Are Pentraxin 3 and Transsignaling Early Markers for Immunologic Injury Severity in Polytrauma?

A Pilot Study

Christian Kleber MD, Christopher A. Becker MD,
Katharina Schmidt-Bleek DVM, PhD,
Klaus D. Schaser MD, Norbert P. Haas MD

Published online: 19 March 2013
© The Association of Bone and Joint Surgeons® 2013

Abstract

Background Inflammatory-related conditions and organ failure (OF) lead to late trauma mortality. Cytokine profiles can predict adverse events and mortality, potentially guiding treatment strategies (damage control surgery versus early total care). However, the specific cytokines to predict the clinical course in polytraumatized patients are not fully identified.

Questions/purposes We investigated the early pentraxin 3 (PTX3), IL-6, soluble IL-6 receptor (sIL-6R), and transsignaling ratio (TSR) in polytraumatized patients to

estimate immunologic injury severity and predict OF and survival.

Methods We prospectively followed 58 patients with severe polytrauma, six patients with minor trauma, and 10 healthy volunteers. The mean Injury Severity Score (ISS) was 43 points and the mean Hannover Polytrauma Score (PTS) was 59 points, with a consequently high mortality rate (30%). Twenty-seven of the 58 polytraumatized patients (46%) developed OF, 67% systemic inflammatory response syndrome, and 38% sepsis.

Results Mean sIL-6R concentrations in polytrauma initially were low. Mean PTX3 concentrations were high and peaked at 24 hours. The mean TSR peaked at 6 hours; at that time, the mean value was higher for nonsurvivors. PTX3 concentrations at admission were associated with injury severity calculated by ISS and PTS. Higher PTX3 serum concentrations 24 hours after admission correlated with lower probability for survival.

Conclusions PTX3, sIL-6R, and TSR were early markers for posttraumatic inflammatory status, OF, injury severity, and TSR for survival after polytrauma. The temporal profile of PTX3 and TSR might be used to anticipate the total injury severity and the clinical course and thereby guide decision making in polytraumatized patients.

Level of Evidence Level II, prognostic study. See Instructions for Authors for a complete description of levels of evidence.

The institution of one or more of the authors (CK) received, during the study period, funding from the Deutsche Forschungsgemeinschaft (German Research Foundation, Bonn, Germany) through the Berlin-Brandenburg School for Regenerative Therapies (GSC 203). Each author certifies that he or she, or a member of his or her immediate family, has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research* editors and board members are on file with the publication and can be viewed on request. Each author certifies that his or her institution approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.

C. Kleber (✉), C. A. Becker, K. Schmidt-Bleek,
K. D. Schaser, N. P. Haas
Center for Musculoskeletal Surgery and Julius Wolff Institute,
Charité–Universitätsmedizin Berlin, Augustenburger Platz 1,
13353 Berlin, Germany
e-mail: christian.kleber@charite.de

C. Kleber
Berlin-Brandenburg Center for Regenerative Therapies,
Charité–Universitätsmedizin Berlin, Berlin, Germany

Introduction

Trauma is the leading cause of death in young adults [14]. The new bimodal distribution of trauma-related deaths is divided into early and late mortality with two “hot spots” of traumatic death: during the prehospital time and during

the time in the intensive care unit (ICU) [14]. Inflammatory-related conditions (systemic inflammatory response syndrome [SIRS], sepsis) and organ failure (OF) are responsible for late trauma mortality [14, 23].

The two types of surgical management in multiply injured patients are generally early total care (ETC) or generally lesser (damage control) orthopaedic surgery (DCO). Whereas ETC involves definitive treatment including internal fixation soon after trauma, DCO means stopping the bleeding and contamination followed by temporary fracture stabilization (eg, external fixator) and ICU treatment.

Early detection or prediction of inflammatory adverse events in the management of polytrauma would give the trauma surgeon the opportunity to adapt individual management strategies and avoid secondary adverse clinical events [23]. The two surgical approaches influence the development of secondary adverse clinical events in distinct ways. DCO reportedly reduces the incidence of OF [8]. However, DCO increases the hospitalization phase, number of needed operations, and total costs [24, 31]. Therefore, to appropriately allocate patients to DCO or ETC, it is important to have diagnostic tools predicting OF and mortality in polytrauma within 24 hours after injury. Anatomic injury severity scores, eg, the Injury Severity Score (ISS) [3], reflect only parts of the real clinical injury severity because no physiologic or immunologic parameters are considered.

