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Ventilator-associated pneumonia and atelectasis: evaluation through bronchoalveolar lavage fluid analysis

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Abstract *Objective:* Surfactant offers protection against alveolar collapse and contributes to the local defense mechanism, but it is unclear if surfactant alterations have a role in the development of atelectasis or ventilator-associated pneumonia (VAP). The present study was undertaken to monitor surfactant, as well as biochemical BAL fluid alterations, during the course of VAP and atelectasis in mechanically ventilated patients without primary cardiopulmonary disease, to elucidate the pathogenesis and to differentiate these two entities. *Design:* Prospective controlled study. *Setting:* 14-bed general ICU of a 750-bed University Hospital. *Patients:* Sixty-one ventilated patients, without primary cardiopulmonary disease–normal initial chest X-ray, satisfactory oxygenation (PaO_2/FiO_2>300 mmHg), and expected time of ventilation exceeding 2 weeks–were initially enrolled. Twelve of them developed VAP and eight lobar or segmental atelectasis during the 2-week study period. *Interventions:* An initial BAL was performed in all patients within 48 h from admission. Patients who developed VAP or atelectasis were subjected to a second and third BAL during and after the resolution of VAP or atelectasis, respectively. *Measurements and results:* VAP and atelectasis resulted in a significant increase of total protein and markers of inflammation, such as PAF and neutrophils, which partially remitted after their resolution. Large surfactant aggregates, which contribute to surface tension decrease, were significantly reduced during both entities and remained low even after their resolution. *Conclusions:* BAL alterations during VAP and atelectasis suggest increased alveolar-capillary permeability, severe surfactant abnormalities, and signs of local inflammatory reaction. These alterations are associated with the observed deteriorated gas exchange and lung mechanics and could predispose to further lung injury in ventilated patients.

Keywords Ventilator-associated pneumonia · Atelectasis · BAL · Lung surfactant · Platelet-activating factor (PAF) · PAF-acetylhydrolase

Introduction

Mechanical ventilation has been associated with a high incidence of respiratory complications, which has been recognized ever since it was introduced into clinical practice [1]. VAP and atelectasis probably represent the most common of these complications, although their exact incidence is difficult to determine. The epidemiological data for VAP incidence remain controversial, which is a problem probably arising from differences in diagnostic criteria and patient populations [2]. Limited epidemiological data exist for atelectasis in mechanically ventilated patients, referring to specific patients populations [3, 4].

VAP is a major problem for patients admitted to intensive care units, being the most common nosocomial infection in mechanically ventilated patients. The impact of VAP on morbidity and mortality remains contradictory with some authors supporting the idea that VAP has an aggravating effect on the above parameters [5, 6, 7], whereas some do not [8, 9]. Although the significance of respiratory tract colonization has been recognized in VAP, the pathophysiology of progression to infection and the role of local risk factors remain unclear.

Atelectasis, besides aggravating gas exchange and lung mechanics, has been demonstrated to provoke high stress forces, which are traumatic for alveolar epithelium in mechanically ventilated lungs [10, 11]. Diffuse microatelectases occur continuously during the course of mechanical ventilation and their prevention largely depends on routine respiratory therapy and ventilator settings. In a significant proportion of cases, lobar or segmental atelectasis, due to obstruction of bronchus, complicates the course of mechanical ventilation. The differentiation between atelectasis and VAP can often be difficult in the clinical setting [12].

Lung surfactant is recovered in BAL fluid in different formations including large and small aggregates. Large aggregates can exhibit an efficient depression in the surface tension protecting alveoli from collapse, while small aggregates are insufficient for this role. Surfactant also contributes to the local defense mechanism. However, it is unclear if surfactant alterations have a role in the development of atelectasis or VAP. Alterations in surfactant that could precede and promote the above complications have not been studied in 'normal' mechanically ventilated human lung. The present study was undertaken to monitor surfactant as well as biochemical parameters of BAL fluid during the course of VAP and atelectasis in mechanically ventilated patients without previous cardiopulmonary disease to elucidate the pathogenesis and to differentiate these two entities. Alterations in protein, phospholipids, as well as markers of inflammation were determined in BAL fluid, before during and after the development of the complications.

