REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO

JOURNAL OF THE SÃO PAULO INSTITUTE OF TROPICAL MEDICINE

ORIGINAL ARTICLE

http://doi.org/10.1590/S1678-9946202264027

String test: a potentially useful tool in the diagnosis of pulmonary tuberculosis in Brazilian children and adolescents

Claudete Aparecida Araújo Cardoso[1], **Andrea Maciel de Oliveira Rossoni**[2], **Joana Moraes de Rezende**[3], **Rafaela Baroni Aurilio**[3], **Roberta Jaime Ferreira Lima dos Santos**[3], **Maria Aparecida Gadiani Ferrarini**[4], **Afranio Lineu Kritski**[5], **Anandi Martin**[6], **Clemax Couto Sant'Anna**[3,5]

ABSTRACT

This study investigated the potential use of the String Test (ST) for the diagnosis of pulmonary tuberculosis (PTB) in children and adolescents. This is a case series of patients aged 4-15 years presenting with clinically presumed PTB and submitted to ST in three pediatric TB referral centers in Brazil, between November 2017 and July 2020. The ST was performed in the morning, after 4-12 h of fasting, followed by ingestion of the capsule by the patient, which was attached to the patient's malar region. The material was collected for simultaneous smear microscopy (acid-fast *bacilli* - AFB), culture and the molecular investigation by the GeneXpert MTB/RIF®. Thirty-three patients with presumed PTB were included and ST was performed in 26 (78.8%) of them and 7 (21.2%) patients could not swallow the cord. The diagnosis of PTB was established in 11 (42.3%) of the 26 patients who underwent the ST. The diagnosis of PTB was confirmed (by culture or GeneXpert MTB/RIF®) in 5 patients, 4 of whom were also positive by the ST. Two of them showed positivity by the GeneXpert MTB/RIF® only in the ST sample. Two other patients had a positive ST following the induced sputum test (AFB, GeneXpert MTB/RIF®, and positive culture in both specimens). Thus, ST was positive in 36.4% of the patients in whom PTB was diagnosed. ST could be a useful test for diagnosing PTB in children and adolescents.

KEYWORDS: Diagnostic techniques and procedures. Pulmonary tuberculosis. Children. String test.

INTRODUCTION

Pediatric tuberculosis (TB) accounts for 12% of all reported cases of TB globally and for approximately 1,200,000 new cases in 2019. Pediatric TB has been considered an underdiagnosed and underreported disease for many decades due to numerous factors, including the lack of specificity of symptoms, difficulty in obtaining samples for laboratory tests in children who frequently struggle to produce effective sputum, in addition to paucibacillary characteristics of pediatric TB[1-3].

Given the difficulty in obtaining adequate clinical samples in children and adolescents, the string test (ST) might be a useful strategy. The ST was initially designed to evaluate enteric diseases and to isolate *Giardia lamblia* and *Helicobacter pylori* from gastric samples[4]. Over the last two decades, its efficacy in detecting *Mycobacterium tuberculosis (Mtb)* in gastric juice by retrieving the sputum swallowed by patients, similar to gastric lavage (GL), has been evaluated[5]. The ST in the pediatric population was well-tolerated by children, parents and the health care staff[6].

[1]Universidade Federal Fluminense, Faculdade de Medicina, Departamento Materno-Infantil, Niterói, Rio de Janeiro, Brazil

[2]Universidade Federal do Paraná, Programa de Pós-Graduação em Saúde da Criança e do Adolescente, Curitiba, Paraná, Brazil

[3]Universidade Federal do Rio de Janeiro, Instituto de Puericultura e Pediatria Martagão Gesteira, Rio de Janeiro, Rio de Janeiro, Brazil

[4]Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, São Paulo, Brazil

[5]Universidade Federal do Rio de Janeiro, Faculdade de Medicina, Rio de Janeiro, Rio de Janeiro, Brazil

[6]Ghent University, Department of Biochemistry and Microbiology, Laboratory of Microbiology, Ghent, Belgium

Correspondence to: Clemax Couto Sant'Anna
Universidade Federal de Rio de Janeiro, Faculdade de Medicina, Rua Bruno Lobo, 50, Cidade Universitária, CEP 21941-912, Rio de Janeiro, RJ, Brazil
Tel: +55 21 2235-3634, +55 21 98814-0597

E-mail: clemax01@gmail.com

Received: 21 October 2021

Accepted: 7 March 2022

The present study is a case series exploring the feasibility and performance of the ST, a noninvasive method, for obtaining clinical specimens for diagnosing pediatric pulmonary TB (PTB).

