Pneumonia and Acute Lung Injury: New Insights on Histopathology

Mustafa El Ebiary, Neus Fàbregas and Antoni Torres

*Serveis de Pneumologia i Al·lèrgia Respiratòria, i Anestesia.
Universitat de Barcelona. Barcelona, Spain.*

Pneumonia is a frequent complication of mechanical ventilation with an incidence that ranges between 9 and 70% [1]. Ventilator-associated pneumonia (VAP) seems to be particularly frequent in patients with acute respiratory distress syndrome (ARDS) in whom several local and systemic factors apparently predispose VAP development [2]. Most of our knowledge about VAP comes from clinical and or microbiological studies that have been published in the last decade. However, the histology of VAP has been only known in recent years mainly through immediate postmortem studies that have been performed to validate techniques used to elicit microbial diagnosis of pneumonia in mechanically ventilated patients [3-8]. These studies have extensively investigated the lungs of patients mechanically ventilated for several days and along with experimental models of pneumonia have allowed to describe the peculiar histological and microbiological characteristics and interactions of human VAP, as well as to confirm the frequent association of this infection with histological evidence of lung injury. From the histological information on VAP important clinical implications have been concluded. In the present review, the histological characteristics of VAP and its association with histological lung injury (diffuse alveolar damage, DAD) are commented in depth.

Classical Definition of Histological Pneumonia

Pneumonias are classified pathologically and radiologically according to the portion of lung that is involved. *Lobar pneumonia* results in diffuse consolidation of most or all of one or more lobes. Historically, it has usually been caused by *Streptococcus pneumoniae*; but other bacteria are occasionally involved. However, lobar pneumonia is an unusual autopsy finding in hospitalized patients. Lobar pneumonia has been shown, in the classic literature, to progress through four characteristic pathological stages: congestion, red hepatization, gray hepatization, and resolution [9]. *Interstitial pneumonias* involve the alveolar walls and peribronchial tissues. These pneumonias are usually caused by viruses and mycoplasma, and are not discussed in this review.

In contrast to lobar pneumonia, *bronchopneumonia* is a patchy inflammatory process that tends to involve multiple lobes of the lung in a multifocal manner. The inflammatory exudate tends to spread into the alveolar parenchyma immediately surrounding terminal and respiratory bronchioles. In Figure 1, a macroscopic view at gross examination of human bronchopneumonia is shown. Microscopically, and during initial periods, alveolar macrophages, are present with few polymorphonuclear leukocytes (PMNL) in addition to serous exudate in early disease. In subsequent periods abundant PMNL rapidly appear, and depending on the severity, fibrin may or may not be present. Many of the aerobic Gram-negative bacilli, in addition to the alveolar lesion, produce necrosis of the alveolar walls, which results in multiple abscesses scattered throughout the affected lung. Focal hemorrhages may be present. The alveolar inflammatory exudate contains both PMNL and mononuclear cells [9].

Although this classification might still be valid from a general scope, postmortem studies in VAP have defined particular features regarding the distribution and evolution of lesions that escape from the classical point of view and that may have clinical implications.

Histological Definition and Classification of VAP

The histological presence of VAP has classically been accepted as the presence of foci of consolidation with intense leukocyte accumulation in bronchioles and adjacent alveoli [5,8,9]. This definition is very simplistic since it does not take into account severity and distribution of lesions. Rouby and coworkers [5], studying whole lungs in patients after mechanical ventilation, could define four different stages of severity and/or extension of VAP as follows: 1) *Bronchiolitis* or early infection, characterized by intense proliferation of PMNL localized within the bronchiolar lumens and associated with purulent mucus plugs and bronchiolar wall alterations (Figure 2); 2) *Focal bronchopneumonia*: scattered neutrophilic infiltrates localized to

Address for correspondence: Dr. A. Torres, Servei de Pneumologia, Hospital Clínic, c/Villarroel 170, 08036 Barcelona, Spain

Fig. 1. Macroscopical aspect of human lung bronchopneumonia. Note the characteristic patchy distribution (arrow). Reprinted with permission from reference 5.

terminal bronchioles and surrounding alveoli (Figure 3a); 3) *Confluent bronchopneumonia*: defined as the extension of these elementary lesions to several adjacent lobes (Figure 3b); and, 4) *Lung abscess*: which is defined as the confluent bronchopneumonia associated with tissue necrosis and disruption of normal lung architecture (Figure 4).

