Respiratory Medicine (2008) **102**, 464–469

ELSEVIER

respiratoryMEDICINE

Evaluation of bronchoalveolar lavage fluid from ARDS patients with regard to apoptosis

Keu Sung Lee[a], Young Hwa Choi[a], Young Sun Kim, Seung Hee Baik, Yoon Jung Oh, Seung Soo Sheen, Joo Hun Park, Sung Chul Hwang, Kwang Joo Park*

Department of Pulmonary and Critical Care Medicine, Ajou University School of Medicine, Suwon, South Korea

Received 1 May 2007; accepted 1 October 2007
Available online 7 November 2007

KEYWORDS
Acute respiratory distress syndrome;
Apoptosis;
Bronchoalveolar lavage;
Chemokine;
Neutrophil;
Epithelial cell

Summary
Background: Apoptosis is thought to play an important role in the development of acute respiratory distress syndrome (ARDS). We evaluated the bronchoalveolar lavage (BAL) fluid from ARDS patients focusing on apoptosis.
Methods: The study enrolled 31 ARDS patients and 20 healthy controls. BAL fluid levels of caspase-cleaved cytokeratin-18 (CK-18) and soluble mediators such as interleukin-8 (IL-8), soluble Fas (sFas), soluble Fas ligand (sFasL), growth-related oncogene-α (GRO-α), granulocyte colony-stimulating factor (G-CSF), and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) were measured using enzyme-linked immunosorbent assay (ELISA).
Results: The BAL fluid caspase-cleaved CK-18 levels in ARDS patients were higher than those in controls, reflecting increased epithelial apoptosis, and were correlated with lung injury scores ($r_s = 0.49$). The BAL fluid levels of all mediators were significantly higher in ARDS patients than in controls. In ARDS patients, the BAL fluid IL-8 level was positively correlated with the levels of sFas ($r_s = 0.57$), GRO-α ($r_s = 0.47$), and TRAIL ($r_s = 0.45$). The BAL fluid IL-8 ($r_s = 0.61$), sFas ($r_s = 0.57$), G-CSF ($r_s = 0.44$), and TRAIL ($r_s = 0.33$) levels were correlated with the BAL fluid neutrophil count. The G-CSF levels were significantly higher in non-surviving than in surviving ARDS patients [median 183.4 pg/mL (interquartile range 76.7–315.9) vs. 63.8 pg/mL (36.2–137.2); $p < 0.05$]. The sFas levels were positively correlated with the PaO_2/FiO_2 ratio ($r_s = 0.40$), and the TRAIL levels were negatively correlated with the multiple organ dysfunction scores ($r_s = -0.37$).

*Corresponding author. Tel.: +82 31 219 5121; fax: +82 31 219 5124.
E-mail address: parkkj@ajou.ac.kr (K.J. Park).
[a]These authors equally contributed to this work.

doi:10.1016/j.rmed.2007.10.001

Conclusions: Among the mediators in BAL fluid from ARDS patients, G-CSF had the most significant prognostic implications, and the sFas and TRAIL levels were correlated with clinical severity.

Introduction

Acute respiratory distress syndrome (ARDS) is characterised by increased permeability across the pulmonary microvasculature and alveolar epithelium.[1] Although various inflammatory cells and mediators participate in the pathogenesis of ARDS,[2] the exact mechanisms of the injury and its evolution are not clearly understood.

Apoptosis is thought to play an important role as one of the basic pathogenic mechanisms in ARDS.[3] Apoptosis in ARDS can be viewed from two perspectives. On one hand, the apoptosis of neutrophils is inhibited so their life span and numbers increase, perpetuating proinflammatory reactions.[3,4] Several soluble mediators are reported to be involved in inhibiting neutrophil apoptosis, including interleukin (IL)-2, IL-8, granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), and growth-related oncogene-α (GRO-α).[5–8] On the other hand, epithelial cells, which are the main cells injured in ARDS, suffer from enhanced apoptosis, disrupting the alveolo-capillary barrier.[4,9] The Fas/Fas ligand (FasL) system plays a major role in the apoptosis of epithelial cells.[10,11] It was reported that Fas and FasL were overexpressed in bronchoalveolar lavage (BAL) cells,[9] and soluble Fas ligand (sFasL) levels in the BAL fluid of patients with ARDS were higher in non-survivors than in survivors.[11] A subsequent study that measured soluble Fas (sFas) and sFasL in the oedema fluids of ARDS showed that sFas, but not sFasL, levels were related to the severity of organ dysfunction.[12]

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), well-known for its pro-apoptotic effects on tumour cells, has also been shown to play a role under nonmalignant conditions, acting on endothelial cells and inflammatory chemokines.[13,14] However, the role of TRAIL in ARDS has not been evaluated.

