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Biochemical parameters of bronchoalveolar lavage fluid in fat embolism

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Abstract *Objective:* To identify diagnostic markers distinguishing between acute lung injury/acute respiratory distress syndrome (ALI/ARDS) due to fat embolism syndrome (FES) and that due to other causes, and to investigate whether phospholipase A_2 and platelet-activating factor (PAF) play a role in the pathogenesis of ALI due to FES. *Design and setting:* A prospective study in a 14-bed ICU. *Patients:* We studied 13 patients with FES, 11 with ALI/ARDS from other causes (6 without trauma, ALI/ARDS group 1; 7 with trauma, ALI/ARDS group 2) and 5 without cardiopulmonary disease. *Measurements and results:* We compared broncholveolar lavage (BAL) fluid alterations in the respective groups. Total BAL protein in FES group was significantly higher compared to in ALI/ARDS group 1 and controls but ALI/ARDS group 2. Higher total phospholipids were found than in other groups. The alterations in individual phospholipid classes were similar to those in ALI/ARDS patients. However, total cholesterol, lipid esters, and monoglycerides were significantly higher in FES than in other groups. The level of PAF in FES was significantly higher and there was an inverse correlation between PAF and PAF-acetylhydrolase. Phospholipase A_2 activity was significantly higher in both FES and ALI/ARDS groups than in control. *Conclusions:* The levels of neutral lipids and especially cholesterol and cholesterol esters in BAL can be used to distinguish patients with FES from ALI/ARDS due to other predisposing factors. Phospholipase A_2 may be involved in the development, and PAF-acetylhydrolase in the downregulation of inflammation in FES.

Keywords Neutral lipids · Phospholipase A_2 · Acute lung injury · Acute respiratory distress syndrome · Platelet activating factor–acetylhydrolase

Introduction

Fat embolism syndrome (FES) is a common posttraumatic complication in patients with serious injuries, especially those with pelvic or long bone fractures. It can also occur as a complication during cardiopulmonary resuscitation or lipid infusion used for parenteral feeding [1]. According to Peltier's [2] interpretation, the neutral fat is embolized after fracture and causes capillary obstruction (physical stage); then the pulmonary lipases hydrolyze the neutral fat and releases free fatty acids which are extremely toxic to the lung (chemical stage). Other authors emphasize the toxicity to the platelet aggregates, the formation of pulmonary capillary thrombi, and red cell sludge [3]. The syndrome is characterized by a classical triad of symptoms that appear 24–72 h after trauma: acute respiratory failure, neurological dysfunction, and petechial rash, but one or more of these findings may be absent [4]. However, in patients with isolated respiratory symptoms the presence of hypoxemia and pulmonary infiltrates are

easily confused with other more commonly encountered problems such as aspiration pneumonia and lung contusion. Furthermore, the neurological dysfunction does not have characteristic clinical features and may be incorrectly attributed to posttraumatic cerebral damage or to other causes. Finally, the appearance of the petechial rash is delayed or not seen at all. Thus the diagnosis of the syndrome is not always evident.

Fat embolism is a predisposing factor for direct acute lung injury–acute respiratory distress syndrome (ALI/ARDS). Trauma patients can develop direct or indirect ALI/ARDS from many other predisposing factors, such as blood transfusion, aspiration, lung contusion, and sepsis. However, massive traumatic tissue injury can directly precipitate or predispose the developing ALI/ARDS. Therefore in this situation it is very difficult to decide whether fat embolism is the cause or one of the causes in developing ALI/ARDS [5].

Several laboratory tests have been contributed to the diagnosis until now, such as the detection of fat globules in urine and blood, measurement of serum lipase activity, and determination of bronchoalveolar lavage (BAL) cells containing fat droplets [1, 4, 6, 7, 8, 9]. However, these tests have poor sensitivity and lack of specificity. A rise in serum free fatty acids (FFA), which are considered to be involved in the pathogenesis of fat embolism, has been observed in a prospective study of patients with low extremity fractures [9]. FFAs can be generated through the activity of lipase on triglycerides as well as of phospholipases on phospholipids. Elevated levels of phospholipase A_2 (PLA_2) and platelet-activating factor (PAF), a potent lipid mediator have been found in BAL fluid of ARDS patients [10].

