Diagnosis of Ventilator-associated Pneumonia by Bacteriologic Analysis of Bronchoscopic and Nonbronchoscopic "Blind" Bronchoalveolar Lavage Fluid[1,2]

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SUMMARY Substantial efforts have been devoted to improving the means for early and accurate diagnosis of ventilator-associated (VA) pneumonia in intensive care unit (ICU) patients because of its high incidence and mortality. A good diagnostic yield has been reported from quantitative cultures of bronchoalveolar lavage (BAL) fluid or a protected specimen brush, both obtained by fiberoptic bronchoscopy. As bronchoscopy requires specific skills and is costly, we evaluated a simpler method to obtain BAL fluid, that is, by a catheter introduced blindly into the bronchial tree. Quantitative cultures from bronchoscopically sampled BAL (B-BAL) and blindly nonbronchoscopically collected BAL (NB-BAL) were assessed for sensitivity, specificity, and predictive value for the diagnosis of VA pneumonia. A total of 40 pairs of samples were examined in 28 patients requiring prolonged mechanical ventilation and presenting a high risk of developing pneumonia. For comparison with bacteriologic data we defined a clinical score for pneumonia ranging from zero to 12 using the following variables: body temperature, leukocyte count, volume and character of tracheal secretions, arterial oxygenation, chest X-ray, Gram stain, and culture of tracheal aspirate. To quantify the bacteria in BAL the bacterial index (BI) was used, defined as the sum of the logarithm of the number of bacteria cultured per milliliter of BAL fluid. A good correlation between clinical score and quantitative bacteriology was observed ($r = 0.84$ for B-BAL and 0.76 for NB-BAL; $p < 0.0001$). Similar to studies in baboons, patients with pulmonary infection could be distinguished by a BI ≥ 5 with a sensitivity of 93% and a specificity of 100% (B-BAL). Quantitative culture of blind sampling of BAL resulted in a slightly lower sensitivity (73%) and a specificity of 96% for the diagnosis of pneumonia. When analyzing pairs of B-BAL and NB-BAL samples we found similar results for both qualitative and quantitative bacteriology even if BAL fluids came from different lobes or the contralateral lung. These results suggest that "blind" sampling of BAL can be of value in clinical practice. Microscopic examination of BAL provided rapid (the day of BAL), sensitive (100%), and specific (88%) results, allowing us to introduce early and specific antibiotic therapy.

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Introduction

Bacterial pneumonia is a frequent complication in patients requiring mechanical ventilation in an intensive care unit (ICU) and carries a significant mortality. Craven and colleagues reported a 20% incidence of nosocomial pneumonia in a general ICU (1), but this figure is higher in certain conditions, such as the adult respiratory distress syndrome (ARDS) (2). The risk of developing ventilator-associated (VA) pneumonia rises linearly by about 1%/day, with most of the pneumonias occurring in the first 8 days after intubation (3, 4). The mortality rate of VA pneumonia reaches 50 to 70% (1, 3–6).

This condition presents a major diagnostic challenge because of the low yield of clinical criteria, such as fever, leukocytosis, or radiology (2, 3, 7). When these parameters are combined the predictive value is higher, but still less than 100%, particularly in ARDS (7). The distinction between tracheobronchial colonization and pulmonary infection is also difficult if the standard semiquantitative method is used for culture of tracheal aspirates (8). Using quantitative cultures with a threshold of 10^7 bacteria/ml allows one to exclude false positive results due to contamination or colonization (9). Salata and coworkers report a significant difference between infected and colonized patients with this method (10^6 versus 10^4 bacteria/ml), but a large overlap between the two groups results in a low specificity of this costly technique (5). The same authors showed that the finding of 3+ bacteria on a Gram stain of bronchial secretions together with the presence of elastin fibers on a KOH stain was associated with a high probability of nosocomial pneumonia.

A more invasive approach has been proposed by Chastre and colleagues (10), that is, bronchoscopy and quantitative culture of a protected specimen brush (PSB). By using 10^3 bacteria/ml as a threshold the sensitivity was almost 90% and the positive predictive value above 75% for the detection of VA pneumonia (3, 11). The disadvantages of a PSB technique are (*1*) high cost, (*2*) significant morbidity (1 to 25% incidence of pneumothorax or bleeding) (7, 11–13), (*3*) false negative results (14, 15), and (*4*) the 24- to 48-h delay in obtaining the results of bacterial cultures (14). To overcome the problem of delay Chastre and coworkers combined PSB with counting polymorphonuclear leukocytes (PMN) containing intracellular bacteria in bronchoalveolar lavage (BAL) fluid (14). VA pneumonia was associated with the presence of more than 15% of infected PMN.

Johanson and colleagues evaluated quantification of bacteria in BAL fluid for the diagnosis of pneumonia in a baboon model (15). In this study the correlation was good between the quantitative culture of bacteria in BAL fluid and both the quantitative cultures of the pulmonary lobe sampled as well as the most infected lobe at the time of sacrifice. These results suggest that there are no

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significant differences in the bacteriologic findings when sampling is done in different sites. This important point remains to be demonstrated in ventilated patients with a radiologically localized infiltrate. Johanson's group also observed a comparable specificity for the results of BAL and PSB in the baboon model, but the sensitivity was clearly superior for BAL (100 versus 62%) (15). Torres and coworkers found an equivalent diagnostic yield with both techniques in mechanically ventilated patients (11). Quantitative culture of bronchoscopic BAL (B-BAL) was used recently in immunocompromised patients. Growth of more than 10^5 bacteria/ml in BAL fluid was associated with a pneumonia (16, 17).

B-BAL needs expertise and training. Nonbronchoscopic, that is, "blind" BAL (NB-BAL) sampling is much simpler and was first used in the acquired immunodeficiency syndrome (18, 19) and then in intubated patients in the ICU (20, 21). Qualitative bacteriologic results from NB-BAL gave results similar to those obtained by PSB or postmortem culture (20, 21). However, no attempt was made to differentiate between colonized and infected patients.

The aims of the present study were first to validate a new, blind sampling method that is less costly and simpler to perform by comparing quantitative bacteriology obtained by BAL with a fiberoptic bronchoscope with BAL sampled blindly with a new catheter. Second, we attempted to determine early criteria for the diagnosis of pulmonary infection by microscopic examination of BAL. Our findings suggest that blind BAL sampling combined with quantitative bacteriology gives an accurate diagnosis of pneumonia. Rapid diagnosis is possible using the Gram stain together with a staining technique for elastin fibers in BAL fluid.

