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Number of sputum specimens during treatment follow-up of tuberculosis patients: two or one?

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Setting: National Institute for Research in Tuberculosis clinics in Chennai and Madurai, India.

Objective: To examine the pattern of serial smears (negative-negative [NN], negative-positive [NP], positive-negative [PN], positive-positive [PP]) during treatment follow-up of culture-confirmed new smear-positive tuberculosis (TB) patients, and the proportion of culture-negatives in each category.

Design: We reviewed the records and extracted follow-up smear (fluorescent microscopy) and culture (Löwenstein-Jensen) results of patients enrolled in clinical trials from January 2000 to August 2012 and treated with the Category I regimen ($2EHRZ_3/4HR_3$). Data entry and analysis were performed using EpiData.

Results: Among 520 patients (176 infected with the human immunodeficiency virus), the proportions of culture-negative patients with NN, discordant (PN or NP) and PP patterns were approximately 98%, 80% and 40%, respectively. The smear-positive culture-negative phenomenon was more frequent in follow-up smear results graded 1+, followed by 2+ and 3+.

Conclusion: There is justification for discontinuing the examination of second specimens during treatment follow-up among TB patients. However, a positive result on the first smear needs to be confirmed by a second positive result before making clinical management decisions. The World Health Organization may need to reconsider its recommendation on this issue.

The World Health Organization (WHO) recommends that two sputum examinations be performed during treatment follow-up of tuberculosis (TB) patients and that a positive result in any one of the samples be considered smear-positive.[1,2] Previous operational research from India and other countries has shown that the incremental yield of a second smear was very low, irrespective of the month of follow-up and category of TB patient, and have questioned the value of a second smear.[3–5] Previous studies have also shown that the specificity of smear microscopy to detect living tubercle bacilli during treatment follow-up is low: more than two thirds of all follow-up smear-positives were culture-negative.[6–9] In addition, whenever only one of the two smears was positive, they were mainly graded as scanty or '1+ positive', possibly indicating fewer and mostly non-viable bacilli.[4] However, we have not found any evidence in the published literature in favour of or against this hypothesis. In this study, we aimed to examine the patterns of serial smears (negative-negative [NN], negative-positive [NP], positive-negative [PN], positive-positive [PP]) during treatment follow-up of TB patients and the proportion of culture-negatives in each of these categories.

STUDY POPULATION, DESIGN AND METHODS

We reviewed the records of new culture-confirmed smear-positive TB patients enrolled into four clinical trials conducted between January 2000 and July 2012 at the National Institute for Research in Tuberculosis (NIRT) in Chennai, India. The patients received the Category I 6-month rifampicin-containing intermittent regimen ($2(EHRZ)_3/4(HR)_3$*) and underwent monthly follow-up sputum examinations on three sputum specimens (two overnight specimens and one spot sample) at every visit. Sputum smear examination was performed using fluorescence microscopy and culture on Löwenstein-Jensen medium (modified Petroff's method). Smears were prepared directly from unprocessed sputum, stained with auramine rhodamine, read under fluorescence microscopy and graded as follows: <6 bacilli/high power field (HPF; 1+), 6–100 bacilli/HPF (2+), and >100 bacilli/HPF or large clumps (3+).[10] We collected information on the results of sputum smear (with grading) and culture during treatment follow-up at months 1–6, including age, sex and human immunodeficiency virus (HIV) status.

Data were double-entered and analysed using EpiData version 3.1 (EpiData Association, Odense, Denmark).

As they were of better quality, we used the two overnight specimens for the primary analysis of the pattern of serial smear results. We also analysed other combinations of importance for the programme: a spot specimen with the first overnight specimen and then separately with the second overnight specimen. The subject was considered culture-negative only if all specimens tested were culture-negative (defined as lack of growth of tubercle bacilli after 3 months). The proportion of smear-positive, culture-negative samples was calculated for every month of follow-up. For the purposes of examining the relationship between smear grading and culture, the unit of analysis was the specimen rather than the patient.

*E = ethambutol; H = isoniazid; R = rifampicin; Z = pyrazinamide. Numbers before the letters indicate the duration in months of the phase of treatment; numbers in subscript indicate the number of times the drug is taken each week.