The details of how numerous mediators are involved in apoptosis in ARDS have not been sufficiently integrated to determine the precise interplay of positive and negative regulators. In fact, the interplay of the above-mentioned mediators may be extremely complicated, and difficulties arise in determining which mediator functions in what direction, that is, whether it is beneficial or detrimental to the lung injury or the clinical outcome.

We measured the levels of several known apoptosis-related and inflammatory mediators in BAL fluid from ARDS patients, and evaluated their interrelationships and clinical significance.

Materials and methods

Study subjects

Thirty-one ARDS patients admitted to the intensive care units of Ajou University Hospital between August 2003 and December 2004 and 20 healthy volunteers were enrolled in this prospective study. The diagnosis of ARDS followed the definition of the American European Consensus Conference for ARDS.[15] Cases with one or more of the following were excluded: age younger than 18 years, pregnant women, evidence of acute myocardial infarction or heart failure, patients medicated with steroids or immunosuppressants for more than 2 weeks within the past 3 months, or immunodeficiency. At the time of enrolment, the acute physiology and chronic health evaluation (APACHE) II scores,[16] sequential organ failure assessment (SOFA) scores,[17] multiple organ dysfunction scores (MODS),[18] and lung injury scores[19] were calculated for the patients. The outcome measure was the 28-day mortality after the diagnosis of ARDS. The protocol was approved by the ethics committee of Ajou University Hospital. Informed consent was obtained from the next of kin.

Bronchoalveolar lavage protocol

BAL was performed on the 31 patients with ARDS within 48 h of its diagnosis, and also on the 20 healthy volunteers. The procedure was performed at the right middle lobe or lingular division of the left upper lobe by instilling five separate 30-mL aliquots of 0.9% NaCl at 21 °C. The BAL aliquots were transported to the laboratory immediately for processing. A portion of the BAL fluid from each patient was used for the cellular analysis. The total protein concentrations in the BAL fluid were measured using the Biuret method (Sicdia TP reagent; Eiken Chemical, Tokyo, Japan). Total cell counts were measured using a haemocytometer. Cytocentrifuge preparations were made using a Shandon Cytopsin-3 (Shandon, Runcorn, UK), and the differential cell count was performed using Wright–Giemsa staining. The BAL fluid was centrifuged, and the supernatant was collected and stored at −80 °C until analysis.

Evaluation of epithelial apoptosis in BAL fluid

To evaluate alveolar epithelial apoptosis in BAL fluid, we measured caspase-cleaved cytokeratin-18 (CK-18) levels using the M30-Apoptosense enzyme-linked immunosorbent assay (ELISA) kit (PEVIVA AB, Bromma, Sweden). CK-18 is a type I intermediate filament protein and the major component of epithelial cells, but it is not contained in BAL cells (bone marrow-derived cells). The M30-ELISA assay is based on the epitope-specific M30 antibody, which only recognises soluble CK18 fragments cleaved at Asp396 by caspases.[20] M30 antigen levels are expressed as Units per litre. One Unit (U) corresponds to 1.24 pmol of a synthesised peptide containing the M30 recognition motif according to the manufacturer.

Measuring mediators

The concentrations of IL-8, sFas, sFasL, GRO-α, and TRAIL in the BAL fluid were measured using quantitative sandwich ELISA kits (R&D Systems, Minneapolis, MN, USA), and the concentration of G-CSF was measured using an ELISA kit (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions.

Statistical analyses

The data were analysed using SPSS for Windows (version 12; SPSS, Chicago, IL, USA) and the results presented as the median (interquartile range) because the data were nonparametric. The Mann–Whitney *U*-test was used to compare the two groups. Spearman's correlation of rank coefficient was used to analyse correlations between parameters, and $p<0.05$ was considered statistically significant.

