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BRIEF REPORT

Systematic Screening for Tuberculosis Using Molecular Testing on Stool Samples in Acutely Malnourished Children: A Pilot Implementation Study in a High-Burden Drug-Resistant Tuberculosis Country

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Systematic screening of tuberculosis (TB) using molecular testing on stool samples detected TB in 5.2% of acutely malnourished children, including cases without evident clinical symptoms. This screening strategy could enhance TB diagnosis and facilitate prompt treatment initiation in this population. Further studies are required to confirm long-term outcomes and cost-effectiveness.

Keywords. acute malnutrition; systematic screening; tuberculosis; Xpert MTB/RIF ultra; stool samples.

Severe acute malnutrition (SAM) during childhood negatively impacts both physical and mental development of those who suffer from it. The weakening of the immune system increases the risk of severe infection contributing to the high mortality found in this population [1]. Tuberculosis (TB) continues to affect this vulnerable population as they face a higher risk of disease progression following the initial infection. Indeed, malnutrition has emerged as one of the main risk factors associated with TB [2].

Microbiological confirmation of TB in children remains low due to several factors. First, pediatric TB usually presents as a paucibacillary disease; second, extrapulmonary forms are more frequent, limiting the retrieval of easy to collect samples; and third, respiratory sample collection can be challenging in children <5 years of age. Furthermore, access to medical equipment such as nasogastric tubes can hamper the collection of high-quality samples [3]. Given these limitations, the World Health Organization (WHO) recommends using scoring systems, such as the Keith-Edwards score, or treatment decision algorithms to initiate anti-TB therapy in children with a clinical suspicion of TB despite not having microbiological confirmation. However, the advent of rapid molecular testing has significantly increased the proportion of microbiologically confirmed TB in children. While respiratory samples are optimal for diagnosis, recent studies have shown good diagnostic accuracy with stool samples (sensitivity around 50%). Consequently, the WHO has recently endorsed rapid molecular testing of stool samples for diagnosing TB in children [4]. This simpler, safer, and more accessible approach has the potential to improve TB diagnosis and increase the proportion of microbiologically confirmed cases in children.

Angola is among the countries with the highest burden of TB and multidrug-resistant TB (MDR-TB) worldwide. The prevalence of malnutrition in children is unacceptably high across the country, with 5% suffering from wasting syndrome and up to 50% experiencing chronic malnutrition [5]. This study aimed to implement systematic TB screening using molecular testing on stool samples in children admitted to the Acute Malnutrition Unit (AMU) of a rural hospital in Benguela province, Angola.

MATERIALS AND METHODS

This was a cross-sectional study including children <5 years of age with acute malnutrition admitted to the AMU at Nossa Senhora da Paz Hospital (Cubal, Angola) between March and June 2023. All children were invited to participate in the study with the exclusion of those who had received TB treatment in the past year to avoid the detection of residual genomic material from the previous episode. Molecular testing for TB diagnosis was performed on stool samples using the Xpert MTB/RIF Ultra assay (Cepheid). We collected sociodemographic data and clinical and anthropometric variables, including weight, height, and mid-upper arm circumference (MUAC).

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Symptoms and signs of TB included cough of any duration, fever, enlarged lymph nodes, abnormal chest auscultation, and lethargy. Weight was measured with an electronic scale, and height was measured using a 2-piece height board. Malnutrition was diagnosed if children presented a weight-for-height z-score ≤ -2 standard deviations (SD) and/or MUAC $\leq$12.5 cm and/or pitting edema or anasarca. SAM was defined as weight-for-height z-score ≤ -3 SD or MUAC <11.5 cm. Participants were offered human immunodeficiency virus (HIV) rapid testing (Alere Determine HIV, Abbott Diagnostic Medical Co). Stool samples were collected within the first 48 hours of admission after obtaining informed consent from the main caregiver. Samples were prepared according to the Simple One-Step method (Supplementary Annex 1) and analyzed within 24 hours [6]. Upon admission, participants were clinically assessed for signs or symptoms of TB with the support of Keith-Edwards score (Supplementary Annex 2).

Data Analysis

The database was designed in Excel and transferred to SPSS software (version 20.0; IBM SPSS) for the statistical analysis. Qualitative variables were expressed as absolute numbers and percentages, and quantitative variables as mean ± SD or median and interquartile range (IQR) depending on the normality of the distribution. The χ^2 test or Fisher exact test was used to compare the distribution of categorical variables, and Student t test or Mann-Whitney U test for continuous variables. Results were considered statistically significant if the 2-tailed P value was <.05. For the comparison analysis, Xpert MTB/RIF Ultra results were considered positive or negative according to the manufacturer instructions and invalid results were classified as negative.