Patients and methods

Patients

Sixty-one mechanically ventilated patients were initially included in the study. The inclusion criteria were: absence of cardiopulmonary disease, normal chest radiograph, PaO_2/FiO_2>300 mmHg, and expected time for mechanical ventilation exceeding 2 weeks. The patients were not given antibiotics, and if antibiotics were given for other reasons than VAP, they were excluded from the protocol. All patients were subjected to BAL during the first 48 h. Nineteen patients were excluded from the study due to death, to interruption of mechanical ventilation, to development of pulmonary embolism, to sepsis or uncertain diagnosis (Control I). Of the remaining 42 patients, twelve developed a clinical picture compatible with ventilator-associated pneumonia (VAP). Diagnosis of VAP: when clinical and laboratory signs, such as fever and leucocytosis, were combined with a new radiographic infiltrate compatible with pneumonia, patients were subjected to a BAL. The diagnosis of VAP was confirmed when quantitative culture of BAL fluid exceeded 10^4 cfu/ml BAL fluid. After the resolution of pneumonia another BAL was performed in the involved bronchopulmonary segment. Pneumonia is considered as resolved when patients were afebrile for at least 48 h, white blood counts in peripheral blood less than $10x10^3$ cells/μl, the shadow in chest X-ray was significantly improved, and quantitative cultures were negative.

Eight patients exhibited lobar atelectasis during the study period, which required bronchoscopy. The diagnosis of atelectasis was based in radiographic criteria. The diagnosis was confirmed with negative quantitative culture of BAL fluid (<10^4 cfu/ml BAL fluid) as well as by endoscopic findings. A BAL followed the bronchoscopic lysis of atelectasis. The lysis of atelectasis was confirmed by chest X-ray. Bronchoscopy was repeated 48–72 h afterwards, at the involved bronchopulmonary segment. The chest radiograph was cleared before the second bronchoscopy.

Twenty-two patients did not develop any complication to the respiratory system during the monitoring period and constituted Control II group of the present study.

The PaO_2/FiO_2 ratio and lung mechanics were measured just before the BAL procedure. The patients were ventilated through a cuffed endotracheal tube with a 900C Siemens ventilator (Siemens-Elema, Solna, Sweden), using pressure control, volume control or pressure support modes. Frequency: 10–18 breaths/min, tidal volume: 9–11 ml/kg body weight, PEEP: 3–5 cmH_2O. $PaCO_2$ was kept between 30–40 mmHg.

Written informed consent was obtained from patient's next-of-kin. The ethics committee of the University Hospital of Ioannina approved the study, and it was performed in accordance with the ethics standards laid down in the 1964 Declaration of Helsinki.

BAL procedure

BAL was performed by fiberoptic bronchoscopy. Patients were ventilated with Control Mechanical Ventilation mode during BAL procedure, FiO_2 was set at 1.0, and PEEP was removed or reduced. They were sedated with midazolam and paralyzed with atracurium. Topical anesthetics were not used. Heart rate, arterial pressure, and arterial oxygen saturation by pulse oximetry were monitored throughout the procedure. Trachea was suctioned before introducing the bronchoscope through an adapter (swivel adapter), which allows the maintenance of mechanical ventilation. The tip of the bronchoscope was then wedged in a segmental or subsegmental bronchus. Six aliquots of 20 ml sterile normal saline were infused through the working channel of the bronchoscope. Processing of microbiological specimens has been described in details [13]. Briefly, the first aspirated fluid, reflecting a bronchial sample, underwent microbiological screening, while the others were divided into two portions: one for quantitative cultures and Gram staining and the other for biochemical evaluation. The second sample of BAL was filtered through sterile gauze and centrifuged at ×500 *g* at 4 °C for 15 min, to remove mucus and isolate cells, respectively. The supernatant of ×500 *g* was used to measure the biochemical parameters. BAL fluids were collected in ice-cold tubes to avoid PAF degradation due to PAF-acetylhydrolase (PAF-AcH) activity. BAL cells differential counts were performed by counting at least 300 cells in cytocentrifuge preparations stained with eosin-hematoxylin.

