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Original article

Performance of Xpert Ultra nasopharyngeal swab for identification of tuberculosis deaths in northern Tanzania

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ABSTRACT

Objective: Numerous tuberculosis (TB) deaths remain undetected in low-resource endemic settings. With autopsy-confirmed tuberculosis as our standard, we assessed the diagnostic performance of Xpert MTB/RIF Ultra (Ultra; Cepheid) on nasopharyngeal specimens collected postmortem.

Methods: From October 2016 through May 2019, we enrolled pediatric and adult medical deaths to a prospective autopsy study at two referral hospitals in northern Tanzania with next-of-kin authorization. We swabbed the posterior nasopharynx prior to autopsy and tested the samples later by Ultra. At autopsy we collected lung, liver, and, when possible, cerebrospinal fluid for mycobacterial culture and histopathology. Confirmed tuberculosis was defined as *Mycobacterium tuberculosis* complex recovery by culture with consistent tissue histopathology findings; decedents with only histopathology findings, including acid-fast staining or immunohistochemistry, were defined as probable tuberculosis.

Results: Of 205 decedents, 78 (38.0%) were female and median (range) age was 45 (0,96) years. Twenty-seven (13.2%) were found to have tuberculosis at autopsy, 22 (81.5%) confirmed and 5 (18.5%) probable. Ultra detected *M. tuberculosis* complex from the nasopharynx in 21 (77.8%) of 27 TB cases (sensitivity 70.4% [95% confidence interval {CI} 49.8–86.2%], specificity 98.9% [95% CI 96.0–99.9%]). Among confirmed TB, the sensitivity increased to 81.8% (95% CI 59.7–94.8%). Tuberculosis was not included as a death certificate diagnosis in 14 (66.7%) of the 21 MTBc detections by Ultra.

Discussion: Nasopharyngeal Ultra was highly specific for identifying in-hospital tuberculosis deaths, including unsuspected tuberculosis deaths. This approach may improve tuberculosis death enumeration in high-burden countries. **Cristina Costales, Clin Microbiol Infect 2022;28:1150.e1–1150.e6**

Abbreviations: TB, Tuberculosis; sSA, sub-Saharan Africa; NPS, Nasopharyngeal swab; CDA, Complete diagnostic autopsy; MITS, Minimally invasive tissue sampling.

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Introduction

Mortality from tuberculosis (TB) in sub-Saharan Africa (sSA) is high. In Tanzania in 2020, the World Health Organization estimated 20 000 TB deaths in HIV-positive persons and 12 000 deaths in HIV-negative persons [1]. Postmortem studies have confirmed a high prevalence of TB at autopsy in sSA, in up to 43% in HIV-positive adults [2] and more than 30% of adults dying at home with an unknown cause of death [3]. Postmortem studies in sSA have also demonstrated under-recognition of TB in HIV-positive persons [4–6]. This under-recognition can stem from nonspecific clinical presentations, especially in disseminated TB among persons living with HIV; but clinical under-recognition of fulminant and fatal TB also belies the challenge of establishing microbiologically confirmed TB in severely ill patients, many of whom present with nonproductive cough [7].

Identifying missed TB deaths is important for accurate measurement of disease burden, especially in endemic settings with limited clinical resources. Enhanced case detection by postmortem testing would improve mortality assessments. Although postmortem pathology examinations are superior to clinical diagnoses for death certification [4], medical autopsy is not readily available in much of sSA [8], and in some settings, next of kin may show reluctance to consent for the procedure [9–11]. In such settings, an accurate, rapid postmortem test for TB administered by minimally trained healthcare staff would be more suitable for assessing deaths in the community and in hospitals.

The Xpert MTB/RIF Ultra assay (Ultra; Cepheid, Sunnyvale, CA, USA) is an updated version of Xpert MTB/RIF for clinical diagnosis of pulmonary and extrapulmonary TB using sputum and cerebrospinal fluid, which detects paucibacillary disease [12,13]. Ultra is widely available among sSA countries with TB control programs. The Xpert MTB/RIF and Ultra demonstrated high diagnostic accuracy for identification of TB in postmortem tissue specimens [14,15] and moderate diagnostic accuracy on nasopharyngeal aspirates in children [16,17]. We sought to evaluate the diagnostic performance of Ultra on nasopharyngeal swabs prospectively collected at autopsy from children and adults who died in hospital compared to autopsy diagnosis of TB based upon mycobacterial culture and histopathology methods.

