Comparison of systemic cytokine levels in patients with acute respiratory distress syndrome, severe pneumonia, and controls

Torsten T Bauer, Concepción Montón, Antoni Torres, Hernan Cabello, Xavier Fillela, Abel Maldonado, Jose-Maria Nicolás, Elisabet Zavala

Abstract

***Background*—The inflammatory response has been widely investigated in patients with acute respiratory distress syndrome (ARDS) and pneumonia. Studies investigating the diagnostic values of serum cytokine levels have yielded conflicting results and only little information is available for the differential diagnosis between ARDS and pneumonia.**

***Methods*—Clinical and physiological data, serum concentrations of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, and quantitative cultures of lower respiratory tract specimens were obtained from 46 patients with ARDS and 20 with severe pneumonia within 24 hours of the onset of the disease and from 10 control subjects with no inflammatory lung disease. Cytokine concentrations were compared between groups and determinants in addition to the diagnosis were tested.**

***Results*—Serum TNF-α levels were significantly higher in ARDS patients (67 (57) pg/ml) than in patients with severe pneumonia (35 (20) pg/ml; p = 0.031) or controls (17 (8) pg/ml; p = 0.007). For IL-1β and IL-6 the observed differences were not statistically significant between patients with ARDS (IL-1β: 34 (65) pg/ml; IL-6: 712 (1058) pg/ml), those with severe pneumonia (IL-1β: 3 (4) pg/ml, p = 0.071; IL-6: 834 (1165) pg/ml, p = 1.0), and controls (IL-1β: 6 (11) pg/ml, p = 0.359; IL-6: 94 (110) pg/ml, p = 0.262). TNF-α (standardised coefficient β = 0.410, p<0.001) and IL-1β (standardised coefficient β = 0.311, p = 0.006) were most strongly associated with the degree of lung injury, even when the diagnostic group was included in the statistical model.**

***Conclusions*—Serum TNF-α levels were higher in patients with ARDS than in those with severe pneumonia or in control subjects. Multivariate results suggest that the levels of systemic TNF-α and IL-1β reflect the severity of the lung injury rather than the diagnosis.**

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Cytokines are widely recognised as mediators of an inflammatory response. While some cytokines promote inflammation by recruitment of cells—for example, interleukin (IL)-6, IL-8, and tumour necrosis factor (TNF)-α—others such as IL-10 counteract this process. Although the role of cytokines in the pathogenesis of acute respiratory distress syndrome (ARDS) has been widely recognised, their importance in the clinical diagnosis has not been clearly defined.[1–12]

Pneumonia and ARDS both produce an inflammatory response locally in the lung, but the appearance and the level of systemically circulating cytokines in the blood may make it possible to differentiate between the two conditions. ARDS can be caused and is often accompanied by a systemic inflammatory response, whereas a systemic inflammatory response is not always present in severe pneumonia.[13] Schütte and co-workers[14] measured cytokine levels in patients with ARDS, severe pneumonia, and cardiogenic pulmonary oedema and found consistently higher levels of IL-8, IL-6, and TNF-α in the bronchoalveolar lavage (BAL) fluid and serum of patients with severe pneumonia and/or ARDS than in those with pulmonary oedema, but they did not find a clear difference between those with severe pneumonia and ARDS. This would have been very helpful because it is often difficult to distinguish between ARDS and severe pneumonia from the clinical presentation but treatment of the two conditions differs considerably. Although ARDS is characterised by bilateral infiltrates on the chest radiograph, pneumonia can follow a similar pattern.[15] Bacteriological data, with or without quantitative cultures, do not definitely identify an infectious aetiology of pulmonary infiltrates, especially when antibiotics have been administered.[16–18] Furthermore, clinical signs such as fever and leucocytosis may be present at the onset of ARDS even in the absence of infection.[19]

A study was undertaken to compare serum levels of pro-inflammatory cytokines in patients with ARDS, severe pneumonia, and controls to test the potential of these parameters to differentiate between the two diseases, and possible determinants of cytokine levels in these subjects were determined.

Methods

PATIENTS

The trial was conducted in a tertiary care hospital between 1 January 1995 and 31 December 1997. All patients with severe pneumonia and controls receiving ventilator support were consecutive patients in one respiratory inten-

sive care unit (RICU). Data on ARDS patients were collected in one RICU and two medical ICUs of the same hospital. Patients with severe pneumonia or ARDS were included in the protocol within the first 24 hours after onset of the disease.

