



Short Communication

Examination of respiratory specimens improves microbiological diagnosis of patients with presumptive extrapulmonary tuberculosis



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ABSTRACT

Objectives: Bacteriological confirmation of extrapulmonary tuberculosis (EPTB) is challenging for several reasons: the paucibacillary nature of the sample; scarce resources, mainly in middle and low-income countries; the need for hospitalization; and unfavorable outcomes. We evaluated the diagnostic role of respiratory specimen examination prospectively in a cohort of patients with presumptive EPTB.

Methods: From July 2018 to January 2019, in a tuberculosis (TB)/HIV reference hospital, a cohort of 157 patients with presumed EPTB was evaluated. Xpert[®] MTB/RIF Ultra or a culture-positive result was considered for bacteriologically confirmed TB.

Results: Out of 157 patients with presumptive EPTB, 97 (62%) provided extrapulmonary and respiratory specimens and 60 (38%) extrapulmonary specimens only. Of the 60 patients with extrapulmonary samples, 5 (8%) were positive. Of those with respiratory and extrapulmonary samples, 27 (28%) were positive: 10 in both the respiratory and extrapulmonary samples, 6 in the extrapulmonary sample only, and 11 in the respiratory sample only. A respiratory specimen examination increased by 6-fold the chance of bacteriological confirmation of TB (odds ratio = 5.97 [1.11–47.17]).

Conclusion: We conclude that respiratory samples should be examined in patients with presumptive EPTB. © 2021 The Author(s). Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Of the 7 million new tuberculosis (TB) cases reported worldwide in 2018, 15% were extrapulmonary tuberculosis (EPTB)

(World Health Organization, 2019). EPTB is usually paucibacillary, and presentation may be atypical; therefore, it is more challenging to diagnose. Invasive procedures may be required, resulting in a delayed diagnosis, need for hospitalization, unfavorable outcomes and higher costs (Norbis et al., 2014).

Extrapulmonary presentations such as lymph node and pleural TB occur with concomitant pulmonary TB (Shaw et al., 2019; Züker et al., 2019). However, most current TB guidelines do not include respiratory specimen examination in the evaluation of

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presumptive EPTB. The new Xpert[®] MTB/RIF Ultra increased the yield of paucibacillary sample examination compared to smear microscopy or the previous generation of the molecular test (Zhang et al., 2020). We evaluated whether the examination of respiratory samples for bacteriological confirmation of TB could improve patients' diagnosis with presumptive EPTB.

Methods

The study is a *post hoc* analysis of a prospective observational cohort study conducted at *Fundação de Medicina Tropical* Doutor Heitor Vieira Dourado (FMT-HVD), an academic reference center for TB/HIV co-infection, located in the Brazilian Amazonian region. Between July 2018 and January 2019, 157 out- and in-patients aged ≥ 18 years with presumptive EPTB were tested for TB in FMT-HVD's mycobacteriology laboratory and included in the study cohort. The laboratory had received 100% proficiency on the biannual INSTAND test (INSTAND, 2021), which assesses the quality of sample processing, for the previous 4 years. The clinical team made the decision on which specimen should be obtained without the researchers' intervention.

We considered the World Health Organization's definition of a bacteriologically confirmed case: a positive result of smear, culture or WHO-approved rapid diagnostics such as the Xpert RIF/MTB Ultra in any sample (World Health Organization, 2020). Clinically diagnosed TB is defined in the presence of symptoms and radiological images compatible with TB without a positive result of any of the tests above (missing or negative). If there was evidence of concomitant involvement of extrapulmonary sites and lung parenchyma or the tracheobronchial tree, the patient was classified as having pulmonary TB (PTB). At FMT-HVD, all biological samples from patients with presumptive TB are submitted to liquid culture and molecular test (Xpert[®] MTB/RIF Ultra, Cepheid, Sunnyvale, CA, USA).

Clinical and demographic data (age, sex, HIV status, CD4 count and viral load) were gathered from patients' electronic medical records. Data on respiratory symptoms (dyspnea, chest pain or cough) and X-ray abnormalities were collected from patients with bacteriologically confirmed TB in any anatomic site. The sensitivity and specificity of Xpert Ultra was calculated using culture as the reference standard.

Written informed consent was obtained from participants. Clinical investigations were conducted following the Declaration of Helsinki. All the information handled by the research team was de-identified.