Methods

Patients

Over a 6-month period from January 1 to June 30, 1989, 28 ventilated patients at high risk for developing a nosocomial pneumonia were studied in the division of surgical intensive care at the University Hospital, Geneva, Switzerland. This is a 20-bed unit with 1,300 admissions/yr. Patients were included in the study if they had been intubated and mechanically ventilated for more than 72 h and had a recognized predisposing pathology, such as multiple trauma, cerebral trauma, major emergency surgery, esophagectomy, or postoperative complications of cardiovascular surgery. Of these 28 patients 19 were studied once (68%), 6 were studied twice several days apart (21%), and 3 were investigated three times (11%). A total of 40 episodes were studied.

The APACHE II score was used to assess the severity of the clinical condition at the time of admission to the ICU (22). The percentage of patients with ARDS and the number of days of prior and total mechanical ventilation were noted. The 1-month mortality was recorded. Postmortem findings (if autopsy was performed) were noted.

The study was approved by the committee for ethics in human research of our institution. Informed consent was obtained from the patient or, if this was not possible because of the clinical condition, by a member of the family or the treating physician.

Clinical Variables

The following variables were recorded for the 2 days before the study and on the day of investigation: (*1*) body temperature; (*2*) blood leukocyte count and number of band forms; (*3*) character of tracheal secretions (purulent or not) and quantity of tracheal aspirates (for each endotracheal aspiration the nurses estimated the quantity of secretions from 0 to 4+s; estimation of the volume of total secretions per day was calculated by adding all the + values recorded over 24 h together; to ensure a certain uniformity of this estimation only nurses having at least 2 yr of continuous training in our ICU participated in this investigation; the total number of tracheal aspirations also was recorded); (*4*) microscopic examination (Gram stain) and semiquantitative culture of the bronchial secretions; (*5*) arterial oxygen tension/inspiratory fraction of oxygen (Pa_{O_2}/FI_{O_2}); and (*6*) chest X-ray. The presence of bacteremia or positive culture of the pleural fluid in the 5 days preceding and the 5 days following BAL were compared with bacteria cultured from sputum and in BAL fluids.

A clinical pulmonary infection score (CPIS) with a range of 0 to 12 was obtained from these clinical parameters (table 1). The range and weighting for temperature, leukocytes, sputum culture, and character as well as chest X-ray were determined from published studies (2, 22–25). The range and weight for the Pa_{O_2}/FI_{O_2} ratio and the sputum volume were determined by retrospective analysis of the frequency of distribution.

Bronchoalveolar Lavage (BAL)

The two BAL techniques were performed in a sequential manner in all patients, starting with bronchoscopic BAL. The heart rate, systemic arterial pressure, and arterial oxygen saturation (Sa_{O_2}) were monitored throughout the procedure. The study was stopped if Sa_{O_2} fell below 90% or if the patient became hypotensive (mean systemic arterial pressure below 60 mm Hg). The majority of the central nervous system trauma patients had intracranial pressure monitoring. The study was stopped if the cerebral perfusion pressure (that is, mean systemic arterial pressure minus intracranial pressure) fell below 60 mm Hg.

Before starting BAL all patients were ventilated with 100% oxygen and sedated with midazolam and morphine intravenously; head-injured patients were given sodium pentothal. All patients received ventilatory support during the study with a 20% increase in tidal volume. The site for B-BAL was chosen according to chest X-ray appearance. B-BAL was performed in an area of localized pulmonary infiltration if present. When a diffuse infiltrate or no infiltrate was seen on X-ray B-BAL was performed in the most inflamed or purulent pulmonary segment determined by visual inspection. If no inflammation or purulent secretions were seen B-BAL was performed in the right lower or middle lobe. B-BAL was performed via the endotracheal tube using a swivel adaptor (Bodai Suction-Safe®, Swivel Y, Sontek Med-

TABLE 1

CPIS USED FOR THE DIAGNOSIS OF VA PNEUMONIA*

1. Temperature °C
   - ≥ 36.5 and ≤ 38.4 = 0 point
   - ≥ 38.5 and ≤ 38.9 = 1 point
   - ≥ 39 or ≤ 36.0 = 2 points
2. Blood leukocytes, mm^{-3}
   - ≥ 4,000 and ≤ 11,000 = 0 point
   - < 4,000 or > 11,000 = 1 point + band forms ≥ 500 = +1 point
3. Tracheal secretions
   - < 14+ of tracheal secretions = 0 point
   - ≥ 14+ of tracheal secretions = 1 point + purulent secretion = +1 point
4. Oxygenation: Pa_{O_2}/FI_{O_2}, mm Hg
   - > 240 or ARDS = 0 point
   - ≤ 240 and no evidence of ARDS = 2 points
5. Pulmonary radiography
   - No infiltrate = 0 point
   - Diffused (or patchy) infiltrate = 1 point
   - Localized infiltrate = 2 points
6. Culture of tracheal aspirate (semiquantitative: 0-1-2 or 3+)
   - Pathogenic bacteria cultured ≤ 1+ or no growth = 0 point
   - Pathogenic bacteria cultured > 1+ = 1 point + same pathogenic bacteria seen on the Gram stain > 1+ = +1 point

* Total points = CPIS (varies from 0 to 12 points).

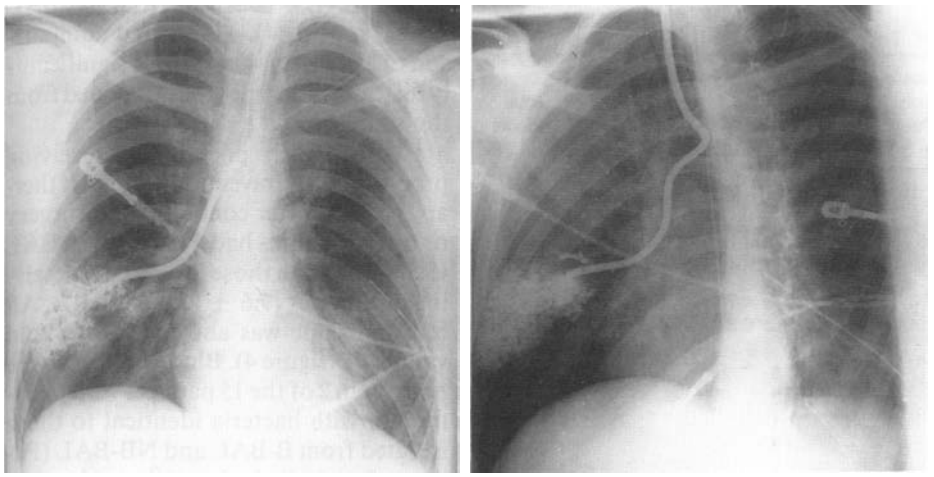

Fig. 1. Chest X-ray showing position of catheter introduced "blindly" and territory sampled by BAL. Films are taken after injection of 5 ml contrast liquid. (*Left*) Antero-posterior view; (*right*), right oblique posterior view.

ical, Hingham, MA). Under visual control the bronchoscope was advanced in the direction of the chosen pulmonary segment until a wedged position was achieved. Lavage was carried out using two aliquots of 50 ml sterile isotonic saline. The bronchoscope was removed and ventilation with an F_{IO_2} of 100% continued for a further 10 min (26).

