Histopathologic and Microbiologic Aspects of Ventilator-associated Pneumonia

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Background: The relationship between microbiology and histology in patients with ventilator-associated pneumonia has been sparsely described.

Methods: Twenty-five patients who died in the intensive care unit after their lungs had been mechanically ventilated for 72 h were studied. Twenty of the 25 died with clinical suspicion of pulmonary infection. A total of 375 immediate postmortem pulmonary biopsies were obtained after death and processed for quantitative microbiology and histology. Four evolutionary stages of pneumonia were defined: early, intermediate, advanced, and resolution.

Results: At least one specimen with histologic evidence of pneumonia was found in all but two patients (92%). Histologic pneumonia was a widespread and frequent process (46% of biopsies examined) involving predominantly the lower lobes (55% of all biopsies with pneumonia) and showing different histopathologic stages of progression coexisting in the same lung lobes. Lung cultures were frequently polymicrobial (149 of 375, 40% of the pulmonary biopsy cultures, and 20 of 25, 80% of the cases) and not always yielding the same pathogen (19 microorganisms) when comparing one lung to the other. Histopathology and microbiologic biopsy cultures showed a weak relationship (28% and 49% of species had counts ≥ 105 cfu/g in samples without pneumonia from patients with and without prior antibiotic treatment, respectively). Histopathologic evolutionary stages were not associated with any differences in quantitative culture results of pulmonary biopsies, independently of prior administration of antibiotics. Higher bacterial concentrations of biopsy cultures were associated with the absence of prior antibiotic treatment.

Conclusions: Ventilator-associated pneumonia is a frequent diffuse and polymicrobial process showing different coexisting degrees of evolution and involving preferentially the lower lobes. Microbiology and histology can be dissociated even in the absence of prior antibiotic treatment. Lung histology appears more reliable than bacteriology as a diagnostic reference test. (Key words: Lung histology. Postmortem biopsy. Pulmonary infection. Ventilator-associated pneumonia.)

VENTILATOR-ASSOCIATED PNEUMONIA is a frequent complication of mechanical ventilation, with an incidence ranging between 9% and 70%.1-8 Crude mortality rates of this complication vary from 25% to 50%, whereas mortality directly attributable is 27%.3 Among several prognostic factors, both inappropriate and prior antibiotic therapy5,7-8 have particular importance and indirectly highlight the need for a correct clinical and microbiologic approach to the management of pneumonia. To validate the different diagnostic techniques available for ventilator-associated pneumonia, microbiology and histology of immediate postmortem pulmonary biopsies have been used.9-12 These have been helpful in establishing different thresholds for bronchosopic samples to distinguish colonization from infection. Although Rouby et al.11 established the multifocal pattern of ventilator-associated pneumonia acquired during mechanical ventilation, the relationship between histology and bacteriology of ventilator-associated pneumonia is incompletely described. This prompted us to obtain multiple immediate postmortem tissue samples from patients whose lungs were being mechanically ventilated and who died in our respiratory intensive care unit to establish the histologic and microbiologic patterns of this pulmonary infection. The relevance of the issue and the need for more data in these difficult-to-repeat studies motivated us to perform the current study. The main differences between this study and others are the number of lungs examined, the number of biopsies taken, and the use of cultures in all the samples.

Materials and Methods

Patients

Over a period of 2 years, 25 patients who died in the intensive care unit were included in the study. None of them had received prior antibiotic therapy, and their inherent pulmonary response, anatomic and functional. In 20 of the 25 patients, death was sudden because of acute respiratory failure (n = 6), congestive heart failure (n = 2), bacteremia (n = 7), and sepsis (n = 6). Twenty-one of the 25 patients (7 cases) or local infiltrates 24 h before death were ventilated with no infiltrates. Cheesman et al.9 studied the day of the study. Two ventilated patients had significant heart and pulmonary infection and pneumonia and 5 cases were ventilated before death, 0.4). General clinical criteria and causes of respiratory death are deep and received prior antibiotic therapy. We included 17 of the remaining 8 patients with pneumonia with symptoms (n = 17) for more than 2 days at the time of death. Patients were pneumonia-free for more than 2 days in 1 case. In 1 case, the patient died of sepsis, pneumonia, and organ failure. Treatment was 9 days. One case gave informed consent for the study, and personal data of our center were collected.

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of biopsies taken, and the performance of quantitative cultures in all the samples obtained.

