Are we justified in treating for multidrug-resistant tuberculosis based on positive follow-up smear results?

R. S. Kumar,* A. M. V. Kumar,† M. Claassens,‡ V. V. Banurekha,* G. Sekar,* P. Venkatesan,* S. Swaminathan*

*National Institute for Research in Tuberculosis, Chennai, †International Union Against Tuberculosis and Lung Disease, South-East Asia Regional Office, New Delhi, India; ‡Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Stellenbosch University, Cape Town, South Africa

SUMMARY

SETTING: National Institute for Research in Tuberculosis, India.

OBJECTIVE: To assess, among new culture-confirmed smear-positive pulmonary tuberculosis (TB) patients, the proportion of follow-up smear-positives that were culture-negative (S\textsuperscript{+}C-) by month of follow-up examination, human immunodeficiency virus (HIV) status, pre-treatment drug susceptibility status and smear grading.

DESIGN: We extracted follow-up smear (fluorescence microscopy) and culture (L\textsuperscript{o}wenstein-Jensen) results of patients enrolled in clinical trials from January 2000 to August 2012 and treated with the WHO Category I regimen (2EHRZ\textsubscript{3}/4HR\textsubscript{3}).

RESULTS: Of 520 patients, including 176 who were HIV-infected, respectively 199, 81, 47 and 43 were smear-positive at months 2, 4, 5 and 6; of these, respectively 138 (69%), 62 (75%), 32 (68%) and 27 (63%) were culture-negative. The S\textsuperscript{+}C- phenomenon was more pronounced among ‘1+ positive’ patients than in 2+ or 3+ positive patients and in ‘pan-susceptible’ patients than in those with any resistance, and did not vary by HIV status.

CONCLUSION: Nearly two thirds of patients with follow-up smears positive at months 5 and 6 were culture-negative. Starting multidrug-resistant TB (MDR-TB) treatment empirically based on smear results, even in resource-limited settings, is incorrect and can have hazardous consequences. There is an urgent need to revisit the WHO recommendation concerning empirical MDR-TB treatment.

KEY WORDS: empiric MDR-TB treatment; S\textsuperscript{+}C- phenomenon; sputum smear; sputum culture; pulmonary tuberculosis

SPUTUM EXAMINATION by smear microscopy and culture is used during follow-up to monitor treatment response among pulmonary tuberculosis (TB) patients. Although culture is ideal, as it provides information on the viability of bacilli and facilitates drug susceptibility testing (DST), it is not widely available and results often take too long to be of value in clinical management. National TB programmes therefore rely on smear microscopy to monitor treatment response, particularly in resource-limited settings. The World Health Organization (WHO) 2010 guidelines for anti-tuberculosis treatment recommend that TB patients whose treatment has failed based on positive smear results at 5 months of treatment or later should be started on an empirical multidrug-resistant tuberculosis (MDR-TB) regimen and continued, if MDR-TB cannot be confirmed by culture and DST.\textsuperscript{1} There have been concerns around this recommendation, particularly in settings using first-line regimens containing rifampicin (RMP) throughout the course of treatment. Traditionally, when a TB patient was treated with a non-RMP-containing regimen, a positive smear correlated well with a positive culture.\textsuperscript{2} With the use of RMP in short-course chemotherapy regimens, this has changed,\textsuperscript{3,4} and results from previous studies indicate that one third to two thirds of follow-up smear-positive results were culture-negative,\textsuperscript{3–9} and starting MDR-TB treatment empirically based on positive smear result may consequently be unnecessary and hazardous for the patient. Most of the evidence regarding smear-positive, culture-negative (S\textsuperscript{+}C-) cases comes from countries with a low TB burden. No studies have been published from India, a high TB burden country, on this issue.

In the present study, we aimed to examine the extent of the S\textsuperscript{+}C- phenomenon in the follow-up sputum examinations among new smear-positive, culture-confirmed pulmonary TB patients receiving an RMP-containing first-line treatment regimen. The
specific objectives were to determine the proportion of follow-up S+C− patients, disaggregated by month of follow-up examination and described by age, sex, baseline DST status, human immunodeficiency virus (HIV) status and pre-treatment smear quantification.