NB-BAL was then performed using a flexible, single-lumen, 14 French polyvinyl catheter with a proximal Luer adaptor, stiffened with a metallic Teflon®-coated guide wire (catheter for duodenography no. PV14.0–75-P-NS-BBD; Cook A.G., Sempach-Station, Switzerland). The catheter was introduced in a blind manner through the same swivel adaptor used for the B-BAL. It was advanced until a resistance (generally at a distance of 50 to 60 cm from the adaptor) was felt and maintained in a wedged position. The guide wire was then removed and the lavage performed with two aliquots of 50 ml sterile isotonic saline. For NB-BAL as well as for B-BAL lavage with a third aliquot of 40 to 50 ml was performed if there was a poor yield from the first 100 ml. In severe pulmonary failure, that is, ARDS patients, BAL was done with a total of 60 to 75 ml. After NB-BAL the catheter was maintained in the wedged position and 5 ml contrast liquid (Omnipaque®-350; Schering A.G. Pharma, Berlin, Germany) was injected to determine the site of BAL. An anteroposterior and oblique chest film were taken (figure 1). The site of NB-BAL was then assessed in a blind manner by a radiologist who had no knowledge of the patient's condition. BAL samples were sent to the bacteriology laboratory within 5 min and processed immediately. The sequence B-BAL followed by NB-BAL was maintained for the whole study because the contrast liquid has bacteriostatic properties and could thereby produce false negative results for the following examination.

Bacteriology

The 40 pairs of BAL samples were treated in a similar manner. To a first part of BAL fluid 2 ml of dithiothreitol (DTT) (Sputolysin® Stat-Pak; Behring Diagnostics, San Diego, CA) was added, vortexed until homogenization, and then centrifuged. Supernatant (50 µl) was plated on solid media using Spiral Plater® volume deposition (Spiral System Instruments, Inc., Bethesda, MD). Sheep blood agar, colistin nalidixic acid (CNA) agar, chocolate agar, and McConkey's agar were incubated in 5% CO_2 for 72 h. Legionella, mycobacteria, mycoplasma, or viruses were not cultured. Colonies were quantified and identified conventionally and sensitivity tests performed. A bacterial index (BI) was calculated for each sample according to Johanson's formula: BI = sum of log quantity of different bacteria cultured (15).

A manual microscopic count of cells was done on BAL fluid using standard techniques. BAL fluid was then cytocentrifuged at 1,200 rpm for 5 min (Cytospin® 2; Shandon Southern Products, Cheshire, UK) and stained with a May-Grünwald-Giemsa for cell identification and differential count. On a Gram stain of cytocentrifuged material the quantity of bacteria was determined with a standard scale of 0 to 3+ according to the number of bacteria seen per oil-power field (5). Gram-negative and gram-positive bacilli and cocci, yeasts, and the percentage of PMN containing intracellular bacteria were determined. The presence or absence of elastin fibers was assessed with an elastin stain followed by microscopic examination.

Statistical Analysis

Data are expressed as means ± standard deviation (SD). An unpaired Student's *t* test was used to compare the mean of two groups, the Mann-Whitney U test to compare groups with variables not normally distributed, and a paired *t* test for paired values. Frequencies and categories were compared with a χ^2 test with the Yates correction. Linear regression was calculated using the method of least squares. Sensitivity, specificity, and predictive values were calculated according to standard formulas.

Results

Patients and BAL Procedures

There was no significant difference in age, sex, diagnostic categories, APACHE II score, percentage of ARDS, or mortality between infected and noninfected patients (table 2).

No serious complications were noted during or after the BAL procedures. A significant decrease in the Pa_{O_2}/F_{IO_2} ratio was observed in 40% of patients, necessitating a temporary increase in F_{IO_2}. All patients recovered baseline values of oxygenation within 12 h. No procedure had to be stopped because of hypoxemia, hypotension, or other complications.

Bacteriologic Analysis of BAL

A significant correlation was found between the CPIS and the BI calculated from Johanson's formula ($r = 0.84$, $p < 0.0001$ for B-BAL and $r = 0.76$, $p < 0.0001$ for NB-BAL; figures 2 and 3) (15). When analyzing B-BAL samples on the plot of CPIS versus BI (figure 2), two groups of results can be identified: one group with a CPIS > 6, in whom 93% of the corresponding fluids had a culture with a BI ⩾ 5 (defined as pulmonary infection) and a second group in whom the CPIS was ⩽ 6 and all corresponding BI were < 5, patients without pulmonary infection. Using a CPIS > 6 as clinical definition of pulmonary infection in ventilated patients, the sensitivity of the quantitative culture of B-BAL fluid assessed by the calculation of Johanson's BI was 93%; the specificity and positive predictive value were 100%. The BI calculated on NB-BAL samples gave slightly lower sensitivity (73%), specificity (96%), and positive predictive value (92%).