Capillary leakage and endothelial dysfunction lead to an overwhelming posttraumatic proinflammatory response with shortened microcirculation, which provokes OF [21]. The proinflammatory IL-6 is basically involved in the regulation of the posttraumatic acute-phase response [11]. The IL-6 interactions have been extensively investigated for several traumatic conditions (burns, surgical interventions, injury severity, soft tissue injury, thoracic injury, OF, mortality) [5, 6, 11, 21, 25, 28, 30, 31], but the role of the agonistic function of the soluble IL-6 receptor (sIL-6R) is unclear [12, 13, 22, 27]. Normally, IL-6 binds to its membrane-bound receptor (IL-6R) regulating intracellular gene transcription (JAK-STAT, MAP-kinase pathway). After alternative splicing (intracellular production of cytokine receptor without transmembrane domain) and shedding (cleavage of the transmembrane domain by bacterial/endogen enzymes), the IL-6R can be disunited from its transmembrane protein gp130, creating the sIL-6R. Remarkably, the sIL-6R in blood serum can bind to IL-6, forming the agonistic IL-6/sIL-6R complex. This complex can directly bind to gp130. gp130 is expressed in almost every cell line, rendering normally IL-6 cytokine nonresponsive cells (no IL-6R) capable of responding to IL-6/sIL-6R, vastly enlarging the spectrum of IL-6 activities. This mechanism is called transsignaling and might be a fundamental explanation for the unspecific overwhelming posttraumatic inflammatory reaction [13]. The transsignaling mechanism is involved in hemorrhagic shock [22], cancer [27],

chronic autoimmune diseases [27], and sepsis [22], thus representing a decisive factor during severe trauma. Based on these observations, we suggest high IL-6 and low sIL-6R concentrations (consumption due to IL-6/sIL-6R complex formation and binding to gp130) could be markers for high inflammatory response. We implement a new ratio, the transsignaling ratio (TSR; see Patients and Methods). We do not believe a single cytokine has the ability to reflect the complex posttraumatic inflammatory status. Therefore, we will likely need to detect key cytokines regulating post-traumatic immune response via cytokine crosstalk and multidirectional signaling pathways to establish an individual cytokine profile for each polytraumatized patient. Pentraxin 3 (PTX3) is one of these promising cytokines. The trauma-relevant interactions of IL-6/sIL-6R and PTX3 are illustrated in a schematic drawing (Fig. 1). PTX3 is an extrahepatic acute-phase protein produced in all trauma-relevant cells (endothelial cells, lung epithelial cells, fibroblasts, adipocytes, synovial cells, monocytes, dendritic cells, myocytes, chondrocytes) and is involved in ischemia [1, 4, 7, 9, 20, 29], reperfusion [29], sepsis [1, 4, 17], and tissue and lung injury [1, 7]. Because of the extrahepatic formation and induction by various trauma-related cytokines (IL-1, IL-6, TNF- α), PTX3 is likely involved in the pathophysiologic mechanisms in trauma, although this has not been confirmed.

We addressed the following questions: (1) What are the temporal profiles of immunologic parameters for polytraumatized patients? (2) Are there differences in the sIL-6R and PTX3 concentrations and TSR among healthy subjects, patients with minor trauma, and patients with polytrauma? (3) Are there differences in the temporal profiles of sIL-6R

Fig. 1 A flowchart shows the trauma-associated IL-6 cytokine family and PTX3 interactions. LPS = lipopolysaccharide.

and PTX3 concentrations and TSR between survivors and nonsurvivors? (4) Are there differences in sIL-6R and PTX3 concentrations and TSR between polytraumatized patients with and without OF, SIRS, or sepsis? And (5) is sIL-6R or PTX3 associated with anatomic injury severity as assessed by the ISS [3] and Hannover Polytrauma Score (PTS) [26]?

