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Usefulness of Sputum Induction with Hypertonic Saline in a Real Clinical Practice for Bacteriological Yields of Active Pulmonary Tuberculosis

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Background: Mycobacterial identification in active pulmonary tuberculosis (APTB) is confirmative, even though successful rates using self-expectorated sputum are limited. Sputum specimens collected by hypertonic saline nebulization showed higher bacteriologic diagnostic sensitivities over those of self-expectoration, mostly studied in smear-negative or sputum-scarce patients. The efficacy of induced sputum was rarely assessed in real clinical settings.
Methods: A prospective randomized case-control study was performed in one hospital. The subjects highly suspicious of APTB were asked to provide 3 pairs of sputum specimens in 3 consecutive days. The first pairs of the specimens were obtained either by self-expectoration (ES) from the next day of the visit or sputum induction with 7% saline nebulization in clinic (SI), and the other specimens were collected in the same way. The samples were tested in microscopy, culture, and polymerase chain reaction (PCR). The outcomes of the bacteriological diagnosis were compared.
Results: Seventy six patients were assigned to either ES (38 subjects, median age of 51, 65.8% male) or SI (38 subjects, median age of 55, 52.6% male). APTB was clinically confirmed in 51 patients (70.8%), 27 in ES and 24 in SI. Among the APTB, more adequate specimens were collected from SI (41/65, 63.1%) than ES (34/80, 42.5%) (p=0.01). Bacteriological confirmation was achieved in 14 (58.3%) patients in SI, and 13 (48.1%) in ES (p=0.46). In the same-day bacteriological diagnosis with microscopy and PCR, there were positive results for 9 patients (37.5%) in SI and 7 patients (25.9%) in ES (p=0.37).
Conclusion: Sputum induction improves sputum specimen adequacy. It may be useful for the same-day bacteriological diagnosis with microscopic examination and PCR.

Keywords: Tuberculosis, Pulmonary; Saline Solution, Hypertonic; Sputum; Diagnosis

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Introduction

The global prevalence of active *Mycobacterium tuberculosis* infection is unacceptably high. Despite efforts to counteract the disease, millions of new cases of active tuberculosis per year are being identified worldwide, more than one-third of which are in low-income countries[1].

People with active pulmonary tuberculosis (APTB) are the main community source of the mycobacteria. Rapid and accurate diagnosis and immediate appropriate chemotherapy of APTB are the key concepts of tuberculosis control, especially in high-burden regions. Microscopic examination of sputum samples for acid-fast bacilli (AFB) and their culture with sub-

sequent drug-susceptibility testing for antituberculosis drugs is the standard diagnostic method for the diagnosis of APTB, although it has insufficient sensitivity and specificity. As a supplement to improve the rapidity and accuracy of diagnosis, nucleic acid amplification, imaging, and histopathologic examination are useful, but are rarely available in low- and middle-income countries[2,3].

Mycobacterial identification in respiratory specimens is confirmative for the diagnosis of APTB. The success rate of the identification depends on the quality of the collection technique, and the quality of specimens collected by patients' self-expectoration (ES) is frequently unsatisfactory. To overcome the relatively low sensitivity of the standard methods in patients in whom active disease is suspected, two or three consecutive daily sputum samples, preferably expectorated early in the morning, are requested and tested in the laboratory by AFB smear and mycobacterial culture[4].

Nebulization with hypertonic saline facilitates sputum expectoration even in patients who usually do not expectorate. This method has been applied in patients with cystic fibrosis to enhance mucus clearance and for identification of infectious agents, and for cytological examination in inflammatory airway disorders[5-7]. When applied to the diagnosis of APTB, sputum induction (SI) by hypertonic saline increases the diagnostic sensitivity, especially in sputumless patients. In most previous studies, SI was applied to patients who had scanty sputum at presentation or who were suspected of having APTB but had a negative AFB smear[8-11]. Its diagnostic sensitivity is higher than that of self-expectorated sputum, nasopharyngeal aspiration, or gastric lavage, and is equal to that of invasive techniques such as bronchoscopic lavage[12-14]. Despite the benefits reported in the literature, SI is not recommended as a supplemental standard method even in patients who spontaneously produce scanty sputum.

In a real clinical practice, we prospectively applied SI with hypertonic saline on the day of the clinic visit for patients suspected of having APTB, and investigated its usefulness compared with the standard method of sputum sample collection for bacteriological confirmation of APTB.

