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Misdiagnosis of tuberculosis and the clinical relevance of non-tuberculous mycobacteria in Zambia

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ABSTRACT

Objective: To determine the accuracy of TB diagnosis of TB in Zambia in the era of increasing HIV prevalence. **Methods:** Sputum of the clinically diagnosed TB cases was additionally subjected to liquid culture and molecular identification. This study distinguished between TB cases confirmed by positive *Mycobacterium tuberculosis* (*M. tuberculosis*) cultures and mycobacterial disease caused by non-tuberculous mycobacteria (NTM). **Results:** Only 49% of the 173 presumptively diagnosed TB cases was *M. tuberculosis* cultured, while in 13% (22) cases, a combination of *M. tuberculosis* and NTM was found. In 18% of the patients only NTM were cultured. In 28%, no mycobacteria was cultivable. HIV positive status was correlated with the isolation of NTM ($P<0.05$). **Conclusions:** The diagnosis of tuberculosis based on symptoms, sputum smear and/or chest X-ray leads to significant numbers of false-positive TB cases in Zambia, most likely due to the increased prevalence of HIV. The role of NTM in tuberculosis-like disease also seems relevant to the false diagnosis of TB in Zambia.

1. Introduction

The increase of tuberculosis (TB) incidence over the past 25 years in sub-Saharan Africa is closely related to the spread of the HIV epidemic[1]. The clinical presentation of TB in HIV-infected individuals varies with the degree of immunosuppression and symptoms are often non-specific, resulting in a delayed- or misdiagnosis[2].

Sputum smear examination for acid-fast bacteria (AFB) in sputum is still the cornerstone of diagnosis of pulmonary TB in Africa[3]. Smear examination by experienced technicians can result in the diagnosis of at most 50-60% of TB cases[4,5]. Moreover, the sputum smears of HIV-positive patients often present as Ziehl-Neelsen (ZN) negative[6]. Unfortunately, although sputum culture is considered the 'gold standard' for the diagnosis of pulmonary TB, this technique is rarely available in Africa. In the last decade, liquid culture methods have become available in the industrialized world, with a reduced turn-around time to 1-3 weeks[7,8].

According to International Standards for Tuberculosis Care, all patients with an unexplained productive cough lasting two weeks or more should be evaluated for TB[3]. These patients should submit at least two and preferably three sputum specimens for microscopic examination. If such patients have a sputum smear positive for acid fast bacilli, they are diagnosed with TB. The diagnosis of sputum smear-negative pulmonary TB is based on at least three negative sputum smears, chest radiography findings consistent with TB, and lack of response to broad-spectrum antimicrobial agents. However, the diagnosis of smear-negative TB is problematic, especially given the atypical presentation of pulmonary TB in HIV-infected patients. Moreover, in low income countries, access to high quality radiography and expert interpretation is often limited[9]. Therefore, patients with a strong clinical suspicion of TB are often treated empirically with a combination of anti-tuberculosis drugs for six to nine months[10].

In addition, non-tuberculous mycobacteria (NTM) can cause infections mimicking tuberculosis, especially in patients with AIDS. In Africa, the contribution of NTM to

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such disease has so far been examined only on a very small scale. To guide the diagnosis in a patient from whom NTM has been isolated, the recently published new diagnostic criteria for non-tuberculous mycobacterial diseases by the American Thoracic Society (ATS) provide support[11]. This is especially important because the treatment of NTM disease differs from that of TB and involves multiple drugs, most of the time including a newer macrolide (e.g. azithromycin, clarithromycin)[11]. The clinical criteria of the ATS include a symptomatic patient with pulmonary symptoms and nodular or cavitary opacities on chest radiograph. In addition, the microbiologic criteria comprise positive culture results from at least two separate sputum samples.

In Zambia the effects of the HIV/AIDS pandemic are devastating, about 15 to 20 percent of the adults are HIV-positive; about 900 000 people are living with AIDS and there are over 550 000 AIDS orphans. Since the advent of AIDS, the TB case rates in Zambia increased from 100~650 per 100 000 people[12].