Materials and Methods

Patients

Over a period of 1 yr, 25 patients whose lungs had been mechanically ventilated for more than 72 h and who died in our respiratory intensive care unit were included in the study. Patients with immunosuppression or hematologic neoplasia were excluded because of their inherent particularities regarding etiology, immune response, and presentation of pulmonary infection.

In 20 of the 25 patients, pulmonary infection on the day of death was clinically suspected. Twelve of the 25 patients were admitted to the intensive care unit because of acute respiratory failure due to pneumonia (n = 6), congestive heart failure (n = 2), alveolar hemorrhage (n = 2), or pulmonary neoplasia (n = 2). Twenty-one of the 25 patients (84%), had either diffuse (7 cases) or localized (14 cases) chest radiograph infiltrates within 24 h before death. In four patients, there were no infiltrates. Chest x-ray results were reviewed on the day of the study. The clinical diagnoses of the pulmonary infiltrates were pneumonia in 14 patients, pneumonia and acute respiratory distress syndrome in 5, congestive heart failure in 1, and alveolar hemorrhage and pneumonia in 1. The mean interval between appearance of infiltrates in chest x-ray and death was 6 ± 1.4 days for patients whose lungs were ventilated <10 days and 16.6 ± 14.2 days for patients whose lungs were ventilated ≥10 days (Mann-Whitney test, P = 0.04).

The general characteristics of the study population and causes of respiratory intensive care unit admission and death are described in Table 1. Seventeen patients received prior antibiotic therapy (group A), whereas the remaining 8 subjects (group B) did not receive antibiotics within the 48 h before death. The indications for antibiotic therapy in group A were pneumonia in 8, aspiration pneumonia in 2, multiple trauma in 2, postoperative prophylaxis in 1, peritonitis in 1, sepsis in 1, purulent tracheobronchitis in 1, and organ donor in 1. The mean duration of antibiotic treatment was 9.5 ± 7.9 days. Family members in each case gave informed written consent for the study to be performed, and permission from the Ethical Committee of our center was granted.

Table 1. General Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Group A (with antibiotics) (n = 17)</th>
<th>Group B (without antibiotics) (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>57.2 ± 17</td>
<td>52 ± 1</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>14/3</td>
<td>2/6</td>
</tr>
<tr>
<td>APACHE II</td>
<td>21 ± 7</td>
<td>19.5 ± 2</td>
</tr>
<tr>
<td>Mechanical ventilation duration (days)</td>
<td>13 ± 13</td>
<td>10 ± 7</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>36 ± 1</td>
<td>36.7 ± 1</td>
</tr>
<tr>
<td>Leukocytes (×10⁹/L)</td>
<td>14.8 ± 7.8</td>
<td>13.3 ± 18.4</td>
</tr>
<tr>
<td>PaO₂/FIO₂</td>
<td>207 ± 126</td>
<td>210 ± 136</td>
</tr>
<tr>
<td>Chest x-ray findings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No infiltrates</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Localized</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Diffuse</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Cause of admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute respiratory failure</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Stroke</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Postoperative</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cranial trauma</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Cause of death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple organ failure</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Hypoxemia</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cerebral death</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

Study Protocol

Immediately (within 45 ± 45 min, range 0–90 min) after death, patients' lungs remained mechanically ventilated with FIO₂ 1.0. Bilateral fiberoptic bronchoscopies were performed using different bronchoscopes for each lung following standard techniques.¹⁰,¹¹ One guided biopsy from each lung, through thoracotomy incisions, was obtained with the aid of the light of the bronchoscope. The bronchoscope was guided to the area of visual maximal inflammation corresponding to chest x-ray infiltrates. In the absence of infiltrates, the bronchoscope was guided to the lower lobes. Furthermore, bedside blinded (not guided by fiberoptic bronchoscope) bilateral pulmonary biopsies, following strict aseptic techniques, were obtained through the same thoracic incisions. The thoracotomy incision was done at the 5th intercostal space from the midclavicular to the midaxillary lines. In organ donors, this procedure was performed in the operating theater; in the remaining patients, the procedure was done in our intensive care unit. Lungs were not removed from the thorax, and sampling of posterior segments was possible because the lungs were partially collapsed as mechanical
ventilation was maintained throughout the study. Three fragments were obtained from both superior and inferior lobes from each lung and 2 from the middle lobe, such that the overall number of biopsies per patient was 16 (2 guided and 14 blinded). The total number of biopsies was 375 (in three patients, one lung only was sampled). In 14 patients (217 samples), samples were obtained within 30 min after death; in 5, within 60 min (62 samples); and in 6, within 90 min (96 samples). Of the 14 patients in whom samples were obtained within 30 min after death, 8 were organ donors, and the period between brain death and obtaining the sample was less than 12–24 h. One organ donor had the sample obtained between 30 and 60 min. The average size of the bronchoscopically guided biopsies and that of the blind biopsy samples was 2 × 2 × 2 cm each. Biopsy samples were obtained from peripheral zones of the lung. Each fragment was sectioned into two pieces, one for quantitative microbiology culture and the other for histopathology processing.