Differential centrifugations of BAL

After the removal of cells, an aliquot of the ×500 *g* supernatant was further centrifuged at ×30,000 *g* at 4 °C for 90 min (Sorvall

RC-5B, Dupont, Canada). The pellet of ×30,000 *g* was suspended in a small volume of saline and was kept at -80 °C until the analysis of total lipid phosphorus and total protein, as described below. The supernatant of ×30,000 *g* was submitted to ultracentrifugation at ×100,000 *g* at 4 °C for 1 h (Beckman L5-65B with SW41 rotor). The pellet and supernatant were kept at -80 °C until the analysis.

Biochemical parameters in BAL

Total protein and albumin were measured according to [14, 15]. Total lipids were extracted from the ×500 *g* supernatant, according to Bligh and Dyer [16], and separated into classes by thin layer chromatography (TLC), after two successive developments on K-6 thin-layer plates (Whatman), in the same direction, using the following solvent systems: A) chloroform-petroleum ether-methanol-acetic acid (50:30:15:10, v/v) to the top; and B) chloroform-methanol-water-acetic acid (65:35:5:10, v/v) to the top. Phospholipids were visualized under ultraviolet lamp, after spraying with 2-(p-toluidinyl)-naphthylene-6-sulfonic acid (TNS). Afterwards they were scraped off the plate and measured as described below.

Total phospholipids and individual phospholipid classes after TLC separation were determined from their lipid phosphorus content after 70% perchloric acid digestion, according to Bartlett [17].

PAF was purified from the lipid extract of BAL fluid with TLC, using chloroform-methanol-water (65:35:7, v/v). The area between authentic sphingomyelin and lyso-phosphatidylcholine, where PAF migrates, was scraped off the plate, extracted, and tested for biological activity. PAF determination was based on the aggregation of washed rabbit platelets, pretreated with CP/CPK, an ADP scavenger, and acetylsalicylic-lysine, a cyclooxygenase inhibitor, according to [18]. The standard curve was assessed using the hexadecyl analog of PAF. The detection limit under our experimental conditions was 40 pg PAF/9 ml BAL fluid. Low temperatures were maintained throughout the BAL treatment to avoid PAF degradation due to PAF-acetylhydrolase.

PAF-Acetylhydrolase activity was determined in BAL fluids fluorimetrically, according to [19]. The specificity of the activity was tested in the presence of EDTA, bromophenacylbromide, and in competition with 0.5×10^{-4} M 1,2-dipalmitoyl-sn-glycero-3-phosphocholine.

Statistics

Data are reported as mean±standard deviation (SD). The initial clinical and biochemical data of Control groups I and II were compared to those with those of patients with VAP and atelectasis by logistic regression analysis. The measurements of the three consecutive BAL fluids were compared by using repeated measures ANOVA. If the results of ANOVA were significant, comparison between groups of data was performed by the Wilcoxon test for paired measurements. The level of significance was defined as a *P* value of less than 0.05. All the *P* values were two-tailed. Data analysis was performed with STATISTICA package.

Results

VAP group

Patients' data

VAP patients, two women and ten men of mean age 49±21 years, with head or spinal trauma, cerebrovascular accident, and neuromuscular disease, were subjected to mechanical ventilation for 7.4±2.4 days before the diagnosis of VAP. The intervals between 1st and 2nd BAL and 2nd and 3rd BAL were 5.7±1.2 and 7.3±2.8 days, respectively. Four out of 12 patients (25%) died but pneumonia was not considered as the cause. The quantitative cultures from the 3rd BAL were sterile. The chest X-ray before the 3rd BAL was very much improved in all cases. During VAP a significant decrease in PaO_2/FiO_2 and respiratory system compliance was observed ($P<0.01$). Clinical data are shown in Table 1.

Protein content

Total protein and albumin increased dramatically after the development of VAP compared to all the other measurements ($P<0.01$). Protein levels in BAL fluid after VAP were reduced compared to levels during VAP, but they were statistically significantly higher compared to the relevant values before the development of VAP ($P<0.05$) (Table 1). The % distribution of total protein in the pellet of ×30,000 *g* was significantly reduced during and after VAP compared to the levels before VAP ($P<0.05$) (Table 2).

