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Inflammation Research

Dynamics of neutrophilic NF-κB translocation in relation to IL-8 mRNA expression after major trauma

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Abstract. *Objective:* Systemic inflammation subsequent to polytrauma is connected to neutrophil (PMN) dysregulation characterized by reduced NF-κB-translocation and cytokine expression. The dynamics of NF-κB-activation as well as its down-stream regulation of IL-8-expression in PMN following major trauma remain unclear. The aim of this pilot study was to analyse NF-κB nuclear translocation in relation to IL-8-mRNA-expression in PMN after major trauma.
Patients and methods: PMN were isolated from blood samples of 15 major trauma patients (New Injury Severity Score, NISS > 16) drawn within 90 min and subsequently 6, 12, 24, 48, 72 h after trauma. NF-κB-translocation was analysed by Electrophoretic Mobility Shift Assay, EMSA and quantified by densitometry [arbitrary units], IL-8-mRNA-expression by RT-PCR, [copies/50 ng RNA]. Additionally, NF-κB-translocation and IL-8-expression in PMN of healthy volunteers were analysed natively (-control) and after LPS stimulation (+control).
Results: NF-κB-translocation and IL-8-mRNA-expression was significantly increased in polytrauma patients (n=15; NISS: 34 ± 8 [mean ± SEM]) initially. In non-survivors, NF-κB-translocation was significantly increased on admission and subsequently reduced within 6 h, while it increased in the survivors group. After 24 h, a second significant increase in NF-κB-activity and IL-8-expression was found in survivors that was subsequently reduced in both groups.
Conclusion: This pilot study has shown that a concomitant initial increase in transcriptional NF-κB-activity and IL-8 mRNA expression was observed in the early posttraumatic period which preceded the down-regulation of the innate immune system.

Key words: NF-κB – EMSA – RT-PCR – IL-8 – PMN – NISS – major trauma – MOF

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Introduction

Mortality after major trauma is associated with insufficiency of the innate immune system [36]. Early destabilisation of the innate immune system after trauma has been described as *Systemic Inflammatory Response Syndrome*, SIRS and can provoke a posttraumatic transition to *Multiple Organ Dysfunction Syndrome*, MODS which can be followed by complete decompensation of organ functions as *Multiple Organ Failure*, MOF [25]. In this context, the macrophage/leukocyte-system was identified to play a pivotal role in the development of the early MODS [12, 23]. Patients suffering from blunt multiple injuries exhibit an increase of pro-inflammatory mediators in the early posttraumatic phase [8, 11].

The synthesis and release of these inflammatory cytokines and chemokines represent only the terminal step of inflammatory pathways and is regulated by nuclear factors such as NF-κB [17]. NF-κB is an inducible cytoplasmatic transcriptional factor which is enabled to translocate into the nucleus upon activation and bind to promoter regions of proinflammtory genes such as IL-1, IL-6, IL-8 and TNF-α In contrast, in posttraumatic immunoparalysis, the capacity of *de novo* cytokine release in immunocompetent cells as well as the amounts of circulating cells could be shown to be diminished after major trauma [25]. Within *in vitro* experiments, diminished apoptosis rate with constitutively increased I-κB phosphorylation could be demonstrated after traumatic challenge.

However, the role of NF-κB in PMN as well as its down-stream regulation of IL-8 expression in major trauma patients remains unclear, so far. Therefore, the aim of this study was to investigate NF-κB nuclear translocation by electrophoretic mobility shift assay, EMSA, and the mRNA expression of IL-8 by RT-PCR in PMN obtained from major trauma patients in the very early posttraumatic period as well as to correlate these results to systemic parameters and to clinical outcome after trauma.

Patients and methods

Patients

After approval by the local ethics committee [Munich, reference number 012/00] this prospective pilot study was conducted at our university level I trauma center including major trauma patients suffering from multiple injuries according to a new injury severity score, (NISS), [4, 31] of at least 16 points. The major cause for blunt multiple injuries were high velocity traffic accidents as well as falls from a height. Signed informed consent was obtained from all patients or in case of unconsciousness from a legal representative. Excluding criteria was isolated traumatic brain injury, anamnestic malignancies and autoimmune dysfunctions. After initial resuscitation and subsequent surgical interventions patients were admitted to the intensive care unit.

Control group

In order to establish positive and negative controls, two blood samples each were taken from eight healthy volunteers. One sample was processed natively without stimulation as described below which served as negative control. As positive control, from the second blood sample PMN were separated after incubation with 10 µl of a 100 ng/ml lipopolysaccharide, LPS-solution (Sigma-Aldrich, Munich, Germany) for 2 h for inflammatory stimulation and NF-κB activation [34].

