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Correlation between Sputum and Bronchoalveolar Lavage Fluid Cultures

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A correlation study of the cultured bacteria from paired sputum and bronchoalveolar lavage fluid samples was performed. The rates of concordant culture-positive paired specimens that were isolated within 1 or 7 days were 93.7% and 96.5%, respectively, suggesting that the culture of readily collectable sputum specimens may result in useful microbiologic diagnosis.

The usefulness of sputum culture has been widely debated. For example, one study found a lack of sensitivity for detecting *Streptococcus pneumoniae* from expectorated sputum samples compared with that from a more invasive specimen collection method (1). However, Gram staining and sputum sample culture have been recommended for the diagnosis of community-acquired pneumonia (CAP) in immunocompetent patients (2), as studies have shown the usefulness of these methods with immunocompromised subjects (3). Complementarity between the two sampling methods was also reported with immunocompromised patients (4). When sputum induction is not possible, such as in ventilator-associated pneumonia (VAP), the value of bronchoalveolar lavage (BAL) fluid culture is significant, as the survival rate is dependent on the empirical therapy (5–7). Finally, sputum provides sufficient information compared to that with bronchoscopy in cystic fibrosis patients (8), particularly in those infected with *Pseudomonas aeruginosa* (9). Here, we compare the results of standard culture techniques from samples from patients who underwent both bronchoalveolar lavage (BAL) fluid and sputum specimen collection in a retrospective study to determine the sputum culture-positive predictive value for BAL fluid positivity.

All samples were recovered from clinical units from the Assistance Publique–Hôpitaux de Marseille (APHM) and were analyzed in a microbiology laboratory from January 2002 to June 2014, according to the Société Française de Microbiologie (SFM) recommendations and EUCAST 2014 (10). Briefly, the specimens were screened for initial quality regarding the presence of white blood cells and epithelial cells, inoculated onto chocolate agar, colistin-nalidixic acid agar, and MacConkey agar plates (bioMérieux, Marcy l'Etoile, France), and incubated aerobically with 5% CO_2 for 48 h. Each colony that grew from the BAL fluid and sputum specimens with a bacterial load of $\geq 10^4$ CFU/ml and $\geq 10^7$ CFU/ml, respectively, was identified. Colony identification was performed until September 2009 using the Vitek 2 apparatus (bioMérieux); thereafter, colony identification was performed using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (11). A total of 25,926 positive samples that were recovered from 6,918 patients between January 2002 and June 2014 were analyzed in this study. Overall, 169,608 sputum samples and 19,536 BAL fluid samples were received, of which 21,760 (12.8%) and 4,166 (21.3%) were positive for microorganisms, respectively. After removing the duplicates, 3,159 positive BAL fluid and 8,470 positive sputum samples were included in the analysis. Of these, 511 of the culture-positive paired specimens were identified and characterized using microbiological analyses of BAL fluid and sputum samples on the same day and again within 7 days. The pairs were obtained from patients who were hospitalized in long-term health care units (436 of 511 pairs) significantly more than those in short-term units (75 of 511 pairs) ($P < 10^{-5}$). When pair sampling was not performed within 24 h, sputum samples ($n = 234$) were obtained significantly before BAL fluid samples ($n = 111$) ($P < 10^{-5}$). For samples in which several microorganisms were found, if one organism was missing from one of the two specimens, the pair was considered mismatched.

In total, 146 different bacterial species were isolated from the sputum samples, and 84 were isolated from the BAL fluid samples. Eight of the 10 most common bacterial species for each sample type were concordant between the types. Regarding the discrepancies, *Moraxella catarrhalis* and *Serratia marcescens* were commonly identified in the sputum samples, whereas *Staphylococcus epidermidis* and *Enterobacter aerogenes* were commonly identified in the BAL fluid samples (Table 1). Using the formula $H' = -\Sigma\ pi \times \ln(pi)$ (12), the Shannon diversity index values were estimated to be 2.73 and 2.75 for the BAL fluid and sputum samples, respectively.