**Histopathologic Processing**

All lung tissue specimens were embedded in paraffin and stained with hematoxylin and eosin, periodic acid Schiff, May-Grünwald Giemsa, and Gram stains. The microscopic evaluation was performed after classification of pneumonia evolution based on the most widespread guidelines for this type of lesion. In the current study, pneumonia was divided into four phases related to the following sequence of events in lung tissue: early, intermediate, advanced, and resolution. Early-phase pneumonia was defined as the presence of capillary congestion with increased number of polymorphonuclear leukocytes at this level; the alveolar spaces usually showed a fibrinous exudate. Intermediate-phase pneumonia was considered when alveoli had presence of fibrin, few erythrocytes, and several polymorphonuclear leukocytes. Advanced-phase was assessed when polymorphonuclear leukocytes filled most of the alveoli, and macrophages were incorporating cellular debris in the cytoplasm. In the resolution-phase, the inflammatory exudate was eliminated because of macrophagic activity of mononuclear cells. In figure 1, the different evolutionary phases are illustrated. Bronchiolitis was defined as an intense proliferation of polymorphonuclear leukocytes localized within the lumen of bronchioles and associated with purulent mucus plugs and bronchiolar wall alterations (fig. 2). Focal, confluent bronchopneumonia and lung abscesses were examined in lung biopsy samples, according to the definition of Rouby et al.11

Other pulmonary findings, such as diffuse alveolar damage,15 were assessed. Diffuse alveolar damage was classified into two phases: The early exudative phase was defined as the presence of interstitial and alveolar edema, with varying degrees of intraalveolar hemorrhage and fibrin deposition and initial development of hyaline membranes. The late stage of diffuse alveolar damage was defined by the presence of fibroblast proliferation within the interstitium, interstitial inflammation and alveolar lining cell hyperplasia remaining prominent, but residual edema or hyaline membrane formation being minimal.15 For clarity, results regarding diffuse alveolar damage were presented as a dichotomous variable (presence or absence).

**Microbiologic Processing and Isolate Identification**

Biopsy samples were placed, with sterile sand and 3 ml of sterile saline, in a mortar (Vidrafloc, Barcelona, Spain) and homogenized. Serial dilutions (10⁻¹, 10⁻², 10⁻³) of each sample were prepared in sterile normal saline. One hundred microliters of each dilution of biopsy samples was inoculated into the following agar media: 5% sheep blood, chocolate, Centers for Disease Control and Prevention blood, McConkey, blood charcoal yeast extract, and Sabouraud-dextrose. All cultures were incubated at 37°C under aerobic and anaerobic conditions and in carbon dioxide-enriched atmosphere. Cultures were evaluated for growth 24 and 48 h later and discarded, if negative, 5 days after, except for Centers for Disease Control and Prevention blood and Wilkins-Chalgren, evaluated at 7 days, and Sabouraud, evaluated at 4 weeks. All microorganisms isolated were identified by standard laboratory methods.16 Results are expressed as colony-forming units per gram of tissue (cfu/g = number of colonies × dilution factor × inoculation factor). Results of biopsy cultures were analyzed first considering individual pulmonary biopsy samples and second considering lung lobes. In the latter, a lobe was considered as compatible with pneumonia if at least one biopsy sample obtained from the same lobe was positive. For the analysis of individual lobes, when bacterial isolates coincided in more than one sample from the same lobe, the species with the highest bacterial count was chosen. Positive lung cultures were considered when one or several microorganisms were isolated in the culture of a lung sample. A false-negative result was considered if no growth was
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Fig. 1. Normal lung parenchyma and phases of pneumonia. (A) Normal alveolar structure. Absence of interstitial and capillary abnormalities (hematoxylin and eosin, ×200). (B) Early phase of pneumonia. Accumulation of polymorphonuclear leukocytes within the capillaries (arrow; hematoxylin and eosin, ×200). (C) Intermediate phase of pneumonia. Polymorphonuclear leukocytes, fibrin, and few erythrocytes in the alveolar lumina (hematoxylin and eosin, ×200). (D) Advanced phase of pneumonia. Polymorphonuclear leukocytes and macrophages filling the alveolar lumina (hematoxylin and eosin, ×200). (E) Resolution phase of pneumonia. Some alveoli show macrophage activity with rests of inflammatory exudate (arrow; hematoxylin and eosin, ×200).
obtained from a lung biopsy culture and the histologic examination demonstrated pneumonia. A false-positive result was defined as a positive bacterial growth in absence of histologic pneumonia. Results also were expressed quantitatively to evaluate the bacterial concentration of pulmonary samples. The threshold of 10^5 cfu/g of tissue was used, as previously recommended. The reference test used to evaluate the sensitivity and specificity of pulmonary biopsy cultures was the histology of these biopsies. Bacteriologic results were analyzed by dividing the microorganisms into those with high pathogenicity and those without or with a lesser degree of pathogenicity (Staphylococci other than Staphylococcus aureus, Streptococci other than Streptococcus pneumoniae, and yeasts).