**METHODS**

*Study design*

This was a retrospective cohort study conducted from June 2012 to May 2013.

*Setting*

The study was conducted at the National Institute for Research in Tuberculosis (NIRT) in Chennai and Madurai, India. For patients recruited into clinical trials, three sputum specimens (two overnight specimens and one spot) were collected at monthly follow-up visits during treatment. Smears were prepared from raw sputum, stained with auramine-rhodamine and read under fluorescence microscopy. They were graded as follows: <6 bacilli/high-power field (HPF) (1+), 6–100 bacilli/HPF (2+) and >100 bacilli/HPF or large clumps (3+). Sputum was decontaminated and concentrated before culture for mycobacteria using the modified Petroff’s method. The cultures were graded on the basis of the quantum of growth in Löwenstein-Jensen (LJ) medium, as follows: actual number up to 19 colonies, 20–100 colonies (1+), >100 colonies (2+), and confluent growth (3+). Positive cultures were identified as *Mycobacterium tuberculosis* using standard methods. DST for isoniazid (INH) and RMP was performed in LJ medium using the minimum inhibitory concentration (MIC) method. MICs of >5 mg/l and >128 mg/l were defined as resistance to INH and RMP, respectively.

*Study population*

All new smear- and culture-positive pulmonary TB patients who were initiated on a 6-month RMP-containing thrice-weekly intermittent regimen WHO Category I 2(EHRZ)3/4(HR)3 enrolled in one of four clinical trials conducted at the NIRT from January 2000 to August 2012 constituted the study population. Patients who were culture-negative at baseline and those with atypical mycobacterial infections were excluded.

*Sample size*

Assuming 40% prevalence (proportion of smear-positive, culture-negative), an absolute precision of 4% and a confidence level of 95%, the sample size was calculated to be ~600. To achieve the sample size, we decided to include all TB patients enrolled as controls in the four clinical trials.

*Data collection, variables, and source*

We collected information on the following variables: age in completed years, sex, pre-treatment DST status, HIV status and results of sputum smear and culture during treatment follow-up at months 1, 2, 3, 4, 5 and 6. These data were extracted from the records of TB patients maintained at the NIRT. Data were double-checked and validated per the protocol at NIRT.

*Data entry and analysis*

Data on age, sex and pre-treatment DST results were extracted from the pre-existing electronic databases. Data on the results of monthly follow-up smears and cultures were captured in an EpiData database (Version 3.1, EpiData Association, Odense, Denmark), dual entered and validated. The two data sets were merged to create a master data set, which was used for analysis using EpiData Analysis (version 2.2.2.180).

*Definitions of key indicators*

A patient was considered smear-positive if any of the three specimens examined during follow-up was smear-positive. To be smear-negative, all three specimens tested had to be smear-negative. Similarly, a person was considered culture-positive if any of the three specimens examined during follow-up was positive for culture, and culture-negative only if all three specimens tested were culture-negative. The proportion of smear-positives that were culture-negative was calculated for every month of follow-up.

*Ethics approval*

Administrative approval to use the data was obtained from the Director of the NIRT. Clinical trials from which the data were extracted for this study were previously approved by the Institutional Ethics Committee of the NIRT. The study protocol was approved by the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (The Union), Paris, France.

**RESULTS**

Data were available for 673 patients. Excluding patients who were smear- or culture-negative at baseline (n = 153), 520 smear- and culture-positive patients were included in the analysis. Among these, 389 (75%) were male and 176 (34%) were HIV-infected. The mean age of the cohort was 34.1 years (standard deviation 10.3). Respectively 316, 199, 118, 81, 47 and 43 patients had at least one positive smear at months 1, 2, 3, 4, 5 and 6. Of the smear-
positives, respectively 53 (17%), 138 (69%), 89 (75%), 62 (77%), 32 (68%) and 27 (63%) were culture-negative (Table 1). SiC- cases did not vary by age, sex, or HIV status, but were more likely among patients whose pre-treatment smear grading was 1+ than in those who were 2+ or 3+, and in patients with bacilli susceptible to all first-line drugs (Table 2) than in those with any resistance. More than 98% of smear-negative patients were culture-negative during follow-up.