The first aim of this study was to determine the accuracy of the clinical diagnosis of TB, based on sputum smears and chest X-ray in Zambia in the era of increasing HIV prevalence. The second aim was to evaluate the clinical relevance of NTM in Zambia.

2. Materials and methods

This study was conducted in St Francis Hospital in Katete, Yeta District Hospital in Sesheke and Our Lady's Hospital in Chilonga. Ethical approval for the study was provided by the research ethical committee of the University of Zambia, the Central Board of Health, and the Ministry of Health in Zambia.

All adults aged 15 years and more, admitted to the internal medicine ward with chronic respiratory complaints lasting two or more weeks, were screened for TB and HIV after informed consent had been obtained. The study was performed between 2001 and 2003 during consecutive time periods of several months.

The medical history of the patients was obtained, and detailed physical examination was performed. During three consecutive days, early morning sputum was collected from patients with a productive cough. The first two sputum specimens were cultured for mycobacteria, and the third one was stored at -20 ℃. Not concentrated sputa specimens were examined in the normal routine flow of the hospital by microscopy for the presence of acid-fast bacteria after Ziehl-Neelsen staining and all were subjected to culture in mycobacteria growth indicator tubes (Becton Dickinson Microbiology Systems, Cockeysville, Md., US). Decontamination of the sputum was done using N-acetyl-L-cysteine (NALC)-NaOH and 6% sulphuric acid after dividing the sputum specimen in two equal parts to compare the decontamination procedures for the detection of mycobacteria[13]. Negative controls were included to exclude a laboratory cross-contamination. Centrifugation for concentration was not performed.

One chest X-ray was made on the day of inclusion and assessed in Zambia. These chest X-rays were re-evaluated in the Netherlands in a blinded manner. Films were scored for mediastinal adenopathy, cavitation, pleural and pericardial fluid, miliary pathology, alveolar infiltration, interstitial pathology, and pathology other than mentioned. The conclusions of the scoring system were defined as: chest X-rays labelled with no pathology, pathology not consistent with TB, or pathology consistent with TB.

Serological testing for HIV was performed using a qualitative immunoassay (Abbott Determine HIV-1/2) and the Vidas HIV DUO assay (Biomérieux, Marcy l'Etoile, France). The Accuprobe culture confirmation test for *Mycobacterium tuberculosis* (*M. tuberculosis*) complex (Accuprobe, bioMérieux, Marcy l'Etoile, France) and/or 16S rRNA gene sequencing of the 151bp variable region A of the 16S rDNA gene were used to identify *Mycobacterium* isolates[14]. A *Mycobacterium* isolate was labelled as 'unidentified' in case the Accuprobe culture confirmation test for *M. tuberculosis* complex was negative and the 16S rRNA gene sequencing was resultless.

3. Results

A total of 320 chronically ill patients were enrolled. The median age of this population was 36 (range 16~80) and 52% were female. Of these patients, 313 were HIV-tested and 75% of them were HIV-positive. The median duration of their complaints before admission to the hospital was eight weeks.

From the 320 patients, 173 were assigned a presumptive diagnosis of TB on the basis of a productive cough lasting two or more weeks, no improvement on two courses of routine empiric antibiotics (e.g. chloramphenicol, cotrimoxazole), and a positive ZN sputum smear or abnormalities on the chest X-ray consistent with TB. At least two sputum smears for AFB were microscopically examined in 156 (92%) patients and at least two sputum specimens were cultured from 144 (83%) patients. In this cohort, the HIV status, median duration of complaints and the median age were comparable between the patients diagnosed with TB and other patients in the studied cohort.

