

Microscopic and Bacteriological Comparison of Paired Sputa and Transtracheal Aspirates

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Ninety-six sputum specimens from patients with pneumonia were microscopically screened for leukocytes and buccal squamous epithelial (BSE) cells. Cultures of these specimens were compared with cultures of paired transtracheal aspirates (TTA). Agreement between sputa with <25 BSE cells per 100× field and TTA was good (79%). Only 27% of the specimens with >25 BSE cells per 100× field agreed with TTA. Sixty-six of the sputa were of group 5 quality, i.e., >25 leukocytes and <10 BSE cells per 100× field. A potential pathogen growing in one of these specimens was 94% predictive of growth in the TTA. If a group 5 sputum was negative for a potential pathogen, there was a 45% chance that a fastidious organism had been overgrown or overlooked. The presence of definite lower tract secretions in group 5 sputa as determined by visualizing bronchial epithelial cells and alveolar macrophages did not significantly increase the diagnostic value of these specimens. Microscopic screening of sputum before culture with rejection of selected specimens can increase the value of sputum in determining the etiology of bacterial pneumonia.

Sputum culture is generally considered inferior to transtracheal aspirate (TTA) culture in the diagnosis of bacterial pneumonia (4). This is because sputum is a variable mixture of upper and lower respiratory tract secretions. The flora of the upper tract secretions may falsely indicate a pulmonary pathogen or may overgrow the actual pathogen.

The quality of the sputum specimen may determine its diagnostic value. Some specimens submitted for culture contain only upper tract secretions, i.e., saliva, whereas others contain variable amounts of lower respiratory material. Thus, microscopic screening and subsequent rejection of selected specimens may increase the diagnostic value of sputum cultures. This conclusion has been inferred from data showing that random sputum samples containing many leukocytes and few buccal squamous epithelial (BSE) cells grow similar numbers of bacterial species and/or pathogens as random TTA (8, 9).

The present study attempts to confirm these observations by comparing cultures of microscopically screened sputa with cultures of paired TTA.

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MATERIALS AND METHODS

One hundred patients admitted to the infectious disease ward with clinical and roentgenographic pneumonia were enrolled in the study. All gave written, informed consent. Eighty-two were recruits in basic training or technical school students just out of basic training. The remainder were other active duty personnel or dependents. The majority of patients were young adults with no significant underlying diseases, who had been admitted for short-term hospitalization.

Sputum expectoration was induced by heated mist inhalation and postural drainage if a specimen could not be produced spontaneously. A transtracheal aspiration was then performed by the method of Kalinske et al. (5). The microbiology technician Gram stained each specimen and coded the slides.

Sputa and TTA cultures were incubated for 24 h under 5% CO₂ on sheep blood, eosin-methylene blue, and chocolate agar plates. Sputa were also cultured on Thayer-Martin medium (Transgrow, Baltimore Biological Laboratories, Cockeysville, Md.) to detect *Neisseria meningitidis*. TTA cultures were incubated anaerobically for 48 h by the GasPak (BBL) system. Organisms were identified with standard microbiological techniques. The sputum plates were read before the TTA to avoid prejudiced interpretations.

The following organisms were considered to be pathogens: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and enteric gram-negative rods. Each slide was read blindly and was microscopically grouped by the method of Murray and Washington (8). The slide is scanned with the 10×

objective (100× magnification), and the most purulent area is graded by its number of leukocytes and BSE cells. Group 1, consisting only of saliva, has >25 BSE cells and <10 leukocytes per field. Group 5, an ideal specimen, has <10 BSE cells and >25 leukocytes per field. Groups 2 to 4 fall in between (Table 1). A group 6 was added, which has <25 BSE cells and <25 leukocytes per field. Additionally, the slides were evaluated for definite evidence of lower respiratory tract secretions by using the oil immersion objective. These are areas devoid of BSE cells that contain alveolar macrophages, bronchial epithelial cells, and leukocytes (7).

After all slides were read, the code was broken. Two patients were eliminated because of obviously contaminated TTA; microscopic examination showed BSE cells and culture grew "normal flora." Two others were eliminated because only anaerobic bacteria grew from the TTA, thus making comparison with sputum impossible.

RESULTS

The cellular composition of the sputum samples is shown in Table 1. The majority (69%) were group 5 specimens. A group 5 specimen did not necessarily contain areas with bronchial epithelial cells and/or alveolar macrophages indicating the presence of lower tract secretions. In fact, 21/66 (32%) did not. The other groups rarely contained identifiable lower tract secretions.