Blood sampling and PMN isolation

A total volume of 30 ml whole blood was drawn into ethylenediaminetetraacetic acid (EDTA) on admission within 90 minutes and 6 h, 12 h, 24 h, 48 h and 72 h after the traumatic event. 42.5 ml red blood cell lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM ETDA, pH 7.2) was added. After lysis time of 10 minutes the samples were centrifuged for 10 minutes at 450xg at 20 °C. Two additional lysis steps were performed with the cell pellet using 5 ml lysis buffer to achieve quantitative lysis of the red blood cells. Thereafter, cells (1×10^8 in 80 µl) were incubated with 200 µl CD15 antibody coated micromagnetic beads (Miltenyi Biotech, Auburn, CA, USA) for 15 min at 4 °C for magnetic cell sorting (MACS). After incubation the volume was adjusted to 15 ml using PBS/EDTA buffer and cells were sedimented by a centrifugation step (450xg, 10 min, 20 °C). The cell pellet was resuspended in 500 µl PBS/EDTA buffer and added to a high gradient magnetic separation column using the magnetic separator MiniMACS system (Miltenyi Biotech, Auburn, CA, USA). The column was washed three times with PBS/EDTA buffer. After removing the column from the magnetic field cell elution was performed by addition of 1 ml PBS/EDTA buffer. To save patients material, the purity of the PMN preparation was controlled by Fluorescence Activated Cell Sorting, FACS analysis of samples from three healthy controls using anti-CD15 antibodies (Beckman Coulter EPICS.XL.MCL., Krefeld, Germany).

Nuclear protein extraction

Nuclear protein was extracted as described before [2]. Briefly, 0.5x 10^8 PMN were lysed in ice-cold lysis buffer [10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, DTT, 1 mM phenylmethylsulfonyl fluoride, PMSF and 1 % protease inhibitor cocktail which contains per ml the following ingredients: 500 µg antipain, 500 µg aprotinin, 500 µg leupeptin, 50 µg pepstatin, 750 µg bestatin, 400 µg phosphoramidone, and 500 µg trypsin inhibitor (ROCHE, Mannheim, Germany). The cell suspension was incubated on ice for 10 minutes in order to induce PMN lysis, and centrifuged at 12000xg for 5 minutes (4 °C). From the supernatant the cytosolic fraction was carefully extracted. Then the nuclear pellet was resuspended in ice cold lysis buffer (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF and 1 % protease inhibitor cocktail, see above) and rocked vigorously for 20 minutes on a shaking platform. The nuclear protein fraction was isolated via centrifugation and total protein concentration was determined by Bradford assay (Coomassie Plus, Pierce, Rockford, IL, USA).

Electrophoretic mobility shift assay (EMSA) Oligonucleotide labeling

The nuclear protein fraction was subjected to electrophoretic mobility shift assay, EMSA, as described before [16]. Briefly, oligonucleotides were end-labeled using a double-stranded consensus sequence of NF-κB (sense: 5´-AGT TGA GGG GAC TTT CCC AGG C-3´, antisense: 3`-TCA ACT CCC CTG AAA GGG TCC G-5´, Promega, Madison, WI, USA) in the presence of [γ-^{32}P]-ATP. The labeling reaction was catalyzed by T_4-polynucleotide kinase and incubated in 10x kinase buffer at 37 °C for 30 minutes. Nuclear extracts (0.8 µg each) were incubated in binding buffer (20 mM HEPES, 1 mM EDTA, glycerol 40 %, 2 mM DTT, 0,5 mM PMSF, 10 mM MgCl, 5 mM EDTA, pH 7 500 mM NaCl, 100 mM TrisHCL, pH 7.5, poly(dI-dC), (ROCHE, Mannheim, Germany) for 15 minutes at room temperature. Thereafter, the radiolabeled nucleotide was added (approx. 50,000 cpm), and the mixture was again incubated for 15 minutes at room temperature. EMSA was performed in a 5 % acrylamide gel in 50 mM Tris-45 mM boric acid-0.5 mM EDTA, pH 8.4. For specific competition, a 50 fold excess of unlabeled, 'cold' oligonucleotides was added, as nonspecific competitor SP1 (sense: 5'-ATT CGA TGC GGG CGG GGC GAG C-3', antisense: 3' –TAA GCT AGC CCC GCC CCG CTC G-5', Promega Madison, WI, USA) was used. For supershift experiments, each sample was incubated with anti-p50 antibody (Santa Cruz, CA, USA). EMSA was performed for 2 h with 100V using 5 % polyacrylamid gels in 0.5 % TBE, [9] (Tris base, boric acid, EDTA), buffer. Gels were dried and exposed to X-ray film (Kodak BioMax MR) for up to 3 days at –80 °C to avoid artifacts. After development of the autoradiographs the intensity of the blackening according to radioactive labeled NF-κB-DNA complexes was quantified by densitometry.