Other histological descriptions of VAP have been recently published, partially modifying the earliest definitions of VAP [6,10]. These published studies used modifications of the classification described by Johanson et al. in a baboon model of VAP [11]. Marquette et al. in a postmortem study in mechanically ventilated patients [6] considers VAP when there is consolidation at the level of secondary lobules with intense accumulation of PMNL, fibrinous exudate, and cellular debris within alveolar spaces, whether or not the lesions were centered on terminal or respiratory bronchioles. Lung abscess was defined when the lesions of pneumonia were associated with tissue necrosis and destruction of the surrounding lung architecture. Another study by Chastre and colleagues [10] examining lungs of patients who have been mechanically ventilated, defined three grades of severity according to a previous animal study [11]: 1) *Mild bronchopneumonia*: the presence of scattered neutrophilic infiltrates localized to terminal bronchioles and some surrounding alveoli. According to these authors, lesions may be non-specific for pneumonia when severe pulmonary underlying disease such as adult respiratory distress syndrome (ARDS) or extensive chronic

Fig. 2. Bronchiolitis with intense proliferation of polymorphonuclear leukocytes localized within the lumen of bronchioles. Reprinted with permission.

Fig. 3. Examples of histologic classification used to quantify severity of bronchopneumonia: (a): Focal bronchopneumonia characterized by an intense PMNL accumulation within the lumen of two distal bronchioles and in the adjacent alveoli. Original magnification: $\times 20$. (b): Confluent bronchopneumonia characterized by the confluence of several foci of bronchopneumonia between adjacent lobules. Original magnification $\times 10$. Reprinted with permission

obstructive lung disease coexist. 2) *Moderate bronchopneumonia*: was defined when the extension of the inflammatory process caused grossly evident confluence of infiltrates between adjacent lobules and when purulent mucus plugs were present in bronchioles. 3) *Severe bronchopneumonia*: was considered when there was extensive confluence of inflammation and this was occasionally associated with tissue necrosis.

In a postmortem study with bilateral multiple biopsy sampling [8], we described four evolution stages of pneumonia: 1) *Early phase* (0–2 days of evolution) which shows the presence of capillary congestion with increased number of PMNL at this level. The alveolar spaces usually show a fibrinous exudate. 2) *Intermediate phase* (3–4 days of evolution) characterized by presence of fibrin, few erythrocytes and several PMNL in the alveoli. 3) *Advanced phase* (5–7 days of evolution) showing PMNL filling up most of the alveoli and macrophages incorporating cellular debris in the cytoplasm. 4) *Resolution phase* (> 7 days of evolution): the

Fig. 4. Left: The low power micrograph shows a microabscess surrounded by congested alveolar walls and focally edematous air spaces ($\times 70$). Right: The PMNL exudate is better visualized ($\times 350$). Reprinted with permission.

inflammatory exudate is eliminated due to phagocytic activity of mononuclear cells. We have also defined two degrees of severity in relation to the extension of the lesion: mild and severe. Figure 5 illustrates the histological features of this classification.

Human and Experimental Postmortem Histological Studies on VAP

Chastre and colleagues [3] were the first to develop a postmortem human model upon critically-ill patients. In the immediate postmortem period (within 30 min) they performed a left thoracotomy, under surgical aseptic conditions, and obtained six superficial small specimens from the anterior segment of the left lower lobe for culture. In addition a 1 cm³ specimen was obtained for histological analysis. They found a good association between histologic and bacteriologic findings (quantitative cultures). Similar results were obtained by Gaussorgues and coworkers [12]. Rouby and colleagues [13] performed a more extensive approach and analyzed histologically two small lung specimens obtained from an area of consolidation of the lower lobe. Another small specimen was cut from the same area for

Fig. 5. Normal lung parenchyma, and phases of pneumonia. Panel A: Normal alveolar structure. Absence of interstitial and capillary abnormalities. Hematoxylin & Eosin ($\times 200$). Panel B: Early phase of pneumonia: Accumulation of polymorphonuclear leukocytes within the capillaries (arrow). Hematoxylin & Eosin ($\times 200$). Panel C: Intermediate phase of pneumonia: Polymorphonuclear leukocytes, fibrin and few R.B.C's in the alveolar lumina. Hematoxylin & Eosin ($\times 200$). Panel D: Advanced phase of pneumonia: Polymorphonuclear leukocytes and macrophages filling the alveolar lumina. Hematoxylin & Eosin ($\times 200$). Panel E: Resolution phase of pneumonia: Some alveoli show macrophage activity with rests of inflammatory exudate (arrow). Hematoxylin & Eosin ($\times 200$). Reprinted with permission from reference 8.

