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Diagnostic value of direct examination of the protected specimen brush in ventilator-associated pneumonia

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ABSTRACT: Interpretation of the protected specimen brush (PSB) technique is based on quantitative bacterial cultures (QC), which unfortunately requires at least 24 h. We prospectively compared the diagnostic value of direct examination (DE) and QC of PSB specimens in 75 patients with suspected pneumonia. We also determined the optimal technique for DE.

QC was performed using the serial dilution technique. From the original suspension, two cytospin slides were obtained and stained by the May-Grünwald Giemsa (MGG) and the Gram method for DE. If the prescreening on the MGG-stained slide was positive, the morphology and the Gram staining of the organisms were assessed on the Gram-stained slide.

Using the 10^3 colony forming units (cfu·ml^{-1}) threshold for defining PSB as positive or negative, DE had a sensitivity of 85% and a specificity of 94%. In a parallel *in vitro* study, 18 pairs of PSB specimens were collected from respiratory secretions inoculated with *S. aureus*. From each pair, one brush was processed as described above and the other was smeared on a glass slide prior to performance of QC. Using direct smear instead of cytocentrifuged preparation, slightly but significantly affected QC.

Direct examination of cytospin slides is highly predictive of quantitative bacterial culture results, and provides rapid information regarding the Gram-stain morphology of the causative organisms. It may therefore guide initial therapy.
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Nosocomial pneumonia is one of the most serious complications in patients receiving mechanical ventilation [1–3]. Unlike the case of community-acquired pneumonia, combinations of clinical and radiological signs have a high rate of misdiagnosis in ventilator-associated pneumonia [4–6]. Thus, for epidemiological, clinical and therapeutic investigations new techniques have been developed, in order to establish a more accurate diagnosis of ventilator-associated pneumonia (VAP).

The protected specimen brush (PSB) technique, combined with quantitative culture techniques, was introduced by Wimberley and co-workers [7, 8] in the late 1970s. It has been extensively studied [6, 9–24], and shown to have an acceptable diagnostic yield in bacterial pneumonia both in non-intubated and intubated patients. It is now recommended as a reference method in the diagnostic assessment of VAP, provided that the diagnostic threshold of 10^3 colony forming units (cfu·ml^{-1}) is used [25–28]. The need for at least 24 h to obtain the result of quantitative culture is, however, the major problem with this technique. Direct examination of PSB specimens could overcome this problem, but the diagnostic yield of direct examination remains controversial [29–34]. Thus, the use of bronchoalveolar lavage (BAL) along with the PSB has been advocated, in order to obtain rapid diagnostic information based on the percentage of cells containing intra-cellular micro-organisms [32, 35, 36]. In fact, direct examination of BAL specimens seems to be an attractive method. However, performing BAL along with PSB carries an additional risk in critically ill patients, and is time-consuming, especially for the laboratory processing of the specimens. This latter drawback prevents the routine use of BAL as a diagnostic tool for VAP in many hospitals.

For these reasons, as suggested at the first International Consensus Conference on Clinical Investigation of Ventilator-Associated Pneumonia [28, 37], the present study was undertaken to prospectively evaluate whether the direct examination of the PSB specimen could predict the result of the quantitative culture and, thereby, help to guide the initial treatment of pneumonia.

Methods

Patient selection

The patients were studied over a 5 month period, from June 15 to November 15, 1992, in three intensive care

units (ICU) at the University Hospital of Lille, France. These ICUs comprise 70 beds and approximately 2,500 admissions·yr^{-1}. Patients were included in the study if they were mechanically-ventilated and if they had clinical signs suggestive of pneumonia. All had fever (>38°C), a new radiographic density, and either macroscopically purulent tracheal aspirates, leucocytosis or leucopenia. Informed consent was obtained from the patient or from the nearest relative.

Specimen collection

Patients were appropriately sedated in order to avoid "fighting" the ventilator. Fractional inspiratory oxygen (F_{I,O_2}) was adjusted to 100% and kept at this level throughout the procedure. Careful endotracheal suctioning was performed prior to bronchoscopy. The fibreoptic bronchoscope was then introduced into the endotracheal tube, through an adaptor designed to minimize air leak (Model 514900, Rüsch AG, Kernen, Germany), and advanced under direct vision next to the orifice of the bronchus selected according to the location of the infiltrate on the chest radiograph. Neither suction, nor topical anaesthetics were used. A plugged telescoping catheter brush (Model 89.01; TAG Medical, Bobigny, France) was passed through the working channel and advanced 2 cm beyond the tip of the fibreoptic bronchoscope. After ejecting the distal plug by protruding the inner cannula, the brush was advanced 2–4 cm beyond the tip of the inner cannula, gently rotated several times, and then retracted a few centimeters into the inner cannula. After removing from the fibreoptic bronchoscope, the whole catheter was immediately transported to the laboratory. The entire sampling procedure lasted less than 1 min.