MATERIALS AND METHODS

This is a case series of patients with symptoms suggestive of PTB seeking medical care. They were prospectively included in the study by convenience sampling from three pediatric TB referral centers in three Brazilian State capitals (Rio de Janeiro, Curitiba and Sao Paulo), from November 2017 to July 2020. Children (aged <10 years) and adolescents aged 10-15 years treated as outpatients or inpatients were included. Patients who were unable to cooperate with the examination due to intellectual disability , difficulty swallowing or unstable clinical status were excluded.

The presumption of PTB was based on the presence of at least two of the following criteria: 1) symptoms compatible with TB: chronic cough, fever, weight loss and pneumonia that did not improve with antibiotic therapy; 2) chest radiograph with lymphadenopathy, cavitations, infiltrates, condensations, and/or miliary pattern, encompassing unilateral or bilateral changes; or 3) history of close contact with an adult diagnosed with PTB.

The ST was performed in the morning, in an outpatient clinic or ward after 4-12 h of fasting. Inpatients underwent other concomitant diagnostic tests for PTB in addition to the ST such as GL, induced sputum (IS) and bronchoalveolar lavage (BAL) according to the age of the patient and tests availability in the services. The decisions on what tests to perform and how many tests to perform were made at the discretion of the attending physician. The outpatient patients underwent the ST alone. Only one sample was obtained from each patient without repeating the ST on consecutive days. The clinical specimen collected for the ST was simultaneously submitted to at least one of the following diagnostic tests: acid-fast bacilli (AFB) smear, culture for *Mtb* and GeneXpert MTB/RIF®.

Patients with presumed PTB were allocated into the "confirmed TB" group if the culture or GeneXpert MTB/RIF® from any clinical specimen was positive[7].

The ST procedure in this study involved a capsule of approximately 1 cm in diameter attached to a thin, 80-cm long string. The material of the capsule is degradable in gastric juice. The capsule was filled with Leinen-Operation szwirn Et.Nr.100 suture (Leinen-ET Heike Dietrich, Mülheim-Saarn, Germany)[8].

The ST was performed by fasting for 4-12 h followed by ingestion of the capsule by the patient, with the string attached to the patient's malar region. In the stomach, the gelatin crumbles and the exposed string are impregnated by gastric secretion, including the pathogens present in the gastric secretion. The string was left inside the child's stomach for 1 h and was then removed after rinsing the mouth with water. During this period, fasting was maintained and the participant was free to perform their usual tasks, including recreational activities. When the string was retrieved, the initial approximately 50 cm were cut and discarded. The remainder (around 30 cm) were stored in a Falcon tube with 2 mL of 0.9% saline solution to cover the string completely in the flask, and the sample was sent on the same day, within 2 h, to the mycobacteriology laboratory of the units participating in the study. The samples were then decontaminated with a 2% NaOH-NaCl solution. The material was processed for direct investigation of the presence of AFB, seeded in the culture medium, and submitted to the Xpert test, according to the manufacturer's recommended procedure and the national guidelines issued by the Brazilian Ministry of Health[9,10]. The teams responsible for performing the procedure were adequately trained through video classes, with communication about the services involved between their respective laboratories to clarify any doubts. Failure during the ST was defined as the inability to swallow the capsule after two attempts, according to the literature[11].

A descriptive analysis of the data was performed using the Microsoft Excel® software (Microsoft, Redmond, WA, USA). All legal guardians of the patients signed an informed consent form before any procedure was performed. Patients aged 7-15 years have also provided a written assent to participate.

The Research Ethics Committee approved the present study in all the units participating in the study (Universidade Federal do Parana, approval Nº 61126816.1.0000.0096-2017, Curitiba, PR).

RESULTS

During the study period, 33 patients with presumed PTB were studied, as shown in Figure 1. Of the 33 patients selected, 21 (64%) were adolescents, and 2 (10%) of these 21 patients could not swallow the capsule. Of the 12 children (aged <10 years), five (42%) could not perform the ST, one of them due to a significant comorbidity (esophageal candidiasis) associated with AIDS.