3.1. Sputum culture

Of the 173 patients presumptively diagnosed with TB on the basis of sputum smears and chest X-ray, 66 (38%) had a positive ZN smear and 164 (95%) had a chest X-ray consistent with TB (Table 1). Only 49% (85) of these 173 patients, *M. tuberculosis* was isolated from one of two sputum specimens. In 36% (63/173) of these patients, exclusively *M. tuberculosis* was isolated and in 13% (22/173) a combination of *M. tuberculosis* and NTM was cultured. In another 18% (32) of these 173 cases, exclusively NTM were cultured. In 28% (48) of the TB cases, the culture for mycobacteria remained negative. In two patients diagnosed with TB and having a positive ZN sputum microscopy, only NTM were isolated.

3.2. HIV-status

Of the 173 diagnosed TB patients, 126 (73%) were HIV-positive. Positive NTM cultures were presented in 36% (45/126) of the HIV-positive patients, significantly more frequent than 19% (9/47) of the HIV-negative patients, (P<0.05). Furthermore, 32% (40/126) of the HIV-positive TB patients had a positive culture for exclusively *M. tuberculosis*, in comparison to 49% (23/47) of the HIV-negative TB patients (P<0.05).

3.3. Anti-tuberculosis treatment

Anti-tuberculosis treatment was initiated in 124 of the 173 (72%) patients in the patient group presumptively diagnosed

with TB on the basis of a positive sputum smear or a chest X-ray consistent with TB. Of the 85 patients with *M. tuberculosis* positive sputa, 80 (94%) were treated. However, also 15 of the 32 (47%) of the patients with only NTM in their sputum and 25 of the 48 (52%) patients with a negative culture for mycobacteria were also treated.

3.4. Diagnosed with TB without a positive culture for M. tuberculosis

There were a total of 88 patients diagnosed with TB on basis of the sputum smear and/or chest X-ray of which the liquid culture for *M. tuberculosis* remained negative (Table 2). The culture was negative for all mycobacteria in 48 (54%) of these patients and in 32 patients NTM were isolated. Four (5%) of these patients had a positive ZN of the sputum and 86 (98%) had abnormalities on the chest X-ray consistent with tuberculosis. HIV-positive status, the median duration of complaints, the median age, as well as percentage of women in this patient group were comparable with the 173 patients diagnosed with TB in the whole study group. A history of TB was documented in 23 (26%) of these patients which was also similar to the entire group of TB patients diagnosed.

Table 1

Ziehl-Neelsen (ZN) of sputum, chest X-ray, culture results and HIV status of diagnosed TB patients.

ZN sputum	Chest X-ray	Culture result					Total
		Negative	*M. tuberculosis*	*M. tuberculosis* and NTM	NTM only	AFB not identified	
Negative	Consistent with TB	47 (36 HIV^+)	18 (16 HIV^+)	6 (5 HIV^+)	30 (23 HIV^+)	6 (5 HIV^+)	107
Positive	No abnormalities	1 (HIV^-)	5 (4 HIV^+)	2 (2 HIV^+)	0	1 (HIV^-)	9
	Consistent with TB	0	40 (20 HIV^+)	14 (13 HIV^+)	2[a] (2 HIV^+)	1 (HIV^-)	57
Total		48 (28%)	63 (36%)	22 (13%)	32 (18%)	8 (5%)	173

[a]One of these two patients with a sputum positive ZN and only NTM isolated from the sputum was started with anti-tuberculosis treatment after inclusion in study.

Table 2

Characteristics of 88 patients diagnosed with TB and a negative *M. tuberculosis* culture.

Culture result		ZN positive	Chest X-ray TB[a]	HIV positive	TB treatment[b]	TB in past[c]	Died
Negative	48 (54%)	1	47	36	25	8	9
Single NTM positive culture	18 (21%)	0	18	13	6	6	4
Different NTM in both sputa	9 (10%)	1	9	7	6	2	0
Same NTM in both sputa	5 (6%)	1	5	5	3	2	3
AFB not identified	8 (9%)	1	7	5	4	5	1
Total	88	4 (5%)	86 (98%)	66 (75%)	44 (50%)	23 (26%)	17 (19%)

[a]Chest X-ray consistent with tuberculosis, [b]Anti-tuberculosis treatment started by clinician without knowing culture results after inclusion in study, [c]History of tuberculosis.