Descriptive data are expressed as proportions with their 95% confidence interval (95% CI) levels or median values with their interquartile range (IQR). We used a multivariate regression model to evaluate the association of having a respiratory sample examined with the diagnosis confirmation using the odds ratios (OR) and 95% CI levels, adjusted for age (in years), sex and CD4 count.

Results

Among 157 patients with presumptive EPTB, the median (IQR) age was 37 (30–43) years, 60% were male, and 75% were people living with HIV. Of these, 62% had a CD4⁺ T-cell count below 200 cells/mm³, and 64% had a detectable viral load. Extrapulmonary specimens examined were cerebrospinal fluid (n = 56), urine (n = 37), skin (n = 29), lymph node (n = 21), pleural fluid (n = 12) and ascitic fluid (n = 2). In total, there were 32 positives results from respiratory or extrapulmonary samples. Sixty patients (38%) provided extrapulmonary specimens only; 5 (8%) were positive. Extrapulmonary and respiratory specimens were provided by 97 (62%) patients; 27 (28%) were positive, 10 in both the respiratory and extrapulmonary specimens, 6 in the extrapulmonary

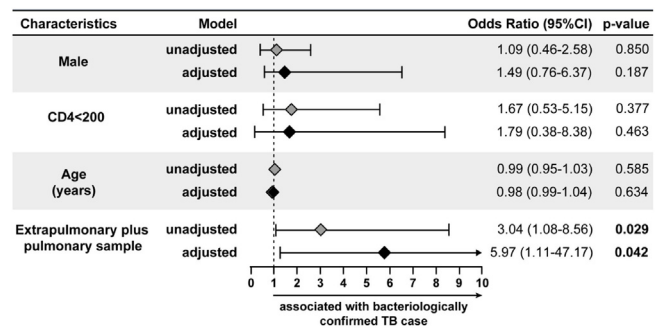


Figure 1. Factors analyzed for the association with bacteriological confirmation of extrapulmonary tuberculosis.

specimen only, and 11 in the respiratory specimen only. The results for patients with respiratory specimen only corresponded to 34% of all positive results and represented an 11% overall added value. Of 21 patients with a positive respiratory sample, 4 (19%) patients had no respiratory symptoms. Adjusted for age, sex and CD4 cell count <200 (Figure 1), having a respiratory specimen examined was associated with bacteriological confirmation of EPTB (OR = 5.97 [1.11–47.17]). A summary of all rapid molecular test results is displayed in Supplementary Table S1.

The overall sensitivity and specificity (95% CI) of Xpert MTB/RIF Ultra compared to *M. tuberculosis* culture were, respectively, 100% (95% CI 73.5–100) and 94.3% (95% CI 88.6–97.7). In one sputum sample, the DNA of a rifampicin-resistant strain was identified using Xpert Ultra and confirmed in the drug susceptibility test against first-line drugs. Information on antiretroviral treatment and respiratory symptoms was only available for 32 patients and was not included in the multivariate analysis.

Discussion

In this cohort of patients with presumptive EPTB, the chance to bacteriologically confirm the diagnosis of TB increased by 6-fold when a respiratory sample was obtained. In poor resource settings, adding a respiratory sample examination to the EPTB workup may increase the chances of rapidly confirming TB and initiating therapy and be more cost-effective than hospitalization for invasive procedures (this analysis was beyond the scope of the current study). Although most patients with a positive respiratory sample did have respiratory symptoms, 1/5 of them did not, suggesting that this test could be useful regardless of symptoms.

Our findings have public health implications as they corroborate previous concerns about EPTB patients' contagiousness and imply the need for contact surveillance and infection control actions, usually neglected in EPTB (Hernández-Garduño et al., 2004; Schirmer et al., 2010).

Finally, in settings where high-quality free-of-charge chest X-rays are unavailable, as in many low- and medium-income countries, Xpert Ultra is now provided by the public health system and more accessible for disadvantage populations. In our study, the sensitivity and specificity of Ultra were high, confirming previous findings (Horne et al., 2019). Relying exclusively on chest X-rays may have adverse consequences regarding transmission, as up to 10% of people living with HIV may have normal radiographs (Pepper et al., 2008; Palmieri et al., 2002).

