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Sputum Screening by Nomarski Interference Contrast Microscopy

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Gram-stained smears of specimens submitted for sputum cultures were compared with direct wet mounts examined by Nomarski interference contrast microscopy (NIM) for enumeration of squamous epithelial cells (EPC) and leukocytes (WBC). The results obtained by the two methods were comparable, but specimens were more rapidly screened and cell types were more readily differentiated by NIM. Specimens submitted for sputum culture over a 3-month period were examined for EPC and WBC by NIM. Twenty-two percent of the specimens had >25 EPC/field or a predominance of EPC (class I), 30% had >25 EPC and >25 WBC/field (class II), and 48% had >25 WBC/field or a predominance of WBC (class III). The clinical relevance of the culture results was determined by reviewing the records of patients whose specimens were included in the study. Class I specimens provided only 30% clinically relevant culture results. Specimens in class II provided useful culture results in 63% of the patients, and 96% of those in class III provided clinically relevant information. The results confirm the value of sputum screening and demonstrate that NIM provides a rapid, simple, and accurate method for sputum screening.

There are an estimated two million cases of pneumonia per year in the United States (9), and, despite modern diagnostic methods and effective antimicrobial therapy, pneumonia remains a leading cause of death. A factor that may contribute to the continuing morbidity and mortality of pneumonia is the frequently poor quality of expectorated sputum specimens received for culture in clinical microbiology laboratories. For example, *Streptococcus pneumoniae*, which is the leading cause of bacterial pneumonia, may be recovered in only 50% of expectorated sputum specimens from patients with documented pneumococcal pneumonia (1).

Microscopic screening of sputum specimens can improve the reliability of culture results (2, 3, 5, 8, 10). Geckler and co-workers (5) have confirmed the value of sputum screening by their demonstration that expectorated sputum specimens, judged to contain lower respiratory tract secretions, gave culture results comparable to specimens obtained by transtracheal aspiration. Gram-stained smears of sputum specimens are normally employed for screening, but Gram staining is time-consuming and cells are often distorted and difficult to identify. In the present study, we evaluated the use of direct wet mounts examined by Nomarski interference contrast microscopy (NIM) for sputum screening.

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MATERIALS AND METHODS

The study included specimens submitted over a 3-month period to the Clinical Microbiology Laboratory of the Veterans Administration Hospital for routine sputum culture. Single expectorated specimens were collected under the supervision of nursing personnel and transported to the laboratory immediately or after not more than 1 h of refrigeration. The specimens were processed for culture immediately upon receipt in the laboratory. Microscopy was generally done at the same time as the culture. When microscopy could not be done immediately, the specimens were refrigerated for not more than 4 h after collection. Refrigeration for this period of time did not change the cellular composition of the sputum. All specimens, except those that could not be examined within 4 h after collection, were included in the study.

The most purulent mucoid or blood-flecked portion of each specimen was carefully selected for culture and microscopy. Direct wet mounts were made by placing a loopful of the selected portion of the specimen on a slide, adding a cover slip, and gently pressing down without allowing the specimen to go beyond the cover slip edges. Gram-stained smears were prepared from the same portions of the specimens. The sputa were cultured on 5% sheep blood agar, colistin-nalidixic acid agar, MacConkey agar, and chocolate agar. Organisms were enumerated semi-quantitatively on streak plate cultures and identified by established procedures.

The direct mounts were examined by NIM (Olympus model BHA), and the Gram-stained smears were observed under normal bright-field optics. All of the microscopic observations were made by the same individual (D.F.W.). In each case the entire slide was first scanned at ×125 magnification to determine the distribution of cell types. Leukocytes (WBC) and buccal epithelial cells (EPC) were then counted in 10 representative fields, and the average number of each cell type was determined.

Medical records of the patients whose specimens were included in the study were reviewed to determine the clinical correlation of sputum culture results with respect to the quality of specimen provided. Clinical and laboratory evidence of lower-respiratory-tract infection included radiographic findings of pneumonia, elevated WBC count, fever, increased sputum production, and positive blood cultures. Evidence against lower-respiratory-tract infection included absence of the above findings and subsequent diagnosis of other noninfectious pulmonary diseases (e.g., neoplasms).