Laboratory processing of specimens

Quantitative cultures. The distal portion of the catheter was wiped clean with 70% ethanol, cut with sterile scissors, and discarded. The brush was advanced beyond the tip of the transected inner cannula, aseptically severed with a wire-cutter and dropped into a sterile glass vial, containing 1 ml of Ringer's solution. The vial was then mechanically vortexed for 60 s. Three successive ten-fold dilutions were prepared, and 0.1 ml aliquots of the original suspension and of each dilution were plated on different media (Pasteur Production, Paris) for quantitative culture and identification: purple lactose agar, 5% blood Columbia agar, 5% blood Columbia agar (to which nalidixic acid (40 mg·l^{-1}) was added for *S. pneumoniae* cultures), and "chocolate" agar (to which bacitracin (30 UI·ml^{-1}) was added for *Haemophilus sp.* cultures). If positive, identification and countings of cfu·ml^{-1} of Ringer's solution were performed for each species. With this quantitative culture technique the threshold of detection of a single bacterial species was 10^1 cfu·ml^{-1} of Ringer's solution (representing a threshold of 10^4 cfu·ml^{-1} of respiratory secretions), since the amount of respiratory secretions collected with the brush is approximately 1 ml [7, 13, 38] and is diluted in 1 ml of holding medium (Ringer's solution).

Direct examination. Three drops of the original suspension were dropped into two cytospins, and centrifuged at 800×g for 5 min. The slides were stained by the May-Grünwald Giemsa (MGG) and the Gram method. The slides were examined at low magnification (25 fold) for the presence of squamous epithelial cells (SEC), polymorphonuclear neutrophils (PMN), ciliated bronchial cells and alveolar macrophages. Screening for micro-organisms was performed at high magnification (×100) on the MGG stained slide. If positive, the morphology and the Gram staining of the micro-organisms were observed on the Gram-stained slide.

In vitro *experiment*

In order to investigate whether the direct smearing of the brush on a glass slide could result in a loss of bacteria and, thereby, affect the quantitative culture results, we performed the following experiment. Eighteen endotracheal aspirates (EA), collected in intubated patients and known to grow sterile, were homogenized by mechanical vortexing after 10 min incubation with an equal volume of sterile 1% N-acetyl-1-cysteine (Mucomyst, Laboratoires Allard, Paris), and were inoculated with various concentrations of a marker organism (*S. aureus*). Eighteen pairs of PSB specimens were then collected from these EA. From each pair, one brush was processed as described above (cytocentrifuged PSB) and the other was first smeared on a glass slide (direct smear PSB). Both were then processed for bacterial culture. Quantitative culture results of cytocentrifuged PSB and direct smear PSB were compared, by using the paired t-test. For each brush, the volume of respiratory secretions released into the holding medium (Ringer's solution) was calculated by dividing the quantitative culture result of the PSB by the quantitative culture result of the original EA specimen.

Data analysis

Diagnostic groups. The patients were divided by the presence or absence of pneumonia according to the criteria defined in table 1. Therefore, a comprehensive work-up was performed, in order to identify the alternative causes of fever and radiographic densities. The diagnosis of atelectasis was defined by the complete disappearance of the radiographic density within 48 h. The diagnosis of left ventricular failure was defined by the presence of a suggestive haemodynamic profile on pulmonary artery catheterization and the resolution of radiographic signs with diuretics, inotropic drugs and/or vasodilators, as appropriate. When suspected, the diagnosis of a pleural-effusion-related opacity was confirmed by pleural echography, and complete resolution of the radiographic abnormality was checked after pleural drainage. The diagnosis of pulmonary contusion was based on a typical history of chest trauma, associated with the presence of active bleeding in the segmental or subsegmental bronchi leading to the lung area concerned.

