

Quantitative differences in sputum smear microscopy results for acid-fast bacilli by age and sex in four countries

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SUMMARY

OBJECTIVE: To examine the influence of age and sex on the grading of tuberculosis (TB) sputum smear microscopy results.

SETTING: Laboratories in Moldova, Mongolia, Uganda and Zimbabwe.

METHODS: Data from nationally representative samples of laboratory registers were double-entered and validated, and discordances were resolved by rechecking against the registers.

RESULTS: The dataset comprised 128 808 examinees from 23 laboratories in Moldova, all 31 in Mongolia, 30 in Uganda and 23 in Zimbabwe, each covering at least one calendar year. Among all examinees, 89 362 had a diagnostic examination, 13 577 (15.2%) of whom were cases. A non-quantified positive result was recorded among 1272 (9.4%) of these. Scanty results were most

frequent in Zimbabwe (8.5%) and most infrequent in Uganda (1.1%). In contrast, the highest grade, of 3+ positive, was most frequent (43.9%) in Mongolia. The lowest proportion of low-grade positivity was recorded for males aged 15–24 years and the highest among females aged ≥ 65 years.

CONCLUSION: Differences in the frequency of low-grade positivity between the four countries were striking. Females tended to have lower bacillary counts, and low-grade positivity was more frequent at the extremes of age. These results reinforce the need for appropriate instructions on how to produce high-quality sputum to improve yield.

KEY WORDS: tuberculosis; laboratory; microscopy; epidemiology

THE PROBABILITY of finding acid-fast bacilli (AFB) in microscopic sputum smear examinations is closely correlated with their concentration in sputum,¹ the quality of sputum produced for examination,² and technical appropriateness, such as examination time³ and staining procedures.⁴ In a wider epidemiologic context, the proportional distribution of sputum smear microscopy grading among tuberculosis (TB) case patients will depend on the extent of the disease at the time of presentation coupled with the patient's ability to produce an adequate specimen. The influence of potential technical shortcomings might be controlled for by a relative comparison of findings in age- and sex-specific subsets of patients in a given setting.

The purpose of this study was to examine the absolute and relative frequency distribution of sputum smear microscopy grading categories, and their variability with age and sex, as recorded in peripheral laboratories in Moldova, Mongolia, Uganda and Zimbabwe.

MATERIALS AND METHODS

Records of examinees in the Tuberculosis Laboratory Register during the period January 1999–December 2003 were utilized for the study in Moldova, Mongolia, Uganda and Zimbabwe. From exhaustive lists of laboratories in each country that offered TB diagnostic services using sputum smear microscopy and having a standard Tuberculosis Laboratory Register, 23 in Moldova, 30 in Uganda and 23 in Zimbabwe were randomly selected to ensure the desired unbiased and representative evaluation of the work in the countries' laboratories. All the 31 laboratories in Mongolia were selected.

Approval for the study was obtained from the Ministry of Health in each of the respective countries.

The data from Moldova and Mongolia were collected from registers completed in 1999; from Uganda registers they comprised 3 years, 1999–2001; and in Zimbabwe the registers had been completed mainly in 2002 and 2003, but a few laboratories contributed

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data from 2001. Only in Uganda were data collected from each laboratory for 3 years; in all other countries, each laboratory contributed a single year (in the case of Zimbabwe, not all laboratories contributed data for the same year).

At the time the laboratories examined the specimens, none of the countries had a formal external quality assessment scheme in place.

Data collection, entry, validation and analysis

Data from these 107 laboratories on sex, age, reason for examination and results of sputum smear examination were electronically captured from the registers and validated by double entry and comparison. Where indicated, identified discordances were rectified and corrected by rechecking the recorded entries in the physical registers. A uniform data entry form was used by all four countries using the freely available EpiData Entry software (version 3.1, EpiData Association, Odense, Denmark, <http://www.epidata.dk>). Details of these procedures have been reported elsewhere.⁵⁻⁸ The validated files from the laboratories were combined for analysis.