Materials and Methods

This prospective, randomized, case-control comparative study was planned in a clinic in a regional referral hospital in a high-income, low-human immunodeficiency virus (HIV)-prevalence country, and the study protocol was approved by the Institutional Review Board of the Jeju National University Hospital in Jeju, Korea (IRB no. 2011-58).

Patients over 18 years old who were clinically suspected of having APTB were enrolled from January 2012 to August 2012. Patients exposed to antituberculosis agents within the previous 3 months were excluded. Clinically suspicious cases was subjectively identified by one of 3 experienced specialists based on the patients' symptoms, physical examination, chest imaging and/or laboratory tests. With informed consent, each patient, regardless of his or her subjective amount of sputum at presentation, was randomized to either SI or ES. Patients were briefed on sputum collection techniques and their further utilization.

Patients who were randomized to SI were, on the day of the clinic or emergency toom (ER) visit (SI D1), required to breathe

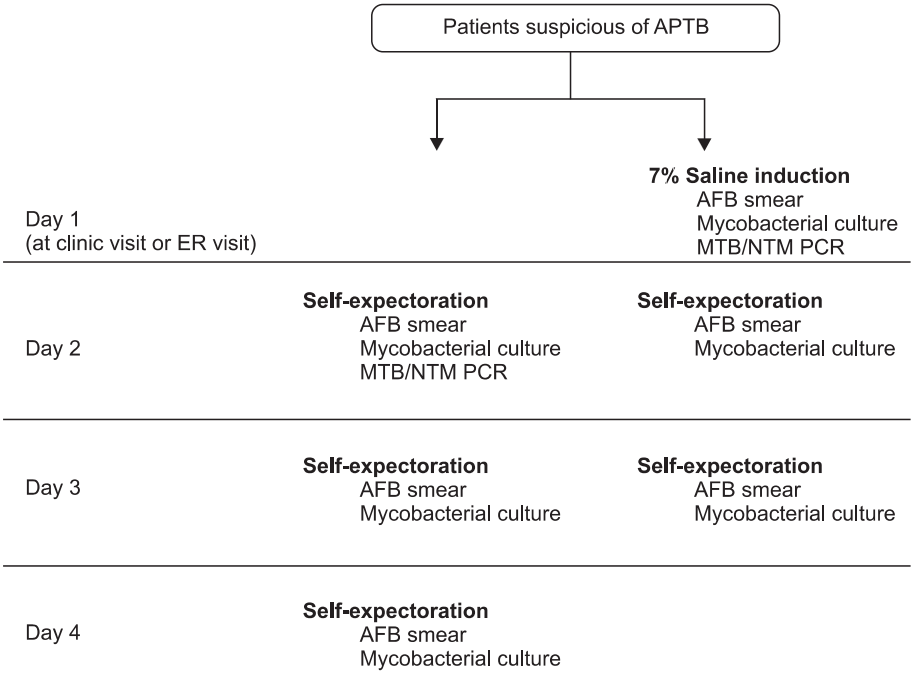

Figure 1. A scheme of the study. APTB: active pulmonary tuberculosis; AFB: acid-fast bacilli; MTB: *Mycobacterium tuberculosis*; NTM: nontuberculous mycobacterium; PCR: polymerase chain reaction; ER, emergency room.

under nebulization (PARI Company, Starnberg, Germany) with 5 mL of 7% NaCl solution for at least 5 minutes, and thereafter to produce 3 sputum samples into aseptic sputum jars for AFB smear, mycobacterial culture, and real-time polymerase chain reaction (PCR) for *M. tuberculosis* and nontuberculous mycobacterium (MTB & NTM kit; BioSewoom Inc., Seoul, Korea). On each of the following two days (SI D2, D3), the SI patients were asked to submit 2 sputum specimens for AFB smear and culture, which were collected early in the morning at home or on the ward.

Patients randomized to ES were asked to submit 3 sputum samples for AFB smear, culture, and PCR collected early in the morning on the day after the clinic or ER visit (ES D2). Other samples were collected on day 3 (ES D3) and day 4 (ES D4) by the same method as those used for SI D2 and SI D3. Patients who for any reason did not submit more than 2 specimens were excluded from the outcome analyses (Figure 1).

The adequacy of the specimens was measured according to microscopic examination. An adequate specimen was defined as sputum that contained >25 neutrophils/low-power field (LPF) and <25 squamous epithelial cells/LPF (grades 4 and 5)[15].