Patients and Methods

In this prospective observational pilot study, we enrolled 58 patients with polytrauma (Group I), six patients with minor trauma (Group II), and 10 healthy volunteers (Group III) from April 2009 to March 2012. Inclusion criteria were: Group I: (1) patients with polytrauma, (2) age of 18 years or more, (3) ISS of greater than 25 points, (4) admission less than 2 hours and transfer less than 6 hours after trauma with a therapy-free interval of less than 30 minutes; Group II: (1) minor trauma, (2) age of 18 years or more, (3) ISS of 15 points or less, (4) admission less than 2 hours and transfer less than 6 hours after trauma with a therapy-free interval of less than 30 minutes; and Group III: (1) healthy subjects, (2) age of 18 years or more, (3) no infectious disease in less than 1 month. The IL-6, sIL-6R, TSR, and PTX3 values of Groups II and III (minor trauma, healthy) were compared to those of Group I (polytrauma) to reveal the impact of injury severity on cytokine expression. Furthermore, Group III was used to verify and determinate the standard values for IL-6, sIL-6R, TSR, and PTX3 in healthy humans. A patient was screened whenever the study team was alerted or aware of admission of a polytraumatized patient to our trauma center and the inclusion criteria were fulfilled. Our series of patients with polytrauma was not consecutively selected. The study cohort (Group I) consisted of patients with severe polytrauma (mean $\pm$ SD: ISS, 43 ± 14 points; PTS, 52 ± 19 points), which was reflected by a high mortality rate ($n = 17$, 30%). In consequence of the severe trauma, 46% developed OF (23% renal/intracranial failure, 42% multiple OF), 67% SIRS, and 38% sepsis. The sepsis origin was wound infection in 50%, pneumonia in 27%, and catheter-associated infection in 23%. SIRS was detected in 56% on the day of trauma, 48% on the first day, 39% on the second day, 32% on the third day, and 25% on the fourth day after trauma. Established critical care scores 24 hours after trauma (Sequential Organ Failure Assessment [SOFA] [10], 8 ± 4 ; Simplified Acute Physiology Score II [SAPS II] [19], 39 ± 18 ; Acute Physiology and Chronic Health Evaluation II [APACHE II] [16], 20 ± 9) underlined the uniquely high injury severity. The overall survival calculated from the Revised Injury Severity Classification (RISC) [18] calculated overall survival was 58%. Compared to the observed survival of 70% in our study cohort, the performed trauma management in our center posed a 12% survival advantage.

To measure the sIL-6R and PTX3 serum concentrations, blood samples from Groups I and II were drawn at admission, prepared, centrifuged, and stored at -80°C . For Group I, additional blood was drawn 6, 24, 48, 72, and 96 hours after admission to reveal the temporal profile of sIL-6R and PTX3 after admission, as well as the posttraumatic changes of C-reactive protein (CRP), procalcitonin (PCT), IL-6, and white blood cell (WBC) count. The sIL-6R (dilution 1:50; Quantikine® Human IL-6sR Immunoassay DR 600; R&D Systems, Wiesbaden-Nordenstadt, Germany) and PTX3 serum concentrations (Quantikine® Human Pentraxin 3/TSG-14 Immunoassay DPTX30; R&D Systems) was quantified in triplicate by ELISA. PTX3 ELISA was established and validated for serum polytrauma blood samples (1:16 dilution) by spike and recovery test (100%, R&D Systems). ELISA readout was performed with a microplate reader at 450 nm, with correction at 570 nm. We evaluated the readings with Microplate Manager™ Version 5.2 software (Bio-Rad Laboratories, Inc, Hercules, CA, USA).

The general IL-6 cytokine activation (cytokine plus agonistic receptor-mediated transsignaling) was estimated by establishing the TSR: quotient of IL-6 (pg/mL) and sIL-6R (ng/mL). TSR was calculated for each time point.

We obtained and/or calculated the following information from the patients charts: sex, age, ISS, PTS, RISC [18], SIRS [2], sepsis, sepsis origin, OF, type and origin of OF, SAPS II [19], APACHE II [16], and SOFA [10].

The mean and SD were calculated. The single IL-6, sIL-6R, TSR, and PTX3 serum concentrations and temporal profile were compared between patients with and without SIRS, sepsis, or OF and between survivors and nonsurvivors. To analyze differences in the sIL-6R and PTX3 concentrations and TSR among healthy subjects, patients with minor trauma, and patients with polytrauma and between survivors and nonsurvivors, we used the Mann-Whitney U test. For differences in the temporal profiles of sIL-6R and PTX3 concentrations and TSR, we used the Wilcoxon test. The differences in sIL-6R and PTX3 concentrations and TSR between polytraumatized patients with and without OF, SIRS, or sepsis were tested by the Mann-Whitney U test. The correlation of sIL-6R or PTX3 with anatomic injury severity was assessed by a correlation test. We performed the statistical analyses using PASW® 20.0 software (SPSS Inc, Chicago, IL, USA).