bacteriological examination. Then, the entire lung was surgically removed and a complete lung autopsy was done. Foci of pneumonia were predominantly distributed to lower lobes and in dependent zones of the lung. The histologic lesions of bronchopneumonia were always located within large zones of altered lung parenchyma. They also demonstrated that a single lung specimen could miss the detection of histological pneumonia in around 30% of cases. The latter finding must be taken into account when interpreting the results of earlier studies which limited histological examination to a single sample. The same group [5] performed another postmortem study analyzing extensively a whole lung from 83 deceased critically-ill patients. Five to 10 slices per lung segment were obtained and examined using the histologic classification alluded to above [5]. They reconfirmed the multifocal nature of VAP and described the frequent association of VAP with bronchiolitis.

Our group studied single bilateral postmortem biopsies in mechanically ventilated patients who died with prior antibiotic treatment [4]. We obtained, from the lower lobe of each lung, one specimen for histological analysis and another for microbiologic quantitative cultures. In this study we observed a poor relationship

between histologic and microbiological results, perhaps due to the administration of prior antibiotic treatment in all patients. In other words quantitative cultures of lung cultures could not easily discriminate the presence or absence of histological pneumonia. This prompted us to perform a subsequent study [8] including patients with and without antibiotic treatment (48 hours at least free of antibiotic treatment). Several specimens from each lobe of the two lungs were aseptically obtained (average of 16 specimens/patient). Applying the evolution classification of VAP described above, we confirmed the disseminated multifocal heterogeneous pattern of VAP, predominantly involving lower lobes. Interestingly, we observed that all the phases alluded to above coexisted in the same patient and in the same lung exhibiting a pleomorphic histological pattern (Figure 6). Overall intermediate and advanced phases were the most common stages of pneumonia observed in this study. As in other studies nonspecific alveolar damage and bronchiolitis were also frequent findings.

In a recently published human postmortem study [10] two large specimens from each anterior segment of the left upper and lower lobes were obtained from 20 patients who had not suffered prior episodes of pneu-

Fig. 6. A schematic representation of ventilator-associated pneumonia. This is a multifocal pattern bilateral process affecting predominantly the lower lobes. Different degrees of evolution and severity coexist at the same time. Reprinted with permission.

monia and were free of recent antibiotic changes. Interestingly, in this study the histologic grade of severity of the two matched segments was discordant in 7 out of 19 patients (36%) in whom the two segments were analyzed.

Marquette and coworkers [6] studied the histological characteristics of the entire fixed lungs and they confirmed the findings of previous studies. Pneumonia was found in 50% of the dependent segments and in 37% of the non-dependent segments. One of the major characteristics of the lesions was their typically scattered pattern of distribution within normal or damaged lung parenchyma. Only 14 of 83 examined lobes (16.8%) had all of their segments involved by the infectious process. The scattering of the lesions was even more prominent at the segmental level, where the infectious alveolar damage ranged from limited foci of pneumonia to large areas of confluent pneumonia. A distinctive finding in several cases was the absence of pneumonia from the peripheral lung samples, while more central areas of the same segment displayed typical foci.

Finally, Papazian and coworkers [7] analyzed an entire lung from 38 patients, using the histologic classification from Johanson and coworkers [11]. They found no sign of bronchopneumonia in 20 cases. Conversely, in the remaining 18 cases they confirmed bronchopneumonia histologically. There was no relationship between the results of the various cultures and the pathology results. Bronchiolitis was noted in 8 patients, out of whom 5 has concomitant histologic signs

of pneumonia. The remaining 3 patients had negative lung cultures. These authors examined the significance of the isolation of *Candida* spp. in their samples. Despite frequent lung colonization by *Candida*, only 2 patients exhibited histologic signs of *Candida* pneumonia. Lung tissue cultures were positive for *Candida albicans* in these 2 patients. This agrees with the results of El-Ebiary's et al. study who found that the incidence of definite *Candida* pneumonia was 8% [14]. Nevertheless, in this study, the incidence of *Candida* isolation from pulmonary biopsies in critically-ill mechanically ventilated, non-neutropenic patients who die was high (40%) indicating that *Candida* is a frequent colonizing agent in very critically ill patients.