BAL fluid contains lung surfactant aggregates, soluble agents such as enzymes and cytokines, and cells. In normal lung, where the alveolar-capillary membrane is practically impermeable, the protein concentration in BAL fluid ranges roughly from 50 to 150 μg/ml, and total phospholipids are approx. 2–3 μg/ml lipid phosphorous. The main phospholipid classes are dipalmitoylphosphatidylcholine (70–80% of total phospholipids) and phosphatidylglycerol (approx. 10% of total phospholipids). Neutral lipids and especially cholesterol exist in low concentrations [11].

The aim of this study was (a) to identify diagnostic markers facilitating the distinction between ALI/ARDS due to fat embolism and other causes through the evaluation of BAL fluid alterations and (b) to investigate whether PLA_2 and PAF play a role in the pathogenesis of ALI due to FES. We studied patients with FES who developed acute hypoxemic respiratory failure requiring mechanical ventilation. BAL characteristics of the patients with FES were compared to those of patients with ALI/ARDS from other predisposing factors except FES as well as to those of a group of mechanically ventilated patients without cardiopulmonary disease.

Materials and methods

Patients and study protocol

The study included 31 mechanically ventilated patients: 13 with hypoxemia due to FES, 13 with ALI/ARDS, and 5 without pulmonary disease (control group). Patients with dyslipidemia, lung contusion and hepatic and renal failure were excluded. None of the patients received parenteral nutrition or sedation with propofol during the study period. The protocol was approved by the Ethics Committee of the University Hospital of Ioannina, and the patients or next of kin gave informed consent to the study.

The 13 patients with FES included ten men and three women aged 41.4±14.3 years, with a PaO_2/FIO_2 ratio of 124.3±22.0 (Table 1). The criteria for FES diagnosis were: (a) hypoxemia, (b) neurological abnormalities, and (c) a petechial rash. All patients included in this study developed the above clinical classical triad within 24–72 h after long bone and/or pelvic fractures and fulfilled the criteria of ALI/ARDS. Criteria of intubation and mechanical ventilation were: (a) severe hypoxemia (PaO_2/FIO_2 <200 mmHg regardless of positive end-expiratory pressure, PEEP) and (b) deterioration in mental status due to FES. Standard criteria for ARDS diagnosis were: (a) acute hypoxemic respiratory failure requiring mechanical ventilation, (b) diffuse bilateral alveolar infiltrates on chest roentgenography, (c) refractory hypoxemia (PaO_2/FIO_2 <200 mmHg regardless of PEEP), (d) pulmonary artery wedge pressure less than 18 mmH_2O or no clinical evidence for left atrial hypertension, and (e) recognized appropriate clinical setting or risk factor for the development of ARDS. Patients with a PaO_2/FIO_2 ratio of 201–300 mmHg were characterized as having ALI.

Patients with ALI/ARDS were divided into two groups: those without trauma (ALI/ARDS group 1, n=6) and those with trauma showing lung contusion but without evidence of FES (ALI/ARDS group 1, n=7). Group 1 included three men and three women aged 48.8±18.6 years, with a PO_2/FIO_2 ratio of 127.3±22.9; group 2 included three men and three women aged 32.3±12 years, with PO_2/FIO_2 ratio of 187±47.8 (Table 2). The latter group excluded patients with head trauma, in whom it is difficult to assess the clinical criteria of FES. None of the ALI/ARDS patients developed petechial rash. They were intubated because of the hypoxemia and not for neurological abnormalities. The ventilatory settings in FES and ALI/ARDS patients were: 10–18 breaths/min, tidal volume 6–7 ml/kg body weight, and PEEP 8–15 cmH_2O. FIO_2 was set at the minimal level at which an arterial oxygen saturation of 90% could be achieved. The plateau pressure was kept below 30 cmH_2O. The $PaCO_2$ level was not a target in the ventilator settings.