Table 1. – Diagnostic criteria for pneumonia in mechanically-ventilated patients

Definite pneumonia (Group I)
Pathogenic evidence of pneumonia on histological examination of the lung tissue obtained by open lung biopsy or at postmortem examination within 3 days of the diagnostic procedure. Pneumonia was defined as an area of consolidation with intense PMN accumulation in the bronchioles and alveolar airspaces, with or without abcess formation.
Definite absence of pneumonia (Group II)
Absence of pathogenic evidence of pneumonia on histological examination of the lung tissue obtained by open lung biopsy or at postmortem examination within 3 days of the diagnostic procedure.
Probable pneumonia (Group III)
III a: presence of positive blood cultures unrelated to an extrapulmonary source within 48 h before or after the respiratory sampling or positive culture of pleural fluid, provided the recovered micro-organism is identical to the organism recovered from a culture of lower respiratory tract secretions; or
III b: chest X-ray or CT-scan evidence of rapid cavitation; or
III c: positive PSB sample ($\geq 10^3$ cfu·ml^{-1}) with a clinical course consistent with pulmonary bacterial infection.
Probable absence of pneumonia (Group IV)
IV a: resolution of fever or radiographic infiltrates without antibiotic therapy; or
IV b: negative PSB sample ($<10^3$ cfu·ml^{-1}) with a definite alternative diagnosis.
Uncertain diagnosis (Group V)
Any situation where the patient could not be classified in one of the previous groups.

PMN: polymorphonuclear neutrophils; CT: computed tomography; PSB: protected specimen brush; cfu: colony forming units.

Pulmonary densities erroneously described as "recently appeared" after first examination on admission in the ICU were retrospectively well classified by careful reviewing of historical chest roentgenograms.

Timing of the suspected pneumonia. Using 48 h after intubation and initiation of mechanical ventilation [39] as a time marker, the patients were divided into three groups, depending on the time elapsed between intubation and the appearance of clinical and radiological signs suggestive of pneumonia. Group 1 (onset of pneumonia prior to intubation: pneumonia was the primary indication for ventilatory support *per se*); Group 2 (early onset pneumonia, <48 h); and Group 3 (late onset pneumonia, >48 h).

Antibiotic (ATB) status. In order to assess the effects of antibiotic treatment on PSB results, patients were divided into four groups, according to the following criteria: Group 1 (patients on antibiotics for more than 48 h before respiratory sampling; Group 2 (antibiotics started or changed less than 48 h before respiratory sampling); Group 3 (patients without previous antibiotics); and Group 4 (antibiotics stopped at least 48 h before respiratory sampling).

Statistical analysis

Predictive value of direct examination (DE) of the PSB specimens on quantitative culture (PSB-QC) results. To assess whether DE could predict the PSB-QC results, the results of DE were divided into four categories. As for PSB a value $\geq 10^3$ cfu·ml^{-1} is considered as a significant level for bacterial pneumonia [7, 28, 29], DE was considered as "truly positive" if each morphotype present was also grown at significant concentration ($\geq 10^3$ cfu·ml^{-1}), as "truly negative" if no organism was present on examination, and quantitative culture was either sterile or positive at low concentration ($<10^3$ cfu·ml^{-1}); as "falsely positive" if a morphotype present on examination was not grown at significant concentration; and as "falsely negative" if a microorganism was grown at significant concentration ($\geq 10^3$ cfu·ml^{-1}) but was not seen on direct examination, even if another morphotype was present on DE. In the case of polymicrobial cultures, all the bacterial isolates grown at significant concentration had to be morphologically present on DE. If not, the case was classified as false negative (*e.g.* patients No. 10 and 12, in table 2c).

Diagnostic yield of PSB-QC for identifying pneumonia. Since the result of PSB-QC was used in some patients to define the presence or the absence of pneumonia (criteria IIIc and IVb, table 1), only the patients in whom the diagnosis did not depend on their PSB-QC results were included for calculating the sensitivity and the specificity of PSB-QC (*i.e.* the patients meeting the criteria I, II, IIIa, IIIb, and IVa, as defined in table 1).

Results

Population studied

Seventy five patients (47 men and 28 women) were included. Mean age was 62±20 (SD) yrs (range 18–90 yrs). The average number of ventilator-days was 12±16 days (range 1–71 days). The primary indication for ventilatory support included exacerbation of chronic obstructive pulmonary disease (n=15), neurological emergencies (n=14), nosocomial pneumonia (n=7), multiorgan failure associated with sepsis (n=9), acute cardiac failure (n=6), postoperative respiratory failure (n=7), multitrauma (n=7), community-acquired pneumonia (n=4), status asthmaticus (n=1), miscellaneous (n=5).

Six patients were classified in Group I as defined in table 1 (definite pneumonia), on the basis of histologic

examination of autopsy specimens (n=5), or open lung biopsy specimens (n=1).

