INT J TUBERC LUNG DIS 12(12):1436–1440

Reducing the string test intra-gastric downtime for detection of *Mycobacterium tuberculosis*

W. H. Bae,* A. Salas,† M. F. Brady,‡ J. Coronel,§ C. G. L. Colombo,§ B. Castro,§ R. H. Gilman,§¶# D. A. J. Moore§¶#

*Harvard College, Cambridge, Massachusetts, USA; †Hospital Nacional Dos de Mayo, Lima, Peru; ‡The Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA; §Universidad Peruana Cayetano Heredia, Lima, Peru; ¶Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA; #Wellcome Trust Centre for Clinical Tropical Medicine, Imperial College London, London, UK

SUMMARY

OBJECTIVES: To explore the potential for reducing the procedural duration of the string test for the diagnosis of tuberculosis (TB) using microscopic observation drug susceptibility (MODS) culture.

METHODS: Twelve patients already diagnosed with pulmonary TB, four each with sputum smear acid-fast bacilli grade 1+, 2+ and 3+, underwent four consecutive string tests of varying intra-gastric downtime (IGDT) of 30 min, 1, 2 and 4 h. Each retrieved string was cut into three—one oesophageal and two gastric sections. Eluates from one of the gastric sections and the oesophageal section were cultured in MODS after a decontamination procedure; eluate from the other gastric section was cultured in MODS with no decontamination.

RESULTS: No significant difference was observed in the retrieval efficacy of *Mycobacterium tuberculosis* ($P = 0.29$) or time to positive MODS culture ($P = 0.80$) among string tests of varying IGDTs. Every patient with a sample that was positive after a 4-h IGDT also had positive culture of a 1-h IGDT sample. A pre-inoculation sample decontamination step significantly reduced culture contamination ($P < 0.001$).

CONCLUSION: In smear-positive patients, reducing the IGDT to 1 h did not affect the *M. tuberculosis* retrieval efficacy of the string test. Future evaluations in non-expectorating human immunodeficiency virus and paediatric populations should include a 1-h IGDT.

KEY WORDS: tuberculosis; diagnosis; string test

MICROBIOLOGICAL DIAGNOSIS of tuberculosis (TB) can be difficult in subjects who cannot produce an adequate sputum sample, such as human immunodeficiency virus (HIV) and paediatric patients. Treatment for these patients is usually administered empirically on clinical grounds, and is therefore inherently imprecise. The string test, in which a string-containing capsule is swallowed by a patient and the string is later withdrawn, has previously been demonstrated to be an effective and well-tolerated diagnostic tool for retrieval of *Mycobacterium tuberculosis* present in the swallowed sputum of these patients.[1,2]

The string test has been used for many years to retrieve enteropathogens such as *Giardia lamblia*, *Salmonella typhi* and *Helicobacter pylori* from the upper gastrointestinal tract.[3–6] The intra-gastric downtime (IGDT) used for the string test, which refers to the time elapsed between swallowing an encapsulated string and its removal, varies from 1 h to 4 h depending on the enteropathogen of interest. Previous studies on retrieval of *M. tuberculosis* using the string test have used the 4-h IGDT. In the present study, we investigated whether this IGDT can be reduced without compromising the retrieval efficacy for *M. tuberculosis*, with the aim of enhancing patient convenience and adherence. This was tested by performing multiple string tests with a range of IGDTs on smear-positive patients. We also determined whether the string test could be used in combination with microscopic observation drug susceptibility (MODS) culture, for which the medium contains an antibiotic cocktail (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin [PANTA]), without the need for prior sample decontamination, by culturing retrieved samples in parallel either without or following sample decontamination.

