

## Smear-negative, culture-positive pulmonary tuberculosis among patients with chronic cough in Cotonou, Benin

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### SUMMARY

**SETTING:** The main tuberculosis (TB) centre in Benin, West Africa, where only 2% of adult pulmonary TB cases are sputum smear-negative, all other pulmonary cases being smear-positive.

**OBJECTIVES:** To assess the burden of smear-negative, culture-positive pulmonary TB among TB suspects in Cotonou, and to estimate the total number of non-smear-positive TB cases at country level.

**DESIGN:** For 1 year, one morning sputum culture was performed for every TB suspect (cough lasting >3 weeks, as defined in Benin's national guidelines) with three negative sputum smears (fluorescence technique).

**RESULTS:** Of 214 TB suspects for whom culture was

performed, only 22 smear-negative, culture-positive cases were identified. During the same period, 831 sputum smear-positive cases were diagnosed. Culture therefore contributed only 2.6% of the total number of bacteriologically proven cases.

**CONCLUSION:** These results show the relatively low input of culture in TB diagnosis among chronic coughers in Cotonou, Benin, and demonstrates that the expected number of non-smear-positive TB cases in Benin is probably much lower than the World Health Organization's current annual estimates.

**KEY WORDS:** tuberculosis; microscopy; culture; Benin

IN MANY COUNTRIES, smear microscopy remains the only tool for the bacteriological diagnosis of tuberculosis (TB). However, compared to culture, the sensitivity of smear microscopy is considered to be lower, stressing the necessity of using another test and/or algorithm to diagnose smear-negative pulmonary TB patients.

In Benin, West Africa, as in most low-resource settings, smear-negative pulmonary TB is generally diagnosed using the following algorithm: 1) at least three sputum specimens negative for acid-fast bacilli (AFB), 2) radiographic abnormalities consistent with active pulmonary TB, 3) no response to a course of non-specific antibiotics, and 4) decision by a clinician to treat with a full course of anti-tuberculosis chemotherapy.<sup>1</sup>

According to the World Health Organization's (WHO's) 2008 report,<sup>2</sup> Benin is one of the African countries with the highest detection rates for sputum smear-positive TB (86%), but with a low detection rate (44%) for TB all forms. This is related to the lowest proportion (7%) of smear-negative pulmonary TB over all new pulmonary cases reported in the Africa region (average 41%, range 7–76).

In the present study, we 1) assess the burden of smear-negative but culture-positive pulmonary TB among TB suspects (i.e., cough lasting >3 weeks, as defined in Benin's national guidelines) in Cotonou, the largest city in Benin, 2) evaluate the missed diagnosis of sputum smear-negative pulmonary TB, and 3) discuss the expected number of non-sputum smear-positive TB cases in Benin.

### MATERIALS AND METHODS

#### Setting

The study was performed at the National Reference Hospital for TB (Centre National Hospitalier de Pneumo-Phtisiologie [CNHPP]) in Cotonou, Benin. The National Reference Mycobacteria Laboratory (Laboratoire de Référence des Mycobactéries [LRM]), is situated in this hospital, and fluorescence microscopy has been systematically performed for all TB suspects for more than 20 years. Culture is routinely performed for research purposes, but not for routine diagnosis in this laboratory, and at the CNHPP the above algorithm is used for the diagnosis of sputum smear-negative pulmonary TB. The percentage of

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smear-negative pulmonary TB cases among all pulmonary cases in 2006 was 6% in CNHPP, while for all the other TB centres in Benin it was 7%.

### Patients

Between August 2006 and August 2007, patients were prospectively and consecutively recruited. Inclusion criteria were cough for  $\geq 3$  weeks (regardless of other symptoms), age  $\geq 15$  years and three sputum samples (spot-morning-spot) with smear-negative results. Patients were excluded if they had previously received treatment for TB. For each patient, demographic data were collected and chest X-ray, human immunodeficiency virus (HIV) serology testing after counselling and culture on the morning sputum specimen were performed. For the purpose of this study, a full 15-day course of erythromycin was then administered to the patients, who were encouraged to return after treatment. Those who did not return were not traced unless they were culture-positive.

Depending on treatment results, smear microscopy was performed again on three sputum samples (spot-morning-spot). If the results were again negative, the patient was clinically and radiologically assessed by a physician who decided whether or not to treat the patient for TB using the routine algorithm. Inclusion steps are summarised in Figure 1.

For the purposes of analysis, the results among the study population were compared with the number of

new sputum smear-positive patients diagnosed at the CNHPP during the same period. As smear examination at this reference centre is performed on all patients with cough, regardless of duration, the definition of 'TB suspect' is much wider than in the official guidelines.

