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BRIEF COMMUNICATION

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Gene-Xpert Ultra for the diagnosis of extrapulmonary tuberculosis in children and adolescents

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ABSTRACT

This prospective study describes the use of Gene-Xpert Ultra for the diagnosis of extrapulmonary tuberculosis (EPTB) in children and adolescents, in Rio de Janeiro, Brazil. Eighteen patients were studied; the final diagnosis of EPTB was established in 13 (72%). Gene-Xpert Ultra results showed detection in 10/13 (77%) of EPTB cases (7 of these 10 with trace-positive results). Gene-Xpert Ultra proved to be a promising method for the diagnosis of childhood EPTB.

KEYWORDS: Extrapulmonary tuberculosis. Children. Adolescents. Diagnoses. Real-time PCR.

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INTRODUCTION

In 2017, the World Health Organization (WHO) endorsed the replacement of Gene-Xpert MTB RIF (Xpert) with Gene-Xpert Ultra (Ultra). When compared to Xpert, Ultra showed a lower detection limit of *Mycobacteruim tuberculosis* (*M. tb.*) from 116 (with Xpert) to 15.6 CFU. Ultra maintains the real-time PCR technique, with the addition of two more *M. tb.* targets and changes in the melting curve, favoring the detection of rifampicin (RMP) resistance. Detection of bacillary load is categorized as high, medium, low, and trace-positive results. The latter category indicates that the *IS1081* and *IS6110 M. tb.* targets were detected, but not in the specific region of TB *rpo*-β gene, resulting in an indeterminate resistance to RMP. Hence, there is a greater sensitivity of Ultra compared to Xpert[1,2]. In Brazil, Ultra was incorporated in December 2019 for the diagnosis in children younger than 10 years old. In extrapulmonary TB (EPTB), trace-positive results indicate positivity to *M. tb*.[3]. To date, we have not identified studies in the literature that used Ultra exclusively for the diagnosis of EPTB in children[4]. The current prospective preliminary study aims at describing the use of Ultra in a reference university hospital for pediatric TB, from April 2020 to February 2021, in Rio de Janeiro, Brazil.

MATERIALS AND METHODS

The study included children (< 10 years old) and adolescents (ages 10 – 19 years) consecutively enrolled with presumed TB whose specimens were submitted to Gene-Xpert Ultra. This study has been approved by the IPPMG-UFRJ Ethics Committee (CAAE 02173518.2.0000.5264). After written informed consent was obtained from participant's parents or caregivers, the sample was collected and submitted

to Ultra analyses. Variables studied were: age, exposure to TB in the last two years, positive tuberculin skin test (TST) results (positive ≥ 5 mm; negative< 5 mm], Ultra results (detected, trace, and undetected), acid-fast bacilli test results (positive and negative) and culture for *M. tb* by the Mycobacterial Growth Indicator Tube (MGIT 960) method (positive, negative and inconclusive due to contamination). In the latter, when the result is positive, an antimicrobial sensitivity test (AST) was performed. The final diagnosis of EPTB was established by laboratory confirmation (using Ultra), clinical data and a favorable clinical response after 60 days of treatment.

RESULTS AND DISCUSSION

From a total of 23 patients initially included in the study, 18 (78%) were children and 5 (22%) were adolescents. Ages ranged from 11 months to 12 years. Of all patients, 18 (78%) were presumed to have EPTB and 5 (22%) were presumed to have pulmonary tuberculosis (PTB). The description of the Ultra results according to TB forms is shown in Figure 1.

The final diagnosis of PTB was established in four out of five patients (80%), in whom 2/4 (50%) were children and 2/4 (50%) were adolescents; Ultra results were trace-positive in all four cases of PTB and all corresponding cultures were negative.

The final diagnosis of EPTB was established in 13 out of 18 patients (72%), and five patients (28%) received other diagnoses; Ultra detected 10 of 13 (7/10 were trace-positive) and culture was positive in only 3 of these 10 positive patients by Ultra. Six of the 13 patients (46%) presented with peripheral enlarged lymph node TB, three (23%) pleural, and four (31%) had other manifestations (one peritoneal, one bone, one sacroiliac and one soft tissue swelling over the sternum). There were no cases of resistance to RMP by Ultra or AST. All patients with Ultra trace-positive results or not detected results had a negative culture, as shown in Table 1.

The positivity of Ultra (detected or trace-positive) in the present study occurred in 77% of EPTB patients. Unlike the latest WHO Manual, these results endorse the recommendation of the Ministry of Health in Brazil in which trace-positive results are considered to point to *M. tb*. positivity in a paucibacillary sample (pediatric and extrapulmonary sample)[3,4]. Ultra detected 67% (4/6) of the patients with enlarged lymph node TB. This percentage is a little higher than in a previous study (56%; 5/9) carried out in our hospital, although the lymph node TB samples were small in both studies[5]. This finding corroborates the Ultra's superiority over Xpert in the diagnosis of paucibacillary forms of TB.