Our study has limitations. Firstly, despite being a prospective study, the analysis was performed *post hoc*. The clinical team managed patients and there were no clear guidelines for respiratory sample collection in patients with presumptive EPTB in Brazil (Ministério da Saúde, 2018). Patients with respiratory

symptoms had more respiratory sample examination than those without respiratory symptoms, leading to a bias in selection; there was also an absence of a TB history that could have affected the analysis. Furthermore, antiretroviral treatment could not be analyzed as the information was missing for most patients. Thus, robust conclusions on the yield of respiratory specimen examination in all EPTB patients are not possible. Despite these limitations, we believe that systematically screening patients with presumptive EPTB using respiratory specimens should be considered in clinical settings while awaiting results from further studies.

Ethical approval statement

The study was approved by the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado Ethics Committee (#2.525.182).

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Conflict of interest statement

None of the authors has any conflict of interest (financial or personal) in this study.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ijid.2021.03.022>.

References

- Hernández-Garduño E, Cook V, Kunimoto D, Elwood RK, Black WA, FitzGerald JM. Transmission of tuberculosis from smear negative patients: a molecular epidemiology study. *Thorax* 2004;59:286–90, doi:<http://dx.doi.org/10.1136/thx.2003.011759>.
- Horne DJ, Kohli M, Zifodya JS, Schiller I, Dendukuri N, Tollefson D, et al. Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults. *CDSR* 2019;(Issue 6)CD009593, doi:<http://dx.doi.org/10.1002/14651858>.
- Norbis L, Alagna R, Tortoli E, Codecasa LR, Migliori GB, Cirillo DM. Challenges and perspectives in the diagnosis of extrapulmonary tuberculosis. *Expert Rev Anti Infect Ther* 2014;12:633–47, doi:<http://dx.doi.org/10.1586/14787210.2014.899900>.
- Palmieri F, Girardi E, Pellicelli AM, Rianda A, Bordi E, Rizzi EB, et al. Pulmonary tuberculosis in HIV-infected patients presenting with normal chest radiograph and negative sputum smear. *Infection* 2002;30(April (2)):68–74, doi:<http://dx.doi.org/10.1007/s15010-002-2062-9>.
- Pepper T, Joseph P, Mwenya C, McKee GS, Haushalter A, Carter A, et al. Normal chest radiography in pulmonary tuberculosis: implications for obtaining respiratory specimen cultures. *Int J Tuberc Lung Dis* 2008;12(April (4))397–403 PMID: 18371265.
- Saúde, Ministério da. Manual de Recomendações para o controle da tuberculose no Brasil. [Accessed 10 October 2020]. 2018. <http://www.aids.gov.br/pt-br/pub/2019/manual-de-recomendacoes-para-o-controle-da-tuberculose-no-brasil>.
- Schirmer P, Renault CA, Holodniy M. Is spinal tuberculosis contagious?. *Int J Infect Dis* 2010;14., doi:<http://dx.doi.org/10.1016/j.ijid.2009.11.009>.
- Shaw JA, Diacon AH, Koegelenberg CFN. Tuberculous pleural effusion. *Respirology* 2019;24:962–71, doi:<http://dx.doi.org/10.1111/resp.13673>.
- Zhang M, Xue M, He J qing. Diagnostic accuracy of the new Xpert MTB/RIF Ultra for tuberculosis disease: a preliminary systematic review and meta-analysis. *Int J Infect Dis* 2020;90:35–45, doi:<http://dx.doi.org/10.1016/j.ijid.2019.09.016>.
- Züker K, Ballif M, Kiertiburanakul S, Chenal H, Yotebieng M, Grinsztejn B, et al. Diagnosis and clinical outcomes of extrapulmonary tuberculosis in antiretroviral therapy programmes in low- and middle-income countries: a multicohort study. *J Int AIDS Soc* 2019;22(9):e25392, doi:<http://dx.doi.org/10.1002/jia2.25392/full>.
- World Health Organization. Definitions and reporting framework for tuberculosis – 2013 revision (updated December 2014 and January 2020). [Accessed 1 March 2021]. 2020. <https://www.who.int/tb/publications/definitions/en/>.
- World Health Organization. Global tuberculosis report. [Accessed 10 October 2020]. 2019. https://www.who.int/tb/publications/global_report/en/.
- INSTAND. [Accessed 29 March 2021].