Comparison of paired BI calculated from B-BAL and NB-BAL fluids showed no statistical difference. This was unaffected by the site of BAL sampling. The difference of means of the BI of samples obtained from the same lobe was 1.6 ($p = 0.23$), from a different lobe 0.5 ($p = 0.39$), from the same lung 0.2 ($p = 0.77$), and from the contralateral lung 0.02 ($p = 0.98$). Also in those cases in whom B-BAL was performed at the site of a radiologically localized pulmonary infiltrate and NB-BAL was sampled from another radiologically healthy site, the BI obtained by the two methods were not significantly different ($n = 12$, mean BI

TABLE 2

CLINICAL DATA AND DIAGNOSIS OF THE 28 PATIENTS STUDIED

	No Pulmonary Infection (*N = 15 Patients, mean ± SD*)	Pulmonary Infection (*N = 13 Patients, mean ± SD*)	p Value	Total Patients (*N = 28*)
Age, yr	51 ± 18	44 ± 21	NS	
APACHE II score	12 ± 5	14 ± 6	NS	
Sex, M:F	10:5	11:2	NS (χ^2 = 0.43)	
Duration of prior ventilation, days	5.3 ± 3.1	6.3 ± 4.7	NS	
Duration of total ventilation, days	8.7 ± 4.9	10.8 ± 6.8	NS	
Diagnosis				
Polytrauma	3	8		11
Cerebral trauma	2	2		4
Complications after cardiovascular surgery	3	1		4
Septic shock	1	1		2
Esophagectomy	1	1		2
Pancreatitis	2	0		2
Peritonitis	3	0		3
ARDS (%)	4 (26)	1 (7)	NS (χ^2 = 0.66)	5 (18)
Mortality rate, %	33	54	NS (χ^2 = 0.51)	42

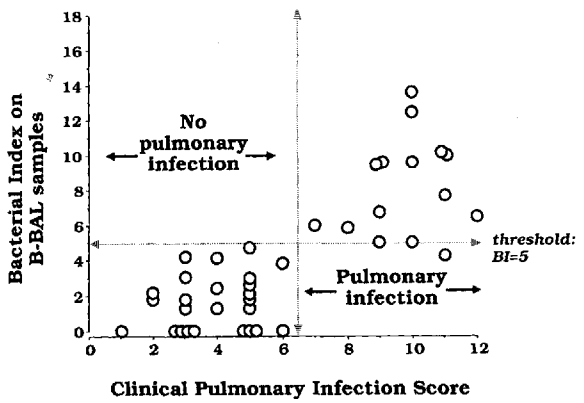

Fig. 2. Relationship between bacterial index, that is, the sum of log quantity of bacteria (15) cultured from BAL obtained by bronchoscopy and the clinical pulmonary infection score determined from 40 investigations in 28 patients.

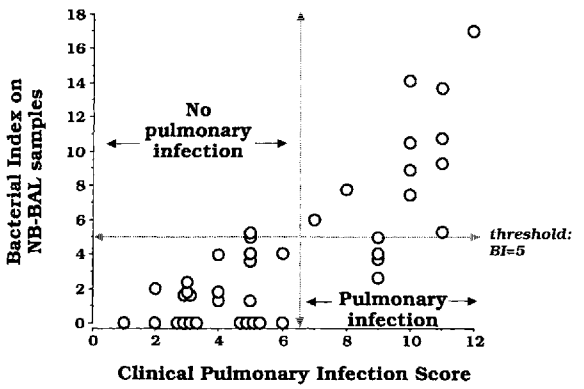

Fig. 3. Relationship between bacterial index obtained by quantitative culture from nonbronchoscopic blind BAL and the clinical pulmonary infection score determined from 40 investigations in 28 patients.

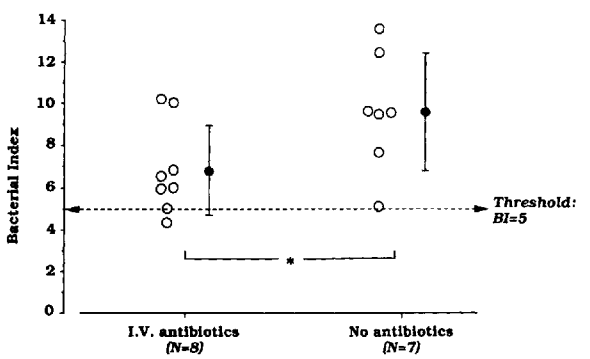

Fig. 4. Bacterial index (BI) in patients with pulmonary infection depending on concomitant antibiotic therapy for surgical prophylaxis or not. Open circles = individual values; closed circles with bar indicate mean ± SD. Asterisk indicates $p < 0.05$.

from B-BAL − mean BI from NB-BAL = 0.5, p = 0.44). The paired comparison of cellularity was also not significantly different if samples were obtained from the same site or a different site.

Patients with pneumonia receiving prophylactic intravenous antibiotic therapy (n = 8) after contaminated surgery or open fractures had a lower BI in BAL (6.8 ± 2.1) than those (n = 7) not receiving antibiotics (9.6 ± 2.9, $p < 0.05$). All but one value was above the threshold value of 5 (figure 4). Blood cultures were positive in 2 of the 15 patients with pneumonia, with bacteria identical to those isolated from B-BAL and NB-BAL (Patients 5 and 10). In 1 patient with pneumonia culture from pleural fluid yielded the same microorganism as BAL fluids (Patient 8). Of the 7 patients who died in the group of pulmonary infections, 3 patients had an autopsy within 8 days after the BAL procedures. The lungs of these 3 patients showed macroscopic and histologic signs of moderate to severe confluent bronchopneumonia. One of these patients also had positive blood cultures with the same organism (*Pseudomonas aeruginosa*) present in sputum and BAL fluids. Of the 5 patients who died in the noninfected group of patients, 3 had an autopsy: 2 of them did not show any sign of pneumonia and the third, who died 12 days after BAL, had signs of mild bronchopneumonia.

The bacteria responsible for pulmonary infection in the group of patients with pneumonia are summarized in table 3. Of the infections 87% were polymicrobial and the predominant bacteria cultured quantitatively were the same in B-BAL and NB-BAL in 14 of 15 samples (93%). In the pneumonia group one identical microorganism was cultured in both BAL fluid and tracheobronchial secretions in 13 of 15 patients (87%), but the predominant bacteria of B-BAL fluid were found in only 11 of 15 tracheobronchial aspirates, resulting in a sensitivity of 73%. Culture of tracheal aspirate resulted in a specificity of only 48% (13 samples with bacterial growth of the 25 from patients without clinical pulmonary infection).

