Diagnosis of tuberculosis in developing countries in the era of high HIV transmission; alternative approaches
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Citation for published version (APA):
Yassin, M. A. (2005). Diagnosis of tuberculosis in developing countries in the era of high HIV transmission; alternative approaches Amsterdam: Rozenberg
Summary

Tuberculosis is a serious health problem in the world and one of the most common infectious causes of morbidity and mortality among adults in developing countries. The situation of TB is compounded by poverty and the spread of HIV. TB control programmes rely on early diagnosis of infectious cases and availability of adequate chemotherapy. Early diagnosis, however, depends on the health seeking behaviour of the patients, accessibility to health facilities and the presence of efficient diagnostic tools. Smear microscopy is the main tool for the diagnosis of TB in resource constrained countries. Direct smear microscopy however is not an ideal test as it is insensitive for the diagnosis of TB.

The studies presented here explored the potential of developing TB diagnostic approaches and techniques, which are more sensitive, and applicable in areas where manpower and resources are scarce and where the majority of TB patients live.

Chapter one presents an overview of the global epidemiology of TB with an emphasis in developing countries. In addition, the impact that HIV has had on the epidemiology, clinical presentation and management of TB is discussed. In line with the increasing number of TB cases associated with HIV, the chapter discusses TB diagnostic options available to National TB Control Programmes (NTCP), their limitations and alternative approaches available.

The diagnosis of PTB requires submission of three sputum samples over 2 days\(^1\). In Chapter two, we present data from 42 TB diagnostic centres in the Southern Region of Ethiopia to explore how many specimens are required to identify these patients. The mean positive results of 15,821 patients with symptoms suggestive of PTB who had submitted three samples each (47,463 smears) in 2000 were reviewed. The median number of patients per health facility was 228 with a range of 10 to 2,955. The proportion of patients who were smear-positive for PTB (two or more positive smears) was 25%, with a range of 17% to 36% per Zone. In our area, every fourth patient examined was smear positive, which is higher than the expected 10% smear positivity. This may indicate an application of a tight screening procedure. A total of 4099 (26%) patients had at least one positive smear with 3753 (91.6%) of the first specimens being positive. A further 303 (7.4%) were negative in the first specimen but positive in the second specimen, and 42 (1%) had two negative specimens fol-
ollowed by a positive third specimen. The value of the third specimen is therefore negligible as 99% of the cases were identified from the first and second specimens. Our finding shows that there is a possibility of reducing the number of specimens required to two or even one in countries where resources are scarce and laboratories are over-burdened.

Sputum samples are usually processed on open bench and safety cabinets are rarely available in most laboratories in developing countries. It has been documented that handling sputum samples in such situations could potentially increase the risk of aerosol transmission\(^2\) though this risk is minimal for direct smears. Moreover, direct smear microscopy, although a good technique to identify the most infectious cases, is not sensitive for diagnosis of TB\(^3\). In Chapter three, we determined the safety and efficacy of short-term digestion of sputum samples with household bleach (NaOCl) before ZN staining. Optimum quality and staining was achieved within 30-45 min bleaching time, which resulted in the liquefaction of the sputum debris. Smears produced from this mixture had a clearer background and bacilli were seen more easily without morphological change. We also determined that bleach kills all bacilli within a 15 minutes contact time, sterilising the samples. Using this contact time, we prepared smears before and after bleach digestion from 200 patients with symptoms suggestive of PTB at Bushullo Major Health Centre (BMHC) in the outskirts of Awassa, Ethiopia to compare the positivity rates obtained by using the standard and digested approaches. Bleached first on-the-spot specimens identified 52 (26%) patients with smear positive PTB compared to 35 (17.5%) identified by the direct smears (p<0.001). More smears were graded as scanty (1-9 AFB/100 fields) by the bleach than the direct smears. The addition of bleach dilutes the number of bacilli in the samples. This effect however seems to be compensated by the ease of reading the slides against a clear background. The results were compared to a case definition for smear positive PTB (at least 2 positive direct smears) proposed by the WHO\(^4\). The bleach-digested smears had a sensitivity of 92.3% and a specificity of 93.4% with 78.3% and 97.7% positive and negative predictive values. Ten patients failed to submit a second or third specimen and six of these patients were positive on either the standard or bleach-digested first on-the-spot smears. Bleach digestion is simple, cheap and kills mycobacteria and its sensitivity is as good as three standard smears. These findings strengthen the possibility of reducing the number of specimens submitted by each patient for the diagnosis of PTB with the potential to improve the efficiency of overburdened services in the developing countries.
HIV co-infection in addition to fuelling the number of TB cases is complicating the diagnosis and further impends the performance of direct smear microscopy as co-infected TB patients rarely form cavities in their lung and hence produce fewer bacilli in their sputum, which may not be detected by direct smear microscopy^5,6. 