DEFINITIONS AND STUDY GROUPS

Acute respiratory distress syndrome (ARDS)

The following criteria for the diagnosis of ARDS were applied as defined by the American-European Consensus Conference: (1) acute onset of the syndrome, (2) Pa_{O_2}/F_{IO_2} ratio $\leqslant$ 200 with positive end expiratory pressure applied (PEEP), (3) generalised pulmonary infiltrates involving at least two quadrants on the chest radiograph, and (4) wedge pressure $\leqslant$18 mm Hg in patients with a pulmonary artery catheter present or no clinical signs of congestive heart failure.[15] In addition, a score of $\geqslant$2.5 according to Murray and co-workers was required.[20] Causes of ARDS were classified as direct (pneumonia, aspiration or thorax trauma) or indirect lung injury (extrapulmonary source of inflammation, sepsis or shock).[21]

Severe pneumonia

Pneumonia was suspected clinically in the presence of new infiltrates on the chest radiograph and two of the following criteria: fever $\geqslant$38.3°C or hypothermia $\leqslant$35.0°C, purulent secretions, or leucocytosis ($\geqslant$12 000/mm^3) or leucopenia ($\leqslant$4000/mm^3). According to the length of hospital stay the episode of pneumonia was classified as either community acquired (<72 h) or nosocomial ($\geqslant$72 h). All patients were intubated and mechanically ventilated in the ICU, thus fulfilling the criteria for severe pneumonia.[22]

Controls

This group included only patients who met the following criteria: (1) intubated and mechanically ventilated, (2) absence of any clinical signs of infection, (3) absence of any of the criteria of the above two groups; (4) bronchoscopic examination indicated for other reasons such as tube malposition, minor haemoptysis, or visual inspection of the tracheobronchial tree.

Exclusion criteria

Patients with positive serology for human immunodeficiency virus (HIV), cerebral injury, presence of immunosuppression, or coagulation disorders were excluded.

In each case family members gave informed consent for the study and the study was approved by the ethical committee of our centre.

SEPTIC SHOCK

Septic shock was defined as a systemic inflammatory response to infection (presence of two or more of the following: temperature >38°C or <36°C, heart rate >90 beats/min, respiratory rate >20/min or Pa_{CO_2} <4.27 kPa, and leucocyte count >12 000/mm^3 or >10% band forms), in addition to hypotension (systolic blood pressure <90 mm Hg or a reduction of >40 mm Hg from baseline in the absence of other causes).[23] Other causes of shock such as hypovolaemia or cardiogenic shock were not considered.

PROTOCOL

The following demographic, clinical, and laboratory data were recorded on entry to the study: age, sex, cause of ICU admission, duration of mechanical ventilation before the study, use of corticosteroid medication (any intravenous administration during the 24 hours before sampling), prior antibiotic use (administered intravenously for more then 24 hours), blood analysis necessary for calculation of the simplified acute physiology score (SAPS II),[24] lung injury score,[20] arterial blood gas tensions, and outcome at ICU discharge or death. The chest radiograph on the day of the study was reviewed for each patient and the extent of pulmonary infiltrates was scored as bilateral, unilateral, or none.

Lower respiratory tract sampling

According to the clinical condition of the patients, lower respiratory tract specimens were obtained either non-invasively by tracheobronchial aspirates (TBAS, standard sputum trap; Proclinics, Barcelona, Spain) or bronchoscopically by protected specimen brush (PSB, Microbiology brush; Mill-Rose Laboratories Inc, Mentor, Ohio, USA) within the first 24 hours after onset of the disease. Fibreoptic bronchoscopy was performed without interrupting mechanical ventilation through the endotracheal tube using a special adapter. The fibreoptic bronchoscope (BF30, Olympus, New Hyde Park, New York, USA) was introduced without bronchial suctioning after adequate sedation and no local anaesthetics were administered. The setting of the ventilator was adapted appropriately during the procedure to ensure ventilation and oxygenation. The PSB was used as described by Wimberley and co-workers.[25]

Microbiological processing

The PSB was aseptically cut into a sterile tube containing 1 ml of Ringer's lactate and shaken for one minute. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}) from each PSB and TBAS sample were prepared in sterile normal saline. One hundred ml of each dilution was inoculated into the following agar media: 5% sheep blood, chocolate, Center for Disease Control (CDC) blood, McConkey, blood charcoal yeast extract (BCYE-a), and Sabouraud-dextrose. All cultures were incubated at 37°C under aerobic and anaerobic conditions. Cultures were evaluated for growth 24 and 48 hours later and discarded five days later if negative, except for CDC and Wilkins-Chalgren media which were evaluated at seven days and Sabouraud medium which was evaluated at four weeks. Gram stains were performed on all samples. All microorganisms isolated were identified by standard laboratory methods.[8] The following thresholds for quantitative cultures were used: PSB $\geqslant 10^3$ cfu/ml, TBAS $\geqslant 10^5$ cfu/ml, and the following microorganisms were considered

potentially pathogenic (PPM): *Pseudomonas* spp, *Acinetobacter* spp, *Stenotrophomonas maltophilia*, *Klebsiella* spp, *Enterobacter* spp, *Escherichia coli*, *Serratia* spp, *Campylobacter* spp, *Proteus mirabilis*, *Neisseria* spp, *Haemophilus influenzae*, *Staphylococcus aureus* (coagulase positive), *Streptococcus pneumoniae*, and *Aspergillus* spp.