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KEY WORDS
smear-positive, culture-negative; sputum smear; sputum culture; pulmonary TB

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TABLE 1 Pattern of serial smear results during TB treatment, by month of follow-up, among patients enrolled in clinical trials at NIRT, India, 2000–2012*

	Month 1 *n* (%)	Month 2 *n* (%)	Month 3 *n* (%)	Month 4 *n* (%)	Month 5 *n* (%)	Month 6 *n* (%)
Two overnight specimens	(*n* = 489)	(*n* = 484)	(*n* = 475)	(*n* = 455)	(*n* = 450)	(*n* = 419)
NN	201 (41)	308 (64)	369 (78)	386 (85)	411 (91)	380 (91)
NP	50 (10)	40 (8)	28 (6)	16 (4)	7 (2)	16 (4)
PN	53 (11)	43 (9)	32 (7)	25 (5)	14 (3)	10 (2)
PP	185 (38)	93 (19)	46 (10)	28 (6)	18 (4)	13 (3)
Spot and first overnight specimen	(*n* = 485)	(*n* = 480)	(*n* = 474)	(*n* = 450)	(*n* = 447)	(*n* = 416)
NN	218 (45)	321 (67)	380 (80)	388 (86)	406 (91)	384 (92)
NP	99 (20)	67 (14)	46 (10)	28 (6)	19 (4)	13 (3)
PN	30 (6)	24 (5)	16 (3)	10 (2)	9 (2)	9 (2)
PP	138 (28)	68 (14)	32 (7)	24 (5)	13 (3)	10 (2)
Spot and second overnight specimen	(*n* = 485)	(*n* = 480)	(*n* = 474)	(*n* = 450)	(*n* = 447)	(*n* = 416)
NN	226 (47)	319 (66)	384 (81)	393 (87)	414 (93)	381 (92)
NP	91 (19)	69 (14)	42 (9)	23 (5)	11 (2)	16 (4)
PN	25 (5)	29 (6)	16 (3)	14 (3)	8 (2)	6 (1)
PP	143 (29)	63 (13)	32 (7)	20 (4)	14 (3)	13 (3)

*The variation in denominators at each month is due to the varying availability of culture and smear results of spot and overnight specimens. Percentages may not total 100% due to rounding.
TB = tuberculosis; NIRT = National Institute for Research in Tuberculosis; NN = negative-negative; NP = negative-positive; PN = positive-negative; PP = positive-positive.

Ethics approval was obtained by the NIRT's Institutional Ethics Committee and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (The Union).

RESULTS

Of 673 patients evaluated, 520 smear- and culture-positive patients were included in the analysis. The rest were excluded because they were smear- and/or culture-negative. Of the 520 patients, 389 (75%) were male, 176 (34%) were HIV-infected and the mean (standard deviation) age was 34 (10) years. The pattern of serial smears by month of follow-up is shown in Table 1 and the proportion of culture-negative results is shown in Table 2. Nearly 80% of the smear-positive samples with discordant smear results (PN or NP) were culture-negative, mainly at months 5 and 6 of treatment. The results remained the same, irrespective of the combination of specimens (overnight and spot) used for analysis. The smear-positive, culture-negative phenomenon was more pronounced in follow-up smear results graded 1+, followed by 2+ and 3+ (Table 3).

DISCUSSION

These results confirm our hypothesis that most of the follow-up smear-positive sputum samples were culture-negative, especially

TABLE 2 Proportion of culture-negative results, according to the serial pattern of smear results during TB treatment among patients enrolled in clinical trials at NIRT, India, 2000–2012*

	Culture-negative results/cultures performed					
	Month 1 *n/N* (%)	Month 2 *n/N* (%)	Month 3 *n/N* (%)	Month 4 *n/N* (%)	Month 5 *n/N* (%)	Month 6 *n/N* (%)
Two overnight specimens						
NN	140/201 (70)	293/308 (95)	362/369 (98)	379/386 (98)	401/411 (98)	372/380 (98)
NP	13/50 (26)	31/40 (76)	24/28 (86)	16/16 (100)	7/7 (100)	11/16 (69)
PN	10/53 (19)	34/43 (79)	27/32 (84)	22/25 (88)	12/14 (86)	8/10 (80)
PP	21/185 (11)	56/93 (60)	29/46 (63)	14/28 (50)	7/18 (39)	5/13 (39)
Spot and first overnight specimen						
NN	138/218 (63)	302/321 (94)	372/380 (98)	382/388 (99)	398/406 (98)	375/384 (98)
NP	18/99 (18)	45/67 (67)	40/46 (87)	24/28 (86)	15/19 (79)	9/13 (69)
PN	13/30 (43)	20/24 (83)	13/16 (81)	9/10 (90)	8/9 (89)	6/9 (67)
PP	13/138 (9)	44/68 (65)	16/32 (50)	12/24 (50)	4/13 (31)	4/10 (40)
Spot and second overnight specimen						
NN	139/226 (62)	299/319 (94)	376/384 (98)	385/393 (98)	405/414 (98)	373/381 (98)
NP	17/91 (19)	48/69 (70)	36/42 (86)	21/23 (91)	8/11 (73)	11/16 (69)
PN	9/25 (36)	26/29 (90)	12/16 (75)	12/14 (86)	6/8 (75)	5/6 (83)
PP	17/143 (12)	38/63 (60)	17/32 (53)	9/20 (45)	6/14 (43)	5/13 (39)
Total†	198/513 (39)	432/508 (85)	460/494 (93)	460/485 (95)	444/468 (95)	432/458 (94)