Chapter four describes the performance of the short-term bleach digestion technique described in chapter three, among patients with and without HIV co-infections. This study was conducted at BMHC one year later. One hundred and ninety eight patients with symptoms suggestive of PTB with a median age of 30 years were enrolled. The first on-the-spot direct and digested smears identified 51 (26%) and 62 (31%) smear positive patients out of 198 TB suspects, respectively. Thirty six (18%) of the digested smears were graded as scanty and 26 (13%) as +, ++ or +++ pluses, following the IUATLD classification^1. Twenty-two (35%) of the 62 patients with positive digested smears were missed by the direct smears. Ten (5%) of the direct smears were graded as scanty and 41 (21%) as +, ++ or ++++. Thirty (19%) patients were HIV positive and 7 (23%) of these fulfilled the WHO case definition for smear positive PTB. Most of their direct smears were negative, scanty or 1+ and 6 digested smears were graded as scanty. HIV co-infected TB patients therefore produced fewer bacilli. Forty-two HIV-negative patients had TB and also had more bacilli in their sputum. A single digested smear identified 86% HIV-positive PTB cases with similar sensitivity and specificity to HIV-negative patients. Hence, the bleach technique could be useful in TB control programmes where TB-HIV co-infection is common.

The studies described in chapter 3 and 4 compared the yield of the bleach digestion technique with the yield obtained with the direct smears and against the WHO case definition for smear positive PTB. However, the gold standard for the diagnosis of TB is culture. We therefore conducted a study to validate the technique against culture. Given the lack of culture facilities, the validation studies were conducted at Zankli Medical Centre in Abuja, Nigeria (Chapter five). A total of 2251 (99%) out of a potential 2268 direct smears, 736 (97%) bleach digested smears and 756 BACTEC 960 cultures were prepared from 756 TB suspects attending the 8 district hospitals in Abuja. Four hundred and fifty five (60%) were culture-positive and 301 culture-negative. Of the 455 culture-positive cases, 222 (49%) of the first on-the-spot specimens, 232 (51%) of the morning and 224 (50%) of the second on-the-spot specimens were positive by direct smear microscopy. Smears prepared from early morning sputum were more likely to be graded as “+++” than on-the-spot smears (p<0.01). Two hundred and twenty six (51%) of the 455 culture-positive patients had positive digested smears. Digested smears had slightly lower grades (“scanty” or “+”) than the
direct smears consistent with the small dilution effect of the digestion process. There was a high level of agreement (Kappa 0.92-0.94) between the direct and digested smears for patients with complete smear pairs. The digested smears identified a higher number of smear positive cases (32%) compared to the direct smears (29% to 30%), although this difference was not significant. A total of 235 (31%) had “definite” PTB based on the WHO case definition for PTB (2 positive direct smears or one positive smear and positive culture). A further 223 (29%) patients were “very likely” to have PTB (positive culture but three negative direct smears). The WHO case definition identified 51% (235/458) of the patients with “definite” or “very likely” PTB. One digested smear detected 219 of the 235 patients with “definite” PTB and 10 further patients who were negative by all the 3 direct smears but positive by culture and had a sensitivity and specificity of 50% (229/458; 95%CI 45%-55%) and 99% (97%-100%) respectively. These findings confirm our earlier findings that a single digested smear is as sensitive and specific as three direct smears for the diagnosis of PTB. This represents an increase in the efficiency of the diagnostic process as the case definition requires the examination of a larger number of smears. This approach is expected to improve case-finding and to result in time and cost savings for the health service and the patient. Despite these advantages, extra care should be taken in handling digested smears as they are often transparent, tend to run off during staining and are difficult to identify the stained side by the naked eye.