RNA preparation

IL-8 mRNA expression was analysed by quantitative RT-PCR as described before [22]. Total RNA preparation from lysed PMN was performed using RNeasy midi kit according to the suppliers instructions. Nucleic acids were digested by DNase (RNase-Free DNase Set; Qiagen). Concentration of isolated RNA was determined by spectophotometry (260nm; Biophotometer, Eppendorf, Hamburg, Germany). Extracted RNA from all samples was stored at –80 °C.

An aliquot of 8.2 µl standardized to 1 µg of RNA was reverse transcribed using avian myeloblastosis virus-reverse transcriptase (RT) and oligo(dT) as primer (First Strand cDNA synthesis kit; Roche, Mannheim, Germany). The obtained cDNA was diluted 1/25 with water and 10 µl were used for amplification. The quantitative analysis of target gene expression was performed on a LightCycler by real time-PCR using the LightCycler FastStart DNA SYBR Green I kit (Roche, Mannheim, Germany) according to the instructions of the supplier. RT-PCR was performed by denaturation and polymerase activation (95 °C, 10 min), amplification of RT-PCR products in a 45 cycle one-step PCR including denaturation (95 °C,10 sec)/annealing (68°–58 °C, –0.5 °C/cycle)/extension (72 °C, 16 sec) for each cycle. The results of negative control samples were set as baseline level. PCR results were standardized to this baseline level and given as [copies/50 ng RNA]. Specificity of the amplification products was verified by melting curve analysis combined with agarose gel electrophoresis. For calculation, data processing and documentation the LightCycler analysis software (Version 2.0) was used.

Table 1. Data concerning age, gender, amounts of transfused red blood cell units, (RB-U), fresh frozen plasma units, (FFP) within 24 h and clinical outcome within 40 days after trauma.

	Age	Gender	Injury pattern	ISS	RBC	FFP	Clinical outcome
I	33	M	Subarachnoidal hematoma, lung con-tusion, rib#, scapula#, open°II fibula #	29	12	10	Neurological disorder due to head trauma
II	59	M	Serial rib fracture, hemato-pneumothorax, pulmonary contusion, diaphragm rupture, myocardial contusion, spleen rupture, arteria mesenterica root rupture, fracture of scapula, ulna, pelvis, femur, tibia	57	10	12	Deceased
III	74	M	TBI°II, complicated °III open humeral fracture, severe pelvic trauma with massive retroperitoneal hematoma	38	24	20	Deceased due to MOF
IV	18	M	TBI°III, pulmonary contusion, cardiac contusion, serial rib fracture, cervical spine fracture, liver rupture°III	57	17	19	Neurological deficits due to head trauma
V	29	M	TBI II°, Le Fort II°, III° optic nerve laceration, lung contusion, hematopneumothorax, serial rib #, severe pelvic trauma, open°III ankle #, metarcarpus #, sacrum #	41	19	12	Complete recovery
VI	34	M	Thoracic trauma, cardiac contusion, blunt abdominal trauma, liver rupture, renal contusion, cervical spine fracture, fractures upper	34	15	22	Complete recovery
VII	33	M	TBI°II, pulmonary contusion, humeral fracture upper, multiple fractures lower extremity	24	8	10	Complete recovery
VIII	74	F	TBI°III, fractures of humerus, pelvis, sacrum	26	26	22	Deceased
IX	29	M	TBI II°, Le Fort II°, III° optic nerve laceration, lung contusion, hematopneumothorax, serial rib #, severe pelvic trauma,°III open fractures of ankle, metarcarpus, fracture of sacrum	41	19	12	Complete recovery
X	33	M	Pulmonary contusion, cardiac contusion, serial rib fracture, lumbar spine fracture, renal contusion, hematoma of the spleen °II	34	16	38	Rehabilitation hospital
XI	33	M	TBI°I, pulmonary contusion, fracture upper extremity, multiple fractures of lower extremity	16	11	32	Complete recovery
XII	18	M	TBI°III, pulmonary contusion, cardiac contusion, serial rib fracture, cervical spine fracture, liver rupture	57	14	16	Neurological deficits due to head trauma
XIII	44	F	TBI°III, spinal channel bleeding, serial rib fracture, hemato-pneumothorax, pulmonary contusion, spleen subcapsula hematoma < 50 % surface	29	5	5	Rehabilitation hospital
XIV	75	M	Moderate head trauma, scalp laceration, lung contusion, serial rib fracture, hematopneumothorax, multiple fractures of pelvis	24	6	2	Complete recovery
XV	78	F	TBI°III, subdural hematoma, fractures of tibia, fibula, pelvis	38	4	0	Neurological disorder due to head trauma

Clinical data

Clinical data concerning age, gender, as well as the New Injury Severity Score, NISS, was recorded on admission. Vital parameters, such as blood pressure, BP, heart rate, HR, ventilation parameters, systemic concentration of C-reactive protein, CRP, procalcitonin, PCT, amounts of white blood cells, WBC, differential cell counts, therapeutic intensive care regime including catecholamines demand, amounts of transfused packed red blood cell units (PRBC-U), fresh frozen plasma (FFP) and platelets (PU) were recorded according to the detection time points during the initial 72 h after trauma.

