

*Diagnostic yield and grading of acid-fast bacilli from serial sputum smears of HIV-infected and uninfected tuberculosis patients in routine microscopy laboratory in Ouagadougou, Burkina Faso**

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Abstract

We compared 100 HIV-infected and 100 non-infected adult patients with pulmonary tuberculosis (TB) to evaluate the association between the HIV status and the microscopic yield, and between the HIV status and the grading of acid-fast bacilli (AFB) sputum smears. We stained specimens by Ziehl-Neelsen hot method. The first serial sputum smears diagnosed 89% HIV-infected and 94% uninfected. The additional yields of the second and third sputum smears identified respectively 10% and 1% among the HIV-infected against 5% and 1% among the patients without HIV. Considering grading of AFB, the HIV-positive patients were more scanty and less positive 2+ and 3+ at the first ($P=0.089$) and the second sputum smears ($P=0.010$). For the second AFB-smears grading, there was a significant difference between HIV-infected and uninfected among the males ($P=0.031$), the group of age ranging from 15 to 44 years old ($P=0.003$) and among the ambulatory patients ($P=0.015$); when we analyzed data for subgroups by HIV serological status, the difference was not significant in the results among the females ($P=0.417$) and the TB-hospitalized ($P=0.501$). In conclusion, the morning sputum smears improved the diagnostic yield in both HIV-infected and uninfected patients. However, globally the frequency of scanty was significantly associated with HIV serological status.

Keywords: Tuberculosis - HIV infection –Microscopy-Grading acid-fast bacilli; HIV infection-Tuberculosis-Burkina Faso.

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INTRODUCTION

Tuberculosis (TB) is a major public health problem in the world. According to the World Health Organization (WHO), 8 millions cases of the disease occur each year, resulting 3 millions deaths. HIV infection accounts for much of the recent increase in the global TB incidence. In sub Saharan Africa, the majority of TB patients were HIV-infected.

Direct microscopy remains the main method of TB diagnosis in the majority of laboratories since the adoption of the Directly Observed Therapy, Short-course (DOTS) strategy. Thus, in Burkina Faso, the guidelines proposed by the National Tuberculosis Program (NTP) are based on the recommended international standards: three sputum specimens (spot-morning-spot) are recommended for all TB-suspect patients complaining about cough for more than two weeks; number of acid-fast bacilli (AFB) is quantified¹. This method allows the detection of AFB in approximately 80% of cases². Nevertheless, it is reported that in HIV/TB-infected patients the detection by direct microscopy becomes less effective.^{3,4,5}

The Microscopic yields of sputum smears for the diagnostic of TB were evaluated in a setting with high HIV prevalence; but no descriptions have so far been made on the grading of AFB serial sputum smears of HIV-associated TB. This prompted us to conduct the present study among the adult patients presenting with initial newly-diagnosed pulmonary TB in Ouagadougou, Burkina Faso.

MATERIAL AND METHODS

Setting

The study was carried out in the laboratory of the National Tuberculosis Centre (NTC) in Ouagadougou, Burkina Faso. This laboratory daily detects new cases, follows the bacteriological response to chemotherapy of TB patients living in the town and strongly supports the activities of the NTP.

Study population

TB-suspect patients aged from 15 years were recruited between April 2006 and May 2007 and their sputum smears were subjected to direct smear microscopy by Ziehl-Neelsen hot method; 100 new pulmonary tuberculosis patients with HIV and 100 others without HIV infection were selected (the denominator had not interest us; it was not to determine HIV seroprevalence). These two groups were stratified by age, sex and ambulatory or hospitalized status. Each patient provided a set of 3 viscous or mucopurulent successive sputum specimens according to the national guidelines (spot-morning-

spot) for analyses. They were declared tuberculosis patients so at least two sputum AFB-smears were positive.

Direct microscopy

Smears were prepared and stained by hot Ziehl-Neelsen method as recommended by the IUATLD¹. The quantification scale used was as follow: negative for 0 acid-fast bacillus (AFB)/100 fields; "Scanty" for 1-9 AFB/100 fields; "+" for 10-99 AFB/100 fields; "++" for 1-10 bacilli/field and "+++" for more than 10 AFB/field.

Supervision visits and rechecking were performed as external quality assurance. The items concerned to evaluate the performance of the laboratory were the sputum smears quality, their conservation, smearing, staining, reading and interpretation. During supervision visits done on a six month basis a sample of 15 smears were collected in a blinded way and rechecking was performed by a first controller in the central level. Discordant smears were rechecked by a second controller and results were sent back to the centre being controlled with recommendations and the discordant smears to be confirmed.