Results

The mean values and temporal profiles of all measured immunologic parameters (IL-6, sIL-6R, PTX3, CRP, PCT, WBC) for the polytraumatized patients (Group I) are shown (Table 1). We observed a typical delayed increase of CRP compared to IL-6 and PTX3. The maximum mean

Table 1. Temporal profiles of immunologic parameters for the polytraumatized patients

Parameter	0 hours	6 hours	24 hours	48 hours	72 hours	96 hours
IL-6 (pg/nL)	376 ± 476	1336 ± 2902	642 ± 858	426 ± 462	210 ± 355	203 ± 419
sIL-6R (ng/mL)	21 ± 8	26 ± 9	23 ± 7	20 ± 6	20 ± 6	27 ± 11
PTX3 (ng/mL)	2 ± 3	9 ± 8	61 ± 69	33 ± 24	42 ± 105	44 ± 153
CRP (mg/dL)	0.5 ± 1	0.4 ± 0.4	5 ± 4	20 ± 8	20 ± 10	19 ± 10
PCT (µg/L)	0.4 ± 0.2	0.8 ± 1	5 ± 9	11 ± 25	11 ± 26	7 ± 10
WBC count (number/nL)	15 ± 9	7 ± 4	9 ± 4	9 ± 5	9 ± 5	9 ± 4

Values are expressed as mean ± SD; sIL-6R = soluble IL-6 receptor; PTX3 = pentraxin 3; CRP = C-reactive protein; PCT = procalcitonin; WBC = white blood cell.

IL-6 and PTX3 concentrations were observed at 6 and 24 hours after admission, respectively. The infectious laboratory parameters CRP (48–72 hours after admission) and PCT (48–72 hours after admission) were late markers for posttraumatic inflammation.

The mean initially measured sIL-6R blood concentration in polytraumatized patients (Group I) at admission (21 ng/mL) was low compared to that of patients with minor injuries (Group II; 27 ng/mL) and the standard value in the healthy volunteers (Group III; 80 ng/mL). Furthermore, the mean sIL-6R concentrations in Group I stayed low during the entire observation period (Fig. 2). In contrast, the mean IL-6 blood concentrations were elevated in Group I for the whole observation period, with peak concentration 6 hours after admission (Fig. 3). The mean PTX3 blood concentrations 24 hours after admission in Group I (61 ± 69 ng/mL) were also high compared to Group II (27 ± 11 ng/mL) and Group III (< 2 ng/mL) (Fig. 4). Similar to IL-6 and sIL-6R, the TSR had its peak mean concentration 6 hours after admission (53 ± 124), followed by a constant decrease over the observation period (Fig. 5).

The mean sIL-6R concentrations increased and decreased over the different time points in survivors but not in nonsurvivors (Fig. 6). The mean TSR 6 hours after admission was higher ($p = 0.04$) in nonsurvivors (182 ± 286) than in survivors (29 ± 39). No differences could be detected for mean IL-6 and sIL-6R concentrations alone between survivors and nonsurvivors.

Higher TSR ($p = 0.04$, 0.03, and 0.01) and IL-6 ($p = 0.02$, 0.03, and 0.005) values were seen 24, 48, and 72 hours after admission in patients with OF. Also, higher TSR ($p = 0.009$) and IL-6 ($p = 0.03$) values 24 hours after admission were detected in patients with sepsis. Similarly, the TSR was elevated ($p = 0.01$, 0.008 and 0.03) in patients with SIRS 6, 24, and 72 hours after admission. In addition to TSR and IL-6, high inflammatory parameters (PCT, WBC, PTX3) 48 hours after admission were all associated with the development of OF. Elevated PTX3 blood concentrations 24 hours after admission were associated ($p = 0.03$) with SIRS. We found no association with survival and sepsis.

The PTX3 blood concentration at admission was associated ($p < 0.001$) with the injury severity as assessed by the ISS and PTS. Furthermore, higher PTX3 serum concentrations after 24 hours correlated ($R^2 = 0.542$; $p < 0.001$) with lower probability for survival as calculated by RISC score.

