear-positive according to the same-day method compared to 427 (19.7%) by the 2-day method (McNemar's test, $P < 0.001$). Of the total 429 TB patients, 26 (6.1%) were missed by the same-day method and 2 (0.5%) by the 2-day method.

Conclusion: Same-day specimen collection for microscopy missed more TB than 2-day collection. In India, missing cases by using same-day microscopy would translate into a considerable absolute number, hindering TB control efforts. We question the indiscriminate switch to same-day diagnosis in settings where patients reliably return for testing the next day.

In 2014, an estimated 9.6 million incident tuberculosis (TB) cases occurred worldwide, including an estimated 2.7 million new smear-positive cases, of which nearly 750 000 were in India.¹ Although high treatment success rates have been achieved globally, the decline in TB incidence has been modest. To address this enormous burden and accelerate the decline in incidence, the World Health Organization (WHO) has adopted a new global strategy with a primary objective of achieving universal access to high-quality diagnosis and patient-centred treatment.²

Despite the recent advances made in the field of TB diagnosis, sputum smear microscopy for the detection of acid-fast bacilli (AFB) remains the most widely used test for the diagnosis of pulmonary TB in developing countries. The conventional 2-day testing method, which consists of collecting sputum on the spot and the next morning, has been associated with drop-out rates of as high as 37%, possibly due to the inconvenience associated with multiple visits.^{2–4} This has led

researchers to conduct studies comparing this 2-day approach with a same-day approach ('spot-spot' specimens) for diagnosing sputum smear-positive TB. There is now evidence from several studies, including a meta-analysis, showing that the same-day approach is as accurate as the 2-day method.^{5–12}

Based on the results of these studies, the WHO recommended that countries using a 2-day specimen collection strategy for TB diagnosis should consider moving to same-day sputum collection.¹³ All of the studies that formed the basis of the WHO recommendation, however, are from trial settings^{5–12} and not from routine programmatic settings. In addition, two studies conducted in tertiary care settings in India have provided conflicting results: one supported the same-day sputum collection strategy⁹ while the other did not.¹⁴ Both studies did, however, recommend that their findings be confirmed on a larger scale and in routine programme settings.

In this study, we aimed to compare the same-day (spot-spot) collection method with the conventional 2-day (spot-morning) method in diagnosing sputum smear-positive TB under routine programme conditions. The specific objectives were to compare between the two approaches 1) the proportion of patients with two specimens collected, 2) the quality of sputum specimens, 3) the proportion of patients with AFB-smear positive results, and 4) the incremental yield of the second smear.

METHODS

Study design

This was a cross-sectional study of all adult (age ≥ 18 years) presumptive TB patients seeking medical care from October to December 2012.

Study setting

Eight districts in Assam were randomly selected from among the state's 27 districts, an area that comprised 13.9 million persons, i.e., 40% of the state's population. The district selected in Tripura comprised 1.7 million persons, 47% of the state's population. The districts were chosen randomly to reflect true programmatic conditions, and not based on the predetermined levels of programmatic performance, the years of individual experience and practice of the laboratory staff, or the prevalence of disease (Figure 1). Sputum samples from these districts were sent to district-level microscopy centres, which were also located in Assam and Tripura. All microscopy centres

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ACKNOWLEDGEMENTS

The authors are grateful to the participants who kindly consented to be enrolled for the study. They also thank all the laboratory technicians of the microscopy centres for collecting, preparing and examining the sputum specimens, the Programme Officers and staff of the 10 District TB Centres for extending their help in compiling all the data, and J Tobias for GIS support and for creating Figure 1. Disclaimer: The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions or policies of the World Health Organization and US Centers for Disease Control and Prevention. Conflicts of interest: none declared.

KEY WORDS

sputum microscopy; India; tuberculosis, spot-spot; spot-morning; AFB; diagnosis

Received 8 August 2016
Accepted 30 September 2016

PHA2016;6(4):232–236
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FIGURE 1 Study districts in Assam and Tripura States, India, 2012.

are staffed by laboratory technicians trained in national TB diagnostic guidelines.