Methods

From October 2016 through May 2019, families of inpatient pediatric and adult medical deaths at Kilimanjaro Christian Medical Centre and Mawenzi Regional Referral Hospital in northern Tanzania were offered an autopsy procedure—complete diagnostic autopsy (CDA) or, if CDA was refused, minimally invasive tissue sampling (MITS) postmortem [11,18]. Decedents with next-of-kin authorization were prospectively enrolled. Bodies of decedents were stored at 4°C from time of death until autopsy, with the goal of less than 24 hours from death to procedure. Death certificates were completed by ward clinicians with knowledge of the patient's clinical course but prior to autopsy and postmortem test result availability.

Postmortem procedure

At initiation of each autopsy, trained study staff advanced a sterile swab (FLOQSwab, COPAN, Brescia, Italy) into one of the decedent's nostrils, through turbinate to posterior nasopharynx by slow rotation, then placed the swab into 3 mL universal transport media (COPAN). HIV rapid serologic and confirmatory testing was performed as per Tanzanian national policies [19]. Autopsy, either CDA or MITS, was then performed by one of three study pathologists (A.R.M., P.T.A., C.C.) using standardized sample collection for aerobic and mycobacterial cultures and for fresh frozen archive and histology. Tissue processing, mycobacterial culture, and Ultra testing were performed according to standardized laboratory procedure. When mycobacterial culture was not performed immediately following autopsy, fresh frozen archival tissue specimens were thawed from −80°C and cultured. See web-only Supplementary Methods for additional laboratory procedure details.

Definition of tuberculosis

A panel of study pathologists and infectious disease clinicians determined cause of death diagnoses by adjudication of medical charts, autopsy gross and histology findings, and culture results, without knowledge of Ultra results. CDA and MITS were considered equivalent in rendering an autopsy TB diagnosis, as prior MITS validation studies in similar populations have shown close to 80% or greater concordance for infectious disease postmortem diagnosis [18,20]. Confirmed TB was defined by *Mycobacterium tuberculosis* complex (MTBc) recovery from mycobacterial culture and histopathology microscopic changes consistent with TB infection. These microscopic changes included necrotizing granulomatous inflammation of any organ [21] or lymphohistiocytic inflammation of the leptomeninges [22], without evidence of alternate etiology. Without MTBc culture growth, TB was considered probable if microscopic changes, with or without Ziehl-Neelsen staining or immunohistochemistry for *Mycobacterium* spp. was observed in any tissue. TB was considered disseminated if MTBc was isolated from either liver or spleen, or by TB microscopic changes in at least two noncontiguous organs, with or without pulmonary involvement [23].

Analysis

Decedents without nasopharyngeal swab or tissue specimens collected were excluded. Sensitivity of nasopharyngeal Ultra was defined as the proportion of decedents with confirmed or probable TB at autopsy compared with any 'MTB detected' result. Specificity was the proportion of patients without TB identified at autopsy compared with an 'MTB not detected' result by Ultra. Statistical analyses were performed in STATA 16 (StataCorp, College Station, TX, USA): significance tests of proportions using chi-squared or, in sample size groups less than 5, the Fisher exact test, and area under the receiver operator characteristic curve analysis to assess overall diagnostic accuracy.

Ethical review

This study received ethics approval from the Duke Health Institutional Review Board, the Kilimanjaro Christian Medical Centre Research Ethics Committee, and the Tanzania National Institutes for Medical Research Ethics Coordinating Committee.

Results

Enrollment and decedent demographics

Of 219 autopsies performed during the study period, 14 (6.4%) were excluded (Fig. 1). Demographic information for 205 decedents included in the analysis summarized in Table 1. The median (range) postmortem interval time was 21.5 hours (4–74 hours) with 115 (56.1%) of autopsies performed within 24 hours.

