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Diagnosis of Ventilator-associated Pneumonia

An Evaluation of Direct Examination and Presence of Intracellular Organisms

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Background: **Ventilator-associated pneumonia (VAP) requires early diagnosis and adequate antibiotic therapy. The aim of this prospective postmortem study was to assess the accuracy of direct examination and quantification of intracellular organisms (ICO) for the diagnosis of VAP.**

Methods: **Total and differential cell counts were performed on fluids recovered using nonbronchoscopic sampling techniques (blind bronchial sampling [BBS], mini-bronchoalveolar lavage [mini-BAL]) and from bronchoalveolar lavage (BAL) performed during fiberscopy. These 3 sampling techniques were done within 15 min of death without discontinuing mechanical ventilation. Quantification of ICO was performed on each sample recovered from the various sampling procedures. Gram reaction and morphology of bacteria were evaluated on Gram smears.**

Results: **The results of each technique were compared with histology and culture of lung tissue specimens obtained by surgical pneumonectomies in 28 patients who died after at least 72 h of mechanical ventilation. Histology was positive in 13 patients and negative in 15 patients. When only VAP with positive lung culture was considered (histologic signs of bronchopneumonia plus positive lung tissue culture), the sensitivity of Gram staining on BAL, mini-BAL, and BBS was 56%, 44%, and 56%, respectively. If all samples were considered, the sensitivity and the specificity of the determination of the percentage of ICO were low (less than 70%) whatever the sampling technique.**

Conclusions: **For initial therapeutic guidance, direct examination and presence of ICO do not contribute for establishing the diagnosis of VAP, essentially because of a lack of sensitivity. However, when positive, Gram staining can obviously guide initial antibiotherapy. (Key words: Pneumonia. Direct examination. Gram. Ventilation. Autopsy.)**

NOSOCOMIAL bacterial pneumonia is a severe and frequent complication in patients whose lungs are mechanically ventilated.[1-3] It requires early diagnosis and adequate antibiotic therapy. Several studies have shown the usefulness of quantitative cultures of specimens obtained using bronchoscopic or nonbronchoscopic sampling techniques.[4-9] Because culture results from these sampling methods require 24-72 h, a more rapid diagnostic procedure would be useful. Chastre *et al.*[10] have described a technique to evaluate the presence of bacteria within cells from Giemsa- and Gram-stained cytocentrifuge preparations of bronchoalveolar lavage (BAL). Fluid obtained from fiberoptic bronchoscopy can provide rapid diagnosis, available 1 h after BAL. In their study, the percentage of bacteria found in recovered cells was correlated with protected specimen brush (PSB) culture results. When more than 7% of recovered cells contained intracellular organisms (ICO), the morphology and Gram reaction of ICO predicted subsequent PSB culture results. The advantage of making an earlier diagnosis of VAP would be to potentially avoid the needless and perhaps inappropriate administration of antibiotics. Restricting antibiotic administration could be associated with less subsequent colonization and infection with antibiotic-resistant pathogens and less cost. Therefore, the purpose of this study was to evaluate the diagnostic performance of direct examination performed on fluids recovered from bronchoscopic and nonbronchoscopic sampling using histologic assessment of pneumonia as "gold standard." The recent study by Fabregas *et al.*[11] strongly challenges the use-

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fulness of quantitative bacteriologic analysis in the diagnosis of VAP.

Methods

Patients

Over a 10-month period, from January 1994 to October 1994, nonimmunocompromised patients whose lungs were mechanically ventilated for more than 72 h were included if they died in the intensive care unit (ICU).

The following variables were prospectively recorded during hospitalization: age, sex, severity scores on admission (Simplified Acute Physiology Score and the APACHE II score),[12,13] antimicrobial therapy administered before death, duration of mechanical ventilation, temperature, leukocyte count, and radiologic classification using Weinberg's score.[14] No patient received oral or nonabsorbable antibiotics during their stay in the ICU.

Protocol

Three sampling techniques (blind bronchial sampling [BBS], mini-bronchoalveolar lavage [mini-BAL], and BAL) were performed within 15 min of death without discontinuing mechanical ventilation. To avoid contamination of the lower airways during bronchoscopy, the nonbronchoscopic procedures (BBS and mini-BAL) were performed first. The exact sequence was BBS, mini-BAL, and BAL.

Blind bronchial sampling was performed using a sterile catheter with a specimen trap kit (Vygon 534-16, Ecouen, France) as previously described.[15] The suction tube was blindly introduced through the intubation or tracheostomy tube and wedged into the tracheobronchial tree before suction. No saline was injected before or during suctioning. The mean duration of suctioning was 12 s.