Chapter six presents the findings of the same validation of the short-term digestion technique stratified by HIV infected and uninfected PTB suspects. In addition to the direct and digested smears and culture, blood samples were tested for HIV using rapid tests. Four hundred and thirteen culture positive patients had complete smear microscopy and HIV tests. Of these, 230 (56%) were HIV positive. Among HIV positive patients, AFB was seen in 49% to 50% of direct smears and in 48% of the digested smears. Among HIV-negative culture-positive patients, AFB were seen in 59% to 62% of the direct smears and 59% of digested smears. There was no difference in the proportion of slides with bacilli across the 4 smears in both cases. Among HIV-positive patients with complete sets of four smears, the digested smears identified 50% (111/221) and the direct smears identified 47% to 48% of the PTB cases. Among HIV-negative patients, the digested smears identified 61% (108/177) and the direct smears 57% to 60% of the PTB cases. Digested smears yielded slightly higher number of smear positive cases than the direct smears although this was not statistically significant. A total of 116 (50%) of 225 HIV-positive and 113 (62%) of HIV-negative patients fulfilled at least one of the case definitions for smear positive PTB.
A single digested smear identified 111 (49%) of the 225 HIV-positive PTB patients and 59% (108/181) of the HIV-negative PTB patients. Hence, a single digested smear yielded an equal number of positive cases as 3 direct smears independently of HIV status. These findings corroborate our previous results on bleach digested smears and reinforce the need for consideration of reducing the number of smears submitted by each TB suspect without compromising sensitivity.

According to the IUATLD and WHO categorizations, smears with ≥10 AFB/100 HPF are positive and smears with 1-9 AFB/100 HPF are considered as “scanty” and for diagnosis of PTB based on the later groups, additional confirmatory specimens are required. Sputum smear results with scanty grades are not infrequent and the proportion is higher among HIV co-infected PTB patients. In Chapter seven we verify smears categorised as scanty as true positives or negatives. From a total of 1068 patients who submitted 3 sputum samples for direct smears and culture, 824 (26%) had positive, 137 (4%) scanty and 2243 (70%) negative smears. Six hundred and eighty (64%) were culture positive. One hundred and seventy (95%) out of 137 scanty and 809 (98%) out of 824 positive smears belonged to patients with positive cultures (p=0.02). Twelve out of 18 patients with a single scanty smear and 51 of 52 patients with ≥2 scanty smears were culture positive. These findings confirm that in TB control programmes with adequate quality control, smears reported as “scanty” are more likely to be true than false positives. Accepting scanty smears as positive would result in less than 1% of the patients being wrongly classified as having PTB (7/1068) and a further 34 patients could have been identified for treatment. This would be particularly useful in African countries with high HIV prevalence, as these populations often have low accessibility to services and are overburdened diagnostic facilities.

Early diagnosis and adequate chemotherapy are crucial to stop the transmission of community acquired TB. However, several factors contribute to the early diagnosis of PTB. In Chapter eight we determine the factors associated with patient delay in presentation to PTB services in rural Ethiopia. Two hundred and forty three consenting patients with symptoms suggestive of PTB attending BMHC were interviewed. Median (mean) transport time was 2 (5) hours, and the mean (SD) cost of the journey was 12 (18) Ethiopian birr (US$1.40). More than half (51%) of the patients had borrowed money before reaching the health centre, and 54 (22%) had to sell personal property. One hundred and two had no prior contact with the health system and 206 (85%) had waited more than 10 days from symptom onset before seeking help. Thirty-seven (15%) had used traditional medicines at some point during their illness.
and 183 (75%) patients were unable to engage in their normal daily routines. Patient delay had a median (mean ±) value of 4.3 (9.8±12.4) weeks and 60% (157) presented more than one month after their symptom onset, with 21% attending after 3 months. There was no difference in patient’s delay between smear positive and negative-patients. Patients who spent more than US$1.40 on transport and those who sold personal belongings to cover the costs of the visit were more likely to delay presentation by more than 8 weeks. Rural residence, transport time > 2 hours, and use of traditional medicine remained independent risk factors for prolonged patient delay. These factors are indicators of financial stress and underline economic barriers to health care. The accessibility of the health facilities should not be overlooked as the time and cost incurred for transportation is critical in this community where more than half of the population live more than 10 km away from any health facility (unpublished report). Knowledge and beliefs of the population about TB, and the stigma associated with it, are crucial determinants for the use of health services. These findings also highlight the need for implementing strategies to make DOTS more decentralized and pro-poor.