Sputum smear microscopy was done in a two-step procedure, where samples were screened by auramine-rhodamine stain and positives were confirmed by classical Ziehl-Neelsen staining. An AFB smear was defined as positive if 1+ (10 to 99 AFB per 100 LPF) or more bacilli were detected. Positive mycobacterial culture was defined as identification of either or both *M. tuberculosis* and nontuberculous mycobacterium in an automated liquid or solid culture system requested in the Korea Institute of Tuberculosis (http://www.kit.or.kr). PCR positive was defined as per the manufacturer's instructions.

Radiological suspicion of APTB was identified by an experienced radiologist blinded to the experienced clinical specialist's opinion.

Bacteriological confirmation of APTB in a patient was defined as when at least one of the AFB smear, culture, or PCR from the patient was positive. A patient who had bacteriological confirmation of APTB or who showed clinical and radiological improvement recognized by one of the specialists after having maintained antituberculosis chemotherapy for longer than 3 months was considered as "a confirmed APTB case."

1. Statistical analysis

All statistical analysis was performed using PASW Statistics ver. 14 (SPSS Inc., Chicago, IL, USA). An α level less than 0.05 was considered to be statistically significant. The χ^2 test was used to compare categorical variables. Continuous variables were analyzed using Student's t-test or the Mann-Whitney U test.

Results

1. Demographic characteristics

Seventy six patients (median age, 53 years; interquartile range [IQR], 39–68 years; male, 57.7%) were randomized to either SI (median age, 55 years; IQR, 34–68 years; male, 52.6%) or ES (median age, 51 years; IQR, 42–67 years; male, 65.8%) (p=0.54 and p=0.24, respectively). Productive cough was reported in 18 patients (47.3%) of the SI group and 17 (44.7%) of the ES group (p=0.82). Four patients (5.3%) did not submit sputum specimens, 1 in the SI group and 3 in the ES group. No HIV-positive patient was included.

Of 209 specimens collected for microscopic examination, 106 (50.7%) adequate sputum specimens were obtained, 48 (45.3%) of 106 specimens from the SI group and 58 (56.3%) of 103 specimens from the ES group (p=0.11). APTB was suspected by the radiologist in 58 patients (76.3%), 26 (68.4%) from the SI group and 32 (84.2%) from the ES group (p=0.11) (Table 1).

2. Comparisons of bacteriological yields in patients with confirmed APTB

Among the 72 patients who submitted more than 2 speci-

Table 1. Demographic characteristics of participants

	Self-expectoration group (n=38)	Sputum-induction group (n=38)	p-value
Males	25 (65.8)	20 (52.6)	0.24
Age, yr	51 (34–68)	55 (42–67)	0.54
Productive cough	17 (44.7)	18 (47.3)	0.82
Failure in specimen collection	3 (7.9)	1 (2.6)	0.30
Specimen adequacy	58/103 (56.3)	48/106 (45.3)	0.11
Radiologic suspicion of APTB	32 (84.2)	26 (68.4)	0.11
APTB confirmed*	27 (71.1)	24 (63.2)	0.46

Values are presented as number (%) or median (25th–75th percentile).
*Includes nontuberculous mycobacteria.
APTB: active pulmonary tuberculosis.

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Table 2. Comparisons of bacteriological confirmation between self-expectoration group and sputum-induction group among patients who were clinically confirmed with active pulmonary tuberculosis

	Self-expectoration group (n=27)	Sputum-induction group (n=24)	p-value
Adequate sputum acquired	34/80 (42.5)	41/65 (63.1)	0.01
Positive microscopy in specimens	9/80 (11.3)	12/65 (18.5)	0.22
in patients	4 (14.8)	6 (25.0)	0.36
Positive culture in specimens	22/80 (27.5)	19/75 (25.3)	0.76
in patients*	13 (48.1)	9 (37.5)	0.52
Positive PCR*	5 (14.8)	8 (33.3)	0.23
Overall bacteriological confirmation in specimens*	35/188 (18.6)	39/154 (25.3)	0.13
Overall bacteriological confirmation in patients*	13 (48.1)	14 (58.3)	0.46

Values are presented as number (%).
*Includes nontuberculous mycobacteria.
PCR: polymerase chain reaction.