Six patients were classified in Group II (definite absence of pneumonia), on the basis of histological examination of autopsy specimens (n=3), or open lung biopsy specimens (n=2). Diagnoses in these patients were pulmonary fibrosis (n=1), pulmonary oedema (n=2), diffuse alveolar damage (n=1), inhalation pneumonia (n=1), bronchiolitis (n=1). The last patient in Group II was a 40 year old man with a severe community-acquired pneumonia requiring mechanical ventilation on the third day of hospitalization (patient No. 1 on table 2b). The diagnosis of legionellosis was established by the positivity of the direct immunofluorescence test performed on the BAL fluid (BAL was performed along with the PSB). Since the present study investigated the usefulness of the PSB in the assessment of pulmonary infections due to pyogenic bacteria (as opposed to other infectious or non-infectious pulmonary disorders) this patient was classified in Group II.

Twenty three patients were classified in Group III (probable presence of pneumonia). Three fulfilled the criteria IIIa, four fulfilled both criteria IIIa and IIIc, two fulfilled the criteria IIIb, and 14 fulfilled the criteria IIIc alone (tables 1 and 2c).

Twenty five patients were classified in group IV (probable absence of pneumonia). Five patients fulfilled the criteria IVa and 24 the criteria IVb (tables 1 and 2d). Alternative diagnoses in these patients were atelectasis (n=8), left ventricular failure (n=8), left ventricular failure with catheter-related septicaemia (n=1), catheter- related septicaemia (n=1), pleural effusion (n=2), pulmonary contusion (n=1), pulmonary sequelae of tuberculosis (n=1), coal-worker's pneumoconiosis related opacities (n=1), and pulmonary haemorrhage (n=1).

The remaining patients (n=5) were classified in group V (uncertain diagnosis) (tables 1 and 2e).

Forty one patients (55%) were on antibiotics by the time of the study (ATB status Group 1 and 2), 39 had received antibiotics for more than 48 h, and in only two patients had antibiotics been started within less than 48 h before respiratory sampling (table 2). Twenty three patients (31%) were free of antibiotics by the time of the study (ATB status Group 3), and in 11 patients antibiotics had been stopped at least 48 h before the respiratory sampling (ATB status Group 4).

Nineteen events were classified as early onset suspected pneumonia ("timing of pneumonia 2", table 2). Forty seven were classified as late onset suspected pneumonias ("timing of pneumonia 3"). In nine patients, the suspected pneumonia under investigation was the primary indication of ventilatory support. Two of these latter cases were severe community-acquired pneumonias (patient No. 1 in table 2b and patient No. 12 in table 2c). Four were suspected nosocomial pneumonias in immunosuppressed patients (patient No. 1 and 15 in table 2c, patient No. 14 in table 2d, and patient No. 2 in table 2e). One was retrospectively classified as a pulmonary contusion (patient No. 18 in table 2d). In the two remaining patients (patient No. 12 and 14 in table 2e) no definite diagnostic could be established.

No serious complication was noted during or after the diagnostic procedures. In two patients, probably because of insufficient sedation, severe coughing occurred, resulting in a traumatic procedure with minor bronchial bleeding.

Results of cytological examination of PSB specimens

Seven PSB specimens (9.3%) demonstrated the presence of squamous epithelial cells on DE, most frequently in small amounts (≤1 per field). As expected, most of the specimens were positive for the presence of PMN (87%) and ciliated bronchial cells (97%). Alveolar macrophages were present in more than half of the specimens (59%).

Predictive value of direct examination of the PSB specimens on quantitative culture results

Using the 10^3 cfu·ml^{-1} threshold for defining a positive or a negative PSB specimen, direct examination had a positive predictive value (PPV) of 88%, a negative predictive value (NPV) of 92%, a sensitivity of 85%, a specificity of 94% and a diagnostic accuracy rate (DAR) of 91%. Eliminating the seven patients with SEC present on direct examination from the analysis did not significantly modify these results. Three out of the four cases classified as false negative *(i.e.* DE did not demonstrate a morphotype which subsequently grew at significant concentration in quantitative culture) were related with *A. baumanii* infections.

Antibiotics did not interfere with the ability of DE to predict the results of quantitative cultures, since the sensitivity, the specificity, the PPV, and the NPV were, respectively, 85%, 96%, 92% and 93% in the patients who were on antibiotics (ATB status Group 1 or 2) and 85%, 90%, 85% and 90% in the patients without antibiotics (ATB status Group 3 or 4).

Diagnostic yield of direct examination of the PSB specimens (DE) for identifying pneumonia

DE was positive in 4 of the 6 patients with definite pneumonia (table 2a), and in 21 of the 29 patients with definite or probable pneumonia (table 2a and 2c). Therefore, the sensitivity of DE for identifying pneumonia was 67% or 72%, according to the stringency of the criteria retained for defining pneumonia. Similarly, DE was negative in 4 of the 6 patients with definite absence of pneumonia (table 2b), and in 27 of the 31 patients with definite or probable absence of pneumonia (table 2b and 2d). Therefore, the specificity of DE for identifying pneumonia was 67% or 87%, according to the stringency of the criteria retained for defining pneumonia.