HIV tests

Prior to HIV test, the positive AFB pulmonary tuberculosis patients were allowed to counseling about the test. After free consent, 4 mL venous blood was collected from each patient. The blood samples were first tested using the Determine® HIV-1/2 (Abbott Diagnoses). Afterwards the positive samples were confirmed by ImmunoComb II HIV 1/2 BisSpot (PBS Organics) test. The results were confidentially transmitted to patients.

Statistical analysis

The results were analyzed using SPSS 9.0. Linear-by-linear association and Likelihood Ratio were used to interpret the values. The statistical significance was set at $P < 0.05$.

RESULTS

Of 200 pulmonary tuberculosis patients, 135 (67.5%) were men, 103 (51.5%) aged from 15 to 44 years old and 66 (33.0%) were hospitalized.

No major errors exceeding the acceptance number predetermined by the NTP were detected among the sample rechecked during the period of the study.

Globally, *i.e.* without considering HIV-infection status, 183 (91.5 %) patients were diagnosed by the first smear, 15 (7.5%) were negative in the first but had a positive second specimen and 2 (1 %) patients had two negative specimens followed by a positive third smear. Among HIV-infected group, the first, second and third sputum smears detected 89 (89%), 10 (10%) and 1 (1%) cases respectively, against 94 (94%), 5 (5%) and 1 (1%) among HIV-uninfected patients respectively.

Data on the HIV serological status and grading of serial diagnostic AFB-smears are shown in Table 1. Roughly, the first serial diagnostic sputum specimen showed higher frequencies of low positive smears (scanty and 1+) in the HIV-infected group and within the HIV- negative group more than 50% of the smears had high positive gradations, 2+ and 3+. However, the difference was not significant ($P=0.089$). Among the morning sputum specimens (second sputum specimen), the frequency of scanty smears was higher in the TB-HIV-positive, while the frequencies of 2+ and 3+ were higher in the HIV-negative ($P=0.010$).

On Table 2, concerning the AFB-microscopy grading of second sputum smears, the analysis revealed a significant difference between HIV-infected and uninfected TB-patients among the males ($P=0.031$), the group of age ranging from 15 to 44 years old ($P=0.003$) and among the ambulatory patients ($P=0.015$); none significant difference between the two HIV serological status among the females ($P=0.417$), the patients aged more than 44 years old and the TB-hospitalized ($P=0.501$).

DISCUSSION

Since the adoption of the DOTS, direct microscopy remained an accepted method for the detection of TB among suspected patients. In the present study, we analyzed three consecutive samples to evaluate the diagnostic yield and investigate the

statistical association between grading obtained by AFB microscopy examination and the HIV serological status. According to the present findings, the morning samples were more sensitive in the detection of TB. This was also observed by several studies. As examples, Ipuge *et al.*⁶, found diagnostic yields of 83.4, 12.2 and 4.4% for the first, the second and the third serial diagnostic sputum specimens respectively in a similar study performed in Tanzania. However, studies done in Zambia and in India revealed the diagnostic yields of 77, 15, 7.9%⁷ and 88.7 10.5 and 0.8%⁸. These authors suggested the examination of two consecutive smears to diagnose pulmonary TB in suspected patients.^{9,10}

Taking into consideration the HIV serological status, studies suggested that the AFB-smears could be less sensitive for the detection of pulmonary TB in the group of HIV positive subjects^{3,4,5}. In our study, the diagnostic yield of the first sputum samples within the HIV-positive group was lower than that observed within the HIV-negative group (89% versus 94%). However, statistically the difference was not significant between the two groups ($P>0.05$). These results agree with those of other authors who mentioned that the diagnostic yields by direct microscopy were not significantly related to the HIV serological status^{11,12}. The difference between yields can be associated to reduced cavities number in lungs; therefore, the patients secrete bacilli intermittently¹³. Only pulmonary radiography of the patients shall allow observing the anomalies.

Globally, at the first and the second serial diagnostic sputum smears, the HIV-infected patients were scantier and less positive particularly at the gradations 2+, and 3+, than the non-HIV-infected. After stratification by variables studied, contrary to the HIV-infected men and ambulatory patients, the HIV-infected among females and hospitalized patients harbored more for any grading sputum smears for the second smears than the HIV-negative (TABLE 2). This indicates a high rate of HIV infection among female subjects and a high concentration of HIV-infected TBs in hospitals. But as HIV co-infected patients have more positive sputum smears may be surprising. We would have expected to regain proportionally more important in gradations 1-9 and 1+. In all probability, our

Table 1 - Association between HIV serological status and the gradations of AFB positive sputum smears.