In two patients with a positive sputum smear who were treated with anti-tuberculosis treatment, no *M. tuberculosis* was isolated. In the first patient only NTM were cultured from the ZN-positive sputa. This patient was HIV positive and had suffered from TB in the past. The chest X-ray was consistent with tuberculosis. The NTM were identified as *Mycobacterium intracellulare* (*M. intracellulare*) and in addition an unidentified *Mycobacterium* was isolated. In the second patient the culture also yielded unidentifiable acid fast bacilli. This patient was HIV-negative and had been treated for TB in the past. The chest X-ray was normal. In another two patients with a positive sputum smear from whom no *M. tuberculosis* was isolated, anti-tuberculosis treatment was not started at time of inclusion. In one of these patient *Mycobacterium lentiflavum*(*M. lentiflavum*) was isolated from both sputum samples and in the other patient the culture remained negative. This last patient was HIV-negative and the chest X-ray was without signs of pathology.

3.5. Characteristics of patients with M. tuberculosis and NTM isolates

There were significant differences between patients with *M. tuberculosis* and NTM isolates regarding possible history of TB and positive BCG vaccination status. Eighty-three percent (68/82) of the patients with *M. tuberculosis* isolates had a BCG-vaccination in the past compared to 57% (17/30) of the patients with exclusively positive NTM cultures ($P<0.05$). A history of tuberculosis was observed in 13% (11/85) of the *M. tuberculosis* culture-positive patients. In the patients with only NTM isolates, this percentage was 31 (10/32) ($P<0.05$).

3.6. Identification of Mycobacterium isolates

The collected sputum samples of all patients were split into two equal parts before decontamination in order to compare the influence of the decontamination method on the yield of mycobacteria[13]. From the 173 diagnosed TB patients, a total of 627 sputum samples were cultured. The results of the cultures are depicted in Table 3. From 363 (58%) of the 627 sputum samples of patients, mycobacteria were isolated. *M. tuberculosis* was found in 228 of the 363 (63%) positive sputum specimens and NTM in 135 (37%). The most frequently encountered NTM were *M. lentiflavum*[20] and *M. intracellulare*[16].

Table 3
Isolated mycobacteria from sputum of patients diagnosed with tuberculosis (*n*,%).

Culture result	Isolates
Negative	264 (42.0)
Mycobacterium tuberculosis	228 (36.0)
Mycobacterium lentiflavum	20 (3.0)
Mycobacterium avium complex	18 (2.8)
Mycobacterium intracellulare	16 (2.5)
Mycobacterium avium	2 (0.3)
Mycobacterium gordonae	4 (0.7)
Mycobacterium chelonae	4 (0.7)
Mycobacterium fortuitum	2 (0.4)
Mycobacterium mucogenicum	2 (0.4)
Various unknown *Mycobacterium* species	30 (5.0)
Various other *Mycobacterium* species[a]	5 (0.8)
Unidentified AFB	50 (8.0)
Total number of sputum samples	627(100.0)

[a]Various other *Mycobacterium* species include *M. goodii*, *M. terrae*, *M. neoaurum*, *M. peregrinum*, and *M. obuense*.

3.7. NTM isolated from both sputum samples

In 54 of the 173 (31%) TB patients diagnosed, NTM were isolated from the sputum; 32 (59%) represented exclusively NTM and 22 (41%) yielded a combination of *M. tuberculosis* and NTM. Two consecutive sputum samples were cultured from 47 of the 54 NTM-positive patients. In 29 of the 47 patients (62%) only a single culture was positive for NTM. The remaining 18 patients had two positive cultures with NTM; 10 patients (21%) had different NTM species in the sputum samples and 8 (17%) patients had the same NTM in both sputum samples. In four of the eight patients with the same NTM in both sputum samples, *M. lentiflavum* was isolated and in one patient *M. intracellulare* (Table 4). In the remaining three patients *M. lentiflavum*[2] and *M. chelonae* were isolated in both sputum samples together with *M. tuberculosis*.

