J. A. Sturm (Ed.)

Adult Respiratory Distress Syndrome

An Aspect of Multiple Organ Failure
Results of a Prospective Clinical Study

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Specific Proteins of Inflammatory Cells and α_1-Proteinase Inhibitor in Alveolar Epithelial Lining Fluid of Polytraumatized Patients: Do They Indicate Posttraumatic Lung Failure?

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Introduction

Despite numerous hints that activated inflammatory cells may trigger lung injury, the causative role especially of the polymorphonuclear (PMN) granulocytes (neutrophils) to promote enhanced microvascular permeability in acute respiratory distress syndrome (ARDS) is still a matter of debate [22, 25, 27, 28, 31]. In the early 1980s, Hammerschmidt et al. [11] demonstrated that complement-mediated neutrophil sequestration in pulmonary capillaries leading to lung vascular endothelial injury was a common feature of patients suffering from acute respiratory distress syndrome (ARDS). Meanwhile, however, a wealth of basic information exists indicating that neutrophil activation occurs to a similar extent in high-risk patients who do not, however, eventually develop ARDS [21]. Moreover, signs of ARDS have been described in neutropenic patients, thus calling into question whether neutrophils are required in the generation of ARDS [19]. Yet, the latter observations may be taken as an indirect evidence for the importance of other inflammatory cells, e.g., activated alveolar macrophages [7], in disturbing the alveolar barrier from the epithelial side of the alveoli, at least in neutropenic patients.

Although the primary role of both types of phagocytes (invading neutrophils, sessile lung macrophages) in the development of ARDS still deserves further clarification, the potentially harmful armament of these cells released during cell activation in response to an inflammatory event (e.g., multiple trauma) is beyond doubt. This potent cellular equipment for causing tissue injury in the microenvironment of the phagocytes include elastolytic proteinases (e.g., neutrophil elastase, macrophage-derived cathepsin B) and

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highly destructive oxygen species [7, 18]. The production of the most effective molecules of the latter – hypochlorous acid, chloramines and hydroxyl radicals – is greatly enhanced during phagocytosis by the catalytic action of the neutrophil lysosomal proteins, myeloperoxidase [18] and lactoferrin [17]. Moreover, extracellularly released lactoferrin is known to increase the adhesiveness of neutrophils to pulmonary endothelial cells, thereby prolonging the destructive action of the phagocytes on the vascular permeability barrier [30].

The harmful efficacy of proteolytic and oxidative substances discharged to the extracellular milieu is normally restricted to and abolished in the close vicinity of phagocytizing cells by numerous proteinase inhibitors [29] and antioxidants [20]. During an overshooting activation process, e.g., caused by a serious trauma event, the local anti-oxidative capacity seems to be overcome, especially in the lung, thereby enabling also the oxidative inactivation of proteinase inhibitors [29]. In this respect α_1-proteinase inhibitor (α_1PI), by far the most effective inhibitor of the neutrophil serine proteinase elastase, is highly sensitive to oxidative destruction [29, 18]. In addition, this inhibitor molecule can easily be degraded by cysteine proteinases (cathepsin B, L) and a metalloproteinase released from activated alveolar macrophages [29]. Just recently, the proteolytic inactivation of α_1PI by a neutrophil metalloproteinase has been also demonstrated [8]. Interestingly, the α_1PI, which is probably degraded proteolytically in the epithelial lining fluid (ELF) of the lung, is inactive against elastase [26, 32], but has proved to be a potent neutrophil chemoattractant [4] and may thus augment the elastase burden of the lung during an inflammatory process.

The increase of the lung's permeability due to the proteolytic/oxidative destruction of the endothelial–epithelial barrier in the early phase of ARDS is characterized by the influx of plasma proteins into the ELF [12]. Whereas this stage of the disease may be reversible, the later fibrotic phase [3] is often associated with a lethal outcome due to an irreversible gas exchange disturbance. As shown recently, activated lung macrophages seem to play an essential role in stimulating fibroblasts to enhanced collagen synthesis via the production of a platelet-derived growth factor-like substance [5]. In addition, the diminished regulatory function of destroyed alveolar epithelial cells also promotes fibroblast activity and, thus, fibrosis [1]. The extracellular occurrence of the procollagen-III-peptide (P-III-P) in the ELF can be taken as a clear indication of such an intensified fibrotic process [6].

Up to now, the evaluation of biochemical changes in the ELF drawn by the broncho-alveolar lavage (BAL) technique [24] has been widely applied to gain more insight into the pathomechanisms taking place during lower respiratory tract inflammation [10, 13, 25, 26, 31, 32]. However, convincing clarification of the significance of the different biochemical factors under discussion as inflammatory mediators has been hampered by the lack of close-meshed follow-up determinations after an event (multiple injuries, septicemia, etc.) which suddenly renders a person a high-risk ARDS patient. Therefore, the aims of our present study were as follows:

- Firstly, to find out whether daily determinations of specific proteins released from inflammatory cells into the ELF of severely injured patients can contribute to elucidate the importance of these cellular products for the development of posttraumatic ARDS.
- Secondly, to examine whether one or more of these biochemical inflammation markers can predict the onset as well as the severity of a posttraumatic ARDS course.

To achieve these aims, three neutrophil lysosomal proteins — elastase, myeloperoxidase, and lactoferrin — as well as the macrophage-derived proteinase cathepsin B, the fibroblast product P-III-P, and the proteinase inhibitor α_1PI were determined in BAL fluid samples drawn daily from multiply injured patients over a generally 14 days posttraumatic observation period.

Methods

Study Population

Multiply injured patients ($n = 27$; Injury Severity Score >40 points) from two surgical centers (17 subjects from Essen, 10 from Hannover) were included in this study with a prospectively fixed observation period of 14 days. The persistent rise of extravascular lung water (EVLW) to above 10 ml/kg body weight about 3–4 days after admission was taken as a reliable sign of an increase in pulmonary microvascular permeability. According to this feature, 12 patients had to be classified as "ARDS" subjects, the other 15 as a "non-ARDS" group [16]. Two of the ARDS patients died within 8 days, and three individuals 12 days post trauma, whereas the three nonsurviving patients in the non-ARDS collective died on the 9th and 12th and after the 14th post-traumatic day, respectively.

In addition, ten healthy subjects served as controls for comparative measurements of the test parameters.

Sampling Procedure

Bronchoalveolar Lavage Fluid (BALF). To obtain ELF from the alveoli, BAL was performed by instilling 100 ml 0.9% saline in 20-ml aliquots via a fiberoptic bronchoscope as described previously [15, 16]. In about half of the traumatized patients, this procedure was carried out daily, whereas due to logistic conditions in the other injured patients, performance of BAL was feasible only every 2nd day up to the patient's death or the end of the 14-day observation period. The healthy individuals were subjected to one BAL each on three successive days [15].