DISCUSSION
These findings confirm our hypothesis that among TB patients treated using RMP-containing regimens, many of the smear-positives identified during follow-up are culture-negative. The phenomenon varied as treatment progressed, with nearly 75% of smear-positives converting to culture-negative at months 2, 3 and 4 and 65% at months 5 and 6. This has many implications. First, given that nearly two thirds of all smear-positive failures were culture-negative, starting empirical MDR-TB treatment (as recommended by the WHO) among such patients without laboratory confirmation could lead to unnecessary treatment with toxic drugs.

Second, the findings caution against the current national guidelines in India of extending the intensive phase of anti-tuberculosis treatment based on positive smear results at month 2 of treatment. A study from Bangladesh showed that the extension of the intensive phase based on smear positivity at the end of the phase did not lead to improved treatment outcomes; this formed the basis of global recommendations on this issue.1,22

Third, this also throws doubt on the reliability of positive smear microscopy results during follow-up in guiding patient management. While a negative smear microscopy result correlated well with culture, a positive smear result should be viewed with caution.

National TB programmes should consider alternative tools for monitoring treatment response in addition to smear microscopy. While culture by phenotypic methods could be an alternative, this method has a long turnaround time, it requires trained personnel and laboratory infrastructure, and is not widely available. The newly WHO-approved rapid molecular diagnostics could be a better alternative for the diagnosis of MDR-TB or RMP resistance among smear-positive individuals with ‘failure’ as outcome. Although these molecular diagnostics cannot indicate whether bacilli are viable, they can identify RMP resistance. Given our study findings that positive smears are most likely to be culture-positive in MDR-TB patients, an RMP-resistant result using molecular tools such as the line-probe assay or automated nucleic acid amplification tests could be safely used to initiate MDR-TB treatment without concerns about the viability of bacilli. Other promising tools yet to be widely validated and approved are vital staining of smears using fluorescein-diacetate23 or slide culture24 techniques.

Strengths
This is the first study from India that fills an important knowledge gap in the S+C- phenomenon, on which limited information is available globally. The smear and culture results were reliable, as the examinations were conducted by trained personnel at NIRT, a supranational WHO reference laboratory for mycobacteriology. Patient adherence to treatment was ensured, which helped in studying the effect of treatment regimens on the S+C- phenomenon. We adhered to STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines in reporting of these data.25

Limitations of the study
All patients enrolled in this cohort were investigated, treated and monitored in a clinical trial setting using fluorescence microscopy (and not Ziehl-Neelsen) for the examination of sputum smears; there may thus be concerns regarding the generalisability of the findings to current programme settings. However, fluorescence microscopy has been introduced in ~200 medical colleges in India, and there are plans to scale

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Follow up smear and culture results of new tuberculosis patients enrolled in clinical trials at the National Institute for Research in Tuberculosis, Chennai, India, January 2000-August 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month*</td>
<td>Smear-positive</td>
</tr>
<tr>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>1</td>
<td>316</td>
</tr>
<tr>
<td>2</td>
<td>199</td>
</tr>
<tr>
<td>3</td>
<td>118</td>
</tr>
<tr>
<td>4</td>
<td>81</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
</tr>
<tr>
<td>6</td>
<td>43</td>
</tr>
</tbody>
</table>

*At Month 1 and 3, respectively 4 (1.3%) and 1 (0.8%) patients were smear-positive; however, culture results were not available. CI = confidence interval.
up the technology to all the high workload centres in the next 5 years under the Revised National Tuberculosis Control Programme (RNTCP) in India. The findings of this study may therefore be applicable in the near future. While overall the sample size was adequate, we had few smear-positive patients, especially at months 5 and 6; confidence intervals for ≥C- results at these months are thus wider, indicating greater uncertainty. Nevertheless, the lower limit was ~50%, indicating that at least half of all smear-positives were culture-negative.