RESULTS

Comparison of screening by NIM and Gram stain. In initial studies, specimens were screened by both NIM and Gram stain to determine the comparability of the two methods. Cell counts with the two methods had 96% agreement. The 4% discrepancies encountered were due to more uniform distribution of cells on the wet mount compared with the smear and to easier recognition of cell types by NIM. NIM produces a three-dimensional image that makes recognition of EPC, WBC, and other cell types straightforward (Fig. 1). By NIM, WBC are smaller than EPC, and they appear spherical. Also, lysosomal granules and phagocytic vesicles are apparent on higher magnification. EPC are characteristically large and flat, with a small central nucleus that is readily apparent by NIM.

Classification of specimens. Specimens were initially graded by the scheme of Murray and Washington (10). However, several specimens contained fewer than 25 cells per field and thus did not fit into the Murray and Washington scheme. Two additional groups were established to accomodate these specimens (Table 1). Group 0 had a predominance of EPC, and group 6 had a predominance of WBC. To simplify this scheme, classes of sputum specimens were established (Table 1). Class I consisted of groups 0, 1, and 2, and it represented specimens having more than 25 EPC and/or a predominance of EPC. Class II included specimens of group 3 that had more than 25 of both EPC and WBC. Class III included groups 4, 5, and 6, which were those specimens having more than 25 WBC and/or a predominance of WBC.

Microbiology of sputum specimens. Normal upper respiratory flora was recovered in 96% of class I specimens but in only 86% of class II and 81% of class III specimens (Table 2). Thus, nearly 20% of class III specimens yielded only potential pathogens without normal flora. In general, a higher percentage of class II and III specimens yielded enteric bacteria and *Pseudomonas aeruginosa*. *S. pneumoniae* was isolated from a total of 30 specimens during the study, and group III specimens accounted for 50% of these isolates. More potential pathogens were isolated from specimens of classes II and III compared with class I. An average of 0.65 potential pathogen was recovered from class I specimens. Cultures of class II specimens had an average of 1.2 potentially pathogenic organisms, and class III specimens yielded an average of 1.0 potential pathogen per specimen.

Clinical correlation of culture results. To determine the clinical significance of the sputum culture results, hospital records of 110 patients included in the study were reviewed for evidence of lower-respiratory-tract infection. Clinically relevant culture results were defined as isolation of a potential pathogen in the presence of clinical evidence of infection or as the absence of a potential pathogen with no evidence of infection. Strikingly different results were obtained with sputum specimens of the three classes (Table 3). Class I specimens had 42% false-positive results and 28% false-negative results, providing clinically useful information from only 30% of the specimens. Sputa of class II provided 63% clinically relevant results. Specimens of class III provided 96% clinically relevant culture results, and no false-positive results were encountered with specimens of this class. Therefore, there are marked differences in the clinical validity of culture results from sputum specimens of the three different classes.

DISCUSSION

Accurate microbiological diagnosis of pneumonia depends upon culture of sputum that represents lower-respiratory-tract secretions. The best methods for obtaining such specimens are transtracheal aspiration (7) or lung biopsy (6). However, because of the invasive nature of these procedures, expectorated sputum is the specimen most often obtained. Microscopic screening of sputum specimens can be used to select for culture those specimens that represent lower-respiratory-tract secretions (5, 8, 10). Previous studies have used Gram-stained smears for such screening, but direct wet mounts, examined under oil immersion by standard light microscopy, have been used to differentiate eosinophilic and neutrophilic WBC in sputum (4). In the present study, we demonstrate that NIM