Among the 26 patients with presumed PTB who were able to swallow the capsule for the ST, 18 (69%) were female, with a median age of 12 years (interquartile range [IQR]: 8.7-13.6). Seventeen (65%) of the 26 patients showed alterations in chest radiographs suggestive of PTB; the remaining 9 patients presented with symptoms and a

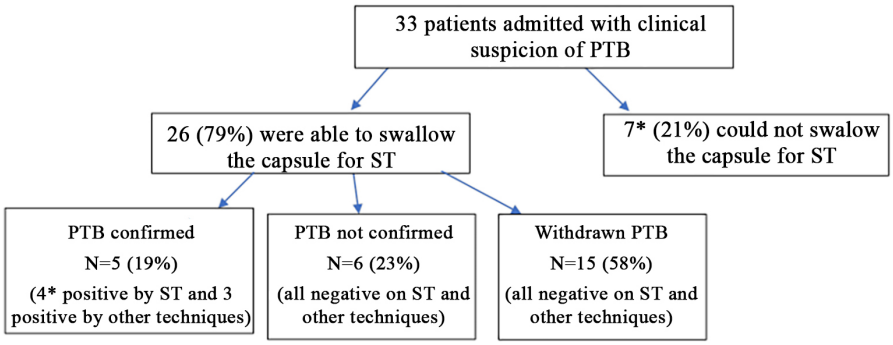

Figure 1 - Flowchart of the inclusion of study participants. PTB = pulmonary tuberculosis; ST = String test; *5 children and 2 adolescents; **Of the 4 diagnosed by ST, in 2 of them the PTB diagnosis was established exclusively by the ST.

history of contact with a person with PTB. Of the 26 patients who underwent ST, 11 (42%), were diagnosed with PTB, with five confirmed as PTB and six with unconfirmed PTB. Of the 11 patients with TB who underwent ST, four (36%) were positive either by AFB, culture or GeneXpert MTB/RIF®. All were adolescents (aged 11- 15 years), three with advanced radiological lesions suggestive of severe PTB, however one had a normal chest radiograph.

Table 1 summarizes the demographic, clinical, radiological and bacteriological characteristics of these 11 patients with PTB. In 15 patients who underwent ST, the diagnosis of PTB was ruled out. All of them showed

Table 1 - Demographic, clinical, radiologicaland bacteriological characteristics of the 11 studied participants with a diagnosis of pulmonary tuberculosis.

Patient	Sex	Age (years)	Tuberculin skin test (mm)	Findings on the chest radiography	String test (AFB/culture/ Xpert	Other procedures (AFB/culture/Xpert)	HIV infection	Final diagnosis: PTB confirmed
01	M	6.4	20	Adenopathy	Neg/Neg/Neg	Not performed	No	No
02	F	12	10	Bilateral condensation and excavation	Neg/Neg/Neg	**Sputum Pos/Pos/Pos**	No	**Yes**
03	F	13.4	20	Adenopathy on the left	Neg/Neg/Neg	Not performed	No	No
04	F	11.8	20	Adenopathy on the right	Neg/Neg/Neg	Not performed	No	No
05	F	14.9	15	Condensation on the right	Neg/Neg/Neg	Sputum induction Neg	Yes	No
06	F	12.1	20	Adenopathy on the right	Neg/Neg/Neg	Not performed	without information	No
07	M	13	10	Bilateral condensation, infiltrate and bilateral excavation	**Pos/Pos/Pos**	**Sputum induction Pos/Pos/Pos**	No	**Yes**
08	F	13.3	10	Bilateral condensation, infiltrate and bilateral excavation	Neg/**Pos**/Neg	**Sputum Pos/Pos/Not fulfilled**	No	**Yes**
09	F	11.4	18	One-sided condensation	Neg/**Pos**/Neg	Not performed	No	**Yes**
10	M	9	0	Bilateral condensation	Neg/Neg/Neg	Sputum and BAL Neg/Neg/Neg	Yes	No
11	F	15.7	21	Normal	Neg/Neg/**Pos**	BAL Neg/Neg/Neg	No	**Yes**

AFB = acid fast bacilli; BAL = bronchoalveolar lavage.

negative ST results, suggesting the procedure´s negative predictive value was high.

Among the seven patients who were unable to swallow the capsule, four (57 %) were female, and the median age was 5.9 years (IQR: 5.4–9.9). One patient was diagnosed with unconfirmed PTB, and the others had the diagnosis of PTB ruled out. The patient diagnosed with unconfirmed PTB was a 13-year-old male adolescent who vomited after two attempts to swallow the capsule. His clinical and radiological parameters established the diagnosis of PTB. A 5-year-old female with AIDS could not swallow the capsule because of dysphagia secondary to fungal esophagitis, without vomiting or nausea. She was diagnosed with bacterial pneumonia on the basis of a clinical improvement with the use of antibiotic therapy for conventional pathogens. Other challenges reported were discomfort at the end of the first hour of the examination (two patients, a 6-year-old boy and an 11-year-old girl). There were no other complaints during the examination. At no time did the participants or their guardians ask to remove the string, nor was the string removed by the patient. No significant signs such as pain facies or other indications of discomfort or pain complaints during the test were reported by the patients, guardians, or observed or detected by the health team in charge.