Cytokine assays

To determine blood concentrations of TNF-α, IL-1β, and IL-6, blood samples were collected in sterile tubes not containing anticoagulants during the first 24 hours after diagnosis, centrifuged at 3000 rpm for 10 minutes, and stored at –70°C until processing. The solid phase enzyme linked immunosorbent assay (ELISA) used was based on the quantitative immunometric sandwich enzyme immunoassay technique on a microtitre plate (EASIA: Enzyme Amplified Sensitivity Immunoassay, Medgenix Diagnostics SA, Fleurus, Belgium) which uses a murine monoclonal antibody specific for the cytokine to be analysed coated onto the microtitre plate to create the solid phase. The results were expressed as pg/ml of serum. The sensitivity of the technique allows the detection of levels as low as 3 pg/ml for TNF-α and 2 pg/ml for IL-1β and IL-6, respectively.

STATISTICAL ANALYSIS

For the comparison of serum cytokine concentrations between patients with ARDS, severe pneumonia, and controls without inflammatory lung disease receiving mechanical ventilation the *t* test was used for comparison of quantitative variables of the two groups. For the comparison of more than two groups an analysis of variance (ANOVA) with post hoc correction was used (Bonferroni). The χ^2 test (Fisher's exact test for comparisons of expected cell frequencies <5) was used to compare proportions.

Comparative evaluation of possible determinants of serum cytokine concentrations in this population by multivariate analysis was performed using a multiple linear regression in a stepwise forward model with cytokine levels (TNF-α, IL-1β, IL-6) as the dependent variable. The diagnosis was recorded (ARDS = 2, severe pneumonia = 1, controls = 0) and tested pairwise with the following severity parameters: extent of pulmonary infiltrates (bilateral = 2, unilateral = 1, none = 0), lung injury score, and the simplified acute physiology score (SAPS II).

The significance level of all analyses was 5%. Results are expressed as mean (SD) and 95% confidence intervals (CI) are given for all comparisons. All data were analysed and processed on SPSS version 8.01.

Results

Forty six patients with ARDS (32 men), 20 with severe pneumonia (17 men), and 10 controls (eight men) were included in the study. The cause of ARDS was direct lung injury in 26 cases (57%; aspiration (n = 5, 19%), community acquired pneumonia (n = 12, 46%), nosocomial pneumonia (n = 8, 31%), other (n = 1, 4%)) and indirect in 20 cases (43%; sepsis (n = 14, 70%), other (n = 6, 30%)). Severe pneumonia was nosocomial in 12 of the 20 patients (60%) and community acquired in eight; 10 of the 20 patients with severe pneumonia had unilateral pulmonary infiltrates and 10 had bilateral pulmonary infiltrates. The demographic characteristics are summarised in table 1. Patients with ARDS were significantly younger than patients with severe pneumonia (mean difference –9.8, 95% CI –8.1 to –1.6), had a higher SAPS II (mean difference 10.9, 95% CI 2.4 to 19.4; p = 0.007), and a higher lung injury score (mean difference 1.1, 95% CI 0.8 to 1.4; p<0.001). The total length of stay in the ICU was similar for the patients with ARDS and those with severe pneumonia, but significantly shorter for control patients than for those with ARDS (mean difference –11.5, 95% CI –23.0 to –0.04; p = 0.049). The causes of ICU admission varied between the diagnostic groups and most patients received antibiotic treatment on the day of the study. Patients with severe pneumonia had received an intravenous dose of corticosteroid medication prior to blood sampling more frequently than those with ARDS (55% versus 26%; mean difference

Table 1 Mean (SD) clinical data of patients with acute respiratory distress syndrome (ARDS), severe pneumonia and controls receiving mechanical ventilation

	ARDS (n = 46)	*Severe pneumonia (n = 20)*	*Controls (n = 10)*
Age (years)	57.2 (15.2)	67.1 (6.4)	65.6 (6.4)
SAPS II	43.8 (13.7)	32.9 (9.9)	34.6 (14.3)
Lung injury score	2.9 (0.9)	1.8 (0.6)	0.9 (0.4)
Pao_2/Fio_2 ratio	114 (47)	251 (102)	428 (102)
Leucocyte count (/mm^3)	16184 (9270)	14573 (7171)	11260 (3589)
Duration of mechanical ventilation until the study (days)	146 (192)	103 (120)	89 (84)
Total length of stay in ICU (days)	23 (16)	22 (11)	12 (6)
Cause of ICU admission (n, %)*			
Community acquired pneumonia	8 (17)	7 (35)	—
Nosocomial pneumonia	9 (20)	—	—
Exacerbation of COPD	2 (4)	3 (15)	4 (40)
Other respiratory disease	2 (4)	1 (5)	1 (10)
Severe sepsis or shock	14 (30)	15 (75)	—
Neurological	2 (4)	2 (10)	5 (50)
Trauma	2 (4)	2 (10)	—
Postoperative	15 (33)	4 (20)	—
Others	—	1 (5)	—

SAPS = simplified acute physiology score; Pao_2 = arterial oxygen tension; Fio_2 = fractional inspired oxygen concentration.
*Total greater than 100% due to multiple causes.

