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Effect of airway clearance techniques on the efficacy of the sputum induction procedure

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ABSTRACT: Sputum induction is used in the early identification of tuberculosis (TB) and pneumocystis infections of the lung. Although manual physiotherapy techniques to clear the airways are often incorporated in the sputum induction procedure, their efficacy in this setting is unknown.

This randomised, crossover trial enrolled adults referred for sputum induction for suspected TB and pneumocystis infections of the lung. All participants underwent two sputum induction procedures, inhaling 3% saline *via* ultrasonic nebuliser. During one randomly allocated procedure, airway clearance techniques (chest wall percussion, vibration, huffing) were incorporated.

In total, 59 participants completed the trial. The airway clearance techniques had no significant effect on how the test was tolerated, the volume expectorated or the quality of the sample obtained (assessed by the presence of alveolar macrophages). The techniques did not significantly affect how often the test identified a suspected organism, nor the sensitivity or specificity of sputum induction.

In conclusion, the study was unable to demonstrate any effect of airway clearance techniques on the sputum induction procedure. The results provide some justification for not including airway clearance techniques as part of the sputum induction procedure.

KEYWORDS: Chest physiotherapy, induced sputum, pneumocystis, tuberculosis

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Early identification of tuberculosis (TB) and pneumocystis infections of the lung is important so that therapy and infection control measures can be instituted appropriately [1, 2]. Sputum induction is one of the ways in which a sample of respiratory tract organisms can be obtained from suspected cases. The reported sensitivity of sputum induction varies widely [3–9], emphasising the importance of technical factors in the collection and processing of the sputum sample.

In a survey of major teaching hospitals in Australia and New Zealand, 75% of centres reported using physiotherapy techniques designed to clear mucus from the airways as part of their sputum induction procedure [10]. However, an electronic search of the Physiotherapy Evidence Database (PEDro), Medline, PubMed, Embase, Cumulative Index of Nursing and Allied Health Literature (CINAHL) and the Cochrane Library, using the search terms ''sputum induction'' and ''induced sputum'', identified no studies on the effect of physiotherapy techniques on the procedure.

A clinical trial was carried out, which aimed to determine whether airway clearance techniques alter the efficacy of the sputum induction procedure. The objectives of the present study were to examine whether adding airway clearance techniques to the sputum induction procedure would: 1) increase sample volume; 2) affect how well and for how long patients tolerate the procedure; 3) improve sample quality assessed by the presence of alveolar macrophages; 4) increase identification of the suspected organism; and 5) affect the sensitivity or specificity of the test.

MATERIALS AND METHODS

Participants

The study was conducted at Royal Prince Alfred Hospital, Sydney, and Princess Alexandra Hospital, Brisbane (both Australia), and was approved by the relevant ethics committees.

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Participants were recruited from inpatients and outpatients referred for sputum induction for suspected pulmonary infection with *Mycobacterium tuberculosis* or *Pneumocystis jiroveci*.

Pneumocystis organisms from different host species have been shown to be multiple species with very different DNA sequences. In recognition of this, the organism that causes human *Pneumocystis* pneumonia is now named *Pneumocystis jiroveci*. The acronym PCP is retained, referring to *Pneumocystis* pneumonia. *P. carinii* now refers exclusively to one of the two *Pneumocystis* spp. found only in rats [11].

In total, 59 participants completed the trial (table 1). Participants were excluded if they were aged <18 yrs or were unable to provide informed consent. Of the participants: 36 (61%) were suspected of *M. tuberculosis* infection only; 10 (17%) were suspected of *P. jiroveci* infection only; and 13 (22%) were suspected of infection with either organism. In total, 33 (56%) had a nonproductive cough and 26 (44%) had produced a spontaneous sputum sample that had been negative for the suspected organism(s).

Study design

A randomised, crossover design was used. Participants attended on 2 days within a 5-day period, undergoing a standard sputum induction procedure on each day. This involved the inhalation of 3% saline *via* an ultrasonic nebuliser for as long as tolerated, for up to 15 min. On one of the days (physio), airway clearance techniques were used during saline delivery. These included manual percussion and vibration of the chest wall during deep breathing and huffing and coughing, interspersed with periods of relaxed diaphragmatic breathing. On the other day (control), participants were advised to breathe slightly more deeply than usual, to occasionally take two to three deep breaths, and to cough at least every 5 min and whenever they felt secretions in their airways. The computer-generated randomisation list was sealed in opaque numbered envelopes by a person not otherwise involved in the study to ensure concealment.