**CONCLUSION**

Nearly two thirds of the follow-up smear-positives at months 5 and 6 were culture-negative. Starting MDR-TB treatment empirically based on smear results even in resource-limited settings is incorrect and can have hazardous consequences, including unnecessary treatment with toxic, less potent, expensive and longer regimens with unwarranted inconvenience to patients and their families. There is an urgent need to revisit the WHO recommendation in this regard. National TB programmes should consider alternative tools for monitoring treatment response in addition to smear microscopy.

**Acknowledgements**

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Conflict of interest: none declared.

**References**


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**Table 2** Smear-positive culture-negative cases by baseline parameters among new TB patients enrolled into clinical trials, National Institute for Research in Tuberculosis, Chennai, India, January 2000–August 2012

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Month 1 (n = 316)</th>
<th>Month 2 (n = 199)</th>
<th>Month 3 (n = 118)</th>
<th>Month 4 (n = 81)</th>
<th>Month 5 (n = 47)</th>
<th>Month 6 (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38/245 (16)</td>
<td>104/152 (68)</td>
<td>73/94 (78)</td>
<td>53/66 (80)</td>
<td>28/38 (74)</td>
<td>26/37 (70)</td>
</tr>
<tr>
<td>Female</td>
<td>15/71 (21)</td>
<td>34/47 (72)</td>
<td>16/24 (67)</td>
<td>9/15 (60)</td>
<td>5/9 (44)</td>
<td>1/6 (17)</td>
</tr>
<tr>
<td>Age group, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–34</td>
<td>26/152 (17)</td>
<td>62/89 (70)</td>
<td>37/48 (77)</td>
<td>29/40 (73)</td>
<td>13/19 (69)</td>
<td>4/8 (50)</td>
</tr>
<tr>
<td>≥35</td>
<td>27/164 (17)</td>
<td>76/110 (70)</td>
<td>52/70 (74)</td>
<td>33/41 (81)</td>
<td>19/28 (68)</td>
<td>23/35 (66)</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>26/74 (35)</td>
<td>41/54 (76)</td>
<td>29/39 (74)</td>
<td>19/24 (79)</td>
<td>10/15 (67)</td>
<td>6/9 (67)</td>
</tr>
<tr>
<td>Negative</td>
<td>27/242 (11)</td>
<td>97/145 (67)</td>
<td>60/79 (76)</td>
<td>43/57 (75)</td>
<td>22/32 (69)</td>
<td>21/34 (62)</td>
</tr>
<tr>
<td>DST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pan-susceptible</td>
<td>46/216 (21)</td>
<td>103/135 (76)</td>
<td>66/77 (86)</td>
<td>50/55 (91)</td>
<td>24/30 (80)</td>
<td>21/28 (75)</td>
</tr>
<tr>
<td>Any resistance other than MDR-TB</td>
<td>7/97 (7)</td>
<td>34/57 (60)</td>
<td>23/37 (62)</td>
<td>12/22 (55)</td>
<td>8/17 (47)</td>
<td>6/15 (40)</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>0/3</td>
<td>1/7 (14)</td>
<td>0/4</td>
<td>0/4</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Smear grading at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥C-</td>
<td>13/96 (14)</td>
<td>41/70 (59)</td>
<td>31/46 (67)</td>
<td>25/35 (71)</td>
<td>11/19 (58)</td>
<td>10/20 (50)</td>
</tr>
<tr>
<td>≥C+</td>
<td>23/154 (15)</td>
<td>66/91 (73)</td>
<td>48/59 (81)</td>
<td>29/37 (78)</td>
<td>18/21 (86)</td>
<td>11/17 (65)</td>
</tr>
<tr>
<td>≥C+</td>
<td>17/66 (25)</td>
<td>31/38 (82)</td>
<td>10/13 (77)</td>
<td>8/9 (89)</td>
<td>3/7 (43)</td>
<td>3/6 (100)</td>
</tr>
</tbody>
</table>

TB = tuberculosis; HIV = human immunodeficiency virus; DST = drug susceptibility testing; MDR-TB = multidrug-resistant TB.
**CONTEXTE :** Institut National de Recherche sur la Tuberculose, Inde

**OBJECTIF :** Evaluer, parmi les nouveaux patients atteints de tuberculose (TB) pulmonaire confirmée par culture de crachats et un frottis positif, la proportion de patients ayant un frottis positif et une culture négative (S+C-) lors de l'examen mensuel de suivi, le statut du virus de l’immunodéficience humaine (VIH), la sensibilité aux médicaments avant le traitement et le grade du frottis.

