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Use of Induced Sputum Specimens for Microbiologic Diagnosis of Infections Due to Organisms Other than *Pneumocystis carinii*

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The optimal diagnostic approach to pneumonia provides a rapid microbiologic identification of pulmonary pathogens by the least invasive means. The technique of sputum induction has been useful in the evaluation of patients with *Pneumocystis carinii* or mycobacterial pneumonia. It is not known whether induced sputum samples are preferable for the detection of pathogens other than *P. carinii* or mycobacteria. Microbiologic yields were evaluated from identically processed induced and conventional sputum samples collected from 509 consecutive patients. No statistically significant differences were found between the microbiologic yields of induced and spontaneous sputum samples. Bacterial pathogens were isolated in 19.6% of induced and 23.5% of routine specimens. Mycobacteria were cultured from 13.1% of routine and 9.4% of induced specimens. Non-*Candida albicans* fungi grew from 24% of routine and 20% of induced specimens. The process of sputum induction with aerosolized hypertonic saline did not alter either the purulence or the bacterial quantitation of Gram-stained sputum specimens. Sputum induction has been useful for the cytologic diagnosis of malignancy, for the diagnosis of pneumocystosis and tuberculosis, and in patients unable to spontaneously produce sputum samples. The use of induced sputum samples for the diagnosis of other infections may not be necessary when routine sputum specimens are available.

Rapid and accurate microbiologic identification of organisms causing pneumonia is essential to successful treatment. Techniques for the induction of sputum specimens by means of instilled or inhaled hypertonic saline have been used in the evaluation of pulmonary disease for over 30 years (3, 6, 15, 19). The earliest uses of sputum induction were in the cytologic diagnosis of lung cancer (3) and in the diagnosis of tuberculosis (8, 10, 22). In these applications, diagnoses achieved by examination of induced sputum specimens reduced the need for invasive diagnostic procedures. Studies comparing the diagnostic yields of mycobacterial cultures of induced sputum samples with those of conventional expectorated sputa or of gastric aspirates did not demonstrate an advantage to any particular method (8, 10, 19, 22). With the incidence of *Pneumocystis carinii* pneumonia observed in individuals with AIDS, sputum induction was coupled to fluorescent antibody staining to enhance the detection of *P. carinii* in AIDS patients and, subsequently, in non-AIDS immunocompromised patients (7, 12, 13, 16, 18, 20). The successful utilization of sputum induction for the diagnosis of *P. carinii* has lead to the routine use of this method for a broader range of potential pulmonary pathogens in both normal and immunodeficient individuals. However, the use of induced sputum specimens for microbiologic diagnoses other than *P. carinii* has not been compared with data obtained with routine expectorated samples from the same patients. The effect of the process of sputum induction itself (e.g., hypertonic saline) on the microbiologic yields of either the induced sputum samples or on subsequent expectorated specimens has also not been addressed. It is also uncertain whether the added expense of the sputum induction procedure is justified for patients in whom the diagnosis of *Pneumocystis* pneumonia is considered unlikely. This study was initiated to compare the microbiologic yields from induced and conventional sputum samples.

MATERIALS AND METHODS

Microbiologic data were recorded according to a standardized protocol for all induced sputum specimens collected between October 1988 and September 1989 at the Massachusetts General Hospital. Results were tabulated without regard to the patients' age, sex, admitting diagnosis, human immunodeficiency virus or immune status, length of hospitalization, or therapeutic regimen. Microbiologic data on expectorated sputum samples collected within 2 days prior to or 2 days after the induced samples were also tabulated. The use of routine samples within 2 days of sputum induction was chosen to minimize the chance that either the acquisition of nosocomial flora or administration of an antibiotic would alter the organisms detected on a Gram-stained smear or by culture.