In order to avoid confounding factors such as antibiotic presence or lung injury described in other studies, Marquette et al. induced pneumonia in pigs free from antibiotics and previous concomitant lung disease secondary to tracheobronchial stenosis [15]. They found that the histologic lesions of pneumonia, as well as the lung bacterial burden were unequally distributed within the lungs and even within the lung segments. Moreover, specimens showing histologic evidence of pneumonia had significantly higher bacterial burden than specimens with bronchial infections and specimens with neither bronchial nor lung infection. However, the authors could not define a clear cut threshold for quantitative cultures to discriminate the presence or absence of pneumonia. This study providing experimental insights into the relationship between microbi-

ologic and histologic features in bacterial pneumonia confirms previous findings in humans [5–8]. However, a prior study from Johanson et al. in baboons [11] could find acceptable correlations between lobar bacterial index (sum in log scale of different species isolated) and the degree of histological pneumonia severity.

In humans, with one exception [10], there is now compelling evidence that quantitative biopsy cultures cannot reliably discriminate between patients with and without evidence of histological pneumonia. The use of a specific threshold to define the presence of pneumonia does not take into account the fact that lung infection occurs along a bacteriologic continuum. Thus, when pneumonia begins or if infectious bronchiolitis is present, the diagnostic threshold may not be met. The same case occurs when prior antibiotic has been given. Another explanation for low quantitative lung cultures in presence of histological pneumonia is the normal functioning of antibacterial lung defenses which clear lung bacterial burden. On the other hand the specificity of lung cultures is low with a high rate of false-positive cultures. In post-mortem studies false-positive lung cultures (without pneumonia) may be due to bacterial colonization, and bronchiolitis. It has been suggested that closely to death, in critically-ill patients, the lung could suffer from massive bacterial colonization which could explain the frequent presence of bacteria in distal airways without histological pneumonia [16]. From all these findings it is clear, due to the poor relationship between quantitative lung cultures and histological examination, that quantitative lung biopsy cultures alone cannot be used to validate *in vivo* diagnostic techniques used to microbiologically investigate VAP.

Clinical Implications of Histological and Microbiological Findings in Postmortem VAP Studies

The histological findings of human postmortem studies have the following clinical implications [17]: 1) Initial phases of VAP that probably need to be treated with antibiotics cannot be detected at portable chest X-ray. 2) Since VAP is a multifocal process the techniques that explore broad lung regions such as bronchoalveolar lavage are clearly preferred to those that only explore a segment (protected specimen brush). 3) Blind diagnostic methods that can sample lung dependent zones are probably as accurate that visually guided methods. 4) Prone position, which is a current popular method to manage refractory hypoxemia, could help to disseminate VAP from posterior to anterior part of the lungs. This hypothesis has not been tested in clinical studies.

The microbiological findings have the following clinical implications: 1) As mentioned above quantitative postmortem lung cultures cannot be used as gold standard to validate microbiological diagnostic tech-

niques. 2) When interpreting quantitative bacteriology at the bedside, the clinician should weigh a number of factors that can modify bronchial and alveolar bacterial burden: presumed stage of bronchopneumonia, administration of antibiotics, technique of distal sampling, natural host antibacterial defenses, duration of mechanical ventilation and presence of acute lung injury. The microbiological complexity of VAP does not support the concept of a standard threshold for the diagnosis of pneumonia. Thus, treatment algorithms based upon definite thresholds of quantitative cultures may lead to undertreat patients. Since early and adequate initial antibiotic treatment is one of the major factors related with prognosis of VAP, the strict compliance of treatment based upon quantitative thresholds without clinical judgement may be hazardous to patients.

Histopathology of Acute Lung Injury

Widespread injury to distal alveoli produces diffuse alveolar damage (DAD) or acute interstitial pneumonitis, while more localized injury to peribronchiolar parenchyma produces bronchiolitis obliterans-organizing pneumonia. The term acute lung injury encompasses these various histologic manifestations. The morphologic picture of the lung in ARDS has been labeled diffuse alveolar damage (DAD). DAD is a descriptive term for the predictable although nonspecific pathologic sequence of events following severe acute lung injury caused by a variety of insults.