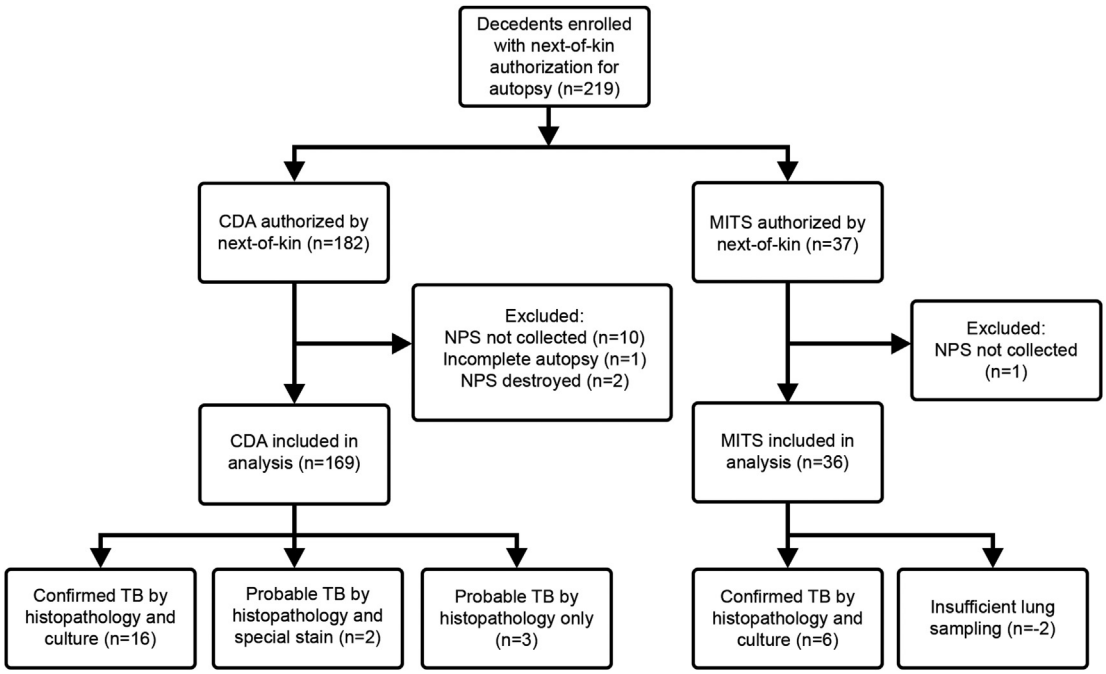

Fig. 1. Autopsy study enrollment from in-hospital pediatric and medical ward deaths with autopsy tuberculosis diagnosis from October 2016 through May 2019, Moshi, Tanzania. CDA, Complete diagnostic autopsy; MITS, minimally invasive tissue sampling; TB, tuberculosis; NPS, nasopharyngeal swab.

Autopsy diagnosed tuberculosis

TB was identified at autopsy in 27 (13.2%) decedents, of which 22 (81.5%) were confirmed and 5 (18.5%) were probable. Six (22.2%) decedents with TB were diagnosed by MITS, all confirmed by MTBc culture. Of 27 decedents with TB, one (4.7%) was adjudicated not to be on the causal pathway to death, which occurred by organophosphate poisoning. For the 26 other instances TB was adjudicated to be the underlying cause of death. Among those with a postmortem TB diagnosis, the median (range) number of tissue types submitted for mycobacterial cultures per decedent was 3 (0–5) and the median (range) number of tissues from which MTBc was isolated was 1 (0–3). Twenty-three (85.2%) of 27 decedents with confirmed or probable TB had disseminated disease; 13 (56.5%) were HIV-seropositive ($p = 0.61$). Of those with non-disseminated TB, one (3.7%) decedent had isolated pulmonary TB, one (3.7%) had isolated thoracic lymph node TB without pulmonary involvement, and two (7.4%) had TB meningitis. Of 69 decedents in which at least one specimen was frozen at −80°C before culture, 3 (7.7%) of 39 liver, 5 (8.1%) of 62 lung, and 1 (20.0%) of 5 cerebrospinal fluid specimens grew MTBc. No mycobacterial cultures were collected from one (20.0%) of five decedents with probable TB. See web-only Supplementary Results for additional culture result details.

