INT J TUBERC LUNG DIS 11(9):959–964

Sputum smear-positive tuberculosis: empiric evidence challenges the need for confirmatory smears

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SUMMARY

OBJECTIVE: To determine the frequency of single scanty or positive sputum smear results and its impact on the surveillance definition of sputum smear-positive tuberculosis (TB).

SETTING: Moldova, Mongolia, Uganda and Zimbabwe.

METHODS: A representative sample of laboratories was selected in each country. Data were double-entered and discordances resolved by rechecking the register.

RESULTS: The dataset comprised 128 808 examinees with valid information from 23 laboratories in Moldova, all 31 in Mongolia, 30 in Uganda and 23 in Zimbabwe, each covering at least one calendar year. The reason for the examination was diagnostic for 89 362, of which 15.2% (n = 13 577) were defined as laboratory cases with at least one bacillus on at least one examination. Cases were confirmed by another examination in 72.6% (n = 9861). Of the 9014 cases who had a full set of three examinations, confirmation was obtained in 92.4% (n = 8325).

CONCLUSION: One quarter of laboratory cases had no confirmatory result, almost entirely attributable to not examining another specimen. The current definition of sputum smear-positive TB requires two positive smears or one positive smear result plus more complex confirmatory evidence. Accepting a single positive examination as sufficient for the definition would greatly increase the sensitivity of the surveillance definition without sacrificing its specificity.

KEY WORDS: tuberculosis; microscopy; diagnosis; surveillance; case definition

WHEN THE MODEL TUBERCULOSIS (TB) program of the International Union Against Tuberculosis and Lung Disease (The Union) was developed in the 1980s in Africa, the result of sputum smear examination was decisive for the decision as to whether the patient was allocated to 'short-course' chemotherapy (sputum smear-positive patients) or to 'standard' chemotherapy of 12 months' duration in the absence of bacteriologic confirmation.[1] When the World Health Organization (WHO) adapted this model as its new strategy for global TB control in the 1990s,[2] the first edition of the treatment guidelines recommended treatment regimens of 6 and 8 months, with or without rifampicin (RMP) throughout, for respectively sputum smear-positive and sputum smear-negative patients.[3] In the third edition of the WHO treatment guidelines, the preferred regimen for all new cases, irrespective of sputum smear microscopy results, is the same 6-month regimen.[4] The main purpose of sputum smear microscopy has thus shifted from guiding treatment decisions to classifying cases as 'definite' or 'other' for the purpose of surveillance.[5]

We therefore examined to what extent the requirement of confirmatory evidence of a sputum smear result with acid-fast bacilli (AFB) by a second smear with AFB was met and how failing to obtaining it impacts on the surveillance definition of sputum smear-positive TB in routine services in two high-burden countries, Uganda and Zimbabwe, and two other high-incidence countries in Asia and Europe, Mongolia and Moldova.

MATERIAL AND METHODS

As reported elsewhere,[6,7] routinely collected data from a representative sample of sputum smear microscopy laboratories were electronically captured. In short, in each country an exhaustive list of sputum smear microscopy laboratories was established from which a random sample of respectively 23, 30 and 23 was

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Article submitted 12 February 2007. Final version accepted 1 June 2007.

[A version in French of this article is available from the Editorial Office in Paris and from the Union website www.iuatld.org]

Table 1 Description of laboratory data set from Moldova, Mongolia, Uganda and Zimbabwe

	Moldova *n* (%)	Mongolia *n* (%)	Uganda *n* (%)	Zimbabwe *n* (%)	Total *n* (%)
Examinees in study	17 865 (100.0)	22 588 (100.0)	55 114 (100.0)	34 744 (100.0)	130 311 (100.0)
Examinees with a valid series*	17 725 (99.2)	22 555 (99.9)	54 050 (98.1)	34 478 (99.2)	128 808 (98.8)
Diagnostic examination	12 515 (70.6)	15 103 (67.0)	36 054 (66.7)	25 690 (74.5)	89 362 (69.4)
Follow-up examination	5 181 (29.2)	7 294 (32.3)	12 555 (23.2)	4 720 (13.7)	29 850 (23.2)
Information missing on reason	29 (0.2)	58 (0.3)	5 441 (10.1)	4 068 (11.8)	9 596 (7.4)
Suspects with at least one AFB†	1 131 (9.0)	1 717 (11.4)	7 280 (20.2)	3 449 (13.4)	13 577 (15.2)