The exact histologic appearance of DAD varies, depending upon the time interval between the onset of symptoms and lung biopsy [18,19]. This picture can be conveniently divided into three phases: 1) *Exudative phase* of edema and hemorrhage; 2) *proliferative phase* or organization and repair; and 3) *fibrotic phase* of end-stage fibrosis. It should be noted that the changes of DAD are not necessarily progressive from the acute to the organizing stage; that is, the destructive process may cease, and recovery can occur at any stage [20]. The exudative phase occupies approximately the first week after the onset of respiratory failure. The earliest changes detectable by light microscopy are capillary congestion, interstitial and alveolar edema, and intra-alveolar hemorrhage. The distinctive histologic features of the exudative phase, however, are dense eosinophilic hyaline membranes that usually are most prominent along the alveolar ducts. Inconstant features of severe endothelial injury include cellular necrosis and disruption with denudation of the capillary basement membrane and intravascular fibrin accumulation. Intracapillary aggregates of neutrophils are focally prominent, especially in diffuse alveolar damage secondary to sepsis or trauma. The proliferative phase is the stage of organization of the intraalveolar and interstitial exudate acquired in the acute phase [21]. Type II cells begin to proliferate along alveolar septa

as early as three days after the onset of clinical ARDS, and fibrosis is apparent by the tenth day. Epithelial cell regeneration heralds the onset of the proliferative phase as draws of cuboidal cells extend along the alveolar wall in an attempt to cover the previously denuded basement membrane. Within the alveolar wall, fibroblasts and myofibroblasts proliferate and subsequently migrate through breaks in the alveolar basement membrane into the fibrinous intra-alveolar exudate. Fibroblasts convert the exudate to cellular granulation tissue and ultimately, by the deposition of collagen, into sparsely cellular dense fibrous tissue. Fibronectin is more prominently seen coating the surface of fibrinous exudate in the exudative phase. Epithelial cells migrate over the surface of the organizing granulation tissue and thereby transform the intra-alveolar exudate into interstitial tissue. This process of fibrosis by accretion is the most important mechanism of lung remodeling in ARDS. This reorganization pattern is identical to that in classic organizing pneumonia and represent an integral, albeit nonspecific, way in which the lung responds to injury.

Diffuse lung injury leading to ARDS is often associated with an increase in the neutrophil counts in the lungs. This finding differs markedly from the pattern of leukocyte accumulation in pneumonia, in which intense collections are found in the regions of terminal bronchioles and attendant alveolar ducts and alveolar sacs. Finally, the fibrotic phase in ventilator-dependent patients who survive beyond 3 or 4 weeks from the onset of

ARDS, the lung is completely remodeled by cellular collagenous tissue. Pulmonary fibroproliferation is a stereotypical reparative reaction to tissue injury and is characterized by the striking accumulation of myofibroblasts and their connective tissue products in the airspaces, interstitium, respiratory bronchioles, and walls of the intraacinar microvessels. Its evolution appears to be determined by the extent of initial inflammatory reaction in the lung (alveolar denudation, basement membrane destruction, and quality of intra-alveolar exudate) and by the presence of a protracted inflammatory response (recurrent injury).

From the histological point of view, diffuse alveolar damage and pneumonia may be confusing, particularly in the initial and resolution phases [8]. Early stages of diffuse alveolar damage show severe abnormalities of capillary alveolar 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 fibrinous exudate. Resolution stage of diffuse alveolar damage is a regenerative process with fibrosis, whereas in pneumonia, there is an intraalveolar lesion that returns to the normal anatomic morphology after phagocytosis. The histological pictures of initial (Figure 7) and resolution (Figure 8) phases of DAD are illustrated. For comparison, please refer to stages shown in Figure 5.

Fig. 7. Initial phase of DAD showing interstitial edema and desquamation of pneumocytes type II ($\times 400$)

Fig. 8. Resolution phase of DAD showing lining cell hyperplasia and interstitial fibrosis