Of the cultures performed within 7 days, 511 sputum-BAL fluid pairs were identified, and the same microorganism was found in 479 cases (93.7%). Finally, the concordance of methicillin susceptibility/resistance for the available *S. aureus* pairs (66 of 117 pairs) was 100%. Of the cultures performed on the same day,

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TABLE 1 Characteristics of the BAL fluid and sputum groups and the 10 most commonly identified bacteria for each specimen type

Microorganism[a]	No. (%) in group[b]: Sputum (n = 8,470)	BAL fluid (n = 3,159)
Staphylococcus aureus	1,883 (22.2)	626 (19.8)
Pseudomonas aeruginosa	1,827 (21.6)	622 (19.7)
Haemophilus influenzae	841 (9.9)	361 (11.4)
Escherichia coli	543 (6.4)	225 (7.1)
Streptococcus pneumoniae	454 (5.4)	180 (5.7)
Klebsiella pneumoniae	399 (4.7)	178 (5.6)
Stenotrophomonas maltophilia	315 (3.7)	98 (3.1)
Staphylococcus epidermidis		101 (3.2)
Enterobacter cloacae	261 (3.1)	97 (3.1)
Serratia marcescens	168 (2)	
Enterobacter aerogenes		88 (2.8)
Moraxella catarrhalis	154 (1.8)	

[a] Microorganisms that were discrepancies between the two groups are in bold type.
[b] The Shannon diversity index values for the sputum and BAL fluid groups are 2.73 and 2.75, respectively.

TABLE 2 Characteristics of the 20 discrepancies observed in this series

Organism in sputum sample	Delay (days) between sputum (reference) and BAL fluid sampling	Organism in BAL fluid sample	Error type
S. aureus[a]	7 (earlier)	*E. aerogenes*	Major
S. aureus	5 (earlier)	***K. pneumoniae***	Not stated
S. aureus	4 (earlier)	*E. coli*	Major
S. aureus	2 (earlier)	*P. aeruginosa*	Major
S. aureus	1 (earlier)	*S. marcescens*	Major
S. aureus	0	***S. pneumoniae***	Not stated
S. aureus	1 (after)	***H. influenzae***	Not stated
Burkholderia cepacia	2 (earlier)	***S. aureus***	Not stated
E. aerogenes	2 (after)	***S. aureus***	Major
E. coli	2 (after)	***S. aureus***	Major
Proteus mirabilis	1 (earlier)	***S. aureus***	Major
Staphylococcus haemolyticus	4 (after)	***S. aureus***	Major
Streptococcus anginosus	5 (earlier)	***S. aureus***	Major
S. pneumoniae	4 (after)	***S. aureus***	Not stated
Citrobacter freundii	0	***H. influenzae***	Major
S. pneumoniae	6 (after)	***H. influenzae***	Not stated
E. coli	7 (earlier)	***K. pneumoniae***	Major
H. influenzae	2 (earlier)	*P. mirabilis*	Major
K. pneumoniae	1 (earlier)	*P. aeruginosa*	Major
P. aeruginosa	1 (earlier)	***S. pneumoniae***	Major

[a] Microorganisms that were major discrepancies between the two groups are in bold type.

285 sputum-BAL fluid pairs were identified, and the same microorganism was found in 275 of the cases (96.5%). These results yielded a positive predictive value (PPV) of 96.5% for sputum culture, considering BAL fluid culture as the gold standard. The correlations were not affected by the identification method; the sputum PPV when the specimens were collected within 1 day was 97.1% when identification was performed using a Vitek 2 apparatus and 96.3% when performed using MALDI-TOF mass spectrometry (MS) (e.g., before and after September 2009, respectively). We then analyzed the discrepancies between the results of the two sampling methods. Finally, we assessed the misdetections of bacteria that were considered strict pathogens for each specimen type, including *S. pneumoniae*, *S. aureus*, *Klebsiella pneumoniae*, *Burkholderia cepacia*, and *Haemophilus influenzae*. Of these 20 discrepancies, 14 were considered major errors, and a specific pathogen was not detected in BAL fluid in 6 cases and in sputum in 8 cases. The errors for cases in which two different microorganisms were identified from each sample were not stated (Table 2).

This 12-year retrospective study shows good agreement between BAL fluid and sputum sample microbiological analyses, which were performed in the same laboratory using the same methods. Indeed, when the analyses were performed within 7 days, the PPV of sputum culture was 93.7%. The interval of 7 days may have been too long to evaluate the PPV of sputum for BAL fluid positivity, leading us to consider a shorter interval of 24 h, for which the PPV was 96.5%. The similar Shannon index values obtained in this study (2.73 versus 2.75), as there was a high correlation of 8 of the 10 most represented bacteria in each group, might reflect the ecology of the medical center due to the biased recruitment of patients who were hospitalized in long-term health care units. Because of respiratory tract flora contamination in sputum samples, BAL fluid has been considered for years to be the best biological sample for identifying a bacterial agent, but these samples may also be contaminated. The major bias of this retrospective study is the inclusion of strictly positive samples. These data show that if microbiological examination of BAL fluid is valuable in the management of VAP (5–7), sputum analysis is more cost-effective and has a similar efficiency to that of invasive sampling methods. This study will be further used prospectively with clinicians to de-escalate antibiotics, if started, and/or to change antibiotic therapy according to the sputum sample culture results.

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