* Valid series: result coded as unknown cannot be followed by a known result. Percentage of total.
† Percentage of examinees with a diagnostic examination.
AFB = acid-fast bacilli.

selected from Moldova, Uganda and Zimbabwe for inclusion in the study. All 31 laboratories from Mongolia were selected. Registers covering at least one full calendar year were selected for each laboratory. In Moldova and Mongolia, the year 2003 was selected for all laboratories (extending in some Mongolian laboratories back to part of December 2002). All three years 1999, 2000 and 2001 were included for each laboratory in Uganda. In Zimbabwe, the year 2001 was selected for one laboratory, the year 2002 for 19 laboratories, and the year 2003 for the remaining three laboratories.

A uniform data entry questionnaire was created using EpiData Entry (EpiData Association, Odense, Denmark; freely available at http://www.epidata.dk). For each examinee in the register, the laboratory serial number, date of registration, age, sex, reason for examination and the results of the three serial examinations were entered, using limits on values that could legally be entered. The data were independently entered twice, the two data files compared and discordances resolved by checking against the physical register with corrections where indicated to produce a finalized data set. EpiData Analysis was used for data analysis.

RESULTS

The combined data set comprised 130 311 records, of which 1503 (1.2%) were excluded because the recorded sequence of serial smears was nonsensical (a missing result followed by a recorded result), leaving 128 808 examinees with a valid sequence of results (Table 1).

The reason for examination was known for virtually all examinees in Moldova and Mongolia, but was missing for more than 10% of examinees in Uganda and Zimbabwe. This analysis considered only the 89 362 examinees with a diagnostic examination (henceforth referred to as suspects), representing 69.4% of the 128 808 examinees with a valid sequence of serial sputum smear examinations. The proportion was lowest in Mongolia (67.0%) and highest in Zimbabwe (74.5%) (Table 1).

For the purpose of the present study, a laboratory case of sputum smear-positive TB was defined as a suspect with at least one AFB in at least one of three possible serial sputum smear examinations. Using this definition, 15.2% (13 577) of suspects were cases, with a large variation in the four countries. The lowest proportion was 9.0% in Moldova, and the highest was 20.2% in Uganda (Table 1).

Only 72.6% (9861) with any AFB of the 13 577 cases had any AFB identified on at least one other smear (Table 2). Thus, 27.4% with any AFB on at least one examination did not meet the international microscopy-based case definition of a sputum smear-positive case. The proportion of cases positive on a single smear examination varied widely in the four countries. The lowest was recorded in Mongolia (5.2% unconfirmed) and highest in Uganda (38.5% unconfirmed). Among scanty results (1–9 AFB/100 fields), 76.1% (611/803) were confirmed, a slightly higher

Table 2 Examinees with a diagnostic examination and at least one scanty or positive result, irrespective of the number of smear examinations performed, Moldova, Mongolia, Uganda and Zimbabwe

	Moldova *n* (%)	Mongolia *n* (%)	Uganda *n* (%)	Zimbabwe *n* (%)	Total *n* (%)
Total	1331 (100.0)	1717 (100.0)	7280 (100.0)	3449 (100.0)	13 577 (100.0)
Confirmed	980 (86.6)	1628 (94.8)	4476 (61.5)	2777 (80.5)	9861 (72.6)
Scanty (1–9/100 fields)	137 (12.1)	126 (7.3)	134 (1.8)	214 (6.2)	611 (4.5)
Positive (1+, 2+ or 3+)	843 (74.5)	1502 (87.5)	4342 (59.6)	2563 (74.3)	9250 (68.1)
Not confirmed	151 (13.4)	89 (5.2)	2804 (38.5)	672 (19.5)	3716 (27.4)
Scanty (1 to 9/100 fields)	27 (2.4)	24 (1.4)	43 (0.6)	98 (2.8)	192 (1.4)
Positive (1+, 2+ or 3+)	124 (11.0)	65 (3.8)	2761 (37.9)	574 (16.6)	3524 (26.0)

Table 3 Examinees with a diagnostic examination and at least one scanty or positive result, with all three examinations performed, Moldova, Mongolia, Uganda and Zimbabwe

	Moldova *n* (%)	Mongolia *n* (%)	Uganda *n* (%)	Zimbabwe *n* (%)	Total *n* (%)
Total	904 (100.0)	1503 (100.0)	3778 (100.0)	2829 (100.0)	9014 (100.0)
Confirmed	812 (89.8)	1448 (96.3)	3594 (95.1)	2471 (87.3)	8325 (92.4)
Scanty and scanty	20 (2.2)	21 (1.4)	22 (0.6)	76 (2.7)	139 (1.5)
Scanty and positive	104 (11.5)	90 (6.0)	102 (2.7)	87 (3.1)	383 (4.2)
Positive and positive	688 (76.1)	1337 (89.0)	3470 (91.8)	2308 (81.6)	7803 (86.6)
Not confirmed	92 (10.2)	55 (3.7)	184 (4.9)	358 (12.7)	689 (7.6)
Single scanty	19 (2.1)	19 (1.3)	17 (0.4)	44 (1.6)	99 (1.1)
Single positive	73 (8.1)	36 (2.4)	167 (4.4)	314 (11.1)	590 (6.5)

proportion than the 72.4% (9250/12 774) among clear positive results (1+, 2+ or 3+ positive).

To examine the extent to which it was actually possible to confirm positive sputum smear results, only the 9014 examinees with a recorded complete series of three serial sputum examination results were analyzed (Table 3). To simplify the various possible sequences of results, the following definitions were chosen to lump groups of patterns. If a series existed of two or three scanty results but no positive result, the result was said to be a scanty result confirmed by another scanty result. If a series had any mixture containing at least one scanty and one positive result, the result was said to be confirmed by a scanty and a positive result. If a series had two or three positive but no scanty results, it was said to be a positive result confirmed by a positive result. Sputum smear results not confirmed had either a single scanty or a single positive result in the series. Of the 9014 cases with a complete series of three results, 92.4% (n = 8325) had at least one other smear result containing AFB, thus meeting the international surveillance definition of a sputum smear-positive case (Table 3). The proportion was lowest in Zimbabwe (87.3%) and highest in Uganda (95.1%). Only 1.1% (n = 99) had an isolated scanty result, ranging from 0.4% in Uganda to 2.1% in Moldova. Of all 621 series with at least one scanty result, 84.1% (n = 522) had another smear with AFB.

DISCUSSION

The present study is based on a very large representative sample of data from sputum smear microscopy laboratories in four countries with thorough data validation procedures. The study demonstrates two aspects of relevance to diagnostic procedures and principles for TB control. First, among those with three sputum smear examinations, one positive will be confirmed in one or two of the other smears for over 90% of patients, indicating that one positive is a very strong predictor of AFB further substantiated by serial smears. Second, there is no confirmatory second examination for a large proportion of those patients with a sputum smear-positive result for AFB, largely because a second examination is not performed. These findings cast considerable doubt on the appropriateness of routinely requiring confirmation of a diagnostic smear examination showing any AFB. The current policy is also questionable because it has no impact on the decision of whether treatment is to be initiated and what kind. Its main effect is that it lowers the sensitivity of the surveillance definition of a sputum smear-positive case of TB, basically because non-confirmed status is a consequence of no additional examinations being made.

There is a wealth of information available on the performance of sputum smear microscopy using the Ziehl-Neelsen (ZN) technique and its potential under more or less controlled conditions.[8] It has also been demonstrated how external quality assessment of sputum smear microscopy[9] can favorably improve the performance of such services.[10,11] Nevertheless, in many high-burden and other high-prevalence countries, such systems have only recently been introduced, often on a very small scale,[12,13] or—more commonly—not at all.

The present study was undertaken in four countries of the latter group, two of which are classified by WHO as high-burden countries.[14] While it is thus not known to what extent results recorded in the laboratory register are accurate, every effort was made to obtain a correct, representative picture of what technicians recorded in their laboratory registers and thus likely forwarded to the health care provider providing the specimen and requesting the examination. To ensure representation for the country, 23 to 30 randomly chosen laboratories in three countries, and all laboratories in one country were selected from an exhaustive list of all microscopy centers in the country using the standard TB laboratory register recommended by WHO and The Union.[15,16] To ensure the accuracy of the computerized data, these were double-entered, compared, and discordances resolved by checking them against the physical register and correcting them where necessary.[6,7]

The internationally agreed definition of a sputum smear-positive TB case is:

1 two or more initial sputum smear examinations positive for AFB, or

2 one sputum smear examination positive for AFB plus radiographic abnormalities consistent with active pulmonary TB as determined by a clinician, or
3 one sputum smear positive for AFB plus sputum culture positive for *Mycobacterium tuberculosis*.[17]

In many resource-poor settings, films for radiography are scarce and culture is rarely available, particularly in rural areas. The purpose of the present study was thus to examine to what extent patients with a diagnostic sputum smear examination met this international case definition solely based on microscopy results under routine program conditions.

The international definition fails to specify whether 'positive' includes scanty positive results or only results to be reported as 1+, 2+ or 3+.[18] For this study, both the numerator and the denominator therefore included scanty positive results to determine the frequency of confirmed smears. While 15% of the TB suspects met the study case definition of at least one AFB in at least one examination, only three quarters of these study cases met the international case definition of at least two scanty and/or positive examinations. In Uganda, only just over 60% met the international case definition, while in Mongolia this figure was 95%. These differences are explained by the fact that, particularly in the presence of one clearly positive result, further examinations are simply not done. This is evidenced by the finding that amongst those who had a full series of three smears, confirmation was readily obtained, even in the presence of scanty positive results.

This study cannot determine the reasons for the reluctance to confirm a scanty or positive smear result with a second examination. It is well known, however, that many sputum smear microscopy laboratories, particularly in sub-Saharan Africa (with two representatives included here, where the failure to do so is particularly conspicuous), have been faced with an increasing amount of work with little or no corresponding adjustment of staffing.[19] Excessive work will almost necessarily lead to circumventing the requirement to examine multiple specimens or, where it is insisted upon, may force technicians to simply copy results, a practice for which there is some evidence.[20]

The findings of this study do not only demonstrate that international recommendations are defied in an appreciable proportion in some settings. More importantly, perhaps, it raises the question as to whether this particular recommendation to define a sputum smear-positive case is appropriate to begin with. While there are still national programs that pursue a different policy for treatment or services depending on sputum smear microscopy results,[21] WHO recommendations clearly advocate the same treatment regimens and services for any TB patient, irrespective of sputum smear results.[4] This marks an important change from the times when the high costs of the most sterilizing anti-tuberculosis medications were of prime concern and the major determinant in prioritizing the allocation of RMP-containing regimens to those patients most likely to transmit *M. tuberculosis* in the community, i.e., sputum smear-positive patients.[1]

There is considerable uncertainty about the accuracy of a TB diagnosis in the absence of bacteriologic confirmation. For surveillance purposes, it is thus essential to know about the magnitude of and trends in the occurrence of 'definitive' cases. The specificity of AFB in spontaneously produced sputum for *M. tuberculosis* is very high in any setting. The purpose of acid-fast microscopy is to identify members of the genus rather than the species. Ascertainment of true specificity thus requires that no positive result be reported if the smear is truly negative. Such a study was conducted using almost 4000 slides in Dar-es-Salaam (Tanzania), Kampala (Uganda) and Nairobi (Kenya), giving a specificity of 99.7%, 96.8% and 98.6%, respectively.[22] The dependence on the qualification of the individual technician was shown in this study and has been confirmed in a study among eight State laboratories in India, where half made no false-positive error, while those that performed most poorly had up to 7% false-positive results.[23] The international requirement for confirmatory smears must thus likely be rooted in the perceived lack of specificity of a single positive smear due to administrative errors. Such errors are nevertheless very rare, and rarer than with sputum culture.[22] While it might be debatable, a minor offset in specificity of the surveillance definition of sputum smear-positive TB imparted by clerical errors would by far be outweighed by the large potential gain in sensitivity if the finding of a single AFB in a single microscopic examination by the ZN method of a spontaneously produced sputum specimen qualified for the definition. This and other studies unambiguously show that such findings could virtually always be confirmed if a second examination were actually done.[24]

Acknowledgements

These studies were conducted as the practical application of operations research courses conducted by the International Union Against Tuberculosis and Lung Disease in Paris in 2003 and 2004. The courses in Paris were financially supported by the United States Agency for International Development under the terms of Award No. HRN-a-00-00-00018-00.