Table 1 Characteristics of the study population and profiles of BAL fluid from the patients and controls.

Parameters	ARDS patients	Controls
n	31	20
Sex (male/female)	16/15	6/14
Age (years)	54.5±18.4	40.2±11.0
APACHE II score	17.5 (11.0–23.8)	
SOFA score	6.0 (4.1–8.0)	
MODS	6.0 (4.8–7.2)	
Lung injury score	3.2 (2.7–3.5)	
BAL parameters		
Protein concentration (μg/mL)	730 (160–1860)	100 (90–110)
Total cell count ($\times 10^4$/mL)	32.0 (13–51)	5.1 (1.8–8.1)
Neutrophil count ($\times 10^4$/mL)	18.0 (2.96–34.86)	0.07 (0–0.12)
Neutrophil percentage (%)	68 (15–77)	2 (0–3)

The data, except for age, are expressed as medians, with the interquartile range in parentheses. Age is expressed as the mean±S.D. APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; MODS: multiple organ dysfunction score.

Results

Characteristics of the study subjects

In ARDS patients, the 28-day mortality was 58% (16/31). The PaO_2/FiO_2 was 109.2 (92.3–140). The causes of ARDS were primary pneumonia ($n=24$) and sepsis of extrapulmonary origin ($n=7$). The baseline characteristics of the patients and controls are presented in Table 1.

Significance of caspase-cleaved CK-18 levels in BAL fluid

BAL fluid caspase-cleaved CK-18 levels were higher in ARDS patients than in the controls [172.3 U/L (99.3–240.4) vs. 52.3 U/L (35.8–88.0); $p<0.01$]. BAL fluid caspase-cleaved CK-18 levels were positively correlated with the lung injury scores ($r_s=0.49$, $p<0.01$) and tended to be negatively correlated with the PaO_2/FiO_2 ratio ($r_s=-0.34$, $p=0.06$), but were not correlated with all the measured mediators.

Comparison of mediators between ARDS patients and controls

The BAL fluid profiles are presented in Table 1. The concentrations of IL-8, sFas, GRO-α, G-CSF, and TRAIL in BAL fluid were all higher in the patients with ARDS than in the controls (Table 2). The sFasL levels were also higher in the ARDS patients than in the controls. However, its concentrations were very low and most were outside the reliable detection range; thus, we did not present the actual concentrations and omitted further analysis of sFasL.

Correlation between mediators and the BAL fluid profiles

The BAL fluid IL-8 level was positively correlated with the levels of sFas, GRO-α, and TRAIL. The sFas level was positively correlated with TRAIL and G-CSF levels. The TRAIL level was positively correlated with GRO-α level (Table 3).

The BAL fluid IL-8 ($r_s=0.61$, $p<0.01$), sFas ($r_s=0.57$, $p<0.01$), G-CSF ($r_s=0.44$, $p<0.05$), and TRAIL ($r_s=0.33$, $p<0.05$) levels were significantly correlated with the neutrophil count in the BAL fluid of ARDS patients.

Table 2 Comparison of the mediators in BAL fluid between ARDS patients and controls.

Mediators	ARDS patients	Controls	*p*-Value
IL-8 (pg/mL)	205.8 (83.9–537.5)	11.9 (9.9–17.9)	<0.01
sFas (pg/mL)	747.9 (178.7–1387.6)	52.7 (38.9–66.9)	<0.01
GRO-α (pg/mL)	759.5 (373.1–1536.9)	343.8 (250.1–499.7)	<0.01
G-CSF (pg/mL)	108.2 (45.3–190.0)	2.0 (1.6–3.9)	<0.01
TRAIL (pg/mL)	99.1 (50.4–177.0)	7.1 (4.0–16.6)	<0.01

The data are expressed as medians, with the interquartile range in parentheses.

Relationships of mediators with clinical parameters

When the levels of mediators were compared between survivors and non-survivors, only the G-CSF levels differed significantly and were higher in non-survivors than in survivors. The BAL fluid IL-8 levels were slightly higher, and the sFas, TRAIL, and GRO-α levels were slightly lower in non-survivors than in survivors without reaching statistical significance (Table 4).