Surfactant phospholipids

There was a significant decrease of total phospholipid content between the levels observed in BAL before and during the development of VAP ($P<0.01$). After the resolution of VAP, total phospholipid levels remained low compared to the initial values ($P<0.01$) (Table 1).

Individual phospholipid alterations were observed as well: phosphatidylcholine and phosphatidylglycerol decreased, while sphingomyelin, phosphatidylinositol, and phosphatidylethanolamine increased during VAP as well as after VAP resolution compared to the initial values. Lyso-phosphatidylcholine increased during VAP and remained high after the resolution, although at lower levels (Table 1).

There was a statistically significant reduction in the % distribution of total phospholipids in the pellet of ×30,000 *g*, which represents the large surfactant aggregates fraction exhibiting good surface properties, during VAP and after its resolution, compared to the values before VAP ($P<0.0001$) (Table 2).

PAF and PAF-AcH

PAF was not detected initially; in contrast, PAF was detected in high levels during VAP ($P<0.001$). The resolution of VAP was followed by a strong decline in PAF levels ($P<0.001$ between during and after VAP values).

Table 1 Clinical and BAL characteristics of all groups of patients. [*VAP* ventilator-associated pneumonia, *ATEL* atelectasis, *NS* non-significant, *Before, During, After* refer to the onset of atelectasis or VAP, *Pa* represents *P* value among baselines (1st or before) BAL parameters from all groups, *Pb* represents *P* value between BAL parameters during VAP and atelectasis, *Pc* represents *P* value between BAL parameters after the resolution of VAP and atelectasis]

	Control I	Control II	VAP	ATEL		VAP	ATEL		VAP	ATEL	
Clinical measurements	1st	1st	Before	Before	Pa	During	During	Pb	After	After	Pc
PO_2/FiO_2 (mmHg)	372±49	359±32	356±32	341±23	NS	247±56[a,b]	256±40[a]	NS	302±40[a,b]	290±36[a]	NS
Compliance (ml/cmH_2O)	69±11	66±5.8	66±5	62±4	NS	51±7[a,b]	49±6[a]	NS	57±6[a,b]	55±5	NS
Maximal temperature		37.8±0.6	37.1±0.4	37.4±0.6	NS	39.2±0.6[a,b]	38.3±0.5	NS	36.9±0.5	37.5±0.5	NS
WBCx10^3/µl (neutrophils %)		9.8±2.1 (73±11)	8.4±0.9 (76±8)	9.0±1.4 (79±6)	NS	15.2±4.1[a,b] (89±6)	12.2±1[a,b] (87±8)	NS	8.7±2.1 (80±7)	10.3±1.7 (75±12)	NS
Measurements in BAL											
Total cell count (x10^3)	168±68	184±38	178±42	186±35	NS	426±175[a,b]	295±89[a,b]	0.01	210±76[b]	207±52[b]	NS
Alveolar macrophages (%)	79±9	83±9	83±7	85±6	NS	56±14[a,b]	61±12[a,b]	NS	77±10[b]	80±8[b]	NS
Neutrophils (%)	9±6	11±4.5	14±5	12±4	NS	42±10[a,b]	36±8[a,b]	NS	21±8[a,b]	14±4[b]	0.05
Total protein (µg/ml)	138±52	148±62	177±58	166±53	NS	1425±1288[a,b]	624±350[a,b]	0.01	347±81[a,b]	278±89[a,b]	0.05
Albumin (µg/ml)	37±23	28±71±3	55±17	51±18	NS	424±301[a,b]	218±118[a,b]	0.001	91±40[a,b]	89±48[a,b]	NS
Albumin/total protein (%)	27±6	28±7	27±3	27	NS	30±4	35	NS	26±4	32	NS
PAF (pg/9ml BAL)	1±3	1±3	0	0	NS	272±192[a,b]	240±223[a,b]	0.05	6±19[a,b]	8±12[a,b]	NS
PAF-AcH (nmmol PAF/ml BAL/min)	0.08±0.02	0.05±0.02	0.06±0.03	0.06±0.03	NS	1.79±1.62[a,b]	0.78±0.43[a]	0.05	0.46±0.21[a,b]	0.11±0.07[a]	0.05
Total phospholipids (µg P/ml BAL)	2.9±1.2	2.7±1.1	2.6±0.8	2.80±0.9	NS	1.16±0.79[a]	1.53±0.68[a]	NS	1.24±0.49[a]	1.77±0.42[a]	NS
Phosphatidylcholine (% of total PL)	73±8	71±5	70±12	68±12	NS	52±6[a]	55±7[a]	NS	59±8[a]	58±6	NS
Phosphatidylglycerol (% of total PL)	10±3	10±3	9±2	8±2	NS	5±2[a]	5±2[a]	NS	6±1[a]	6±3	NS
Sphingomyelin (% of total PL)	6±1	6±1	5±1	6±2	NS	19±6[a]	19±6[a]	NS	15±4[a]	15±2[a]	NS
Phosphatidylethanolamine (% of total PL)	4±1	4±1	4±1	4±1	NS	6±2[a]	6±1[a]	NS	6±1[a]	6±2[a]	NS
Phosphaditylserine (% of total PL)	4±2	4±2	5±1	5±2	NS	6±1	6±2	NS	6±2	6±2	NS
Phosphatidylinositol (% of total PL)	5±2	5±2	5±1	5±1	NS	7±1[a]	7±2[a]	NS	7±2[a]	7±1[a]	NS
Lyso-phosphatidylcholine (% of total PL)	0	0	0	0	NS	3±1[a,b]	2±2	NS	1±1[a,b]	2±1	NS