Extracellular bacteria were seen in 93% of samples from patients with pulmonary infection but in only 12% of samples in the control group. Using semiquantitative determination on a Gram stain of BAL, gram-negative bacilli and gram-positive cocci were found more frequently in both B-BAL ($p < 0.0001$) and NB-BAL ($p < 0.0002$) from patients with a pul-

TABLE 3

BACTERIOLOGY OF BAL FLUIDS OF 13 PATIENTS WITH 15 EPISODES OF PULMONARY INFECTION

Patient	Semiquantitative Culture of Tracheal Aspirate	Quantitative Culture of B-BAL (*cfu/ml*)	BI B-BAL	Quantitative Culture of NB-BAL (*cfu/ml*)	BI NB-BAL	CPIS	Elastin Fibers in BAL Fluid	X-ray–localized Infiltrate	Presence of PMN with Intracellular Bacteria in BAL Fluid
1	*H. influenzae* + + *S. aureus* + + +	260 *H. influenzae* 240 *Streptococcus* sp. 400 *S. pneumoniae* 160 *C. albicans*	9.6	20 *S. aureus* 20 *Neisseria* sp.	2.6	9	Absent	No	Yes*
2	*H. influenzae* + + + *S. aureus* + +	10^6 *H. influenzae* 4 × 10^3 *S. aureus*	9.6	10^6 *H. influenzae* 800 *S. aureus*	8.9	10	Absent	No	No
3	Buccal flora + + + *S. aureus* + + +	1.2 × 10^3 *Streptococcus* sp. 640 *S. aureus*	5.9	10^3 *Streptococcus* sp. 10^3 *S. aureus* 60 *H. influenzae*	7.8	8	Absent	No	No
4	Buccal flora + + *M. morganii* + + +	5 × 10^3 *M. morganii* 600 *Streptococcus* sp.	6.5	10^6 *M. morganii* 10^6 *Streptococcus* sp. 10^5 *H. parainfluenzae*	17	12	Absent	Yes	Yes
5	*P. aeruginosa* + + + *S. aureus* +	10^5 *P. aeruginosa* 60 *S. aureus*	6.8	10^4 *P. aeruginosa*	4	9	Present	Yes	Yes
6	*H. influenzae* + + + *E. faecalis* + +	10^6 *H. influenzae*	6	10^6 *H. influenzae*	6	7	Present	Yes	Yes
7	Buccal flora + + +	10^4 *Streptococcus* sp. 760 *H. influenzae* 400 *Acinetobacter* sp.	9.5	5 × 10^3 *Streptococcus* sp.	3.7	9	Present	Yes	Yes*
8	*H. influenzae* + *P. vulgaris* + *C. albicans* + +	10^4 *H. influenzae* 120 *P. vulgaris* 40 *C. albicans*	7.7	10^6 *H. influenzae* 540 *P. vulgaris* 100 *C. albicans*	10.7	11	Present	Yes	Yes
9	*H. influenzae* + + *P. vulgaris* + +	160 *H. influenzae* 120 *P. aeruginosa*	4.3	10^4 *H. influenzae* 20 *P. aeruginosa*	5.3	11	Absent	Yes	No
10	*P. aeruginosa* + + +	2.8 × 10^3 *P. aeruginosa* 40 *C. albicans*	5.1	10^5 *P. aeruginosa* 300 *C. albicans*	7.5	10	Present†	Yes	Yes
11	*S. marscesens* + + +	10^6 *P. aeruginosa* 10^4 *E. coli*	10	10^6 *P. aeruginosa* 2 × 10^3 *E. coli*	9.3	11	Present	Yes	Yes
12	Buccal flora + + *K. oxytoca* + + +	5 × 10^4 *H. influenzae* 240 *K. oxytoca* 1.2 × 10^3 *Streptococcus* sp.	10.1	10^5 *H. influenzae* 5 × 10^3 *K. oxytoca* 10^5 *Streptococcus* sp.	13.7	11	Absent	Yes	Yes
13	*S. pneumoniae* + + + *H. influenzae* + *N. meningitidis* + + +	5.6 × 10^3 *S. pneumoniae* 10^6 *H. influenzae* 10^3 *N. meningitidis*	13.6	6 × 10^4 *S. pneumoniae* 500 *H. influenzae* 10^3 *N. meningitidis*	10.5	10	Absent	Yes	Yes
14	*C. albicans* + +	10^5 *C. albicans*	5	10^5 *C. albicans*	5	9	Present	Yes	No
15	*H. influenzae* + *S. aureus* + + *S. pneumoniae* + +	5 × 10^4 *H. influenzae* 180 *S. aureus* 5.6 × 10^3 *S. pneumoniae*	12.5	5 × 10^4 *H. influenzae* 400 S. aureus 7 × 10^4 *S. pneumoniae*	14.2	10	Present	Yes	Yes

Definition of abbreviations: *H. influenzae* = *Haemophilus influenzae*; *S. aureus* = *Staphylococcus aureus*; *S. pneumoniae* = *Streptococcus pneumoniae*; *C. albicans* = *Candida albicans*; *M. morganii* = *Morganella morganii*; *H. parainfluenzae* = *Haemophilus parainfluenzae*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *E. faecalis* = *Enterococcus faecalis*; *P. vulgaris* = *Proteus vulgaris*; *S. marscesens* = *Serratia marscesens*; *K. oxytoca* = *Klebsiella oxytoca*; *N. meningitidis* = *Neisseria meningitidis*; plus signs = semiquantitative estimation of number of bacteria (i.e., colony-forming units on agar plates).

* Only found in B-BAL.

† Only found in NB-BAL.

TABLE 4

B-BAL AND NB-BAL*

	Bronchoscopic BAL (*N* = 40)			Nonbronchoscopic BAL (*N* = 40)		
	No Pneumonia (*N* = 25, *Mean ± SD*)	Pneumonia (*N* = 15, *Mean ± SD*)	p Value	No Pneumonia (*N* = 25, *Mean ± SD*)	Pneumonia (*N* = 15, *Mean ± SD*)	p Value
Cellularity, mm³	1,200 ± 1,500	3,100 ± 3,400	0.02	1,500 ± 1,500	2,900 ± 2,300	0.08
PMN cells, mm³	650 ± 900	2,400 ± 2,700	0.006	650 ± 900	2,400 ± 2,200	0.001
Macrophages, mm³	290 ± 380	340 ± 270	> 0.05	260 ± 210	240 ± 220	> 0.05
Lymphocytes, mm³	260 ± 550	440 ± 520	> 0.05	540 ± 1,470	241 ± 240	> 0.05
Gram stain†						
Gram positive cocci	0.08 ± 0.28	0.6 ± 0.5	0.0002	0.08 ± 0.28	0.87 ± 0.9	0.0003
Gram negative bacilli	0.04 ± 0.2	1.1 ± 0.9	< 0.0001	0.12 ± 0.28	1.1 ± 1	< 0.0001
Yeasts	0.08 ± 0.28	0.2 ± 0.5	> 0.05	0.08 ± 0.27	0.13 ± 0.35	> 0.05
% PMN with intracellular bacteria	0	8.3 ± 11.5	0.0008	0	11.7 ± 14	0.0001
Presence of PMN with intracellular bacteria (%)	0/25 (0)	11/15 (73)	< 0.0001 (χ^2 = 21.4)	0/25 (0)	9/15 (60)	< 0.0001 (χ^2 = 16.1)
Presence of elastin fibers (%)	2/25 (8)	7/15 (47)	< 0.01 (χ^2 = 5.9)	3/25 (12)	8/15 (53)	< 0.01 (χ^2 = 6.1)
BI‡	1.7 ± 1.6	8.1 ± 2.8	< 0.0001	1.6 ± 1.8	8.4 ± 4.2	< 0.0001
95% Confidence interval	0.9–2.1	6.6–9.7		0.85–2.3	6.1–10.7	
Range	0–4.2	4.3–13.6		0–5.2	2.6–17	

* Analysis of cellularity, Gram stain, and culture of the BAL fluid (40 investigations in 28 patients).
† Semiquantitative: 0-1-2 or 3+.
‡ Σ log of cultured bacteria.

monary infection than from noninfected patients (table 4).