Induced specimens were obtained according to standard protocol by one of two respiratory therapists. The subjects were hydrated orally (one to two glasses of water unless contraindicated) prior to induction. Patients inhaled nebulized 3% saline for 20 min from a DeVilbiss Ultra-Neb 99 ultrasonic nebulizer in a closed system via a mouthpiece. Patients were encouraged to cough every 5 min, occasionally with gentle chest percussion. Albuterol inhaler (two 100-μg puffs) was administered once to bronchospastic subjects. Specimens were collected in sterile containers and transported immediately to the microbiology laboratory of the

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TABLE 1. Comparison of microbiologic yield by method of collection

Collection method	No. of specimens (%)						
	Aerobic and facultative bacteria		Mycobacteria		Fungi		
	Cultured	Positive[a]	Cultured	Positive[b]	Cultured	Positive[b]	Positive for non-*C. albicans*[c]
Induction	153	30 (19.6)	107	10 (9.4)	25	20 (80)	5 (20.0)
Routine	153	39 (23.5)	107	14 (13.1)	25	19 (76)	6 (24.0)

[a] 2+ to 4+ growth of potential pathogens by culture. All specimens were cultured regardless of Gram-stain results. *P* value was calculated by two-sided *t* test. $P > 0.05$ (not significant).
[b] Any amount of growth. $P > 0.05$ (not significant).
[c] Significance was not evaluable, given the number of subjects.

hospital, where they were processed as requested by the attending physician.

Induced and routine sputum samples were Gram stained when requested, and all specimens were cultured for bacteria by using Brucella with 5% horse blood and MacConkey agar plates for recovery of aerobic and facultative organisms. Following incubation at 35°C in 5% CO_2, the plates were examined at 24 and 48 h and all potentially pathogenic bacteria were identified by previously described methods (2). For the purposes of this study, bacterial growth present in greater than rare amounts (2+ to 4) was tabulated (Tables 1 and 2). Isolated organisms were considered significant in relation to ongoing pneumonitis when moderate or abundant growth (3+ to 4+) of a single organism occurred in a culture from a sample containing ≥4 polymorphonuclear leukocytes per oil immersion field (magnification, ×1,000) by examination of Gram-stained smears (based on the criteria of Bartlett et al. [4]). High-power (×1,000) microscopic evaluation of Gram-stained sputum specimens allows the assessment of the cellular composition and of the predominant bacterial forms in and around inflammatory cells. Growth of coagulase-negative staphylococci, enterococci, nonpneumococcal alpha-hemolytic streptococci, and nonhemolytic streptococci was excluded except in the presence of purulent sputum in the absence of the detection of other pathogens by culture.

Fungal culturing of induced and routine specimens was performed by the inoculation of Sabouraud-Emmonds, inhibitory-mold, and brain heart infusion III agars (DiMed Co., St. Paul, Minn.). Following incubation at 30°C, the plates were examined for growth every 48 h for a maximum of 4 weeks. Yeast and filamentous fungi were identified as described elsewhere (2). Any amount of growth of fungus was included as a positive culture result.

Mycobacterial culturing of induced and routine specimens was performed by using Middlebrook 7H-11, Lowenstein-Jensen, and Mycobactosel agar slants (Becton Dickinson, Cockeysville, Md.). Following incubation at 37°C, specimens were examined for growth at weekly intervals for 8 weeks. All mycobacterial species recovered were identified as described elsewhere (11), and any amount of growth was included as a positive culture result.

Induced sputum specimens were examined for the presence of *P. carinii* with fluorescent antibody stain (Genetic Systems, Sanofi Diagnostics Pasteur, Inc., Chaska, Minn.) as described elsewhere (20). Routine, expectorated samples were not processed for *P. carinii*.

For the purposes of this study, evaluable data were limited to those culture results for individual patients for whom both routine and induced samples were obtained and were cultured for the same pathogens (i.e., bacteria, fungi, or mycobacteria). Statistical analysis of data was performed by a two-sided *t* test with SysStat Inc. (Evanston, Ill.) software.

TABLE 2. Distribution of organisms cultured from sputum specimens by method of collection