After withdrawal (recovery of more than 60% of the instilled saline), cellular components and fluid phase of the combined lavage suspensions were immediately separated by centrifugation [15, 16]. The unconcentrated BALF (on average containing more than 60-fold diluted ELF) was then kept frozen at $-70°C$ until determination of specific proteins and urea.

Citrated Plasma. At each performance of BAL, venous blood was drawn simultaneously from a central line, processed to citrated plasma and deep-frozen at $-70°C$ until selected plasma components had been assayed.

Protein Determinations

The neutrophil lysosomal proteins elastase (complexed with $\alpha_1 PI$), myelo-peroxidase, and lactoferrin in BALF and plasma samples were determined by specific enzyme-linked immunoassays (established in the Biochemical Department of E. Merck, Darmstadt, FRG) as indicated elsewhere [14].

To quantify proteolytically active neutrophil elastase in BALF, one part of each BALF sample was incubated with normal plasma containing enough $\alpha_1 PI$ for total elastase inhibition and then reassayed for an increase in the elastase–$\alpha_1 PI$ complex. After subtracting the amount of the complex added with the normal plasma and correcting for the dilution effect, a rise of more than 10% in elastase–$\alpha_1 PI$ complex over the complex concentration already present in the untreated portion of the same BALF sample was taken as reliable proof for the occurrence of proteolytically active elastase in the alveolar epithelial environment.

The macrophage-derived lysosomal cysteine proteinase cathepsin B was quantified only in pilot measurements as described by Assfalg-Machleidt et al. [2] by estimating the enzyme-catalyzed cleavage of a convenient fluorogenic substrate.

Determination of the fibroblast product P-III-P was carried out with a commercially available radioimmunoassay (RIA-gnost Prokollagen-III-Peptid, Behringwerke, Marburg, FRG).

The immunologically detectable amount of $\alpha_1 PI$ was measured using a nephelometric method as described by Dwenger et al. [9].

Urea Measurement and Correction of the Dilution Effect in BALF

Urea concentrations in corresponding BALF and plasma samples were enzymatically determined as described elsewhere [9]. These measurements were performed to correct the concentrations of the protein components quantified in BALF samples to their original amount in the ELF. Since it is suspected that urea diffuses without restriction between the blood/air compartments of the lung, the ratio of the urea concentration between blood and BALF is supposed to indicate the dilution of ELF by saline. According to

Rennard et al. [23] the following calculation was applied to evaluate concentrations (C) of various proteins in ELF:

$$C_{\text{protein in ELF}} = \frac{C_{\text{protein in BALF}} \times C_{\text{urea in plasma}}}{C_{\text{urea in BALF}}}$$

Diagrams

Data are presented as follow-up curves, either for an individual subject or as mean ± SEM for the two study groups (ARDS; non-ARDS) if not otherwise stated.

Statistical analysis was performed using the Mann-Whitney U-test.

Results

BALF Data of Healthy Subjects

BAL repeated daily in healthy subjects triggered a distinct influx of PMN granulocytes (mean number of cells/ml BALF was 5700 in the first lavage and 27600 in the third lavage) to the alveolar space and a significant release of lysosomal proteins, which indicated local neutrophil activation. This was confirmed not only by the increase in extracellular complexed elastase from an average concentration of 0.77 µg/ml ELF (first BAL) (range: 0.37–1.0) up to 4.2 µg/ml ELF (third BAL) (range: 2.2–6.9), but also by the rise in the specific amount of the elastase complex from 4.8 ng up to 10.4 ng/1000 PMN cells. Yet, no proteolytically active elastase could be demonstrated in any of these BALF samples. Remarkably, the concentration of complexed elastase in the ELF of healthy subjects was approximately tenfold that of normal plasma levels (80 ng/ml)

The lysosomal neutrophil proteins, myeloperoxidase and lactoferrin, were discharged in a similar manner, although in a somewhat lower amount in the case of myeloperoxidase (up to 4.2 µg/ml ELF in the third BAL) and a clearly higher amount in the case of lactoferrin (up to 17.2 µg/ml ELF in the third BAL) than elastase. Since the release of such quantities of neutrophil proteins did not entail any pathological pulmonary disturbances (e.g., influx of blood proteins) in the volunteers, an extracellular concentration of 7 µg complexed elastase or myeloperoxidase as well as 20 µg lactoferrin/ml ELF might be suggested as the upper physiologically tolerable limit.

The average immunologically determined concentration of 200 µg/ml ELF of the main elastase inhibitor, α_1PI, corresponds to about 10% of the plasma level. This level did not change during repeated BAL procedures, thus indicating the absence of an increase in pulmonary permeability. Interestingly, this inhibitor amount equals maximally to only about a 140-fold molar excess over the extracellular concentration of the elastase measurable

in the ELF under physiological conditions, whereas in the plasma of healthy subjects, it is more than 10 000-fold.

With the assay system applied (lower detection limit: 1 ng/ml) no P-III-P could be demonstrated in the BALF samples of the healthy volunteers.

Due to logistic conditions, BALF samples had not been available for the evaluation of normal values of the macrophage proteinase cathepsin B in the ELF.

BALF Data of Traumatized Patients

As shown in Fig. 1, a repeated release of elastase (measured as the total amount of complexed elastase after the addition of inhibitorily active α_1PI) up to more than 60-fold normal values could be seen in all patients during the whole observation period, with a tendency to larger amounts on days 4–6 post trauma. This period was generally associated with the manifestation of the increase in the amount of EVLW in the ARDS group. Surprisingly, however, the neutrophil proteinase was discharged to an even higher, though not statistically different, degree in the non-ARDS patients. The same held
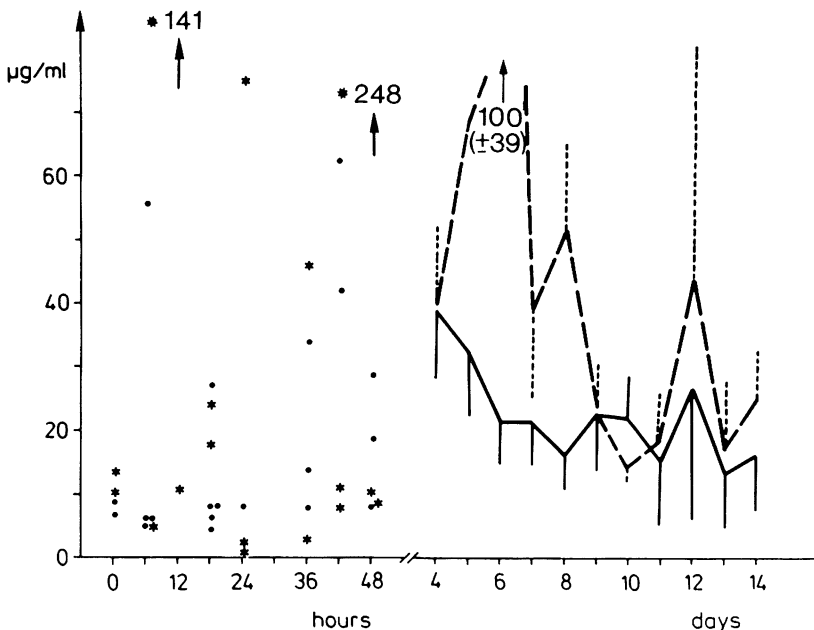
Fig. 1. Posttraumatic course of the overall released neutrophil elastase (E), measured as E–α_1PI complex after the addition of inhibitorily active α_1-proteinase inhibitor (α_1PI) to the alveolar epithelial lining fluids (ELF) of ARDS (*solid line*; *asterisk*; $n \leqslant 12$) and non-ARDS (*broken line*; *circle*; $n \leqslant 15$) patients. Data are given as individual measurements in the early, and as mean ± SEM in the later posttraumatic phase