Mini-BAL was performed with a single-sheathed plugged telescoping catheter according to the technique described by Rouby *et al.*[16] A 50-cm sterile catheter, 1.7-mm internal diameter and occluded by a polyethylene glycol plug (Combicath Plastimed, Saint-Leu-La-Forêt, France), was inserted into the endotracheal tube, blindly advanced into the distal airways, and wedged in a peripheral bronchus. The plug was expelled with 10 ml of air, and a second sterile catheter (58 cm in length, 0.8-mm internal diameter) was then passed through the first catheter and advanced to the sampling site. A 20-ml aliquot of physiologic saline was injected *via* the internal catheter, and at least 2 ml were suctioned for microbiologic examination.

Bronchoalveolar lavage was performed through a fiberoptic bronchoscope. The tip was positioned in the most prominent radiographic infiltrate whenever such an area was present. After wedging the bronchoscope in the bronchial orifice of a lung segment, BAL was performed by infusion of six 20-ml aliquots of sterile, nonbacteriostatic 0.9% NaCl. The first aliquot, which is supposed to be a bronchial wash, was discarded, and subsequent aliquots were pooled, mixed, measured, and then analyzed. The mean volume recovered from the last 5 aliquots was 52 ± 22 ml of the instilled fluid.

Specimen Processing

The same procedure was performed for BBS, mini-BAL, and BAL. The sample was strained through cotton gauze to remove mucus. An aliquot of less than 1 ml of this uncentrifuged, pooled fluid was used to determine the total cell count and cell viability. Cytocentrifuge preparations (Cytospin, Shandon Elliot, Shandon Scientific Company, London, UK) were made by cytocentrifuging aliquots of original fluid containing approximately 5×10^4 cells at 8,000 *g* for 10 min and stained with May-Grünwald Giemsa (MGG) stain and Gram stain. The stained cytospin preparations were examined under oil-immersion microscopy. Differential cell counts were made by examining at least 500 cells. An additional 300 polymorphonuclear leukocytes (PMNs) were examined at a magnification of 1,000 x for the presence or absence of ICO, and the percentage was recorded. Quantification of "infected cells" was expressed as a percentage of PMN recovered by BAL, mini-BAL, and BBS. This parameter was evaluated on cytocentrifuge slides stained with MGG stain because the recognition of intracellular organisms was considerably easier on these preparations compared with Gram-stained preparations. When the number of altered neutrophils was more than 75%, these preparations were considered as not interpretable. When neutrophils are lysed, it is difficult to determine if organisms are intra- or extracellular. Gram reaction and morphology of bacteria were then evaluated on Gram smears. Slides were evaluated by a staff consultant in cytology without previous knowledge of the patient's history or culture results.

Reference Method

Pneumonectomy was performed at bedside during surgical conditions within 30 min after death while

maintaining mechanical ventilation. Depending on which side the radiographic infiltrates were predominant, the right or left lung was removed *via* thoracotomy (fifth intercostal space). If there were no radiographic densities, the right lung was removed. Prevailing French legislation allows removal of organs for scientific research barring express opposition by the patient before death (Law No. 76-1181, December 22, 1976, followed by Statuatory Order No. 78-501, March 31, 1978, and the Implementation Order dated April 4, 1978).

After careful examination to localize regions of lung that were grossly diseased, 6-10 specimens (1-2 g each) were cut superficially to avoid bronchial contamination. They were aseptically collected, weighed, and homogenized in peptone broth to obtain a final dilution of 1:10. Serial 10-fold dilutions (10^{-2}-10^{-6}) of this preparation were made and cultured. Results were expressed as colony-forming units per gram of tissue (CFU/g). Histologic examination was performed by an experienced pathologist (LG) with no previous knowledge of the results of cultures. The entire lung was sectioned into 5- to 10-mm thick sections to localize suspicious areas. Sections were fixed in 10% formalin for 24 h, embedded in paraffin, and cut into 4-μm slices. After removing paraffin, each slice was stained with hematoxylin-eosin-safran. Sections were classified according to Johanson *et al.*[17]

- *Mild bronchopneumonia:* presence of scattered neutrophilic infiltrates localized in terminal bronchioles and surrounding alveoli;
- *Moderate bronchopneumonia:* extension of the process causing grossly evident confluence of infiltrates between adjacent lobules (purulent mucus plugs often are present in bronchioles);
- *Severe bronchopneumonia:* process extensively confluent grossly and microscopically with or without tissue necrosis.

Classification of each lung was based on the worst category observed in that lung.

Statistical Analysis and Definitions

All data are expressed as means ± SD. Depending on the size of the sample, a chi-square test with Yates correction or a Fischer's exact test was used to compare qualitative data.

Ventilator-associated pneumonia was defined as VAP with positive lung culture (VAP_{PLC}) if lung histology exhibited signs of bronchopneumonia and if lung tissue culture was positive with at least one microorganism (whatever the quantity).

Ventilator-associated pneumonia was defined as VAP with negative lung culture (VAP_{NLC}) if histology was positive but lung culture was sterile.