PMN with intracellular bacteria were found in 73% in B-BAL and 60% in NB-BAL fluids from infected patients. No samples in the control group had PMN with intracellular bacteria ($p < 0.0001$). The percentage of PMN containing bacteria in B-BAL fluid was 8.3 ± 11.5% and in NB-BAL 11.7 ± 14% of all cells in patients with pulmonary infection.

Elastin fibers were found in 47% of pulmonary infections compared with 8% in controls in B-BAL fluid ($p < 0.01$). In NB-BAL samples they were found in 53% of infected patients versus 12% of control subjects ($p < 0.01$). In infected patients, elastin fibers were associated with gram-negative bacilli and radiologically localized pulmonary infiltrates (table 3). The false positive results, that is, elastin fibers seen in patients without pulmonary infection, were sampled from two patients with severe ARDS.

Extracellular or intracellular bacteria or elastin fibers were seen in 100% of samples taken from patients with pulmonary infection but in only 12% of the samples of the group without infection.

There was a significant difference between the total cellularity ($p < 0.05$) and quantity of PMN ($p < 0.01$) recovered in BAL fluid from infected and noninfected patients, but not in the number of macrophages and lymphocytes (table 4).

Clinical Variables

Of the clinical variables recorded on the day of BAL and included in the clinical pulmonary infection score (tables 5 and 6), four show a significant difference when comparing infected with noninfected patients: temperature, number of bronchial aspirations that had to be performed per day, quantity of tracheal secretions, and Pa_{O_2}/FI_{O_2} ratio, but not radiology, percentage of patients with purulent tracheal secretions, blood leukocyte count, or band forms.

An analysis of the change in clinical parameters between 2 days before BAL and the day of BAL showed that patients with pulmonary infection had an increase in the volume of secretions ($p < 0.005$), a decrease in the Pa_{O_2}/FI_{O_2} ratio ($p < 0.05$), a nonsignificant rise in body temperature, and no change in blood leukocyte count or band forms (table 6).

Discussion

The management of VA pneumonia requires a rapid and accurate diagnosis. The gold standard for the diagnosis is histology, but this is rarely available in ICU patients. Most investigations of VA pneumonia have therefore defined pulmonary infection using a number of clinical variables. If taken separately these variables do not permit one to distinguish patients with colonization from those with pneumonia. This is particularly true for patients presenting with ARDS and those requiring long-term ventilatory support for acute exacerbation of chronic obstructive pulmonary disease (COPD) (2, 10). Fever, leukocytosis, or X-ray abnormalities are often due to a noninfectious cause in ICU patients, explaining their poor sensitivity and specificity (4, 7, 10). The diagnostic yield of these variables increases, however, when they are combined.

In this report we described a CPIS that uses six easily obtained variables. This score expands clinical judgment by including elements of chest X-ray and bacteriology and permits us to quantify signs of pneumonia. A good correlation was observed between this score and the BI of quantitative culture of BAL fluid calculated according to Johanson's method in VA pneumonia in baboons (15). Two groups of patients can be distinguished by drawing a line between the CPIS values of 6 and 7. We suggest that patients with a CPIS > 6 have a pneumonia, and all their bronchoscopically obtained BAL samples except one had a BI ⩾ 5. The sensitivity of detecting pulmonary infection by quantitative culture of B-BAL was therefore 93%. The only false negative result was obtained in a patient treated for sinusitis with antibiotics active against bacteria found in his lower respiratory tract and presenting

TABLE 5

CLINICAL VARIABLES OBTAINED ON THE DAY OF STUDY*

	No Pulmonary Infection (*N = 25, Mean ± SD*)	Pulmonary Infection (*N = 15, Mean ± SD*)	p Value
Temperature, ° C	38.1 ± 0.8	38.8 ± 0.8	< 0.01
Blood leukocytes/mm³	13,500 ± 5,500	14,300 ± 5,500	NS
Band forms, %	10 ± 12	12 ± 14	NS
Band forms/mm³	1,400 ± 2,100	1,700 ± 2,000	NS
Number of aspirations/day	4.7 ± 2.4	7.2 ± 2.5	< 0.005
Number of + tracheal secretions/day	10 ± 7.5	19 ± 4.5	< 0.001
Purulent tracheal secretions (%)	11/25 (44)	12/15 (80)	0.057 (χ^2 = 3.6)
Pa_{O_2}/FI_{O_2}, mm Hg†	270 ± 100	190 ± 45	< 0.01
Pulmonary radiography (%)			
No infiltrate	8 (32)	3 (20)	0.063‡
Localized infiltrate	5 (20)	12 (80)	(χ^2 = 3.45)
Diffuse infiltrate	12 (48)	0	
Bacteriologic examination of tracheal secretions			
Gram stain§			
Polymorphonuclear cells	1.7 ± 0.7	2.4 ± 0.7	< 0.005
Gram-positive cocci	0.2 ± 0.4	0.8 ± 1	< 0.01
Gram-negative bacilli	0.08 ± 0.2	1.7 ± 1.2	< 0.05
Total bacteria seen‖	0.5 ± 0.9	3.1 ± 2	< 0.0001
Culture§			
Total bacteria cultured‖	0.7 ± 1	3.7 ± 1.7	< 0.0001

* Forty investigations in 28 patients.
† ARDS not included.
‡ Between no and localized infiltrate.
§ Semiquantitative: 0-1-2 or 3+.
‖ Gram-positive + gram-negative rods and cocci + yeasts.