Discussion

Inflammatory-related conditions such as SIRS, sepsis, and OF are responsible for late trauma mortality. Cytokine profiles have the potential to predict adverse events and mortality and guide treatment strategies (DCO versus ETC). We therefore asked the following questions: (1) What are the temporal profiles of immunologic parameters for polytraumatized patients? (2) Are there differences in the sIL-6R and PTX3 concentrations and TSR among healthy subjects, patients with minor trauma, and patients with polytrauma? (3) Are there differences in the temporal profiles of sIL-6R and PTX3 concentrations and TSR between survivors and nonsurvivors? (4) Are there differences in sIL-6R and PTX3 concentrations and TSR between polytraumatized patients with and without OF, SIRS, or sepsis? And (5) is sIL-6R or PTX3 associated with anatomic injury severity as assessed by the ISS and PTS?

Limitations of our study include the following. First, we used a single-center approach. Since the type and quality of initial trauma management influence evolving adverse clinical events and subsequent survival rates, this point may limit the applicability to other hospitals with less experience in polytrauma management and lower patient load. Berlin has the shortest rescue times throughout Germany (average time from accident until hospital admission, 52 minutes). Therefore, we admit patients in our resuscitation room who would not reach the hospital alive in rural areas of Germany. This results in higher mean ISSs and increased rates of early mortality and OF compared to regions with longer rescue times and less specific rescue systems [15]. Therefore, our results may not be representative of other regions and cities. On the other hand, our polytrauma management improves

Fig. 2 The temporal profile of the sIL-6R blood concentration in patients with polytrauma is shown. Low sIL-6R concentrations are seen, without a clear maximum. Horizontal line = time point after admission; box = concentration with SD; whiskers = CI.

Fig. 3 The temporal profile of the IL-6 blood concentration in patients with polytrauma is shown. High IL-6 blood concentrations are seen, with a maximum concentration at 6 hours after admission. Horizontal line = time point after admission; box = concentration with SD; whiskers = CI.

the calculated survival (RISC), potentially lowering the incidence of adverse events and maybe secondary increase of cytokines due to septic complications. Therefore, we might record higher rates of early mortality and OF and higher injury severity compared to regions with longer rescue times and rescue systems without emergency physicians. Second, we could not include all patients meeting our

inclusion criteria, thus introducing the possibility of selection bias. Comparing the characteristics of our study collective with our data from the German Trauma Registry since 2005, the included patient collective mirrors our typical trauma collective with high injury severity, trauma mechanism, mortality rate, incidence of OF, and distribution of anatomic injuries. Therefore, we can exclude a huge

Fig. 4 The temporal profile of PTX3 blood concentration in patients with polytrauma is shown. Peak expression is seen 24 to 48 hours after admission. Horizontal line = time point after admission; box = concentration with SD; whiskers = CI.

Fig. 5 The temporal profile of TSR in patients with polytrauma is shown. Peak expression is 6 hours after admission. Horizontal line = time point after admission; box = TSR values with SD; whiskers = CI.

selection bias, justifying our results as representative of our trauma center. Third, a general problem with interpretation of our data and previously published data is the typically huge variations in cytokine concentrations between patients at single time points. We suspect these deviations are an expression of individual, genetic-based, sex- and age-dependent

immune responses. We believe the only way to minimize this natural deviation is to generate an immunologic fingerprint of our polytraumatized patients by using, eg, IL-1/IL-4/IL-6/IL-10 and CD4/CD8 ratios combined with physiologic parameters (lactate, base deficit, body temperature, fluid balance, oxygenation, ventilation). Together with the

Fig. 6 The temporal profiles of TSR in survivors and nonsurvivors are compared. Higher ($p = 0.04$) TSR 6 hours after admission is seen in nonsurvivors compared to survivors. Horizontal line = time point after admission; box = TSR values with SD; whiskers = CI.

anatomic injury severity and physiology, the trauma surgeon gets a total overview and impression of the polytraumatized patient, assisting decision making in the future. Further prospective studies are needed to reveal cutoff points for sIL-6 and PTX3 after trauma. Fourth, while dilution may result in low cytokine levels in the early phase after trauma due to massive volume resuscitation, we believe this unlikely because IL-6 and PTX3 blood concentrations increase at the same time in the same blood sample, while the sIL-6R concentration decreases.