29%, 95% CI 3.8 to 54.2; p = 0.0473). The percentage of patients who had received antibiotic treatment prior to sampling was similar in all groups (ARDS, 100%; severe pneumonia, 100%; controls, 80%; p = NS).

BACTERIOLOGY

In patients with ARDS the microorganisms recovered most frequently from respiratory specimens were *Pseudomonas* spp (n = 13), *S aureus* (coagulase negative, n = 17), and *Candida* spp (n = 16). In patients with severe pneumonia *Pseudomonas* spp (n = 8), *S aureus* (coagulase positive, n = 6), and *S pneumoniae* (n = 6) were among the most common pathogens. The microorganisms recovered from controls consisted predominantly of non-pathogenic microorganisms such as *Candida* spp (n = 4) and *Strep viridans* (n = 3), and in four cases the samples were sterile.

When only potentially pathogenic microorganisms were taken into account the recovery was 20/46 (44%) in patients with ARDS, 11/20 (55%) in patients with severe pneumonia, and 2/10 (20%) in controls. In control patients these were *Pseudomonas* spp and *Proteus mirabilis*, respectively. With potentially pathogenic microorganisms above the defined threshold these figures were 13/46 (28%) in ARDS patients, 10/20 (50%) in patients with pneumonia, and 1/10 (10%) in controls.

CYTOKINE LEVELS

TNF-α and IL-6 were detected in all patients with ARDS and severe pneumonia and in nine of the 10 control patients. IL-1β was detectable in 44 of 46 patients with ARDS (96%), nine of 20 patients with severe pneumonia (45%), and in five of the 10 controls.

The raw data and mean levels of all three cytokines for the three groups are shown in fig 1. Serum concentrations of TNF-α were significantly higher in ARDS patients (67 (57) pg/ml) than in patients with severe pneumonia (35 (20) pg/ml; mean difference 32.2, 95% CI 2.3 to 62.3; p = 0.031) or controls (17 (8) pg/ml; mean difference 52.3, 95% CI 11.2 to 89.3; p = 0.007). The serum concentration of IL-1β showed a large variation within groups and the differences observed were therefore statistically not significant when patients with ARDS (34 (65) pg/ml) were compared with those with severe pneumonia (3 (4) pg/ml; mean difference 31.8, 95% CI –1.9 to 65.5; p = 0.071) and controls (6 (11) pg/ml; mean difference 28.2, 95% CI –15.7 to 72.2; p = 0.356). IL-6 concentrations also showed a large interindividual variation and were not significantly different between ARDS patients (712 (1058) pg/ml), those with severe pneumonia (834 (1165) pg/ml; mean difference –124.6, 95% CI –795.5 to 546.3; p = 1.0), or controls (94 (110) pg/ml; mean difference 618.0, 95% CI –256.0 to 1491.9; p = 0.262).

The serum concentrations of cytokines in patients with ARDS were not significantly different when patients with an indirect cause were compared with those with a direct cause of ARDS (TNF-α: 67 (53) versus 67 (60) pg/ml, mean difference –0.74, 95% CI –35.1 to 33.6; p = 0.965; IL-1β: 22 (29) versus 44 (82) pg/ml; mean difference 22.3, 95% CI –61.2 to 16.9; p = 0.256; IL-6: 643 (1068) versus 765 (1068) pg/ml, mean difference –122.4, 95% CI –762.6 to 517.9; p = 0.702).

