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Negative sputum smear results in HIV-positive patients with pulmonary tuberculosis in Lusaka, Zambia

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SUMMARY. **During recruitment to a prospective study of tuberculosis patients in Lusaka, Zambia, 109 had pulmonary disease proven by sputum culture for *Mycobacterium tuberculosis*, of whom 72 were HIV-1 antibody-positive and 37 were HIV- negative. Among these culture-proven cases, 43% of the HIV-positive patients had a negative sputum smear, compared with 24% of the HIV-negative cases. There was a strong trend towards lower grade or negative sputum smear in the HIV-positive group (P=0.003). HIV-positive cases also had lower colony counts on culture and colonies took longer to appear. The findings imply that cases of HIV-associated pulmonary tuberculosis may frequently be missed and emphasise the need for new diagnostic methods.**

RÉSUMÉ. **Lors du recrutement en vue d'une étude prospective de malades tuberculeux à Lusaka, Zambie, 109 avaient une maladie pulmonaire prouvée par culture des crachats pour *Mycobacterium tuberculosis*, dont 72 étaient positifs pour les antigènes VIH-1 et 37 étaient séronégatifs. Parmi les cas prouvés par culture, 43% des malades séropositifs étaient frottis négatifs, comparé à 24% des cas VIH-. Il y avait une forte tendance à un frottis de positivité moindre ou négatif chez les VIH+ (P=0,003). Les cas VIH+ avaient aussi un nombre moindre de colonies à la culture, et les colonies étaient plus lentes à pousser. Ces observations impliquent que l'on peut souvent méconnaître des cas de tuberculose pulmonaire associée à l'infection VIH, et soulignent le besoin de nouvelles méthodes de diagnostic.**

RESUMEN. **En Lusaka, Zambia, en un reclutamiento de enfermos tuberculosos para un estudio prospectivo, 109 sujetos tenían una tuberculosis pulmonar confirmada por presencia de *Mycobacterium tuberculosis* en el cultivo de esputo, de los cuales 72 eran VIH-1 positivos y 37 negativos. En estos casos confirmados por cultivo, el 43% de los pacientes VIH-positivos tenía una baciloscopia negativa, comparado con 24% en los casos VIH-negativos. Se constató una fuerte tendencia hacia los grados más bajos o hacia la negatividad de la baciloscopia en el grupo VIH-positivo (P=0,003). Los casos VIH-positivos tenían también un recuento de colonias más bajo al cultivo y las colonias demoraban más en aparecer. Los resultados sugieren que con cierta frecuencia puede haber una omisión diagnóstica en los casos de tuberculosis pulmonar asociada con infección VIH, y se insiste en la necesidad de nuevos métodos diagnósticos.**

INTRODUCTION

Pulmonary tuberculosis in patients infected with the Human Immunodeficiency Virus (HIV) differs from the disease in immunocompetent people in that it is less often restricted to the upper lobe and less often associated with cavitation.[1,2,3] However, there have been conflicting reports of the effect of concurrent infection with HIV on bacillary load in the sputum of patients with pulmonary tuberculosis.[2–5] This is a critical issue: firstly for diagnosis, as sputum smear is the only method available to confirm the diagnosis in many places where tuberculosis is common: and secondly for infectiousness, because tuberculosis is spread through droplets formed from infected sputum.

We report the bacteriological findings at presentation for a group of tuberculosis patients recruited to a cohort study in Lusaka, Zambia.

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METHODS

249 new adults cases of tuberculosis, comprising a representative sample of all such cases presenting to medical wards and the chest clinic of the University Teaching Hospital, Lusaka, Zambia, were recruited to a prospective study between April and December 1989.[6]

Patients with a productive cough were asked to provide 3 specimens of sputum for examination before anti-tuberculosis therapy was started. These were processed at the Chest Diseases Laboratory of the Ministry of Health of the Republic of Zambia, Chelston, Lusaka.

Sputum was examined for acid, alcohol-fast bacilli on direct smear using auramine stain and fluorescence microscopy with a x25 objective and x6.5 eye pieces. The numbers of bacilli seen were classified as scanty (1+), moderate (2+) or heavy (3+) by comparison with standard smears.

For each sputum sample, duplicate cultures were set up on Lowenstein-Jensen medium and cultures were examined weekly until growth occurred, to a maximum of 8 weeks. The number of colonies seen was recorded: 1–19 (the number was recorded), 20–100 (1+), innumerable discrete colonies (2+), confluent growth (3+).