Statistical Analysis
EPIinfo (version 5.01) software was used for the statistical analysis. Results are expressed as mean ± SD. Analysis of variance for repeated measures was performed for multiple comparisons. When significant F statistic was observed, the Scheffé test was applied. The t-test was employed for the comparison of quantitative variables (Mann-Whitney test if the distribution was not normal). The chi-square test (Fisher's exact test when needed) was used to compare proportions. The significance levels were set to 0.05. Results of microbial cultures of biopsy samples were categorized into true-positives, false-positives, true-negatives, and false-negatives. These were used to determine the sensitivity and the specificity according to Bayes' rules.

Results

Histologic Findings

Pneumonia. The incidence of histologic pneumonia in the study population was 92% (23 of 25 patients). Two subjects had no histologic evidence of pneumonia, 3 had unilateral pneumonia, 17 others (68%) had bilateral pneumonia, and the remaining 3, in whom the study was restricted to one lung, had pneumonia also. A total of 47 lungs (24 left and 23 right) from the 25 patients were examined. Overall, 168 biopsies were obtained from the 24 left lungs, 79 of which showed histopathologic evidence of pneumonia (47%). A similar percentage of histopathologic features of pneumonia (46%, 95 of 207 biopsies) was obtained from the 23 right lungs. Thus, the cumulative percentage of histologic pneumonia was 46% (174 of 375). There were no differences in the percentage of histologic pneumonia when comparing guided (53%) versus blinded biopsies (45%). Histologically proven pneumonia involved predominantly lower lobes (57%) compared to upper (30%) or middle lobes (13%) (P < 0.0001 (χ^2 test).

In 14 (30%) of 47 histologic lung examinations, there was a single histologic pattern of evolution, predominantly the intermediate-phase (64%). Early phases of pneumonia alone were observed in two patients (five biopsy samples), and resolution phase alone was not observed. Different histologic evolution phases coexisted in 26 (55%) lungs. In seven lungs (15%), histologic pneumonia was not shown. The incidence of bronchiolitis was 52% (13 of 25 patients), corresponding to 33 biopsy samples (9%). Regarding positive biopsy cultures without histologic presence of pneumonia, bronchiolitis was observed in 11 of 136 samples (8%). Large bronchi (with bronchial smooth muscle and cartilage) were never observed in our biopsies. Focal bronchopneumonia was observed in 128 (34%) samples, corresponding to 15 patients. Confluent bronchopneumonia was observed in 46 (12%) samples, corresponding to 2 patients. Six patients had focal bronchopneumonia in one lung and confluent bronchopneumonia in the other. Two patients had no pneumonia.

Among the four patients with normal chest x-ray results, three had histologic pneumonia. Among the 21 patients with chest x-ray infiltrates, the incidence of histologic pneumonia was 95% (20 of 21). The percentage of samples showing histologic pneumonia was similar in the 14 patients with infiltrates and in the 7 patients with diffuse infiltrates.