DISCUSSION

The present study was a Brazilian multicenter survey that sought to explore the feasibility and performance of using ST for obtaining clinical specimens from children and adolescents with presumed PTB. We evaluated recovery of *Mtb* from ST, comparing it with other diagnostic methods already established in the literature, including spontaneous sputum, gastric lavage (GL), induced sputum (IS), and bronchoalveolar lavage (BAL). Despite the small sample studied, the rate of success of this study (79%) was higher than that reported in the literature: 64% among adolescents, and 58% among children. In the study by Tafur *et al.*[12], approximately 80% of the children aged 4-10 years could not swallow the capsule. The importance of this procedure is its applicability in children. Above this age, it is easier to obtain an adequate sputum sample, and their PTB is more similar to the adult pattern, making the *Mtb* isolation and PTB diagnosis easier[4,13].

ST studies in the HIV-infected pediatric population can have a great utility because of increased difficulties in diagnosing TB in such children. In HIV-infected patients, the clinical presentation of PTB can be highly heterogeneous. The tuberculin skin test (TST) is often nonreactive due to the patient's immune system inability to react, and the radiological image is often atypical[13]. Therefore, this population would undoubtedly benefit from such a test[14].

In the present study, no signs of intense discomfort or pain were observed and no participant removed the string before the pre-decided time, corroborating the findings of other studies that observed a good acceptance of the ST in the pediatric age group[6]. Some authors have described that the moments of swallowing the capsule and removing the string were the least tolerable in children; however, the patients recovered quickly[6,8,12]. In this study, four patients who underwent ST showed *Mtb* positivity in the smear microscopy, culture, or GeneXpert MTB/RIF®. All were adolescents, three of them had advanced radiological forms of PTB, but the other patient had normal chest radiography results. Thus, the diagnostic contribution of ST on this occasion is still clear. The appropriate procedure duration in children to increase the sensitivity of ST is also still unclear in the literature. In some studies, the time with the string positioned intragastrically varied between 1 and 4 h[6,8,12]. Considering that the patient should be fasting during the examination, 4 h would most likely reduce the compliance and efficacy of ST in children. Bae *et al.*[15] used different durations for performing the ST in adults with sputum positivity for *Mtb*, and concluded that the time could be reduced to 60 min without compromising its effectiveness.

The present study has limitations including the small sample size and the collection of only one clinical specimen from each patient. This single sample might have contributed to the low positivity of ST in the samples studied. The literature shows that ST has a similar diagnostic accuracy of IS[4,8,11]. However, there are few studies that have compared ST with GL. The only report comparing both methods had a small sample size and showed similarity in the retrieval of *Mtb* in both techniques[8].

Some studies evaluated whether the ST can compete with other methods, especially IS and GL, for the retrieval of bacilli in children who cannot expectorate, in terms of accuracy, safety and acceptance. All methods require specific training of the healthcare staff, especially nurses[12,16]. The disadvantage of ST is the inherent risk of bronchospasm in the patient, especially in children aged < 2 years, and the risk of transmission of bacilli via aerosols when the patient coughs[11,17,18]. In our case series, there were no cases of bronchospasm and the acceptance was satisfactory. In general, the ST is an excellent tool to be applied in low-resource settings. The material to be examined can be prepared by the service staff as in this study and in previous studies from Peru[6,12]. Although the costs for performing large-scale ST were not calculated in this study, it is assumed that it has financial advantages

over other procedures like IS and GL. Additionally, the possibility of the ST being performed in an outpatient setting, as in the present study, reduces the hospitalization costs compared to GL. We thus point to the benefits of this technique as being outpatient-friendly, noninvasive and with no occupational hazards, compared to other available methods.

CONCLUSION

Increasing the sample size is necessary to obtain more robust data regarding the benefits of using this laboratory tool for diagnosing TB in the pediatric age group, thereby reducing costs related to hospitalization, morbidity and mortality in the affected population, even more if the specimen can be subjected to the Xpert Ultra, admittedly more sensitive than the GeneXpert MTB/RIF®.

ACKNOWLEDGMENTS

To the pediatric infectious disease and the laboratory of the Complexo Hospital de Clinicas da UFPR teams at the time of the research, without whom it would be impossible to carry out this study at our center. In particular to Tony Tannous Tahan, Tatiane Emi Hirose, Tyane de Almeida Pinto, Renata Rolim Sakiyama da Silva, Laura de Almeida Lanzoni, and Gislene Maria Botão Kussen. To Thomas Cardoso Chagas-Neto, Biomedical Supervisor of Microbiology at the Central Laboratory of Hospital Sao Paulo, for his availability, enthusiasm and partnership in this work. To Editage for editing the article.