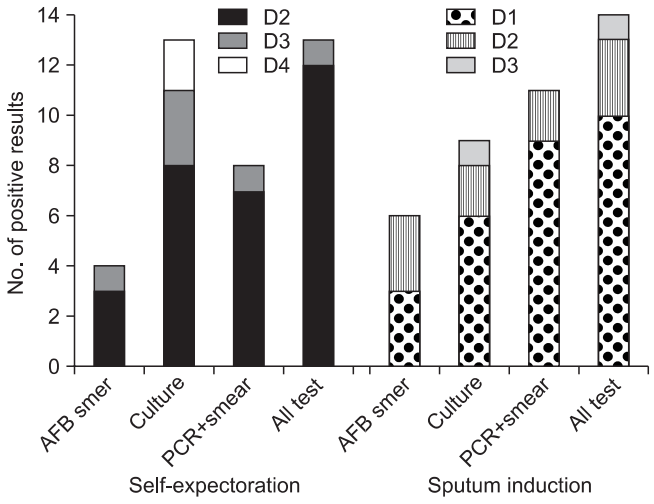

Figure 2. The incidence of bacteriological yields in self-expectoration and sputum-induction groups. New positive cases were counted by excluding the positive cases from the previous day's samples. AFB: acid-fast bacilli; PCR: polymerase chain reaction.

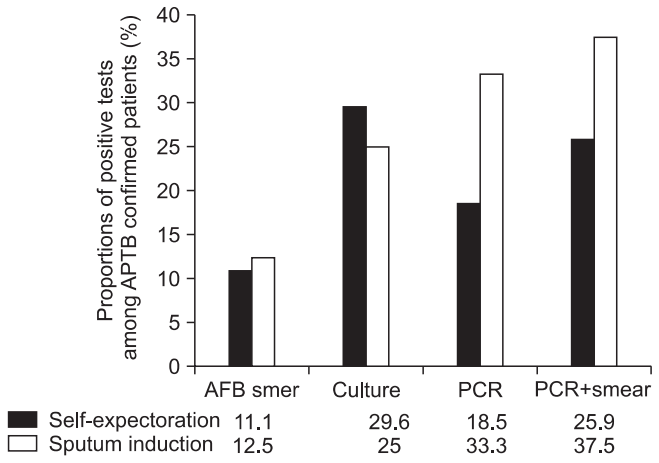

Figure 3. The proportions of the 1st day's bacteriological yields among active pulmonary tuberculosis (APTB)-confirmed cases according to the collection method. AFB: acid-fast bacilli; PCR: polymerase chain reaction.

mens, APTB was clinically confirmed in 51 patients (70.8%), 24 of 37 (64.7%) patients in the SI group, and 27 of 35 (77.1%) in the ES group (p=0.25). In the SI group, of 65 samples that underwent microscopic examination, 41 specimens (63.1%) were noted as adequate sputum specimens, whereas in the ES group only 34 (42.5%) of 80 specimens were adequate (p=0.01). In the SI group, 39 individual sputum specimens (25.3%) of 154 samples were positive for *M. tuberculosis* or nontuberculous mycobacteria, whereas in the ES group, 35 (18.6%) of 188 samples were positive (p=0.13).

Among the 51 confirmed APTB cases, *M. tuberculosis* or nontuberculous mycobacteria was identified in one or more samples from 27 (52.9%) patients, 14 (58.3%) from the SI group and 13 (48.1%) from the ES group (p=0.46). The first samples from each group (SI D1, ES D2) included positive samples from 22 (43.1%) patients, 10 (41.7%) in the SI group and 12 (44.4%) in the ES group. Of the second samples from each group (SI D2, ES D3), 4 additional patients (7.8%) showed positive results, 3 (12.5%) in the SI group and 1 (3.7%) in the ES group. Of the third day samples, only one additional patient (2.0%) from the SI group was newly positive (Table 2, Figure 2).

3. Comparisons of the bacteriological yields among the first-day's sputum samples from APTB-confirmed cases

The first-day sampling methods were the only difference between the two groups (SI D1 vs. ES D2). Among the confirmed APTB cases, mycobacteria were identified in 30 first-day

samples (15 samples from each group). In the first-day samples from the SI group (SI D1), 3 were microscopy positive, 6 culture positive, and 6 PCR positive (15/24, 62.5%). In the first-day samples from the ES group (ES D2), 3 were microscopy positive, 8 culture positive and 4 PCR positive (15/27, 55.6%).

The AFB smear and PCR techniques give prompt bacteriological diagnosis, referred to as same-day diagnostics. In 16 patients (31.4%), *M. tuberculosis* or nontuberculous mycobacteria was detected in at least one of microscopy or PCR, 9 patients from the SI group and 7 from the ES group (37.5% and 25.9%, respectively; p=0.37) (Figure 3).