Finally, patients without histologic signs of VAP nor positive lung tissue culture were classified as "no VAP."

For those with VAP_{PLC}, the Gram stain was defined as negative if no microorganisms were seen on the samples obtained by BBS, mini-BAL, or BAL and if lung tissue culture was sterile. It was considered as positive if microorganisms were identified on the samples obtained by BBS, mini-BAL, or BAL and correlated with what grew in lung tissue culture. VAP_{NLC} were not considered for the evaluation of diagnostic accuracy of Gram staining because lung cultures were negative. Therefore, it is difficult to evaluate Gram staining when lung tissue culture exhibits no growth.

Table 1. Characteristics of the Patients

	VAP	No VAP	Total
Number	13	15	28
Age (yr)	66 ± 9	61 ± 15	64 ± 13
Sex (M/F)	9/4	10/5	19/9
SAPS on admission in ICU	13 ± 5	15 ± 4	14 ± 4
APACHE II on admission in ICU	18 ± 6	22 ± 5	20 ± 6
Duration of mechanical ventilation (days)	11 ± 6	31 ± 28	22 ± 23
Body temperature (°C)*	38.7 ± 1.0	37.5 ± 1.0	38 ± 1.0
WBC (10^9/L)*	19 ± 13	16 ± 9	17 ± 11
Radiologic score*	8 ± 3	7 ± 3	7 ± 3
Not on antibiotics or off antibiotics for more than 48 h before death	8	9	17

VAP = ventilator-associated pneumonia; SAPS = Simplified Acute Physiology Score; APACHE = Acute Physiology and Chronic Health Evaluation score.

* Last result before death.

Results

During the study period, 49 patients died after at least 72 h of mechanical ventilation. Of these 49 patients, 28 were included in the study. Their characteristics are summarized in table 1. The lungs of 15 patients were mechanically ventilated for postoperative complications. There were two trauma patients. The remaining 11 patients presented medical problems (community-acquired pneumonia, 3; acute exacerbation of chronic

obstructive pulmonary disease, 3; coma, 2; polyneuritis, 1; cardiac insufficiency, 1; and rhabdomyolysis,1). The reasons that 21 patients were not included in the study included: autopsy procedure not available within 30 min of death (n = 5), sampling techniques not performed within 15 min of death (n = 11), immunocompromised hosts (n = 3), and medicolegal problem contraindicating autopsy (n = 2).

Histology and Culture of Lung Tissue

Histology revealed no sign of bronchopneumonia in 15 patients. Conversely, in the remaining 13 patients, histology confirmed bronchopneumonia, which was moderate in five patients and severe in eight. Bronchiolitis was noted in six patients. Four of these six patients had concomitant histologic signs of pneumonia. Additional histologic findings were fibrosis in six patients and diffuse alveolar damage, the hallmark feature of adult respiratory distress syndrome, in four patients.

Lung tissue cultures were positive for at least one microorganism in nine patients. All nine were histologically positive and were thus classified as VAP_{PLC}. The remaining four patients showing typical histologic features of bronchopneumonia had negative cultures and were thus classified as VAP_{NLC}. Lung cultures were negative in all 15 histologically negative cases (no VAP).

Gram Staining

Gram stains (table 2) of the BAL effluents were negative in 6 of 13 patients with VAP (sensitivity, 54%). Gram stains of the mini-BAL specimens were negative in 7 of 13 patients with VAP (sensitivity, 46%). Gram stains of the BBS specimens were positive in 8 of 13 patients with VAP (sensitivity, 62%). If only the diagnosis of VAP_{PLC} was considered, the sensitivity of BAL, mini-BAL, and BBS was 56%, 44%, and 56%, respectively. The specificity of Gram staining of these three sampling techniques was 100%, 87%, and 73%, respectively. If only the 17 patients without antibiotics for at least 48 h were considered, the sensitivity of the various sampling techniques for the diagnosis of VAP was respectively 63%, 50%, and 63% for BAL, mini-BAL, and BBS. The specificity was 100% for BAL, 89% for mini-BAL, and 67% for BBS. Despite a higher value, BAL specificity was not statistically different from mini-BAL and BBS specificity.

Viability, Total Cell Count, and Differential Counts

Eighty-six percent of either BAL or mini-BAL specimens were classified as excellent quality by the laboratory. As for BBS, 46% of the specimens were not interpretable because of the great number of altered neutrophils. Contamination by bronchial epithelial cells was less than 1% of the total number of cells recovered by BAL and mini-BAL in all patients. The proportion of bronchial epithelial cells was less than 5% of the total number of cells recovered by BBS in all patients.

Total and differential counts of lavage cells were not useful in distinguishing patients with and without bacterial pneumonia, whatever the sampling technique used (table 3). The number of total cells and neutrophils recovered by BBS was moderately increased in patients with pneumonia compared with that recovered from patients without pneumonia, but no threshold existed that clearly separated the two groups (table 4).