CONFLICT OF INTERESTS

None.

FUNDING

CCS (grant Nº 302973/2019-2) and CAAC (grant Nº 314042/2018-0) were supported by Conselho Nacional de Pesquisa e Desenvolvimento (National Council for Scientific and Technological Development). AM was supported by the Walloon Region (Belgium, convention N° 1920238).

REFERENCES

1. Perez-Velez CM, Marais BJ. Tuberculosis in children. N Engl J Med. 2012;367:348-61.
2. Kumar P, Kumar A, Lodha R, Kabra SK. Childhood tuberculosis in general practice. Indian J Pediatr. 2015;82:368-74.
3. World Health Organization. Global tuberculosis report 2020. Geneva: WHO; 2020. [cited 2022 Mar 8]. Available from: https://www.who.int/publications/i/item/9789240013131
4. Nansumba M, Kumbakumba E, Orikiriza P, Muller Y, Nackers F, Debeaudrap P, et al. Detection yield and tolerability of string test for diagnosis of childhood intrathoracic tuberculosis. Pediatr Infect Dis J. 2016;35:146-51.
5. Vargas D, García L, Gilman RH, Evans C, Ticona E, Navincopa M, et al. Diagnosis of sputum-scarce HIV-associated pulmonary tuberculosis in Lima, Peru. Lancet. 2005;365:150-2.
6. Chow F, Espiritu N, Gilman RH, Gutierrez R, Lopez S, Escombe AR, et al. La cuerda dulce: a tolerability and acceptability study of a novel approach to specimen collection for diagnosis of paediatric pulmonary tuberculosis. BMC Infect Dis. 2006;6:67.
7. Graham SM, Cuevas LE, Jean-Philippe P, Browning R, Casenghi M, Detjen AK, et al. Clinical case definitions for classification of intrathoracic tuberculosis in children: an update. Clin Infect Dis. 2015;61 Suppl 3:S179-87.
8. Imperiale BR, Nieves C, Mancino B, Sanjurjo M, Tártara S, Di Giulio AB, et al. String test: a new tool for tuberculosis diagnosis and drug-resistance detection in children. Int J Mycobacteriol. 2018;7:162-6.
9. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual nacional de vigilância laboratorial da tuberculose e outras micobactérias. Brasília: Ministério da Saúde; 2008. [cited 2022 Mar 8]. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_laboratorial_tuberculose.pdf
10. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. Manual de recomendações para o controle da tuberculose no Brasil. 2ª ed. atual. Brasília: Ministério da Saúde; 2011. [cited 2022 Mar 8]. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/manual_recomendacoes_controle_tuberculose_brasil_2_ed.pdf
11. Atwine D, Nansumba M, Orikiriza P, Riera M, Nackers F, Kamara N, et al. Intra-gastric string test: an effective tool for diagnosing tuberculosis in adults unable to produce sputum. Int J Tuberc Lung Dis. 2015;19:558-64.
12. Tafur KT, Coit J, Leon SR, Pinedo C, Chiang SS, Contreras C, et al. Feasibility of the string test for tuberculosis diagnosis in children between 4 and 14 years old. BMC Infect Dis. 2018;18:574.
13. Marais BJ, Donald PR, Gie RP, Schaaf HS, Beyers N. Diversity of disease in childhood pulmonary tuberculosis. Ann Trop Paediatr. 2005;25:79-86.
14. Venturini E, Turkova A, Chiappini E, Galli L, de Martino M, Thorne C. Tuberculosis and HIV co-infection in children. BMC Infect Dis. 2014;14 Suppl 1:S5.

15. Bae WH, Salas A, Brady MF, Coronel J, Colombo CG, Castro B, et al. Reducing the string test intra-gastric downtime for detection of mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2008;12:1436-40.
16. Marais BJ, Pai M. New Approaches and emerging technologies in the diagnosis of childhood tuberculosis. Paediatr Respir Rev. 2007;8:124-33.
17. Scheicher ME, Terra Filho J, Vianna EO. Sputum induction: review of literature and proposal for a protocol. Sao Paulo Med J. 2003;121:213-9.
18. Reid MJ, Saito S, Fayorsey R, Carter RJ, Abrams EJ. Assessing capacity for diagnosing tuberculosis in children in sub-saharan African HIV care settings. Int J Tuberc Lung Dis. 2012;16:924-7.