Fig. 2. Posttraumatic course of neutrophil lactoferrin in the alveolar epithelial lining fluids (ELF) of ARDS (*solid line; asterisk; n ≤ 7*) and non-ARDS (*broken line; circle; n ≤ 10*) patients. Data are given as individual measurements in the early, and as mean ± SEM in the later posttraumatic phase

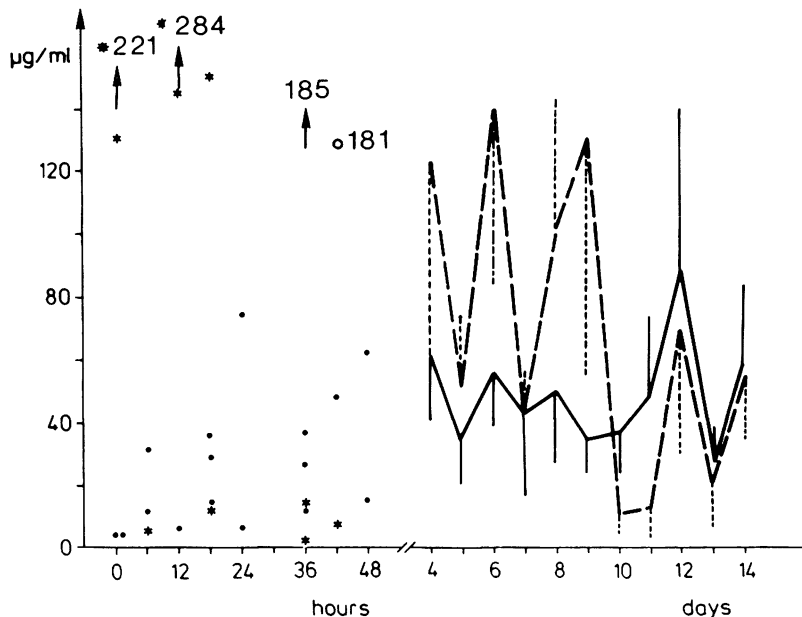true for myeloperoxidase (not depicted here) and lactoferrin (Fig. 2). The simultaneous release of all three lysosomal proteins, especially during the manifest elevation of EVLW, is exemplarily shown in Fig. 3. The data are from a patient who finally overcame the ARDS phase.

Sequential mean α_1PI concentrations were distinctly more elevated above normal in the ELF of the ARDS collective (on an average between 600 and 1200 µg/ml) than in the non-ARDS group who generally showed a 1.5– to 3–fold (300–600 µg/ml) increase (Fig. 4). Despite scarce data available for the first 48 h post trauma, α_1PI concentrations well above 300 µg/ml ELF in 6 of 12 ARDS patients and 2 of 15 non-ARDS patients indicate a very early trauma-induced pulmonary capillary permeability leakage which was clearly more pronounced in the ARDS group. This is confirmed by the observation that during the early posttraumatic phase, α_1PI in the ELF of ARDS patients reached concentrations which were already as high as in the later course of the disease. In contrast, the plasma levels of α_1PI were clearly lower at the beginning of the observation period (below 2000 µg/ml) and increased up to fivefold (to approximately 6000 µg/ml) within 6 days after the traumatic event (Fig. 5; in the graphs, the plasma α_1PI levels of traumatized patients not subjected to BAL for clinical reasons are also included). Assuming that with an intact blood/air barrier the influx of α_1PI to the
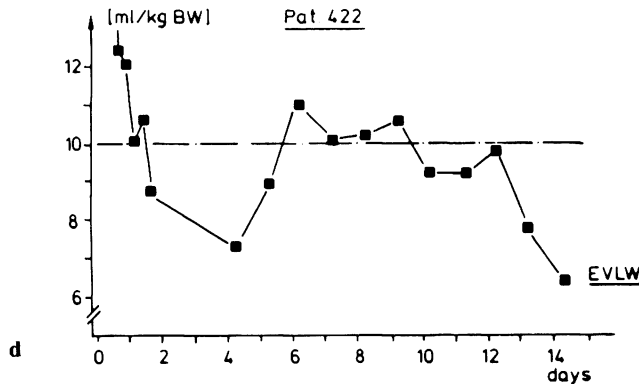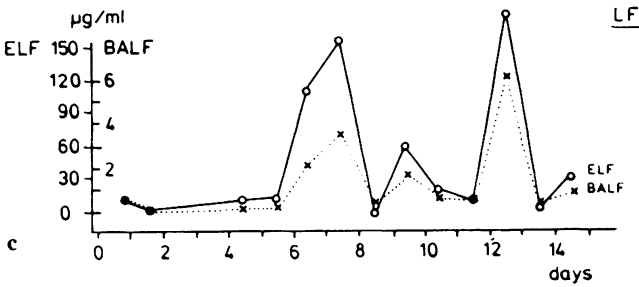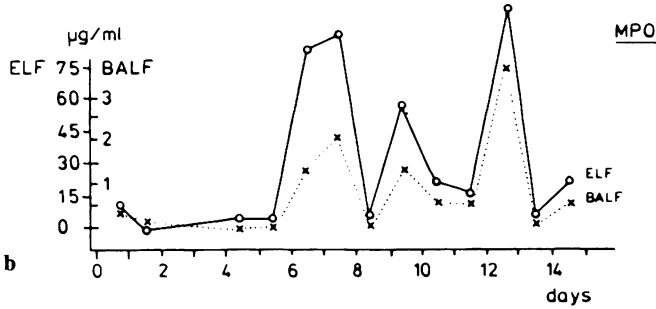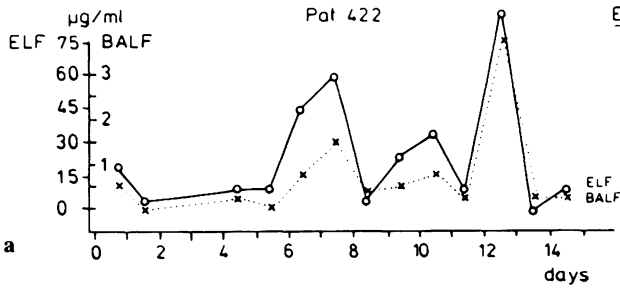
Fig. 3a–d.
Posttraumatic courses of neutrophil **a** complexed elastase (E-α_1PI), **b** myeloperoxidase (MPO), and **c** lactoferrin (LF) in the alveolar epithelial lining fluids (ELF; *solid line*) and the bronchoalveolar lavage fluids (BALF; *broken line*) of an ARDS patient. **d** ARDS is indicated by the manifest elevation in extravascular lung water (EVLW) above 10 ml/kg body weight between the fifth and ninth posttraumatic day
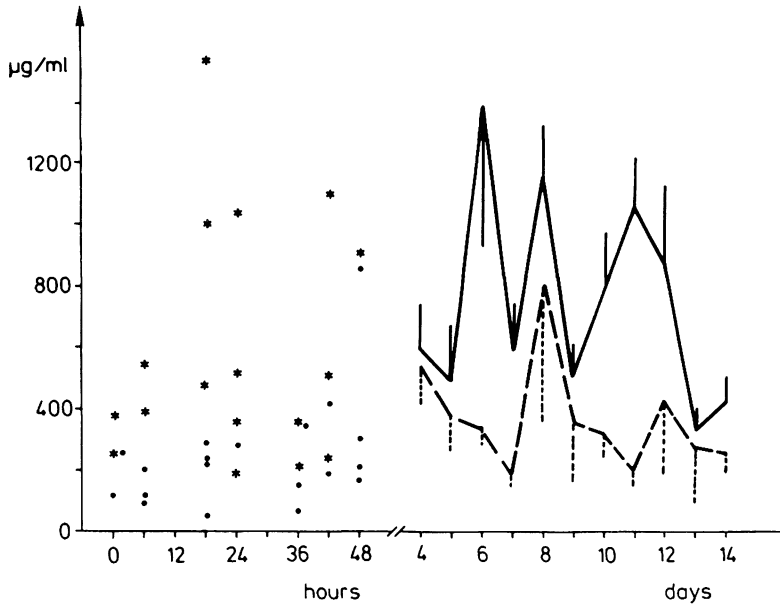
Fig. 4. Posttraumatic course of α_1-proteinase inhibitor (α_1PI) in the alveolar epithelial lining fluids (ELF) of ARDS (*solid line; asterisk; $n \leq 12$*) and non-ARDS (*broken line; circle; $n \leq 15$*) patients. Data are given as individual measurements in the early, and as mean ± SEM in the later posttraumatic phase.
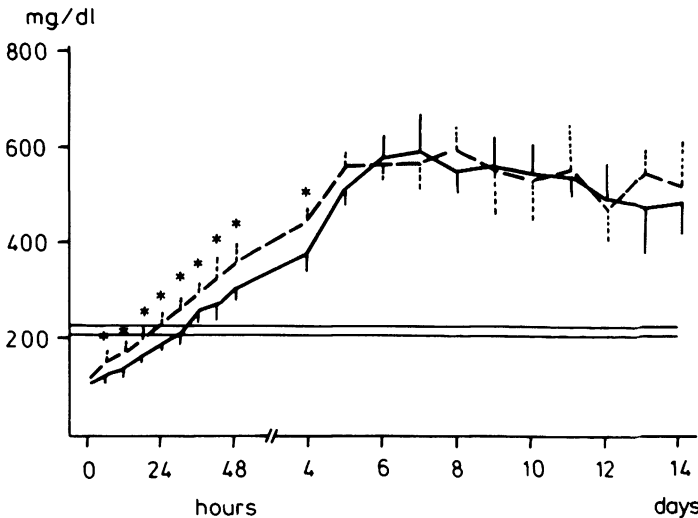