Microscopic Evaluation of Organisms

Quantitative assessment of intracellular organisms present on cytocentrifuge preparations made from the various samples was not useful in separating patients with and without VAP_{PLC} except for BBS (figs. 1 and 2). If various thresholds were considered, no technique exhibited a sensitivity greater than 70% for an acceptable specificity (tables 5 and 6). If only interpretable samples were considered, the diagnostic performance of BBS increased. Therefore, with a cutoff of 10% of cells containing intracellular organisms to identify patients with pneumonia, microscopic examination of bacteria in BBS specimens had a sensitivity of 100% and a specificity of 86% for the diagnosis of VAP_{PLC}. Further, the morphology and Gram stain reaction of intracellular bacteria observed in patients with pneumonia correlated with lung tissue culture results. Overall, lung tissue culture results were accurately predicted by the presence or absence of ICO in 11 of 12 (92%) evaluable patients. When Gram staining and ICO were considered together, the sensitivity of BAL, mini-BAL, and BBS for the diagnosis of VAP_{PLC} was 57%, 38%, and 60%, respectively, whereas the specificity was 54%, 54%, and 57%, respectively.

Discussion

The use of fiberoptic bronchoscopy for the evaluation of VAP has developed, in part, because of the proven inaccuracy of clinical judgment for establishing the presence or absence of this diagnosis.[18,19] Nonbronchoscopic techniques also have been developed for the evaluation of VAP. They have been advocated as poten-

Table 2. Gram Stain Results (Excluding Patients with VAP with Negative Lung Culture)

Patient No.	Histologic Grade	Quantitative Lung Culture		Gram Examination		
		Bacterial Isolate	cfu/g	BBS	BAL	Mini-BAL
1*	Moderate	*Pseudomonas aeruginosa*	2.5×10^2	0	0	0
		Stenotrophomonas maltophilia	2×10^4	0	0	0
2*	Severe	*Enterobacter aerogenes*	10^2	0	0	0
3*		—	—	GPC	0	GPC
4		—	—	0	0	0
5		—	—	0	0	GNB
6		—	—	0	0	0
7*		—	—	0	0	0
8*		—	—	0	0	0
9*		—	—	0	0	0
10*	Severe	*Streptococcus pneumoniae*	7×10^4	GPC	GPC	GPC
11*	Severe	*Pseudomonas aeruginosa*	10^2	GNB	GNB	0
13	Moderate	*Candida albicans*	10^2	0	0	0
14	Severe	*Pseudomonas aeruginosa*	3×10^4	GNB	GNB	GNB
		Escherichia coli	10^5	GNB	GNB	GNB
15*		—	—	0	0	0
17*	Severe	*Pseudomonas aeruginosa*	10^2	GNB	GNB	GNB
20		—	—	0	0	0
21*	Severe	*Staphylococcus aureus*	2×10^2	GPC	GPC	GPC
22*		—	—	0	0	0
23*		—	—	GPC	0	0
24*		—	—	0	0	0
25		—	—	0	0	0
26*		—	—	GPC	0	0
27		—	—	GNB	0	0
28*	Moderate	*Klebsiella pneumoniae*	2×10^3	0	0	0

0 = negative; GNB = Gram-negative bacilli; GPC = Gram-positive cocci.

* Not on antibiotics or off antibiotics for more than 48 h before death.

tially better alternatives because of their minimal invasiveness, wide availability, and relative inexpensiveness compared with bronchoscopy.[8,20,21] Some investigators have found that bronchoscopic methods have unacceptably high false-negative and false-positive rates.[5,8,20,21] These limitations, along with the lack of data showing improvements in patient outcomes after the use of these bronchoscopic methods, have resulted in requests for their validation in prospective clinical trials before their general acceptance.[22,23]

The length of time required to obtain the results of quantitative cultures (24 h) and to start antimicrobial therapy can be harmful for critically ill patients unless broad-spectrum antibiotics are empirically started soon after the diagnostic procedure. Direct examination of BAL specimens would seem to be an attractive method,

Table 3. Comparison of the Total Cell Count and the Differential Cell Counts of Cytocentrifuge Preparations from Analysis of Three Different Cell Suspensions

	Total Cell Count ($\times 10^6$/ml)			Neutrophils (%)			Lymphocytes (%)			Macrophages (%)		
	VAP_{PLC}	VAP	No VAP	VAP_{PLC}	VAP	No VAP	VAP_{PLC}	VAP	No VAP	VAP_{PLC}	VAP	No VAP
BAL	1.6 ± 2.4	1.6 ± 2.2	1.7 ± 2.2	73 ± 30	77 ± 27	77 ± 26	2 ± 3	2 ± 3	1 ± 1	23 ± 29	20 ± 26	19 ± 27
Mini-BAL	0.6 ± 1.1	0.6 ± 1.0	0.7 ± 1.1	91 ± 12	91 ± 10	83 ± 15	1 ± 1	1 ± 1	1 ± 1	2 ± 2	2 ± 2	3 ± 3
BBS	4.5 ± 6.7	3.7 ± 5.6	3.8 ± 6.5	96 ± 3	97 ± 3	91 ± 20	1 ± 1	1 ± 1	1 ± 1	2 ± 2	2 ± 2	3 ± 3

Data are mean ± SD.