Procedure

All adult presumptive TB patients who sought care for a cough lasting >2 weeks and were advised to submit sputum for smear microscopy during October–December 2012 were invited to participate in the study. After providing written informed consent, the patients were asked to submit three sputum samples: an immediate spot sample, an additional spot sample collected 1 h after the first sample on the same day, and an early morning sample the next day. The sputum samples were then subjected to Ziehl-Neelsen staining followed by light smear microscopy by trained laboratory technicians working under a system of external quality assurance.¹⁵ The sputum smears were graded in accordance with programme and WHO guidelines.^{15,16} The results of the first and second spot sample comprised the same-day approach, while the results of the first spot sample and the early morning sample comprised the 2-day approach. A patient was considered smear-positive if at least one specimen was positive.

Sputum quality was graded as 1) mucopurulent if either of two specimens was mucopurulent, 2) blood-stained if one of the specimens was blood-stained and the other blood-stained or salivary, and 3) salivary when both specimens were salivary. To assess grading where there were two positive specimens, the higher grade (in the hierarchical order of scanty, 1+, 2+ and 3+) was taken into account.

Programme laboratories have a standardised mechanism for ensuring quality through on-site evaluation (OSE) and random blinded cross checking (RBCC). RBCC is the process of re-reading

a statistically valid sample of slides from a given laboratory to assess whether that laboratory has an acceptable level of accuracy in diagnosis. OSE and RBCC were conducted once a month by sub-district level senior tuberculosis laboratory supervisors (STLS), and once yearly by state-level microbiologist visits from the Intermediate Reference Laboratory to each of the District TB Centres and TB Units. OSE visits include a comprehensive assessment of laboratory safety procedure, conditions of equipment, adequacy of supplies, as well as technical performance assessments of AFB sputum smear microscopy. Any deficiencies in quality or substandard performance were corrected during the OSE.

Data entry and analysis

Data were double-entered, validated and analysed using EpiData software v. 3.1 for entry and v. 2.2.2.182 for analysis (EpiData Association, Odense, Denmark). The incremental yield of the second sputum sample was compared between the two approaches. Incremental yield was defined as the number of additional positive patients diagnosed with the second sample (the second spot sample for the same-day approach and the morning sample for the 2-day approach) who were not diagnosed by the first spot, expressed as a percentage of all smear-positive patients. Because the observations were paired, McNemar's test was used to assess marginal homogeneity between the two approaches for positive smear results (i.e., to determine whether the row and column marginal frequencies are equal) at an alpha of 0.05. Pearson's χ^2 tests were used to determine significant differences in proportions between the test results of the two methods with regard to the quality of the sputum samples, smear grading and the incremental yield of the second sputum sample.

FIGURE 2 Enrollment of presumptive TB patients and diagnosis of sputum-positive TB in nine districts of Assam and Tripura, India, 2012. TB = tuberculosis.

Ethics

Ethical approval was obtained from National Tuberculosis Institute, Bangalore, India, and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease, Paris, France. Additional review by the institutional review board of the US Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) was not required because the CDC investigators were determined to not be engaged in human subject research as defined by CDC standards. All study participants provided written informed consent. All patients with positive results were linked to treatment services under India’s Revised National TB Control Programme (RNTCP). All data were safeguarded to protect patient confidentiality, and no individual patient identifiers were retained in the study database.

RESULTS

Of the 2283 adult presumptive TB patients eligible for the study, 41 (1.8%) did not consent to participate, 51 (2.2%) were excluded because they provided only one sputum sample, and 23 (1.0%) provided two same-day samples only and did not return to provide a morning sample the following day (Figure 2). These 23 were excluded from the main analysis but their results were noted further below.

Among the 2168 patients who provided all three samples, smear microscopy using the same-day approach yielded 403 (18.6%) smear-positive results as compared to 427 (19.7%) with the 2-day approach. With either method, a total of 429 smear-positive results was obtained. Among these, 26 (6.0%) cases were missed by the same-day approach as compared to only 2 (0.5%) missed by the 2-day approach (McNemar’s χ^2 statistic = 20.6, $P < 0.001$) (Table 1). Among the 23 patients who provided same-day samples only and did not return the following morning, three had smear-positive results. All of these three

were smear-positive on both the first and second same-day spot sputum.