[a] Statistical significance from the baseline (1st or before) BAL
[b] Statistical significance between BAL during and after VAP or atelectasis

Table 2 Differential centrifugations of BAL fuid in VAP patients. (*VAP* ventilator-associated pneumonia)

Measurements	Pellet (×30,000 *g*)	Pellet (×100,000 *g*)	Supernatant (×100,000 *g*)
Protein (% of total)			
Before VAP	19.6±3.7	8.8±1.1	68.7±15.5
During VAP	12.3±1.2	9.1±1.0	77.2±15.4
After VAP	11.2±1.8[a]	8.2±1.1	79.6±17.9
Lipids (% of total)			
Before VAP	83.6±16.2	9.8±2.3	5.2±1.5
During VAP	38.3±12.6[a]	10.9±1.1	49.5±15.8[a]
After VAP	35.6±11.0[a]	12.9±3.1	46.8±14.8[a]

[a] Denotes statistical significance ($P<0.05$) between 1st (before) BAL and the BAL during or after VAP

Table 3 Differential centrifugations of BAL fluid in atelectasis patients

Measurements	Pellet (×30,000 *g*)	Pellet (×100,000 *g*)	Supernatant (×100,000 *g*)
Protein (% of total)			
Before atelectasis	17.5±3.9	9.5±1.6	70.4±18.8
During atelectasis	10.9±1.4[a]	10.5±1.9	75.4±14.8
After atelectasis	11.9±2.5[a]	8.5±1.7	79.2±21.2
Lipids (% of total)			
Before atelectasis	85.1±14.7	8.5±1.6	8.3±1.8
During atelectasis	35.5±14.7[a]	10.0±2.5	53.5±17.5[a]
After atelectasis	38.1±7.9[a]	10.9±2.4	49.8±19.8[a]

[a] Denotes statistical significance ($P<0.05$) between 1st (before) BAL and the BAL during or after atelectasis

PAF-AcH was detected in low levels at the initial BAL. During VAP, PAF-AcH increased significantly ($P=0.007$ compared to initial values). The resolution of VAP was followed by a decrease of PAF-AcH levels compared to levels during VAP ($P=0.026$) (Table 1).

Cells

There was a significant increase in total cell counts recovery in BAL fluid during VAP compared to counts recovery in BAL obtained before the VAP ($P<0.05$). The resolution of VAP was followed by total cell counts similar to before the VAP values. The percentage of neutrophils increased during VAP, and decreased after its resolution ($P<0.05$) (Table 1).