Of the 27 decedents with TB, 16 (59.3%) were aged 15 to 49 years with an overall median (range) age of 40 years (4–75), 15 (55.6%) were HIV-seropositive, 22 (81.5%) were febrile admissions, and 3 (11.1%) had TB meningitis, of which 2 were probable TB diagnoses in HIV-seronegative decedents. Of 48 HIV-seropositive decedents in this analysis, 15 (31.2%) had confirmed or probable TB at postmortem; the odds of TB among HIV-seropositive persons was 5.3 (95% CI 2.1–13.6, $p < 0.01$). Nine (33.3%) TB decedents had an antemortem diagnosis of TB and five (18.5%) were receiving anti-TB therapy at time of death.

Autopsy versus clinical tuberculosis diagnosis

Death certificates were available from 186 (90.7%) of 205 decedents, of which 12 (6.5%) listed TB as a diagnosis associated with the cause of death. Of these 12 TB death-certified decedents, 6 (50.0%) had TB confirmed at autopsy. Among 25 decedents with postmortem confirmed or probable TB and available death certificate, 6 (24.0%) listed TB as cause of death diagnosis on the death certificate. The two decedents with an autopsy TB diagnosis but without death certificates had no mention of TB as a suspected diagnosis in the medical chart. Thus, 18 (66.6%) TB decedents at autopsy had no documented antemortem diagnosis of TB. Fifteen (8.4%) of 178 decedents without TB identified at autopsy had death certificates with TB listed as the cause of death or a medical chart diagnosis.

Ultra detection of tuberculosis

Nineteen (70.4%) of 27 decedents with confirmed or probable TB had MTBc detected by Ultra nasopharyngeal swab (Table 2). The median (range) time interval from death to collection of nasopharyngeal swab for those with Ultra MTBc detection was 18 (7–47) hours and 22 (4–74) hours for all Ultra undetected, without significant difference (p value: 0.13). No rifampicin resistance was detected.

As summarized in Table 3, this postmortem Ultra approach showed sensitivity of 81.8% (95% CI 59.7–94.8%) and specificity of 98.4% (95% CI 95.3–99.7%) for confirmed TB diagnosis, with separate calculations for confirmed or probable TB diagnoses. No significant difference was observed for Ultra sensitivity when comparing confirmed or probable TB to confirmed TB (p value: 0.35). The Ultra receiver operator characteristic curve areas were 0.90 (95% CI 0.82–0.98) for confirmed TB and 0.85 (95% CI 0.76–0.93) for confirmed and probable TB.

Table 1
Demographic information for 205 in-hospital deaths from the pediatric and medical wards included in the assessment of postmortem nasopharyngeal Ultra detection of fatal tuberculosis, October 2016 through May 2019, Moshi, Tanzania

Characteristics	Total, (%)		TB Diagnosis,* (%)	
Total postmortems	205		27	13.2
Female	78	38	6	22.2
Age, years, <5	29	14.1	1	3.7
5–14	7	3.4	1	3.7
15–49	76	37.1	17	63
50–69	55	26.8	6	22.2
≥70	38	18.5	2	7.4
Febrile at admission	156	76.1	22	81.5
HIV seropositive	48	23.4	15	55.6
Minimally invasive tissue sampling	36	17.6	6	22.2

* TB diagnosis includes confirmed and probable diagnoses at autopsy. TB, Tuberculosis; Ultra, Xpert MTB/Rif Ultra assay.

Table 2
Ultra nasopharyngeal swab test results from 205 in-hospital pediatric or medical ward deaths from October 2016 through May 2019, Moshi, Tanzania

	Ultra MTBc results					
	High	Medium	Low	Very Low	Trace	Negative
Confirmed TB	2	4	5	5	2	4
Probable TB	0	0	1	0	0	4
TB Negative	0	0	0	1	1	176
Total Ultra results	2	4	6	6	3	184

TB, Tuberculosis; MTBc, *Mycobacterium tuberculosis* complex; Ultra, Xpert MTB/Rif Ultra assay.