Fig. 5. Posttraumatic course of α_1-proteinase inhibitor (α_1PI) in plasma samples of ARDS (*solid line; $n \leq 26$*) and non-ARDS (*broken line; $n \leq 31$*) patients. Data (including those of patients not subjected to bronchoalveolar lavage for clinical reasons) are given as mean ± pseudostandard error (PSE). *Asterisks* mark significant differences between the two patient groups ($p < 0.05$)

alveolar epithelial compartment approaches at most 10% of the blood level, an early and/or late pulmonary permeability increase could be seen in 11 of the 12 ARDS patients, whereas such a disturbance could be verified only in 3 of the 15 non-ARDS subjects.

Taking 300 µg α_1PI/ml ELF – if reached within 48 h post trauma – as a threshold level for the prognosis of later ARDS, the following predictive parameters were calculated: 63% for sensivity, 71% for specificity, 80% for the positive predictive value, and 58% for the negative predictive value.

Although the immunologically measurable α_1PI concentration was highly elevated in the ELF of ARDS patients, the molar excess of α_1PI over the total amount of released elastase varied generally in the later posttraumatic phase only between 8.6- to 39.6-fold (Fig. 6) compared with about 140-fold under normal conditions. This reduced ratio does not seem to be enough to inactivate the whole amount of elastase discharged from the stimulated invading neutrophils. As shown in Fig. 7a, up to 30% of the released elastase could be detected as an active enzyme in the ARDS group. Moreover, due to the lower amount of α_1PI in the ELF of non-ARDS subjects and the clearly