Atelectasis group

Patients' data

Patients with atelectasis, four women and four men, mean age 54±20 years, with head or spinal trauma, cerebrovascular accident, and neuromuscular disease, were subjected to mechanical ventilation for 8.4±2.4 days before the diagnosis of atelectasis. The intervals between 1st and 2nd BAL and 2nd and 3rd BAL were 6.5±1.3 and 2.9±1.5 days, respectively. The chest X-ray before the 3rd BAL was clear. Three of the eight (37.5%) patients died. Three out of the eight patients developed atelectasis between the 5th and 7th day of mechanical ventilation, and the rest during the second week. During atelectasis a significant decrease in PaO_2/FiO_2 and respiratory system compliance was observed ($P<0.01$) (Table 1). The quantitative cultures were considered negative, yielding $<10^3$, between 0 cfu/ml and 10^2 cfu/ml. Clinical data are shown in Table 1.

Protein content

There was a statistical difference in total protein levels and albumin in the ×500 *g* supernatant BAL fluid between the samples obtained before and during atelectasis, ($P=0.0013$ and $P<0.001$ for total protein, and albumin, respectively) (Table 1). The lysis of atelectasis resulted in a fall of total proteins and albumin compared to the values obtained during atelectasis ($P<0.01$). Total protein was elevated after atelectasis compared to the values before ($P<0.01$) (Table 1). The % distribution of total protein in the pellet of ×30,000 *g* was significantly reduced during and after atelectasis compared to initial BAL ($P<0.05$) (Table 3).

Surfactant phospholipids

Total phospholipid content of the ×500 *g* supernatant was decreased during and after atelectasis compared to the before values ($P<0.01$). The lysis of atelectasis was followed by a slight insignificant increase in the total

Table 4 Initial clinical and BAL characteristics of the patients. (*VAP* ventilator-associated pneumonia, *ATEL* atelectasis, *PL* phospholipids,*P* lipid phosphorus, *Before, During, After* refer to the onset of atelectasis or VAP

Measurements	Without VAP or atelectasis	VAP & ATEL atelectasis	Excluded
Outcome	6/22 (27%)	7/20 (35%)	8/19(42)[a]
PO_2/FiO_2	359±32	349±28	372±49
Compliance (ml/cmH_2O)	66±5.8	63±5	69±11
Total cell count (x10^3)	184±38	182±72	168±68
Alveolar macrophages (%)	83±9	84±7	79±9
Neutrophils (%)	11±4.5	13±5	9±6
Total protein (μg/ml)	148±62	169±55	138±52
Albumin (μg/ml)	40±19	53±17	37±23
Albumin/total protein (%)	28±7	27±3	27±6
PAF (pg/9ml BAL)	1±3	0	1±3
PAF-AcH (nmmol PAF/ml BAL/min)	0.05±0.02	0.06±0.03	0.08±0.02
Total phospholipids (PL) (μg P/ml BAL)	2.7±1.1	2.7±0.8	2.9±1.2
Phosphatidylcholine (% of total PL)	71±5	69±13	73±8
Phosphatidylglycerol (% of total PL)	10±3	9±2	11±3
Sphingomyelin (% of total PL)	6±1	5±1	6±1
Phosphatidylethanolamine (% of total PL)	4±1	4±1	5±1
Phosphaditylserine (% of total PL)	4±2	5±2	4±2
Phosphatidylinositol (% of total PL)	5±2	5±1	6±3
Lyso-phosphatidylcholine (% of total PL)	0	0	0

[a] Denotes statistical significance between patients without VAP or atelectasis and excluded patients

phospholipid values compared to the values obtained during atelectasis, but they were still reduced in comparison with initial values ($P<0.01$).

An alteration of surfactant phospholipids profile during atelectasis was also observed: phosphatidylcholine and phosphatidylglycerol decreased, while sphingomyelin, phosphatidylinositol, and phosphatidylethanolamine increased, compared to initial values ($P<0.05$). All these changes were still observed after lysis of atelectasis. Lyso-phosphatidylcholine was detected during, as well as after atelectasis (Table 1).

The % distribution of total phospholipids in the pellet of ×30,000 *g*, representing surfactant with good surface properties, was significantly reduced in the BAL during and after atelectasis compared to the initial one ($P<0.01$) (Table 3).

