Correspondence: W. Worodria, Dept of Medicine, Mulago Hospital, Makerere University, Kampala, Uganda; MU-UCSF Research Collaboration, Kampala, Uganda. E-mail: worodria@yahoo.com

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Sputum induction *versus* gastric washing for the diagnosis of pulmonary mycobacterial disease

To the Editors:

The diagnosis of pulmonary mycobacterial disease is based primarily on the isolation of mycobacteria in sputum specimens. The collection of at least three samples of spontaneously expectorated sputum on three different days is the standard diagnostic procedure for patients with suspected pulmonary tuberculosis and non-tuberculous mycobacterial lung disease [1, 2]. For patients who are unable to expectorate, bronchial secretions, aspirated by fiberoptic bronchoscopy, or gastric washings (GWs) are collected [1, 3]. These procedures require the patient to be hospitalised, are fastidious and unpleasant for the patient, and the yield of GWs for the diagnosis of pulmonary tuberculosis is considered to be rather low [3].

Another option is sputum induction (SI) with nebulised hypertonic saline [4, 5]. SI does not necessitate the patient to be hospitalised. However, it is a complicated procedure that requires trained staff and respiratory isolation conditions [4].

The diagnostic value of this procedure is still debated: several studies have reported that sputum induction has a rather low diagnostic yield [6]; other studies have found that SI compares favourably with GW and bronchoalveolar lavage in patients with suspected pulmonary tuberculosis [4]. We therefore decided to conduct a prospective study to compare the diagnostic value of SI with that of GW in adult patients with suspected pulmonary mycobacterial disease.

The study population was comprised of consecutive patients admitted to the Dept of Pulmonary Diseases of Ambroise Paré University Hospital (Boulogne, France) between January 2003 and March 2009 because of a clinical suspicion of pulmonary mycobacterial disease. The study was approved by the Ethics Committee for the Protection of Subjects Participating in Biomedical Research at our institution. Patients were included in the study if a spontaneously expectorated sputum sample could not be obtained or was insufficient in volume.

A total of 295 patients were enrolled in the study. 64% of the patients were male and the mean age was 53.3 yrs. The HIV status was positive in nine patients, negative in 154 and unknown in 132. Approximately 45% of the patients were of French origin and 36% were of African origin. Based on clinical, radiological, biological and microbiological criteria, a total of 116 (39%) patients were considered to have mycobacterial disease: active tuberculosis (n=85), latent tuberculosis (n=9), previously healed tuberculosis (n=5), nontuberculous mycobacterial disease (n=17).

A single SI procedure was performed within the first 3 days of admission, and GWs were obtained on three consecutive mornings at approximately 06:00 h following a night in hospital (one procedure per day). SI was performed under the supervision of a trained physical therapist. First, all subjects were administered 0.5 mg ipratropium *via* an ultrasonic nebuliser (LS 290; Systam, Villeneuve-sur-Lot, France). A 5.85% hypertonic saline (Proamp, Lyon, France) was then delivered using the same nebuliser for ~10 min. GWs were performed by nasal introduction of tubing into the stomach, injection and aspiration of ~50 mL of sterile normal saline. A randomly chosen subgroup of 61 French-speaking patients was asked to assess the diagnostic procedure and to indicate a preference for GW or SI.

Respiratory samples were immediately transported to the microbiology laboratory, where microscopic examination, culturing and identification of mycobacterial species were performed by standard procedures [1]. Species of the *Mycobacterium tuberculosis* complex were identified using the Accuprobe® system (bioMérieux, Marcy-l'Etoile, France), whereas non-tuberculous mycobacteria (NTM) were identified by amplification and sequencing of part of the *hsp65* gene as previously described [7]. Statistical analysis was performed using the MacNemar test. A p-value <0.05 was considered to be statistically significant.

Sputum induction was successful in all but 16 patients (95% successful). 22 patients underwent two sputum induction procedures. A total of 273 (93%) patients had at least one GW. Three specimens were obtained from GW in 199 (67%) patients, and two specimens were obtained from GW in 53 (18%) patients. Overall, 259 (88%) patients had at least one SI procedure and one GW procedure. No serious adverse reaction attributable to the procedure occurred during or after SI or GW. 53 (87%) out of the 61 patients who were asked if they had a preference for SI or GW preferred SI, whereas only eight (13%) patients favoured the GW procedure.

A total of 1,047 respiratory specimens were obtained from the 295 patients: 746 from GW and 301 from SI. Overall, 71 specimens from 29 patients stained positive (27 with *M. tuberculosis*, one with *Mycobacterium intracellulare,* and one with *Mycobacterium xenopi*). A positive culture was obtained from 178 (17%) specimens from 69 (23%) patients: 152 grew *M. tuberculosis*, whereas NTM were isolated from 26 specimens. The 152 culture samples positive for *M. tuberculosis* were obtained from 55 patients, whereas the 26 cultures positive for NTM were obtained from 14 patients (*M. avium*: n=5; *M. intracellulare*: n=2; *M. xenopi*: n=4; *Mycobacterium chelonae*: n=2; and *Mycobacterium mucogenicum*: n=1). No smear-positive, culture-negative specimens were detected.