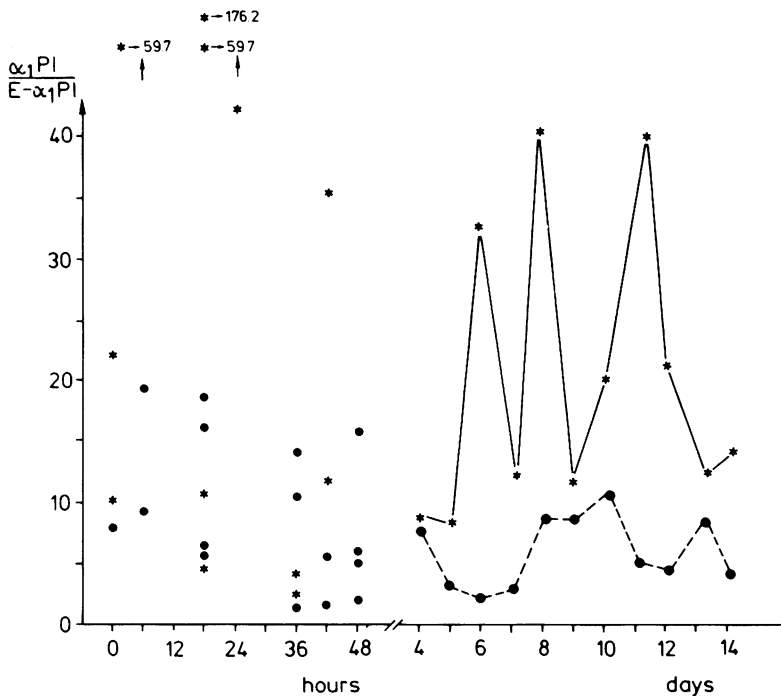
Fig. 6. Posttraumatic course of the calculated molar ratio of α_1-proteinase inhibitor to the overall released neutrophil elastase (see Fig. 1) in alveolar epithelial lining fluids (ELF) of 12 ARDS (*solid line; asterisk*) and 15 non-ARDS (*broken line; circle*) patients. In the ELF of healthy subjects this ratio is approximately 140-fold. Data are given as individual ratios in the early, and as ratio of mean values in the later posttraumatic phase
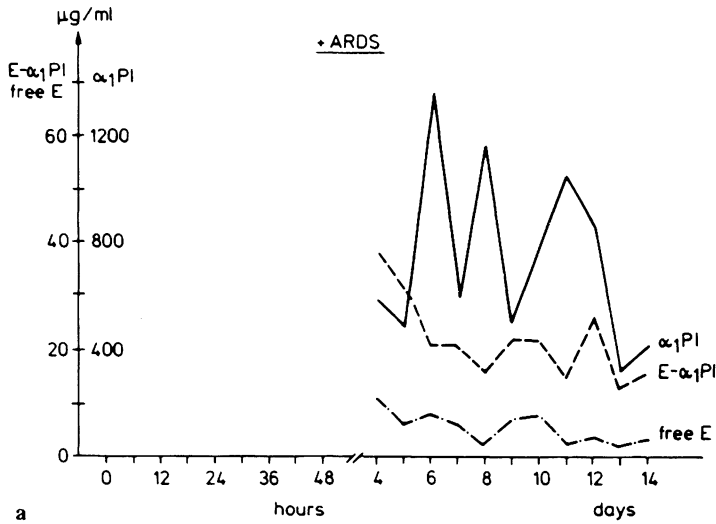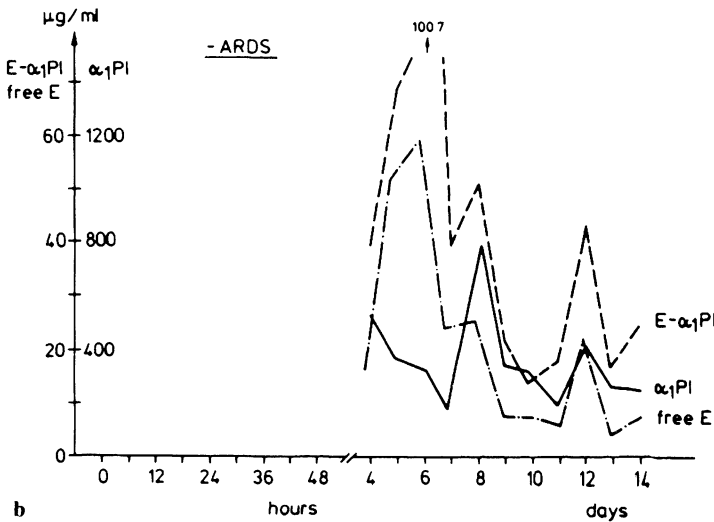
Fig. 7a,b. Posttraumatic courses of overall released neutrophil elastase (E-α₁PI; see Fig. 1.), proteolytically active elastase (*free E*) and α₁-proteinase inhibitor (α₁PI) in the alveolar epithelial lining fluids (ELF). Data are given as means for **a** 12 ARDS patients and **b** 15 non-ARDS patients in the later posttraumatic phase

higher release rate of elastase especially in the later posttraumatic phase, the molar surplus of α_1PI in these subjects was only 1.9- to 12.8-fold (Fig. 6). This might be responsible for the observation that, on average, more than 50% of the extracellular neutrophil proteinases were not inhibited by the locally available epithelial α_1PI (Fig. 7b) in the non-ARDS collective.
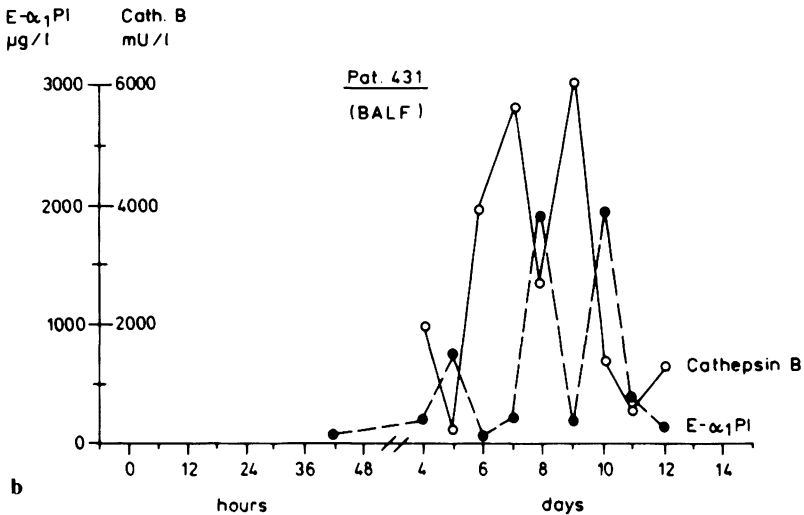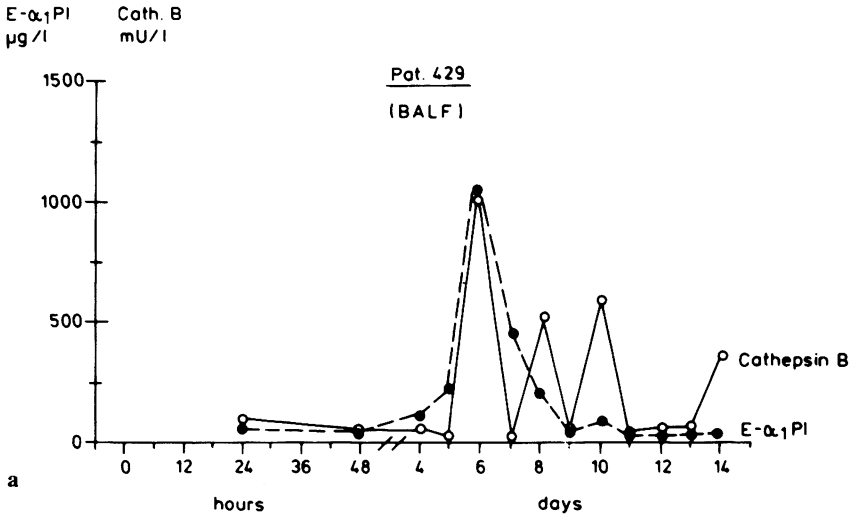
Fig. 8a,b. Individual posttraumatic courses of overall released neutrophil elastase measured as E-α₁PI complex and macrophage-derived cathepsin B in the bronchoalveolar lavage fluids (BALF) of individual patients. **a** Pat. 429 without ARDS, and **b** Pat. 431 with ARDS