Organism type and species	No. (%)[a] of isolates positive	
	Induced	Routine
Aerobic and facultative bacteria[b]	(*n* = 69)	(*n* = 80)
S. aureus	17 (24.6)	18 (22.2)
P. aeruginosa	15 (21.7)	14 (17.3)
K. pneumoniae	9 (13.0)	11 (13.6)
H. influenzae	4 (5.8)	4 (4.9)
Enterobacter cloacae	3 (4.4)	4 (4.9)
β-*Streptococcus* (group G)	3 (4.4)	3 (3.7)
S. pneumoniae	2 (3.0)	5 (6.2)
Escherichia coli	2 (3.0)	8 (5.5)
β-*Streptococcus* (*Streptococcus milleri*)	2 (3.0)	3 (3.7)
H. parainfluenzae	2 (3.0)	2 (2.5)
X. maltophilia	2 (3.0)	2 (2.5)
M. catarrhalis	0 (0)	4 (4.9)
β-*Streptococcus* (group A)	0 (0)	2 (2.5)
Neisseria meningitidis	0 (0)	2 (2.5)
Mycobacteria[c]	(*n* = 11)	(*n* = 14)
M. avium-M. intracellulare complex	5 (45.5)	8 (57.1)
M. tuberculosis	5 (45.5)	6 (42.9)
Mycobacterium gastri	1 (9.1)	0 (0)
Fungi	(*n* = 24)	(*n* = 28)
C. albicans	19 (76)	18 (72)
Penicillium species	2 (8)	0 (0)
Candida pseudotropicalis	2 (8)	2 (8)

[a] Numbers in parentheses represent the percentage of the total number of isolates demonstrated for each organism type.
[b] Organisms listed are the most frequent isolates from each sampling method (2+ to 4+ growth) from patients with purulent sputum by Gram stain.
[c] Any amount of growth.

RESULTS

Data were collected for 509 consecutive patients who had undergone sputum induction. Of these, both induced and routine sputum samples were processed for routine bacterial cultures for 153 patients, for mycobacteria for 107 patients, and for fungi for 25 patients. The microbiologic yields from

the induced and routine sputum samples from each individual were compared and are presented in Table 1. Thirty-nine (23.5%) routine and 30 induced (19.6%) samples of the 153 evaluable pairs of specimens demonstrated diagnostic bacterial growth. Mycobacteria were cultured from 13.1% of routine and 9.4% of induced specimens. For eight patients, mycobacteria were isolated in routine sputa and not in induced specimens from the same patients. For four patients, induction provided a mycobacterial diagnosis not provided by a routine specimen. Fungi other than *Candida albicans* were cultured from 24% of routine and 20% of induced specimens. None of these differences were statistically significant (i.e., $P > 0.05$).

The distribution of potential pathogens cultured from these sputum specimens is shown in Table 2. *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were the three most frequently isolated organisms and represented over half of the total isolates from cultures of sputum obtained by either method. Among pathogens commonly involved in community-acquired pneumonias, *Haemophilus influenzae* comprised 4.9% of the positive cultures of the induced specimens and 5.8% of the positive routine sputa. *Streptococcus pneumoniae* occurred in 6.2% of the routine and 3.0% of the induced specimens. None of these differences reached statistical significance.

A comparison of the diagnostic yields for purulent sputa obtained by routine expectoration or induction also showed no advantage to either method. Cultures of induced sputum specimens identified a pathogenic organism for 17 patients (of 139 total, 12.2%) whose expectorated specimens were nondiagnostic (13 obtained after and 4 obtained before the routine sample). Routine expectorated samples also provided microbiologic diagnoses for 17 (of 139) patients (11 obtained before and 6 obtained after the induced specimen) when an induced specimen failed to provide a diagnostic culture result. Routine expectorated sputum samples obtained after sputum induction produced a better total microbiologic yield (50 versus 33%, $P < 0.01$) than routine specimens obtained prior to induction. Additional diagnoses were obtained in 25.1% of patients by virtue of having both routine and induced sputum samples processed.

The process of sputum induction itself did not alter the microscopic detection of purulence in Gram-stained sputum smears compared with smears from routine, expectorated specimens obtained from the same patients. Of 127 patients for whom Gram stains were performed by laboratory personnel on both types of specimens, the number of polymorphonuclear leukocytes was equal in 25, greater by induction in 51, and greater in the routine specimen in 51.

Bacterial pathogens were frequently (22 of 42, 52%) isolated when induced sputum specimens also contained *P. carinii*: *Mycobacterium avium-Mycobacterium intracellulare* complex (4), *P. aeruginosa* (3), *S. aureus* (3), *S. pneumoniae* (2), *H. influenzae* (2), *Haemophilus parainfluenzae* (2), *K. pneumoniae* (2) and *Mycobacterium catarrhalis*, *Xanthomonas maltophilia*, *Mycobacterium scrofulaceum*, and β-*Streptococcus* group G (one each). *P. carinii* was present in 42 (14%) of the induced samples evaluated. Secondary isolates were also present in patients with *Mycobacterium tuberculosis*: *Enterobacter aerogenes* (2), *H. influenzae*, *S. aureus*, and *K. pneumoniae* (1 each). *M. avium-M. intracellulare* was cultured in association with *K. pneumoniae*, *X. maltophilia*, and an *Alternaria* species (one patient each).