Diagnostic yield of PSB with quantitative cultures (PSB-QC) for identifying pneumonia

Thirty four patients in whom the diagnosis partly relied on the result of PSB-QC were excluded from this

Table 2a. – Group I (definite pneumonia); results of quantitative culture and direct examination of protected specimen brush (PSB) specimens

Pt No.	ATB status	Timing of pneumonia	Bacterial isolate	Quantitative culture cfu·ml^{-1}	Direct examination	PVDE	Blood culture
1	1	3	*S. aureus*	1×10^4	GPC+GNB	FP	*S. aureus*
2	1	3	–		*	TN	
3	3	2	*E. coli*	5×10^4	GPC+GNB	TP	*E. coli*
			S. pneumoniae	2×10^3			
4	3	3	*S. aureas*	7.5×10^3	GPC	TP	
			α-haemolytic streptococci	8×10^4			
5	3	2	*S. aureus*	2×10^3	GPC	TP	
6	3	3	–		*	TN	

Antibiotic (ATB) status: 1) on antibiotics for more than 48 h before respitratory sampling; 2) antibiotics started or changed within less than 48 h before respiratory sampling; 3) patients without previous antibiotics; 4) antibiotics stopped at least 48 h before respiratory sampling. Timing of pneumonia: 1) onset of pneumonia prior to intubation; 2) early onset pneumonia (≤48 h): 3) late onset pneumonia (>48 h). Bacterial isolate: (–) no growth on quantitative bacterial cultures. Direct examination: (GPC) Gram-positive cocci; (GNB) Gram-negative bacilli; (GNCB) GRam-negative coccobacilli; (*) no micro-organism. PVDE: predictive value of direct examination of the PSB specimens on quantitative culture (PSB-QC) results (see Statistical analysis in the text): (TP) true positive; (TN) true negative; (FP) false positive; (FN) false negative.

Table 2b. – Group II (definite absence of pneumonia); results of quantitative culture and direct examination of PSB specimens

Pt No.	ATB status	Timing of pneumonia	Bacterial isolate	Quantitative culture cfu·ml^{-1}	Direct examination	PVDE	Blood culture
1	1	1	–		o	TN	
2	1	2	*P. aeruginosa*	1.5×10^4	GNB	TP	*S. aureus*
3	1	3	*K. pneumoniae*	1×10^6	GNB	TB	
4	1	2	–		*	TN	
5	3	3	–		*	TN	
6	4	3	*A. baumannii*	7×10^2	*	TN	

For explanation and definition of ATB status; Timing of pneumonia; Bacterial isolate; Direct examination; PVDE; see legend to table 2a. o: positive direct immunofluorescence for legionellosis on BAL; *: no micro-organisms.

Table 2c. – Group III (probable presence of pneumonia; results of quantitative culture and direct examination of PSB specimens

Pt No.	Diagnostic subgroup	ATB status	Timing of pneumonia	Bacterial isolate	Quantitative culture cfu·ml^{-1}	Direct examination	PVDE	Blood culture
1	a+c	1	1	*P. auruginosa*	5×10^6	GNB	TP	*P. aeroginosa*
2	a+c	1	3	*A. baumannii*	1.5×10^4	GNB	PN	*A. baumannii*
				Pseudomonas sp.	10^4			
3	a+c	1	2	*E. coli*	2.5×10^4	GNB	TP	*E. coli*
4	c	1	3	*P. aeruginosa*	3×10^3	GNB	TP	
				X. maltophilia	1×10^2			
5	c	1	3	*P. aeruginosa*	4×10^4	GNB	TP	
				X. maltophilia	1.9×10^4			
6	c	1	3	*S. mitis*	2.5×10^4	GPC	TP	
7	b	1	3	–		*	TP	
8	c	1	3	*P. aeruginosa*	2×10^3	GNB	TP	
9	c	1	3	*P. aeruginosa*	1.8×10^4	GNB	TP	
10	c	1	3	*A. baumannii*	3.5×10^5	GPC	FN	
11	c	1	3	*A. baumannii*	1×10^3	GNCB	TP	
12	a	2	1	–		*	TN	*S. pneumoniae*
13	a	3	2	*S. aureus*	5×10^1	*	TN	*S. aureus*
14	b	3	2	–		*	TN	
15	c	3	1	*P. aeruginosa*	5×10^3	GNB	TP	
				C. albicans	4×10^3			
16	c	3	3	*S. aureus*	1.2×10^4	GPC	TP	
17	c	3	3	*S. aureus*	1.8×10^3	GPC	TP	
				Streptococcus sp.	9×10^3			
18	c	3	2	*P. aeruginosa*	1×10^3	GNB	TP	
				S. aureus				
19	a	3	3	*S. aureus*	2×10^2	*	TN	*S. aureus*°
20	c	4	3	*S. aureus*	1.6×10^5	GPC	TP	
21	c	4	3	*P. mirabilis*	1.2×10^3	*	FN	
22	a+c	4	3	*S. aureus*	6×10^5	GPC	FN	*S. aureus*
				P. aeruginosa	8×10^5	GNB		
				A. baumannii	3.2×10^6			
23	c	4	3	*P. aeruginosa*	1.3×10^5	GNB	TP	
				K. pneumoniae	1×10^2			