PAF and PAF-AcH content

PAF in BAL fluid was strongly elevated during atelectasis ($P<0.001$). PAF was not detected initially. The lysis of atelectasis was followed by a significant reduction of PAF.

PAF-AcH levels followed PAF changes. They were significantly increased during atelectasis ($P<0.001$) compared to the initial values. PAF-AcH levels strongly remitted after the lysis of atelectasis (Table 1).

Cells

There was a significant increase in total cell counts recovery in BAL fluid between the BAL before atelectasis and the counts obtained during atelectasis. The lysis of atelectasis was followed by total cell counts comparable to the initial values, before atelectasis. The percentage of neutrophils significantly increased during atelectasis, and decreased after its lysis (Table 1).

Comparison between groups

There were no significant differences in the initial BAL fluid samples from Control groups I and II, and from patients who developed VAP or atelectasis. The mortality was higher in patients who were excluded from the study (Control I) compared to patients without VAP or atelectasis ($P<0.05$) (Table 4). Moreover, there were no statistically significant differences between the control groups and VAP or atelectasis group in predisposing factors, including age, severity of disease, use of antibiotics prior to VAP, sedation and paralysis, position of the patients, nutrition, and gastroprophylaxis. No independent biochemical risk factors for the development of VAP and atelectasis were identified.

The differences among the 1st BALs of controls, VAP, and atelectasis were not statistically significant. The alteration of clinical and biochemical parameters were in the same direction in VAP and atelectasis. The increase in BAL total cells, proteins, PAF, and PAF-Ach were higher during VAP compared to atelectasis. The

differences in proteins and PAF-Ach remained after the resolution of VAP and atelectasis (Table 1).

Discussion

Although to different degrees, during the course of both VAP and atelectasis, there was a similar pattern of BAL fluid alterations, characterized by a significant increase in BAL total protein, severe qualitative surfactant alterations, as well as strong signs of local inflammation. The higher increase in BAL proteins, total cells count, and PAF in the VAP group compared to atelectasis suggested a higher degree of inflammation. However, the distribution of the values does not allow any differentiation between these groups.

Mechanical ventilation could induce a pro-inflammatory reaction as well as surfactant alterations, especially in previously injured lungs. Although our groups of patients had normal lung, participation to some degree in BAL alteration of the mechanical ventilation inflammatory effect cannot be easily excluded, given that the 2nd and 3rd BAL analysis, in the time corresponding to the VAP and atelectasis BALs, are not available in the control group. However, the mechanical ventilation should have affected both groups in a similar way.

VAP

The incidence of VAP, especially in patients without cardiopulmonary disease, has not been defined so far. In our study, the incidence was determined to be 28% for the 2-week observation period. This percentage seems rather high. However, the composition of our patient population could have contributed to this. Both trauma and central nervous system disease have been associated with high incidence of VAP. Cook et al. [20] calculated that the risk ratio for development of VAP was 5 in trauma patients and 3.4 for CNS patients. There were no statistically significant differences between the control group, comprising patients who did not develop any respiratory complication, and the VAP group. We were not able to identify any clinical risk factor associated with VAP, but the relatively small size of our sample as well as the similar therapeutic interventions in both groups did not facilitate this effort. All the cases of VAP developed after the 5th day, signifying a late-onset VAP. The absence of early-onset VAP could be due to the initial exclusion of patients with previous pulmonary disease, abnormal admission X-rays, as well as witnessed aspiration. It should be noted, that our study population were not given preventive therapy with antibiotics, which constitutes a risk factor for VAP development [21]. This finding is in accordance with those of two French studies, which included patients with acute lung injury (ALI), where VAP occurred late in the course of ALI [22, 23]. The VAP did not seem to influence the outcome of the patients. The resolution of VAP occurred after 6–8 days and was accompanied by improvement of oxygenation and respiratory system compliance; these results are consistent with those of Dennesen et al. [24].