The BAL fluid sFas levels were positively correlated with the PaO_2/FiO_2 ratio ($r_s = 0.40$, $p < 0.05$), and the TRAIL levels were negatively correlated with the MODS ($r_s = -0.37$, $p < 0.05$). The TRAIL levels were also negatively correlated with APACHE II and SOFA scores, although statistical significance was not achieved.

Discussion

Neutrophils and epithelial cells are two major objects of apoptosis in ARDS. Delayed apoptosis of intra-alveolar neutrophils was previously demonstrated.[6] Structural alterations due to increased apoptosis of alveolar epithelial cells have also been documented.[21] In the present study, we found increased alveolar epithelial apoptosis and observed its correlation with the severity of lung injury, indirectly through BAL fluid analysis.

Various apoptosis-related mediators measured using *in vivo* and *in vitro* methods play roles in ARDS.[4] This study examined the up-regulation and interrelationship of apoptosis-related mediators in ARDS, and documented their clinical implications.

Table 3 Spearman correlation coefficients between the mediators in BAL fluid from ARDS patients.

	IL-8	s-Fas	GRO-α	G-CSF	TRAIL
IL-8	1				
s-Fas	0.57†	1			
GRO-α	0.47†	NS	1		
G-CSF	NS	0.55*	NS	1	
TRAIL	0.45*	0.49†	0.40*	NS	1

NS, not significant.
*$p < 0.05$.
†$p < 0.01$.

The CXC chemokines IL-8 and GRO-α not only have a role in neutrophil chemotaxis, but also inhibit neutrophil apoptosis.[5,7,8] In addition, pleomorphic cytokine G-CSF has specific effects on the proliferation, differentiation, and activation of the neutrophil granulocyte lineage.[22] It was reported that levels of IL-8, G-CSF, and GRO-α were correlated with the neutrophil count in BAL fluid from ARDS patients.[5,23,24] In our study, the levels of IL-8 and G-CSF, but not GRO-α, were correlated with the BAL neutrophil count. In addition, the sFas and TRAIL levels were correlated with the neutrophil count. It has been suggested that sFas decreases neutrophil apoptosis in postoperative patients,[25] and the Fas/FasL system was shown to induce IL-8 production, which may result in the recruitment of neutrophils indirectly.[26] Membrane-bound TRAIL induces apoptosis of neutrophils, whilst the soluble form of TRAIL is not known to have any direct effects on neutrophils.[27] The significant correlations between mediators and the neutrophil count do not necessarily imply direct actions of these mediators on neutrophil recruitment or apoptosis. From a different perspective, neutrophils are important sources of sFas[28] and TRAIL,[29] so some correlations might be mere secondary phenomena arising from the increased number of neutrophils.

The Fas/FasL system plays an important role in epithelial cell apoptosis in ARDS.[11,30] Fas antigen, also called APO-1 or CD95, is a membrane receptor for FasL that belongs to the tumour necrosis factor (TNF)/nerve growth receptor family. Fas antigen is expressed on various cells and tissues, including lung.[12] FasL is a transmembrane protein in the TNF family that induces apoptosis of susceptible cells by cross-linking its receptor, Fas. Membrane-bound FasL is converted into sFasL by a matrix metalloproteinase-like enzyme.[31] Unlike FasL, sFas is produced by alternative splicing and inhibits cell apoptosis by competing with membrane-bound Fas receptors to bind to FasL.[32] Alveolar and airway epithelial cells express Fas on their surfaces,[33] and the alveolar epithelial injuries in ARDS are caused by enhanced apoptosis due to local activation of the Fas/FasL system.[12]

We evaluated the soluble forms of Fas and FasL in BAL fluid from patients with ARDS. A previous report compared the concentrations of sFas and sFasL levels in oedema fluids between ARDS and congestive heart failure, and it was found that levels of sFas were far higher than those of sFasL.[12] That result was considered unexpected, given that sFas is essentially anti-apoptotic and sFasL is pro-apoptotic for alveolar epithelial cells, and the apoptosis of alveolar epithelial cells in response to soluble mediators such as

Table 4 Comparison of the levels of mediators between survivors and non-survivors in patients with ARDS.