Diffuse Alveolitis

At least one pulmonary alveolitis sign was consistent with evidence of lung injury. This sign was found in all patients in the control group and in 93% (27 of 29 patients) in the treated group. The percentage of patients with diffuse alveolitis was significantly lower in the control group than in the treated group (P = 0.004).

Histologic Findings

Among the 12 patients with acute respiratory distress syndrome, the incidence of histologic pneumonia was 167 (13%) had diffuse alveolitis. In the remaining patients, the reason for acute respiratory distress syndrome was different. The percentage of patients with diffuse alveolitis was significantly lower in patients with acute respiratory distress syndrome than in the other patients (P = 0.004).

Discussion

The percentage of patients with diffuse alveolitis was significantly lower in the control group than in the treated group (P = 0.004). The percentage of patients with diffuse alveolitis was significantly lower in patients with acute respiratory distress syndrome than in the other patients (P = 0.004).

In conclusion, the use of corticosteroids in patients with acute respiratory distress syndrome might be beneficial in reducing the incidence of diffuse alveolitis.

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patients with diffuse infiltrates (50% and 62%, respectively).

The percentage of lung biopsies consistent with the diagnosis of pneumonia was similar in the 17 patients of group A receiving antibiotics and in the 8 patients of group B not receiving antibiotics: 49% (133 among 272 lung biopsy samples) versus 40% (41 among 103 lung biopsy samples). However, as shown in Table 2, the early-phase process was more frequent in group A than group B. In patients receiving antibiotics, pneumonia was more frequent among those with documented microbial antibiotic resistance: In the 10 patients in whom microbial antibiotic resistance was demonstrated, 56% of pulmonary biopsy samples (81 of 144) demonstrated histologic evidence of pneumonia, whereas in the 7 patients in whom no microbial antibiotic resistance could be evidenced, pneumonia was present only in 41% (52 of 128) of the samples examined ($P = 0.01, \chi^2$ test).

**Diffuse Alveolar Damage.** Eight patients (32%) had at least one pulmonary biopsy sample showing histologic signs consistent with diffuse alveolar damage. Six of these patients were bilateral and two unilateral chest x-ray infiltrates. The total number of biopsy samples showing diffuse alveolar damage was 44 of 375 (12%). Diffuse alveolar damage coexisted always with the presence of histologic pneumonia in each individual. Among the 44 samples with diffuse alveolar damage, 32 (73%) showed histologic signs of pneumonia in the same specimen.

**Relationship between Mechanical Ventilation and Histopathology.** When analyzing patients whose lungs were mechanically ventilated for <10 days (n = 14), 12 (86%) showed histologic signs of pneumonia (79 of 199 (40%) biopsy samples), whereas all patients’ lungs (n = 11) with >10 days of mechanical ventilation showed pneumonia (95 of 176 samples (54%); $P = 0.005$). Four patients whose lungs were mechanically ventilated <10 days and another four whose lungs were mechanically ventilated >10 days showed diffuse alveolar damage in 20 of 199 (10%) and 24 of 176 (14%) biopsy samples, respectively ($P = 0.28$).

**Histologic Findings and Respiratory Failure.** In the 12 patients who were initially admitted because of acute respiratory failure, 102 of 167 biopsies (61%) showed histologic evidence of pneumonia and 22 of 167 (13%) had diffuse alveolar damage (5 patients). In the remaining 13 patients who were not admitted because of acute respiratory failure, these percentages were 72 of 208 (35%) for pneumonia and 22 of 208 (10%: 3 patients) for diffuse alveolar damage ($P = 0.0001$ and 0.32, for pneumonia and diffuse alveolar damage, respectively).

**Microbiologic Data.** All the serologies yielded negative results. Two hundred eighty of 375 (77%) biopsies yielded positive cultures, for a total of 470 microorganisms. Among them, 149 (40%) cultures were polymicrobial, obtained from 20 patients (80%). Forty percent (190 of 470) of the isolates were Gram-negative bacilli, of which 72% were *Pseudomonas* species. Thirty-eight percent of the isolates were Gram-positive cocci (38% *Staphylococcus aureus*), and the remaining 22% included yeasts, fungi (45% were *Candida albicans*), and other nonpathogenic microorganisms. In Table 3, the different specific microorganisms are listed in relation to the presence or absence of histologic pneumonia. Samples without pneumonia from group B (without antibiotics) grew a large number of *Staphylococcus aureus* compared to the remaining samples (31% vs. 6%, $P = 0.0001$). On the other hand, *Staphylococcus epidermidis* grew more frequently in samples from patients receiving antibiotics (16% vs. 3%, $P = 0.001$).