*Three sputum specimens (one spot and two overnight) were collected during treatment follow-up.
†Total patients for whom culture examinations were performed and results were available. This is irrespective of the availability of smear results and thus may not match the column total.
TB = tuberculosis; NIRT = National Institute for Research in Tuberculosis; NN = negative-negative; NP = negative-positive; PN = positive-negative; PP = positive-positive.

TABLE 3 Proportion of culture-negative results by smear grading at treatment follow-up among TB patients enrolled in clinical trials at NIRT, India, 2000–2012

	Culture-negative results/cultures performed					
Smear grade*	Month 1 *n/N* (%)	Month 2 *n/N* (%)	Month 3 *n/N* (%)	Month 4 *n/N* (%)	Month 5 *n/N* (%)	Month 6 *n/N* (%)
1+	149/563 (27)	252/333 (76)	145/184 (79)	89/122 (73)	43/65 (66)	40/67 (60)
2+	4/57 (7)	10/20 (50)	5/14 (36)	2/9 (22)	2/11 (18)	1/6 (17)
3+	1/5 (20)	0/1 (0)	0/3 (0)	0/2 (0)	0/1 (0)	0/0 (0)

*1+: <6 bacilli/high power field (HPF); 2+: 6–100 bacilli/HPF; 3+: >100 bacilli/HPF or large clumps.
TB = tuberculosis; NIRT = National Institute for Research in Tuberculosis.

when only one of the two smears was positive. This has important policy implications.

First, the study provides convincing evidence that the examination of one sputum specimen is sufficient during follow-up smear microscopy: a negative smear result on the first specimen indicates that it is likely to be culture-negative. However, a positive result on the first specimen should be viewed with caution and needs confirmation by a second sputum examination. If the second result is negative, it is most likely to be culture-negative. If both specimens are positive, only then should the patient be considered smear-positive and need to be investigated further. Previous studies have demonstrated the low incremental yield of the second specimen and the potential to reduce laboratory workload and costs for the programme by examining only one specimen.[3] Given the benefits for the patient of eliminating the additional visit required for the second examination, we strongly recommend the following change in global policy: a switch from two specimens to one specimen during follow-up and confirmation of a positive smear by a second positive smear before declaring a patient a bacteriological failure. This is in line with the recommendation of The Union.[11]

Second, there was a clear trend for specimens with sputum smear grading 1+ to be more likely to be culture-negative than those graded as 2+ or 3+, although nearly 40% of 1+ specimens at month 5 and 6 were culture-positive. This makes it difficult to make a clear recommendation based on smear grading.

Third, even among patients with both smear results positive, nearly 40% were culture-negative at months 5 or 6. There is therefore no justification for recommending empiric treatment for multidrug-resistant (MDR) TB based on positive smear results at 5 months or later.[12] Patients with smear-positive failures need to be tested for culture and drug susceptibility and a confirmed diagnosis of MDR-TB made before initiating treatment. Given the long turnaround time associated with culture and drug susceptibility testing and other challenges related to decentralisation of technology, it may be better to use one of the rapid molecular technologies such as the line probe assay or automated nucleic acid amplification tests.[12] While it is clear that molecular tests will not be helpful in assessing the viability of the bacilli, it may be useful for assessing whether the patient is harbouring drug-resistant bacilli. We hypothesise that, if the patient is harbouring bacilli found to be resistant by molecular tests, they are most likely to be live bacilli; we recommend further research to test this hypothesis.

Fourth, the results of this study indicate the need to clearly distinguish diagnostic smear microscopy from follow-up smear microscopy. The emphasis in diagnostic microscopy is on increasing the sensitivity of the approach and detecting more cases. This justifies recent recommendations to reduce the positivity threshold from ⩾10 acid-fast bacilli (AFB) per 100 fields to ⩾1 AFB/100 and define a case as smear-positive if ⩾1 smear is AFB-positive.[13]

In contrast, given the smear-positive, culture-negative phenomenon observed during follow-up,[6–8,14–21] we need to be cautious in our approach in follow-up smear microscopy: the emphasis should be on increasing specificity rather than sensitivity. The recommendation to reduce the number of specimens to be examined from two to one and the need for confirmation of a positive result will help us increase the specificity and thus improve the accuracy of the decisions we are likely to make on the basis of the smear results.