### Smear microscopy

For each sputum sample obtained, a smear was performed, air-dried, heat-fixed and stained using 0.3% auramine for 10 min, 74% alcohol containing 1% hydrochloric acid for 4 min and 0.1% potassium permanganate for 30 s.<sup>3</sup> Apart from standardised quality control of all reagents prepared, two systems are routinely used to ensure the quality of fluorescence microscopy performed in the laboratory:

- 1 Daily quality control: every morning a known smear-negative and a known 1+ smear-positive sample are stained and read before routine work begins.
- 2 A rereading quality control system: every week, a supervisor technician randomly selects five routine slides. These slides are restained and read blind by an experienced technician not involved in routine fluorescence microscopy during the control period. In case of discrepancy between the first control and routine work, another technician rereads the same slide on the same day. All the results are reviewed during the quarterly supervisory visits.

### Culture

The morning sputum sample was decontaminated using the modified Petroff method and cultured in 7H9 and on Löwenstein-Jensen (LJ) media. All colonies grown were confirmed using the paranitrobenzoic acid and catalase test.<sup>4</sup>

### Human immunodeficiency virus testing

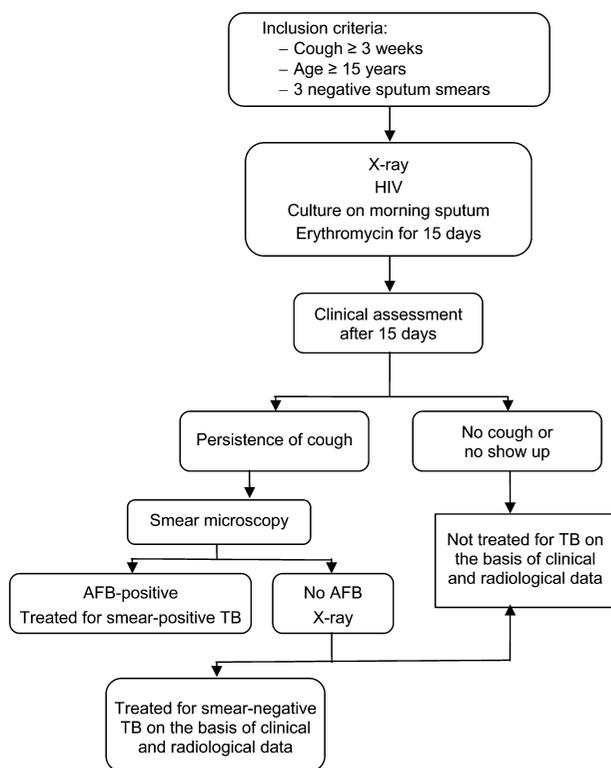
HIV testing was performed using a rapid test (Determine HIV-1/2®, Abbott Diagnostics, Chicago, IL, USA). Seropositive samples were confirmed by a discriminatory HIV1/2 test (Genie II HIV1/HIV2®, Biorad, Marnes-la-Coquette, France). All laboratory tests were performed at the LRM.

### Data handling

Data were electronically captured, validated by double entry and compared using EpiData Entry software version 3.1 (EpiData Association, Odense, Denmark, <http://www.epidata.dk>); analysis was performed using EpiData Analysis (version 2.2, EpiData).

### Ethical considerations

All patients gave informed consent. All bacteriologically proven TB patients were treated for TB. The study was approved by the Board of the Benin National TB Programme (NTP).



**Figure 1** Algorithm for patient inclusion. HIV = human immunodeficiency virus; AFB = acid-fast bacilli; TB = tuberculosis.

## RESULTS

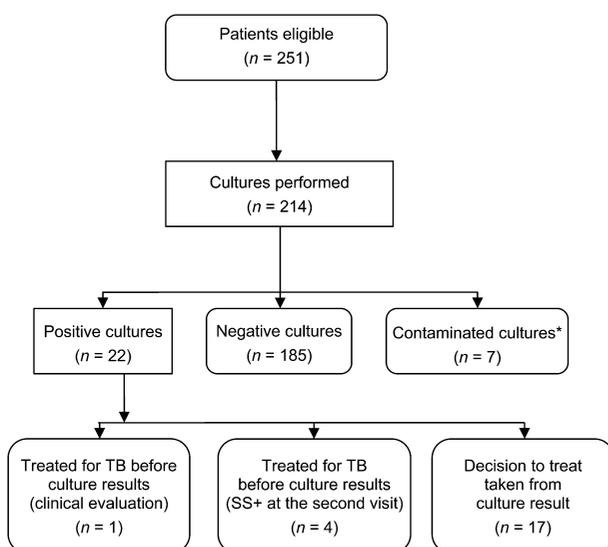
In total, 251 patients were eligible for the study; culture results were available for 214 (for 37 patients the sputum for culture was lost; the sex and age distribution of the missing cases were not different from those for whom culture was performed). Culture was positive for 22 (10.3%), negative for 185 (86.4%) and contaminated for seven (3.3%). The results were identical for both culture methods, except for two sputum specimens where the culture was positive on liquid media but negative on solid media. One culture-positive patient was diagnosed as having smear-negative TB on the basis of clinical and radiological data and put on anti-tuberculosis treatment before the culture results became available.