4. Adverse events

There were no patients who did not tolerate the procedure of 5 minutes' respiratory exposure to a mist of 7% saline. As expected, mild adverse events including cough or salty taste in the mouth and the nose were noted in most patients. There were no serious adverse events including shortness of breath experienced by any of the 38 patients. SI by 7% hypertonic saline was found to be tolerable and safe.

Discussion

Human sputum consists mainly of water and mucin proteins that are highly glycosylated and condensed in the mucin-secreting cells. Once secreted, the physical properties of mucins, including their viscosity, are determined by the content of water and bicarbonate in the airway lumen. The water content in the airway originates from the blood flow surrounding the airway, and diffuses into the lumen through an osmotic pressure that is mainly determined by sodium and chloride ion concentration[16,17]. Salt delivered by nebulization increases the osmotic pressure in the airways and draws more water into the lumen. As a consequence, mucins in the airways are diluted, facilitating sputum expectoration. In addition to its dilutional effects, hypertonic saline also has a protussive effect by stimulating cough reflex, which is believed to facilitate sputum expectoration[18]. Although there is no standardized method noted in the literature, nebulization with 20 mL 3% saline, which takes 40–60 minutes, is most frequently used[11,19,20]. This time-consuming method may be unpleasant for both the patient and the observer of the procedure. Nausea, vomiting, and, rarely, bronchospasm related to this procedure have been observed[20]. Because the observers are exposed to the risk of airborne infection from patients, a facility with a ventilated booth or at least performance of the procedure in the open air is required. Peter et al.[21] suggested that sputum collection facilitated by instructions from a healthcare worker may be more feasible than SI in circumstances where facilities are limited because of low income.

Nonetheless, SI by hypertonic saline is considered to be a safe, cheap, and well-tolerated procedure. If applied in clinical practice, it should result in more compliant sputum collection than collection of early morning specimens at home. Same-day diagnosis may be possible, allowing immediate initiation of treatment. In this study, nebulization with 5 mL of 7% saline for 5 minutes was applied. A higher concentration of saline can shorten the duration of the procedure and achieve a similar sputum-inducing effect. No serious adverse events were noted in our study.

In a low-income, high-burden country, Atiq-ur-Rehman et al.[19] compared 164 paired collections of self-expectorated sputum and sputum induced with 3% saline for 20 minutes. Although adequate specimens were successfully obtained by induction in two-thirds of sputumless patients, the study failed to demonstrate any statistical benefits of SI over ES in bacteriological confirmation of APTB[19]. In a recent study in which the efficacy of induced sputum collection was compared with that of health-care worker-instructed collection in sputumless patients, sputum adequacy and culture positivity were significantly higher with induction. However, the rates of same-day diagnosis by microscopic examination and molecular diagnostics were similar in the two groups[21]. In a systemic review of 23 studies, the overall success rates of sputum collection by saline nebulization were generally high, from 76% to 100%, with few adverse events. In only 8 studies, the results of microscopic examination were compared with those of mycobacterial culture. Higher yields in induced sputum were generally shown, but the sensitivities ranged widely from 0% to 100%[12].

In the present study, the usefulness of hypertonic saline SI for improving bacteriological yield was investigated exclusively in a real clinical practice. Although the results consistently supported the benefits of induced sputum for bacteriological yield, especially in the same-day samples obtained at the clinic visit, this study failed to demonstrate any significant differences, except in the rate of collection of adequate sputum specimens. This is mainly because of the size of the study, low participant numbers and because of a relatively lower rate of culture positivity in patients undergoing SI. We cannot exclude the possibility that airway exposure to high-concentration saline might influence the viability of mycobacteria in respiratory specimens. Mycobacterial culture is important for discriminating nontuberculous mycobacterial infection and for drug-susceptibility tests, so patients with nontuberculous mycobacteria were included in this study. The incidence and proportion of nontuberculous mycobacteria in respiratory samples is consistently increasing in Korea[22]. Needless to say, treatment strategies for nontuberculous mycobacteria are different from those for APTB, but in most cases nontuberculous mycobacterial infection can barely be discriminated clinically or radiologically from APTB. In this study, the third-day samples for AFB smear and culture provided little additional bacteriological yield, with only a 2% increase in confirmed APTB cases. Sampling on two separate days might be sufficient.

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In conclusion, to increase the diagnostic yield in a patient with suspected APTB, it is suggested that in a real clinical practice 1) at the clinic visit, SI with hypertonic saline is applied for same-day diagnosis with microscopic examination and PCR and 2) for the next 2 days, morning self-expectorated specimens are collected for mycobacterial culture. A follow-up larger-scale randomized trial should be performed.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

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