Some of the sputum results used in the study were obtained from the routine service records, but records were kept separately for a sub-group of 150 of the patients who gave sputum specimens. For this group more detailed information was available on the number of samples given and the outcome of culture. Laboratory procedures were otherwise the same for all patients.

For each patient, the highest figure for both sputum smear and culture and the shortest number of weeks to a positive culture were used in the analysis.

Sera were collected from all patients for examination for HIV-1 antibody by competitive recombinant enzyme linked immunosorbent assay (ELISA) (Wellcozyme, Wellcome Diagnostics, Dartford, Kent) and antiglobulin recombinant ELISA (Dupont de Nemours, Belgium).

All patients with pulmonary disease had a chest X-ray. Radiological features, including the zones involved and the presence or absence of cavities, were recorded by the physician at the time of recruitment, usually before the HIV status and outcome of sputum bacteriology were known.

Ethical approval for the study was given by the Research and Ethics Committee of the University Teaching Hospital, Lusaka.

RESULTS

Of the 249 patients recruited to the study 182 (73%) were HIV-1 antibody-positive by Wellcozyme ELISA; overall findings at presentation are presented elsewhere.[6] The relationship between site of disease and HIV status for the whole group is shown in Table 1.

Not all patients with a productive cough provided all of the 3 sputum samples requested, and the number of specimens provided was known only for the 150 patients in the sub-group whose records were kept separately. In this group, 3 samples or more were provided by 25/39 (64%) of HIV-negative and 60/111 (54%) of HIV-positive patients (χ^2=0.81, df=1; P=0.37). Of a total of 972 cultures set up from the sub-group 138 (14%) were contaminated.

Table 1. Site of disease and HIV status

Site	Number (%) of patients HIV-negative n=67	HIV-positive n=182
Pulmonary	48 (72%)	72 (40%)
Extrapulmonary	11 (16%)	62 (34%)
Both	8 (12%)	48 (26%)

176 patients were considered to have pulmonary disease on the basis of sputum smear and/or culture results, bronchoscopy or radiological findings. In 109 of these (62%) *Mycobacterium tuberculosis* was isolated from sputum. In a further 32 cases the diagnosis was supported by a positive sputum smear (19 smear-positive, culture-negative; 13 smear-positive, culture result not available), and in 8 cases by confirmation of tuberculosis at another site. All positive cultures were identified as *M. tuberculosis*. No isolates of *M. bovis* or other species of mycobacteria were grown from samples collected during this study.

The findings among the 109 patients with positive sputum culture for *M. tuberculosis* are included in this analysis.

Among patients with a positive sputum culture, those who were HIV positive more often had a negative or low grade sputum smear (Table 2 (A), exact test for trend, P=0.003).

Of the 109 patients with a positive sputum culture, 87

Table 2. Bacteriological findings in patients with positive sputum culture for *M. tuberculosis*

	Number (%) of patients HIV-negative	HIV-positive
(A) Smear grade for all patients with positive sputum culture (109 cases)		
	n=37	n=72
0	9 (24%)	31 (43%)
1+	4 (11%)	15 (21%)
2+	3 (8%)	8 (11%)
3+	21 (57%)	18 (25%)
(B) Number of colonies for patients with details of low counts (87 cases)		
	n=29	n=58
1–19	4 (14%)	15 (26%)
20–100	5 (17%)	8 (14%)
innumerable discrete	1 (3%)	10 (17%)
confluent	19 (66%)	25 (43%)
(C) Number of weeks to visible growth (77 cases)		
	n=25	n=52
1–2	7 (28%)	5 (10%)
3–4	12 (48%)	23 (44%)
5–6	5 (20%)	12 (23%)
7–8	1 (4%)	12 (23%)

Table 3. Relation of HIV-1 antibody status to sputum smear grade, number of colonies and time to positive culture for patients with positive sputum culture: crude odds ratios and odds ratios adjusted for presence of cavitation.