**SCHEMA :** Nous avons recueilli les résultats des frottis (en microscopie à fluorescence) et des cultures (sur Loewenstein-Jensen) de patients enrôlés dans des essais cliniques de janvier 2000 à août 2012 et traités par le protocole OMS de Catégorie 1 (2EHRZ/4HR3).

**RÉSULTATS :** Sur 520 patients, dont 176 étaient infectés par le VIH, 199, 81, 47 et 43 respectivement avaient un frottis positif à 2, 4, 5 et 6 mois ; parmi eux, 138 (69%), 62 (75%), 32 (68%) et 27 (63%) avaient une culture négative. Ce phénomène S+C- était plus prononcé chez les patients positifs classés 1+ que chez ceux classés 2+ ou 3+ et chez les patients sensibles à tous les médicaments que chez ceux présentant une résistance quelconque ; le statut VIH n’avait pas d’influence.

**CONCLUSION :** Près de deux tiers des patients à frottis positifs à 5 et 6 mois avaient une culture négative. Mettre en route un traitement pour la TB multirésistante (TB-MR) en se basant seulement sur un résultat de frottis, est incorrect, surtout dans un contexte de ressources limitées, et peut avoir des conséquences fâcheuses. Il est urgent de revoir les recommandations de l’OMS relatives au traitement empirique de la TB-MR.

**RESUMEN**

**MARCO DE REFERENCIA :** El Instituto Nacional de Investigación en Tuberculosis, Chennai, India.

**OBJETIVO :** Evaluar a los pacientes con diagnóstico reciente de tuberculosis (TB) pulmonar y baciloscopia positiva, confirmado por cultivo, la proporción de casos con baciloscopia positiva y resultados negativos al cultivo, según el mes de seguimiento, la situación frente al virus de la inmunodeficiencia humana (VIH), el resultado de la sensibilidad a los medicamentos antes del tratamiento y la clasificación de la baciloscopia.

**MÉTODO :** Se obtuvieron los resultados de las baciloscopias (microscopia fluorescente) y los cultivos de seguimiento (Löwenstein-Jensen) de los pacientes incorporados a los ensayos clínicos entre enero del 2000 y agosto del 2012 y que recibieron un esquema de tratamiento de Categoría 1 de la Organización Mundial de la Salud (2EHRZ/4HR3).

**RESULTADOS :** De los 520 pacientes (176 infectados por el VIH), 199 presentaron una baciloscopia positiva a los 2 meses, 81 pacientes a los 4 meses, 47 a los 5 meses y 43 a los 6 meses y de ellos, se obtuvo un cultivo negativo en 138 (69%), 62 (75%), 32 (68%) y 27 pacientes (63%), respectivamente. El fenómeno de obtener un resultado positivo en la baciloscopia y un cultivo negativo fue más pronunciado en los pacientes con una baciloscopia positiva 1+ en comparación con las baciloscopias 2+ o 3+ y en los pacientes sensibles a todos los medicamentos comparados con los pacientes con resistencia a algún medicamento y no se modificó en función de la situación frente al VIH.

**CONCLUSIÓN :** Cerca de dos tercios de los pacientes con baciloscopias de seguimiento positivas al mes 5 y 6 presentaron cultivos negativos. Comenzar el tratamiento de la TB multidrogorresistente (TB-MDR) de manera empírica con base en los resultados de la baciloscopia, incluso en los entornos con recursos limitados, es inadecuado y puede acarrear consecuencias peligrosas. Es urgente revisar las recomendaciones de la OMS en materia de tratamiento empírico de la TB-MDR.