Due to logistic conditions, the release of the alveolar macrophage-derived cysteine proteinase cathepsin B could be determined only in pilot measurements. Fig. 8 depicts the discharge pattern of cathepsin B compared with that of neutrophil elastase in the BALF of a non-ARDS (Pat. 429) and an ARDS subject (Pat. 431), respectively. Both proteinases showed a re-
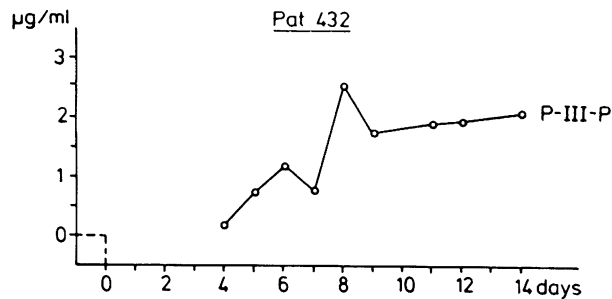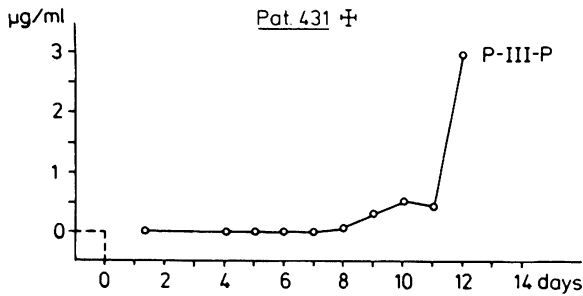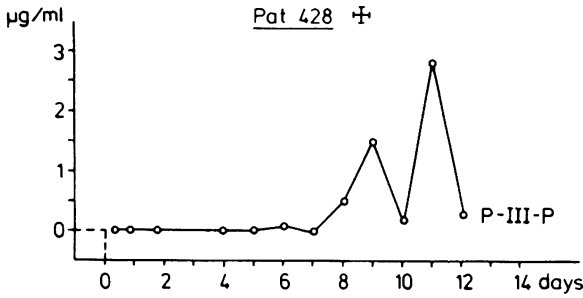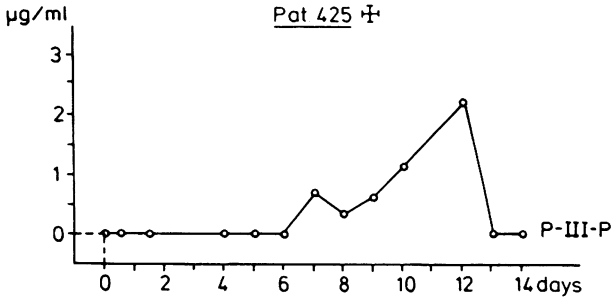
Fig 9. Individual posttraumatic courses of the procollagen-III-peptide (P-III-P) in the alveolar epithelial lining fluids (ELF) of four ARDS patients. Three patients (+) did not survive the organ failure

peated, though not congruent, release which indicates more or less permanent posttraumatic activation of the phagocytic cells. In contrast to the above-given elastase data (combined in mean values for each study group), the diagrams of the individual patient's data point to a distinctly lower stimulation of both types of phagocytes in the non-ARDS subject.

For measuring the fibrosis marker P-III-P, sequential BALF samples had been available only from a subgroup of the study population (6 ARDS and 7 non-ARDS subjects from Essen). Up to the 4th posttraumatic day, P-III-P was not elevated above the lower detection limit (1 ng/ml) of the applied assay in any of the BALF specimens. Between the 4th and 9th posttraumatic days, P-III-P started to increase in all ARDS patients reaching maximum values of 2.2–4.9 µg/ml ELF. Because of the small number of and great individual variation in the data, it is not feasible to present these as mean values. Therefore, four separate diagrams are depicted in Fig. 9. In contrast to the high and longer lasting (more than 5 days) elevation of P-III-P in all ARDS subjects, in 4 of the 7 non-ARDS patients, no P-III-P could be detected in any of the BALF samples drawn daily during the whole observation period. The other three subjects exhibited only a short-term (2–3 days) slight P-III-P increase (maximum values: 0.2–0.6 µg/ml ELF) in the late posttraumatic phase. Although P-III-P levels above 1 µg/mg ELF were always associated with ARDS, they did not predict the outcome of the disease.

Discussion

In the present study, we intended to contribute to the understanding of the ambiguous role of PMN granulocytes in the pathogenesis of ARDS by measuring extracellularly released neutrophil proteins in BALF samples drawn daily from polytraumatized high-risk patients. The conversion of the protein concentrations quantified in BALF to the actual amounts present in alveolar ELF by using the plasma/BALF concentration ratio of urea [23] was expected to allow a sufficiently exact follow-up observation of the locally discharged neutrophil products.

Applying this method, in healthy volunteers the amount of neutrophil lysosomal constituents, elastase, myeloperoxidase, and lactoferrin, which could be determined in ELF, was five- to tenfold the amount found in plasma levels measured simultaneously. This may be taken as a clear hint of considerable, intensive, local activation of PMN granulocytes, which occurs even under physiological conditions. Although repeated BAL was associated with an increased alveolar influx and stimulation of neutrophils, the quantity of proteolytically and oxidatively active cell products released thereby did not apparently disturb the blood/air barrier. Likewise, the amount of $\alpha_1 PI$, though only available in a 140-fold molar surplus over elastase as compared to a more than 10 000-fold excess in plasma, seemed to be enough to achieve

complete inactivation of its discharged target enzyme. From the given results we may conclude that concentrations up to 7 µg complexed elastase/ml ELF are without pathophysiological relevance.

Moreover, taking the rise in EVLW as an unequivocal sign of ARDS [16], it became obvious in our study that even neutrophil movement into the airspaces of patients at high risk did not necessarily cause an increase in alveolar capillary membrane permeability. Although these results are in agreement with those reported by others [21], some paradoxical phe- nomenons shown here deserve further explanation.

As outlined, the amounts of neutrophil lysosomal proteins repeatedly discharged into the ELF of non-ARDS patients were distinctly higher than those in ARDS subjects, whereas in the latter cases the local plasma-derived $\alpha_1 PI$ was clearly elevated, especially in the early posttraumatic phase. Considering the recent findings of Dwenger et al. and Zilow et al. (see this volume), this discrepancy may be explained as follows. The severe traumatic event triggered the influx of neutrophils to the alveolar environment of all patients at risk to develop ARDS. During adhesion to the endothelial layer and the subsequent passage through the alveolar capillary barrier, the cells of patients who eventually developed ARDS could have been considerably more activated, probably by the neutrophil-stimulating complement compo- nents, C3a and C5a, which were present in clearly augmented amounts in the circulation of such patients.

Thereby, the aggressive neutrophil substances, which were also released in higher quantities into the circulation, may have contributed to the more or less simultaneous destruction of the pulmonary endothelial and epithelial cell layer, giving rise to increased permeability not necessarily associated with an elevation in EVLW. This early disturbance of the blood/air barrier is, however, reflected by the significantly higher ELF concentrations of plasma-derived $\alpha_1 PI$ found in ARDS patients than those found in non-ARDS patients and healthy volunteers.