Mediators	Survivors ($n = 15$)	Non-survivors ($n = 16$)	p-Value
IL-8 (pg/mL)	161.8 (83.9–454.5)	213.3 (88.9–1057.6)	NS
sFas (pg/mL)	759.5 (233.2–1388.1)	463.4 (79.0–1373.8)	NS
GRO-α (pg/mL)	843.1 (368.5–1670.6)	533.6 (435.2–1328.6)	NS
G-CSF (pg/mL)	63.8 (36.2–137.2)	183.4 (76.7–315.9)	<0.05
TRAIL (pg/mL)	106.5 (55.7–178.4)	68.8 (46.7–181.7)	NS

The data are expressed as medians, with the interquartile range in parentheses.

sFasL is up-regulated in ARDS.[3] By contrast, the sFasL levels were comparable or higher than the sFas levels in other pulmonary diseases.[34] In our study, BAL fluid sFas levels were over 50 times higher than those of sFasL, although the actual values are not given, since they were too low to be reliable. Furthermore, the BAL fluid sFasL levels were about 10 times lower than those of a similar form of ligand, TRAIL. In terms of concentrations, sFasL may not be sufficient as a single major contributor in the development of ARDS. One previous study reported the prognostic significance of intrapulmonary sFasL levels,[11] whilst another did not.[12] Although the system plays a major role in the pathogenesis of ARDS, its soluble forms only constitute a small portion of the Fas/FasL system, so they could not account for all of the apoptotic events in the milieu of injured alveoli.

Apart from significant interrelations amongst various mediators and their relationship with intra-alveolar neutrophils, the clinical implications should be very important for the utility of the measured mediators from a practical perspective. From our analysis, G-CSF levels had the most significant relationship with survival. The other parameters only differed marginally. BAL fluid G-CSF was also found to be a significant prognostic factor in one study,[5] but not in another smaller study.[22]

In this study, IL-8 levels were higher in non-survivors than in survivors, but did not reach statistical significance. Likewise, previous reports did not find consistently significant differences in the BAL fluid IL-8 levels between survivors and non-survivors.[5,35,36]

In analysing the relationships with clinical features, the sFas levels were correlated with the PaO_2/FiO_2 ratio, although not with the final clinical outcome. This implies a possible protective role of sFas in ARDS. This result is plausible because sFas binds to and neutralises FasL, thereby functionally antagonising the Fas/FasL pathway.[32] However, Albertine et al.[12] reported somewhat contradictory results. They showed that sFas levels in oedema fluid from ARDS patients were elevated more in patients with sepsis, shock, or organ failure than in those without, although no direct correlation with oxygenation status (PaO_2/FiO_2) was presented and no significant relationship with clinical outcome was revealed. Further studies need to address this discrepancy regarding the clinical implications of sFas.

The BAL fluid levels of TRAIL were negatively correlated with the clinical severity scores, suggesting its favourable role in the systemic inflammatory response and organ dysfunction. TRAIL, like FasL, belongs to the TNF family and has structural similarity to FasL.[37] TRAIL, which consists of membrane-bound and soluble forms, has well-known pro-apoptotic effects on tumour cells.[38] However, other functions of TRAIL in benign conditions have been recently introduced. It was reported that TRAIL promoted the survival and proliferation of endothelial cells, and down-regulated the inflammatory chemokines.[13,14] These effects may underlie the negative correlations of TRAIL with clinical severity scores. However, the role of TRAIL has not yet been defined in ARDS, and further evaluation is needed to clarify the significance of TRAIL in ARDS, including measurements of serum levels.

We acknowledge several limitations of our study. First, as noted, the small sample size prevented us from further subgroup analysis. Second, we did not measure the serum levels of the parameters. The data might have been more meaningful if the serum levels had been measured and compared with the levels in BAL fluid. Third, some bias occurred in the control group apart from the age difference. Mechanical ventilation itself causes an inflammatory reaction and the release of mediators, so the differences in mediator levels are overestimated, and the reliability of the comparison between patients and controls is thus limited.

In conclusion, we have shown that apoptosis-related mediators are elevated in BAL fluid from ARDS patients. Furthermore, they are interrelated with each other and correlated with the BAL fluid neutrophils. Of the mediators, G-CSF had prognostic implications, whilst sFas and TRAIL were related to the clinical parameters. Further studies are needed to elucidate the detailed pathophysiologic roles and interplay of the mediators.