Control patients included four men and one woman aged 45.4±20.8 years, with a PO_2/FIO_2 ratio of 354.8±39.6 (Table 2). Inclusion criteria for control patients were: (a) normal chest radiography, (b) PaO_2/FIO_2 higher than 300 mmHg, and (c) hemodynamic stability. Controls were subjected to bronchoscopy for differential diagnosis of the chest radiography shadow, which was confirmed as atelectasis (chest radiography was improved and quantitative culture was negative). The ventilator settings in control patients were: 10–18 breaths/min, tidal volume 7–9 ml/kg body weight, and PEEP of 3–5 cmH_2O, to minimize the risk of atelectasis.

BAL procedure

BAL was performed by fiberoptic bronchoscopy as described previously [9]. This was performed within the first 3 days from the development of the disease for FES and ALI/ARDS groups and from the intubation time for the control group, when patients were heamodynamically stable. Patients were ventilated with a control mechanical ventilation mode; they were sedated (midazolam) and paralyzed (atracurium) during the BAL procedure and measurements. Propofol was not used. Briefly, six aliquots of 20 ml sterile

Table 1 Clinical data of patients with FES

No.	Age (years)	Sex	Fractures	PO_2/FIO_2	Outcome	Petechial rash	Retinopathy	Lipiduria	Neurological abnormalities
1	17	F	Long bone (2), pelvic	104	Alive	Yes	Yes	Yes	Yes
2	35	F	Long bone (3)	122	Alive	Yes	–	Yes	Yes
3	43	M	Long bone (1), pelvic	130	Alive	Yes	–	–	Yes
4	62	M	Long bone (2), pelvic	140	Alive	Yes	–	–	Yes
5	55	M	Long bone (3), pelvic	148	Alive	Yes	Yes	–	Yes
6	22	M	Long bone (5),	88	Alive	Yes	–	–	Yes
7	48	M	Long bone (3),	140	Alive	Yes	–	Yes	Yes
8	50	F	Long bone (3), pelvic	90	Alive	Yes	–	–	Yes
9	43	M	Long bone (2), pelvic	132	Died	Yes	Yes	Yes	Yes
10	22	M	Long bone (3),	140	Alive	Yes	Yes	–	Yes
11	38	M	Long bone (1),	96	Alive	Yes	–	–	Yes
12	44	M	Long bone (3),	142	Alive	Yes	–	–	Yes
13	59	M	Long bone (2), pelvic	144	Alive	Yes	–	Yes	Yes

normal saline 37°C were infused through the working channel of the bronchoscope, and the aspirated fluid was collected in ice-cold tubes. BAL was then filtered through sterile gauze to remove mucus, and soon thereafter it was centrifuged at 500 *g* for 15 min at 4°C to separate cells and liquid. The supernatant of 500 *g* was used to measure all the biochemical parameters. The lipid content of the cells was examined immediately using the Sudan black B staining procedure. All measurements were performed in samples without diagnosis indication (blinded).

Biochemical parameters

Total lipids were extracted according to Bligh and Dyer [12], and the phospholipid content was determined after perchloric acid digestion according to Bartlett [13]. PAF was purified from the lipid extract of BAL fluid by thin-layer chromatography (TLC) using chloroform-methanol-water (65:35:7, v/v) as solvent system. The area between authentic sphingomyelin and lyso-phosphatidylcholine, where PAF migrates, was scrapped off the plate, extracted, and tested for biological activity. PAF determination was based on the aggregation of washed rabbit platelets, pretreated with creatine phosphate/creatine phosphokinase, an ADP scavenger and acetylsalicylic lysine, a cyclooxygenase inhibitor, as described previously [14, 15]. Total BAL fluid protein was determined by the method of Bradford [16], using bovine serum albumin as a reference compound.

Total phospholipids were separated into classes with TLC. For the development two consecutive systems were used: petroleum ether- diethyl ether-methanol-acetic acid (50:30:15:10, v/v) and chloroform-methanol-water-acetic acid (65:35:5:10, v/v). Phospholipids were visualized under ultraviolet lamp after spraying with 2-(*p*-toluidinyl)-naphthylene-6-sulfonic acid. Then they were scraped off the TLC plate and quantified from their phosphorous content.