Patients with symptoms suggestive of PTB are expected to visit health facilities repeatedly to complete the diagnostic process. Multiple visits however are not usually feasible in areas with limited access. The direct and indirect costs incurred for the visits are significant and may hinder patients from completing the diagnostic process. In Chapter nine, we assess the possibility of completing the diagnostic process in a single day by submitting two on-the-spot sputum samples with the second specimen being submitted one hour after the first sample for the diagnosis of PTB. We enrolled 243 TB suspects over a 3-month period at BMHC. Of these, 52 (21%) had at least one positive smear in the set of four smears. If TB was diagnosed on the bases of at least 1 positive smear, 51 patients were detected using the standard method and 49 by the same-day approach. The three cases missed by the same-day approach were detected by the second-day smears. The same-day approach detected one case missed by the three-standard approach. Using the standard case definition (≥2 positive direct smears) as true positives, the sensitivity for the same-day and standard methods were 87% (95% CI 74%-94%) and 89% (95% CI 74%-94%) respectively. If only one AFB-positive smear is accepted as diagnostic, then the sensitivity of the same-day and standard method increased to 94% and 98% respectively; essentially resulting in the same patient yield. Reducing the number of smears per patient and the time required for specimen collection would improve the TB case detection rate as the workload, requirements in the laboratories and patients’ cost will be
SUMMARY

reduced with no dropout from the diagnostic process. Making smear microscopy as feasible as possible and providing results as soon as possible could avoid "provider delay".

Although PTB cases are very important for NTCP and policy makers, as they are sources of infection, extra-pulmonary TB (EPTB) is equally important for the patients and clinicians who manage them. The proportion of EPTB is increasing primarily due to the HIV co-infection which results in a disproportional increase in the rate of EPTB and smear negative PTB in some areas⁹. The diagnosis of EPTB is more challenging than PTB especially in rural areas where there are no facilities and expertise to confirm the diagnosis. We present in Chapter ten the findings of a study aiming to improve the diagnosis of EPTB in rural Ethiopia where it accounts for more than 40% of all registered TB cases. Of 147 patients with chronic lymphadenitis, cervical lymph nodes (LN) are commonly affected accounting for 80% (118/147) of the cases and the duration of the enlargement of LN was >16 weeks for 67% (99/147) of the cases confirming the chronic nature of the lymphadenitis at presentation. Fine-needle aspirate (FNA) specimens were examined after staining by Haematoxylin and Eosin and ZN for cytology and AFB respectively. One hundred and five (71.4%) specimens had a cytology suggestive of TB (TB lymphadenitis = TBLN) and 28 (19%) were positive for AFB on direct smear. Of the 40 cases without cytological features diagnostic for TBLN (Non-TBLN), 37 (92.5%) had marked neutrophil aggregates and 3 (7.5%) were diagnosed as chronic non-specific lymphadenitis based on the cytological findings. Twenty-four of the 107 (22.4%) cases with TBLN and 9 of 40 (22.5%) Non-TBLN cases were HIV positive. HIV status did not affect the cytology or the AFB results (p>0.05). Moreover, HIV positive and negative cases did not differ in their general manifestations. Since the clinical features were similar in the TBLN and Non-TBLN groups as defined by cytology and AFB staining, polymerase chain reaction (PCR) was done on those samples with sufficient specimens for DNA extraction. Accordingly, 71% (34/48) TBLN and 65% (15/23) of Non-TBLN were PCR positive (p>0.05). Although HIV contributes to increased proportion of EPTB cases in general, in our study group, there could be other factors contributing to the increased rate of EPTB among all diagnosed TB cases. These findings show that EPTB could be diagnosed at local level by performing FNA in more than 71% of the cases and if it is combined with a clinical algorithm, the diagnosis could be improved in places where performing whole LN biopsy is not feasible. A considerable number (65%, 15/23) of the cases who were considered as non-TBLN by cytology but only 10% (15/147) of overall cases were additionally positive for M.
tuberculosis specific PCR, indicating the need for further diagnostic criteria or referral of these group before the final diagnosis or exclusion of TB in rural areas.