M. tuberculosis was cultured from 49 SI samples (49 patients) and 103 GW samples (first GW sample in 43 patients and up to three samples in 50 patients). NTM were grown in 12 SI samples and 14 GW samples. A positive stain was observed more often with SI (46% of 61 culture-positive samples) than with GW (38% of 117 culture-positive samples), but this difference was not statistically significant. Among the 27 patients with tuberculosis and a positive sample upon direct examination, a positive stain was observed with both GW and SI in 21 (78%) patients; GWs were positive but SI was negative in one (4%) patient, and SI was positive whereas GWs were negative in five (18%) patients.

Among the 279 patients who underwent SI, a positive culture was obtained in 61 (22%). Among the 273 subjects who underwent GW, the first sample grew mycobacteria in 45 (16%) patients. Taking into account the positivity of any of three GWs increased the number of patients with positive GW cultures from 45 to 56 (21%). Assuming that in our population of 295 patients, the 69 patients with at least one positive mycobacterial culture from SI or GWs represented all patients with active mycobacterial disease, the sensitivity of SI was 88% compared with 65% for the first GW procedure and 81% for up to three GW procedures. Assuming that the 55 patients with at least one culture positive for *M. tuberculosis* from SI or GWs represented all patients with active tuberculosis, the sensitivity of SI was 89% compared with 78% for the first GW procedure and 91% for up to three GW procedures.

The comparison of the diagnostic yields of SI and of GW in the 259 patients who underwent both procedures is presented in table 1. Sputum induction displayed a significantly higher yield than the first GW (20.5% *versus* 16.2%; p=0.03; OR 3.2, 95% CI 1.1–11.2). The diagnostic yields of SI and GW were similar when up to three GW procedures were compared with one SI procedure (20.5% *versus* 20.1%; p=0.81; OR 1.1, 95% CI 0.4–3.1). We found no statistically significant difference in the clinical characteristics of patients with a positive culture using one procedure and a negative culture with the other.

Only three published studies have compared the performance of GW and SI in the diagnosis of mycobacterial lung disease

TABLE 1	Comparison of sputum induction with the first gastric washing and with up to three gastric washings in the 259 patients who underwent both procedures		
	Sputum induction		**p-value**
	Positive culture	**Negative culture**	
First gastric washing			
Positive culture	37	5	0.03
Negative culture	16	201	
Up to three gastric washings			
Positive culture	43	9	0.81
Negative culture	10	197	

Data are presented as n, unless otherwise stated.

using a modern SI technique that includes hypertonic saline [4, 8, 9]. ZAR *et al.* [8] studied a total of 250 children with suspected pulmonary tuberculosis in South Africa and reported that the diagnostic yield from SIs was better than that from GWs. However, this study compared three SI with three GW procedures. HATHERILL *et al.* [9] reviewed the database records of 1,654 children with suspected pulmonary tuberculosis in South Africa. They observed that the diagnostic yield of a single SI sample was equivalent to that of a GW sample for culture confirmation of tuberculosis. The authors attributed the discrepancy between the results of these two studies to the fact that the patients in their study had milder tuberculosis compared to those in the study by ZAR *et al.* [8]. BROWN *et al.* [4] assessed the yield of specimens obtained using SIs and GWs in 140 adults with suspected tuberculosis who were unable to expectorate. They found that the use of three to five SI samples resulted in the detection of more cases than the use of three GWs (39% *versus* 30%), suggesting that SI was a more sensitive technique than GW. Our study extends these results by directly comparing a single SI procedure with one GW, and reveals the superior sensitivity of SI. However, according to several authors, the yield of multiple SIs is better than that of a single procedure [5, 8, 9]. Therefore, even though our results found no statistical difference between a single SI procedure and three GWs in terms of diagnostic yield, the fact that nine patients had a negative result with SI and a positive result with GWs suggests that at least two adequate SI samples should be obtained from patients with suspected pulmonary mycobacterial disease who are unable to expectorate. In addition to a higher diagnostic yield, SI has the advantage that it can be performed in an outpatient setting, several times on the same day and its cost is relatively low [4, 9, 10]. However, careful environmental control should be undertaken to prevent nosocomial transmission of tuberculosis to patients and healthcare workers, especially the physical therapists who perform the SI procedures [11].

In conclusion, we have shown that SI has a significantly higher diagnostic yield for pulmonary mycobacterial disease than GW in patients who cannot produce sputum. In addition, SI was preferred to GW by the majority of the surveyed patients. These results indicate that SI should be the standard noninvasive technique to obtain specimens for the microbiological diagnosis of pulmonary mycobacterial disease.

B. Heym*, A. Beauchet#, M.T. Ngo¶, J-L. Gaillard* and T. Chinet¶

*Microbiology Dept, #Dept of Public Health and Medical Informatics, and ¶Dept of Pulmonary Disease, Hôpital Ambroise Paré, AP-HP, Faculté de Médecine Paris Ile de France Ouest, Université de Versailles Saint Quentin en Yvelines, Boulogne, France.

Correspondence: T. Chinet, Service de Pneumologie, AP-HP, Centre Hospitalier Universitaire Ambroise Paré, 9 avenue Charles de Gaulle, 92104 Boulogne-Billancourt, France. E-mail: thierry.chinet@apr.aphp.fr

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