Relationship Between VAP and Acute Lung Injury

Of all nosocomial infections, pneumonia is the one most likely to lead to death of the patients, particularly when pneumonia complicates ARDS. It has been reported that approximately one third of all patients died in the hospital had a nosocomial infection, but when this infection was determined to be causally related to death, pneumonia was present 60% of the time. In patients with ARDS, fatality rates for pneumonia may be even higher, and the presence of infection of any type has been associated with a poor outcome [22]. For many years there is the clinical belief that ARDS predisposes to pneumonia acquisition. This concept comes from classical necropsy studies that found a high rate of pneumonia (73%) in the lungs of patients that had been mechanically ventilated by ARDS [23]. In fact, this is supported by studies that have demonstrated the impairment of lung neutrophil function in ARDS [24] indicating a predisposition to acquire pneumonia. However, when the diagnosis of nosocomial pneumonia is based on either clinical or bacteriological criteria, the incidence is not so high and varies among studies. The most recent and complete information on this issue has been provided by Shuterland et al [25] who evaluated the incidence of pulmonary infections in ARDS and their impact on survival. They performed sequential protected specimen brush or bronchoalveolar lavage

samples by fiberoptic bronchoscopy in 105 patients with ARDS. Only 16 (16%) patients met quantitative bacteriologic criteria for pneumonia. The clinical incidence of possible or probable pneumonia was 33%. The mortality of patients with bacteriological confirmed pneumonia was not different (37%) compared to that with negative bacterial results (45%). The authors concluded that pneumonia based on quantitative cultures is uncommon in ARDS. This study, although partially biased by prior antibiotic treatment, makes the issue of VAP acquisition during ARDS still controversial.

Another problem in ARDS-VAP relationships are the difficulties in diagnosing VAP in the clinical arena during ARDS evolution. It is important for the clinician to be aware that fever and systemic inflammatory response syndrome can develop exclusively from fibroproliferation in patients with late ARDS and that the former is clinically indistinguishable from a nosocomial infection [20]. Bacteriological quantitative sampling may not help to diagnose VAP during ARDS as demonstrated by Sutherland et al [20]. Not even the measurement (particularly in bronchoalveolar) of biological markers of inflammation is helpful. The release of cytokines in the lung, such as tumor necrosis factor (TNF- α), interleukin 1 (IL-1 β), or interleukin 8 (IL-8), constitutes a critical part of the early inflammatory response in most patients with ARDS and pneumonia. This inflammatory release of cytokines in lung is similar for both processes (ARDS and pneumonia) in the initial periods

[26]. However, some authors have demonstrated that cytokines detected in bronchoalveolar lavage (IL6 and IL8) are persistently high in ARDS patients and decrease after three days in pneumonia [27].

Experimental Studies of Acute Lung Injury and VAP

There are few animal data on severe lung injury complicated by bacterial pneumonia, for the simple reason that few investigators have studied animal models of lung injury so severe that long-term mechanical ventilation is required. Animal models can be useful to further clarify the relationship between the severity of lung damage and the local burden of infection in bacterial pneumonia.

In baboons, breathing 80% oxygen causes a mild inflammatory pneumonitis, and breathing 100% oxygen causes severe diffuse lung injury associated with hyaline membrane formation and clinical respiratory distress. Superinfection of animals breathing 80% oxygen with *Pseudomonas aeruginosa* converts the mild pneumonitis into a severe lung injury indistinguishable from that caused by 100% oxygen [28].

Campbell and colleagues [29] developed a baboon model with bacterial superinfection after lung injury produced by intravenous administration of oleic acid. In those animals without VAP, a deterioration of lung function and pulmonary hemodynamics developed that reversed after 3 days. However, in animals with concomitantly presented VAP a markedly different course characterized by deterioration of all parameters of lung function, erosive gastritis and hemorrhage, thrombocytopenia, and a hyperdynamic circulation with low systemic vascular resistance and high cardiac output. These observations indicate that superinfection of bacterial pneumonia on a non specific lung injury markedly worsened lung function and was associated with systemic signs of sepsis.

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. Seidenfeld and coworkers [30] studied experimental lung injury produced in hamsters by injecting 20 mg/Kg paraquat intraperitoneally; control animals received saline vehicle. Three days later, *Pseudomonas aeruginosa* 10⁸ microorganisms in 0.25 ml, or saline, were inoculated intratracheally. Lung and systemic antibacterial defenses were studied at death 24 hours later. Paraquat alone produced focal interstitial pneumonitis and neutrophilic alveolitis. *Pseudomonas aeruginosa* alone caused focal pneumonia and no deaths. Animals receiving both agents had extensive diffuse alveolar damage characterized by alveolar hemorrhage, edema, influx of neutrophils, and vasculitis. Paraquat injury impairs lung antibacterial defenses by uncertain mechanisms. Superinfection of paraquat-injured lungs by *Pseudomonas aeruginosa*

appears responsible for defects in intrapulmonary and systemic antibacterial defenses.