Outcome measures

The severity of cough, dyspnoea, wheeze and chest tightness was recorded by the participant on a visual analogue scale (VAS) before and immediately after the test. The volume of expectorate and the duration of the test were recorded by the investigator immediately after the procedure. The sample was examined for the presence of alveolar macrophages by the cytologist and then microbiological testing was carried out by microbiological scientists for the suspected organism(s). The cytologist and scientists were blinded to the randomisation order. To test for the presence of alveolar macrophages, four slides were smeared with sputum and fixed in 95% ethanol. They were then stained using the Papanicolaou method and screened. If five or more alveolar macrophages were found, the specimen was deemed to contain alveolar macrophages. PCR assays were used for the identification of *P. jiroveci*, and standard smear and culture for *M. tuberculosis* were performed.

TABLE 1 Characteristics of the 59 study participants

Age yrs	48 (19–81)
Admission status inpatient:outpatient	43:16
Male:female	38:21
HIV positive:negative	21:47

Data are presented as mean (range) or ratios.

Statistical analyses

Data were analysed by intention to treat. Differences in volume of expectorate, symptom severity and test duration were assessed using the paired t-test or, where not normally distributed, the Wilcoxon signed-rank test. The presence of alveolar macrophages and identification of the suspected organism were compared using McNemar's test.

The sensitivity of the sputum induction procedure was calculated as the probability of a positive test among participants with the infection according to a reference standard. The specificity of the procedure was calculated as the probability of a negative test among participants without the infection according to the same reference standard. The following reference standards were used.

For TB two of the following three criteria were required: 1) clinical symptoms suggesting TB (*e.g.* cough, fever, night sweats); 2) acid-fast bacilli visible on smear by Ziehl-Nielsen staining or *M. tuberculosis* cultured from sputum (not including those samples used in the study), bronchoalveolar lavage (BAL) fluid or biopsy samples; and 3) a chest radiograph independently interpreted as highly suggestive of TB. For patients without microbiological confirmation, a clinical and/or radiological response to anti-TB medications was also required.

This definition was used in the trial by FITZGERALD *et al*. [12], and is based on the definition of the American Thoracic Society's diagnostic standards for TB [13].

For PCP a positive BAL or other lung sample (*e.g.* lung biopsy/autopsy) was required. In the absence of any such samples having been tested, two of the following three criteria were required: 1) clinical symptoms suggesting PCP (*e.g.* cough, fever, dyspnoea); 2) bilateral interstitial infiltrates and/or progression to alveolar pattern on chest radiography; and 3) response to anti-PCP therapy.

A respiratory physician determined whether participants met these definitions based on a review of the participant's medical record 6 months after sample collection. The physician was blinded to the test results of the samples obtained at the two study visits. The extended McNemar's test [14] was used to compare the difference in the sensitivities or specificities of the two test conditions.

Sample size calculation

Sample size calculation was based on the outcome of sample quality, assessed by the presence of alveolar macrophages. To determine the required sample size, data from previous sputum induction procedures at Royal Prince Alfred Hospital, where airway clearance techniques were incorporated, was used. The rate at which adequate quality samples were obtained with sputum induction was 92%. If omitting airway clearance techniques reduced this proportion to 60%,

this would be adequate justification for the continued use of physiotherapy as part of the procedure. With a power of 80% and $\alpha=0.05$, the current authors calculated that 50 patients (25 per group) would be required to complete this aspect of the study [15].

RESULTS

Tolerance of the test

Table 2 shows the comparability of participants' ratings of symptoms on the VAS before commencing the sputum induction procedure on each of the 2 study days.

The inclusion of airway clearance techniques did not significantly affect the change in symptoms that occurred during the sputum induction procedure: cough p=0.31; dyspnoea p=0.96; wheeze p=0.46; chest tightness p=0.57 (fig. 1).

There was no significant difference in how long the test was tolerated on the 2 days, p=0.98 (fig. 2). The volume of expectorate produced on the 2 days was not significantly different, p=0.42 (fig. 3). Two participants were nonproductive on both days.

Sample quality

Samples from the first 49 consecutive participants at the Royal Prince Alfred Hospital were assessed for the presence of alveolar macrophages. McNemar's test showed that the airway clearance techniques did not significantly affect how often the test achieved a sample with alveolar macrophages, p=0.11 (difference 0.12 in favour of control; 95% confidence interval (CI) -0.01–0.25; table 3).