DISCUSSION

The prompt and accurate identification of pathogens causing pneumonia has many implications for medical care, including the optimization of antibiotic therapy and a potential shortening of the duration of hospitalization. Noninvasive identification of pulmonary pathogens may make more invasive procedures unnecessary. Sputum induction has gained widespread use in many hospitals, especially those providing care to immunocompromised patients. In an attempt to optimize the diagnostic yield, the use of sputum induction has been extended to the assessment of non-AIDS patients. However, the procedure requires specialized technical personnel and some additional cost. There are few data on the relative efficacy of cultures from induced and routine sputum specimens.

In this series, the microbiologic yields of most routine bacterial pulmonary pathogens from cultures of specimens obtained by sputum induction and expectoration are equal. In a quarter of the patients, the cultures of both routine and induced sputum samples provided an additional bacteriologic diagnosis. This may reflect the advantage of examining multiple sputum samples regardless of the technique. The induction protocol did not alter the purulence of Gram-stained specimens or the growth of common pathogens. The most commonly isolated bacteria (*S. aureus*, *K. pneumoniae*, and *P. aeruginosa*) reflect the frequency of nosocomially acquired pulmonary infections in a large, tertiary-care referral center. Common pathogens of community-acquired pneumonia (e.g., *H. influenzae* and *S. pneumoniae*) are probably underrepresented in the group of patients undergoing sputum induction. This may reflect the ease of diagnosis of these infections by sputum Gram stain or the rapid response to common empiric antimicrobial therapies. The isolation of *S. pneumoniae* was reduced from patients receiving hypertonic saline aerosol induction compared with those providing routine specimens; this difference did not achieve statistical significance.

The efficient diagnosis of mycobacterial infection is of increased importance in light of the growing incidence of antibiotic-resistant tuberculosis and of *M. avium-M. intracellulare* complex infections (17). Early studies of sputum induction for the diagnosis of tuberculosis showed a benefit to patients unable to produce sputum spontaneously. No diagnostic benefit was observed for comparisons of cultures following sputum induction and routine expectorated specimens (8, 10, 22). In this study, the yields of routine and induced specimens for mycobacterial diagnoses were equivalent. In 12 of 25 patients, however, mycobacteria were detected in cultures of sputa obtained by one or the other method but not by both. This result probably reflects the advantage of examining multiple sputum samples for mycobacterial isolation rather than an additive benefit of multiple technologies (5, 14).

Fungal cultures are frequently contaminated by colonizing flora from the upper respiratory tree. *C. albicans* represented approximately 60% of all fungal isolates in this study. The clinical significance of many of the fungal isolates is difficult to assess (1, 21). Sputum induction provided a fungal isolate for only one patient not diagnosed by the routine specimen. In this small group of patients, sputum induction did not provide an advantage for the diagnosis of non-*Candida*, fungal pneumonia.

Sputum induction has been useful for the cytologic diagnosis of malignancy and for mycobacterial diagnosis in patients unable to produce sputum spontaneously. The use

of sputum induction for the routine identification of bacterial pathogens does not appear to be justified. The microbiologic yield from routine specimens was enhanced by prior induction, i.e., hydration and inhalation of saline. It is, therefore, likely that gentle hydration would improve the quality of many routine specimens. Variability in sputum induction techniques between technicians and institutions alters the yields for *P. carinii* (7, 12, 13, 16, 18, 20) and may do so for other pathogens as well. Similarly, the degree of hypertonicity of saline, the duration of inhalation, the use of glycerin or of mucolytic agents, and the type of vaporizer used in the procedure may also impact the viability and growth of some organisms and/or the preservation of neutrophils on stained smears. However, in this study, neither growth nor purulence was altered by sputum induction. The judicious use of sputum induction and the assessment of the value of the technique at each institution will enhance microbiologic diagnosis while minimizing unneeded expenditures on diagnostic procedures.

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