Data analysis

For EMSA results, the densitometric data is given in [arbitrary units] representing the blackening area of the X-ray film, for RT-PCR, data is given as [copies/50 ng total RNA] as mean ± SEM. The Sigma Stat® 3.0 software package (SPSS® Inc., Chicago, USA) was employed for all statistical analysis. Statistical significance between groups was determined by independent two-tailed t-test, ANOVA on ranks and SNK-test. Clinical data according age, NISS, MOF, amounts of transfused red blood cell units and fresh frozen plasma units as well as EMSA results are given as mean ± SEM. P-values below 0.05 were considered significant.

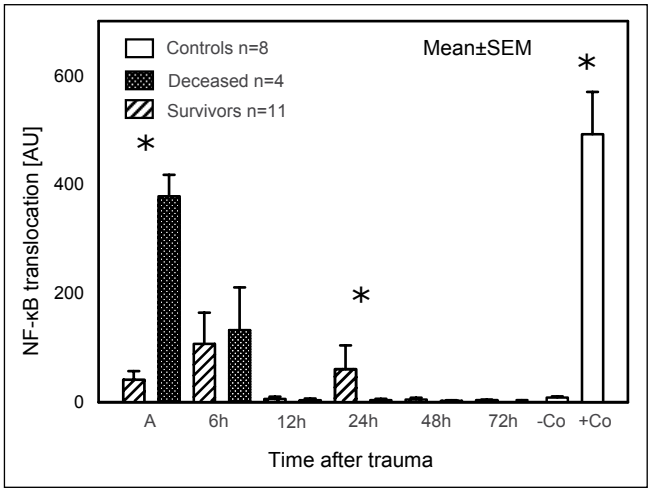

Fig. 1. NF-κB activity is significantly increased biphasically in the early posttraumatic period (* p = 0.038; U-test; SNK-test vs. baseline of negative control and later time points; # p = 0.04 U-test; SNK-test vs. admission[A]).

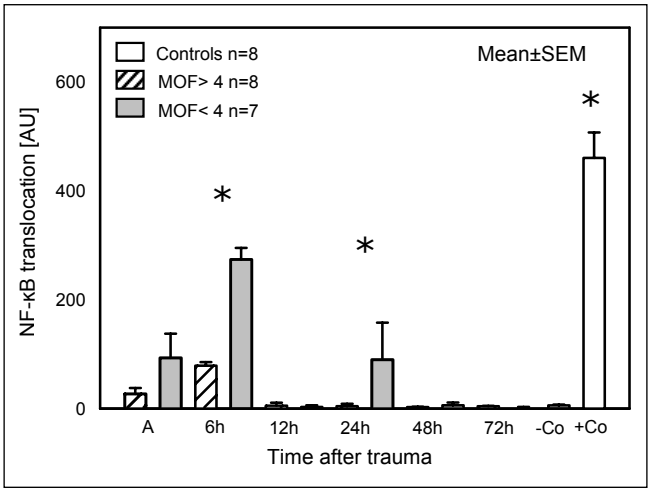

Fig. 2. Patients with higher MOF (>4 points) showed reduced NF-κB activity during the whole analyzed time. NF-κB translocation was significantly increased in the lower MOF group after 6 and 24 h (p = 0.029; ANOVA on ranks, t-test).

MOF

Multiple organ failure, MOF scores of the enrolled patients were recorded according to the sampling time points, respectively and were defined following the score by Goris in the modification of Lefering [15, 24] which scores 'O' for unaffected function of the vital organs such as lung, heart, kidney, liver and blood. One point is assigned to each organ system if the following functional parameters are presented by the patient: mechanical ventilation with a PEEP < 10 and FiO_2 < 0.4; blood pressure > 100 mmHg with low dose of vasoactive drugs, serum creatinine > 2mg/dl; SGOT > 25U/l, bilirubin > 2mg/dl; leukocytes > 30,000/µl, platetes < 50,000/µl. The maximum of 2 points is scored if patients exhibit more severe organ dysfunction such as mechanical ventilation with a PEEP > 10 and FiO_2 > 0.4; blood pressure < 100 mmHg and/or high dose of vasoactive drugs, hemofiltration or hemodialysis; SGOT > 50U/l, bilirubin > 6mg/dl; leukocytes > 60,000 or < 2,500/µl.