Ethical Considerations and Patient Consent Statement

Written informed consent was obtained from the main caregiver of participants. The study was designed, implemented, and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study was approved by the ethics committee of the Health Ministry of the Republic of Angola (number 34/2023).

RESULTS

Overall, 154 children with acute malnutrition were admitted to the AMU and all were included. The median age was 14 (IQR, 12–24) months and 78 (50.6%) were male. Twenty-four (15.6%) reported a family history of TB in the household. None of them had been previously treated for TB. Two (1.3%) children had been previously admitted due to SAM. On admission, 120 (77.9%) participants presented at least 1 symptom or sign of TB besides malnutrition and 8 (5.2%) of them presented a Keith-Edwards score ≥7. Eight (5.2% [95% confidence interval, 1.7%–8.7%]) participants were diagnosed with TB after a positive Xpert MTB/RIF Ultra result on stool. Two (25%) participants with a positive Xpert MTB/RIF Ultra result presented a Keith-Edwards score ≥7 at admission. Two (25%) participants with a positive Xpert MTB/RIF Ultra result had no clinical signs or symptoms of TB but malnutrition. Xpert MTB/RIF Ultra semi-quantitative results were as follows: 2 (25%) trace, 2 (25%) low, and 4 (50%) medium. Rifampicin resistance was detected in 2 (25%) cases. Immediate TB treatment, according to resistance pattern, was initiated for children diagnosed with Xpert MTB/RIF Ultra. Twenty-four (15.6%) participants had an invalid Xpert MTB/RIF Ultra test result. HIV testing revealed 4 (2.6%) infections; none had a positive molecular TB test result. We observed significant differences on weight-for-height z-score between children with TB and children without TB ($P = .03$). Table 1 presents the sociodemographic and clinical characteristics of the participants, with a comparison between children with a Xpert MTB/RIF Ultra positive and negative result.

DISCUSSION

The latest WHO guidelines for systematic screening of TB recommend actively screening populations with a prevalence >0.5% and/or those with risk factors for TB, such as malnourished children [7]. In line with this recommendation, our study found a 10-fold TB prevalence (5.2%) among our sample. Interestingly, no clinical characteristics, epidemiological characteristics, or nutritional status indicators effectively distinguished between children with and without microbiologically confirmed TB. In fact, the significant differences detected in weight-for-height z-score suggested a greater prevalence of TB among those with a better nutritional status. This finding may be attributed to the misclassification of children with edematous malnutrition based on weight-for-height z-score [8], as well as the inclusion of only a few cases of mildly malnourished participants.

In resource-limited settings, where microbiological confirmation of TB is often difficult to obtain, clinical criteria, treatment decision algorithms, and scoring systems such as the Keith-Edwards score are essential tools for initiating treatment, particularly for those who do not improve after nutritional supplementation, deworming, and antibiotic therapy. However, most of these scores have not been validated and all of them are primarily time-dependent, which can delay the onset of TB therapy and have devastating consequences for this highly vulnerable population [9]. In our study we found that only 2 participants with a positive Xpert MTB/RIF-Ultra result presented a Keith-Edwards score ≥7 upon admission. Additionally, if the diagnosis had relied solely on clinical criteria, 2 of the Xpert MTB/RIF Ultra–positive participants would have been initially missed. Finally, addressing rifampicin susceptibility at the time of diagnosis is especially relevant in Angola due to the high burden of MDR-TB. The 2 cases of

Table 1. Sociodemographic and Clinical Characteristics of Participants and Comparison Between Children With an Xpert MTB/RIF Ultra Negative or Positive Result