METHODS

Study population

Patients recently diagnosed with pulmonary TB on the basis of positive sputum acid-fast bacilli (AFB) smears

Correspondence to: David Moore, Laboratorio de Investigación de Enfermedades Infecciosas, Laboratorio de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Urb Ingeniería, San Martín de Porres, Lima 31, Peru. Tel (+511) 382 3398. Fax (+511) 464 0781. e-mail: davidajmoore@msn.com

Article submitted 13 March 2008. Final version accepted 10 July 2008.

were recruited before initiation of treatment at Hospital Nacional Dos de Mayo in Lima, Peru. The study population comprised 12 patients, four patients each with sputum smear AFB grade 1+, 2+ and 3+.[7] Those aged <18 years and those with haemoptysis were excluded from participation. Participation in the study resulted in delaying treatment by a maximum of 24 h.

Clinical procedures

Each participant underwent four consecutive string tests on the same day, with IGDTs of 30 min, 1 h, 2 h and 4 h, starting in the morning after an overnight fast. Half of the patients in each AFB sputum grade were given the string tests in increasing order of IGDT (30 min, 1 h, 2 h and 4 h), and the other half in decreasing order (4 h, 2 h, 1 h and 30 min). Each encapsulated string was prepared in-house by coiling a 90 cm cotton string inside a gelatine capsule along with a small metal ball bearing as a weight. In each test, a patient swallowed an encapsulated string, and the trailing end was taped to the patient's cheek. After the designated IGDT, the patient was asked to wash the mouth with water, after which the string was retrieved.

The first 40 cm of the retrieved string, approximating the section between the taped end of the string and the part sitting in the pharynx, was cut and discarded (Figure). The remaining string of 50 cm in length was cut into one oesophageal section of 20 cm and two gastric sections each of 15 cm. Each section of the string was placed in its own 15 ml Falcon tube containing 2 ml of 0.9% saline, transported on the day of collection to the laboratory, and refrigerated overnight.

Laboratory procedures

Secretions absorbed by each section of the string were eluted the following day by running tweezers along the length of the string to squeeze it as dry as possible. For one of the two gastric sections, this was done directly on the sample received and the eluate was cultured directly in MODS without prior decontamination. For the other gastric section and the oesophageal section, a decontamination step was used prior to inoculation. Briefly, 2 ml of 2% NaOH-NALC solution was added to the tube containing the string in 2 ml of saline and left for 10 min. The string was then removed from the tube and squeezed with tweezers to retain as much eluate as possible, and the mixture was left in the tube for a further 5 min, after which an aliquot was removed for auramine AFB staining and the remainder was centrifuged at 3000 g for 15 min. The pellet was resuspended in Middlebrook 7H9-OADC-PANTA, as per the MODS methodology,* and inoculated into MODS plates. MODS cultures for detection of *M. tuberculosis* and direct isoniazid (INH) and rifampicin (RMP) susceptibility testing were performed as described previously.[8]

* www.modsperu.org

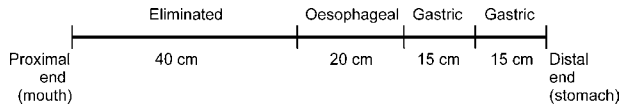

Figure Clinical and laboratory procedures of a retrieved string. The first 40 cm of the retrieved string was eliminated, and the rest of the string was cut into three sections: a 20 cm oesophageal section and two 15 cm gastric sections. One of the two gastric sections was decontaminated before culture and the other was cultured directly, with no decontamination step.

Statistical methods

Statistical analyses were performed using Stata version 9.0 (Stata Corp, College Station, TX, USA), and power calculations were done using Power and Precision version 2.1 (Biostat Inc, Portage, MI, USA). Rates of *M. tuberculosis* detection and contamination between groups were compared using logistic regression, Fisher's exact test for independent samples, and McNemar's test and Cochran's *Q* for dependent samples. Median time to culture positivity was compared using the Kruskal-Wallis test and the Wilcoxon rank-sum test.

Ethical approval

The study was approved by the institutional review boards of Universidad Peruana Cayetano Heredia and Hospital Nacional Dos de Mayo. All participants were provided with verbal and written information and signed an informed consent form. Each participant was compensated for the time and inconvenience of the test with a single payment of 50 Peruvian Nuevo Soles (approximately US$17).