Results

Patient collective and clinical data

15 major trauma patients including 12 males and 3 females were enrolled with a mean age of 49 ± 13 [ranging from 18 to 78 years] who presented a mean NISS of 34 ± 8 in a range of 17 to 57. 11 patients survived, four patients died within the posttraumatic period due to MOF after 26.3 ± 11.2 days.

All patients presented signs of posttraumatic SIRS: The temperature was 34.8 ± 1.5 °C on admission, 35.4 ± 1.4 °C after 6 h and 36.6 ± 1.4 °C after 12 h. The amount of WBC on admission was 12,600 ± 2,200/µl, 14,100 ± 2,800/µl 12 h after trauma and 13,800 ± 4,200/µl after 24 h. Seven patients presented a leukopenia (1,100 ± 270/µl), six patients developed a leukocytosis (18,700 ± 6,300). In 76 % a significant left shift occurred between three and eight hours after trauma verified by differential blood count. At 24 h after trauma, 86 % of the surving but none of the deceased patients presented a left shift. After 12 h, CRP was elevated to 5.6 ± 1.5mg/dl, PCT increased to 3.3 ± 0.8ng/ml. Analysis of WBC, CRP and PCT adduced no significant differences. In the higher MOF group, elastase was inconstantly increased to 151 ± 43 µg/l.

All patients required mechanical ventilation during at least 72 h. The heart rate on admission was 98 ± 25 and 96 ± 24 after 6 h. In ten patients, the mean arterial blood pressure was kept above 68 ± 21 mmHg by catecholamine therapy. The initial base excess [–4.3 ± 1.2] obtained from blood gas analysis provided no significant difference. Lactate concentration in survivors was significantly lower on admission (p = 0.02), after 12 h (p = 0.002) and 24 h (p = 0.002) as compared to non-survivors. In all trauma patients, creatinine kinase was increased to 1001 ± 256U/l.

The MOF score was 3.8 ± 0.7 on admission, 4.0 ± 0.9 after 6 h, 3.9 ± 0.6 after 12 h, 4.1 ± 0.9 after 24 h, 3.6 ± 0.8 after 48 h and 3.6 ± 0.8 after 72 h. Amounts of transfused packed red blood cell units (PRBC-U; approx. volume 300 ml) and fresh frozen plasma units (FFP; approx. volume 250 ml) within 72 h after trauma as well as clinical outcome within 90 days are depicted in the Table 1.

Methodological aspects

In control experiments, the cell isolation system using CD15 antibody coated micro magnetic beads enabled a PMN cell fractionation purity of 92 ± 3 % (n = 3, mean ± SEM) controlled by FACS. From a total number of $0.5x10^8$ cells of each sample a variable total nuclear protein fraction ranging from 2 µg up to 8 µg was isolated.

NF-κB activity

NF-κB translocation increased significantly to 476 ± 148 [AU] after LPS-stimulation (positive control) (p = 0.028).

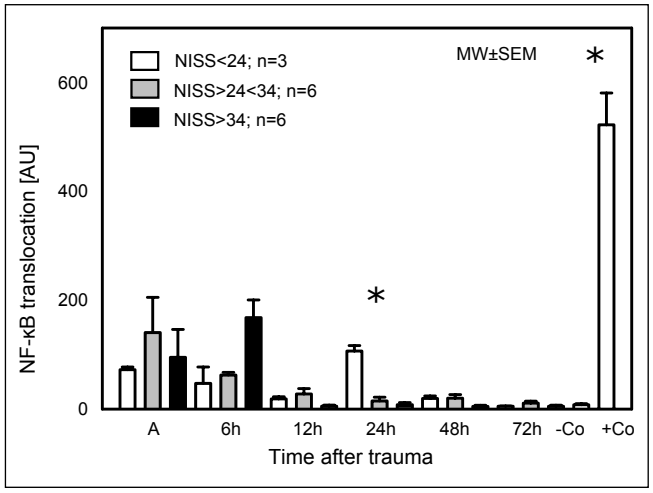

Fig. 3. While NF-κB activity was relatively constant within the first 6 h, it increased in the high NISS group (>34) to be diminished in all groups after 12 h. In patients presenting moderate injury severity, a significant increase of NF-κB translocation was identified at 24 h after trauma ($p = 0.014$).