Analysis was performed using EpiData Analysis (version 2.1, EpiData). For the purpose of the study,

a case of TB was defined as a suspect with at least one AFB in at least one of the serial sputum smear examinations. While the dataset comprised examinees with one to three examinations each, the unit of measurement for the analysis was individual sputum smear examination, to avoid bias resulting from examinations with incomplete series of examinations. To analyze quantitative differences in sputum smear microscopy results for AFB, non-quantified positive results were excluded. Quantified results were stratified by country, age and sex.

RESULTS

Of a total of 130 311 records from 107 laboratories in the four countries, 1503 (1.2%) were excluded because of improper recording practices in the register (e.g., 'No result recorded' followed by a valid result). Of the 128 808 examinees, 89 362 (69.4%) were recorded as TB suspects with a diagnostic examination.

A total of 13 577 suspects (15.2%) met the case definition criteria for a TB case. These cases had a total of 30 766 positive smear examinations. The distribution of positivity grading of these smear examinations by country is summarized in Table 1. The

Table 1 Distribution of graded microscopy results by country

Quantified microscopy result	Moldova		Mongolia		Uganda		Zimbabwe		Total	
	<i>n</i>	Col %	<i>n</i>	Col %	<i>n</i>	Col %	<i>n</i>	Col %	<i>n</i>	Col %
Positive, not quantified	80	2.8	9	0.2	1068	7.1	1750	21.0	2907	9.4
Scanty positive	220	7.8	216	4.6	229	1.5	492	5.9	1157	3.8
1+ positive	1060	37.7	206	4.4	3756	25.1	2012	24.2	7034	22.9
2+ positive	612	21.8	2519	53.7	5717	38.3	1868	22.5	10716	34.8
3+ positive	839	29.8	1745	37.2	4173	27.9	2195	26.4	8952	29.1
Total	2811	100.0	4695	100.0	14943	100.0	8317	100.0	30766	100.0

Col = column.

Table 2 Distribution of graded microscopy results by country, sex and age group

	Scanty positive		1+ positive		2+ positive		3+ positive		Total
	<i>n</i>	Row %	<i>n</i>	Row %	<i>n</i>	Row %	<i>n</i>	Row %	
Total	1157	4.2	7034	25.2	10716	38.5	8952	32.1	27859
Study country									
Moldova	220	8.1	1060	38.8	612	22.4	839	30.7	2731
Mongolia	216	4.6	206	4.4	2519	53.8	1745	37.2	4686
Uganda	229	1.7	3756	27.1	5717	41.2	4173	30.1	13875
Zimbabwe	492	7.5	2012	30.6	1868	28.4	2195	33.4	6567
Examinee's sex									
Female	483	4.3	2871	25.4	4381	38.8	3556	31.5	11291
Male	663	4.2	4000	25.1	6102	38.2	5202	32.6	15967
Sex unknown	11	1.8	163	27.1	233	38.8	194	32.3	601
Age group, years									
0-14	28	5.3	147	27.8	221	41.8	133	25.1	529
15-24	184	3.6	1061	21.0	2168	43.0	1630	32.3	5043
25-34	352	4.1	2136	25.1	3262	38.3	2767	32.5	8517
35-44	269	4.7	1506	26.3	2058	35.9	1897	33.1	5730
45-54	147	5.0	817	27.7	1025	34.7	961	32.6	2950
55-64	48	3.9	264	21.3	500	40.4	425	34.4	1237
≥65	37	5.9	173	27.5	225	35.8	194	30.8	629
Age unknown	92	2.9	930	28.8	1257	39.0	945	29.3	3224

Table 3 Distribution of low- and high-grade positive results by sex, age group and country