Conflict of interest

The authors have no conflict of interest financial or otherwise regarding this manuscript.

References

1. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000;**342**:1334–49.
2. Bhatia M, Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J Pathol* 2004;**202**:145–56.
3. Martin TR, Nakamura M, Matute-Bello G. The role of apoptosis in acute lung injury. *Crit Care Med* 2003;**31**:S184–8.
4. Matute-Bello G, Martin TR. Science review: apoptosis in acute lung injury. *Crit Care* 2003;**7**:355–8.
5. Aggarwal A, Baker CS, Evans TW, Haslam PL. G-CSF and IL-8 but not GM-CSF correlate with severity of pulmonary neutrophilia in acute respiratory distress syndrome. *Eur Respir J* 2000;**15**:895–901.
6. Lesur O, Kokis A, Hermans C, Fulop T, Bernard A, Lane D. Interleukin-2 involvement in early acute respiratory distress syndrome: relationship with polymorphonuclear neutrophil apoptosis and patient survival. *Crit Care Med* 2000;**28**:3814–22.
7. Kettritz R, Gaido ML, Haller H, Luft FC, Jennette CJ, Falk RJ. Interleukin-8 delays spontaneous and tumor necrosis factor-alpha-mediated apoptosis of human neutrophils. *Kidney Int* 1998;**53**:84–91.
8. Glynn PC, Henney E, Hall IP. The selective CXCR2 antagonist SB272844 blocks interleukin-8 and growth-related oncogene-alpha-mediated inhibition of spontaneous neutrophil apoptosis. *Pulm Pharmacol Ther* 2002;**15**:103–10.
9. Hashimoto S, Kobayashi A, Kooguchi K, Kitamura Y, Onodera H, Nakajima H. Upregulation of two death pathways of perforin/granzyme and FasL/Fas in septic acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2000;**161**:237–43.
10. Matute-Bello G, Liles WC, Frevert CW, Nakamura M, Ballman K, Vathanaprida C, et al. Recombinant human Fas ligand induces alveolar epithelial cell apoptosis and lung injury in rabbits. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**:L328–35.
11. Matute-Bello G, Liles WC, Steinberg KP, Kiener PA, Mongovin S, Chi EY, et al. Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). *J Immunol* 1999;**163**:2217–25.
12. Albertine KH, Soulier MF, Wang Z, Ishizaka A, Hashimoto S, Zimmerman GA, et al. Fas and fas ligand are up-regulated in