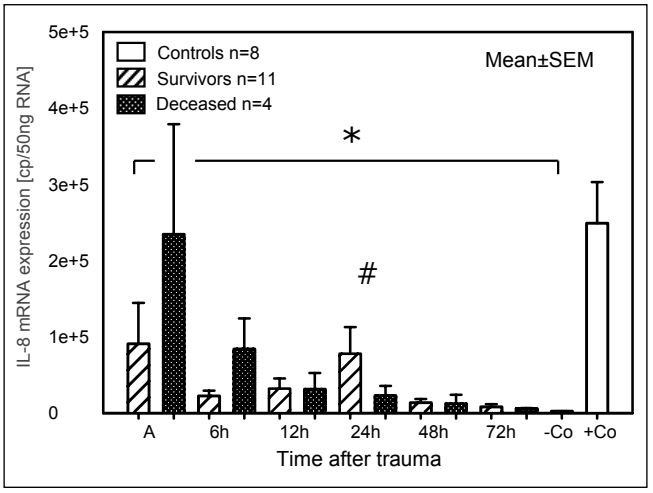

Fig. 4. In the survivors group, IL-8 mRNA expression was significantly elevated within 90 minutes after trauma as compared to the negative control and the following detection time points (*$p < 0.05$). After 24 h, the survivors exhibited significantly increased IL8 expression as compared to the deceased patients (#$p = 0.04$).

Supershift experiments by p50 antibodies exhibited reduced blackening (data not shown). In all patients, the translocation level of NF-κB increased significantly to 84 ± 25 [AU] [mean ± SEM] on admission and to 102 ± 26 [AU] at 6 h after trauma as compared to healthy volunteers ($p = 0.04$). Transcriptional activity over all patients was diminished at 12 h (5 ± 1 [AU]). Interestingly, nuclear presence increased again at 24 h (46 ± 13 [AU]) to return to healthy volunteers level (48h: 6 ± 2; 72h: 4 ± 1[AU]).

Mean transcriptional NF-κB activity in non-survivors was elevated significantly on admission (378 ± 189 vs. 46 ± 21; $p = 0.002$) and (132 ± 66 vs. 107 ± 32) after 6 h as compared to surviving patients. After 12 h, the transcriptional level was diminished fast in both groups (4 ± 2 vs. 6 ± 2) to increase again in the survivors group at 24 h (65 ± 18 vs. 8 ± 5 [AU]; $p = 0.004$) and to be further on reduced in both groups (48h: 6 ± 2 vs. 2 ± 1; 72h: 2 ± 1 vs. 3 ± 1 [AU]) (Figure 1).

NF-κB nuclear translocation was reduced in patients with higher MOF scores during the whole analyzed time. After 6 h, patients who presented a MOF score over 4 presented significantly reduced NF-κB activity as compared to the lower MOF group (55 ± 18 vs. 134 ± 46 [AU]; $p = 0.029$). While NF-κB was reduced in both groups in 12 h, a second activity peak was present after 24 h in the lower MOF group.

Patients presenting a MOF score less than 4 points revealed increased neutrophilic NF-κB activity on admission and after 6 h (276 ± 104 vs. 53 ± 18 [AU]; $p = 0.029$) as compared to the higher MOF group (Figure 2).

Patients presenting low and moderate NISS values (NISS <24; >24<34) presented relatively constant NF-κB activity for the first 6 h (Figure 3). In all groups, NF-κB activity decreased at 12 h after trauma to increase again significantly in the lower NISS group after 24 h (NISS <24: 107 ± 6 vs. NISS >34: 14 ± 3 [AU]; $p = 0.014$).

IL-8 mRNA expression

IL-8 mRNA expression of natively processed PMN was 48,010 ± 16,974 [cp/50 ng/RNA]. After LPS stimulation, IL-8 mRNA expression increased significantly to 249,140 ± 88,084 [cp/50 ng/RNA]; ($p = 0.001$) as compared to the negative control. In major trauma patients, IL-8 mRNA expression was significantly elevated as compared to healthy volunteers ($p < 0.01$) during the whole analyzed time. After major trauma, a significant increase of IL-8 mRNA expression was found within 90 minutes (182,769 ± 41290 [cp/50 ng/RNA]) as compared to the subsequent detection time points (12h: 29,342 ± 7,576; $p = 0.036$; 48h: 11,799 ± 3,046; $p = 0.011$; 72h: 7,644 ± 1,973 [cp/50 ng/RNA]; $p = 0.022$).

On admission, IL-8 mRNA expression was significantly elevated in non-survivors (202,873 ± 61,168 vs. 22,071 ± 11,036 [cp/50 ng/RNA]; $p < 0.05$; Figure 4). After 6 h, mean IL-8 expression was significantly diminished in surviving patients (22,488 ± 6,780 vs. 84,210 ± 42,105 [cp/50 ng/RNA]; $p = 0.002$). IL-8 expression was reduced in both groups (32,062 ± 9667 31,611 ± 15,806 [cp/50 ng/RNA]) at 12 h after trauma, to be significantly increased in the survivors group after 24 h (103,631 ± 31,246 vs. 26,609 ± 13,305 [cp/50 ng/RNA]; $p = 0.043$).