TABLE 6

CHANGES IN CLINICAL VARIABLES BETWEEN 2 DAYS BEFORE BAL AND THE DAY OF BAL*

	No Pulmonary Infection (*Mean ± SD, N = 25*)	Episodes of Pulmonary Infection (*Mean ± SD, N = 15*)	p Value
Body temperature, ° C	0.37 ± 0.61	0.89 ± 0.95	0.072
Blood leukocyte count, mm³	450 ± 6,000	−1,400 ± 9,000	0.46
Band forms, mm³	−200 ± 3,000	−90 ± 1,000	0.89
Volume of tracheal secretions, NB of +	−0.3 ± 6.7	6.8 ± 5.6	< 0.005
Pa_{O_2}/FI_{O_2} mm Hg	−8 ± 57	−57 ± 83	< 0.05

Definition of abbreviation: NB = number.
* Forty investigations in 28 patients.

with clear-cut clinical and radiologic signs of pneumonia resulting in a CPIS of 11. In patients with a CPIS ⩽ 6 the BI was consistently < 5, corresponding to a specificity and a positive predictive value of 100% for the B-BAL. Sensitivity, specificity, and predictive values are slightly lower for NB-BAL but still seem valid for clinical use.

Our threshold value for BI = 5 is close to that found by Johanson's group (15). In their study on ventilated baboons a BI ⩾ 6 was always associated with moderate to severe pneumonia on histology, but no or only mild signs of pneumonia were observed when the BI was < 6. Our index of 5 is also similar to the threshold value of 10^5 bacteria/ml cultured from B-BAL fluid reported in immunocompromised patients (16, 17), resulting in a sensitivity of 85 to 89% and a specificity of 100% in the detection of bacterial pneumonia.

As in Johanson's study, we observed a high incidence (87%) of polymicrobial cultures in VA pneumonia. The use of the BI, taking into account more than one microorganism in BAL, seems to be important when comparing different samples. The reported incidence of polybacterial VA pneumonia seems lower when the diagnosis is made by PSB (3). PSB probably recovers only a small local sample, which is less representative of the whole infected region, whereas BAL allows collection of the microflora of an entire lung segment (27). Chastre and colleagues observed false negative results using PSB with a threshold value of 10^3 bacteria/ml, both positive or negative results being obtained in clinically infected patients (14). This group did not find a clear threshold value using Johanson's BI on BAL samples to differentiate colonization from pneumonia. The mean BI was 11.7 ± 6.2 in the 5 patients with proven pulmonary infection, with all five values above our threshold value of 5. However, the specificity of the BI was low, with a high rate of false positive results (14). These different results are probably due to other criteria used for the diagnosis of pneumonia, resulting in a different classification of other types of lower respiratory tract infection, such as tracheobronchitis, possibly recorded as bronchial colonization (17, 27–29). In our study 3 of the 15 episodes of pulmonary infection were not associated with infiltrates on chest X-ray but with clear clinical signs of infection (CPIS of 8, 9, and 10) and BI of 5.9, 9.6, and 9.6, respectively, consistent with purulent tracheobronchitis (30). The second reason for the discrepancy between our results of BAL cultures and those of Chastre's group (14) is a difference in the populations studied. Our population consisted mainly of young multiple trauma patients, whereas Chastre's group studied mostly patients with COPD in whom heavy bronchial colonization is common and could explain the high false positive rate found using the BI of BAL samples in their study.

As in all studies of nosocomial pneu-

monia we found a clear predominance of aerobic gram-negative bacilli as causative bacteria. *Haemophilus influenzae* was detected in 53% of patients in our study, a well-recognized miroorganism in "early-onset nosocomial pneumonia" as a primary endogenous infection, originating from oropharyngeal flora. Pollock and coworkers found *H. influenzae* in 65% of nosocomial pneumonia patients (30), Reusser and colleagues in 53% of head trauma patients (31), and Fagon and coworkers in 33% of VA pneumonia patients (3). Chastre and colleagues reported a lower incidence of early-onset pneumonia, probably because their patients had a longer period of respiratory support before BAL (15 ± 11 versus 6.3 ± 4.7 days in our study).

We observed a good agreement for the type of microorganism between B-BAL and NB-BAL. The predominant bacteria cultured were identical in 93%, and no significant differences were found for the BI or the cellularity in the two samples. This was also true when B-BAL was performed at the site of a localized pulmonary infiltrate and NB-BAL in a different lobe or the contralateral and radiologically healthy lung. This is in agreement with the data of Johanson's group obtained in baboons, demonstrating that BI calculated on B-BAL samples correlated with both the culture from the lavaged lung lobe and the most infected lobe, that is, the greatest BI (15). This suggests that in ventilated patients bacteriology and cellularity are similar in all pulmonary lobes and both lungs even when a localized infiltrate is present on chest X-ray. This is interesting and may explain the good sensitivity and specificity of a "blind" sampling technique like BAL.

Results from quantitative cultures of BAL can only be obtained after 24 to 48 h, but the proportion of PMN with intracellular bacteria seen on a Gram stain provides a more rapid diagnosis. In pneumonia more than 15% of PMN contain intracellular bacteria, but this was seen in only 1 of 13 BAL samples of patients without pneumonia (14). In our study no intracellular bacteria were found in patients without pneumonia but in 73% of samples from patients with pneumonia. The presence of bacteria within PMN in BAL fluid seems specific (100%) but lacks sensitivity (73%). Adding extracellular and/or intracellular bacteria and/or presence of elastin fibers improved the sensitivity in the detection of pulmonary infection to 100%, but the specificity fell to 88%. Elastin fibers in BAL fluid were associated with gram-negative pulmonary infection and infiltrates on chest X-ray. False positive results were obtained in two cases of severe ARDS in whom damage to pulmonary parenchyma is common. Our findings on elastin fibers in BAL fluid are similar to those reported by Salata and coworkers (5) in sputum of ventilated patients.

Analysis of clinical criteria taken separately resulted in significant differences in the mean value for several variables between the patients with and without pneumonia, with marked overlaps as reported previously (14). In our study, however, the association of clinical variables in a score (CPIS) allowed us to discriminate patients with a high concentration of bacteria in BAL from those with low amounts of bacteria.

Standard cultures of tracheal aspirates were of poor value to separate infected from noninfected patients (positive predictive value and specificity were 48%), confirming the results of earlier studies (8, 11).