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RÉSUMÉ

OBJECTIF : Déterminer la fréquence d'un résultat unique positif ou très faiblement positif des frottis de crachats et son impact sur la définition de la surveillance des tuberculoses à bacilloscopie positive des crachats.

CONTEXTE : Moldavie, Mongolie, Ouganda et Zimbabwe.

MÉTHODES : Un échantillon représentatif des laboratoires a été sélectionné dans chaque pays. Les données ont été encodées en double et les discordances résolues par réexamen du registre.

RÉSULTATS : L'ensemble des données comprend 128 808 sujets examinés avec information valide provenant de 13 laboratoires en Moldavie, de l'ensemble des 31 laboratoires en Mongolie, de 30 en Ouganda et 23 au Zimbabwe, chacun s'étendant au moins sur une année de calendrier. La raison de l'examen a été le diagnostic pour 89 362 sujets, parmi lesquels 15,2% (n = 13 577) ont été définis comme des cas de laboratoire avec au moins un bacille au cours d'au moins un examen. Les cas ont été confirmés par un deuxième examen dans 72,6% des cas (n = 9861). Parmi les 9014 cas qui avaient eu un ensemble complet de trois examens, on a confirmé le diagnostic dans 92,4% des cas (n = 8325).

CONCLUSION : Un quart des cas de laboratoire n'ont pas bénéficié d'un résultat de confirmation, ceci étant presqu'entièrement attribuable à l'absence d'examen d'un deuxième échantillon. La définition actuelle des tuberculoses à bacilloscopie positive des frottis de crachats exige deux examens de frottis positifs ou un examen positif accompagné de preuves de confirmation plus complexes. Le fait d'accepter qu'un seul examen positif soit suffisant pour répondre à la définition augmenterait considérablement la sensibilité de cette définition de surveillance sans sacrifier beaucoup sa spécificité.

RESUMEN

OBJETIVO: Determinar la frecuencia de resultados de baciloscopia con un bacilo acidorresistente con escasos bacilos o francamente positivas y su repercusión en la definición de casos de tuberculosis (TB) con baciloscopia positiva del esputo, con fines de vigilancia.

MARCO DE REFERENCIA: La República Moldova, Mongolia, Uganda y Zimbabwe.

MÉTODOS: Se escogió una muestra representativa de laboratorios de cada país. Los datos se consignaron en duplicado y se resolvieron las discordancias, verificando el registro.

RESULTADOS: El conjunto de datos comprendió 128 808 pacientes examinados con información válida, provenientes de 23 laboratorios de la República Moldova, de los 31 laboratorios de Mongolia, los 30 de Uganda y los 23 de Zimbabwe ; la información cubrió como mínimo un año civil. El motivo del examen fue diagnóstico en 89 362 casos, de los cuales 15,2% (n = 13 577) se definieron como casos para el laboratorio con al menos un bacilo en por lo menos una de las baciloscopias. Se confirmaron 72,6% (n = 9861) de los casos con un nuevo examen. De ellos, 9014 casos contaron con la serie completa de tres baciloscopias y se confirmó el diagnóstico en 92,4% (n = 8325).

CONCLUSIÓN: En un cuarto de los casos diagnosticados en el laboratorio no se contó con un resultado de confirmación y en su mayoría debido a la falta del examen de otra muestra. La definición vigente de TB con baciloscopia positiva exige dos resultados positivos o una baciloscopia positiva con otra prueba clínica confirmatoria. Si se acepta como criterio suficiente para la definición de caso solo un resultado positivo de baciloscopia, se aumentaría en forma considerable la sensibilidad de la definición con fines de vigilancia, sin mayor detrimento de su especificidad.