Similar data for TTA are listed in Table 2. In these specimens, a group 5 cellularity predicted the presence of lower tract secretions. However, 41% of the aspirates were group 6. These specimens contained sparsely cellular mucus or scattered cellular elements with minimal mucus. A small proportion 14/39 (36%) of group 6 aspirates contained identifiable lower tract areas.

Table 3 shows the results of sputum culture compared with that of the paired TTA. The same pathogen or no pathogen in a pair constitutes agreement. Groups 4, 5, and 6 demonstrated good overall agreement: 100, 74, and

TABLE 1. Cellular composition of 96 sputum samples

Group	Cells (no./field) ^a		No. of specimens	LT+ ^b	LT- ^b
	BSE cells	Leuko-cytes			
6	<25	<25	14 (15) ^c	2	12
5	<10	>25	66 (69)	45	21
4	10-25	>25	5 (5)	1	4
3	>25	>25	6 (6)	1	5
2	>25	10-25	1 (1)	0	1
1	>25	<10	4 (4)	0	4

^a 100× magnification.

^b Presence (+) or absence (-) of lower respiratory tract secretions.

^c Number in parentheses represents percentage of total.

TABLE 2. Cellular composition of 96 TTA

Group	Cells (no./field) ^a		No. of specimens	LT+ ^b	LT- ^b
	BSE	Leuko-cytes			
6	<25	<25	39 (41) ^c	14	25
5	<10	>25	57 (59)	57	0

^a 100× magnification.

^b Presence (+) or absence (-) of lower respiratory tract secretions.

^c Number in parentheses represents percentage of total.

93%, respectively. The diagnostic value of a group 5 sputum is reflected in this table. Of 66 group 5 sputa, 33 grew a potential pathogen. Thirty-one (94%) of the corresponding TTA were positive for the same pathogen. Of the 33 other samples negative for pathogens, 15 (45%) of the corresponding TTA grew a pathogen. Groups 4 and 6 had a comparable diagnostic value. Groups 1 to 3 showed poor agreement (27%); the pairs that did agree grew no pathogens. Of the four pathogens growing in the group 1 to 3 sputa, none grew in the corresponding TTA. Of the 96 sputa, 12 grew pathogens not found in the corresponding TTA, resulting in a 12.5% false-positive rate. Four of eleven (36%) group 1 to 3 sputa were falsely positive as compared to eight of eighty-five (9%) group 4 to 6 specimens. Five of the latter contained two pathogens, only one of which appeared in the TTA.

Since group 5 sputa were the most common, they were evaluated to determine whether cultural agreement with TTA was related to the the presence or absence of lower tract areas in the sputum Gram stain (Table 4). Considering all pairs, it seemed to make no difference whether lower tract areas could be found (75 versus 72% agreement). If group 5 sputa with a pathogen in the paired TTA are examined separately, the overall agreement falls to 66%. However, the presence of lower tract areas then seems to have a favorable effect (73 versus 54% agreement). However, this difference is not statistically significant ($P > 0.10$ by chi-square test).

Table 5 illustrates the overall data tabulated as mean number of pathogens per specimen. No striking differences are noted between group 4 to 6 sputa (range 0.60 to 0.79) and TTA (0.71). Sputum groups 1 to 3 contained fewer pathogens (0.36) than TTA. The most common pathogens isolated were *H. influenzae*, *N. meningitidis* group Y, and *S. pneumoniae*.

DISCUSSION

The value of sputum culture in the diagnosis of bacterial pneumonia is questionable. High rates of false positivity have been found when sputum and TTA cultures are compared (4).

TABLE 3. Comparison of 96 sputa and TTA

Group	Total	Presence of pathogen				Overall agreement ^c
		Absent in both	Present ^a in both	Present ^b in TTA only	Present in sputum only	
6	14	4	9	0	1	13/14 (93)
5	66	18	31	15	2	49/66 (74)
4	5	2	3	0	0	5/5 (100)
1-3	11	3	0	4	4	3/11 (27)

^a Five sputa contained an additional pathogen not present in the TTA.

^b Three TTA contained two pathogens not in the corresponding sputum.

^c Pathogen present or absent in both specimens.

^d Number in parentheses represents percentage.

TABLE 4. Comparison of paired group 5 sputa and TTA

Pairs	Agreement		
	Overall	LT+ ^a	LT- ^a
All pairs	49/66 (74) ^b	34/45 (75)	15/21 (72)
Pairs with pathogen in TTA	31/47 (66)	24/33 (73)	7/13 (54)

^a Presence (+) or absence (-) of lower respiratory tract secretions in sputum.

^b Number in parentheses represents percentage.