RESULTS

Study population

Ten of the 12 participants were male; the median age of the study population was 25 years (range 18–47). HIV testing was not performed; no subjects were known to be HIV-positive (<2% of TB patients in Peru are HIV-positive).

Overall detection and drug susceptibility testing

MODS culture of the gastric samples obtained from the string test detected *M. tuberculosis* in all 12 patients in a median of 7 days after inoculation. Of 144 cultures, 10 were contaminated, all of which were from gastric samples cultured with no decontamination step. Among the remaining 134 samples, nine samples, including eight from one patient, were culture-negative. Results from MODS cultures stratified by sputum AFB grades, string sections and IGDTs are summarised in Table 1. The raw data, including the auramine stain results, can be seen in Table 2. One patient had isolated RMP monoresistance, and three patients were found to have multidrug resistance (resistance to at least both RMP and INH).

Table 1 *M. tuberculosis* detection and median time to positive culture of each string section with MODS, stratified by the IGDT and the sputum AFB smear grade

		Proportion of *M. tuberculosis* detection				Median time to positive culture, days			
		Sputum smear grade				Sputum smear grade			
IGDT	String section	AFB 1+	AFB 2+	AFB 3+	All	AFB 1+	AFB 2+	AFB 3+	All
30 min	Oesophageal	1.0	0.75	1.0	0.92	7.5	7	6.5	7
	Gastric decontaminated	0.75	0.75	1.0	0.83	7	7	5.5	6.5
	Gastric not decontaminated	1.0	0.5	0.75	0.75	7.5	9	6	7
1 h	Oesophageal	1.0	1.0	1.0	1.0	7	7.5	5	7
	Gastric decontaminated	1.0	0.75	1.0	0.92	7.5	7	5	7
	Gastric not decontaminated	1.0	0.5	0.5	0.67	7	7.5	9	7
2 h	Oesophageal	1.0	1.0	1.0	1.0	7	7.5	5	7
	Gastric decontaminated	1.0	1.0	1.0	1.0	7	8	5	6.5
	Gastric not decontaminated	0.75	0.25	0.75	0.58	8	9	5	8
4 h	Oesophageal	1.0	1.0	1.0	1.0	7.5	7.5	6.5	7.5
	Gastric decontaminated	1.0	0.75	1.0	0.92	7.5	9	5.5	7
	Gastric not decontaminated	1.0	0.75	0.75	0.83	7.5	7	8	7.5

MODS = microscopic observation for detection and susceptibility; IGDT = intra-gastric downtime; AFB = acid-fast bacilli.

Oesophageal vs. gastric samples

Forty-seven of the 48 oesophageal samples (97.9%) were culture-positive; median time to culture positivity was 7 days. Forty-four of the 48 (91.7%) decontaminated gastric samples yielded positive cultures, also with a median time to culture positivity of 7 days. There was no significant difference in culture positivity between the oesophageal and decontaminated gastric samples (exact McNemar test for dependent samples $P = 0.25$, Fisher's exact test for independent samples $P = 0.083$), and post-hoc power analysis showed that there was 80% power to detect a difference in sensitivities of 21% at alpha = 0.05 (Casagrande and Pike approximation for Fisher's exact test).

Effect of intra-gastric down-time on recovery of M. tuberculosis

Overall, there was no statistical difference between detection sensitivity and time to positive culture among the different IGDTs. With culture positivity as the outcome, IGDT was not statistically significant as a predictor when treated as a continuous variable (logistic regression, $P = 0.29$) or as a categorical variable (Cochran's Q, $P = 0.097$). The time to positive culture among different IGDTs was also non-significant by the Kruskal-Wallis rank test ($P = 0.80$). In every instance where the decontaminated gastric culture was positive with a 4-h IGDT, it was also positive at 1 h. Similarly, patients in whom oesophageal samples were culture-positive after a 4-h IGDT always also had positive oesophageal cultures after a 1-h IGDT.