Since preactivated neutrophils, which had invaded the epithelial compart- ment, had evidently exhausted their lysosomal protein equipment, they may have responded to supplementary local stimuli (bacteria, cell debris, complement split products, etc.) only with a diminished release of cellular constituents. In addition, in the later posttraumatic course a growing portion of immature granulocytes with a distinctly lower content of lysosomal proteins appeared in the circulation of ARDS patients and was stimulated to pass the alveolar capillary barrier. This altogether may have been responsible for the generally lower amount of neutrophil proteins present in ELF of ARDS than in that of non-ARDS subjects.

In the later course of ARDS (from the 4th posttraumatic day onwards) the epithelial wall seemed to be sealed again by fibrotic processes, as is partly demonstrated by the appearance of the fibroblast product P-III-P in ELF specimens, whereas the endothelial layer continued to be progressively destroyed. This pathological situation was now associated with a manifest

increase in EVLW in the ARDS patients. Despite reparative processes on the epithelial side of the alveoli, the repeated influx of plasma-derived $\alpha_1 PI$ well above the regular 10% ELF/plasma ratio of this protein can be taken as a further indication that the blood/air barrier was still disturbed.

In contrast, in non-ARDS subjects a similar permanent pulmonary impairment could not be verified, neither by a persistent influx of $\alpha_1 PI$ nor by the rise in EVLW. Here, an early transitory inflow of $\alpha_1 PI$ into the alveolar space distinctly exceeding the physiological 10% ELF/plasma ratio was followed later by a two- to threefold elevation in ELF-$\alpha_1 PI$, which can be explained by the simultaneously rising plasma concentration of the inhibitor as an acute phase protein.

Although the $\alpha_1 PI$ concentration was far above the normal value in all ELF specimens of the traumatized patients, this was apparently not sufficient to inhibit all neutrophil elastase released in the local epithelial milieu of the alveoli. Therefore, the obviously deficient inhibitory capacity of $\alpha_1 PI$ may have been caused by two synergic effects. On the one hand, despite the increase of $\alpha_1 PI$ in ELF, the inhibitor amount equalled only a considerably lower molar surplus (in general two- to 40-fold) over its released target enzyme compared to the 140-fold excess found under physiological conditions. This by itself might give rise to a locally incomplete proteinase inhibition. On the other hand, the simultaneously discharged neutrophil lysosomal proteins myeloperoxidase and lactoferrin – both of which are known to be involved in oxidatively destructive processes [18, 17] – as well as the macrophage-derived cathepsin B, presumably together with the even more potent $\alpha_1 PI$-degrading cathepsin L and metalloproteinases (for references see [8]), may have contributed to an additional decrease in the elastase inhibitory capacity, which was not previously sufficient. Applying special assay systems, both oxidatively and proteolytically inactivated $\alpha_1 PI$ could be demonstrated by Schraufstatter et al. [26] in some individual BALF samples drawn only once during manifest ARDS courses. Moreover, these authors also detected active elastase despite the presence of a surplus of immunologically quantified $\alpha_1 PI$ in those BALF specimens. As shown recently, proteolytically degraded $\alpha_1 PI$ is a potent neutrophil chemoattractant [4] and may thus augment neutrophil influx to the alveolar spaces, resulting there in an increased elastase burden during a pathophysiological situation. However, other authors have not been able to demonstrate elastase activity against high molecular weight protein substrates in BALF samples of ARDS patients [13, 32]. This might be for two reasons: first an insufficiently sensitive assay system, which probably did not allow detection of small amounts of free elastase in the highly diluted BALF samples, and second, the lack of enough sequentially drawn BALF specimens during the development and manifestation of ARDS. As pointed out here, the repeated release of phagocyte constituents in to the alveolar space requires a close-meshed sampling procedure to enable sufficient insight into the local inflammatory cell-induced pathomechanisms.

Although the distinctly higher amount of free elastase in the BALF specimens of non-ARDS patients could be due to an augmented local elastase/α_1PI imbalance, these observations seem to contradict the importance which is generally assumed for this neutrophil enzyme in the development of ARDS. In this respect, our findings support data published by other authors (for review of literature see [21]) indicating an equivalent or even higher influx and activation of neutrophils in high-risk patients not generating ARDS. Yet, it should be kept in mind that both the ARDS and non-ARDS patients required artificial ventilation throughout the observation period. This indicates persistent lung failure in all severely traumatized subjects. Therefore, the considerably higher amounts of neutrophil products released to the alveolar compartments of both patient groups may indeed have contributed to lung dysfunction in comparison with the situation evaluated in healthy volunteers. Moreover, as it can be deduced from the high standard deviations of mean, the presentation of data combined in mean values for each group may have concealed somehow the individual situation. This is exemplarily depicted in Figs. 3 and 8. The diagrams demonstrate the existence of a clear correlation between the augmented release of phagocyte constituents and elevated EVLW. However, the definition of ARDS as an increase of EVLW above 10 ml/kg body weight [16] might not be reliable enough to classify exactly the degree of severity of lung function disturbances, because those subjects are excluded whose inflammatory cell-induced pulmonary dysfunction is already indicated by a smaller elevation in EVLW. Therefore, final proof of presumed pathomechanisms awaits further follow-up studies with higher numbers of a homogeneous (e.g., only traumatized) high-risk patients collective, which may allow an exact classification of lung dysfunctions according to various clinical and biochemical parameters.

Regarding the second aim of our study, of the inflammation markers measured so far, only α_1PI in amounts above 300 μg/ml ELF in the early posttraumatic phase heralded the later onset of ARDS with a clinically acceptable degree of sensitivity and specificity. These findings are in agreement with results published by Fowler et al. [10] who could also show permeability changes in patients at high risk for ARDS prior to development of the syndrome. Moreover, manifestation of ARDS was clearly confirmed in our study by ELF concentrations of the fibrosis parameter P-III-P exceeding 1 μg/ml. Yet, neither this nor the other inflammation markers determined here were able to indicate the outcome of the disease.

Conclusion

Daily drawn BALF samples of multiply injured patients and healthy volunteers allowed us to confirm a significantly higher influx of neutrophils into the alveolar space as well as an increased local discharge of phagocyte

(neutrophil; macrophage) constituents (elastase, myeloperoxidase, lacto-ferrin; cathepsin B) in subjects at high risk to develop ARDS. Hence, these aggressive components of inflammatory cells may indeed contribute to the occurrence of lung dysfunctions in all severely traumatized patients.

Taking EVLW elevation above 10 ml/kg body weight as an unambiguous sign of ARDS manifestation, in patients showing this sign 3–4 days post trauma, the permeability increase of the blood/air barrier was heralded much earlier (within 48 h after the traumatic event) by the enhanced inflow of plasma-derived α_1PI to the alveolar epithelial environment. ELF concentrations of α_1PI above 300 µg/ml in this initial posttraumatic phase predicted the later occurrence of ARDS with a clinically acceptable degree of sensitivity (63%) and specificity (71%). Moreover, concentrations of the fibrosis marker, P-III-P, exceeding 1 µg/ml ELF confirmed ARDS manifestation. However, none of the inflammation markers studied indicated the outcome of posttraumatic lung dysfunctions.