HIV co-infection modified the clinical presentation, diagnosis and management of TB cases. The knowledge of the local HIV prevalence among TB patients could alert the staff in taking precautions and help policy makers to launch a concerted effort to control the dual infections. In Chapter eleven, we present the findings of a cross sectional survey of HIV infection among patients with confirmed TB in 5 predominantly rural hospitals of the Southern Region of Ethiopia. Five hundred TB patients; 489 (98%) new and 11 previously treated patients were enrolled. The average HIV prevalence was 19% (97/500) ranging from 15% to 25% across the hospitals, which is significantly higher than the prevalence of HIV among mothers attending antenatal clinics in the same hospitals, which ranged from 2.3% to 12% (p<0.01). There was a strong association between the area of residence and HIV, with 15% (47/329) of the rural and 30% (45/150) of the urban patients being HIV positive (p<0.001). The highest number of TB cases occurred in the age group 15 to 24 years. TB-HIV co-infection however peaked in children and in 25 to 34 year old adults, which was later than the peak for TB. The HIV prevalence was 19% (51/261) and 26% (36/137) among smear-positive and smear-negative PTB cases respectively. These prevalences were higher than in patients with EPTB (11%) although this was only marginally significant. The proportion of patients with smear-negative PTB was higher in HIV-positive than HIV-negative patients in all the centres. However, the proportion of cases with smear-positive PTB among HIV-negative cases was higher in the centres with the highest maternal HIV prevalence, and the hospital with the lowest maternal HIV positivity (2.3%) had the highest proportion of EPTB. These findings highlight the importance of determining the prevalence of TB-HIV co-infection among different groups and diseases categories at a local level as the findings could differ across areas. Areas with higher HIV prevalence could register higher numbers of smear positive TB cases and not necessarily higher EPTB or smear-negative TB, and conversely, areas with lower HIV prevalence could have disproportionately higher EPTB cases. These differences could be explained in part by the difference in diagnostic procedures, catchment population (rural vs. urban) and time since the introduction of HIV into an area. Information on the proportion of TB patients co-infected with HIV and the changing clinical presentation of TB could help plan concerted efforts to prevent and control dual infection. This planning however needs to take into consideration other factors which influence the epidemiology of TB and HIV.
Conclusions and recommendations

The present studies clearly demonstrate the need for a simple, robust and feasible diagnostic approach for the control of TB, especially in developing countries. Implementation of the short-term bleach digestion method for the diagnosis of TB improves the quality of the sputum smears and reduces the time spent reading the smears. It has been consistently demonstrated in different locations that one bleach-digested smear identifies as many patients with PTB as the routine three-sample approach. Reducing the number of sputum specimens submitted by patients with symptoms suggestive of PTB would have several advantages for the health service and for patients. The sputum digestion approach is equally useful for the diagnosis of TB in patients co-infected with HIV. Although one digested smear could be sufficient in most situations, National TB Control Programmes could benefit at most by examining two smears on the same-day to save patient cost and provide with improved sensitivity. These two consecutive smears could be bleach-digested, which may result in an even higher yield than the current two-day approach. This approach would be critical in areas where HIV is fuelling the number of TB cases.

We believe that the international recommendations for the diagnosis of TB should be revisited and modified based on new evidence as presented in this Thesis. This approach could facilitate achieving the WHO target of detecting 70% of smear positive cases.
Reference: