Burden of tuberculosis at post mortem in inpatients at a tertiary referral centre in sub-Saharan Africa: a prospective descriptive autopsy study

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Summary

Background **Patients with subclinical tuberculosis, smear-negative tuberculosis, extrapulmonary tuberculosis, multidrug-resistant tuberculosis, and asymptomatic tuberculosis are difficult to diagnose and may be missed at all points of health care. We did an autopsy study to ascertain the burden of tuberculosis at post mortem in medical inpatients at a tertiary care hospital in Lusaka, Zambia.**

Methods **Between April 5, 2012, and May 22, 2013, we did whole-body autopsies on inpatients aged at least 16 years who died in the adult inpatient wards at University Teaching Hospital, Lusaka, Zambia. We did gross pathological and histopathological analysis and processed lung tissues from patients with tuberculosis through the GeneXpert MTB/RIF assay to identify patients with multidrug-resistant tuberculosis. The primary outcome measure was specific disease or diseases stratified by HIV status. Secondary outcomes were missed tuberculosis, multidrug-resistant tuberculosis, and comorbidities with tuberculosis. Data were analysed using Pearson χ^2, the Mann-Whitney *U* test, and binary logistic regression.**

Findings **The median age of the 125 included patients was 35 years (IQR 29–43), 80 (64%) were men, and 101 (81%) were HIV positive. 78 (62%) patients had tuberculosis, of whom 66 (85%) were infected with HIV. 35 (45%) of these 78 patients had extrapulmonary tuberculosis. The risk of extrapulmonary tuberculosis was higher among HIV-infected patients than among uninfected patients (adjusted odds ratio 5·14, 95% CI 1·04–24·5; p=0·045). 20 (26%) of 78 patients with tuberculosis were not diagnosed during their life and 13 (17%) had undiagnosed multidrug-resistant tuberculosis. Common comorbidities with tuberculosis were pyogenic pneumonia in 26 patients (33%) and anaemia in 15 (19%).**

Interpretation **Increased clinical awareness and more proactive screening for tuberculosis and multidrug-resistant tuberculosis in inpatient settings is needed. Further autopsy studies are needed to ascertain the generalisability of the findings.**

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Introduction

Accurate data on the burden of tuberculosis are not available from countries with high tuberculosis endemicity in Asia, eastern Europe, and Africa because of inadequate microbiology and pathology services, which do not have the resources, expertise, or capacity to accurately diagnose extrapulmonary tuberculosis, subclinical tuberculosis, sputum-smear-negative tuberculosis, and multidrug-resistant tuberculosis. These cases are difficult to diagnose and can be easily missed at all points of care.

WHO estimated that worldwide there were 9 million new cases of tuberculosis in 2013, with 1·3 million deaths, of which 480 000 were from sub-Saharan Africa.[1] The actual burden of multidrug-resistant tuberculosis in most African countries remains undefined. WHO estimates that 3 million people worldwide have active tuberculosis and multidrug-resistant tuberculosis but remain undiagnosed and untreated.[1] However, all WHO data are based on estimates from national tuberculosis returns, verbal autopsy studies, and death certificate records, all of which are inaccurate.

The universal gold standard for identifying specific causes of death is by undertaking an autopsy, which offers valuable insights into the accuracy of earlier clinical diagnoses and can identify previously undiagnosed disease burden.[2] However, autopsies are not done routinely and a general decline has been noted worldwide, including in the UK, USA, and Europe.[3] Autopsy studies[4–8] are difficult to undertake in any geographical setting because of cultural objections, shortages of trained personnel, and underdeveloped pathology services and infrastructure.

We did a study of patients who died in the inpatient adult general medical wards at a tertiary care referral centre in Lusaka, Zambia, to assess the burden of pulmonary tuberculosis, extrapulmonary tuberculosis, undiagnosed subclinical tuberculosis, and multidrug-resistant tuberculosis at post-mortem examination.

Methods

Study design and setting

Between April 5, 2012, and May 22, 2013, we did a prospective autopsy study of patients aged at least

16 years, irrespective of admission diagnosis, who died in the adult general medical inpatient wards at the University Teaching Hospital (UTH), a tertiary care referral centre in Lusaka, Zambia. UTH receives patients from across the country, although most are referred from Lusaka's network of primary and secondary care community clinics. Patients presenting to UTH casualty with a medical disorder (communicable or non-communicable disease with or without co-morbidity) who need inpatient medical care are admitted to one of six general medical adult (≥16 years) inpatient admission wards (three for men and three for women) in the department of general medicine. There are no specialist admissions. Patients suspected of having tuberculosis on admission are nursed in a side room.

Recruitment was undertaken by dedicated clinical officers who are experienced in grief counselling. Next of kin were approached by the attending physician and study clinical officer to obtain consent. Only patients with next of kin were eligible for inclusion. Study information was given verbally and through information leaflets in local languages—chi Nyanja and chi Bemba—and in English. Next of kin were told that although the study could be of no benefit to the deceased, information gathered from the autopsy could potentially help improve health services for the community. Relatives were not told that this study was specifically focused on tuberculosis or any other disease and were told that the reason for undertaking the study was to find out what disease or diseases may have caused death. Relatives were given the option to consult with other relatives and to return with further questions. Reasons given for refusing consent were recorded. Diagnostic counselling and testing for HIV at UTH is standard, using WHO-approved testing kits. A nominal remuneration (US$10) was given to the relative or next of kin who signed the consent form to assist with transportation for burial. The study and these remunerations were approved by the University of Zambia Biomedical Research Ethics Committee.

Procedures

A whole-body post-mortem examination was undertaken as soon as possible after consent from the next of kin was obtained, so as to cause as little delay as possible to burial proceedings and to avoid autolysis of tissues. Pathological examination comprised two stages. First, gross pathology, in which all organs were weighed and dissected, was done by the study consultant pathologists (VM and AS). When gross pathological abnormalities were detected, sampling was directed towards observed pathological changes or lesions. For healthy tissues, samples were collected in accordance with routine procedures. Samples for histopathology and cryopreservation were taken from the lungs, brain, lymph nodes, thyroid, heart, liver, spleen, intestines, kidneys, bladder, and pancreas. A new set of sterile gloves and blades were used for each organ sampled. Second, histopathological examination was done, with the histopathologists (VM and AS) masked to clinical information. Tissue sections were examined after initial staining with haematoxylin and eosin, Ziehl-Neelsen, and Grocott-Gomori's methenamine silver stains. Special stains were applied when necessary.[9] Specific pathological abnormalities and diseases identified on examination were recorded. Patient case notes and death certificates were reviewed for (1) information regarding whether tuberculosis was suspected by the admitting physician, (2) laboratory investigations, (3) clinical diagnosis, (4) treatment status, and (5) the time from admission to death, where recorded.

We used the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) on tissue samples from patients with tuberculosis detected on histopathological examination at autopsy to detect rifampicin-resistant tuberculosis (which, by proxy, defines multidrug-resistant tuberculosis). Tissues were processed using the GeneXpert MTB/RIF assay,[10] a real-time PCR-based diagnostic assay that detects both *Mycobacterium tuberculosis* complex and rifampicin resistance simultaneously.[11] This assay is recommended for use on samples other than sputum. Rectangular tissue samples from diseased areas, about 30×5×5 mm in dimension, were cut and cryopreserved at –80°C. These samples were processed in a category 3 biosafety cabinet and a 10×5×5 mm section was cut using a single-use sterile surgical blade. 1 mL of distilled deionised RNase and DNase-free water was added and the sample was macerated, transferred to a 10 mL glass screw cap tube containing glass beads, and topped up with 2 mL of deionised water. After vortexing for 5 min, the tube was left to stand for 30 min. 1 mL of supernatant was transferred into a 50 mL Falcon tube containing 3 mL of GeneXpert reaction mix, vortexed, and then left to stand for 20 min. 2 mL of the clear reactant–sample mixture was added to the GeneXpert MTB/RIF cartridge and processed through the GeneXpert machine.

Outcomes

The primary outcome measure was specific disease or diseases stratified by HIV status. Secondary outcomes were missed tuberculosis, multidrug-resistant tuberculosis, and comorbidities with tuberculosis.

	Population*	Study	p value†
Age (years)	38 (30–46)	35 (29–43)	0·182
Men	97/173 (56%)	80/125 (64%)	0·169
HIV infected	119/163 (73%)	101/125 (81%)	0·123
Receiving anti-tuberculosis treatment	71/186 (38%)	65/104 (63%)	<0·0001

Data are median (IQR) or n/N (%). *Adult mortalities at University Teaching Hospital surveyed for 8 weeks before study commencement. †Binary variables: χ^2 test; age: Mann-Whitney *U* test.

Table 1: **Comparison of key descriptive data between study and population deaths**

Statistical analysis

We did data entry in Epidata, with subsequent analysis in SPSS version 21. We did univariate and multivariate binary logistic regression analyses, controlling for age, sex, anti-tuberculosis therapy, and extrapulmonary tuberculosis status, on unweighted data, to assess the amount to which HIV infection might affect the odds of the main pathological abnormalities identified. All odds ratios (ORs) are given with 95% CIs and the level of significance was 0·05. To estimate point prevalence for the main pathological abnormalities noted within the target population, we weighted the dataset (prevalence population/prevalence sample) on the basis of age distribution, sex, HIV status, and tuberculosis treatment status. The resulting four weighting variables were then multiplied together to make a composite weight for each case, which was then divided by the mean weight so that the weighted dataset matched the study sample size. Data on deaths within the inpatient population were collected via an 8-week survey of admission records before study commencement, noting the number of deaths, sex, age, HIV status, and whether or not patients were on anti-tuberculosis therapy.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and MB and VM had final responsibility for the decision to submit for publication.

Results

812 families were approached for consent, of which 125 (15%) consented to autopsy. 1372 deaths occurred during the study period, giving a coverage of 9% (125 of 1372). Median time from admission to death was 7 days (IQR 5–14), median age was 35 years (IQR 29–43), 80 (64%) patients were men, and 101 (81%) were HIV positive. These demographics were consistent with those of other inpatient deaths; however, study participants were more likely to be on anti-tuberculosis therapy (63% *vs* 38%; p<0·0001; table 1). Of the 687 families who refused to consent, 238 (35%) refused because of religious and other belief reasons, 145 (21%) said the autopsy would be of no benefit to them, 138 (20%) expressed time constraints on burial proceedings, and 52 (8%) said they were opposed to the cadaver being mutilated by autopsy; 114 families did not give a reason.

	Unweighted			Weighted*
	Overall (n=125)	HIV infected (n=101)	HIV uninfected (n=24)	Overall (n=125)
Lung pathological abnormalities	123 (98%)	100 (99%)	23 (96%)	100 (80%, 3·6)
Tuberculosis (all forms)†	78 (62%)	66 (65%)	12 (50%)	71 (57%, 4·4)
Extrapulmonary‡	35 (28%)§	33 (33%)	2 (8%)	21 (17%, 3·4)§
Pulmonary only	43 (34%)	33 (33%)	10 (42%)	50 (40%, 4·4)
Pyogenic pneumonia	46 (37%)	39 (39%)	7 (29%)	41 (33%, 4·2)
Pulmonary oedema	10 (8%)	6 (6%)	4 (17%)	3 (2%, 1·4)
Interstitial pneumonia	8 (6%)	8 (8%)	0	3 (2%, 1·4)
Interstitial fibrosis	5 (4%)	5 (5%)	0	3 (2%, 1·4)
Cytomegalovirus pneumonia	3 (2%)	3 (3%)	0	2 (2%, 1·3)
Other¶	5 (4%)	4 (4%)	1 (4%)	4 (3%, 1·7)
CNS pathological abnormalities	12 (10%)	9 (9%)	3 (13%)	11 (9%, 2·5)
Meningitis	9 (7%)	7 (7%)	2 (8%)	8 (6%, 2·2)
Other‖	3 (2%)	2 (2%)	1 (4%)	3 (2%, 1·3)
Cancers	11 (9%)	8 (8%)	3 (13%)	11 (9%, 2·5)
Kaposi's sarcoma	6 (5%)	6 (6%)	0	5 (4%, 1·7)
Other**	5 (4%)	2 (2%)	3 (13%)	6 (5%, 2·0)
Cardiac pathological abnormalities	9 (7%)	6 (6%)	3 (13%)	10 (8%, 2·4)
Congestive cardiac failure	4 (3%)	3 (3%)	1 (4%)	4 (3%, 1·7)
Cardiomegaly	3 (2%)	3 (3%)	0	3 (2%, 1·4)
Other††	3 (2%)	1 (1%)	2 (8%)	4 (3%, 1·5)
Other pathological abnormalities‡‡	9 (7%)	5 (5%)	4 (17%)	NA

Data are number (%) or number (%, SE). NA=not applicable. *Weighted for age, sex, HIV status, and anti-tuberculosis therapy status. †Pulmonary or extrapulmonary tuberculosis, or both. ‡All also had pulmonary tuberculosis. §Unweighted *vs* weighted Pearson χ^2 p=0·034. ¶Emphysema (one HIV positive, one HIV negative), pulmonary candida (one HIV positive), pulmonary schistosomiasis (one HIV positive), and *Pneumocystis jirovecii* pneumonia (one HIV positive). ‖Meningoencephalitis in two patients (one HIV positive and one HIV negative) and hepatic encephalopathy in one patient (HIV positive). **Lung carcinoma in two patients (both HIV negative), brain tumour in two patients (one HIV positive and one HIV negative), and lymphoma in one patient (HIV positive). ††Biventricular heart failure in two patients (one HIV positive and one HIV negative) and pericarditis in one patient (HIV negative). ‡‡Liver cirrhosis in three patients (one HIV positive and two HIV negative), septicaemia in two patients (one HIV positive and one HIV negative), gastrointestinal ulcers in one patient (HIV negative), kidney failure in two patients (both HIV positive), and oral thrush in one patient (HIV positive).

Table 2: **Main autopsy findings**

Table 2 shows the major findings at autopsy. Infectious diseases were more common than non-communicable diseases (114 [91%] *vs* 31 [25%] of 125; p<0·0001). Apart from HIV infection (in 101 [81%] of 125 patients), which was diagnosed before death, tuberculosis was the most common finding at autopsy, with 78 patients (62%) diagnosed by histopathology, of whom 35 (45%) had extrapulmonary tuberculosis, all of whom also had pulmonary tuberculosis. The risk of extrapulmonary tuberculosis was higher among HIV-infected patients than among uninfected patients (adjusted OR 5·14, 95% CI 1·04–24·5; p=0·045). Of 78 patients with tuberculosis, 20 (26%) were only diagnosed after they died and 13 (17%) had undiagnosed multidrug-resistant tuberculosis.

Pyogenic pneumonia was the third most common finding at autopsy, occurring in 46 (37%) of 125 patients. CNS pathological abnormalities were noted in 12 patients (10%) and meningitis in nine (7%). No cases of tuberculosis meningitis were reported. Non-communicable disease causes of death were less common. 11 (9%) of 125 patients had cancer: six had Kaposi's sarcoma, two had lung carcinoma, two had brain tumour, and one had lymphoma. Cardiac pathological abnormalities occurred in nine (7%) of 125 patients. The proportion of patients with extrapulmonary tuberculosis was lower after than before adjusting for age, sex, HIV status, and anti-tuberculosis therapy (17% *vs* 28%; p=0·034). Small differences between unweighted and weighted data were noted with respect to other pathological abnormalities (table 2).

The attending physician noted pulmonary or disseminated tuberculosis as a cause of death in 47 (60%) of 78 patients. 20 (26%) of 78 patients with tuberculosis were not on tuberculosis treatment when they died. Ten (8%) of 125 patients were not suspected of having tuberculosis on admission and were undiagnosed and untreated. Admission diagnoses for the ten missed patients with tuberculosis were as follows: suspected meningitis (five), renal dysfunction (two), pneumonia (one), suspected toxoplasmosis (one), and cancer of the conjunctiva (one). Median time from admission to death for these missed patients with tuberculosis was 7 days (IQR 3–8).

HIV treatment status was available in 96 (95%) of 101 HIV-infected patients, 48 (50%) of whom were on antiretroviral therapy. In 35 patients for whom data were available, the median duration of antiretroviral therapy was 6 weeks (IQR 2–35). Among HIV-infected patients, neither status nor duration of antiretroviral therapy had a significant association with tuberculosis (data not shown). Extrapulmonary tuberculosis was significantly more prevalent among patients with HIV than among those without (p=0·017; table 3), with a five times higher odds of extrapulmonary tuberculosis in HIV-infected patients than in uninfected patients (adjusted OR 5·14, 95% CI 1·04–24·5; p=0·045; table 4). Pyogenic pneumonia and pulmonary oedema did not differ between those with and without HIV infection (tables 2 and 4). Two patients had emphysema (one HIV positive and one HIV negative) and lobar pneumonia (one HIV positive and one HIV negative). Normal lungs were noted in two patients (one HIV positive and one HIV negative). Interstitial pneumonia (eight), interstitial

	Overall (n=125)	HIV infected (n=101)	HIV uninfected (n=24)	p value
Tuberculosis (all forms)*	78 (62%)	66 (65%)	12 (50%)	0·16
Extrapulmonary†	35 (28%)	33 (33%)	2 (8%)	0·017
Pulmonary only	43 (34%)	33 (33%)	10 (42%)	0·40

Data are number (%), unless otherwise specified. *Pulmonary or extrapulmonary tuberculosis, or both. †All also had pulmonary tuberculosis.

Table 3: **Overall prevalence of tuberculosis at autopsy**

	OR (95% CI)*	p value	Adjusted OR (95% CI)†	p value
Tuberculosis (all types)	1·89 (0·77–4·63)	0·17	1·48 (0·54–4·04)	0·45
Pulmonary tuberculosis	0·68 (0·27–1·69)	0·41	0·94 (0·32–2·76)	0·91
Extrapulmonary tuberculosis	5·34 (1·18–24·1)	0·029	5·14 (1·04–24·5)	0·045
Pyogenic pneumonia	1·53 (0·58–4·02)	0·39	2·25 (0·79–6·44)	0·13
Pulmonary oedema	0·32 (0·08–1·22	0·095	0·65 (0·09–4·82)	0·67

OR=odds ratio. *Univariate binary logistic regression analysis undertaken on non-weighted data. †Multivariate binary logistic regression analysis undertaken on non-weighted data adjusting for age, sex, anti-tuberculosis therapy, and extrapulmonary tuberculosis status.

Table 4: **Effect of HIV infection on odds of common pathological findings**

	Overall (n=78)	HIV uninfected (n=12)	HIV infected (n=66)
Any comorbidity	56 (72%)	10 (83%)	46 (70%)
Pyogenic pneumonia	26 (33%)	5 (42%)	21 (32%)
Anaemia	15 (19%)	2 (17%)	13 (20%)
Pyogenic meningitis	7 (9%)	2 (17%)	5 (8%)
Interstitial pneumonitis	4 (5%)	0	4 (6%)
Pulmonary oedema	4 (5%)	1 (8%)	3 (5%)
Brain tumour	3 (4%)	1 (8%)	2 (3%)
Cytomegalovirus pneumonia	3 (4%)	0	3 (5%)
Kaposi's sarcoma	3 (4%)	0	3 (5%)
Interstitial fibrosis	2 (3%)	0	2 (3%)
Candidiasis	1 (1%)	0	1 (2%)
Emphysema	1 (1%)	1 (8%)	0
Liver cirrhosis	1 (1%)	1 (8%)	0
Meningoencephalitis	1 (1%)	0	1 (2%)
Pericarditis	1 (1%)	1 (8%)	0
Pneumocystis jirovecii pneumonia	1 (1%)	0	1 (2%)
Pulmonary embolism	1 (1%)	1 (8%)	0
Schistosomiasis	1 (1%)	0	1 (2%)

Data are number of patients (%).

Table 5: **Comorbidity of tuberculosis cases, stratified by HIV infection**

fibrosis (five), cytomegalovirus (three), *Pneumocystis jirovecii* pneumonia (one), pulmonary schistosomiasis (one), and candida (one) were noted only in HIV-infected patients (table 5). Prevalence of HIV did not differ between patients with and without tuberculosis (85% *vs* 75%; p=0·16).

Comorbidity within patients with tuberculosis was common, occurring in 56 (72%) of 78 patients (table 5). The most common findings were pyogenic pneumonia in 26 patients (33%), anaemia in 15 (19%), and pyogenic meningitis in seven (9%; table 5). We did not detect any cases of cryptococcal meningitis. The prevalence of the most common comorbidities were similar between HIV-infected and HIV-uninfected patients.

All 78 patients with tuberculosis were GeneXpert positive for mycobacterial DNA and 13 (17%) were positive for rifampicin resistance (multidrug-resistant tuberculosis by proxy). On case note review of these 13 patients, ten were on inappropriate standard quadruple therapy for drug-sensitive tuberculosis and three were not on tuberculosis treatment. Median time from admission to death for patients with multidrug-resistant tuberculosis was 8 days (IQR 6–12; data not shown).

Panel: Research in context

Systematic review

We searched PubMed for articles published between Dec 1, 1990, and Dec 1, 2014, with the search terms "autopsy or post mortem" and "Africa" and "tuberculosis or TB". No language restrictions were used. Case reports and verbal autopsy studies were also excluded. We identified 21 autopsy studies in which mortality was reported, 12 of which were undertaken on patients older than 16 years. Insufficient autopsy data exist, with just five adult studies undertaken since the rollout of antiretroviral therapy,[5,7,8,12,13] and there is a need for more autopsy data to assess the undiagnosed burden of disease and comorbidities during life, improve mortality statistics, and assist with improvements to diagnostic algorithms at referral centres. Previous autopsy studies have shown that many patients continue to die of treatable infectious diseases. Respiratory infectious diseases, particularly tuberculosis and pyogenic bronchopneumonia, are treatable, but remain important causes of mortality in adult inpatients since they are difficult to diagnose because of overlapping symptoms. Previous studies have focused on specific patient groups (maternal deaths,[5] HIV-infected cases only,[8] or members of a specific community, such as miners[7,12]).

Interpretation

We present findings from a cross-sectional autopsy study of both HIV-infected and HIV-uninfected African adults in which we used the Xpert MTB/RIF assay to estimate the burden of multidrug-resistant tuberculosis at post mortem. Although our study had limitations of sample size and unavoidable bias, we found a substantial burden of tuberculosis (78 [62%] of 125 patients autopsied), undiagnosed tuberculosis (20 [16%] of 125), and multidrug-resistant tuberculosis (13 [17%] of 78 patients with tuberculosis), none of whom were on multidrug-resistant tuberculosis treatment before they died. This high burden of undiagnosed tuberculosis and multidrug-resistant tuberculosis in a busy inpatient ward, without isolation, is a risk to other patients and staff, and demands an enhanced tuberculosis case finding approach at our centre, but also poses an important question to other similar units across the region. The high proportion of inpatients dying of treatable infections despite effective diagnostics and treatment being available is probably a result of shortcomings in the cascade of health care. Further autopsy studies are needed to assess the generalisability of these findings, both nationally and regionally.

Discussion

In this study, we show that a large burden of tuberculosis, in all its clinical forms, was identified in medical inpatients. A substantial proportion of tuberculosis cases were missed clinically and were not diagnosed before death. Extrapulmonary tuberculosis was common, was found in nearly half of cases of tuberculosis, and was associated with HIV infection. Cases of multidrug-resistant tuberculosis were missed before death and patients with multidrug-resistant tuberculosis were not on appropriate therapy. Only 50% of patients with tuberculosis who were co-infected with HIV were receiving antiretroviral therapy (panel).

Our study has several limitations. The data have to be interpreted in light of the generic limitations faced by all autopsy research studies.[2–8,11–15] Autopsy studies are confounded by low levels of consent and recruitment, because of various cultural and religious beliefs and socioeconomic factors.[4] Thus, findings from subgroups with low sample sizes might not be representative of the overall study population.[16] Our study was designed to ascertain the burden of tuberculosis at autopsy in medical inpatient deaths and was done at a tertiary referral centre, and thus does not represent causes of death at other levels of health care services. To avoid further sampling bias and recruitment enrichment of patients with tuberculosis, relatives were not told that this study was specifically focused on tuberculosis or on any other disease and that the reason for the study was to find out the causes of death. We would have expected relatives of patients who died whilst on tuberculosis therapy or who already had a clinical diagnosis of tuberculosis not to consent since the cause of their disease was already known and relatives would see no point in doing the autopsy and delaying burial proceedings. Contrary to that argument, the recruiting team noted that poorer families were more likely to consent to take part in the study, which might explain the higher burden of tuberculosis within the study group compared with the population.

Binary logistic regression ORs are exaggerated for low sample sizes,[16] which should be taken into consideration when interpreting findings of small studies. Therefore, before recruitment, we specifically collected data on the population of inpatient deaths and adjusted for the bias towards patients on tuberculosis treatment. Mycobacterial culture of autopsied lung, lymph node, and other tissues was not done and would probably have identified additional tuberculosis cases, adding further to the burden of tuberculosis.

Our results are similar to data from a previous study of inpatients in adult general medical wards at UTH,[17] which showed that proactive screening for tuberculosis in all inpatients who could produce sputum for analyses,

irrespective of their admission diagnosis, can yield a substantial number of patients with undiagnosed, unsuspected, or subclinical tuberculosis. The fact that patients continue to die of tuberculosis at a tertiary care referral centre reveals several shortcomings in clinical awareness, recognition, and diagnosis of tuberculosis, coupled with poor laboratory services and an inadequate cascade of tuberculosis health care, from peripheral clinics up to the tertiary care level. Making an accurate clinical diagnosis of pulmonary and extrapulmonary tuberculosis can be difficult since the symptoms of active tuberculosis disease vary and overlap with clinical manifestations and presentations of other bacterial and viral causes of respiratory tract infections. In many developing countries, including Zambia, diagnostic services at primary and secondary care health centres are basic and treatment of respiratory tract infections is empiric in most patients. Thus, many patients with underlying pulmonary tuberculosis, extrapulmonary tuberculosis, and subclinical tuberculosis are easily missed during their presentation along the cascade of health care. Thus, an intensification of calls for more proactive screening for tuberculosis and multidrug-resistant tuberculosis are needed worldwide.[18]

Annual reports from WHO, which delineate the global burden of tuberculosis, drug-resistant tuberculosis, and associated mortality, present data that are only estimates since no accurate verifiable data on causes of death are available. Present mortality statistics are based on national government annual returns data on vital statistics and verbal autopsy studies, both of which are inaccurate. Several challenges of collecting good quality vital statistics that better inform national tuberculosis control programmes, WHO, and international agencies include the accuracy of clinical diagnosis, poor laboratory infrastructure, poor record keeping, inconsistent reporting, and a scarcity of autopsy studies. The 2014 WHO report[1] also states that the incidence of drug-resistant tuberculosis is increasing, with an estimated 480 000 new cases of multidrug-resistant tuberculosis in 2013. This estimate also seems to be an underestimate, since, as our study suggests, multidrug-resistant tuberculosis can be easily missed before death. Additionally, collection of accurate data on the true burden of drug-resistant tuberculosis across sub-Saharan Africa, Asia, and eastern Europe[1,19,20] is impaired by the shortage of drug-resistance testing services at health-care facilities, which has led to a scarcity of proactive screening for multidrug-resistant tuberculosis.

The GeneXpert MTB/RIF assay[10,21] was designed to detect pulmonary tuberculosis from sputum specimens and to screen for rifampicin resistance at the same time (proxy for multidrug-resistant tuberculosis). It is now recommended for use on non-sputum samples for the diagnosis of extrapulmonary tuberculosis.[22] This assay provided us with an opportunity to examine tissue obtained at autopsy to detect *M tuberculosis* DNA and, by proxy, multidrug-resistant tuberculosis through detection of rifampicin resistance genes.[10,22] Previous studies have shown that the GeneXpert MTB/RIF assay detects *M tuberculosis* DNA from dead bacilli in sputum of patients with tuberculosis who have been treated or in those with latent tuberculosis infection.[6–8] Thus, we used the GeneXpert MTB/RIF assay to assess the possible prevalence of multidrug-resistant tuberculosis in tuberculosis cases detected on autopsy. The GeneXpert MTB/RIF assay was not used for diagnosis of additional tuberculosis cases. Ten of the patients with multidrug-resistant tuberculosis were on inappropriate standard quadruple therapy and three were not on any tuberculosis treatment. Our findings are comparable to those from a previous necropsy study,[23] which used fine-needle sampling to obtain post-mortem tissue samples and processed them through culture-based drug sensitivity testing to identify people with multidrug-resistant tuberculosis.