Table 4. Intracellular Organism Percentage of Cytocentrifuge Preparations from Analysis of Three Different Cell Suspensions

	VAP with Positive Lung Culture (%)	All VAP (%)	No VAP (%)
BAL	6.4 ± 5.4	5.4 ± 4.7	5.3 ± 5.4
Mini-BAL	6.3 ± 2.9	6.1 ± 3.2	5.7 ± 5.5
BBS	17.4 ± 6.1*	12.6 ± 8.3	6.7 ± 8.4

* $P < 0.02$ *versus* no VAP.

although performing BAL with PSB carries an additional risk in critically ill patients and is time-consuming, especially for the laboratory processing of specimens. This latter drawback prevents the routine use of BAL as a diagnostic tool for VAP in many hospitals. For these reasons, the present study was undertaken to prospectively evaluate whether the direct examination of the BAL, mini-BAL, and BBS specimens could predict the results of histopathologic examination of the lung combined with lung tissue culture.

The diagnostic accuracy of direct examination performed on BAL, mini-BAL, and BBS was prospectively evaluated in a series of 28 patients whose lungs were mechanically ventilated and who died in the ICU. Complete postmortem lung examination and lung tissue culture were used as the "gold standard" for establishing the diagnosis of VAP. The main findings of this study were the following: (1) The sensitivity of Gram stain

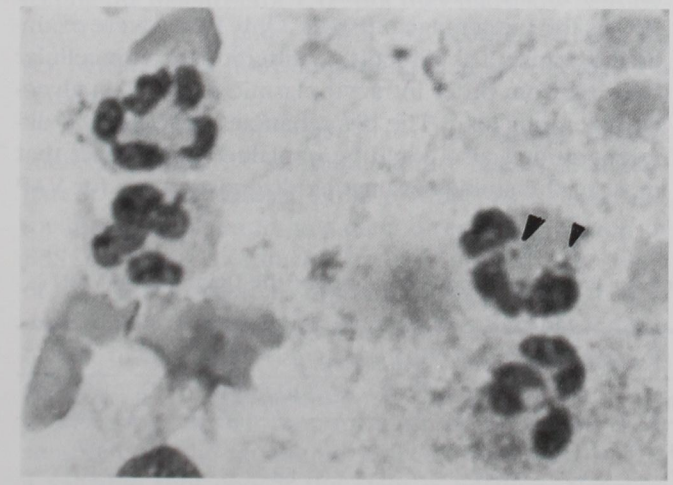

Fig. 1. Interpretable sample. Well-preserved neutrophils. The cytoplasmic membrane is evident. Intracellulars organisms are visible (*arrows*). (magnification, × 1000; May-Grünwald Giemsa stain).

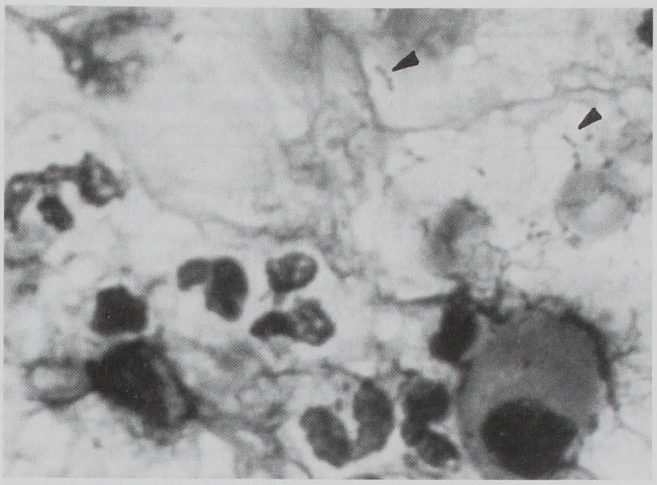

Fig. 2. Not interpretable sample. Altered neutrophils. The cytoplasmic membrane is not preserved. Organisms are not intracellular (*arrows*) (magnification, × 1000; May-Grünwald Giemsa stain).

was respectively 56%, 44%, and 56%, for BAL, mini-BAL, and BBS, and the specificity was 100%, 87%, and 73%; and (2) The determination of ICO with a threshold at 10% in BBS samples was useful (sensitivity, 100%; specificity, 86%) for detecting VAP, only when direct examination was possible (54% of patients).