We found the mean sIL-6R was lower in polytraumatized patients compared to monotraumatized patients and healthy individuals. sIL-6R concentration showed no increase or decrease over the different time points in nonsurvivors compared to survivors. Analyzing the early sIL-6R profile may assist in anticipating the outcome of patients with severe polytrauma. The low sIL-6R concentrations in nonsurvivors may reflect exhaustion of the immune system, resulting in an inadequate early immune response. To consolidate already known cytokine patterns and their clinical interactions, we tried to achieve a valid and practicable cytokine profile for daily clinical use, including sIL-6R. Furthermore, the sIL-6R concentrations seem to have a functional relationship with the injury severity. In contrast, PTX3 concentrations were higher in polytraumatized compared to monotraumatized and healthy individuals.

According to the above-mentioned pathophysiologic considerations, we fused the new knowledge of sIL-6R with the old knowledge of IL-6 and calculated a TSR for each time point of the observation period. TSR and IL-6 were associated with OF (24–72 hours) and sepsis (24 hours). TSR was the only parameter we tested that was capable of dividing nonsurvivors from survivors, with higher values in the nonsurvivor group. TSR is revealed as an early parameter to predict survival after trauma. The simultaneous measurement of IL-6 and sIL-6R within the first 24 hours after injury can assist the trauma surgeon in predicting survival, OF, and inflammatory adverse events.

While the TSR and sIL-6R could be markers to predict the impact of inflammation, sepsis, and OF, PTX3 appears as an early marker to estimate the total, anatomic, and inflammatory injury severity and survival probability. In contrast to sIL-6R, the PTX3 blood concentrations were increased, with peak expression 24 hours after polytrauma. Taking the known PTX3 blood concentrations for endotoxic shock, sepsis, and other inflammatory and infectious diseases (200–800 ng/mL) into account, the trauma-induced increases were moderately elevated [20]. The well-established inflammatory parameters CRP and PCT in our study had later peak levels (48–96 hours) and were slow markers of inflammation in polytrauma (Table 1). Measurement of CRP and PCT is not useful in the early phase of polytrauma.

management (< 48 hours). Therefore, PTX3 may allow detection of early inflammation in polytrauma. This supports our findings concerning sIL-6R and TSR, keeping in mind that IL-6 is a strong inducer of PTX3. Thus, extrahepatic PTX3 is predominately synthesized in cell lines involved in soft tissue injury (adipocytes, fibroblast, endothelial cells, muscle cells) after trauma [1, 20]. These observations lead us to suggest that PTX3 concentration may reflect the immunologic trauma impact caused by soft tissue injury. Based on this idea, we detected an association of PTX3 concentration at admission and the anatomic injury severity as assessed by the ISS and PTS. PTX3 concentration at admission appears to be a marker for assessing injury severity, and peak PTX3 expression 24 hours after admission correlates with the RISC-calculated survival. This underlines the unique pathophysiologic involvement of PTX3 in the posttraumatic inflammatory response. PTX3 may explain why anatomic scores, independent of age, preconditions, and physiologic status, can predict survival. The interactions of PTX3, induced by the most important trauma-relevant cytokines and produced by all cell lines involved in tissue trauma, are shown (Fig. 1). PTX3 stands at the crossroad of several cytokine-signaling pathways and has the ability to reflect a more common activation of inflammation. Therefore, PTX3 might be a promising cytokine for estimating inflammatory and anatomic injury severity. Because of its pathophysiologic interactions with atherosclerotic diseases, PTX3 might also reflect endothelial damage and dysfunction after trauma. Further studies are needed to prove PTX3 as a surrogate parameter for soft tissue damage.

Apart from specific differences of the sIL-6R concentration in survivors and nonsurvivors, we also found higher PTX3, sIL-6R, and TSR levels in patients with OF, SIRS, and sepsis at different time points. These changes underscore the pathophysiologic role of these cytokines in the posttraumatic immune response and their capability to potentially use them in an immunologic bedside test aimed to predict the clinical course after multiple trauma.