	No. of patients	Odds ratio for HIV infection (exact 95% confidence interval) Crude		Adjusted *	
Smear grade (109 cases)					
0	40	1	P=0.004†	1	P=0.137‡
2+	30	0.95	(0.27, 3.50)	1.11	(0.29, 4.52)
3+	39	0.25	(0.08, 0.72)	0.43	(0.11, 1.73)
Number of colonies for patients with details of low counts (87 cases)					
1–19	19	1	P=0.067†	1	P=0.423‡
20-innumerable	24	0.80	(0.14, 4.16)	1.34	(0.20, 10.38)
confluent	44	0.36	(0.07, 1.37)	0.47	(0.09, 2.20)
Number of weeks to visible growth (77 cases)					
1–2	12	1	P=0.024†	1	P=0.181‡
3–4	35	2.62	(0.58, 13.00)	2.35	(0.47, 12.84)
5–8	30	5.33	(1.05, 30.64)	3.59	(0.63, 21.61)

* Adjusted for presence of cavitation; † exact test for trend; ‡ asymptomatic test for trend.

were in the sub-group with detailed results available. In this group colony counts were available for isolates where less than 20 colonies grew, and in 77 of the 87 cases the number of weeks to visible growth was recorded. The results are shown in Table 2 (B,C). Culture of sputum produced fewer colonies in HIV-positive cases, although this difference was not statistically significant (exact test for trend, P=0.14). In HIV-positive cases the cultures took longer to grow (exact test for trend, P=0.01).

Chest X-rays were available for 107 of the 109 sputum culture positive cases. These showed cavitation in 24/37 (65%) of the HIV-negative cases, but in only 25/70 (36%) of the HIV-positive cases (χ^2=7.15; df=1; P=0.008). The total number of zones seen to be involved on X-ray was 4 or more out of 6 in 20/37 (54%) of the HIV-negative patients and 28/70 (40%) of the HIV-positive patients (χ^2=1.41, df=1; P=0.24). 33/37 (89%) HIV-negative patients showed involvement of upper zones compared with only 43/70 (61%) HIV-positive cases (χ^2=7.77; df=1; P=0.005).

When the association between HIV status and sputum smear, number of colonies and time to growth of visible colonies were stratified for presence of cavitation the observed trends were still present, but were less marked and ceased to be statistically significant (Table 3).

DISCUSSION

In this study we have found a lower bacillary load in the sputum of HIV-positive than of HIV-negative tuberculosis patients. HIV-positive patients with positive sputum culture of *M. tuberculosis* more often had low grade or negative sputum smears, and cultures had fewer colonies and took longer to grow. Furthermore, the occurrence of a low bacillary load in the sputum appeared to be partly related to absence of cavitation on X-ray.

This supports the findings of our earlier cross-sectional study in Lusaka,[2] and of a study in Haiti,[4] that HIV-positive patients with pulmonary tuberculosis often have a negative sputum smear, but contrasts with other careful studies, including one in Nairobi[7] and one in Haiti.[5] The most likely explanation is that, in contrast to the present study, patients in both these studies were referred for inclusion specifically on the basis of earlier diagnosis or clinical suspicion of pulmonary tuberculosis, a procedure which may have selected against patients with a lesser degree of pulmonary involvement and without cavitation on X-ray, as the authors acknowledge.

HIV-positive patients with pulmonary tuberculosis vary with regard to level of immunodeficiency and prior exposure to tuberculosis. It is probable that these differences account for the spectrum of disease observed, ranging from cavitary, sputum smear positive disease (similar to typical, post-primary disease in HIV-negative patients) to atypical, non-cavitary, sputum smear negative forms. In some HIV-positive cases the pattern of disease is suggestive of primary infection, but it is not known whether this is indeed the result of new infection, or rather of impaired immunity with reactivation of prior infection.

In this study, the HIV-positive patients gave all 3 sputum samples requested less often than did the HIV-negative patients. This may have led to an underestimation of the proportion with bacilli detectable on smear microscopy in the HIV-positives, but probably indicates that HIV-positive patients more often had a dry cough.

Our findings re-emphasise the danger that the diagnosis of tuberculosis may be missed or delayed in patients with HIV,[8] but more intensive use of the sputum smear may not provide the solution. Culture for *M. tuberculosis* is seldom available in Africa and, in any case, delays in obtaining results limit the value of culture in diagnosis. Clearly, new, simple, quick diagnostic methods are needed.

Where tuberculosis and HIV are common, a pragmatic approach is suggested. Since HIV-positive patients with pulmonary tuberculosis may have a negative sputum smear, suspected cases who fail to respond to broad-spectrum antibiotics may be started on a trial of antituberculosis therapy.

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