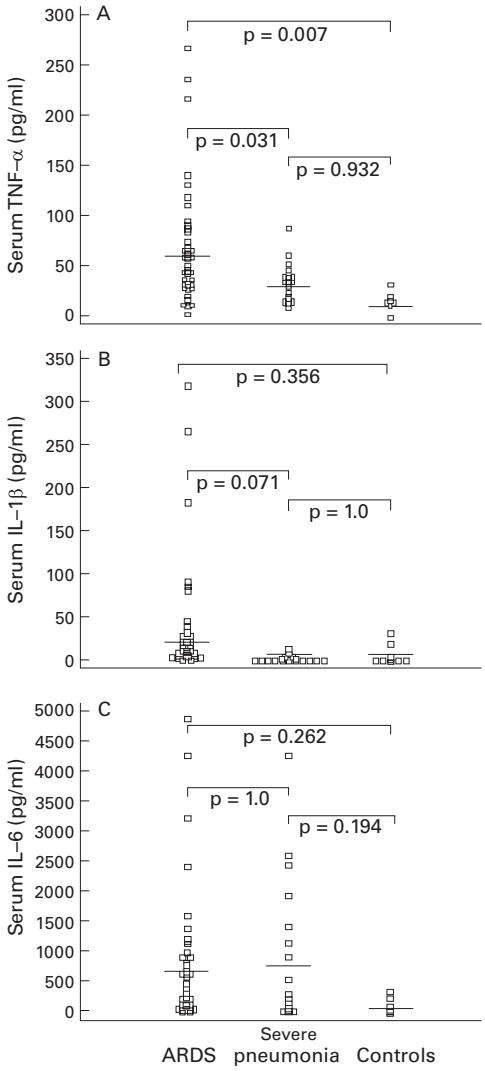

Figure 1 Individual data and mean serum concentrations of (A) TNF-α, (B) IL-1β, and (C) IL-6 for patients with acute respiratory distress syndrome (ARDS), severe pneumonia, and controls on mechanical ventilation without inflammatory lung disease. The mean values were compared by analysis of variance (ANOVA) with Bonferroni's correction for multiple comparisons. Exact levels of significance are given for all comparisons.

MULTIVARIATE ANALYSIS

The results of the multiple regression analyses are summarised in table 2. The serum concentrations of TNF-α and IL-1β were most strongly associated with the degree of lung injury as measured by the lung injury score, even when the diagnostic group was included in the statistical model. The extent of pulmonary infiltrates was no better associated with the serum level of TNF-α and IL-1β than in the diagnostic group. When the SAPS II score was also entered into the model in the diagnostic group it improved only the approximation of TNF-α serum levels but not those of IL-1β. IL-6 levels were inconsistent. The diagnostic

Table 2 Results of multivariate linear regression for cytokine concentrations and diagnostic group (ARDS, severe pneumonia, controls) tested pairwise with the extent of the pulmonary infiltrate (bilateral, unilateral, none), the lung injury score, and the SAPS II score

	TNF-α		*IL-1β*		*IL-6*	
Diagnostic group + extent of pulmonary infiltrates	0.394 (<0.001)	—* (0.734)	0.251 (0.029)	—* (0.531)	0.499 (0.039)	0.722 (0.003)
Diagnostic group + lung injury score	—* (0.469)	0.410 (<0.001)	—* (0.728)	0.311 (0.006)	—*	—*
Diagnostic group + SAPS II	0.321 (0.005)	0.235 (0.037)	0.244 (0.035)	—* (0.681)	—* (0.751)	0.292 (0.011)

Results are presented as standardised coefficients (β) with p values in parentheses.
ARDS = acute respiratory distress syndrome; TNF-α = tumour necrosis factor α; IL = interleukin; SAPS = simplified acute physiology score.
*Parameter not selected in the stepwise forward linear regression model.

Table 3 Mean (SD) cytokine levels of survivors and non-survivors for all patients and for patients with ARDS, severe pneumonia, and control

	Survivors	*Non-survivors*	*p value (mean difference, 95% CI)*
All patients			
TNF-α	50 (45)	53 (52)	0.834 (−2.4, −25.6 to 20.7)
IL-1β	25 (65)	21 (44)	0.770 (3.7, −21.2 to 28.5)
IL-6	468 (776)	791 (1163)	0.185 (−323.3, −766.5 to 120.0)
ARDS			
TNF-α	80 (51)	62 (59)	0.323 (18.5, −18.8 to 55.9)
IL-1β	51 (94)	28 (50)	0.270 (23.8, −19.1 to 66.7)
IL-6	666 (904)	731 (1125)	0.854 (−64.7, −770.5 to 641.1)
Severe pneumonia			
TNF-α	34 (25)	35 (15)	0.956 (−0.5, −19.4 to 18.4)
IL-1β	3 (4)	2 (4)	0.793 (0.5, −3.4 to 4.4)
IL-6	464 (823)	1210 (1371)	0.158 (−745.1, −1807.6 to 317.4)
Controls			
TNF-α	18 (10)	14 (3)	0.603 (3.2, −11.6 to 17.0)
IL-1β	5 (12)	8 (11)	0.786 (−2.2, −20.6 to 16.2)
IL-6	106 (132)	67 (28)	0.634 (39.3, −84.4 to 163.0)

ARDS = acute respiratory distress syndrome; TNF-α = tumour necrosis factor α; IL = interleukin.

group appeared to be associated with IL-6 levels when the extent of pulmonary infiltrates was included in the multivariate model. However, this was not the case in the analysis of the lung injury or SAPS II scores.

MORTALITY

Forty six of the 76 patients investigated (61%) died during their stay in the ICU. The mortality among patients with ARDS (33/46, 72%) was significantly higher than in the control group (3/10, 30%; mean difference 42%, 95% CI 10.8 to 73.2) but was not significantly different from that in patients with severe pneumonia (10/20, 50%; mean difference 22%, 95% CI –3.5 to 47.5; p = 0.158). Cytokine levels were not significantly different between survivors and non-survivors, nor were any differences seen when patients were analysed as the whole group or as the separate diagnostic subgroups (table 3).