Characteristic	Low-grade positive: Scanty/ 1+ positive		High-grade positive: 2+/3+ positive		Total
	<i>n</i>	Row %	<i>n</i>	Row %	
Total	8191	29.4	19668	70.6	27859
Study country					
Moldova	1280	46.9	1451	53.1	2731
Mongolia	422	9.0	4264	91.0	4686
Uganda	3985	28.7	9890	71.3	13875
Zimbabwe	2504	38.1	4063	61.9	6567
Examinee's sex					
Female	3354	29.7	7937	70.3	11291
Male	4663	29.2	11304	70.8	15967
Sex unknown	174	29.0	427	71.0	601
Age group, years					
0-14	175	33.1	354	66.9	529
15-24	1245	24.7	3798	75.3	5043
25-34	2488	29.2	6029	70.8	8517
35-44	1775	31.0	3955	69.0	5730
45-54	964	32.7	1986	67.3	2950
55-64	312	25.2	925	74.8	1237
≥65	210	33.4	419	66.6	629
Age unknown	1022	31.7	2202	68.3	3224

proportion of cases with such non-quantified positive results was lowest in Mongolia (0.2%) and highest in Zimbabwe (21.0%).

Of the 13 577 suspects, 1250 had one or more non-quantified positive examination result. These 2907 (9.4% of the 30 766) non-quantified positive smear results were excluded from further analysis, leaving 27 859 quantified, positive smear examinations for further analysis.

The distribution by the four grading categories (scanty positive, 1+ positive, 2+ positive and 3+ positive) of the 27 859 positive results retained is summarized by country, sex and age group in Table 2. Scanty results were most frequently recorded in Moldova (8.1%) and most infrequently in Uganda (1.7%).

To simplify grade grouping, the four grades were aggregated into two categories. Scanty positive and 1+ positive findings were grouped into the low-grade category, and 2+ and 3+ positive findings were grouped into the high-grade category (Table 3). The most pronounced difference in the frequency of low-grade positivity was between countries, ranging from a low 9.0% in Mongolia to 46.9% in Moldova. In this crude analysis, there was no difference between the sexes. By age, the youngest and the oldest appeared to have the highest frequencies of low-grade positivity.

To further evaluate the influence of age and sex, only the 10 633 cases (86.4% of the total number of specimens with quantified positive results) with complete information on age and sex were analyzed by stratification. These case patients had a total of 20 549 positive, quantified sputum smear results.

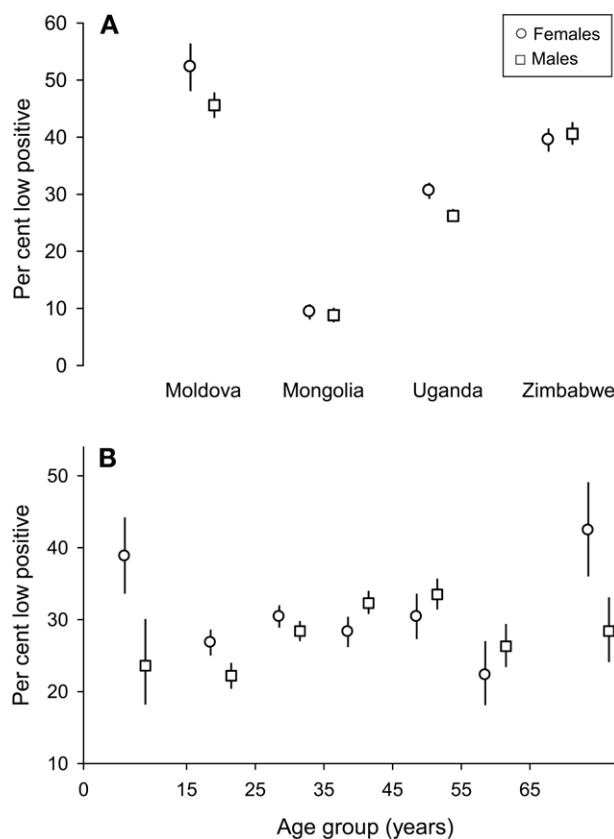


Figure 1 Proportion of low-grade positive (scanty or 1+) microscopy results by sex and country (A) and by sex and age group (B), based on examinations from cases with at least one positive examination, Moldova, Mongolia, Uganda, and Zimbabwe.

Except for Zimbabwe, females had greater, if only slightly so, proportions of low-positive results than males (Figure 1A). Stratification by age and sex showed that females aged <35 and >64 years had a greater proportion of low-positive results, while between 35 and 64 years the inverse was the case (Figure 1B). Finally, case smears were stratified by age, sex and country to account for the large difference between countries and to obtain a relative measure within each country for comparison (Figure 2). In all four countries, women aged ≥65 years had a higher proportion of low-grade results than men, although the difference was significant only in Moldova. In Uganda, girls had a significantly greater proportion of low-grade positive results than boys.