For diagnostic subgroups see legend to table 1. For explanation and definitions of ATB status; Timing of pneumonia; Bacterial isolate; Direct examination; and PVDE; see legend to table 2a. °: micro-organism isolated from pleural fluid.

Table 2d. – Group IV (probable absence of pneumonia): results of quantitative culture and direct examination of PSB specimens

Pt No.	Diagnostic subgroup	ATB status	Timing of pneumonia	Bacterial isolate	Quantitative culture cfu·ml-1	Direct examination	PVDE	Blood culture
1	b	1	2	–		*	TN	
2	b	1	3	–		*	TN	
3	b	1	3	–		*	TN	*S. aureus*
4	b	1	2	–		*	TN	
5	b	1	3	–		*	TN	
6	b	1	3	–		*	TN	
7	b	1	3	–		*	TN	
8	a	1	3	–		*	TN	
9	b	1	2	–		*	TN	
10	b	1	2	–		*	TN	
11	b	1	3	*P. aeruginosa*	1×10^{1}	*	TN	
				A. baumannii	3×10^{1}			
12	b	2	2	–		*	TN	
13	a+b	3	2	–		*	TN	
14	b	3	1	–		GNB	FP	*E. coli*
15	b	3	2	–		*	TN	
16	a+b	3	2	*S. aureus*	2×10^{2}	*	TN	
				P. mirabilis	3×10^{1}			
17	b	3	2	–		*	TN	
18	a+b	3	1	–		*	TN	
19	b	3	3	–		*	TN	
20	b	4	3	*C. albicans*	3×10^{5}	Yeasts	TP	
21	b	4	3	–		*	TN	
22	b	4	3	–		*	TN	
23	b	4	3	–		*	TN	
24	a+b	4	3	–		*	TN	
25	b	4	3	*P. aeruginosa*	1×10^{2}	*	TN	
				S. marcescens	3×10^{2}			

For diagnostic subgroups see legend to table 1. For explanation and definition of ATB status; Timing of pneumonia; Bacterial isolate; Direct examination; and PVDE, see legend to table 2a.

Table 2e. – Group V (uncertain diagnosis); results of quantitative culture and direct examination of PSB specimens

Pt No.	Diagnostic subgroup	ATB status	Timing of pneumonia	Bacterial isolate	Quantitative culture cfu·ml-1	Direct examination	PPDE	Blood culture
1		1	2	–		*	TN	
2		1	1	*E. faecalis*	1×10^{5}	GPVC	TP	*S. agalectiae*
3		1	3	–		*	TN	
4		1	3	–		*	TN	
5		1	3	–		*	TN	
6		1	3	–		*	TN	
7		1	3	*S. aureus*	2×10^{1}	*	TN	
8		1	3	–		*	TN	
9		1	3	–		*	TN	
10		1	3	*S. aureus*	2×10^{1}	*	TN	
11		1	3	–		*	TN	*S. aureus*
12		3	1	*P. aeruginosa*	9×10^{2}	GNB	FP	
				E. cloacae	1×10^{2}			
				S. aureus	8×10^{1}			
13		3	3	*S. aureus*	7×10^{3}	GPC	TP	
				M. mirabilis	1×10^{2}			
				A. baumannii	3×10^{2}			
				E. coli	2×10^{1}			
14		3	1	–		*	TN	
15		3	2	–		*	TN	

For diagnostic subgroups see legend to table 1. For explanation and definition of ATB status; Timing of pneumonia; Bacterial isolate; Direct examination and PVDE, see legend to table 2a.

analysis (*cf.* statistical analysis in methods). Sensitivity of PSB-QC for identifying pneumonia was 53% and specificity was 82%, if only the 12 patients with a definite diagnosis and the 14 patients (9 and 5 patients from Group III and IV, respectively) in whom the diagnosis did not depend on the PSB-QC result were considered.