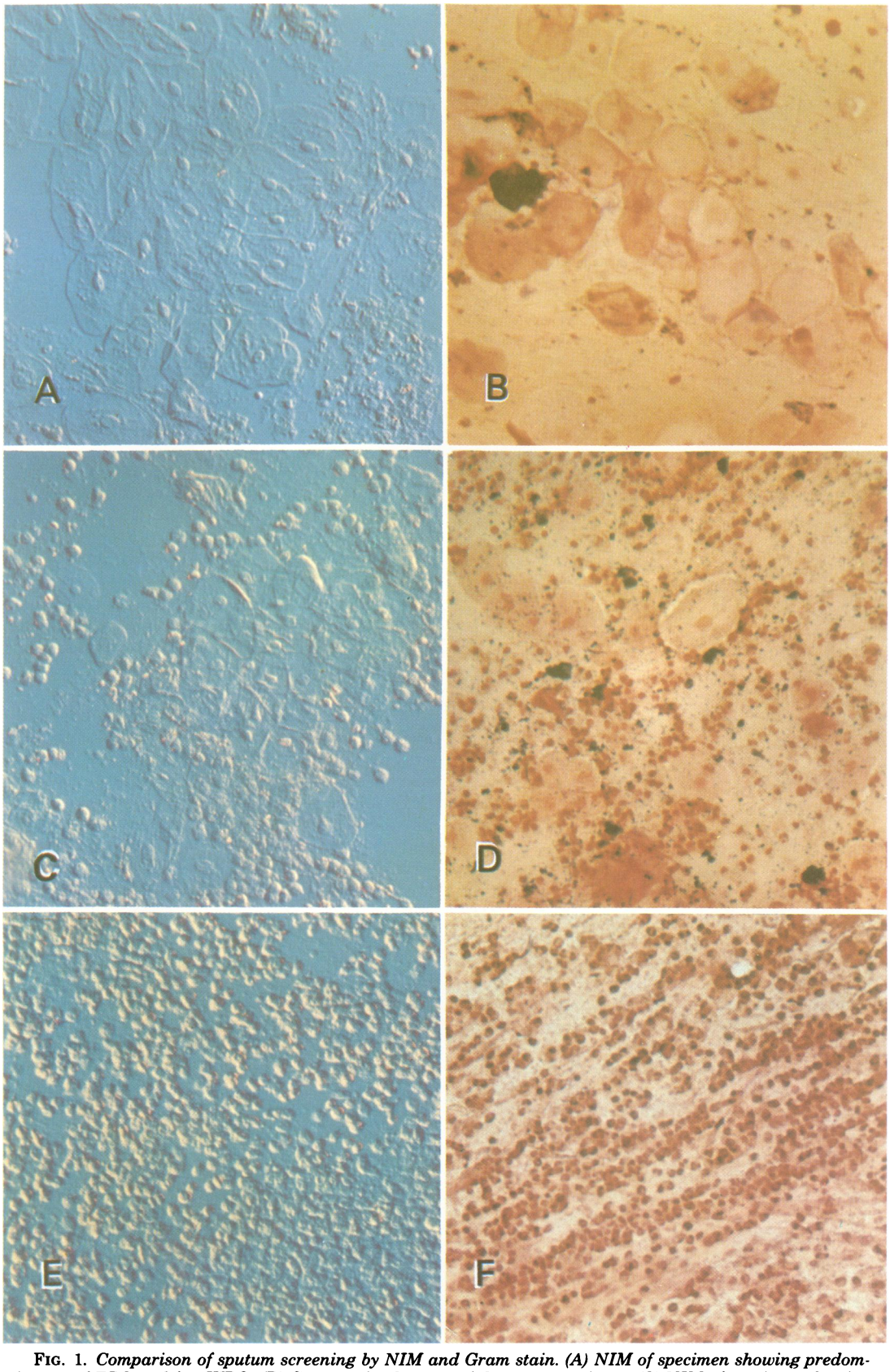

FIG. 1. *Comparison of sputum screening by NIM and Gram stain. (A) NIM of specimen showing predominance of EPC and few WBC; (B) Gram-stained smear of the same specimen; (C) NIM of specimen showing large numbers of both EPC and WBC; (D) Gram-stained smear of the same specimen; (E) NIM of specimen showing predominance of WBC and few EPC; (F) Gram-stained smear of the same specimen. ×125.*

TABLE 1. *Classification of sputum specimens*

Class	Group	EPC[a]	WBC[b]
I	0	<25 (EPC > WBC)	<25
	1	>25	<10
	2	>25	10–25
II	3	>25	>25
III	4	10–25	>25
	5	<10	>25
	6	<25	<25 (WBC > EPC)

[a] Number of EPC per ×125 field (average of 10 fields).