TABLE 2 Baseline visual analogue scale scores (0–100 mm) of symptoms on each study day

	Physio. day	Control day
Cough	3 (0–60)	10 (0–64)
Dyspnoea	4 (0–66)	1 (0–66)
Wheeze	1 (0–47)	0 (0–48)
Chest tightness	2 (0–50)	0 (0–45)

Data are presented as median (95% range). Physio.: physiotherapy.

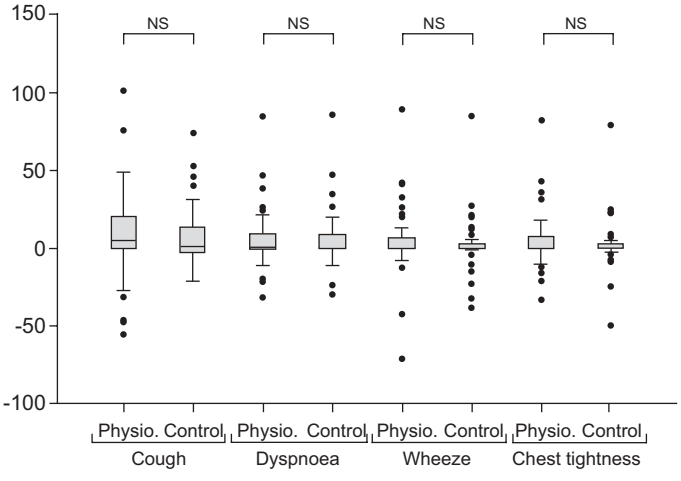

FIGURE 1. Box plots to show the change in cough, dyspnoea, wheeze and chest tightness during the sputum induction procedure with (physio.) and without (control) airway clearance techniques incorporated between the 10th and 90th percentiles. ●: outlier. Physio.: physiotherapy; NS: nonsignificant. n=48.

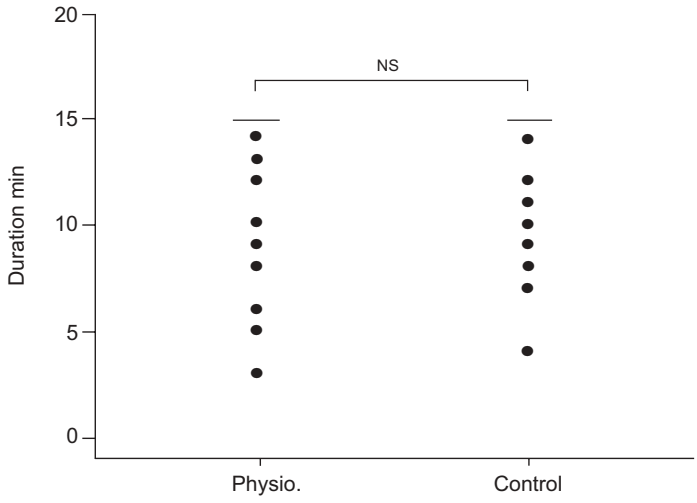

FIGURE 2. Mean duration for which the test was tolerated. ——: median containing interquartile range; ●: outlier. Physio.: physiotherapy; NS: nonsignificant. n=59.

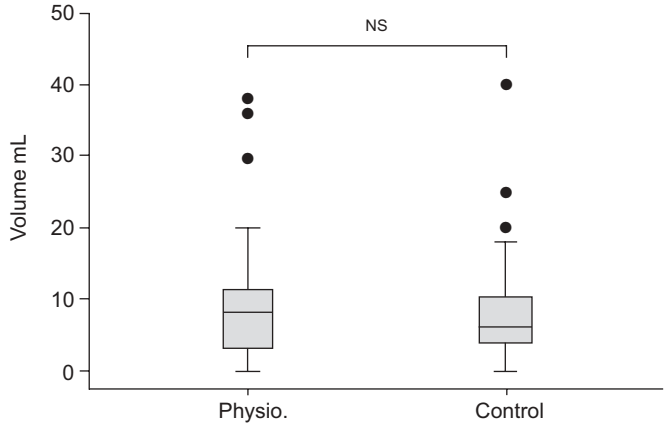

FIGURE 3. Box plots showing volume of expectorate produced during the test between the 10th and 90th percentile. ●: outlier. Physio.: physiotherapy; NS: nonsignificant. n=59.