DISCUSSION

This study showed large differences between the four countries in the proportion of low-grade positive results, ranging from 10% to 50%. Within each country, minor differences were found by age and sex, with the youngest and oldest showing a tendency towards lower grades among positive sputum smears, and women frequently showing a tendency towards higher

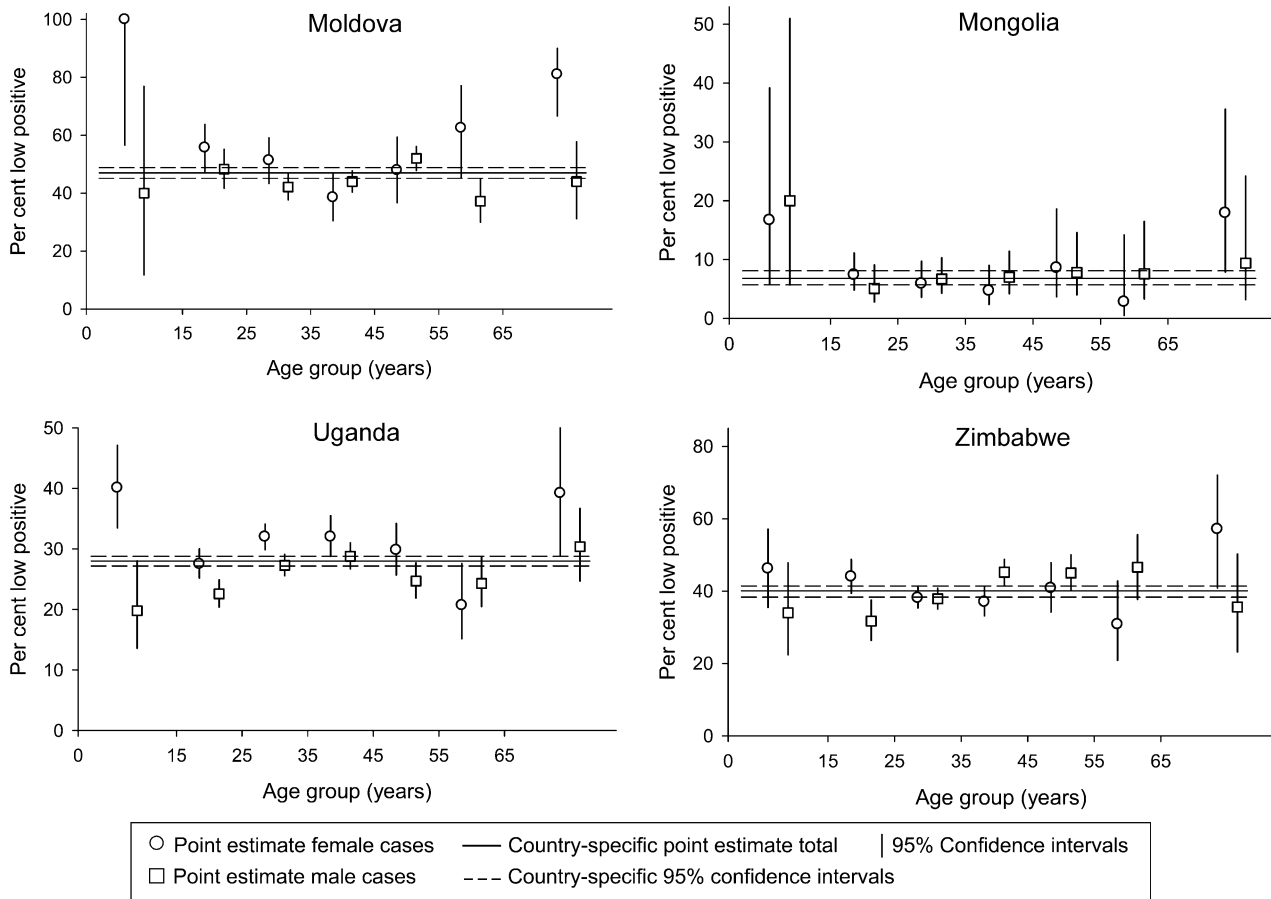


Figure 2 Proportion of cases with a low-positive sputum smear microscopy result (scanty or 1+ positive) among all suspect cases, by age, sex, and country, based on examinations from cases with at least one positive examination, Moldova, Mongolia, Uganda, and Zimbabwe.