Patients developing moderate MOF showed increased IL-8 expression level within the first 24 h (Figure 5). After 6 h, IL-8 expression was significantly increased as compared to the higher MOF group (36,893 ± 13,044 vs. 166,147 ± 62,798 [cp/50 ng/RNA]; $p = 0.019$).

Regarding the anatomic injury severity according to a NISS <24 points significantly increased IL-8 expression 12h after trauma was detected (45,115 ± 13,043 [cp/50 ng/RNA]) as compared to the higher NISS group (NISS <34: 11,588.7 ± 2,992.0; NISS >34: 22,544.9 ± 6,582.2 [cp/50 ng/RNA]; $p = 0.02$). The lower NISS group exhibited a second significant increase of IL-8 expression at 72 h after trauma

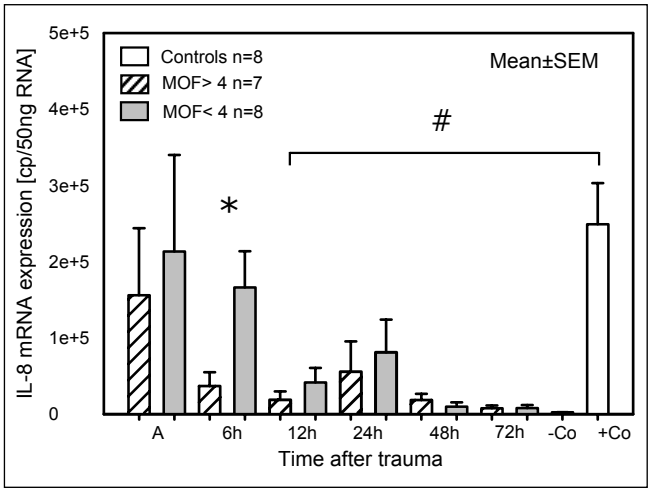

Fig. 5. After 6 h, IL-8 mRNA expression was significantly reduced in patients presenting a MOF score >4 points, (*p = 0.019). In the higher MOF group, IL-8 mRNA expression was significantly reduced within 12–72 h after trauma as compared to the positive control (#p = 0.03).

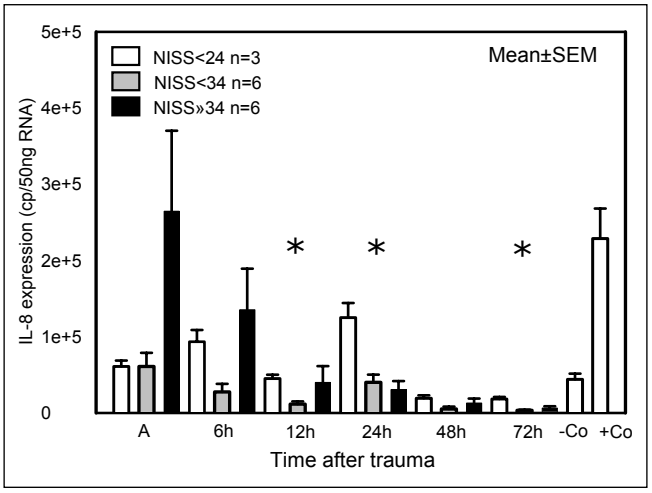

Fig. 6. Patients with moderate injury severity revealed significantly increased IL-8 mRNA expression at 12 h and 24 h after trauma as compared to the higher NISS groups (NISS < 34: NISS > 34: p = 0.02). In the lower NISS group, IL-8 expression increased significantly 72 h after trauma as compared to higher affected patients (NISS > 24 < 34 p = 0.04).

(14,106.7 ± 3,642.2 [cp/50 ng/RNA]) as compared to highly injured patients (NISS > 24< 34: 3,035.9 ± 783.9; NISS > 34: 6,531.3 ± 1,686.4 [cp/50 ng/RNA]; p = 0.04) (Figure 6).