Microbial cultures of bronchoscopically guided biopsies were coincident in both lungs in 12 patients and did not coincide in 10. In three patients, this comparison was not possible, because they were pneumonectomized. When comparing cultures from both lungs in the same patient, cultures coincided in 34 microbial species but did not in 19 microbial species. The noncoincident microorganisms were *Candida*
species in five, *Aspergillus* species in three, *Pseudomonas* species in three, *Staphylococcus epidermidis* in three, *Corynebacterium* species in two, *Acinetobacter* species in one, *Serratia marcescens* in one, and meticillin-resistant *S. aureus* in one case. Twelve microorganisms retrieved from pulmonary samples from group A (with antibiotics) were resistant to the given antibiotics. The most frequent microorganisms resistant to antibiotics were meticillin-resistant *S. aureus*, *Pseudomonas* species, *Xanthomonas maltophilia*, *Enterococcus faecalis*, and *Candida krusei* and *Aspergillus fumigatus*.

The sensitivity of positive biopsy samples, considering all isolated microorganisms, was 80% in patients receiving antibiotics and 100% in whom antibiotics were withdrawn (false-negative rate of 20% and 0%, respectively). Specificity was 30% and 4%, respectively, in both groups. Thus, the rate of false-positive results was 70% and 96%, respectively. When nonpathogenic microorganisms were excluded from the analysis, the sensitivity was 93% and 72%, respectively, and specificity was 15% and 40%. Regarding the delay between death and the extraction of pulmonary biopsies, we found 20% of false-positive results in patients studied within 30 min, 21% in patients studied within 30 to 60 min, and 6% in those studied between 60 and 90 min.

**Quantitative Aspects.** In figure 3, the relationships between microbiology and histopathology in patients with and without antibiotic treatment are illustrated and analyzed according to the recommended threshold of $10^3$ cfu/g. As shown in figure 3A, biopsy samples obtained from patients of group A receiving antibiotics were characterized by a reduction of the number of species with counts $>10^3$ cfu/g ($P < 0.0001$ for biopsy samples demonstrating pneumonia and $P = 0.002$ for biopsy samples without antibiotics) when compared to group B patients without antibiotics. Interestingly, in patients not receiving antibiotics, 49% of species isolated from biopsy samples without histologic pneumonia were found in $>10^3$ cfu/g. As shown in figure 3B, when considering whole pulmonary lobes, the results were similar to those obtained when analyzing individual samples.

In figures 3C and 3D, the number of bacterial species ($< or $\geq 10^3$ cfu/g), excluding nonpathogenic microorganisms, is expressed in relation to the presence or absence of histologic pneumonia in patients from groups A and B. Taking into account individual pulmonary biopsies (fig. 3C) or pulmonary lobes (fig. 3D). The percentage of species with counts $>10^3$ cfu/g in samples without histologic pneumonia in patients without antibiotics (group B) was 62% and 67% when considering individual microorganisms and lung lobes, respectively.

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**Table 3. Isolated Microorganisms from the 375 Pulmonary Biopsy Specimens**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>With Antibiotics (Group A)</th>
<th>Without Antibiotics (Group B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pneumonia</td>
<td>No Pneumonia</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>44 (29)</td>
<td>33 (27)</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>4 (3)</td>
<td>5 (4)</td>
</tr>
<tr>
<td><em>Xanthomonas maltophilia</em></td>
<td>9 (6)</td>
<td>13 (11)</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>9 (6)</td>
<td>—</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>16 (10)*</td>
<td>7 (6)†</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>25 (16)¶</td>
<td>15 (12)§</td>
</tr>
<tr>
<td><em>Streptococci</em></td>
<td>13 (9)</td>
<td>20 (17)</td>
</tr>
<tr>
<td><em>Corynebacterium spp.</em></td>
<td>4 (3)</td>
<td>5 (4)</td>
</tr>
<tr>
<td><em>Candida and Aspergillus</em></td>
<td>28 (18)</td>
<td>21 (17)</td>
</tr>
</tbody>
</table>

Values are no. (%). P values are determined by chi-square test.

* P $= 0.001$
† P $= 0.01$
‡ P $= 0.001$
§ P $= 0.0002$

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biopsies and lung lobes, respectively. These percentages were high when compared to those obtained when considering all microorganisms (49 and 44%, respectively).