proportions of low-grade sputum smear results. Infection with the human immunodeficiency virus (HIV) is known to result in a shift from higher to lower grade positivity in sputum smear examinations.⁹ The prevalence of HIV infection varies greatly among the four countries analyzed here. As such data were not and are not routinely collected in sputum smear microscopy laboratories, this study could not determine the influence of HIV infection on the result in individual patients. It is noteworthy, however, that the largest proportion of cases with low-grade positivity was found in Zimbabwe, the country estimated to have the highest HIV prevalence among TB patients of the four countries considered here,¹⁰ while Mongolia, the country with possibly the lowest prevalence of HIV among TB patients, had the lowest proportion of low-grade smears. Nevertheless, while HIV infection may contribute to this observation, it is unlikely to be the only explanation, as other factors, such as time of presentation as TB suspect and quality of sputum smear examination, may play a much more decisive role.

Grading of sputum smear microscopy results¹¹ serves several purposes. As a direct reflection of bacil-

lary counts that may be aerosolized, it is a predictor of transmissibility of case patients¹² and predicts the speed of bacteriologic conversion on chemotherapy.¹³ Low counts are more difficult than higher counts to find in a microscopic sputum examination, and thus are more likely to be missed.¹⁴ The relative frequency of low-count grading results has thus been considered for use as an indicator of the quality of sputum smear microscopy.¹⁵

The proportional distribution of different grading results may be determined by the extent of disease in the community, the quality of the sputum produced,² by using compromised stains or a poor staining technique,¹⁶ the integrity of the microscope's optical system,¹⁷ or the amount of time spent on examination.³ A multitude of factors thus influences the distribution of different grading results among newly identified TB cases.

In this study of records of a selection of nationally representative microscopy laboratory registers, we were interested in evaluating whether and to what extent such differences were observed in graded sputum smears in the four countries. Stratification by age and sex within each country was used to show the

relative influence of these two characteristics in a given country setting.

The largest difference was found between countries. In Mongolia, only about 10% of all cases had a low-positive result, while half of all positive results were in this category in Moldova. Next in importance appeared to be age, with the extremes of age being particularly prone to low-grade positive results. In some settings and age groups, females had low-grade positive results more frequently, but overall, the influence of sex was of minor importance.

A study in Pakistan has demonstrated that the yield of positive results can be improved by appropriate instruction, with a striking gain in the yield of sputum smear-positive cases among women.² While the findings in our study of a frequent female preponderance of low-positive smear microscopy results in some of the settings does not necessarily indicate that the quality of sputum was poorer among women, the above study from Pakistan strongly suggests that improved instructions could contribute to balancing any such discordance. It is remarkable that in all four countries the highest frequencies of low-positive results were found at the extremes of age. In children, this observation is likely to be related to both a generally lesser extent of cavitory disease and/or more difficulties in obtaining a good sputum specimen in this age group. While the clinical presentation of TB in the elderly does not seem to differ substantially from that in younger age groups, the radiographic presentation is frequently different.¹⁸ In a meta-analysis of studies on TB in the elderly, cavitation was shown to be less common than among younger patients, but there was no important difference in the recovery of AFB between the groups, although no information was given on graded results.¹⁹

This study has shown striking differences in the frequency of low-grade positivity between the four countries examined. Differences by sex were minor on average, but there was a tendency for females to have lower bacillary counts on microscopic examination and a strong indication that low-grade positivity was more frequent at the extremes of age. While the reasons for these findings are manifold, a practical implication is that particular emphasis should be given to appropriate instructions on how to produce high-quality sputum, demonstrated elsewhere to result in a much improved yield among female suspects.² The large differences found between Moldova and Mongolia are unlikely to be explained solely by differences in the quality of examination, and may reflect important differences in ready accessibility to diagnostic services, resulting in the identification of TB in relatively early or advanced stages of the disease, respectively.