Sputum series	HIV status	AFB positive smears gradations n (%)					P value
		Total	1-9	1+	2+	3+	
First series	+	89 (48.6)	22 (56.4)	20 (55.6)	17 (47.2)	30 (41.7)	0.089
	-	94 (51.4)	17 (43.6)	16 (44.4)	19 (52.8)	42 (58.3)	
	Total	183 (100.0)	39 (100.0)	36 (100.0)	36 (100.0)	72 (100.0)	
	Total	183 (100.0)	Scanty	All positivity			
	+	89 (48.6)	22 (56.4)	67 (46.5)			0.237
	-	94 (51.4)	17 (43.6)	77 (53.5)			
	Total	183 (100.0)	39 (100.0)	144 (100.0)			
Second series	Total	188 (100.0)	1-9	1+	2+	3+	P value
	+	90 (47.9)	23 (71.9)	13 (48.1)	16 (40.0)	38 (42.7)	0.010
	-	98 (52.1)	9 (28.1)	14 (51.9)	24 (60.0)	51 (57.3)	
	Total	188 (100.0)	32 (100.0)	27 (100.0)	40 (100.0)	89 (100.0)	
Total	188 (100.0)	Scanty	All positivity			P value	
	+	90 (47.9)	23 (71.9)	67 (42.9)			0.003
	-	98 (52.1)	9 (28.1)	89 (57.1)			
	Total	188 (100.0)	32 (100.0)	156 (100.0)			

Notes: P value linear – by – linear association; Scanty = 1-9 AFB/Field; All positivity = (1+, 2+, 3+).

patients had been diagnosed early. Considering the sex, some investigators stipulated that the microscopy technique used was itself biased as they observed a difference in detecting AFB between women and men, the female sex being underprivileged.¹⁴

CONCLUSION

The morning sputum smears improved the diagnostic yield in both HIV-infected and

uninfected patients. When we analyzed data for subgroups by HIV serological status, the difference was significant in the second sputum smears results, globally, among the males ($P=0.031$), the group of age ranging from 15 to 44 years old ($P=0.003$) and among the ambulatory patients ($P=0.015$); this does not cast doubt on the microscopic diagnosis of TB because, as the patient is weak or strongly AFB positive, the chemotherapy remains the same.

Suporte diagnóstico e classificação de bacilos álcool-ácido resistentes em baciloscopia de pacientes infectados pelo HIV e não infectados pela tuberculose em microscopia de rotina laboratorial em Ouagadougou, Burkina Faso

Resumo

Cem pacientes infectados pelo HIV e 100 pacientes adultos não-infectados pela tuberculose pulmonar (TB) foram comparados no intuito de avaliar a associação entre o status dos pacientes HIV+ e o

Table 2 - Association between HIV serological status and the gradations of AFB positive second sputum smears after stratification by sex, age group, hospitalization and ambulatory status.

Variables studied	HIV status	Microscopy: number and frequency of AFB positive smears at the second series of sputum					P
		Total	1-9	1+	2+	3+	
Male sex	+	52 (40.0)	13 (65.0)	6 (40.0)	10 (34.5)	23 (34.8)	0.031
	-	78 (60.0)	7 (35.0)	9 (60.0)	19 (65.5)	43 (65.2)	
	Total	130 (100.0)	20 (100.0)	15 (100.0)	29 (100.0)	66 (100.0)	
Female sex	+	38 (65.5)	10 (83.3)	7 (58.3)	6 (54.5)	15 (65.2)	0.417
	-	20 (34.5)	2 (16.7)	5 (41.7)	5 (45.5)	8 (34.8)	
	Total	58 (100.0)	12 (100.0)	12 (100.0)	11 (100.0)	23 (100.0)	
15-44 years	+	70 (50.4)	20 (80.0)	11 (55.0)	10 (35.7)	29 (43.9)	0.003
	-	69 (49.6)	5 (20.0)	9 (45.0)	18 (64.3)	37 (56.1)	
	Total	139 (100.0)	25 (100.0)	20 (100.0)	28 (100.0)	66 (100.0)	
45 ≥ years	+	20 (40.8)	3 (42.9)	2 (28.6)	6 (50.0)	9 (39.1)	0.824*
	-	29 (59.2)	4 (57.1)	5 (71.4)	6 (50.0)	14 (60.9)	
	Total	49 (100.0)	7 (100.0)	7 (100.0)	12 (100.0)	23 (100.0)	
Hospitalization	+	40 (67.8)	13 (68.4)	7 (77.8)	9 (75.0)	11 (57.9)	0.501
	-	19 (32.2)	6 (31.6)	2 (22.2)	3 (25.0)	8 (42.1)	
	Total	59 (100.0)	19 (100.0)	9 (100.0)	12 (100.0)	19 (100.0)	
Ambulatory	+	50 (38.8)	10 (76.9)	6 (33.3)	7 (25.0)	27 (38.6)	0.015*
	-	79 (61.2)	3 (23.1)	12 (66.7)	21 (75.0)	43 (61.4)	
	Total	129 (100.0)	13 (100.0)	18 (100.0)	28 (100.0)	70 (100.0)	