PLA_2 and PAF-acetylhydrolase (AcH) were measured fluorimetrically by a method developed in our laboratory [17]. Briefly, standard incubation mixture for PLA_2 contained 10 mM Tris HCl buffer, pH 7.4, with 2 mM Ca^{2+} and 5mM C_{12}-NBD-PC as a substrate. For PAF-AcH the incubation mixture contained 10 mM Tris HCl buffer, EDTA 10 mM, pH=7.4 and 5mM C_6-NBD-PC as a substrate. The reactions started with the addition of 100 µl BAL at 37°C, and were continuously monitored with a Perkin Elmer LS-3 spectrofluorimeter, equipped with xenon lamp and RCA 931 photomultiplier. Excitation and emission wavelengths were adjusted to 475 and 535 nm, respectively [16].

Neutral lipids were separated by TLC using petroleum ether-diethyl ether-acetic acid (80:20:1, v/v). Total cholesterol was determined according to Courchaine et al. [18]. Individual neutral lipid esters (mono-, di-, triglycerides and cholesterol esters) were analyzed and quantified by gas-liquid chromatography (GLC) after transesterification of the acyl-chain moieties with BF_3/MeOH, according to Morrison and Smith [19]. A Shimadzu 14A gas chromatograph equipped with a 10% Silar 10C on Q11, 10 feet (Altech) capillary column was used. FAME mix GLC 20 and 30 (Supelco) were used as standards. Cholesterol and neutral lipid esters are expressed in micrograms per micrograms of lipid phosphorous of BAL to emphasize to the relationship of the amounts of neutral lipids with total phospholipids avoiding an additional error due to the dilution of the alveolar fluid in BAL.

Statistics

Statistical analysis was performed using the Statistical Package for Social Sciences version 12 for Windows. Data were tested for normality by the Kolmogorov-Smornov test. The three groups were compared using one-way analysis of variance followed by post hoc comparisons with Bonferroni's method for normally distributed variables and the Kruskall-Wallis test followed by the Mann-Whitney test for post hoc comparisons for nonnormally distributed variables.

Results

BAL protein content

Total BAL protein was higher in the FES group than in ALI/ARDS group 1 ($p<0.03$) and controls ($p<0.0001$). No statistically significant difference was observed between FES and ALI/ARDS group 2 (Table 3).

Total phospholipids and individual phospholipid classes

The total BAL phospholipid level was higher in the FES group than in both ALI/ARDS groups and in controls ($p<0.05$). Alterations in the proportion of individual phospholipid classes were similar to those observed in ALI/ARDS patients: phosphatidylcholine and phos-

Table 2 Demographic characteristics of ARDS and control patients (*group 1* ARDS without trauma, *group 2* ARDS with trauma *H/S* head and/or spine trauma, *LC* lung contusion, *RF* rib fracture, *SF* sternum fracture, *LBF* long bone fracture, *CVA* cerbrovascular accident, *NM* neuromuscular

No.	Age (years)	Sex	Disease	PO_2/FIO_2	Outcome
ALI/ARDS group 1					
1	23	F	Pneumonia	108	Alive
2	49	M	Aspiration	140	Alive
3	67	F	Sepsis	90	Died
4	52	F	Aspiration	148	Alive
5	32	M	Sepsis	138	Alive
6	70	M	Sepsis	140	Died
ALI/ARDS group 2					
1	32	M	LC, RF,	168	Alive
2	25	M	RF, LC, LBF (1)	230	Alive
3	24	M	LC,	142	Died
4	18	M	LC, RF	210	Alive
5	52	M	LC, LBF (2)	264	Alive
6	44	M	LC, RF, SF	152	Alive
7	31	M	LC, RF	144	Died
Controls					
1	52	M	CVA	360	Alive
2	46	F	H/S	420	Died
3	19	M	H/S	320	Alive
4	75	M	CVA	346	Died
5	35	M	NM	328	Alive

Table 3 Biochemical parameters in BAL fluid (*PAF* platelet-activating factor, *AcH* acetylhydrolase, PLA_2 phospholipase A_2)