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RÉSUMÉ

OBJECTIF : Examiner l'influence de l'âge et du sexe sur le degré de positivité des résultats de l'examen microscopique des frottis de crachats.

CONTEXTE : Laboratoires en Moldavie, Mongolie, Ouganda et Zimbabwe.

MÉTHODES : On a utilisé les données provenant d'échantillons représentatifs au niveau national des registres de laboratoire qui ont été introduites en double, validées et dont les discordances ont été résolues par un nouveau contrôle des registres.

RÉSULTATS : L'ensemble des données comporte 128 808 sujets examinés provenant de 23 laboratoires en Moldavie, des 31 en Mongolie, de 30 en Ouganda et de 23 au Zimbabwe, pour chacun d'entre eux pendant au moins une année calendrier. Parmi tous les sujets examinés, 89 362 ont bénéficié d'un examen de diagnostic, et parmi ceux-ci 13 577 (15,2%) ont été considérés comme des cas. Un résultat positif non-quantifié a été enregistré

chez 1272 (9,4%) de ces derniers. Les résultats très faiblement positifs ont été les plus fréquents au Zimbabwe (8,5%) et les plus rares en Ouganda (1,1%). Au contraire, c'est en Mongolie qu'on a enregistré le plus fréquemment les taux de positivité les plus élevés des frottis (3+). La proportion la plus basse d'un faible degré de positivité a été enregistrée chez les hommes âgés de 15 à 24 ans et la plus élevée chez les femmes âgées de ≥ 65 ans.

CONCLUSION : Il existe des différences frappantes de fréquence du faible degré de positivité entre les quatre pays. Dans l'ensemble, les femmes ont tendance à avoir des décomptes bacillaires plus bas ; la positivité de faible degré est plus fréquente aux âges extrêmes. Ces résultats renforcent la nécessité d'instructions appropriées sur la façon de produire des crachats de bonne qualité pour améliorer le rendement.

RESUMEN

OBJETIVO : Examinar la influencia de la edad y del sexo en la calificación de los resultados de las baciloscopias de esputo.

MARCO DE REFERENCIA : Los laboratorios en Moldavia, Mongolia, Uganda y Zimbabwe.

MÉTODOS : Se capturaron informáticamente los datos sobre muestras representativas de la escala nacional en los registros de laboratorio y se validaron mediante entrada doble, verificación de las discordancias y reverificación cuando fue necesario en los registros físicos.

RESULTADOS : La base de datos comportó 128 808 personas examinadas en 23 laboratorios de Moldavia, los 31 de Mongolia, 30 en Uganda y 23 en Zimbabwe ; cada registro cubría como mínimo un año civil. De todas las personas, 89 362 contaban con un examen diagnóstico y el 15,2% (13 577) cumplía con la definición de caso de tuberculosis. Se consignó un resultado positivo sin cuantificación en 1272 de ellos (9,4%). La mayor

frecuencia de resultados 'bacilos escasos' se encontró en Zimbabwe (8,5%) y la menor frecuencia en Uganda (1,1%). En contraste, Mongolia presentó con mayor frecuencia los resultados con calificaciones positivas más altas de '3+' (43,9%). La proporción más baja de positividad de bajo grado se observó en los hombres entre los 15 y los 24 años de edad y la proporción más alta en las mujeres a partir de los 65 años de edad.

CONCLUSIÓN : Se encontraron diferencias sorprendentes en la frecuencia de la positividad de bajo grado en los cuatro países. Se observó una tendencia hacia recuentos más bajos de bacilos en las mujeres y la positividad de bajo grado fue más frecuente en las edades extremas. Estos resultados refuerzan la necesidad de establecer recomendaciones apropiadas sobre la técnica de recogida muestras de esputo de alta calidad, a fin de mejorar el rendimiento diagnóstico.