Figure 4 shows the mean concentrations of microorganisms obtained from lung biopsies demonstrating pneumonia according to the different evolution phases. Biopsy cultures from patients without antibiotic treatment yielded higher microbial concentrations in all evolution phases ($P < 0.001$). However, when considering groups A and B separately, there were no differences among evolution phases.

Figure 5 shows the mean concentrations of microorganisms obtained in lung biopsies according to the histologic grade. There were no differences among the mean bacterial concentrations obtained from lung biopsies without pneumonia, focal bronchopneumonia, or confluent bronchopneumonia, independent of prior antibiotic treatment. The only significant difference was observed comparing samples with bronchiol-
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![Graph showing log10 CFU/g and different phases of pneumonia](image)

*Fig. 4. Geometric means (± SD) of lung biopsy cultures (expressed as log10 CFU/g) according to the different evolution phases. Open circles = patients receiving antibiotics (group A); solid circles = patients without antibiotics (group B). P < 0.01 when comparing bacterial counts from biopsy cultures in different evolution phases in patients with and without antibiotics. pNS when comparing different phases within the same group (ANOVA).*

Analysis of mechanical ventilation (more than 10 days). This observation agrees with clinical studies that demonstrated that the risk of ventilator-associated pneumonia increases with the length of mechanical ventilation. Moreover, histologic pneumonia was more common in patients who were admitted with acute respiratory failure. Adult respiratory distress syndrome and severe respiratory failure have been shown to be predisposing factors for ventilator-associated pneumonia.

In the current study, pneumonia showed several important histologic features. First, ventilator-associated pneumonia was found to be a nonhomogeneous multifocal process frequently involving both lungs, especially lower lobes. This histologic pattern may be due to the flow and volume patterns generated by mechanical ventilation that probably favors the distribution of bacteria from central to distal airways. Rouby et al. who sampled only one lung, described a similar observation. Rouby et al.’s and our results support the concept that a single lung biopsy cannot exclude pneumonia. Second, ventilator-associated pneumonia is a heterogeneous process characterized by different phases of evolution at the same time. We are unable to explain this finding, but we believe that studying these aspects may be worthwhile in future postmortem research. As far as we know, our group is the first to report on the different evolution phases of pneumonia. Previous human and animal studies only evaluated the degree of widespread distribution of ventilator-associated pneumonia with visually guided procedures. None of them showed that, when using clinical data, the risk of ventilator-associated pneumonia was higher in patients with severe and/or extensive infection and focal lesion. In our study, patients with bronchiolitis alone were never observed, and bronchiolitis was found in two patients with bronchopneumonia. These findings indicate that the interpretation of histologic findings and microbiology of ventilator-associated pneumonia may be subject to the evolution of the initial lesion and the patient's condition. Severe bronchopneumonia was found in patients with extirpation of the lung in a background of normal anatomy. The combination of bronchial dilatation, fibrosis, and polymorphonuclear cells is characteristic of diffuse alveolar damage. In the interstitium, there is an intense infiltrate of inflammatory cells, and there is no destruction of the normal structure. The presence of this finding is highly specific for diffuse alveolar damage. In the interstitium, there is an intense inflammatory infiltrate.
Histopathologic and microbiologic aspects of VAP

Both the widespread distribution and the heterogeneity of ventilator-associated pneumonia may explain why blind endobronchial sampling has similar accuracy compared with visually guided samples.22,23 It is important to note that, when using a histologic classification of extension and/or severity, only two categories (lack of lung infection and focal bronchopneumonia) were well represented in our study. Confluent bronchopneumonia and bronchiolitis were less frequent and lung abscesses were never observed. In other words, we have only found in our biopsies mild degrees of ventilator-associated pneumonia, and this must be considered during the interpretation of the relationship between histology and microbiology.