	FES	ALI/ARDS 1	ALI/ARDS 2	Control
Total phospholipids (µg/ml BAL)	3.7±1.2*,**	2.7±0.9*	2.46±0.8*	2.28±0.5
Phosphatidylcholine (%)	53.7±8.3**	50.9±6.6**	44.6±6**	76±5.2
Phosphatidylglycerol (%)	5.1±1.8**	5.3±1.1**	4.2±1.9**	8.7±2.1
Sphingomyelin (%)	13.7±5.9**	15.7±5.9**	11.4±6.7**	2.3±0.8
Phosphatidylethanolamine (%)	5.7±2.2	6.9±1.2	4.6±1.4	4.2±0.6
Phosphaditylserine (%)	6.9±2.9	7.7±2.8	6.7±3.1	4.4±0.6
Phosphatidylinositol (%)	7.5±2.2**	8.3±3.2**	9.1±2.5**	4.1±1.1
Lyso-phosphatidylcholine (%)	2.8±1.6*,**	1.9±1.5**	0.8±0.9*,**,***	–
Total protein (µg/ml)	1911±1395*,**	876.7±276*,**	1210±1250**	137±32
PAF (pg/9 ml)	1089±364*,**	189±38**	27±19	28±49
PAF-AcH (nmol m^{-1} min^{-1})	0.06±0.04*,**	5.45±3.1**	8.9±4.5**	1.2±0.6
PLA_2 (nmol ml^{-1} min^{-1})	0.51±0.13**	0.65±0.32**	0.9±0.3**	0.01±0.01
Esters (µg/γphospholipid) 0.035±0.07	0.44±0.22*,**	0.015±0.01*	0.012±0.026*	
Cholesterol (µg/µg phospholipid)	10.2±5.3*,**	4.38±0.75*,**	3. 7±0.5*,**	1.97±1.22

*$p \leq 0.05$ FES vs. ALI/ARDS group 1 and/or 2, **FES vs. Control, ***ALI/ARDS groups 1 vs. 2

phatidylglycerol were reduced and sphingomyelin and phosphatidylinositol increased. Lysophosphatidylcholine was higher than in both ALI/ARDS and in controls ($p<0.05$ and 0.001 respectively; Table 3).

Total cholesterol

As shown in Table 3 and Fig. 1, the concentration of total cholesterol in BAL fluid from FES patients was significantly higher than in ALI/ARDS groups and in controls ($p<0.001$).

Total lipid esters in BAL

The content of lipid esters in BAL fluid from patients with FES was significantly higher than in ALI/ARDS and control groups ($p<0.0001$ for all groups; Table 3, Fig. 2).

Neutral lipids

As shown in Fig. 3, the band corresponding to total neutral lipids was much more intense in FES patients than in ALI/ARDS and control groups ($p<0.05$). This difference was due, at least in part, to total cholesterol which

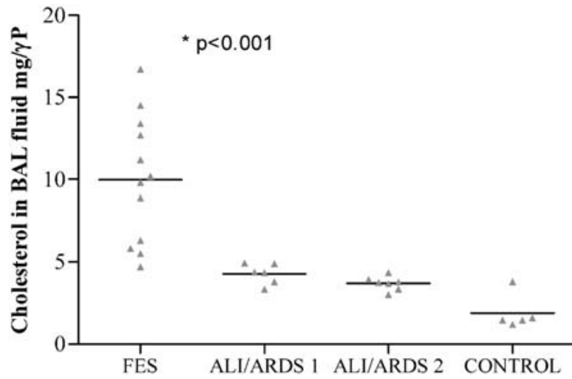

Fig. 1 Cholesterol levels in BAL fluid from FES, ALI/ARDS group 1, ALI/ARDS group 2, and control. *Horizontal lines* Mean cholesterol levels. *p<0.001 FES vs. other groups