Within this subgroup of 26 patients, analysing separately the patients according to their "antibiotic status" did not significantly modify the diagnostic yield of PSB-QC. Indeed, sensitivity and specificity were respectively 57% and 60% in patients on antibiotics (ATB status Group 1 or 2) and 50% and 100% in patients off antibiotics (ATB status Group 3 or 4). Given the small sample size, these differences are not statistically significant. The only case where antibiotics clearly influenced the result was patient No. 12 in Group III (table 2c). This patient was admitted because of severe community-acquired pneumonia and received antibiotics (amoxycillin) 12 h before the respiratory sampling. Blood culture grew *S. pneumoniae* but PSB was negative both by direct examination and culture, although bronchoscopy demonstrated frank purulent respiratory secretions.

In vitro *experiment*

Cytocentrifuged PSB obtained slightly but significantly ($p<0.05$) higher bacterial counts than direct smear PSB. The mean (±SD) difference was 0.26±0.3 $\log_{10}$ (range 0.9–1.08 $\log_{10}$). In all but one pair this difference was less than one $\log_{10}$, which is the maximal precision affordable with quantitative bacterial cultures. The mean (±SD) amount of respiratory secretions released into the holding medium by the cytocentrifuged PSB was 2.37±2.07 µl (range 0.05–9.23 µl). The mean (±SD) amount of respiratory secretions released into the holding medium by the direct smear PSB was 1.68±1.77 µl (range 0.02–3 µl). The mean (±SD) amount of respiratory secretions lost for quantitative culture by the direct smear procedure was 0.68±0.75 µl (range 0–3 µl).

Discussion

The present study investigated the usefulness of direct examination of respiratory specimens obtained with the protected specimen brush technique. Two conclusions emerge from this study. Firstly, direct examination correctly predicts the results of quantitative bacterial cultures and can partially identify (Gram-stain morphology) the micro-organisms subsequently growing at significant concentrations. Secondly, cytocentrifuged preparations are preferable to direct smears, since this latter technique can affect the results of quantitative cultures.

PSB with quantitative cultures is considered to be a reliable tool for identifying pneumonia in mechanically-ventilated patients. However, the length of time necessary to obtain the result of quantitative cultures (≥24 h) and to start specific antimicrobial therapy may be harmful to critically ill patients, unless broad-spectrum antibiotics are empirically started soon after the diagnostic procedure. The low sensitivity of Gram-stain of PSB specimens [31, 32] prompted several authors to use microscopic analysis of BAL along with quantitative cultures of PSB specimens [32, 35, 36]. Although the advantages and limits of BAL need to be further established, it seems that microscopic analysis of BAL provides both rapid identification of patients with pneumonia (diagnosis based on the presence of intracellular micro-organisms within a certain percentage of cells, which still needs to be clearly defined), and bacteriological information (morphology on Gram-stain) allowing the early initiation of appropriate therapy. These potential advantages of BAL must yet be weighed against two limits. Firstly, performing BAL carries an additional risk in severely hypoxic patients or in patients with underlying chronic obstructive pulmonary disease (COPD) where the percentage of retrieved fluid may be very low. Secondly, microscopic analysis of BAL is much more complex to perform than PSB, thus preventing its routine use in many centres. As PSB is easy to perform, and since only few data are available regarding the usefulness of direct examination of PSB specimens in VAP, the first International Consensus Conference on Clinical Investigation of Ventilator-Associated Pneumonia underscored the need of further evaluation of this technique [28, 37].

From the results of this prospective study, it appears that direct examination of PSB specimens is a useful adjunct to quantitative culture, since it accurately identifies the patients where PSB specimens will grow at levels considered significant for bacterial pneumonia (10^3 cfu·ml^{-1}) and also provides reliable bacteriological information regarding the morphotypes of the organisms subsequently cultured at significant concentration. Direct examination can, however, hardly predict infections due to *A. baumanii*. This is due to the fact that, depending on its state of development, these Gram-negative coccobacilli can present either as Gram-negative bacilli or as Gram-positive cocci [40].