References

1. Adamson IY, Young L, Bowden D (1988) Relationship of alveolar epithelial injury and repair to the production of pulmonary fibrosis. Am J Pathol 130:377
2. Assfalg-Machleidt I, Jochum M, Klaubert W, Inthorn D, Machleidt W (1988) Enzymatically active cathepsin B dissociating from its inhibitor complexes is elevated in blood plasma of patients with septic shock and some malignant tumors. Biol Chem Hoppe Seyler 369 [Suppl]: 263
3. Auler JO, Cahlheiros DF, Brentani MM, Santello JL, Lemos PC, Saldiva PH (1986) Adult respiratory distress syndrome: evidence of early fibrogenesis and absence of glucocorticoid receptors. Eur J Respir Dis 69:261
4. Bander MJ, Rice AG, Griffin GL, Senior RM (1988) α_1-Proteinase inhibitor is a neutrophil chemoattractant after proteolytic inactivation by macrophage elastase. J Biol Chem 263:4481
5. Brody AR, Bittermann PB (1988) The lung matrix and inflammation: Part II. Biochemical and molecular mechanisms of fibrogenesis: Implications of environmental lung disease. Am Rev Respir Dis 138:1056
6. Cantin AM, Boileau R, Begin R (1988) Increased procollagen III aminoterminal peptide – related antigens and fibroblast growth signals in the lungs of patients with idiopathic pulmonary fibrosis. Am Rev Respir Dis 137:572
7. Chapman HA Jr, Stone OL (1984) Comparison of live human neutrophil and alveolar macrophage elastolytic activity in vitro. J Clin Invest 74:1693
8. Desrochers PE, Weiss SJ (1988) Proteolytic inactivation of alpha-1-proteinase inhibitor by a neutrophil metalloproteinase. J Clin Invest 81:1646
9. Dwenger A, Schweitzer G, Regel G (1986) Bronchoalveolar lavage fluid and plasma proteins, chemiluminescence response and protein contents of polymorphonuclear leukocytes from blood and lavage fluid in traumatized patients. J Clin Chem Clin Biochem 24:73

10. Fowler AA, Hyers TM, Fisher BJ, Bechard DE, Centor RM, Webster RO (1987) The adult respiratory distress syndrome. Cell populations and soluble mediators in the air spaces of patients at high risk. Am Rev Respir Dis 136:1225

11. Hammerschmidt DW, Weaver LJ, Hudson LD, Craddock PR, Jacobs HS (1980) Association of complement activation and elevated plasma-C5a with adult respiratory distress syndrome: pathophysiologic relevance and possible prognostic value. Lancet 1:947

12. Holter JF, Weiland JE, Pacht ER, Gadek JE, Davis WB (1986) Protein permeability in adult respiratory distress syndrome. J Clin Invest 78:1513

13. Idell S, Kucich U, Fein A, Kueppers F, James HL, Walsh PN, Weinbaum G, Colman RW, Cohen AB (1985) Neutrophil elastase-releasing factors in bronchoalveolar lavage from patients with adult respiratory distress syndrome. Am Rev Respir Dis 132:1098

14. Jochum M, Fritz H (1989) Pathobiochemical mechanisms in inflammation. In Faist E, Ninnemann JL, Green DR (eds) Immune consequences of trauma, shock and sepsis. Springer, Berlin Heidelberg New York Tokyo, p 165

15. Joka Th, Nakhosteen JA, Obertacke U, Herrmann J, Coenen Th, Brand M, Jochum M, Zilow G, Dwenger A, Kreuzfelder E (1988) Beeinflußt die bronchoalveoläre Lavage das Milieu in der Alveole? Prax Klin Pneumol 42:705

16. Kreuzfelder E, Joka Th, Keinecke H-O, Obertacke U, Schmit-Neuerburg K-P, Nakhosteen JA, Paar D, Scheiermann N (1988) Adult respiratory distress syndrome as a specific manifestation of a general permeability defect in trauma patients. Am Rev Respir Dis 137:95

17. Nemet K, Simonovits I (1985) The biological role of lactoferrin. Haematologia 18:3

18. Nowak D (1988) The comparative study of reactive oxygen species generated by polymorphonuclear leukocytes as α_1-proteinase inhibitor inactivators. Possible application for antioxidant prevention of emphysema. Arch Immunol Ther Exp 36:723

19. Ognibene FP, Martin SE, Parker MM (1986) Adult respiratory distress syndrome in patients with severe neutropenia. N Engl J Med 315:547

20. Pacht ER, Davis WB (1988) Role of transferrin and ceruloplasmin in antioxidant activity of lung epithelial lining fluid. J Appl Physiol 64:2092

21. Parsons PE, Worthen GS, Moore EE, Tate RM, Henson PM (1989) The association of circulating endotoxin with the development of the adult respiratory distress syndrome. Am Rev Respir Dis 140:294

22. Regel G, Dwenger A, Seidel J, Nerlich ML, Sturm JA, Tscherne H (1987) Die Bedeutung der neutrophilen Granulozyten bei der Entstehung des posttraumatischen Lungenversagens. Unfallchirurg 90:99

23. Rennard SJ, Basset G, Lecossier D, O'Donnell KM, Pinkston P, Martin PG, Crystal RG (1986) Estimation of volume of epithelial lining fluid recovered by lavage using urea as a marker of dilution. J Appl Physiol 60:532

24. Reynolds HY (1987) State of the art: bronchoalveolar lavage. Am Rev Respir Dis 135:250

25. Rinaldo JE (1986) Mediation of ARDS by leukocytes. Chest 89:590

26. Schraufstatter I, Revak SD, Cochrane CG (1984) Biochemical factors in pulmonary inflammatory disease. Federation Proc 43:2807

27. Shale DJ (1987) The adult respiratory distress syndrome – 20 years on. Thorax 42:641

28. Thommasen HV (1985) The role of the polymorphonuclear leukocyte in the pathogenesis of the adult respiratory distress syndrome. Clin Invest Med 8:185

29. Travis J, Salvesen GS (1983) Human plasma proteinase inhibitors. Am Rev Biochem 52:655

30. Varani J, Fligiel SEG, Till GO (1985) Pulmonary endothelial cell killing by human neutrophils. Possible involvement of hydroxyl radical. Lab Invest 53:565

31. Weiland JE, Davis WD, Holter JF, Mohammed JR, Dorinsky PM, Gadek JE (1986) Lung neutrophils in the adult respiratory distress syndrome. Clinical and pathophysiologic significance. Am Rev Respir Dis 133:218

32. Wewers MD, Herzyk DJ, Gadek JE (1988) Alveolar fluid neutrophil elastase activity in the adult respiratory distress syndrome is complexed to alpha-2-macroglobulin. J Clin Invest 82:1260