Notes: P value linear – by – linear association; * likelihood Ratio

suporte microscópico laboratorial e entre o status dos pacientes HIV+ e da classificação dos bacilos álcool-ácido resistentes (AFB) pela baciloscopia. As amostras foram coradas pelo método de Ziehl-Neelsen. A primeira série baciloscópica diagnosticada foi de 89% infectados pelo HIV e 94% não infectados. O aumento da produtividade da segunda e terceira baciloscopia identificaram respectivamente 10% e 1% entre os infectados pelo HIV contra 5% e 1% entre os pacientes sem HIV. Considerando a classificação da AFB, os pacientes HIV positivos foram mais escassos e menos positivos 2+ e 3+ na primeira baciloscopia ($P = 0,089$) e na segunda baciloscopia ($P = 0,010$), respectivamente. Para a segunda classificação de esfregaços AFB, houve uma diferença significativa entre os HIV-infectados e não infectados quando analisamos os dados de subgrupos por status sorológico entre os homens ($P = 0,031$), o grupo de idade entre 15 a 44 anos ($P = 0,003$) e entre os pacientes ambulatoriais ($P = 0,015$). Contudo, a diferença não foi significativa nos resultados entre as mulheres ($P = 0,417$) e nas TB-hospitalizadas ($P = 0,501$). Em conclusão, a baciloscopia melhorou o suporte diagnóstico em ambos os infectados pelo HIV e pacientes não infectados. No entanto, globalmente, a frequência dos valores amostrais estava significativamente associada com o status sorológico do HIV.

Palavras-chave: Tuberculose - HIV- Microscopia; HIV-Tuberculose -Burkina Faso.

REFERENCES

- 1 ENARSON, D.A. et al. *Prise en charge de la tuberculose*. 5.ed. Paris: IUATLD, 2000.
- 2 TOMAN, K. *Tuberculosis case finding and chemotherapy: question and answers*. Geneva: WHO, 1979.
- 3 ELIOT, A.M. et al. *The impact of HIV on infectiousness of pulmonary tuberculosis: a community study in Zambia*. *AIDS*, London, v.7, p.981-987, 1993.
- 4 SAMB, B. et al. *Risk factors for negative sputum acid-fast bacilli smears in pulmonary tuberculosis: results from Dakar, Senegal, a city with low HIV seroprevalence*. *Int. J. Tuberc. Lung Dis.*, Paris, v.3, p.330-336, 1999.
- 5 HARGGREAVES, N.J. et al. *What causes smear-negative pulmonary tuberculosis in Malawi, an area of high HIV seroprevalence?* *Int. J. Tuberc. Lung Dis.*, Paris, v.5, p.113-122, 2001.
- 6 IPUGE, Y.A.I.; RIEDER, H.L.; ENARSON, D.A. *The yield of acid-fast bacilli from serial smears in routine microscopy laboratories in rural Tanzania*. *Trans. R. Soc. Trop. Med. Hyg.*, London, v.90, p.258-261, 1996.
- 7 WALKER, D. et al. *An incremental cost-effectiveness analysis of the first, second and third sputum examination in the diagnosis of pulmonary tuberculosis*. *Int. J. Tuberc. Lung Dis.*, Paris, v.4, p.246-251, 2000.
- 8 GOPI, P.G. et al. *Smear examination of two specimens for diagnosis of pulmonary tuberculosis in Tuuvallur District, South India*. *Int. J. Tuberc. Lung Dis.*, Paris, v.8, p.824-828, 2004.
- 9 CRAMPIN, A.C. et al. *Comparison of two versus three smears in identifying culture-positive tuberculosis patients in rural African setting with high HIV prevalence*. *Int. J. Tuberc. Lung Dis.*, Paris, v.5, p.994-999, 2001.
- 10 HARRIES, A.D. et al. *Screening tuberculosis suspects using two sputum smears*. *Int. J. Tuberc. Lung Dis.*, Paris, v.4, p.36-40, 2000.
- 11 LONG, R. et al. *The impact of HIV on the usefulness of sputum smears for the diagnosis of tuberculosis*. *Am. J. Public Health*, Washington, DC, v.81, p.1326-1328, 1991.
- 12 SMITH, R.L. et al. *Factors affecting of acid-fast sputum smears in patients with HIV and tuberculosis*. *Chest*, Northbrook, v.106, p.684-686, 1994.
- 13 BAH, B. et al. *Dans une ville d'Afrique de l'Ouest, indices évoquant l'existence d'une tuberculose pulmonaire à bacilloscopie négative*. *Int. J. Tuberc. Lung Dis.*, Paris, v.6, p.592-598, 2002.
- 14 KIVIHYA-NDUGGA, L.E. et al. *Sex-specific performance of routine TB diagnostic tests*. *Int. J. Tuberc. Lung Dis.*, Paris, v.9, p.294-300, 2005.

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