Discussion

The results of this study show that serum concentrations of TNF-α are significantly higher in patients with ARDS than in those with pneumonia (p = 0.03) or controls (p = 0.007) and, in multivariate analysis, the serum concentrations of TNF-α and IL-1β were better approximated by the lung injury score than by the diagnostic group. Serum cytokine levels in survivors and non-survivors obtained during the first 24 hours of the disease were not significantly different.

Concentrations of systemic inflammatory cytokines reported in patients with ARDS have varied depending on the sample investigated, the timing of the sample collection, and the assay used. Some authors have described increased TNF-α concentrations in patients with ARDS[2 21 26] while others found no differences when compared with a group of patients at risk of developing ARDS.[3–6] In our study TNF-α concentrations were significantly higher in patients with ARDS than in those with pneumonia and even higher than in control subjects. TNF-α is mainly produced by monocytes and macrophages but may also be released by lymphocytes or mast cells. It triggers the release of other inflammatory mediators and stimulates recruitment of polymorphonuclear neutrophils from the vascular compartment.[27] The role of TNF-α in patients with ARDS is not fully understood. Persistently high levels of this cytokine caused by extrapulmonary sources of infection such as sepsis may, on the one hand, induce fever and increased capillary permeability in the lung which is part of the pathogenesis of ARDS. On the other hand, ARDS may be induced by direct lung injury such as aspiration and serum TNF-α levels would only reflect the systemic response to the initial local challenge.[21] However, in our study we did not find any differences between serum TNF-α concentrations in patients with direct or indirect lung injury. Although Gattinoni and co-workers recently established differences in pulmonary mechanics, the inflammatory response in the serum does not seem to be helpful for differentiating between patients with these two aetiologies of ARDS.[28]

In patients with pneumonia the lung is the site of the initial inflammatory response to respiratory pathogens and there is clear indication that TNF-α is a key mediator.[29–32] In contrast to ARDS there is evidence from animal studies that the inflammatory response in pneumonia, as measured by TNF-α release, is initially confined to the compartment of the lung and systemically circulating cytokines may not be present.[33] In subjects with unilateral pneumonia Dehoux and co-workers found higher TNF-α levels in the BAL fluid of the involved lung than in the non-involved lung.[34] However, with increasing severity circulating cytokines may also be present in the serum of patients with pneumonia. Detectable levels of TNF-α in serum have been reported in patients with community acquired or severe pneumonia.[14 35] In our study all patients with severe pneumonia had detectable TNF-α levels, thus confirming TNF-α as a severity marker in pneumonia.

In the multivariate analyses the lung injury score was the factor which agreed most closely with TNF-α serum levels. Two major factors may have contributed to these results. Firstly, in some cases a positive differential diagnosis between ARDS and severe pneumonia with bilateral pulmonary infiltrates cannot always be easily established. This problem has also been recognised by the American-European Con-

sensus Conference on ARDS which states that pulmonary infection may be considered to be ARDS if the physiological criteria are met.[15] Furthermore, direct lung injury due to pulmonary infection is a common aetiology of ARDS.[36] Although in this study every attempt was made to keep these two clinical entities apart, a diagnostic overlap cannot be entirely ruled out. Secondly, systemically circulating TNF-α might have little or no discriminating potential between ARDS and severe pneumonia. Schütte and co-workers also acknowledged this diagnostic overlap and analysed an intermediate group of patients with ARDS and signs of pulmonary infection separately from those with a clear aetiology.[14] However, even then they could not find any differences in systemic TNF-α release on the first day of the disease between patients with pneumonia and those with ARDS. In our study serum TNF-α concentrations were higher in patients with ARDS but the degree of lung injury was clearly a better predictor of TNF-α levels. This measurement includes the radiological extent of the infiltrates but also takes into account additional measurements such as the Pa_{O_2}/F_{IO_2} ratio, the level of positive end expiratory pressure (PEEP), and the pulmonary compliance.[20] This supports the view that the level of circulating TNF-α increases with the degree of lung injury, which can be similar in patients with ARDS and severe pneumonia, but it not does not help to clarify the aetiology of the lung injury.[22 37] TNF-α and IL-1β have similar effects on cellular functions and are usually classified together, according to their position in the inflammatory cascade, as proximal cytokines.[38] Their association with the severity markers tested in the multivariable analysis was found to be similar (table 2).

In this study interpretation of the serum IL-6 concentrations was difficult because the results showed a large variation and the multivariable testing yielded inconsistent results (table 2). IL-6 is located more distally in the inflammatory cascade (distal cytokine) and its main physiological effects differ from those of TNF-α and IL-1β. The systemic effects of IL-6 include induction of fever, production of acute phase proteins in the liver such as C reactive protein, and regulation of the immune response. This might explain, at least in part, why serum levels of IL-6, unlike those of TNF-α and IL-1β, were also associated with the SAPS II score.