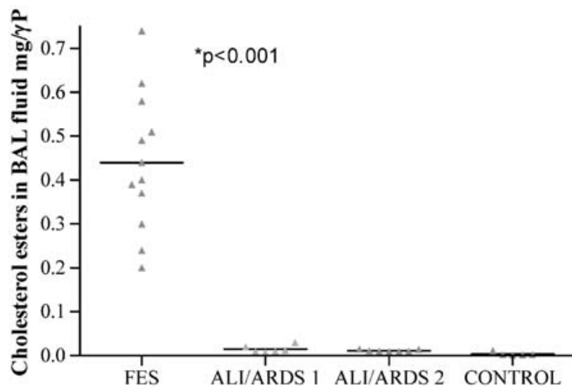

Fig. 2 Cholesterol ester levels in BAL fluid from FES, ALI/ARDS group 1, ALI/ARDS group 2, and controls. *Horizontal lines* Mean cholesterol ester levels. *p<0.001 FES and other groups

was found significantly higher in patients with FES (Table 3). Moreover, in these patients the percentage of cholesterol esters and monoglycerides were found to be significantly increased (p<0.05). In contrast, in patients with FES the percentage of triglycerides was significantly lower than in ALI/ARDS and control groups (p<0.001; Table 4). The proportion of free fatty acids in FES was higher than in controls (p<0.05) but significantly lower than in ALI/ARDS group 1 (p<0.05). No difference was found between FES and ALI/ARDS group 2.

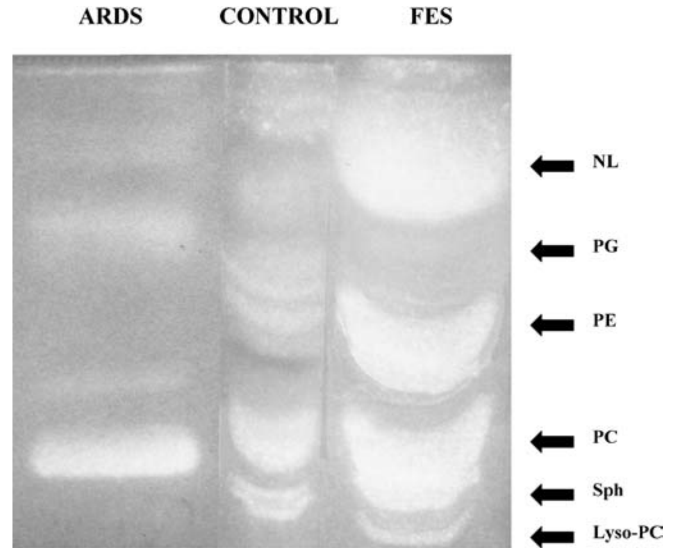

Fig. 3 Representative thin layer chromatographic separation of total BAL lipids from ARDS, control, and FES patients. The silica gel G TLC plates were developed twice in the same direction with two consecutive solvent systems: petroleum ether-diethyl ether-methanol-acetic acid (50:30:15:10, v/v) or chloroform-methanol-water-acetic acid (65:35:5:10, v/v). Lipids were visualized under ultraviolet lamp after spraying with 2-(p-toluidinyl)-naphthylene-6-sulfonic acid. *NL* Neutral lipids; *PG* phosphatidylglycerol; *PE* phosphatidylethanolamine; *PC* phosphatidylcholine; *Lyso-PC* lysophosphatidylcholine

Alveolar macrophage with fat droplets

The proportion of cells stained by Sudan black B were 34±23.3%, 13±8%, 15.2±10.3%, and 2±3.2% in FES, ALI/ARDS group 1, ALI/ARDS group 2, and controls, respectively. The number of stained cells was significantly higher in FES than in controls (p<0.05) while no significant difference was found between FES and ARDS (Table 5).

PAF levels, PAF-AcH and PLA_2 activities

The level of PAF in patients with FES was significantly higher than that in ALI/ARDS and control groups (p<0.0001 for all cases; Table 3). PAF-AcH activity was far lower in patients with FES than in ALI/ARDS and control groups (p<0.0001). An inverse correlation be-

Table 4 Composition of neutral lipid esters in BAL fluid (percentages)

	FES	ALI/ARDS 1	ALI/ARDS 2	Control
Monoglycerides	42.3±21.56*,**	23.65±21.35*	29.9±18.2	20.1±3.2
Diglycerides	9.5±2.4	13.6±21.35	8.4±4.5	13.3±8.8
Triglycerides	10.06±4.55*,**	43.7±14.3*,**	51±12.8*,**	58.1±11.0
Free fatty acids	10.75±3.17*,**	15.3±7.9*,**	5.9±5.4**,***	1.2±6.8
Cholesterol esters	34.8±21.4*,**	2.6±1.2*,**	4.3±2.9*	7.3±3.2