Ultra has also contributed to the diagnosis of pleural TB in our study. Conversely, in a study in Shanghai (China) with patients over 16 years old, the positivity of Ultra in tissue aspirates (lymph node, thoracic or abdominal wall, skin, crissum and bone) was higher (79%) than that of pleural fluid samples (43.7%), probably because the bacillary load in tissues or abscesses is greater than in serous liquids; 23 of the 30 patients showed trace-positive results in Ultra with concomitant negative cultures[6]. Our patients with trace-positive results presented negative cultures as well, perhaps due to the very low bacillary load detected by Ultra.

In patients of different ages and in different clinical specimens, Ultra sensitivities ranged from 90 to 95%, and specificities ranged from 86 to 99%[7-9]. We did not perform a comparison between Xpert and Ultra, as Xpert is no longer available in Brazil.

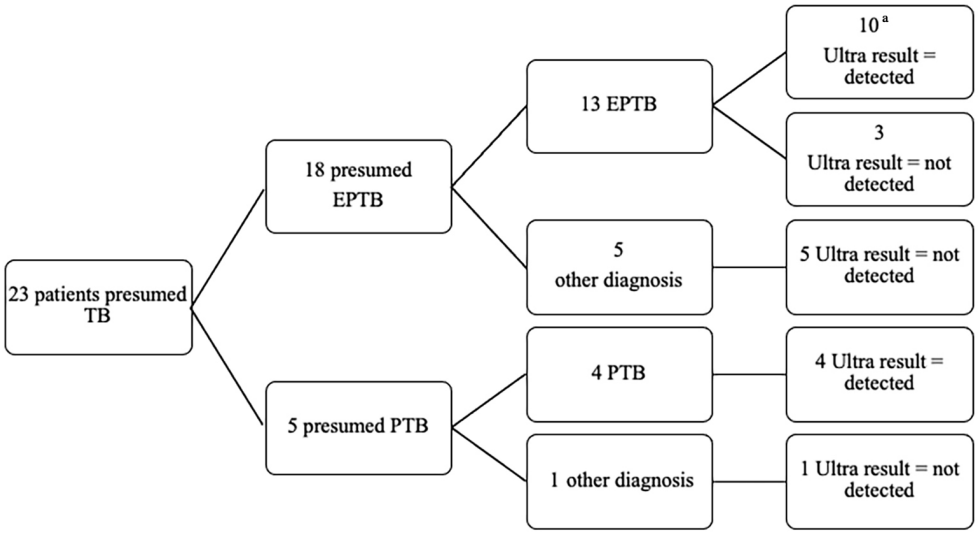

Figure 1 - Description of the Ultra results according to the final diagnosis. [a]7/10 = trace-positive results; TB = tuberculosis; ETBP = extrapulmonary tuberculosis; TBP = pulmonary tuberculosis.

Table 1 - Description of patients with a final diagnosis of extrapulmonary tuberculosis (n=13).

Patient number	Age (years)	Close contact with TB	TST	Sample	Ultra result	RMP-resistance	AFB	Culture	Final diagnoses
1	0.9	Yes	Positive	Lymph node	Not detected	No	Negative	Negative	Peripheral lymph node TB
2	1.5	No	Not performed	Lymph node	Detected	No	Negative	Positive[a]	Peripheral lymph node TB
3	2	No	Positive	Soft tissue swelling over the sternum	Detected	No	Negative	Positive[a]	Bone TB + Pulmonary TB
4	2.1	Yes	Positive	Lymph node	Trace-positive	Indeterminate	Negative	Negative	Peripheral lymph node TB
5	6.5	No	Negative	Lymph node	Trace-positive	Indeterminate	Negative	Negative	Peripheral lymph node TB
6	8.5	No	Negative	Pleural effusion	Trace-positive	Indeterminate	Negative	Negative	Pleural TB
7	8.9	No	Positive	Lymph node	Detected	No	Negative	Positive[a]	Peripheral lymph node TB
8	10	WI	Negative	Peritoneal fluid	Trace-positive	No	Negative	Negative	Peritoneal TB
9	10.5	No	Negative	Bone	Trace-positive	Indeterminate	Negative	Negative	Osteoarticular TB
10	10.9	No	Positive	Pleural effusion	Trace-positive	Indeterminate	Negative	Negative	Pleural TB
11	11.2	No	Negative	Sacroiliac abscess	Trace-positive	Indeterminate	Negative	Negative	Osteoarticular TB + Pulmonary TB
12	11.3	No	Positive	Lymph node	Not detected	No	Negative	Negative	Peripheral lymph node TB[b]
13	11.5	Yes	Positive	Pleura	Not detected	No	Negative	Negative	Pleural TB[c]

WI = without information; TST = Tuberculin skin test; [a]Sensitive to first-line drugs; EPTB = extrapulmonary tuberculosis; [b]Diagnosis made by histopathological exams compatible with TB; [c]Diagnosis based on clinical history and TST result.

Among the limitations of this study, we acknowledge that the small number of patients with EPTB could have acted as a selection bias in the results and the collection of only one sample per patient may have reduced the positivity of Ultra which, regardless, was high. As the study was carried out in a reference center for TB, patients of greater complexity and with a greater pretest probability for active TB may have been selected.

Ultra proved to be a promising method in the diagnosis of EPTB in children, but we emphasize that negative results do not exclude the diagnosis[3,4]. Studies with a larger pediatric population are necessary, before it can be inferred that Ultra should be used in clinical practice.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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