According to different authors, total and differential cell counts proved to have little value in identifying patients with pneumonia who were receiving ventilation.[10,24,25] Recent data have shown that microscopic identification of organisms within cells recovered by lavage may provide a sensitive and specific means for early and rapid diagnosis of VAP.[10,24-26] The implications of ICO in cells recovered by lavage from lungs in ventilated patients with clinical suspicion of nosocomial bacterial pneumonia have been assessed in different studies. Different diagnostic thresholds for pneumonia have been proposed. Chastre *et al.*[10] obtained cytospin preparation of BAL effluent for quantitative examination of the presence of ICO in recovered cells in lungs of 21 ventilated patients clinically suspected of nosocomial pneumonia. Quantitative PSB cultures and further confirmatory data were positive in 5 of 21 patients. Chastre *et al.*[10] found more than 25% of cells with ICO in all five patients with VAP and less than 15% of cells with ICO in all patients without lung infection. None of the patients had received antimicrobial therapy before bronchoscopy. Subsequently in another article, Chastre *et al.*[27] reported the results of BAL and PSB cultures in 61 patients suspected of having nosocomial pneumonia.

Table 5. Diagnostic Performance of the Determination of the Percentage of Intracellular Organisms

		VAP with Positive Lung Culture						All VAP					
		n	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	n	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy
Interpretable cases	BAL 4%	20	71	46	42	75	55	24	55	46	46	55	50
	Mini-BAL 5%	21	75	62	55	80	67	24	64	62	58	67	63
	BBS 10%	12	100	86	83	100	92*	15	63	86	83	67	73
All cases	BAL 4%	24	56	40	33	58	46	28	46	40	38	47	43
	Mini-BAL 5%	24	67	53	46	73	58	28	54	53	50	57	54
	BBS 10%	24	56	40	36	60	46	28	38	40	36	43	39

n = number of cases; PPV = positive predictive value (true positive/true positive + false positive); NPV = negative predictive value (true negative/true negative + false negative).

* Fisher exact test BBS *versus* BAL ($P < 0.05$).

No data regarding antibiotic use were reported. Of 14 patients with confirmed pneumonia (the gold standard was a PSB culture $\geq 10^3$ CFU/ml), 12 had more than 7% of recovered cells by BAL containing ICO. Of 47 patients who were considered to be free of lung infection, fewer than 2% of recovered cells contained ICO. More than 7% of recovered cells containing ICO were found in only two patients who were considered to be free of lung infection. Thus, using a cutoff of 7% of cells containing ICO to identify patients with presumed pneumonia, microscopic examination of the bacteria and lavage fluid had a sensitivity of 86% and a specificity of 96%. In our study, with a cutoff at 7% of recovered cells by BAL containing ICO, sensitivity was 33% and specificity 67%. Therefore, the determination of the percentage of ICO performed on BAL fluid cannot be proposed in the diagnosis of VAP. On the other hand, determination of the percentage of ICO on BBS fluid could be proposed in the diagnosis of VAP. When the sample was interpretable (*i.e.*, less than 75% of lysed neutrophils), the sensitivity of BBS for the diagnosis of VAP_{PLC} reached 100% with a specificity of 86% for a threshold at 10% of ICO.

However, when the determination of ICO is not possible, it can be necessary to perform BBS again. Because of the methodology used in the present study (surgical pneumonectomy performed immediately after BBS, mini-BAL, and BAL), it was impossible to evaluate the diagnostic performance of repeated BBS. Pugin *et al.*[28] evaluated ICO from bronchoscopic and "blind" BAL in 28 surgical ICU patients whose lungs were ventilated. Of 13 positive bacterial cultures of BAL effluent (bronchoscopic or blind BAL), 10 (77%) demonstrated ICO. The results of ICO were not correlated with antibiotic use. Meduri *et al.*,[29] using a protected balloon-tipped catheter for BAL, showed ICO in more than 2% of the alveolar cells recovered in all but two patients with pneumonia and in none of the patients without pneumonia. In the present study, we preferred to express ICO as the percentage of PMNs. Only PMNs were examined because it is difficult to differentiate intracellular bacteria from other intracytoplasmic particles in alveolar macrophages.[25] The discrepancies between the different studies also could be explained by the fact that most of the studies evaluating diagnostic tests for VAP

Table 6. Comparison of the Various Thresholds of ICO Determined for Each Sampling Technique for the Diagnosis of VAP with Positive Lung Culture (n = 24)

	BAL		Mini-BAL		BBS	
Thresholds (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
2	67	13	89	7	56	7
4	56	40	67	47	56	20
5	44	47	67	53	56	27
7	33	67	33	60	56	27
10	11	73	11	73	56	40

did not use objective criteria to define pneumonia. It has been recommended that diagnostic tests for VAP be systematically evaluated with definitive criteria for VAP based on lung-tissue examination.[30] After the results of the study of Fabregas *et al.*,[11] who noted a weak relationship between histopathology and microbiologic biopsy cultures, we classified VAP as VAP_{PLC} and VAP_{NLC}. VAP_{NLC} can be related to the bactericidal effects of antibiotics (three of the four patients with VAP_{NLC} were receiving antibiotics at the time of death). Other possible explanations for the negativity of lung tissue culture are the natural host defenses against infection, which can sterilize lungs or pulmonary sampling for microbiologic examination performed in a noninfected area. However, in the present study, a precise histologic analysis was performed on lung regions close to those cultured and, in the four patients with VAP_{NLC}, demonstrated histologic signs of VAP.