Overall, 83 patients returned for the second visit after a 15-day course of erythromycin; of these, four had positive smears at the second visit and were culture-positive at the first visit (Figure 2). The decision to treat was always taken before culture results were available; retrospectively, not a single patient with negative culture was treated for TB. During the study period, of 4662 suspects who underwent microscopy examination for diagnostic purposes at the CNHPP, 831 (18%) new smear-positive cases were diagnosed.

HIV serology results were available for 208 patients: 91 (43.8%) were positive. Of 164 patients with an interpretable culture result, 10/75 (13%) HIV-positive patients and 7/89 (8%) HIV-negative patients were smear-negative, culture-positive; the difference was non-significant. Of the total 22 culture-positive cases (Figure 2), 10 were HIV-positive.

## DISCUSSION

Among the 22 culture-positive cases, four were smear-positive at the second visit (TB treatment was initi-



**Figure 2** Bacteriological results. \*Contaminated on both culture methods. TB = tuberculosis; SS+ = sputum smear-positive.

ated before culture results), and one patient was treated immediately after the first consultation on the basis of clinical and radiological signs. During the same period, 831 sputum smear-positive cases were diagnosed. The incremental number of bacteriologically proven cases of TB who would not have been treated if culture had not been performed is therefore only 17, i.e., 2%.

No special technique was used for the performance of microscopy examination, as the objective was to assess the burden of smear-negative, culture-positive pulmonary TB among TB suspects in Cotonou under routine conditions. The fluorescence technique has been used routinely for many years in CNHPP, with regular rereading quality control.

The high sensitivity of the microscopy examinations observed here is probably due to the use of fluorescence microscopy, which usually has higher sensitivity than the Ziehl-Neelsen technique.<sup>5</sup> It is also certainly due to the high quality of the work of the laboratory technicians and the fact that in Benin, as in a lot of African settings, many patients seek care late and already have extensive pulmonary lesions when they present at a health centre. Taking culture as the gold standard, this high sensitivity is similar to that reported in other African countries for HIV-positive or -negative patients: 98% in Uganda,<sup>6</sup> 89% in Kenya<sup>7</sup> and 90% in Zimbabwe.<sup>8</sup> However, the sensitivity of microscopy is not always as high among HIV-positive patients in other sub-Saharan African countries.<sup>9,10</sup> It should also be noted that the sensitivity of smear microscopy in Africa is always much higher than in industrialised countries, where usually less than 50% of culture-positive sputum samples are smear-positive.<sup>11</sup> This is very probably related to the severity of the disease: in developing countries, there may be significant delays before patients seek treatment.<sup>12</sup>

Among the 208 patients with chronic cough who had three negative sputum smear examinations, 91 (43.8%) were HIV-positive, a very high proportion compared to the HIV positivity rate (17%) found among patients treated for TB and the 2% estimate in the general population, but much lower than in Zimbabwe (83%),<sup>8</sup> showing once again that the TB and HIV epidemics are much lower in Benin than in Southern Africa. The rates of *M. tuberculosis* culture positivity were not significantly higher among HIV-positive patients (13%) than in HIV-negative patients (8%), further evidence of the high sensitivity of smear microscopy in Cotonou, even among HIV-positive patients.

This study has several limitations: 1) only one sputum was cultured; however, morning sputum samples have been shown in many studies to contain more bacilli.<sup>13,14</sup> 2) The culture technique may not have been optimal; however, the LRM has more than 20 years of experience, with regular visits from the Antwerp Supranational Laboratory; furthermore, two different

media (solid and liquid) were used to increase the sensitivity of the test. 3) The study was performed at the CNHPP, the national referral hospital for TB, and the situation in the rest of the country could be different from that in Cotonou. Nevertheless, the quality of the microscopy network within the NTP is regularly assessed and is generally excellent. 4) Although culture results were missing for 37 patients, and for seven it was contaminated, the sex and age distribution of the missing cases were not statistically different, and we do not expect any bias from the non-inclusion of these patients. 5) Finally, the official definition of a TB suspect was strictly adhered to in this study (cough for >3 weeks). However, as the CNHPP is a referral centre, the policy is to look for AFB in every patient with cough, regardless of the duration of cough. Clearly many microscopy-positive TB patients (the proportion is unknown) complained of cough of <3 weeks, and it is likely that more smear-negative, culture-positive cases would have been diagnosed if culture had been performed for every patient who underwent microscopy. Nevertheless, considering the limited incremental yield provided by culture among patients with chronic cough, it is unlikely that many more cases of smear-negative, culture-positive TB would have been detected among the total number of patients with cough. We therefore believe that the results of this study are reliable and that there are several important consequences for Benin's NTP.