In summary, this study describes a valuable clinical pulmonary infection score calculated from six easily obtained variables. This score correlated with the quantitative culture of BAL obtained by both bronchoscopy and a catheter introduced blindly into the bronchial tree. The clinical score allowed us to differentiate between infected and colonized patients. As previously suggested in a study on ventilated baboons, we confirmed that the site of BAL sampling is not important for making a correct diagnosis. Quantitative bacteriology on BAL samples gave identical results irrespective of the pulmonary segment sampled, even in the presence of a localized infiltrate on the chest X-ray. Quantification of extracellular and intracellular bacteria observed on the Gram stain and the search of elastin fibers in the BAL fluid allowed rapid and accurate diagnosis, available within 1 h after BAL. Further studies should now be done to evaluate if early and accurate diagnoses of VA pneumonia will affect the outcome of these patients.

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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. Andrews CP, Coalson JJ, Smith JD, Johanson WG. Diagnosis of nosocomial bacterial pneumonia in acute, diffuse lung injury. Chest 1981; 80: 254–8.
3. Fagon J-Y, Chastre J, Domart Y, *et al.* Nosocomial pneumonia in patients receiving continuous mechanical ventilation. Prospective analysis of 52 episodes with use of a protected specimen brush and quantitative culture techniques. Am Rev Respir Dis 1989; 139:877–84.
4. Langer M, Mosconi P, Cigada M, Mandelli M, the Intensive Care Unit Group of Infection Control. Long-term respiratory support and risk of pneumonia in critically ill patients. Am Rev Respir Dis 1989; 140:302–5.
5. Salata RA, Lederman MM, Shlaes DM, *et al.* Diagnosis of nosocomial pneumonia in intubated, intensive care unit patients. Am Rev Respir Dis 1987; 135:426–32.
6. Stevens RM, Teres D, Skillman JJ, Feingold DS. Pneumonia in an intensive care unit. Arch Intern Med 1974; 134:106–11.
7. Fagon J-Y, Chastre J, Hance AJ, *et al.* Detection of nosocomial lung infection in ventilated patients. Use of a protected specimen brush and quantitative culture techniques in 147 patients. Am Rev Respir Dis 1988; 138:110–6.
8. Johanson WG, Pierce AK, Sanford JP, Thomas GD. Nosocomial infections with gram-negative bacilli. The significance of colonization of the respiratory tract. Ann Intern Med 1972; 77:701–6.
9. Wilson MJB, Martin DE. Quantitative sputum culture as a means of excluding false positive reports in the routine microbiology laboratory. J Clin Pathol 1972; 25:697–700.
10. Chastre J, Viau F, Brun P, *et al.* Prospective evaluation of protected specimen brush for the diagnosis of pulmonary infections in ventilated patients. Am Rev Respir Dis 1984; 130:924–9.
11. Torres A, Puig de la Bellacasa J, Xaubet A, *et al.* Diagnostic value of quantitative cultures of bronchoalveolar lavage and telescoping plugged catheter in mechanically ventilated patients with bacterial pneumonia. Am Rev Respir Dis 1989; 140:306–10.
12. Cartier F. Strategy in immunocompromised patients with pulmonary infiltrates. Intensive Care Med 1987; 13:87–8.
13. Papin TA, Lynch JP, Weg JG. Transbronchial biopsy in the thrombocytopenic patient. Chest 1985; 88:549–52.
14. Chastre J, Fagon J-Y, Soler P, *et al.* Diagnosis of nosocomial bacterial pneumonia in intubated patients undergoing ventilation: comparison of the usefulness of bronchoalveolar lavage and protected specimen brush. Am J Med 1988; 85:499–506.
15. Johanson WG, Seidenfeld 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.
16. Thorpe JE, Baughman RP, Frame PT, Wesseler TA, Staneck JL. Bronchoalveolar lavage for diagnosing acute bacterial pneumonia. J Infect Dis 1987; 155:855–61.
17. Kahn FW, Jones JM. Diagnosing bacterial respiratory infection by bronchoalveolar lavage. J Infect Dis 1987; 155:862–9.
18. Caughey G, Wong H, Gamsu G, Golden J. Nonbronchoscopic bronchoalveolar lavage for the

diagnosis of *Pneumocystis carinii* pneumonia in the AIDS. Chest 1985; 88:659–62.
19. Mann JM, Altus CS, Webber CA, Smith PR, Muto R, Heurich AE. Nonbronchoscopic lung lavage for diagnosis of opportunistic infection in AIDS. Chest 1987; 91:319–22.
20. 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.
21. Gaussorgues P, Piperno D, Bachmann P, *et al.* Comparison of nonbronchoscopic bronchoalveolar lavage to open lung biopsy for the bacteriologic diagnosis of pulmonary infections in mechanically ventilated patients. Intensive Care Med 1989; 15:94–8.
22. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985; 13:818–29.
23. Ledingham IM, Eastaway AT, McKay IC, Alcock SR, McDonald JC, Ramsay G. Triple regimen of selective decontamination of the digestive tract, systemic cefotaxime, and microbiological surveillance for prevention of acquired infection in intensive care. Lancet 1988; 1:785–90.
24. Stoutenbeek CP, van Seane HFK, Miranda DR, Zandstra DF. The effect of selective decontamination of the digestive tract on colonisation and infection rate in multiple trauma patients. Intensive Care Med 1984; 10:185–92.
25. Unertl K, Ruckdeschel G, Selbmann HK, *et al.* Prevention of colonization and respiratory infections in long-term ventilated patients by local antimicrobial prophylaxis. Intensive Care Med 1987; 13:106–13.
26. Klech H, Pohl W. Technical recommendations and guidelines for bronchoalveolar lavage (BAL). Eur Respir J 1989; 2:561–85.
27. Faling LJ. New advances in diagnosing nosocomial pneumonia in intubated patients. Am Rev Respir Dis 1988; 137:253–5.
28. Garner JS, Jarvis WR, Emori TG, *et al.* Center for disease control definition for nosocomial infections. Am J Infect Control 1988; 16:129–40.
29. Brun-Buisson C, Legrand P, Rauss A, *et al.* Intestinal decontamination for control of nosocomial multiresistant gram-negative bacilli. Study of an outbreak in an intensive care unit. Ann Intern Med 1989; 110:873–81.
30. Pollock HM, Hawkins EL, Bronner JR, Sparkman T, Bass JB. Diagnosis of bacterial pulmonary infections with quantitative protected catheter cultures obtained during bronchoscopy. J Clin Microbiol 1983; 17:255–9.
31. Reusser P, Zimmerli W, Scheidegger D, Marbet GA, Buser M, Gyr K. Role of gastric colonization in nosocomial infections and endotoxinemia: a prospective study in neurosurgical patients on mechanical ventilation. J Infect Dis 1989; 160:414–21.