A significantly greater proportion of patients provided mucopurulent sputum from the 2-day method than the same-day method (79.2% vs. 62.5%, $P < 0.001$) (Table 2). No significant difference between the two methods was found in the grading of positive smears ($P = 0.745$). The second sputum sample resulted in an incremental yield of 16 cases (4.0%) with the same-day method and 40 cases (9.4%) with the 2-day method, a significant increase ($P < 0.001$).

DISCUSSION

We found that implementing the same-day approach for sputum specimen collection in programmatic settings missed 6.1% of the smear-positive cases found using the conventional 2-day method. When extrapolated to a populous high-burden country like India, this would translate into a large absolute number of missed sputum-positive cases. In 2012, an estimated 930000 sputum smear-positive TB cases were diagnosed in India.¹⁷ Applying these findings to the RNTCP performance context, we would have missed approximately 50000 cases if the same-day strategy had been implemented.

Several studies have evaluated the performance of the same-day approach compared with the 2-day approach and argued that reducing the number of visits may help optimise smear microscopy and that same-day sputum smear microscopy was as sensitive as the conventional 2-day approach. All of these studies, however, were carried out in trial settings where the technicians were likely to have spent more time reading the smears and tended to capture more sputum smear-positive results than technicians in a routine laboratory.¹³ Previous studies have thus recommended that their findings be confirmed on a larger scale under routine programmatic conditions. In contrast, a recent study from India conducted under routine programme settings has shown that the same-day approach missed 17% of smear-positive TB cases.¹⁸

The discrepancy between studies that found a significant difference and those that observed no difference between the same-day and 2-day approaches may be due to differences in the intensity of grading of the smear results. If most of the smear-positive results were high positives (i.e., 3+ or 2+), it is possible that the spot specimen itself classified the patient as sputum smear-positive, with little additional yield from the early morning specimen. A previous study in Ethiopia reported no ‘scanty positive’ TB patients, and cited this as a possible reason for the lack of difference between the two approaches.⁷ In contrast, in our study, over half of the sputum smear-positive TB patients were low positives (scanty or 1+), and a more sensitive early morning specimen might thus have added value and driven the results.

TABLE 1 Performance of same-day and 2-day approaches in diagnosing smear-positive TB in nine districts of Assam and Tripura, India, 2012 ($n = 2168$)

	2-day approach	
	Smear-positive	Smear-negative
Same-day approach		
Smear-positive	401	2
Smear-negative	26	1739

McNemar’s χ^2 statistic = 20.6; $P < 0.001$.
TB = tuberculosis.

TABLE 2 Comparison of same-day and 2-day sputum collection approaches in diagnosis of smear-positive TB in Assam and Tripura, India, 2012 ($N = 2168$)

Parameters	Same-day sputum collection <i>n</i> (%)	2-day sputum collection <i>n</i> (%)	χ^2 <i>P</i> value
Quality of sputum samples*			
Mucopurulent	1354 (62.5)	1716 (79.2)	<0.001
Blood-stained	24 (1.1)	22 (1.0)	
Saliva	790 (36.4)	430 (19.8)	
Smear grading†‡			
3+ positive	113 (28.0)	113 (26.5)	0.745
2+ positive	76 (18.9)	73 (17.1)	
1+ positive	148 (36.7)	172 (40.3)	
Scanty positive	66 (16.4)	69 (16.2)	0.001
Incremental yield of second smear†	16 (4.0)	40 (9.4)	

*Sputum quality was visually graded as: 1) mucopurulent if either of two specimens was mucopurulent, 2) blood-stained if one of the specimens was blood-stained and the other was blood-stained or salivary, and 3) salivary when both specimens were salivary.

†Denominator is the total number of smear-positive cases diagnosed by the respective approach among patients with three samples.

‡To assess grading in case of two positive specimens, the higher grade was considered.

TB = tuberculosis.

Another finding of note was that the incremental yield of the second same-day specimen was 4%. This is not trivial; it shows that in situations where an early morning specimen cannot be obtained, a second spot specimen should be obtained and examined. Loss to follow-up is of major concern with the 2-day approach. In our study the proportion of patients returning with the next day morning specimen was exceptionally high (99.0%). In settings where patients do not return for testing on the second day, these potential losses should be considered, and dual same-day testing may be preferable. Other factors, such as patient preference and convenience, and the availability of more sensitive technologies such as Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA), should also be considered when changing diagnostic policy. Our findings may also be different from those in settings with high rates of human immunodeficiency virus infection, as the eligibility selection, based on symptomology and onset, differs between immune-competent and immunocompromised individuals. This information was not collected for the present study.