*p≤0.05 FES vs. ALI/ARDS group 1 and/or 2, **FES vs. Control, ***ALI/ARDS groups 1 vs. 2

Table 5 Absolute number and differential cell count of BAL

	FES	ALI/ARDS1	ALI/ARDS 2	Control
Total cell count (×10^3/ml)	256±77*	328±69*	392±105*	122±23
Alveolar macrophages (%)	52±9.3*	47±7.5*	55±11.4*	83±5.8
Neutrophils (%)	38±6.6*	44±7.3*	41±7.4*	9±5.5
Sudan black B staining (%)	34±23.0*	13±8.0*	15.2±10.3*	2±3.2

*p<0.05 FES, ALI/ARDS 1 and ALI/ARDS 2 vs. Control

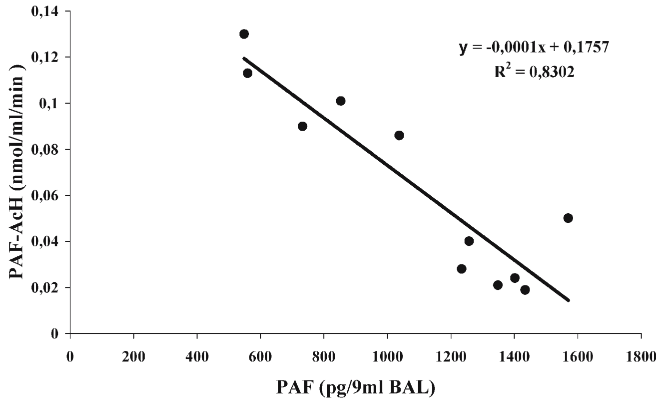

Fig. 4 Correlation between PAF and PAF-AcH in FES patients. An inverse correlation between PAF and PAF-AcH was observed (p<0.0001)

tween PAF and PAF-AcH was found in the FES group (R^2=0.8302, p<0.0001; (Fig. 4), but no correlation was observed in the ALI/ARDS or control groups. PLA_2 activity was significantly higher in FES and ALI/ARDS patients than in controls (p<0.0001). No difference was observed between FES and ALI/ARDS groups (Table 3).

Discussion

This study compared patients fulfilling all the clinical criteria for FES with patients having ALI/ARDS and patients without pulmonary disease exhibiting low probability of FES. Differentiation between ALI/ARDS due to FES and other predisposing causes was possible by lipid evaluation in BAL fluid. However, one limitation of the study was that in patients with an uncertain clinical picture there was no other way (e.g., histological examinations) to confirm or to exclude FES diagnosis, and therefore a new laboratory test could not be unequivocally evaluated.

A significant finding of our study was the markedly higher concentration of neutral lipids and especially that of total cholesterol and cholesterol esters in BAL from all FES patients than in ARDS and control groups. To our knowledge, neither cholesterol nor cholesterol esters have been studied in BAL from patients with FES. As the levels of total cholesterol and cholesterol esters in FES patients did not overlap with those of patients having ALI/ARDS from other causes, we could differentiate these two entities. This method can be applied in mechanically ventilated patients where BAL can be easily performed, but it is less relevant in patients with mild manifestation of FES who do not need invasive mechanical ventilation. Although the presence of fat droplets in the alveolar macrophages has been proposed as a diagnostic marker of fat embolism, most studies have found the sensitivity and specificity of this test to be low, thus limiting its diagnostic value [1, 2, 3, 4, 5, 6, 7, 8, 9, 20, 21]. In our study, in contrast to BAL cholesterol content, the number or the percentage of alveolar macrophages containing fat droplets did not clearly distinguish FES from ALI/ARDS due to other causes. There is no obvious explanation for the lack of correlation between the number of macrophages containing fat and the BAL lipid content. This phenomenon could be due to a different activation state of macrophages or even to the different neutral lipid composition between the studied groups.

