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**SECOND VIENNA  
SHOCK FORUM**

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## **Vienna Shock Forum Series**

**Series Editors: Günther Schlag and  
Heinz Redl**

**First Vienna Shock Forum**

**Part A: Pathophysiological Role of Mediators and  
Mediator Inhibitors in Shock**

**First Vienna Shock Forum**

**Part B: Monitoring and Treatment of Shock**

**Second Vienna Shock Forum**

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# SECOND VIENNA SHOCK FORUM

Proceedings of the Second Vienna Shock Forum held May 12–14, 1988

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Editors

**Günther Schlag**

**Heinz Redl**

Ludwig Boltzmann Institute  
for Experimental Traumatology  
Vienna, Austria

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## POSTTRAUMATIC PLASMA LEVELS OF MEDIATORS OF ORGAN FAILURE

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### INTRODUCTION

Severe traumatic events are often followed by the development of acute respiratory distress syndrome (ARDS) or even multiorgan failure in case of additionally occurring septic complications (Nuytinck et al., 1986). In recent years, a nearly illimitable variety of humoral and cellular mediators has been described which all may contribute more or less to the posttraumatic organ failure (Schlag and Redl, 1987). Among these factors activated inflammatory cells such as polymorphonuclear leukocytes (PMNL), monocytes/macrophages, lymphocytes or fibroblasts are supposed to play an essential role in the initiation and perpetuation of inflammatory processes (Dittmer et al., 1986; Dwenger et al., 1986; Nuytinck et al., 1986; Joka et al. 1987; Redl et al., 1987; Lammers et al., 1988).

In the early posttraumatic phase mainly PMNL and monocytes are attracted into the wound area and stimulated to phagocytosis of damaged tissue and invasive organisms. During this physiological repair, however, the phagocytizing cells release destructive enzymes and oxygen free radicals from

their phagolysosomes also extracellularly thus contributing considerably to the consumption of the body's antiproteolytic and antioxidative defence mechanisms. Especially liberated proteinases (e.g. elastases, cathepsins) may overcome locally the inhibitory potential of their main antagonists,  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ -PI),  $\alpha_2$ -macroglobulin and cysteine proteinase inhibitors, thereby being able to destroy vital structural as well as humoral proteins. Such pathobiochemical reactions are suggested to be, at least in part, conducive to the maintenance of inflammation (Lang and Fritz, 1986). As shown previously (Jochum et al., 1986), the extracellular amount of neutrophil elastase complexed to  $\alpha_1$ -PI in plasma can be taken as a reliable likeness of the PMNL activation in the wounded or infected area, whereas stimulation of monocytes/macrophages is reflected by the serum concentration of neopterin, a specific guanosinetriphosphate metabolite excreted from activated mononuclear cells (Huber et al., 1987 a,b; Redl et al., 1987).

Fibroblasts also play a dual role during the posttraumatic inflammatory response. On the one hand the recruitment of fibroblasts into the wound initiates the reparative phase of wound healing, whereby among other substances high amounts of type I and III collagens are synthesized to restore connective tissue matrices. Those fiber-forming collagens are produced as procollagens with additional propeptide extensions at both ends. Before the mature molecules are deposited into the tissue the propeptides are cleaved off and liberated into the extracellular fluid. Only recently it could be demonstrated that the increase in the synthesis rate of type III collagen in the granulation tissue after major abdominal surgery is high enough to be reflected by the increasing amount of the N-terminal procollagen-III-peptide (P-III-P) in serum (Haukipuro et al., 1987). On the other hand, several lines of evidence suggest that the conversion of functional organ tissue (e.g. in liver or lung) to connective tissue is also indicated by elevated serum P-III-P levels and allows a rough quantification of the fibrosis grade of these organs (Surrenti et al., 1987; Mc Cullough et al., 1987; Lammers et al., 1988; Kirk et al., 1984).

Here we describe a close follow-up measurement of plasma or serum levels of complexed neutrophil elastase, neopterin and P-III-P which may be indicative of organ failure subsequently to severe multiple trauma.

## PATIENTS AND METHODS

24 multiply injured patients (Injury Severity Score more than 30 points) with predisposition to ARDS were prospectively studied up to 14 days after trauma. Increase of extravascular lung water (EVLW) above 10ml/kg body weight (pulmonary arterial wedge pressure below 15mmHg) was taken as a main criterion of ARDS (Joka et al., 1987). Septic complications and hepatic failure (total serum bilirubin above  $34\mu\text{mol/l}$ ) were diagnosed according to Goris et al. (1985).