Table 3
Diagnostic performance of Ultra on postmortem nasopharyngeal swabs from 205 decedents with in-hospital deaths from October 2016 through May 2019, Moshi, Tanzania

	TB Confirmed, (95% CI)		TB Confirmed or Probable Combined, (95% CI)	
Sensitivity	81.8	(59.7–94.8)	70.4	(49.8–86.2)
Specificity	98.4	(95.3–99.7)	98.9	(96.0–99.9)
Positive Predictive Value	85.7	(63.7–97.0)	90.5	(69.6–98.8)
Negative Predictive Value	97.8	(94.5–99.4)	95.7	(91.6–98.1)
Positive Likelihood Ratio	49.9	(16.0–156.0)	62.6	(15.4–254.0)
Negative Likelihood Ratio	0.19	(0.08–0.45)	0.30	(0.17–0.40)

TB, Tuberculosis; CI, confidence interval; Ultra, Xpert MTB/Rif Ultra assay.

Two false positive Ultra results were identified, one with 'MTB trace' detection and the other with 'MTB very low' detection, both from decedents with negative MTBc cultures—one an infant CDA and the other an adult MITS procedure with inadequate lung sampling; neither with an antemortem diagnosis of TB. Eight (29.6%) of the 27 TB decedents had no MTBc detected by nasopharyngeal Ultra. Of these eight Ultra false negatives, four (50.0%) were from probable TB decedents with negative mycobacterial cultures: one with nondisseminated pulmonary TB on anti-TB therapy at the time of death, another with TB restricted to thoracic lymph nodes, and two with probable TB meningitis. Three (37.5%) of eight Ultra false negatives were from decedents with TB meningitis. Fourteen (66.7%) of the 21 MTB detections by Ultra occurred in deaths without TB as a death certificate diagnosis.

Discussion

Using simple-to-collect, noninvasive nasopharyngeal swabs, we demonstrate that the Ultra assay had high specificity for identifying TB-related deaths at two referral hospitals in northern Tanzania. Our postmortem study is notable in that systematic histologic tissue diagnosis and extensive mycobacterial culturing was used as a comparator to the Ultra. While this nasopharyngeal Ultra approach showed only moderate sensitivity for detecting confirmed TB, the overall accuracy by AUROC analysis was 90% for identifying confirmed TB in this autopsy series. As many resource-limited settings with moderate-to-high TB incidence have access to Ultra through government-funded TB programs, our findings have important public health implications: postmortem nasopharyngeal sampling to confirm TB-related deaths in suspected cases and in those with unknown cause of death has the potential to be a powerful diagnostic tool requiring minimal additional investment from individual care settings or health systems. Evaluating the diagnostic performance of the Ultra on nasopharyngeal specimens from living patients may also be of interest, particularly in populations where lower respiratory tract samples are difficult to obtain, such as patients living with HIV with disseminated disease, those unable to expectorate due to severe illness, and children. Indeed, while the pathogenesis of MTBc colonization of the nasopharynx is not well established, our results are consistent with studies showing that nasopharyngeal aspiration is a comparable alternative to obtaining bronchial secretions for TB testing in children [16,17,24].

That a low proportion of decedents with TB were diagnosed prior to death underscores the value of postmortem cause of death assessment, without which TB deaths may be underestimated in mortality data and clinical under-recognition might continue unchecked. African postmortem studies have shown similarly high proportions of clinically missed TB diagnoses in the antemortem inpatient period [6,25], justifying the need for accurate postmortem methods for identifying TB-related deaths.

The majority of decedents with TB at autopsy had disseminated infections. The high proportion of disseminated TB among HIV-seropositive decedents was consistent with both prior autopsy series in sSA [2] as well as bloodstream infection studies in sSA, which show disseminated bacteremic TB occurs predominantly in persons with advanced HIV [26,27]. Meanwhile, 10 (43.5%) of the disseminated TB cases in our study occurred in HIV-seronegative decedents, 9 of whom did not have an antemortem TB diagnosis. This suggests that the index of suspicion for disseminated TB should not be limited to persons living with HIV.