[b] Number of WBC per ×125 field (average of 10 fields).

TABLE 2. *Microbiology of sputum specimens*

Culture result	Sputum class (%)[a]		
	I	II	III
Normal throat flora[b]	96	86	81
Streptococcus pneumoniae	7	8	15
Streptococcus pyogenes	2	10	2
Staphylococcus aureus	11	23	11
Haemophilus influenzae	11	12	12
Escherichia coli	7	22	9
Klebsiella pneumoniae	2	9	9
Enterobacter aerogenes	4	1	4
Proteus mirabilis	2	3	3
Pseudomonas aeruginosa	6	1	11
Candida albicans	12	19	13
Other	4	6	10

[a] Percentage of cultures of each sputum class that yielded the indicated organism. Sputum classes are defined as follows: I, >25 EPC or predominance of EPC (57 specimens); II, >25 EPC and >25 WBC (77 specimens); III, >25 WBC or predominance of WBC (122 specimens).

[b] Alpha-hemolytic streptococci, diphtheroids, *Neisseria* sp., nonhemolytic streptococci, *Staphylococcus epidermidis*, *Hemophilus* sp., and yeasts.

provides a rapid and accurate method for screening wet mounts of sputum. Examination of wet mounts has the additional advantage that Quellung reactions can be performed, where indicated, by the addition of antiserum. Also, under ×450 or ×1,000 magnification, erythrocytes, pulmonary macrophages, ciliated epithelial cells, fungi, parasites (e.g., *Trichomonas tenax*), and bacteria can be observed. These observations are often difficult to make on Gram-stained preparations.

We found that sputum specimens could be divided into three classes based on the relative numbers of EPC and WBC in the specimens, and the culture results varied with the sputum class. Our results are similar to those of Van Scoy (11) and Murray and Washington (10), which suggest that sputum specimens with a predominance of EPC (class I) have an average of less than one potential pathogen, and specimens with many WBC or a predominance of WBC (classes II and III) average about one potential pathogen per culture. As suggested by Van Scoy (11), these results indicate that class I specimens should be rejected as upper respiratory in origin, and only class II and III specimens should be cultured. This conclusion is further substantiated by our observations that 70% of cultures of class I sputum specimens fail to provide clinically relevant results, but most specimens of classes II and III do provide useful results. These findings support the concept that sputum specimens containing mostly EPC do not provide valid results and that only specimens with many WBC or a predominance of WBC should be cultured.

TABLE 3. *Clinical correlation of sputum culture results*

Sputum class[a]	Clinical correlation[b]				Clinically valid results (%)[c]
	True positive	True negative	False positive	False negative	
I	15	15	42	28	30
II	49	14	19	18	63
III	83	13	0	4	96

[a] Definitions of sputum classes: I, >25 EPC or predominance of EPC (26 specimens); II, >25 EPC and >25 WBC (37 specimens); III, >25 WBC or predominance of WBC (47 specimens).

[b] Percentage of specimens from each class that provided: true-positive results, potential pathogen isolated and evidence of lower-respiratory tract infection; true-negative results, no potential pathogen isolated and no evidence of infection; false-positive results, potential pathogen isolated and no evidence of infection; or false-negative results, no potential pathogen isolated and evidence of infection.

[c] Percent true-positive results plus percent true-negative results.

The results of this study demonstrate that microscopic sputum screening is a valuable procedure that can significantly enhance the validity of sputum culture results. Screening of sputa by NIM provides several advantages over the examination of Gram-stained smears, and Nomarski optics can be added to most microscopes at relatively modest cost. Microscopes with Nomarski optics can also be used for routine microscopy and, therefore, need not be dedicated to sputum screening. In addition, NIM is very useful in other areas of clinical microbiology, such as virology, mycology, and parasitology. NIM should be considered as a simple, rapid,

and accurate method for determining the cellular composition of sputum specimens.

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