Blood samples were drawn 4 times per day up to 48 hours post trauma and thereafter once a day till to the end of the observation period. The specimens were processed either to plasma or to serum and kept frozen at  $-70^{\circ}\text{C}$  until use. Complexed neutrophil elastase in plasma was assayed by an ELISA test kit (PMN Elastase, E. Merck, Darmstadt; upper normal range: 180 ng/ml). The RIA technique was applied for the determination of D(+)neopterin in serum (Neopterin-RIAacid/serum, Henning, Berlin; normal range: 6-10nmol/l) as well as of P-III-P in plasma (RIA-gnost Prokollagen-III-Peptid, Behringwerke, Marburg; normal range: 3-15ng/ml). Total serum bilirubin was quantified with a test combination of Boehringer, Mannheim (upper normal range:  $17\mu\text{mol/l}$ ).

## RESULTS

Twelve of the 24 patients studied had to be allocated to the ARDS group according to the rise of EVLW above the prospectively established limit of 10ml/kg b.w. during the early (48 hours) or late (from day 4 onwards) observation period. Whereas all patients of this group developed moderate to severe septic complications and hepatic failure (total bilirubin well above  $34\mu\text{mol/l}$ ), only 4 patients without acute respiratory disease showed minimal transient signs of bacterial infection. However, in about 80% of the non-ARDS patients total serum bilirubin was moderately elevated above normal indicating impairment of liver function.

As demonstrated in Fig. 1 increased release of neutrophil elastase could be assayed in both groups already in the first blood sample (time 0) taken at least within two hours after trauma. In plasma of patients without development of ARDS maximal elastase liberation was evident 6 hours later followed by a rapid normalization. ARDS patients showed highly elevated



plasma levels of complexed elastase up to the 7th posttraumatic day. Even at the end of the observation period these values did not decline to the normal range.

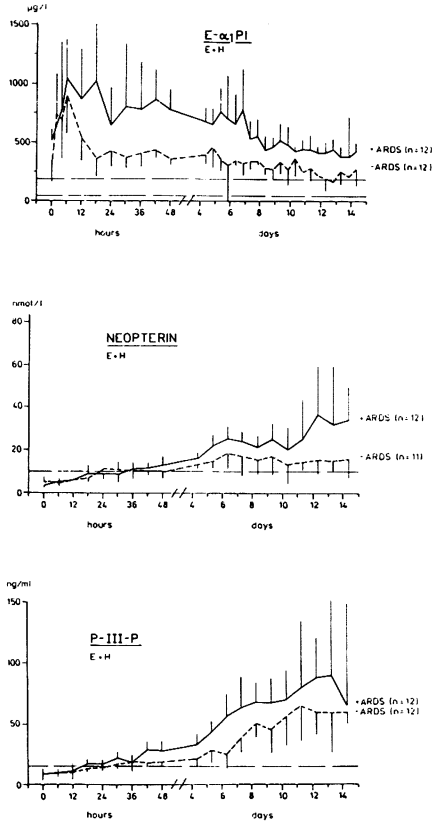


Fig. 1: Posttraumatic plasma/serum levels (median  $\pm$  pseudo SE) of elastase in complex with  $\alpha_1$ -proteinase inhibitor (E- $\alpha_1$ PI), neopterin and procollagen-III-peptide (P-III-P) in patients with or without acute respiratory distress syndrome (ARDS).

Increase of neopterin respectively P-III-P levels above normal was evident only from the second posttraumatic day onwards (Fig.1). Neopterin serum concentrations reached a first

maximum about 6 days post trauma in both groups; thereafter an additional significant rise was seen in ARDS patients till the end of the study period, whereas in the non-ARDS collective a slight decrease was measurable. P-III-P levels increased steadily without significant differences in both patient groups up to the 14th posttraumatic day.

Case Reports

Fig. 2 shows the sequential plasma/serum levels of the above mentioned parameters in 4 individual cases.

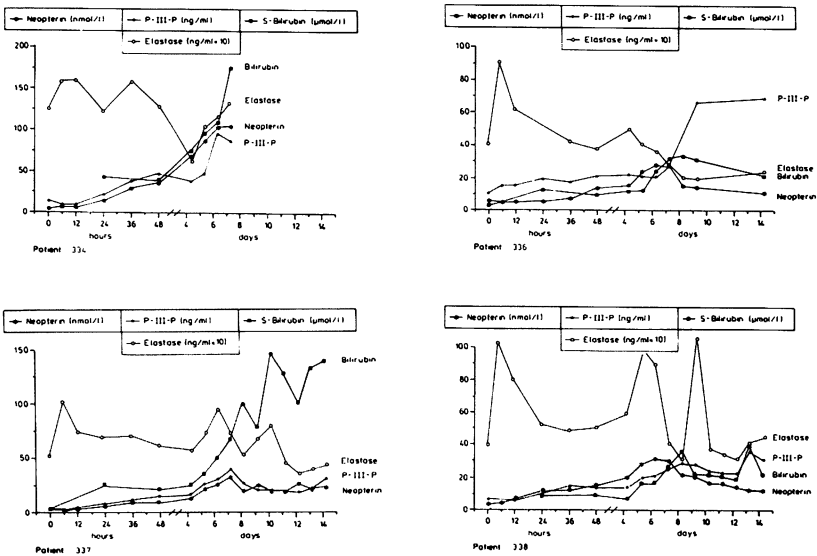


Fig. 2: Posttraumatic plasma/serum levels of complexed elastase, neopterin, procollagen-III-peptide (P-III-P) and bilirubin in individual polytrauma patients.