3.8. Predictive value of routine algoritm for diagnosing TB

In this study the positive predictive value of the algorithm for diagnosing TB on the basis of a productive cough for two or more weeks, no improvement on two courses of empiric antibiotics, and a positive sputum smear or pathology on the chest X-ray consistent with TB was 50% (88/176) (Table 5). Of these 88 patients, 72 were diagnosed because they did not improved on two courses of routine empiric antibiotics, without positive smear and/or sign of TB on X-ray. Five out of these 72 patients had a culture positive for M. tuberculosis (with or without NTM) which makes the negative predictive value of this algorithm 93%. The sensitivity and specificity were 95% (88/93) and 43% (67/155), respectively.

Table 4
Cases with the same NTM in two consecutive sputum samples.

Case	Isolate sputum	ZN	Gender	Age	BMIa	HIV	Tempb	Complaints	Duration(wks)[d]	Chest X-ray	Died	Remarks
1 (11)	*M. lentiflavum*	–	Female	50	20	+	?[e]	resp	21	TB	yes	*M. lentiflavum* also in urine,started with TB treatment
2 (13)	*M. lentiflavum*	+	Male	35	24	+	39.0	resp	34	TB	no	––
3 (15)	*M. lentiflavum*	–	Female	30	18	+	38.5	resp	5	TB	no	TB in past, started with TB treatment
4 (34)	*M. lentiflavum*	–	Female	25	?[e]	+	38.5	resp	4	TB	yes	TB in past
5 (684)	*M. intracellulare*	–	Male	32	15	+	36.8	resp	17	TB	yes	––

[a]BMI = Body mass index, [b]Temp = Temperature at time of inclusion, [c]Complaints = Reasons for visiting the hospital, Resp = Complaints/symptoms of the tractus respiratorius, [d]Duration = Duration of complaints at the point of visiting hospital and inclusion in study. ?[e] = Temperature or BMI not known.

Table 5
Performance of algorithm for diagnosing TB on basis of Ziehl-Neelsen sputum smear and/or chest X-ray.

	M. tuberculosis culture (+)	*M. tuberculosis* culture (–)	Total
ZN^+ and/or $X\text{-}ray^+$	88	88	176
ZN^- and $X\text{-}ray^-$	5	67	72
Total	93	155	248

4. Discussion

The results of this study show that *M. tuberculosis* was not cultured from the sputum of 49% of the patients presumptively diagnosed with TB according to the local algorithm. In 46% of the cases exclusively NTM or a negative culture was found. In the remaining 5%, acid fast organisms which could not be identified to the species level were found. Among these patients presumptively diagnosed with TB an extremely high rate of HIV was observed: 73%.

The spectrum of HIV-related morbidity in adults in sub-Saharan Africa shows some differences to that observed in Europe and North America; a higher incidence of tuberculosis and of bacterial diseases in general, but a lower incidence of *Pneumocystis jirovecii* pneumonia[15,16]. However, sufficient knowledge on the effect of HIV on the occurrence of NTM disease is lacking[17-26]. Nevertheless, the results in this study indicate that NTM may be important pathogens regarding the diagnosis and perhaps the aetiology

of tuberculosis-like infections in Zambia. A positive HIV status was significantly correlated with isolation of NTM from sputum. In patients presumptively diagnosed with TB, the culture was exclusively positive for NTM in 18% of the cases. In another 13% of the 'TB' cases NTM together with M. tuberculosis was isolated. Therefore, the role of NTM in tuberculosis-like disease deserves more attention in Zambia.

In this study probably five patients would meet the criteria for NTM pulmonary disease according to the ATS. Two consecutive sputum samples of these HIV-positive patients yielded twice *M. lentiflavum* for one case and a double isolate of *M. intracellulare* for the other. These patients presented with a productive cough and the chest X-ray showed abnormalities compatible with tuberculosis. According to criteria of the ATS for diagnosing NTM, these patients suffered from lung disease. However, these sputa were not additionally tested with molecular amplification techniques for the presence of *M. tuberculosis*. Furthermore, due to liquid cultures it was not possible to count the number of colony-forming units cultured from the sputum to discriminate between colonization and infection/disease.