Our finding that extrapulmonary tuberculosis was linked to co-infection with HIV is consistent with findings from other studies in sub-Saharan Africa.[12,14] Although the rollout of antiretroviral therapy has dramatically reduced HIV-associated mortality,[24] the most common causes of death in our autopsy study—tuberculosis and bacterial pneumonia—are still major causes of death.[25,26] WHO recommends proactive screening for tuberculosis and multidrug-resistant tuberculosis in high-risk groups such as prisoners,[27] health-care workers,[28] and miners.[29] The prevalence of tuberculosis in Zambia's prisons is 7·6%, with all new inmates screened on entry.[30] The neglected burden of tuberculosis among hospital inpatients might be greater than in these groups. Inpatients in health-care settings in high tuberculosis endemic areas should be classed as high risk and screened routinely for tuberculosis and multidrug-resistant tuberculosis, irrespective of the admission diagnosis. This additional screening would go some way to detecting a proportion of the 3 million missed cases of tuberculosis and undiagnosed multidrug-resistant tuberculosis.[1] Referral centres with inadequate tuberculosis diagnosis and isolation facilities have probably become centres for the transmission of tuberculosis and multidrug-resistant tuberculosis. Heightened clinical awareness of multidrug-resistant tuberculosis and more widespread screening among inpatients—by use of rapid diagnostic instruments such as the GeneXpert MTB/RIF assay or Hain test—could facilitate rapid detection of multidrug-resistant tuberculosis, early initiation of appropriate treatment, and initiation of appropriate infection control measures. With a high tuberculosis load, we suggest screening for tuberculosis on admission for all inpatients, irrespective of admission diagnosis, with the rapid GeneXpert MTB/RIF assay on sputum, if available, or on other clinical specimens. Further studies are needed to assess whether mortality can be reduced through prompt initiation of appropriate treatment.

Pyogenic pneumonia was the second most common cause of death in our autopsy study. This finding lends

support to the present calls for more investment into the development of rapid point-of-care diagnostics instruments that can screen simultaneously for all microbial causes of respiratory tract infections (not only tuberculosis) and their antibiotic resistances.[31] With the widespread emergence of multi-antibiotic-resistant bacterial species, which cause respiratory tract infections, further studies from developing countries are needed to delineate local and regional antibiotic resistance patterns of common bacterial causes of respiratory tract infections and to heighten clinical awareness of this problem.

We did not detect any cases of cryptococcal meningitis and selection bias might have contributed to this. Since our study of 230 cases of cryptococcal meningitis from UTH in 2001,[32] the widespread rollout of antiretroviral drugs and the increase in ability of community clinics to provide antiretroviral treatment and monitoring has led to a substantial reduction in the number of patients with cryptococcal meningitis presenting at tertiary referral centres. Many patients with HIV who present to community clinics with fever receive empiric antibacterial antibiotics and fluconazole, and those admitted to hospital with meningitis are empirically treated with antifungals (fluconazole and flucytosine combined) and broad-spectrum antibiotics. In southern Africa, community prevalence of cryptococcal meningitis, as ascertained by serum cryptococcal antigen tests, is less than 3%,[33] and thus undertaking an autopsy on more patients would probably reveal more deaths due to cryptococcal meningitis.

In the present study, we focused on primary findings at autopsy and further autopsy studies are needed to define comorbidities, especially in HIV-infected individuals; these studies should include detailed molecular analyses, including next-generation sequencing of biobanked waxed blocks and cryopreserved tissues. Such studies would also enable genetic analysis of co-infections with several strains of *M tuberculo*sis. Data from autopsy studies present an opportunity to improve civil registration and the collection of vital statistics, as emphasised by the adjustment of estimated data on global tuberculosis morbidity and mortality statistics in the 2014 WHO annual tuberculosis report.[1] We argue for greater investment into pathology services so that routine autopsies can be introduced in all African countries.

Contributors

AZ obtained funding for the study and designed and initiated the study with MB, VM, and PM. MB coordinated the study as part of the tuberculosis, HIV, and respiratory portfolio of the University of Zambia and University College London Medical School Research and Training Programme. VM and AS were study pathologists who did the autopsies and histopathology. JK, JT, MK, CC, LC, and MC were the study team responsible for counselling, recruitment, molecular analysis, and day-to-day management. NK, MH, MM, and PM were part of the advisory and monitoring group and provided input into data analyses and interpretation. MB and AZ wrote the first and subsequent drafts of the manuscript. All authors contributed to finalisation of the manuscript.

Declaration of interests

We declare no competing interests.

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