pulmonary edema fluid and lung tissue of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Pathol* 2002;**161**:1783–96.
13. Secchiero P, Gonelli A, Carnevale E, Milani D, Pandolfi A, Zella D, et al. TRAIL promotes the survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. *Circulation* 2003;**107**:2250–6.
14. Secchiero P, Corallini F, di Iasio MG, Gonelli A, Barbarotto E, Zauli G. TRAIL counteracts the proadhesive activity of inflammatory cytokines in endothelial cells by down-modulating CCL8 and CXCL10 chemokine expression and release. *Blood* 2005; **105**:3413–9.
15. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994;**149**:818–24.
16. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985;**13**:818–29.
17. Vincent JL, de Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on ''sepsis-related problems'' of the European Society of Intensive Care Medicine. *Crit Care Med* 1998;**26**:1793–800.
18. Marshall JC, Cook DJ, Christou NV, Bernard GR, Sprung CL, Sibbald WJ. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. *Crit Care Med* 1995;**23**:1638–52.
19. Murray JF, Matthay MA, Luce JM, Flick MR. An expanded definition of the adult respiratory distress syndrome. *Am Rev Respir Dis* 1988;**138**:720–3.
20. Kramer G, Erdal H, Mertens HJ, Nap M, Mauermann J, Steiner G, et al. Differentiation between cell death modes using measurements of different soluble forms of extracellular cytokeratin 18. *Cancer Res* 2004;**64**:1751–6.
21. Bardales RH, Xie SS, Schaefer RF, Hsu SM. Apoptosis is a major pathway responsible for the resolution of type II pneumocytes in acute lung injury. *Am J Pathol* 1996;**149**:845–52.
22. Wiedermann FJ, Mayr AJ, Hobisch-Hagen P, Fuchs D, Schobersberger W. Association of endogenous G-CSF with anti-inflammatory mediators in patients with acute respiratory distress syndrome. *J Interferon Cytokine Res* 2003;**23**:729–36.
23. Villard J, Dayer-Pastore F, Hamacher J, Aubert JD, Schlegel-Haueter S, Nicod LP. GRO alpha and interleukin-8 in Pneumocystis carinii or bacterial pneumonia and adult respiratory distress syndrome. *Am J Respir Crit Care Med* 1995;**152**: 1549–54.
24. Wiedermann FJ, Mayr AJ, Kaneider NC, Fuchs D, Mutz NJ, Schobersberger W. Alveolar granulocyte colony-stimulating factor and alpha-chemokines in relation to serum levels, pulmonary neutrophilia, and severity of lung injury in ARDS. *Chest* 2004;**125**:212–9.
25. Iwase M, Kondo G, Watanabe H, Takaoka S, Uchida M, Ohashi M, et al. Regulation of Fas-mediated apoptosis in neutrophils after surgery-induced acute inflammation. *J Surg Res* 2006;**134**: 114–23.
26. Hagimoto N, Kuwano K, Kawasaki M, Yoshimi M, Kaneko Y, Kunitake R, et al. Induction of interleukin-8 secretion and apoptosis in bronchiolar epithelial cells by Fas ligation. *Am J Respir Cell Mol Biol* 1999;**21**:436–45.
27. Renshaw SA, Parmar JS, Singleton V, Rowe SJ, Dockrell DH, Dower SK, et al. Acceleration of human neutrophil apoptosis by TRAIL. *J Immunol* 2003;**170**:1027–33.
28. Marsik C, Halama T, Cardona F, Wlassits W, Mayr F, Pleiner J, et al. Regulation of Fas (APO-1, CD95) and Fas ligand expression in leukocytes during systemic inflammation in humans. *Shock* 2003;**20**:493–6.
29. Cassatella MA. On the production of TNF-related apoptosis-inducing ligand (TRAIL/Apo-2L) by human neutrophils. *J Leukoc Biol* 2006;**79**:1140–9.
30. Kitamura Y, Hashimoto S, Mizuta N, Kobayashi A, Kooguchi K, Fujiwara I, et al. Fas/FasL-dependent apoptosis of alveolar cells after lipopolysaccharide-induced lung injury in mice. *Am J Respir Crit Care Med* 2001;**163**:762–9.
31. Kayagaki N, Kawasaki A, Ebata T, Ohmoto H, Ikeda S, Inoue S, et al. Metalloproteinase-mediated release of human Fas ligand. *J Exp Med* 1995;**182**:1777–83.
32. Cheng J, Zhou T, Liu C, Shapiro JP, Brauer MJ, Kiefer MC, et al. Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. *Science* 1994;**263**:1759–62.
33. Hamann KJ, Dorscheid DR, Ko FD, Conforti AE, Sperling AI, Rabe KF, et al. Expression of Fas (CD95) and FasL (CD95L) in human airway epithelium. *Am J Respir Cell Mol Biol* 1998;**19**: 537–42.
34. Kuwano K, Kawasaki M, Maeyama T, Hagimoto N, Nakamura N, Shirakawa K, et al. Soluble form of fas and fas ligand in BAL fluid from patients with pulmonary fibrosis and bronchiolitis obliterans organizing pneumonia. *Chest* 2000;**118**:451–8.
35. Baughman RP, Gunther KL, Rashkin MC, Keeton DA, Pattishall EN. Changes in the inflammatory response of the lung during acute respiratory distress syndrome: prognostic indicators. *Am J Respir Crit Care Med* 1996;**154**:76–81.
36. Meduri GU, Kohler G, Headley S, Tolley E, Stentz F, Postlethwaite A. Inflammatory cytokines in the BAL of patients with ARDS. Persistent elevation over time predicts poor outcome. *Chest* 1995;**108**:1303–14.
37. Kwon B, Youn BS, Kwon BS. Functions of newly identified members of the tumor necrosis factor receptor/ligand superfamilies in lymphocytes. *Curr Opin Immunol* 1999;**11**:340–5.
38. Chaudhari BR, Murphy RF, Agrawal DK. Following the TRAIL to apoptosis. *Immunol Res* 2006;**35**:249–62.