The qualitative alterations of surfactant in FES were similar with those in ARDS patients, and they probably share the same pathogenic mechanisms. Such alterations could directly affect surfactant biophysical activity [22].

In a previous work we observed higher total phospholipids during the early phase of ARDS than in the late phase [14]. This could be due to a local production, hyperventilation, or even a defect in reuptake of phospholipids by type II alveolar cells [23, 24, 25]. Total phospholipid content in FES was found even higher than in ALI/ARDS. Several reasons may account for this difference. It might, at least partially, represent differences in the time course of the illness. Furthermore, phospholipids from the bone marrow fat of FES patients could pass into the alveolar space through the increased alveolar-capillary membrane permeability. Finally, PAF, according to some investigators could increase surfactant production and/or secretion [26]. It is of note that PAF was found in very high levels in the BAL fluid from our FES group.

Elevated PAF levels, also encountered in ARDS patients from other risk factors, could be implicated to a local inflammatory reaction. PAF is rapidly inactivated by circulating PAF-AcH [27]. The fact that PAF-AcH was inversely correlated with PAF suggests that PAF-AcH is also locally produced. PLA_2, which was increased in both FES and ARDS patients, is involved in PAF production via the remodeling pathway. This enzyme can affect pulmonary function either directly by hydrolyzing lung surfactant phospholipids and increasing surface tension [28, 29] or indirectly through the production of biologi-

cally active molecules such as PAF, lyso-phosphatidylcholine, and eicosanoids. Alveolar macrophages are considered as major source of PLA_2 in the lung [30, 31]. In addition, various cell types, such as platelets, mast cells, fibroblasts, macrophages, and neutrophils produce and secrete PLA_2 following stimulation [32]. The involvement of PLA_2 in ARDS has been shown in a study with animal models, where the enzyme was increased in correlation with the severity of lung injury [29]. It has been reported that plasma PLA_2 remained unchanged in an animal model of fat embolism. Thus the high levels of PLA_2 in BAL fluid in our study could signify a local production of the enzyme [33]. PLA_2 catalyzes the degradation of phosphatidylcholine to lyso- phosphatidylcholine, and free fatty acids. Therefore the presence of lyso-phosphatidylcholine strongly indicate that PLA_2 is implicated in the pathogenesis of FES. In contrast to our expectations, however, the percentage of free fatty acids in FES patients was lower than in ARDS patients and higher than in our normal group. A possible explanation for this is the formation of cholesterol esters, which were found significantly increased, after esterification with the liberated fatty acids of cholesterol, possibly via an acyl-coenzyme A–cholesterol acyltransferase. The free fatty acids used as substrates in this procedure could possibly derive from: (a) triglycerides which are converted to monoglycerides through a triglyceride lipase activation (for review, see [2]); this interpretation is consistent with the increased monoglycerides and decreased triglycerides in BAL from FES patients or (b) from phospholipids and especially from phosphatidylcholine that are cleaved by phospholipase A_2.

Finally, the increased protein observed in BAL fluid can be attributed either to an increase in the alveolar fluid volume recovery in BAL due to an increase in lavaged area or, even, to increased alveolar protein concentration. This distinction, however, is not feasible because of the absence of reliable alveolar fluid volume markers [34]. Alveolar fluid reabsorption could be an additional mechanism for the increase in protein levels [35, 36]. However, the increase in protein levels in the BAL fluid of patients with FES was a consistent finding, most probably signifying that the high concentration protein was due to an increase in alveolar-capillary permeability.

In conclusion, the high levels of protein concentration in BAL fluid from FES patients suggest increased capillary-alveolar membrane permeability. This confirms that FES is indeed a predisposing actor for primary (direct) acute lung injury. The biochemical alterations, such as the high levels of PAF and PLA_2 encountered in BAL fluid from FES and ARDS groups, could indicate a common pathogenetic pathway after a certain stage in the development of ARDS. PLA_2 could play an important role in the development of inflammation in FES, and PAF-AcH in its attenuation. Finally, the increased level of neutral lipids and especially cholesterol and cholesterol esters in BAL fluid could clearly distinguish patients with ALI/ARDS due to FES from ALI/ARDS due to other causes.

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