Finally, determination of ICO on BAL specimens did not have a diagnostic yield superior to that of direct examination of BBS or mini-BAL specimens. This point should be noted because until now, the major advantage claimed for BAL has been its ability to permit immediate recognition of the causative organisms, thereby helping therapeutic decisions. We did not observe any correlation between the quantitative lung culture and Gram staining. The most likely hypothesis is that bactericidal inoculum present in the distal bronchial tree is much greater than the bacterial burden present in the lung parenchyma. The Gram stains of the BAL specimens in this study were highly specific (100%) in establishing the diagnosis of VAP, but they had a low sensitivity (56%), even in patients who did not receive antibiotics. Sensitivity is more important than specificity for a screening test.

The desirability of a test that could rapidly predict subsequent positive sampling culture results seems apparent. From a practical point of view, we believe that for initial therapeutic guidance, direct examination of BBS specimens is a valuable tool if it shows the presence of ICO. It is possible to reperform this sampling procedure if BBS is not interpretable, but because of the design of the study (lung surgically removed immediately after performing directed and blind samplings), we can just suggest that reperforming BBS would improve sensitivity of the technique. For Gram staining, BAL is a valuable tool only if the stain is positive. Negative Gram staining on BAL specimens does not exclude VAP.

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References

1. Craven DE, Kunches LM, Kilinsky V, Lichtenberg DA, Make BJ, McCabe WR: Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am Rev Respir Dis 1986; 133:792-6

2. Papazian L, Bregeon F, Thirion X, Gregoire R, Saux P, Denis JP, Perrin G, Charrel J, Auffray JP, Gouin F: Effect of ventilator-associated pneumonia on mortality and morbidity. Am J Respir Crit Care Med 1996; 154:91-7

3. Chevret S, Hemmer M, Carlet J, Langer M: European cooperative group on nosocomial pneumonia. Incidence and risk factors of pneumonia acquired in intensive care units. Intensive Care Med 1993; 19:256-64

4. Chastre J, Viau F, Brun P, Pierre J, Dauge MC, Bouchama A, Akesbi A, Gibert C: Prospective evaluation of the protected specimen brush for the diagnosis of pulmonary infections in ventilated patients. Am Rev Respir Dis 1984; 130:924-9

5. Pham LH, Brun-Buisson C, Legrand P, Rauss A, Verra F, Brochard L, Lemaire F: Diagnosis of nosocomial bronchopneumonia in mechanically ventilated patients. Comparison of a plugged telescoping catheter with the protected specimen brush. Am Rev Respir Dis 1991; 143:1055-61

6. Rouby JJ, Martin De Lassale E, Poete P, Nicolas MH, Bodin L, Jarlier V, Le Charpentier Y, Grosset J, Viars P: Nosocomial bronchopneumonia in the critically ill. Am Rev Respir Dis 1992; 46:1059-66

7. Marquette CH, Georges H, Wallet F, Ramon P, Saulnier F, Neviere R, Mathieu D, Rime A, Tonnel AB: Diagnostic efficiency of endotracheal aspirates with quantitative bacterial cultures in intubated patients with suspected pneumonia. Comparison with the protected specimen brush. Am Rev Respir Dis 1993; 148:138—44

8. Torres A, El-Ebiary M, Padro L, Gonzalez J, Puig De La Bellacasa J, Ramirez J, Xaubet A, Ferrer M, Rodriguez-Roisin R: Validation of different techniques for the diagnosis of ventilator-associated pneumonia. Comparison with immediate postmortem pulmonary biopsy. Am J Respir Crit Care Med 1994; 149:324-31

9. Papazian L, Martin C, Meric B, Dumon JF, Gouin F: A reappraisal of blind bronchial sampling in the microbiologic diagnosis of nosocomial bronchopneumonia. Chest 1994; 103:236-42

10. Chastre J, Fagon JY, Soler P, Bornet M, Domart Y, Trouillet JL, Gibert C, Hance AJ: Diagnosis of nosocomial bacterial pneumonia in intubated patients undergoing ventilation: Comparison of the usefulness of bronchoalveolar lavage and the protected specimen brush. Am J Med 1988; 85:499-506