This is the first study to fill the important knowledge gap on the correlation between the pattern of serial smear results and culture results during follow-up of TB patients. The strengths of the study are the quality-assured smear and culture results conducted by trained personnel at NIRT, a supranational WHO reference laboratory, and ensuring patient treatment adherence as part of the clinical trials. One limitation is the possible lack of generalisability of the findings from a clinical trial setting (using fluorescence microscopy and not Ziehl-Neelsen to examine sputum smears) to current programme settings. This is changing, however, and fluorescence microscopy is likely to be used more frequently in the near future.[22] We could not assess whether the smear-positive, culture-negative phenomenon was related to drug susceptibility patterns during follow-up. This could be a topic for future research.

In conclusion, there is justification for discontinuing the examination of a second specimen during treatment follow-up among TB patients. However, all positive results should be confirmed by a second positive result before declaring a patient a bacteriological failure. The WHO needs to reconsider its recommendation on this issue.

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Contexte : Dispensaires de l'Institut National de Recherche sur la Tuberculose (NIRT) à Chennai et Madurai, Inde.
Objectif : Examiner le type des frottis successifs (négatif-négatif [NN], négatif-positif [NP], positif-négatif [PN], positif-positif [PP]) au cours du suivi du traitement des nouveaux cas de tuberculose (TB) à frottis positif confirmés par la culture ainsi que la proportion de cultures négatives dans chacune de ces catégories.
Schéma : Nous avons revu les dossiers et extrait les résultats des frottis de suivi (examen microscopique par fluorescence) et des cultures (Löwenstein-Jensen) chez les patients enrôlés dans des essais cliniques entre janvier 2000 et août 2012 et traités par un régime de Catégorie 1 ($2EHRZ_3/4HR_3$). On a utilisé EpiData pour entrer les données et les analyser.
Résultats : Sur 520 patients, dont 176 infectés par le virus de l'immunodéficience humaine, les proportions de cultures négatives parmi les patients avec le type NN, le type discordant (PN ou NP) et le type PP ont été respectivement d'environ 98%, 80% et 40%. Le phénomène de culture négative avec frottis positif est plus prononcé dans les résultats des frottis de suivi classés comme 1+, suivis par 2+ et 3+
Conclusion : Il est justifié d'interrompre l'examen du deuxième échantillon au cours du suivi du traitement chez les patients TB. Toutefois un résultat positif du premier frottis impose une confirmation par un deuxième résultat positif avant de prendre des décisions de prise en charge clinique. A cet égard, l'Organisation Mondiale de la Santé pourrait devoir revoir ses recommandations.

Marco de referencia: Los consultorios del Instituto Nacional de Investigación en Tuberculosis de Chennai y Madurai, en la India.
Objetivo: Examinar el patrón de los resultados de los exámenes microscópicos seriados de esputo (NN, NP, PN, PP; [N = negativo, P = positivo]) durante el seguimiento del tratamiento de los casos nuevos de tuberculosis (TB) con baciloscopia positiva y determinar la proporción de casos con cultivo negativo en cada categoría.
Métodos: Se analizaron las historias clínicas y se extrajeron los resultados de las baciloscopias (por microscopia fluorescente) y los cultivos (en medio Löwenstein-Jensen) de los pacientes que participaron en estudios clínicos entre enero del 2000 y agosto del 2012 y que recibieron tratamiento con un régimen de Categoría I ($2EHRZ_3/4HR_3$). Se utilizó el programa EpiData en la entrada de los datos y su análisis.
Resultados: En los 520 pacientes incluidos (176 infectados por el virus de la inmunodeficiencia humana), la proporción aproximada de cultivos negativos en los pacientes con baciloscopia NN fue 98%; en los casos discordantes, PN o NP fue 80%; y en los PP la proporción fue 40%. El fenómeno de obtener un resultado positivo de la baciloscopia con un cultivo negativo fue más frecuente con las baciloscopias clasificadas de grado 1+, seguidas por los grados 2+ y 3+.
Conclusión: Está justificado suspender el examen de la segunda muestra de esputo durante el seguimiento del tratamiento de los pacientes con TB. Sin embargo, un resultado positivo en la primera baciloscopia se debe confirmar con un segundo resultado antes de tomar decisiones clínicas en materia de manejo. Tal vez sería necesario que la Organización Mundial de la Salud examinase de nuevo sus recomendaciones al aspecto.

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