VAP was associated with a significant increase in total protein and albumin of BAL fluid. Protein and albumin levels were extremely increased during VAP, and these levels were comparable with those measured during ARDS by our group [13]. An increase in permeability of capillary-alveolar membrane and an increase of protein contamination of alveolar fluid from local inflammation, or possibly both, could explain this increase. This explanation is compatible with the increased PAF and neutrophils in BAL fluid, which partially remitted after VAP resolution. In any case, the existence of an increased protein level implies an increased quantity of alveolar fluid and some form of pulmonary edema. The reversal of total protein after VAP to values similar to those preceding VAP shows that some aspects of epithelial and endothelial function seem to improve rather quickly with the resolution of pneumonia [25].

Total phospholipids, as well as certain phospholipid classes, decreased during and after VAP in comparison to the initial levels. VAP was associated with a significant reduction of total phospholipids recovered at the pellet of ×30,000 *g*, which represents the large aggregates. The decreased proportion of total phospholipids and large aggregates recovered during VAP could be due to a direct insult of the alveolar cells, dilution by edema fluid or removal by activated macrophages.

It is not the first time that pneumonia has been associated with surfactant alterations [26], but to our knowledge this study is the first effort to monitor these changes during the course of VAP. The observed surfactant alterations during VAP predispose to edema formation, while at the same time the proteinaceous fluid in the alveoli inactivates surfactant. This vicious cycle might be further provoked by the action of phospholipases, resulting in the formation of lysophospholipids [27], which further inactivate surfactant and damage the alveolar epithelium absorbing properties. Lyso-phospholipids, lipids with a well-established injurious effect on alveolar epithelium [28], were detected both during and after VAP in BAL indicating the action of phospholipase A_2.

VAP's resolution was not followed by an improved phospholipid pattern. Although total protein and inflammatory markers levels had returned to pre-VAP values in the BAL, surfactant alteration persisted, indicating a continuing depression of alveolar type II cell function. Besides total phospholipids, which remained extremely low, indirect markers of alveolar cell injury, such as the reduced fraction of large aggregates and the abnormal phosphatidylglycerol /phosphatidylinositol ratio, suggest

that some aspects of alveolar type II cell function recur with a significant delay.

Atelectasis

Eight patients (19%) in our study population developed lobar or segmental atelectasis, which was not resolved with physiotherapy and position maneuvers. Increased total protein and albumin levels were observed right after the lysis of atelectasis, compared to BAL values that preceded it. An increase of permeability of capillary-alveolar membrane or local inflammation could have contributed to this finding. The parallel variation between BAL total protein and local inflammatory markers (PAF, total cells, neutrophils percentage) favors this explanation. Finally, the re-expansion of atelectatic regions could have evoked local edema with increased permeability [29], offering another possible explanation.

The surfactant alterations in the BAL that followed the lysis of atelectasis, especially the structural surfactant alterations and the fact that these persisted 48–72 h after the lysis of atelectasis – a time that exceeds the predicted life-cycle of surfactant – indicates a prolonged alveolar cell injury. It is not obvious whether a surfactant alteration is the cause or the result of atelectasis. However, these phospholipid and surfactant abnormalities could predispose for micro-atelectasis, introducing a vicious cycle.

The BAL fluid analysis following bronchoscopic lysis of atelectasis revealed the presence of inflammatory markers, such as increased PAF levels, increased total cell count, and the proportion of neutrophils. Forty-eight to seventy-two hours after the lysis of atelectasis, PAF levels as well as the proportion of neutrophils in the involved segment returned to the pre-atelectasis values, a finding supporting the link between atelectasis and inflammation. Our clinical data are consistent with experimental data supporting that prolonged and extended contact of epithelial surfaces can provoke inflammation [30]. The opening of the bronchus and the subsequent reperfusion of the lavaged area could have contributed, to a lesser extent, to the local inflammation observed.

In conclusion, the present study demonstrated BAL fluid alterations that occurred during and after VAP or atelectasis. Both entities are associated with local edema and severe surfactant abnormalities, and trigger local inflammatory reactions resulting in deteriorated gas exchange and lung mechanics and can predispose to further injury in mechanically ventilated patients. Therefore, the differential diagnosis between these two entities based on BAL parameters is not feasible. However, the elucidation of the alterations associated with these complications could contribute in understanding their pathophysiology. Surfactant alterations in the development of VAP as well as atelectasis, could have a significant role and the exogenous surfactant administration could prove to be useful in the prevention or treatment of these complications.

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