Our results are in accordance with those studies where direct examination was performed on slides prepared directly from the brushes. TEAGUE *et al.* [30] used two brushes, one for bacterial culture and one for direct examination (direct smear of the brush onto a glass slide) and showed that Gram-stain correlated with the growth of bacteria in concentration ≥10^6 cfu·ml^{-1} (corresponding to a growth ≥10^3 cfu·ml^{-1} of Ringer's solution), and they failed to show bacteria in all cases in which growth on culture was ≤10^5 cfu·ml^{-1} (which corresponds to a growth of ≤10^2 cfu·ml^{-1} of Ringer's medium). Similarly POLLOCK *et al.* [29] reported a 76% sensitivity when smearing the brush directly onto a glass slide before putting it into the holding medium. In contrast, studies using cytocentrifuged Gram-stain reported a lower sensitivity of the method [31, 34]. We believe that our good results using cytocentrifuged specimens can be accounted for by the prescreening technique. Indeed, detecting microorganisms on a Gram-stained slide full of bronchial ciliated cells, alveolar macrophages and polymorphonuclear cells is long and tedious and has, therefore, a low sensitivity. The prescreening for bacteria on MGG-stained slides, at high magnification, is much easier. We therefore performed a prescreening for bacteria on the MGG-stained

slides and, if positive, we examined the Gram-stained slide in order to precisely determine the morphology of the bacteria detected. The whole procedure, including serial dilutions for quantitative culture and preparation of cytocentrifuged stains, takes about 45 min, with 2–5 min devoted to the direct examination *per se*.

It is noteworthy that, in this series, antibiotics did not interfere with the close correlation between DE and quantitative culture results. The strict antibiotic policy in our institution may explain this finding. Actually, with the exception of two patients, antimicrobial therapy was never inadvertently started or modified before the diagnostic procedure (table 2). If a suspected pneumonia developed whilst a patient was receiving antibiotics, we either performed the diagnostic procedure without modifying the ongoing therapeutic regimen, or we stopped it for at least 48 h before the bronchoscopy. If the patient was not on antibiotics, the diagnostic procedure was planned before starting antimicrobial therapy. We cannot exclude the possibility that without following such a strict policy the result would have been different.

Unfortunately, DE of PSB specimens does not do better than quantitative culture for identifying patients with pneumonia. Indeed, unlike microscopic analysis of BAL specimens, DE of PSB specimens does not provide additional helpful diagnostic information, such as the presence of intracellular organisms. Eventually, from a clinical standpoint, the advantage of DE consists in rapid and reliable prediction of quantitative culture results, which in turn allows early and appropriate therapeutic decisions in most of the patients.

In this study, quantitative culture of PSB specimens (PSB-QC), using the 10^3 cfu·ml^{-1} recommended diagnostic threshold, demonstrated an acceptable specificity but a relatively low sensitivity, compared to other studies [6, 11]. This limitation of our study can be explained by several factors. Firstly, the study design was primarily planned in order to compare DE and quantitative culture results, and not to assess the ability of either technique for identifying pneumonia *per se*. Secondly, the operating characteristics of PSB-QC were deliberately calculated from a small sample of patients meeting very stringent diagnostic criteria *(i.e.* criteria I, II, IIIa, IIIb, and IVa, as defined in table 1), which was not the case in many previous studies. Thirdly, even in the patients with a pneumonia proven at autopsy, the respiratory specimens were obtained before death (within 3 days), and, therefore, were perhaps more "realistic" than postmortem collected specimens, which have been reported 100% sensitive [11]. Finally, the intrinsic variability of the PSB technique [41], which can result in borderline quantitative culture results [24] even in real cases of pneumonia, can account for a somewhat low sensitivity, especially when this index is calculated from a small sample size.

By the end of this study, the high diagnostic yield of direct examination prompted us to simplify the technique by using direct smears instead of cytocentrifuged preparations. However, unless two brushes for each individual patient are used, as Teague *et al.* [30], direct smearing of the brush carries two risks. Firstly, the procedure can potentially contaminate the specimen which is subsequently cultured, although a careful technique can overcome this problem. Secondly, and more importantly, the loss of a significant amount of bacteria on the glass slides can affect the result of quantitative culture. We could confirm this latter risk in an *in vitro* experiment by demonstrating that about 0.68 µl of the collected specimen is released onto the glass slide from the brush. This slightly, but significantly, affected quantitative cultures. Using direct smears instead of cytocentrifuged preparations would, therefore, be an additional factor affecting the intrinsic variability of the PSB technique [41].

In summary, direct examination of cytocentrifuged PSB specimens is a useful adjunct to quantitative culture in the assessment of bacterial pneumonia, provided that a prescreening on MGG-stained slides is performed before examining the Gram-stained slides. This technique appears to be highly predictive of quantitative culture results and provides rapid information (Gram-stain morphology) about the causative organisms. Therefore direct examination of PSB specimens may help to guide initial therapeutic decisions.

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