First, large-scale implementation of culture in Benin does not seem necessary to increase the detection of pulmonary TB cases (<2%). This is good news, considering the complexity of the test and the infrastructure, costs and well-trained, motivated technicians culture requires. Second, the ratio of 1 smear-positive to 1.3 non-smear-positive patients used by the WHO to estimate the total number of expected TB cases<sup>2</sup> appears excessively high, and it is necessary to revise these numbers to deal with more realistic figures. Third, given the high rate of HIV positivity among patients with a cough of >21 days, even non-smear-positive patients should be tested for HIV and those who are positive addressed to the appropriate clinical service.

## CONCLUSION

As stated by Mitchison, 'smear examination of several specimens from each patient is almost as efficient as culture examinations in clinics in developing countries'.<sup>12</sup> This study confirms that this strategy is still

working in Benin and that the development of culture for routine diagnosis does not appear to be a priority. The very low percentage of sputum smear-negative, culture-positive patients corresponds to NTP reports, and the WHO may need to revise its estimations of the number of non-sputum smear-positive cases expected in Benin.

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## R É S U M É

**CADRE :** Au principal centre antituberculeux au Bénin, en Afrique de l'Ouest, seulement 2% des tuberculoses (TB) pulmonaires sont à microscopie négative chez les patients adultes.

**OBJECTIF :** Evaluer le nombre de cas de TB pulmonaire à microscopie négative mais culture positive à Cotonou et estimer le nombre global des cas de TB pulmonaire à microscopie négative attendus au niveau national.

**MÉTHODES :** Pour cette étude, pendant un an, on a effectué systématiquement une culture chez tous les suspects (selon les recommandations nationales, patients présentant une toux chronique de plus de 3 semaines) avec trois examens microscopiques négatifs (sur technique de fluorescence). La culture a été faite sur le premier crachat du matin.

**RÉSULTATS :** Sur 214 suspects pour lesquels on a fait une culture, on n'a trouvé que 22 cas à culture positive, alors que pendant la même période 831 cas à frottis positifs ont été diagnostiqués. La culture a ainsi contribué pour 2,6% seulement à l'identification des cas prouvés par la bactériologie.

**CONCLUSION :** Nos résultats montrent que la culture joue un rôle peu important dans le diagnostic de la TB chez les patients présentant avec une toux chronique à Cotonou, Bénin. Par ailleurs, le nombre de cas de TB qui ne sont pas à frottis positifs publié annuellement par l'Organisation Mondiale de la Santé semble beaucoup trop élevé.

## R E S U M E N

**MARCO DE REFERENCIA:** El principal centro de atención de la tuberculosis (TB) en Benin, África occidental, donde solo 2% de los casos de TB pulmonar presentan baciloscopia negativa del esputo.

**OBJETIVOS:** Evaluar la proporción de casos de TB pulmonar con baciloscopia negativa y cultivo de esputo positivo en los pacientes con presunción clínica de TB en Cotonou y evaluar el número total de casos de TB sin baciloscopia positiva que se pueden prever a escala nacional.

**MÉTODO:** Durante un año, se practicó el cultivo de una muestra matinal de esputo en cada paciente con presunción clínica de TB (tos de >3 semanas de duración, según lo definen las directrices nacionales) y tres baciloscopias de esputo negativas en el examen por microscopía de fluorescencia.

**RESULTADOS:** De los 214 pacientes con presunción de TB en quienes se practicó cultivo del esputo, solo se detectaron 22 casos con cultivo positivo y baciloscopia negativa. Durante el mismo tiempo se diagnosticaron 831 casos de TB con baciloscopia positiva. Por lo tanto, el cultivo solo contribuyó con 2,6% de los casos confirmados bacteriológicamente.

**CONCLUSIÓN:** Estos resultados ponen en evidencia el escaso aporte de los cultivos al diagnóstico de la TB en los pacientes tosedores crónicos en Cotonou; además, se calcula que el número de casos de TB sin baciloscopia positiva en Benin puede ser mucho más bajo que los actuales cálculos anuales de la Organización Mundial de la Salud.