Histologic signs of diffuse alveolar damage and pneumonia may be confusing, particularly in the initial and resolution phases. Early stages of diffuse alveolar damage show severe abnormalities of capillary walls and pneumocytes, with thickening of the alveolar septa and accumulation of cell debris in the alveoli, which precedes the hyaline membrane formation. This morphologic picture is quite different from the initial phases of pneumonia in which there is no destruction of any lung structure but capillary dilatation, fibrinous exudate, and accumulation of polymorphonuclear leukocytes. Resolution stage of diffuse alveolar damage is a regenerative process in the interstitium with fibrosis, whereas in pneumonia, there is an intraalveolar lesion that returns to the normal anatomic morphology after macrophage activity.14,15 The hypothetical overlap between initial and resolution phases of pneumonia and diffuse alveolar damage could induce an overestimation in the presence of histologic pneumonia. This does not apply to our results, because early phases of pneumonia alone (not coexisting with other phases) were found only in 2 of the 25 patients, and resolution phases were not found alone in any of the patients studied.

Diffuse alveolar damage was found in 32% of the study patients. This figure is low compared with other work11 and may be due to the differences in population studied. All patients having diffuse alveolar damage also showed foci of pneumonia, and in 73% of the samples, diffuse alveolar damage was combined with histologic pneumonia. Our results confirm data reported by Rouby et al.11 These observations support the concept that ventilator-associated pneumonia is often a complication of diffuse alveolar damage and vice versa. Damaged lungs are at high risk of bacterial infection, as shown in experimental and clinical circumstances, probably due to the impairment of intrapulmonary antibacterial defenses. Intratracheal inoculation of Pseudomonas aeruginosa was followed by superinfection of the lower airways in an animal model of paraquat lung injury.24

The specificity of lung cultures in this study was invariably low, regardless of the prior administration of antibiotics. Furthermore, when using the recommended threshold of $10^5$ cfu/g, this resulted in poor value for differentiating colonization from true alveolar infection. In a previous study9 from our group using a single biopsy per lung and in another by Rouby et al.,11 this finding was also confirmed. Our results have important clinical implications, because quantitative bacterial thresholds are used by most Intensivists for the diagnosis of ventilator-associated pneumonia.1

In this study, there was a poor relationship between histology and microbiology of lung cultures, even in absence of prior antibiotic administration. Moreover, when analyzing microbiologic data, excluding microorganisms with low virulence, results did not vary markedly. These findings are consistent with the view that postmortem biopsy cultures do not represent a valid reference for the evaluation of quantitative cultures of bronchoscopic and nonbronchoscopic techniques. This probably happens because, in the complex setting of the critically ill patient, many factors may interfere and influence bacterial concentration of the lung. We believe, based on the available data, that the only valid reference test for the diagnosis of ventilator-associated pneumonia is the histologic confirmation of the disease.15

The rate of false-positive biopsy cultures was high in the current study. In postmortem studies false-positive lung cultures may be due to lung contamination after death or poor aseptic techniques during thoracotomy, bronchial colonization, and bronchiolitis. As regards contamination after death, some authors have reported a high incidence of positivity of lung cultures despite the absence of any relationship with in vivo processes of pulmonary infection.25 The delay between death and pulmonary sampling could explain some false-positive results. However, the percentage of false-positive results in this study was less in samples obtained within 90 min, compared with those obtained within 30 and 60 min. This could be explained because the majority of patients in whom the extraction procedure was performed within 30 min after death were organ donors. In those patients, brain death occurred 12–24 h before

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the study protocol, and perhaps this fact favored the presence of pulmonary colonization. Concerning the technique of thoracotomy, this was performed under strict aseptic methods, minimizing contamination during the procedure. Bronchial colonization does not explain the false-positive results, because the pulmonary biopsy samples were taken from the periphery of the lung, making the presence of bronchial fragments into the biopsy unlikely. Finally, bronchiolitis is thought to contribute to some of the false-positive results. Bronchiolitis accounted for 8% of the false-positive cultures in our study.

A possible association between antibiotic treatment and bacterial growth was evaluated. A relationship was observed between the high bacterial counts and the absence of antibiotic treatment in all pneumonia stages. A significant reduction in distal airway bacterial concentration was shown after 3 days of antibiotic treatment. These data strengthen the importance of distal airway sampling before starting antibiotic therapy. However, the impact of prior use of antibiotics on lung cultures is difficult to assess with our study design due to the lack of control patients.

In summary, ventilator-associated pneumonia was found to be a polymicrobial, multifocal, and dynamic histologic process with little relationship to the bacterial culture results. These histologic and microbiologic features of ventilator-associated pneumonia may explain the difficulties in validating both invasive and noninvasive methods for the diagnosis of this common pulmonary complication related to mechanical ventilation.

The authors thank Dr. H. K. F. van Saene, for suggestions during the preparation of this manuscript.

References

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