Only two nasopharyngeal swabs gave false positive Ultra results among 179 autopsies without TB identified. The adult false positive decedent likely had TB missed by autopsy due to inadequate lung sampling by the MITS procedure. This decedent was HIV seropositive, not on antiretroviral or TB therapy, and presented to hospital with a massive pleural effusion and renal failure. The reduced efficiency of MITS for pulmonary infection diagnosis is a described limitation of the method [18]. The infant false positive showed histologic findings more consistent with a viral pneumonia and TB was not clinically suspected.

Four false negative Ultra results occurred in probable TB decedents in the absence of positive mycobacterial cultures. Two of these decedents had positive Ziehl-Neelsen tissue staining for acid fast bacilli. Thus, the false negative cases may represent nontuberculous mycobacterial (NTM) infections with similar

microscopic tissue changes and no growth in culture [28]. Alternatively, these may represent paucibacillary MTBc infections below the limit of detection of Ultra. TB molecular testing directly from post-mortem tissues should be considered for future studies to confirm culture-negative paucibacillary MTBc infections [15].

Our study had several limitations, including that a subset of the mycobacterial cultures were performed on thawed tissues or body fluids. While culture of thawed tissue specimens yielded many positive results, sensitivity was likely reduced compared to standard processing. The false positive Ultra infant and one of five probable TB decedents had thawed lung and liver tissue processed for culture, both without mycobacterial growth. For Ultra performance analysis, we considered autopsy TB diagnosis by CDA or MITS as the gold standard comparator, from previous MITS validation studies that showed 93% (pediatric) and 79% (adult) diagnostic concordance for infectious disease postmortem diagnoses [18,20]. While all six MITS decedents with TB diagnosis were confirmed by culture, TB may have been missed in other MITS cases due to sampling error, in particular the adult decedent classified as a false-positive Ultra detection. We excluded 11 decedents from analysis due to deviation from swab collection protocol, introducing a source of potential bias. Excluding decedents with an extended postmortem interval may have increased sensitivity of Ultra. However, the interval from death to swab collection may more closely reflect the circumstances of utilizing the test in non-research, resource-constrained settings. The total number of TB decedents at autopsy was low ($n = 27$) with relatively wide confidence intervals noted for Ultra calculated sensitivities, such that our findings must be confirmed in the context of a larger TB study population. Importantly, even with low overall numbers, our approach identified 18 TB deaths that would have otherwise gone unidentified.

In conclusion, the Ultra assay by nasopharyngeal swab collected postmortem showed moderate sensitivity and high specificity for the diagnosis of fatal TB. For both in-hospital deaths and deaths in the community, the nasopharyngeal Ultra may provide an accessible tool for critical assessment of TB-associated mortality missed during clinical evaluation. Enhanced case detection using this approach would likely benefit national TB prevention efforts in high-burden settings. Further research is warranted to assess the utility of nasopharyngeal Ultra testing among severely ill adults suspected of having disseminated TB and ambulatory adults with suspected pulmonary TB.

Transparency declaration

The authors declare that they have no conflicts of interest. This work was supported by the US National Institutes of Health, National Institute of Allergy and Infectious Diseases [R01 AI121378, K23 AI116869] and Fogarty (Vanderbilt-Emory-Cornell-Duke) Consortium for Fogarty Global Health Fellows [D43 TW009337], through the Fogarty International Center at the US National Institutes of Health. The authors thank Copan for their donation of Universal Transport Media and swabs. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Author contributions

MPR, CC, JAC, MC, AMN, and MJM were responsible for study design. BTM and VPM provided administrative support. CC, MPR, ARM, PTA, BTM, VPM, NHK, KGK, GK, BFL, FL, AM, RM, CM, MC, DM, AMN, EM, and SRZ performed data collection; CC and MPR performed data analysis; and CC, MPR, and JAC handled data interpretation. CC, MPR, and JAC did the writing. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.03.027.

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