CONCLUSION

In our setting, the same-day sputum collection strategy was less sensitive in diagnosing smear-positive TB than the conventional 2-day strategy. We recommend that the WHO revisit their recommendation and caution countries to study the epidemiological and programmatic factors in their own settings before adopting a same-day strategy for wider scale-up.

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Contexte : Neuf centres de microscopie de district dans les états d'Assam et de Tripura, Inde.

Objectif : On recommande maintenant une microscopie de frottis le même jour pour le diagnostic de la tuberculose (TB). Nous avons comparé cette méthode par rapport à l'approche conventionnelle en 2 jours dans un contexte de programme de routine.

Méthodes : Entre octobre et décembre 2012, tous les patients adultes présumés atteints de TB ont été invités à fournir trois échantillons de crachats (lors de la visite initiale, 1 heure après le premier échantillon et le matin suivant) pour un examen par microscopie de frottis selon Ziehl-Neelsen. La détection de bacilles acido-alcool-résistants dans un quelconque échantillon constituait le diagnostic. Les premier et deuxième échantillons ont constitué l'approche du même jour et le premier échantillon plus celui du matin suivant constituaient l'approche en 2 jours.

Résultats : Sur les 2168 patients présumés TB, 403 (18,6%) étaient frottis-positifs selon la méthode du même jour comparés à 427 (19,7%) par la méthode en 2 jours (test de McNemar $P < 0,001$). Sur ce total de 429 patients TB, 26 (6,1%) ont été manqués par la méthode du même jour et 2 (0,5%) par la méthode en 2 jours.

Conclusion : Le recueil d'échantillons le même jour pour une microscopie a manqué plus de cas de TB que le recueil en 2 jours. En Inde, une stratégie de microscopie le même jour à ce taux se traduirait par un grand nombre absolu de cas manqués, entravant les efforts de lutte contre la TB. Nous remettons en question le fait de passer sans discrimination au diagnostic du jour même dans des contextes où les patients reviennent de manière fiable pour un test le jour suivant.

Marco de referencia: Nueve centros distritales de microscopía de Assam y Tripura en la India.

Objetivo: Actualmente se recomienda en el diagnóstico de la tuberculosis (TB) practicar la baciloscopia del esputo el mismo día de la consulta. En el presente estudio se comparó este método con la estrategia corriente de baciloscopia en dos días que se aplica en las prácticas programáticas.

Métodos: De octubre a diciembre del 2012 se solicitó a todos los adultos con presunción diagnóstica de TB que aportaran tres muestras de esputo (en el momento de la consulta inicial, una hora después y a la mañana siguiente), con el fin de practicar el examen microscópico con la coloración de Ziehl-Neelsen. El criterio diagnóstico fue la detección de bacilos acidorresistentes en cualquiera de las muestras. La primera y la segunda muestras inmediatas constituyeron la estrategia del mismo día y la primera muestra inmediata y la muestra del día siguiente constituyeron la estrategia de 2 días.

Resultados: En los 2168 pacientes con presunción de TB, se confirmó el diagnóstico en 403 casos (18,6%) con el método del mismo día y en 427 (19,7%) con la estrategia de 2 días (prueba de McNemar $P < 0,001$). Del total de 429 pacientes con diagnóstico de TB, se pasaron por alto 26 casos cuando se recogieron las muestras el mismo día (6,1%) y 2 casos cuando se recogieron durante 2 días (0,5%).

Conclusión: La recogida de muestras destinadas a la baciloscopia en el mismo día omitió el diagnóstico de más casos de TB que la recogida de muestras en 2 días. En la India, pasar por alto el diagnóstico de casos con el examen microscópico realizado el mismo día tendría como consecuencia omitir una cifra absoluta de pacientes muy alta, que entorpecería los resultados de las iniciativas de control de la TB. En el presente artículo se pone en duda la utilidad de un cambio indiscriminado hacia el método de diagnóstico en el mismo día, en los entornos donde se puede confiar en que los pacientes regresarán al día siguiente para completar las muestras de esputo.