Presence of suspected organisms

The 57 productive participants had their samples assessed for the presence of the suspected organism(s). McNemar's test showed that the airway clearance techniques did not significantly affect how often the test identified a suspected organism on microbiological testing, p=0.45 (difference 0.05 in favour of physio.; 95% CI -0.04–0.14; table 4).

Sensitivity and specificity

After application of the reference standards, the sensitivity of the procedure with airway clearance techniques was 57% and without was 50%. This 7% difference in the sensitivities of the

TABLE 3 Quality of sample determined by the presence of alveolar macrophages on cytology[#]

	Control		
	Alv mac -	Alv mac +	Total
Physio.			
Alv mac -	4	8	12
Alv mac +	2	35	37
Total	6	43	49

Alv mac -: alveolar macrophages not present; Alv mac +: alveolar macrophages present; Physio.: physiotherapy. [#]: McNemar's test p=0.11; n=49.

TABLE 4 Successful identification of a suspected organism on microbiological testing[#]

	Control		
	Sus org-	Sus org +	Total
Physio.			
Sus org -	43	2	45
Sus org +	5	7	12
Total	48	9	57

Sus org -: suspected organism not present; Sus org +: suspected organism present; Physio.: physiotherapy. [#]: McNemar's test p=0.45; n=57.

two test conditions was nonsignificant (95% CI -24–38%). The specificity of the procedure with airway clearance techniques was 94% and without was 100%, which was also nonsignificant (95% CI -3–15%).

DISCUSSION

The present study found that the identification of a suspected organism with sputum induction was not significantly altered by the inclusion of airway clearance techniques. In addition, airway clearance techniques did not significantly alter factors which might be expected to improve the efficacy of the diagnostic test, such as the likelihood of obtaining a sample.

As an indication of baseline comparability, the participants' symptoms before the commencement of the sputum induction procedure were reasonably consistent across the 2 study days, with differences in median VAS scores of $\leqslant$7 mm on a 100-mm scale. These differences were accounted for by examining change in VAS scores.

There was little support for the mechanisms by which airway clearance techniques might improve the efficacy of the sputum induction procedure. The techniques did not significantly affect the test duration or the volume of expectorate. The CI for sample quality excluded the possibility that airway clearance techniques make a clinically worthwhile impact on the likelihood of obtaining a sample with alveolar macrophages.

Airway clearance techniques did not significantly affect the change in symptoms that occurred during the sputum induction procedure. Apart from a few outliers, most participants reported little change in symptoms with either test.

The identification of the suspected organism was not significantly altered by the inclusion of airway clearance techniques. A clinically important effect size was not prespecified, but the authors acknowledge that the CI did not completely exclude the possibility of a clinically important benefit from airway clearance techniques.

The difference in sensitivities of the tests was 7% better with airway clearance techniques. The CI here was wide enough to contain clinically important effects in either direction. Further research in populations with a higher prevalence of *M. tuberculosis* and *P. jiroveci* infection may help determine any effect on sensitivity. The difference in specificities of the test was 6% better without airway clearance techniques. The narrow CI indicates that airway clearance techniques are unlikely to improve the specificity of the procedure to a clinically important extent.

Airway clearance techniques have been shown to accelerate mucus clearance in diseases such as cystic fibrosis [16] and bronchiectasis [17], where there is substantial retention of mucus with abnormal rheological properties. Accordingly, airway clearance techniques are designed to dislodge sticky mucus and use airflow to move it more centrally, from where it may be eliminated with cough or forced expiratory techniques. However, sputum samples induced with hypertonic saline are of small volume and have more favourable rheology for clearance [18], which may render the airway clearance techniques unnecessary. Some centres report using 7, 10 and even 20% saline for the procedure [10]. Higher saline concentrations cause a greater improvement in mucus rheology [19], so airway clearance techniques are unlikely to be more effective in such protocols.

In conclusion, airway clearance techniques did not significantly improve the efficacy of the sputum induction procedure in the present study. Some confidence intervals contained differences that could be considered clinically important. Therefore, the results of this study do not completely exclude the possibility that airway clearance techniques may be a clinically useful adjunct to the procedure. However, the lack of evidence for a mechanism by which airway clearance techniques might improve the efficacy of the procedure does not support this scenario. Further studies are required to definitively determine the effect of airway clearance techniques on some aspects of the test, particularly sensitivity. In the interim, the current results provide some justification for not including, or for discontinuing the use of, airway clearance techniques as part of the sputum induction procedure.

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