The mechanisms underlying the worsening of lung function when bacterial infection is superimposed on a nonspecific lung injury are not known with certainty. Possible interactions between pneumonia and acute lung injury are outlined in Table 1 [31]. Pulmonary edema causes an impairment of lung antibacterial defenses [32], decreasing the inactivation of bacteria that reach the distal lung. As bacteria proliferate, an intense chemotactic signal for neutrophils is generated. This may be initiated by macrophages, but recent evidence suggests that interleukin-8 released from neutrophils provides a powerful amplifying mechanism [32]. Bacterial pneumonia increases the permeability of the alveolar vessels, presumably because of the action of proteolytic enzymes and oxygen radicals released from PMNL. The distribution of pulmonary blood flow also is markedly altered. Alveolar filling processes are associated with markedly decreased ventilation and decreased regional perfusion. This diversion of blood flow away from consolidated lung is due in large part to hypoxic pulmonary vasoconstriction.

Finally, bacteria that gain entrance to the periphery of the lung may have access to the systemic circulation. It might be speculated that bacteria would be released from injured lungs at lower concentrations than from normal lungs. Bacteria may migrate through the lymphatics. In lung injury there is an increase in lung permeability and lung lymph flow, both conditions that may enhance the release of bacteria from lungs [33].

Human Histological Studies

Human postmortem studies on VAP have clarified the relationship between acute lung injury and pneumonia. Rouby and colleagues [5] examined the lungs of 83 patients who had been mechanically ventilated. Most of them had diffuse pulmonary infiltrates. Bronchopneumonia was found in 52% of lungs. Non specific alveolar damage was noted in nearly 80% of the lungs: 70% of those without pneumonia and 81% of lungs with pneumonia.

Table 1. Effects of pneumonia in acute lung injury

Acute effects:
Increased leukocyte recruitment
Increased permeability and edema
Decreased hypoxic vasoconstriction
Altered blood flow distribution
Activation of systemic inflammation
Chronic effects:
Lung destruction and remodelling
Increased lung fibrosis
Airway inflammation and hyperactivity
Bronchiectasis

Modified from reference 31

We performed a postmortem study upon 25 patients who died after being mechanically ventilated for several days [8]. Examining both lungs from each patient we obtained a total of 375 biopsies. The incidence of VAP was 92%. Eight patients (32%) had at least one pulmonary biopsy sample showing histologic signs consistent with DAD. The total number of biopsy samples showing diffuse alveolar damage was 44 of 375 (12%). DAD 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 (Figure 9). The incidence of DAD did not change in relation to the period of mechanical ventilation (> 10 days or > 10 days; 14 and 10%, respectively). However, the incidence of pneumonia was higher in patients with longer mechanical ventilation periods (54 vs 40%). In the 12 patients who were initially admitted because of acute respiratory failure, 102 of 167 biopsies (61%) showed histological 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). The hypothetical overlap between initial and resolution phases of pneumonia and diffuse alveolar damage could induce an overestimation in the presence of his-

tologic pneumonia. This does not apply to our results, because early phase of pneumonia alone (not coexisting with other phases) was found only in 2 of the 25 patients, and resolution phases were not found alone in any of the patients studied. Our results confirm data reported by Rouby and coworkers [5]. These observations support the concept that VAP 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. Interestingly we did not find that the incidence of lung was influenced by the period of mechanical ventilation.

Diffuse interstitial fibrosis often follows severe acute lung injury. Lamy et al. [34] examined the lung tissue from 45 patients either with an open lung biopsy or at autopsy, and correlated the histological findings with various physiological parameters. They noted that lung fibrosis was most likely to occur in association with infection.

In summary, human postmortem studies confirm that pneumonia is a very common finding in the lungs from mechanically ventilated patients. The acquisition of pneumonia worsens lung function in ARDS. Postmortem studies have shown that DAD is less frequent than pneumonia. However, when present it coexists always along with pneumonia, suggesting that the latter is a frequent complication of ARDS. Nevertheless,

Fig. 9. Left: Characteristic pneumonia pattern in patient with ARDS. Hyaline membranes, edema, and abundant PMNL specially in the alveoli subjacent to the bronchus are present. Right: Higher magnification of the alveolar exudate. (H&E: left $\times 70$, right $\times 350$). Reprinted with permission.

in the clinical setting this issue is still controversial and needs further investigations.

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