Significant numbers of false-negative cultures have been found in bacteremic *S. pneumoniae* (1) and *H. influenzae* (6) pneumonias. This may be related to the fact that many sputum specimens submitted for culture are improperly collected and contain predominantly upper respiratory or oropharyngeal secretions. To avoid this, Bartlett (2), in 1974, suggested the use of a microscopic screening system based upon the relative numbers of leukocytes and BSE cells in the specimen. The presence of leukocytes is graded +1 and +2, whereas BSE cells are counted as -1 and -2. The scores are added, and specimens with zero or less are rejected. This system was not evaluated on a day-to-day basis.

In 1975 Murray and Washington (8) compared 382 sputa with 47 random TTA and found that sputum samples with <10 BSE cells per 100× field grew the same numbers of organisms as TTA. This system utilized groups 1 to 5 as listed in Table 1. The data were later reanalyzed to consider only potential lower respiratory pathogens (9). It then appeared that groups 3 to 5, i.e., specimens with >25 leukocytes per 100× field, were similar to TTA.

Porschen, in 1976, reported the screening of 2,000 sputum specimens (R. K. Porschen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C31, p. 31). He used the above groupings, but added a group 6: <25 leukocytes and <25 BSE cells. The following indicates the percentage of these spec-

TABLE 5. Relationship between specimen group and mean number of pathogens isolated

Group	No. of specimens	Mean no. of pathogens
Sputum group		
6	14	0.79
5	66	0.60
4	5	0.60
1-3	11	0.36
TTA group		
Total	96	0.71

imens that contained potential pathogens: group 5, 54.4%; group 4, 42.7%; group 3, 22.8%; group 2, 16.2%; group 1, 18.4%; and group 6, 13.0%.

The conclusions of the above studies were that group 4, 5, and possibly 3 sputum specimens should be accepted and other groups rejected for culture. These observations have been shown not to apply to isolation of mycobacteria (3).

It seems reasonable to believe that sputa with many BSE cells and few leukocytes may be unreliable indicators of organisms present in lower respiratory secretions. Conversely, specimens with many leukocytes and few BSE cells should be more reliable. The above studies only imply that this is true, since the sputa and TTA were all random.

The present study directly compares sputa with paired TTA. These data indicate that group 4 to 6 sputum cultures show good overall agreement with TTA. These specimens contain variable numbers of leukocytes, but less than 25 BSE cells per field. Though the numbers are small, it appears that group 1 to 3 specimens (>25 BSE cells per 100× field) are unreliable and should be rejected for culture or cultured only at specific request.

All TTA must be cultured because of the invasive procedure required for collection. Microscopic examination should be performed to document the presence of oropharyngeal contamination. This could occur if the tracheal

catheter is misdirected or if the patient has recently aspirated oral secretions. The growth of "normal flora" or multiple organisms from the TTA could be more easily explained if the microscopic examination of the specimen had shown BSE cells. An uncontaminated TTA will fall into group 5 or 6. Group 5 specimens have many leukocytes and evidence of lower tract cells. Group 6 aspirates contain only sparsely cellular mucus or are diluted with saline injected into the trachea during specimen collection.

Ideally only group 5 sputa should be cultured. Other groups are often sent to the clinical microbiology laboratory because collection techniques are inadequate. Rejection of poor specimens has been shown to increase the number of good specimens submitted for culture. In the study of Murray and Washington, the proportion of group 4 and 5 sputa rose from 25 to 55%. Porschen reported a rise from 28 to 45%. Under optimal conditions, we obtained 69% group 5, 74% group 4 and 5, and 89% group 4, 5, and 6. Thus, only 11% of the specimens were unsatisfactory.

The presence of lower tract secretions can be confirmed by careful examination of the sputum specimen by using the oil immersion objective. Group 5 sputa with lower tract areas were not significantly more accurate in predicting TTA growth than those without such areas. This was true even if those pairs with pathogens were examined separately. Thus, this more tedious and difficult determination is not routinely necessary.

A group 5 sputum culture positive for a potential pathogen is indicative of a lower respiratory source. Ninety-four percent of group 5 sputa growing a potential pathogen had corresponding TTA cultures positive for the same organism. On the other hand, if a group 5 sputum did not grow a pathogen, there was a good chance (45%) that the paired TTA would be positive.

Thus, if a potential pathogen grows from a group 5 sputum, a TTA would probably be positive. However, if a group 5 sputum is negative for potential pathogens, it is quite likely a fastidious organism has been overlooked.

This study again documents the superiority of TTA over sputum culture in identifying lower respiratory pathogens. The problem of false positive and false negative sputa has also been reconfirmed. Microscopic screening, along with rejection of unsatisfactory specimens, can minimize the unreliability of sputum culture. This will also encourage better sputum collection techniques.

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