Association between sputum smear AFB grades and recovery of M. tuberculosis

For oesophageal and decontaminated gastric samples, no association was observed between sputum AFB grades and *M. tuberculosis* detection (Fisher's exact $P = 0.12$), although at a median of 5 days, culture times were significantly shorter in samples from patients with AFB 3+ sputum than in those with either AFB 2+ or AFB 1+ sputum, for whom the median time to a positive culture was 7 days (Kruskal-Wallis rank test $P = 0.0042$), with follow-up pair-wise comparisons using the two-sample Wilcoxon rank-sum test showing that AFB 1+ and AFB 2+ groups were not significantly different from each other ($P = 0.38$), while both were significantly different from the AFB 3+ group ($P = 0.0037$ and $P = 0.0049$, respectively).

Effect of NaOH-NALC decontamination

Gastric samples cultured with no prior decontamination had a lower detection rate of *M. tuberculosis* due to a high rate of culture contamination (20.8%, 10 cultures), compared to only 2.1% ($n = 1$) of cultures of decontaminated gastric samples ($P = 0.0063$ by the exact McNemar test). The contaminated culture from a decontaminated gastric sample yielded a positive culture when its original sample was recultured with MODS after a second decontamination step.

DISCUSSION

We found no previous data on an adequate IGDT for the string test. The 4-h IGDT currently being used does not seem to have a published scientific rationale and we found no evidence to support it, at least for the retrieval of *M. tuberculosis* in our study. This study demonstrates that the IGDT can be reduced to 60 min without compromising its retrieval efficacy, at least in this subject group, thereby making it more convenient and tolerable for patients, making it effectively a brief out-patient procedure. Based on results obtained from cultures of various sections of the string, we recommend culture of the entire lower 50 cm of a string, including the oesophageal section, with NaOH-NALC decontamination prior to culture to maximise retrieval efficacy and minimise MODS culture contamination.

Although all the participants in this study were

Table 2 Raw data for auramine and culture testing of every study sample

AFB grade, patient	Section	Auramine AFB stain of E, GD or GN sample				MODS			
		30 min	1 h	2 h	4 h	30 min	1 h	2 h	4 h
1+									
1	E	−	−	−	−	+	+	+	+
	GD	−	±	−	−	c (+)*	+	+	+
	GN	−	−	−	−	+	+	+	+
2	E	−	±	±	±	+	+	+	+
	GD	±	−	±	±	+	+	+	+
	GN	±	−	±	−	+	+	+	+
3	E	±	±	±	±	+	+	+	+
	GD	±	±	−	±	−	+	+	+
	GN	−	−	−	−	+	+	+	+
4	E	−	±	±	±	+	+	+	+
	GD	±	±	±	+	+	+	+	+
	GN	+	±	+	−	+	+	c	+
2+									
5	E	±	+	±	±	+	+	+	+
	GD	+	−	−	−	+	+	+	+
	GN	−	−	−	−	+	c	c	+
6	E	−	−	±	±	+	+	+	+
	GD	±	−	±	−	+	+	+	+
	GN	−	±	±	−	+	+	+	+
7	E	±	−	±	±	−	+	+	+
	GD	+	±	±	±	−	−	+	−
	GN	±	±	±	±	−	−	−	−
8	E	+	+	+	+	+	+	+	+
	GD	+	+	+	+	+	+	+	+
	GN	+	+	+	+	c	+	c	+
3+									
9	E	−	−	−	±	+	+	+	+
	GD	−	±	±	+	+	+	+	+
	GN	±	−	−	−	+	+	+	+
10	E	±	+	+	+	+	+	+	+
	GD	−	±	±	+	+	+	+	+
	GN	±	+	+	±	+	c	+	+
11	E	±	+	±	±	+	+	+	+
	GD	+	+	±	±	+	+	+	+
	GN	±	±	−	±	+	+	c	+
12	E	+	+	+	+	+	+	+	+
	GD	+	+	+	+	+	+	+	+
	GN	+	+	+	+	c	c	+	c