Discussion

PMN play a pivotal role in the first response to injury. Additionally to superoxide and elastase synthesis [7, 8), PMN are enabled to synthesize inflammatory mediators such as IL-8. PMN are characterized by a high turn-over which is especially regulated by apoptosis. Physiologically, PMN having fulfilled their function in the tissue, undergo apoptosis. Within the posttraumatic period a delayed apoptosis was demonstrated which can last up to 3 weeks after trauma [30]. NF-κB translocation was shown after various artificial stimulation such as hypoxia, LPS, TNFα and IL-6 [27, 32, 33]. While TNFα as well as IL-1ß were found elevated at the beginning of the posttraumatic inflammatory cascade, they both induce augmented secretion of IL-8 which leads to leukocyte recruitment and activation [18]. Cytokine synthesis is increased in the early posttraumatic period followed by hyporesponsiveness. Neutrophilic NF-κB induction with consecutive cytokine synthesis was shown after external stimulation [32]. NK-κB dependent inhibition of posttraumatic apoptosis was shown after *in vitro* stimulation by sera of major trauma patients [27]. Differential blood analysis provided a significant left shift in surviving patients. Such a left shift was not found in non-survivors later than 24 h post trauma. Surviving patients might exhibit lower apoptotic effects with consecutive stimulation of bone marrow towards increased granulopoesis [20]. Further more RelA was shown to play a key role in apoptotic regulation. Rel A deficient mice exhibited significantly increased apoptosis rate and embryonic lethality [20]. Apoptosis leads to diminished phagocytic capacity and synthesis of pro-inflammatory mediators while pro-inflammatory reaction followed by reduced apoptosis rate in PMN and consecutive phosphorylation of I-κB was demonstrated [29].

Following the transcriptional NF-κB signalling, IL-8 expression was increased on admission to be rapidly reduced. IL-8 expression was shown to be mainly induced by NF-κB [3, 14]. Over the whole analysed period, significantly increased neutrophilic IL-8 mRNA expression was found after major trauma as compared to healthy volunteers. The initial increase of IL-8 mRNA expression over all patients appeared stronger than the up-stream NF-κB translocation. This phenomenon might be due to initial posttraumatic priming effects as well as to alternative transcriptional pathways responsible for IL-8 induction such as AP1 [38]. Further more, NF-κB undergoes degradation within 90 minutes after trauma. Reduction of pro-inflammatory mediators release due to I-κB stabilization following major trauma has been previously described [40]. While p*ost mortem* analysis of plasmatic IL-8 concentration revealed significantly higher values in traumatically deceased patients [19], significantly elevated plasma IL-8 level was recorded in respect of developing MODS after major trauma [13]. Following our results, IL-8 mRNA expression was significantly reduced in patients exhibiting highly affected organ functions (MOF >4). In accordance, significant induction of IL-8 mRNA expression was found in surving patients as well as in patients with moderate MOF.

Increased neutrophilic ROS were investigated in septic patients as well as in septic shock due to higher apoptosis rate [25]. While elastase synthesis seems to be significantly influenced by trauma and hemorrhage (5, 35], in our hands, systemic release of elastase was only inconstantly increased in the higher MOF group but not in non-survivors.

In vitro effects of IL-8 on PMN are increased chemotaxis, adherence and degranulation [39]. Within posttraumatic ARDS as well as within ischemia-reperfusion syndrome, IL-

8 was demonstrated to increase PMN migration in the tissue as well as to prolong inhibition of apoptosis by these tissue damaging effects [21]. No specific inhibitors of IL-8 have been identified up to now [26, 28]. IL-8 expression after elective surgery was shown to be constant [40]. Following our analysis, no change of cytokine expression could be investigated due to surgical interventions in the posttraumatic period

In densitometric analysis of EMSA results, a significant increase of NF-κB translocation was found within the first 90 minutes after trauma and after 6 h. The second peak after 24 h fits well to the previously described biphasic immunological activation in monocytic cells after trauma [20].

No NF-κB activity in monocytic cells was found by initial detection on day one after trauma [1]. NF-κB reduction was shown to be preceded by an initial significant posttraumatic increase as compared to healthy volunteers [6, 34].

For validation of injury severity the NISS in the modification of *Osler et al* was used which reflects the sum of squares of the most severe traumatic lesions regardless of the anatomic region [4, 31]. In accordance to the valid paradigm [10] but in contrast to further investigation of I-κB in major trauma patients, significantly increased NF-κB activity as well as increased down-stream IL-8 expression was found in patients exhibiting a moderate injury severity (NISS < 24).

As including criteria a NISS over 16 points was chosen as a parameter of critical increased incidence for the development of posttraumatic MOF [37]. Within a retrospective analysis of 1,278 multiple trauma patients an 80 % incidence for SIRS development as well as for MODS (85 %) could be shown in correlation with high NISS.

In conclusion we investigated neutrophilic transcriptional activity of NF-κB in relation to IL-8 mRNA expression briefly after major trauma within this pilot study regarding the anatomical injury severity, the development of organ failure as well as the clinical outcome. Thereby, a concomitant initial increase of transcriptional activity and the IL-8 mRNA expression could be elucidated in the early posttraumatic period which precedes the down-regulation of the innate immune system. The demonstrated biphasic increase of the nuclear factor κB as well as of the down-stream IL-8 expression fits well to the previously described biphasic dynamic immune response to injury. We demonstrated a correlation of high NF-κB, IL-8 levels and increased mortality after trauma.

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