In patient 334 the accident caused severe lung and liver contusion. ARDS and sepsis was diagnosed at the 4th posttraumatic day; the patient died at the 7th day due to irreversible lung, liver and kidney failure. The severe clinical course is closely reflected by the highly elevated and repetitively increasing elastase levels during the whole observation period

as well as by the tremendously rising concentrations of neopterin, bilirubin and P-III-P from the second day onwards.

In contrast, patient 336 did not develop ARDS despite severe lung contusion. Apart from tracheal germs, which were sufficiently treated with antibiotics from the 6th posttraumatic day till recovery, no septic complications or organ failure occurred. The initial trauma-induced elastase release was followed by a rapid normalization, the minor signs of the local infection seem to be reflected by a small additional increase of complexed elastase and a more retarded transient extracellular secretion of neopterin. Although the slightly elevated bilirubin levels between day 4 and 8 indicate only minor liver dysfunction, the P-III-P plasma concentrations rose remarkably until the end of the study phase.

Patients 337 and 338 also sustained severe lung contusions. In patient 337 the traumatic event was followed immediately by manifestation of ARDS and was further complicated by liver dysfunction - the latter being indicated by a steadily increase in total serum bilirubin - were present till to the end of the study period. The development of the infectious complications are indicated by the consistently high elastase levels in the early phase and the repeatedly release of the PMNL protease in the later posttraumatic phase. A significant increase of the neopterin and P-III-P levels is evident between day 5 and 8. The patient recovered from the multiorgan dysfunction about 7 weeks after trauma. Patient 338, in contrast, did not develop life-threatening long-term organ failure. After an early clinical normalization, which is reflected also in the rapid decline of extracellular elastase release, a transient septic period from day 5 to 11 was accompanied with moderate respiratory insufficiency. Thereafter the patient convalesced without further complications. The sepsis-like phase was paralleled by increasing elastase levels as well as by a slight elevation of neopterin and P-III-P in the circulation. Total serum bilirubin was only modestly and temporarily increased.

## DISCUSSION

In recent years, a great number of studies have focused on the role of neutrophils as a prominent source of powerful mediators in the acute inflammatory process initiated by polytrauma or major surgery. With respect to the proteases released extracellularly from the activated PMNL cells, sequen-

tial measurements of complexed elastase in plasma turned out to be a helpful tool in early diagnosis of septic complications and the grading of the severity of septicemia (Jochum et al.,1986, Nuytinck et al.,1986, Inthorn and Jochum,1988). As shown in this paper, the primary activation of the PMNL immediately after the polytraumatic event is followed by repetitive increases of elastase in plasma in those patients who developed ARDS and additional organ failure. Since this multiple organ insufficiency in our patients was mainly due to septic complications, we cannot confirm the former statement of Nuytinck et al.(1986) and Redl et al.(1987) that ARDS per se is indicated by elevated plasma levels of complexed PMNL elastase. In agreement with these authors, however, the involvement of the monocyte/macrophage system in sepsis-related ARDS could be proven. Neopterin, an activation marker of the lymphocyte/macrophage axis as well as of the direct action of bacterial endotoxins on the mononuclear cells (Huber et al.,1987b), significantly increased in the circulation even before severe sepsis became manifest. Transient signs of infection were indicated only by a minor rise of the neopterin serum levels. Neither the traumatic event per se nor the severity of the trauma was reflected by neopterin which is in contrast to the behaviour of PMNL elastase as demonstrated recently by Dittmer et al.(1986).

Conflicting results have also arisen concerning P-III-P excretion to the circulation as a valuable sign of liver (Surrenti et al.,1987; Mc Cullough et al.,1987) and/or lung fibrosis (Kirk et al.,1984; Lammers et al.,1988) in acute and chronic diseases. Moreover, elevated posttraumatic P-III-P plasma levels may only indicate physiological wound healing (Haukipuro et al.,1987). From our data the latter can be deduced at least for those patients who did not develop organ failure in the posttraumatic course. On the other hand, in most of the patients with infaust multiorgan insufficiency P-III-P levels increased clearly above the maximal median value (60 ng/ml) of the non-ARDS group some time before lethal outcome. Therefore, highly elevated P-III-P plasma concentrations may be a reliable marker of bad prognosis due to massive organ fibrosis. The threshold value, however, has to be evaluated in further studies.

In conclusion, measurement of sequential plasma levels of cell-derived inflammation mediators turned out to be a helpful tool for early diagnosis of severe posttraumatic multiorgan failure.

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