Odd-numbered patients underwent four consecutive string tests in increasing order of IGDT, and even-numbered patients in decreasing order of IGDT. Auramine staining of inoculated samples was done at the start of MODS culture. Auramine stain with >5 AFB was coded as '+', 1–4 AFB as '±', and no AFB as '−'. A sample that yielded contaminated culture is marked 'c.'
*This culture of a decontaminated gastric sample initially became contaminated. The original sample was decontaminated again and recultured, which yielded a positive result.
AFB = acid-fast bacilli; E = oesophageal; GD = gastric decontaminated; GN = gastric not decontaminated; MODS = microscopic observation drug susceptibility assay; IGDT = intra-gastric downtime.

sputum producers who had already been diagnosed with TB with positive sputum smears, no association was detected between AFB grade and *M. tuberculosis* detection. The study was intended as an exploratory proof-of-principle exercise, and we cannot therefore exclude the possibility that the small sample size led to a type II error in which we simply did not test enough patients to detect a difference; however, the lack of a gradient in time to culture positivity related to sputum bacillary load between AFB 2+ and AFB 1+ suggests, without proving, that this is unlikely to be the case.

Although we believe these data establish the principle that a shorter IGDT down to 1 h does not necessarily compromise retrieval efficiency, an important limitation of this study is that the target patient group for the string test is patients who do not expectorate adequately. The 4-h IGDT string test has been shown to be superior to sputum induction for diagnosing TB in HIV patients who cannot produce adequate sputum.[1] Such findings now warrant further evaluation with a shorter 1-h IGDT, including investigation of whether more than one string test culture provides incremental diagnostic sensitivity, an important but as yet unanswered question. The string test has been shown to be well-tolerated by TB suspects as young as 4 years old, suggesting that it could be a useful addition to the paediatric TB diagnostic armamentarium if detection performance observed among HIV-positive adults is reproduced in paediatric patients.[2] If a single 1-h IGDT string test yielded as much information as a 4-h IGDT test or two or three 1-h tests, and if this sensitivity was comparable with multiple gastric washings or sputum inductions, ambulatory diagnostic sample retrieval would be significantly facilitated.

An important strength of this study is the collection of multiple samples from the same patients and the resulting ability to directly compare the retrieval efficacy of varying IGDTs within each patient. In addition, all samples were collected before initiation of treatment, as would be the case in patients undertaking the string test in the usual clinical setting.

In summary, these data indicate that in this patient group, the IGDT of the string test for detection of *M. tuberculosis* with MODS can be reduced to 60 min from 4 h, significantly increasing patient convenience without compromising retrieval efficacy. Future comparative studies of the string test with other sample retrieval methodologies should include a 1-h IGDT.

Acknowledgements

The authors wish to thank all the participants, without whom this study would not have been possible, and the staff of the Estrategia Sanitaria Nacional de Prevención y Control de Tuberculosis at Hospital Nacional Dos de Mayo, Lima, for their essential collaboration and assistance. D A J Moore is a Wellcome Trust Clinical Research Fellow in Tropical Medicine and Reader in Infectious Diseases at Imperial College London (Fellowship award number 078067/Z/05). W H Bae was supported by the David Roux Grant and the Summer Internship Grant by David Rockefeller Center for Latin American Studies, both at Harvard University. M F Brady contributed as an National Institutes of Health/Fogarty International Center Research Fellow.

References

1 Vargas D, Garcia L, Gilman R H, et al. Diagnosis of sputum-scarce HIV-associated pulmonary tuberculosis in Lima, Peru. Lancet 2005; 365: 150–152.
2 Chow F, Espiritu N, Gilman R H, 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.

3 Jones J E. String test for diagnosing giardiasis. Am Fam Physician 1986; 34: 123–126.
4 Gilman R H, Hornick R B. Duodenal isolation of *Salmonella typhi* by string capsule in acute typhoid fever. J Clin Microbiol 1976; 3: 456–457.
5 Gilman R H, Islam S, Rabbani H, Ghosh H. Identification of gallbladder typhoid carriers by a string device. Lancet 1979; 1: 795–796.
6 Perez-Trallero E, Montes M, Alcorta M, Zubillaga P, Telleria E. Non-endoscopic method to obtain *Helicobacter pylori* for culture. Lancet 1995; 345: 622–623.
7 World Health Organization. Laboratory services in tuberculosis control. Part II: microscopy. WHO/TB/98.258. Geneva, WHO: 1998.
8 Moore D A, Evans C A, Gilman R H, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. N Engl J Med 2006; 355: 1539–1550.