Various attempts have been made to associate the cytokine concentrations with the prognosis but most studies have failed to establish a clear relationship between the two, at least during the early stages of the disease. In a very selected population of patients with ARDS Meduri and co-workers found significantly higher levels of cytokines in non-survivors during the early course of the disease but the prognostic value improved when the time course of the inflammatory response was taken into account.[21 39] Since cytokines were only measured in our study at the onset of the disease, this might explain why neither univariate nor multivariate analysis supported any prognostic value for serum cytokine levels.

Three possible limitations of this study should be addressed. Firstly, the use of corticosteroids is known to interfere with serum cytokine levels. Meduri and co-workers found that corticosteroid rescue treatment led to an improvement in the lung inflammatory response in patients with ARDS and, in particular, decreased levels of cytokines in plasma.[40] Although we recorded the prior use of intravenous corticosteroids, we did not investigate the actual and cumulative doses. Secondly, the prognostic properties of serum cytokine levels were not a primary or secondary objective of this study and the data provided should be interpreted with caution. Thirdly, cytokines are part of an inflammatory cascade and biological effects are difficult to interpret without appreciation of the entire network of the inflammatory response. However, since one objective of this study was to investigate the possible discriminatory potential of cytokines, we focused in this report on pro-inflammatory cytokines which may be present in most clinical settings.

In conclusion, we found increased levels of TNF-α in patients with ARDS compared with patients with severe pneumonia or intubated control patients without inflammatory lung disease. However, this reflects the extent of pulmonary inflammatory disease rather than a diagnostic entity. Serum levels of IL-1β also increased with the extent of lung injury but were not significantly different between the diagnostic groups.

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1 Millar AB, Foley NM, Singer M, *et al*. Tumour necrosis factor in bronchopulmonary secretions of patients with adult respiratory distress syndrome. *Lancet* 1989;ii:712–4.
2 Marks JD, Marks CB, Luce JM, *et al*. Plasma tumor necrosis factor in patients with septic shock: mortality rate, incidence of adult respiratory distress syndrome and effects of methylprednisolone administration. *Am Rev Respir Dis* 1990;**141**:94–7.
3 Hyers TM, Tricomi SM, Dettenmeier PA, *et al*. Tumor necrosis factor levels in serum and bronchoalveolar lavage fluid of patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 1991;**144**:268–71.
4 Roten R, Markert M, Feihl F, *et al*. Plasma levels of tumor necrosis factor in the adult respiratory distress syndrome. *Am Rev Respir Dis* 1991;**143**:590–2.
5 Parsons PE, Moore FA, Moore EE, *et al*. Studies on the role of tumor necrosis factor in adult respiratory distress syndrome. *Am Rev Respir Dis* 1992;**146**:694–700.
6 Suter PM, Suter S, Giradin E, *et al*. High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, and elastase, in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. *Am Rev Respir Dis* 1992;**145**:1016–22.
7 Donnelly SC, Haslett C, Dransfield I, *et al*. Role of selectins in development of adult respiratory distress syndrome. *Lancet* 1994;**344**:215–9.
8 Goodman RB, Strieter RM, Martin DP, *et al*. Inflammatory cytokines in patients with persistence of the acute