11. Fabregas N, Torres A, El-Ebiary M, Ramirez J, Hernandez C, Gonzalez J, Puig de la Bellacas J, de Anta J, Rodriguez-Roisin R: Histopathologic and microbiologic aspects of ventilator-associated pneumonia. ANESTHESIOLOGY 1996;84:760-71

12. Legall JR, Loirat P, Alperovitch A, Glaser P, Granthil C, Mathieu D, Mercier P, Thomas R, Villers D: A simplified acute physiology score for ICU patients. Crit Care Med 1984; 12:975-7

13. Knaus WA, Draper EA, Wagner DP, Zimmerman JE: APACHE II: A severity of diseases classification system. Crit Care Med 1985; 13:818-29

14. Weinberg PF, Matthay MA, Webster RO, Roskos KV, Goldstein IM, Murray JF: Biologically active products of complement on acute lung injury in patients with the sepis syndrome. Am Rev Respir Dis 1984; 130:791-6

15. Papazian L, Martin C, Albanese J, Saux P, Charrel J, Gouin F: Comparison of two methods of bacteriologic sampling of the lower

respiratory tract: A study in ventilated patients with nosocomial bronchopneumonia. Crit Care Med 1989; 17:461–4

16. Rouby JJ, Rossignon MD, Nicolas MH, Martin De Lassale E, Cristin S, Grosset J, Viars P: A prospective study of protected bronchoalveolar lavage in the diagnosis of nosocomial pneumonia. ANESTHESIOLOGY 1989; 71:179–85

17. Johanson WG, Seidenfeild JJ, Gomez P, De Los Santos R, Coalson JJ: Bacteriologic diagnosis of nosocomial pneumonia following prolonged mechanical ventilation. Am Rev Respir Dis 1988; 137:259–64

18. Andrews CP, Coalson JJ, Smith JD, Johanson WG: Diagnosis of nosocomial bacterial pneumonia in acute, diffuse lung injury. Chest 1981; 80:254–8

19. Fagon JY, Chastre J, Hance AJ, Domart Y, Trouillet JL, Gibert C: Evaluation of clinical judgment in the identification and treatment of nosocomial pneumonia. Chest 1993; 103:547–53

20. Papazian L, Thomas P, Garbe L, Guignon I, Thirion X, Charrel J, Bollet C, Fuentes P, Gouin F: Bronchoscopic or blind sampling techniques for the diagnosis of ventilator-associated pneumonia. Am J Respir Crit Care Med 1995; 152:1982–91

21. Marquette CH, Copin MC, Wallet F, Neviere R, Saulnier F, Mathieu D, Durocher A, Ramon P, Tonnel AB: Diagnostic tests for pneumonia in ventilated patients: Prospective evaluation of diagnostic accuracy using histology as a diagnostic gold standard. Am J Respir Crit Care Med 1995; 151:1878–88

22. Niederman MS, Torres A, Summer W: Invasive diagnostic testing is not needed routinely to manage suspected ventilator-associated pneumonia. Am J Respir Crit Care Med 1994; 150:565–9

23. Cook DJ, Brun-Buisson C, Guyatt GH, Sibbald WJ: Evaluation of new diagnostic technologies: Bronchoalveolar lavage and the diagnosis of ventilator-associated pneumonia. Crit Care Med 1994; 22:1314–22

24. Dotson RG, Pingleton SK: The effect of antibiotic therapy on recovery of intracellular bacteria from bronchoalveolar lavage in suspected ventilator-associated nosocomial pneumonia. Chest 1993; 103:541–6

25. Solé-Violan J, Rodriguez de Castro F, Rey A, Martin-Gonzalez JC, Cabrera-Navarro P: Usefulness of microscopic examination of intracellular organisms in lavage fluid in ventilator-associated pneumonia. Chest 1994; 106:889–94

26. Aubas S, Aubas P, Capdevilla X, Darbas H, Roustan JP, Du Cailar J: Bronchoalveolar lavage for diagnosing bacterial pneumonia in mechanically ventilated patients. Am J Respir Crit Care Med 1994; 149:860–6

27. Chastre J, Fagon JY, Soler P, Domart Y, Pierre P, Dombret MC, Gibert C, Hance AJ: Quantification of BAL cells containing intracellular bacteria rapidly identifies ventilated patients with nosocomial pneumonia. Chest 1989; 95:190s–2s

28. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM: Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic "blind" bronchoalveolar lavage fluid. Am Rev Respir Dis 1991; 143:1121–9

29. Meduri GU, Beals DH, Maijub AG, Baselski V: Protected bronchoalveolar lavage. A new bronchoscopic technique to retrieve uncontaminated distal airway secretions. Am Rev Respir Dis 1991; 143:855–64

30. Baselski VS, El-Torki M, Coalson JJ, Griffin JP: The standardization of criteria for processing and interpreting laboratory specimens in patients with suspected ventilator-associated pneumonia. Chest 1992; 102:571s–9s