RÉSUMÉ

CONTEXTE : Explorer la possibilité de réduction de la durée d'exécution du test à la ficelle lors du diagnostic de la tuberculose (TB) par l'évaluation de susceptibilité dans les crachats des malades par observation microscopique des milieux de culture (MODS).

OBJECTIF : Chez 12 patients où le diagnostic de TB pulmonaire avait déjà été porté, quatre avec des degrés de positivité de chacun des frottis 1+, 2+ et 3+ ont subi quatre tests à la ficelle consécutifs avec des durées de séjour intragastrique (IGDT) respectivement de 30 min, 1 h, 2 h et 4 h. Chaque ficelle retirée a été coupée en trois parties (une section oesophagienne et deux sections gastriques). Les éluats provenant d'une des sections gastriques et de la section oesophagienne ont été cultivés sur MODS après une procédure de décontamination, et l'éluat de la seconde section gastrique a été mis en culture sur MODS sans décontamination.

RÉSULTATS : On n'a pas observé de différence significative dans l'efficacité de la mise en évidence de *Mycobacterium tuberculosis* ($P = 0{,}54$) ou dans la durée avant la positivité de la culture MODS ($P = 0{,}92$) parmi les tests à la ficelle avec diverses IGDT. Chaque patient dont un échantillon était positif après 4 h d'IGDT avait également une culture positive sur l'échantillon retiré après 1 h. Une étape de décontamination de l'échantillon avant l'inoculation a réduit de manière significative la contamination de la culture ($P < 0{,}001$).

CONCLUSION : Chez les patients à bacilloscopie positive des frottis, la réduction à 1 h de l'IGDT n'a pas affecté l'efficience du test à la ficelle pour la mise en évidence de *M. tuberculosis*. Des évaluations ultérieures dans les populations séropositives pour le virus de l'immunodéficience humaine ou pédiatriques sans expectorations devraient inclure un test d'IGDT durant 1 h.

RESUMEN

OBJECTIVOS : Evaluar las posibilidades de reducir la duración del procedimiento de la prueba de la cuerda para el diagnóstico de tuberculosis (TB), utilizando la tecnología microscópica de observación de fármacos (MODS).

MÉTODOS : Doce pacientes ya diagnosticados con TB pulmonar, cuatro de cada uno de ellos con baciloscopia de esputo grado 1+, 2+ y 3+, se sometieron a cuatro pruebas consecutivas de la prueba de la cuerda en diversos tiempos intra-gástricos (IGDT) de 30 min, 1 h, 2 h y 4 h. Cada cuerda recuperada fue cortada en tres secciones —una esofágica y dos gástricas. Los eluidos de una de las secciones gástricas y la esofágica se cultivaron en MODS después un proceso de descontaminación ; el eluido de la otra sección gástrica fue cultivado en MODS sin descontaminación.

RESULTADOS : No hubo diferencia significativa observada sobre la eficacia en la recuperación de *Mycobacterium tuberculosis* ($P = 0{,}29$) o el tiempo para un cultivo positivo con MODS ($P = 0{,}80$) entre las pruebas de la cuerda de los diversos IGDT. Todos los pacientes con una muestra positiva después del IGDT de 4 h también tuvieron un cultivo positivo del IGDT de 1 h. La descontaminación de la muestra antes de la inoculación redujo significativamente la contaminación del cultivo ($P < 0{,}001$).

CONCLUSIÓN : En pacientes con baciloscopia positiva, la reducción del IGDT a 1 h no afectó la eficacia de recuperación de *M. tuberculosis* de la prueba de la cuerda. Futuras evaluaciones a poblaciones de pacientes con virus de inmunodeficiencia humana y niños que no expectoran deben incluir el IGDT de 1 h.