respiratory distress syndrome. *Am J Respir Crit Care Med* 1996;**154**:602–11.
9 Armstrong L, Millar AB. Relative production of tumour necrosis factor alpha and IL-10 in adult respiratory distress syndrome. *Thorax* 1997;**52**:442–6.
10 Headley AS, Tolley E, Meduri GU. Infections and the inflammatory response in acute respiratory distress syndrome. *Chest* 1997;**111**:1306–21.
11 Kiehl MG, Ostermann H, Thomas M, *et al*. Inflammatory mediators in bronchoalveolar lavage fluid and plasma in leukocytopenic patients with septic shock-induced acute respiratory distress syndrome. *Crit Care Med* 1998;**26**: 1194–9.
12 Pugin J, Verghese G, Widmer MC, *et al*. The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. *Crit Care Med* 1999;**27**:304–12.
13 Montón C, Torres A, El-Ebiary M, *et al*. Cytokine expression in severe pneumonia: a bronchoalveolar lavage study. *Crit Care Med* 1999 (in press).
14 Schütte H, Lohmeyer J, Rosseau S, *et al*. Bronchoalveolar and systemic cytokine profiles in patients with ARDS, severe pneumonia and cardiogenic pulmonary oedema. *Eur Respir J* 1996;**9**:1858–67.
15 Bernard MS, Artigas A, Brigham KL, *et al*. Report on the American-European Consensus Conference on acute respiratory distress syndrome: definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994;**149**:818–24.
16 El-Ebiary M, Torres A, González J, *et al*. Quantitative cultures of endotracheal aspirates for the diagnosis of ventilator-associated pneumonia. *Am J Respir Crit Care Med* 1993;**147**:1552–7.
17 Torres A, El-Ebiary M, Padro L, *et al*. Validation of different techniques for the diagnosis of ventilator-associated pneumonia: comparison with immediate postmortem pulmonary biopsy. *Am J Respir Crit Care Med* 1994;**149**:324–31.
18 Chastre J, Fagon JY, Bornet-Lesco M, *et al*. Evaluation of bronchoscopic techniques for the diagnosis of nosocomial pneumonia. *Am J Respir Crit Care Med* 1995;**152**:231–40.
19 Seidenfeld JJ, Pohl DF, Bell RC, *et al*. Incidence, site, and outcome of infections in patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 1986;**134**:12–6.
20 Murray JF, Matthay MA, Luce JM, *et al*. An expanded definition of the adult respiratory distress syndrome. *Am Rev Respir Dis* 1988;**138**:720–3.
21 Meduri GU, Headley S, Kohler G, *et al*. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. *Chest* 1995;**107**:1062–73.
22 American Thoracic Society. Hospital-acquired pneumonia in adults: Diagnosis, assessment, initial therapy, and prevention: a consensus statement. *Am J Respir Crit Care Med* 1996;**153**:1711–25.
23 Bone RC, Balk RA, Cerra FB, *et al*. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/ Society of Critical Care Medicine. *Chest* 1992;**101**:1481–3.
24 Le Gall JR, Lemeshow S, Saulnier F. A new simplified acute physiology score (SAPS II) based on a European/North American multicenter study. *JAMA* 1993;**270**:2957–63.
25 Wimberley L, Falling LJ, Bartlett JG. A fiberoptic bronchoscopy technique to obtain uncontaminated lower airway secretions for bacterial culture. *Am Rev Respir Dis* 1979;**119**:337–43.
26 Calandra T, Baumgartner JD, Grau GE, *et al*. Prognostic values of tumor necrosis factor/cachectin, interleukin-1, interferon-α and interferon-γ in the serum of patients with septic shock. *J Infect Dis* 1990;**161**:982–7.
27 27. Kelley J. Cytokines of the lung. *Am Rev Respir Dis* 1990; **141**:765–788.
28 Gattinoni L, Pelosi P, Suter PM, *et al*. Acute respiratory distress syndrome caused by pulmonary and extrapulmonary disease. *Am J Respir Crit Care Med* 1998;**158**:3–11.
29 Nelson S, Mason CM, Kolls J, *et al*. Pathophysiology of pneumonia. *Clin Chest Med* 1995;**16**:1–12.
30 Nelson S, Noel P, Bokulic R, *et al*. Murine recombinant tumor necrosis factor enhances pulmonary host defense against *Staphylococcus aureus*. *Am Rev Respir Dis* 1989;**139**: A357.
31 Kolls JK, Lei D, Nelson S, *et al*. Exacerbation of murine *P carinii* infection by adenoviral-mediated gene transfer of a TNF soluble receptor. *Am J Respir Crit Care Med* 1995;**151**:A13.
32 Maus U, Rosseau S, Knies U, *et al*. Expression of pro-inflammatory cytokines by flow-sorted macrophages in severe pneumonia. *Eur Respir J* 1998;**11**:534–41.
33 Fox-Dewhurst R, Alberts M, Kajikawa O, *et al*. Pulmonary and systemic inflammatory responses in rabbits with Gram-negative pneumonia. *Am J Respir Crit Care Med* 1997;**155**:2030–40.
34 Dehoux MS, Boutten A, Ostinelli J, *et al*. Compartmentalized cytokine production within the human lung in unilateral pneumonia. *Am J Respir Crit Care Med* 1994;**150**:710–6.
35 Moussa K, Michie HJ, Cree IA, *et al*. Phagocyte function and cytokine production in community-acquired pneumonia. *Thorax* 1994;**49**:107–11.
36 Sloane PJ, Gee MH, Gotlieb JE. A multicenter registry of patients with acute respiratory distress syndrome. *Am Rev Respir Dis* 1992;**146**:419–26.
37 Ewig S, Ruiz M, Mensa J, *et al*. Severe community-acquired pneumonia: assessment of severity criteria. *Am J Respir Crit Care Med* 1998;**158**:1102–8.
38 Rinaldo JE, Christman JW. Acute respiratory distress syndrome: pathogenesis. In: Fishman AP, Elias JA, Fishman JA, *et al*, eds. *Fishman's pulmonary diseases and disorders*. 3rd ed. New York: McGraw-Hill, 1998: 2537–48.
39 Meduri GU, Kohler G, Headley S, *et al*. Inflammatory cytokines in the BAL of patients with ARDS: persistent elevation over time predicts poor outcome. *Chest* 1995;**108**: 1303–14.
40 Meduri GU, Headley S, Tolley E, *et al*. Plasma and BAL cytokine response to corticosteroid rescue treatment in late ARDS. *Chest* 1995;**108**:1315–25.