Characteristic	Overall (n = 154)	Negative Xpert MTB/RIF Ultra (n = 146)	Positive Xpert MTB/RIF Ultra (n = 8)	*P* Value
Sex, male	78 (50.6)	73 (50)	5 (62.5)	.7
Age, mo, mean ± SD	18.4 ± 11.8	17.7 ± 11.1	23.5 ± 17.4	.1
Age >24 mo	40 (25.9)	36 (24.7)	4 (50)	.2
Household, mean ± SD	5.3 ± 2.2	5.3 ± 2.2	5.8 ± 1.4	.6
Household >5 people	84 (54.5)	67 (45.9)	3 (37.5)	.7
Known TB contact (household)	24 (15.6)	21 (14.4)	3 (37.5)	.1
Exclusive breastfeeding 6 mo	33 (21.4)	31 (21.2)	2 (25)	1
Ongoing breastfeeding	51 (33.1)	47 (32.2)	4 (50)	.2
Nutritional status, mean ± SD				
Weight, kg	6.3 ± 2.5	6.4 ± 2.4	5.8 ± 3.7	.4
Height, cm	72.1 ± 8.2	71.9 ± 7.6	73.7 ± 16.2	.5
MUAC, cm	10.7 ± 1.3	10.8 ± 1.3	10.5 ± 1.6	.5
Weight-for-height *z*-score				.07
0[a]	5 (3.2)	5 (3.4)	0	
−1	5 (3.2)	4 (2.7)	1 (12.5)	
−2	17 (11)	14 (9.6)	3 (37.5)	
−3	50 (32.5)	49 (33.6)	1 (12.5)	
−4	77 (50)	74 (50.7)	3 (37.5)	
Acute malnutrition status according to weight-for-height *z*-score				.03
No malnutrition[a]	5 (3.2)	5 (3.4)	0	
Mild	5 (3.2)	4 (2.7)	1 (12.5)	.
Moderate	17 (11)	14 (9.6)	3 (37.5)	
Severe	127 (82.5)	123 (84.2)	4 (50.0)	
Acute malnutrition status according to MUAC (n = 148)				1
No or mild (>12.5 cm)	10 (6.7)	9 (6.4)	0	
Moderate (11.5–12.5 cm)	34 (23)	34 (24.1)	2 (28.6)	
Severe (<11.5 cm)	104 (70.3)	98 (69.5)	5 (71.4)	
Clinical findings				
Fever	91 (59.1)	86 (58.9)	5 (62.5)	1
Cough	75 (48.7)	70 (47.9)	5 (62.5)	.4
Edema	59 (38.3)	54 (37.0)	5 (62.5)	.1
Diarrhea	101 (65.6)	96 (65.8)	5 (62.5)	.5
Lethargy	14 (9.1)	13 (8.9)	1 (12.5)	.5
Enlarged lymph nodes	6 (3.9)	5 (3.4)	1 (12.5)	.2
Pathological chest auscultation	21 (13.6)	19 (13.0)	2 (25.0)	.3
At least 1 symptom or sign of TB[b]	120 (77.9)	114 (78.1)	6 (75)	1
Keith-Edwards score ≥7	8 (5.2)	6 (4.1)	2 (25)	.06

Data are shown as No. (%) unless otherwise indicated.

Abbreviations: MUAC, mid-upper arm circumference; SD, standard deviation; TB, tuberculosis.

[a]Weight-for-height *z*-score can underestimate malnutrition in cases of anasarca.

[b]At least 1 symptom or sign of TB besides malnutrition including fever, cough, lethargy, enlarged lymph nodes, and pathological chest auscultation.

genotypic resistance to rifampicin detected would have been missed, or the initiation of MDR-TB treatment would have been delayed, if only clinical criteria would have been used.

Furthermore, it is important to highlight the great acceptance of the strategy. The simplicity of stool collection and testing ensures its practical implementation and scalability. Finally, a late diagnosis of TB is often linked to a prolonged admission, which can lead to higher costs for the healthcare system and undoubtedly contributes to catastrophic costs for families [10]. In fact, most children admitted to the AMU come from the most disadvantaged segments of the population.

Therefore, systematic TB screening in this population, along with timely treatment initiation, may promote faster recovery, earlier discharge, and a lower risk of incurring catastrophic costs.

The primary limitation of our strategy is the significant number of invalid results, which could be both related to errors in the processing of samples and the inhibition of the polymerase chain reaction by substances present in feces such as complex polysaccharides [11]. Recent studies have reported up to 8% invalid MTB/RIF Ultra results on stool samples, so it is plausible that in up to half of the cases the invalid results were due to

incorrect sample processing [12, 13]. Further research and training are needed to improve the processing of stool samples and to reduce the high number of invalid results, which could have a negative impact on the cost-effectiveness of the strategy. Other important limitations of the study were the low median age of our patients and the lack of prospective follow-up to actually assess how many participants would have finally started TB treatment, as well as the outcomes of the participants.

In conclusion, we found that 5% of children with acute malnutrition tested positive for TB using systematic molecular testing on stool samples upon admission to the AMU in a rural hospital located in a country with a high TB burden. A recent study conducted in similar settings, which implemented systematic screening for TB in children with severe pneumonia, suggested a decrease in mortality only among malnourished children [14]. Given these findings, we advocate for the implementation of active systematic screening at the programmatic level, accompanied by a rigorous evaluation of its long-term outcomes and associated costs.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Note

Potential conflicts of interest. All authors: No reported conflicts of interest.

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