Different NTM species were isolated from consecutively collected sputa of 10 patients. Probably these 10 patients were transiently colonized with different NTM. This contrasts another part of the patients from whom the same NTM were repeatedly isolated from consecutive sputum samples. The latter 8 patients were probably truly infected with NTM and in some of these last patients indeed disease was diagnosed.

The presentation of NTM infections typically mimics TB, thereby confounding the diagnosis of TB. In this study, in two of the 66 (3%) patients with a positive sputum smear only NTM was isolated. In the light of this, methods for distinguishing *M. tuberculosis* and NTM should be implemented on a broader scale in Africa preferably through direct detection and identification of *M. tuberculosis* in clinical specimens are[27-29]. However, the performance of the test is good in clinical respiratory specimens that are AFB smear-positive (sensitivity 95% and specificity 98%) but far less in specimens that contain fewer organisms, or are AFB-negative (sensitivity 50% and specificity 95%)[7]. The challenge to overcome is the implementation of such delicate techniques in African settings.

NTM are widely spread all over the world[11,30,31]. *M. avium* complex (MAC) is the most common NTM species causing disease, but many other NTM species have been found associated to severe infections. In Africa little is known on the prevalence of different NTM species and their clinical importance. In this study, the most isolated NTM in the population of patients diagnosed as TB were *M. lentiflavum* and MAC. Interestingly, a part (22%) of the NTM found in Zambia could not yet been identified to the species known in the Western world. This suggests the distribution of NTM in Africa may be different from Europe and the USA and the evolutionary divergence of phylogenetic sub-lineages of bacteria within species may merit more detailed taxonomic studies. In addition, also other micro-organisms can cause respiratory illness in HIV-positive patients mimicking TB, like e.g. *Streptococcus pneumoniae* and *Haemophilus influenzae*, several viruses and *Pneumocystis jirovecii*. In this study it was not possible to identify these micro-organisms.

Another reason for negative cultures may be found in the general lack of sensitivity of culture; this amounts 80-85% for mycobacteria (with a specificity of approximately 98%)[32,33]. Furthermore, the sputum samples were not concentrated by centrifugation prior to the sputum smear preparation and culture, as this is uncommon in health centres and hospitals in Zambia. Probably this could have an effect on the results and validation of the algorithm TB, as concentration logically increases the sensitivity of direct microscopy and thereby improving the diagnosis of TB[34]. However, it will probably also increase the percentage of positive smears in patients infected with NTM and therefore confuse the diagnosis of TB more.

Anti-tuberculosis treatment was initiated in 124 of the 173 (72%) patients diagnosed with TB. In patients who were *M. tuberculosis* culture positive, treatment was started in 94% of the cases. This suggests that in patients with positive *M. tuberculosis* cultures, the clinical suspicion of TB was higher than in the other patients. In contrast, only approximately half of the patients with positive NTM cultures (47%) or a negative culture for mycobacteria (52%) were put on treatment. The clinicians' diagnosis of TB and the treatment decision often deviated from the protocol used in Zambia, especially for the patients with NTM or a negative culture for mycobacteria. In this study significantly more patients with a positive culture for NTM instead of *M. tuberculosis* had a history of TB. It is likely that the knowledge that the patient had TB in the past discouraged the clinician from re-starting the anti-tuberculosis treatment.

Moreover, significantly fewer patients with a positive culture for NTM had received a BCG-vaccination in the past (57%) than the cases with a positive culture for *M. tuberculosis* (83%). Probably the BCG-vaccination may provide cross-immunity protection against NTM infection, as suggested in the literature[35,36].

With the changing situation in Africa, diagnostic scoring systems and algorithms should be further developed and validated to assist clinicians working in resource-poor settings with a high prevalence of HIV.

Conflict of interest statement

We declare that we have no conflict of interest.

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