We detected specific differences in the sIL-6R, PTX3, and TSR levels in polytraumatized patients with OF, SIRS, and sepsis. The temporal profile of the sIL-6R concentration distinguished between survivors and nonsurvivors but was not associated with OF, SIRS, or sepsis. In contrast, the combination of sIL-6R and IL-6 concentrations in the TSR was higher in patients with OF (24, 48, 72 hours after admission), SIRS (6, 24, 72 hours after admission), and sepsis (24 hours after admission). These results underline the power of combined interpretation of cytokine profiles and the weakness of single cytokine measurement. High PTX3 concentrations at 24 hours after admission were associated with SIRS and might be used to predict proinflammatory status of polytraumatized patients. PTX3 concentration 48 hours after admission was additionally associated with OF.

sIL-6R, TSR, and PTX3 are promising markers to establish a bedside cytokine quick test to estimate the risk of OF and predict survival. PTX3, sIL-6R, and TSR are early markers for the posttraumatic inflammatory status, OF, injury severity, and survival after polytrauma. The temporal profiles of PTX3 and TSR might help to anticipate the clinical course and can guide decision making. Further studies will need to confirm these novel findings.

Acknowledgments The authors thank Norma Schulz and Prof. M. Klouche MD for their support, advice, and discussion.

References

1. Alberti L, Gilardini L, Zulian A, Micheletto G, Peri G, Doni A, Mantovani A, Invitti C. Expression of long pentraxin PTX3 in human adipose tissue and its relation with cardiovascular risk factors. *Atherosclerosis*. 2009;202:455–460.
2. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med*. 1992;20:864–874.
3. Baker SP, O'Neill B, Haddon W Jr, Long WB. The Injury Severity Score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma*. 1974;14:187–196.
4. Doni A, Peri G, Chieppa M, Allavena P, Pasqualini F, Vago L, Romani L, Garlanda C, Mantovani A. Production of the soluble pattern recognition receptor PTX3 by myeloid, but not plasmacytoid, dendritic cells. *Eur J Immunol*. 2003;33:2886–2893.
5. Flohe S, Lendemann S, Schade FU, Kreuzfelder E, Waydhas C. Influence of surgical intervention in the immune response of severely injured patients. *Intensive Care Med*. 2004;30:96–102.
6. Giannoudis PV, Harwood PJ, Loughenbury P, Van Griensven M, Krettek C, Pape HC. Correlation between IL-6 levels and the systemic inflammatory response score: can an IL-6 cutoff predict a SIRS state? *J Trauma*. 2008;65:646–652.
7. Han B, Mura M, Andrade CF, Okutani D, Lodyga M, dos Santos CC, Keshavjee S, Matthay M, Liu M. TNF α -induced long pentraxin PTX3 expression in human lung epithelial cells via JNK. *J Immunol*. 2005;175:8303–8311.
8. Harwood PJ, Giannoudis PV, van Griensven M, Krettek C, Pape HC. Alterations in the systemic inflammatory response after early total care and damage control procedures for femoral shaft fracture in severely injured patients. *J Trauma*. 2005;58:446–452; discussion 452–444.
9. Inoue K, Sugiyama A, Reid PC, Ito Y, Miyauchi K, Mukai S, Sagara M, Miyamoto K, Satoh H, Kohno I, Kurata T, Ota H, Mantovani A, Hamakubo T, Daida H, Kodama T. Establishment of a high sensitivity plasma assay for human pentraxin3 as a marker for unstable angina pectoris. *Arterioscler Thromb Vasc Biol*. 2007;27:161–167.
10. Janssens U, Graf C, Graf J, Radke PW, Konigs B, Koch KC, Lepper W, vom Dahl J, Hanrath P. Evaluation of the SOFA score: a single-center experience of a medical intensive care unit in 303 consecutive patients with predominantly cardiovascular disorders. Sequential Organ Failure Assessment. *Intensive Care Med*. 2000;26:1037–1045.
11. Jones SA. Directing transition from innate to acquired immunity: defining a role for IL-6. *J Immunol*. 2005;175:3463–3468.
12. Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM. The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *FASEB J*. 2001;15:43–58.

13. Jones SA, Richards PJ, Scheller J, Rose-John S. IL-6 transsignaling: the in vivo consequences. *J Interferon Cytokine Res.* 2005;25:241–253.
14. Kleber C, Giesecke MT, Tsokos M, Haas NP, Schaser KD, Stefan P, Buschmann CT. Overall distribution of trauma-related deaths in Berlin 2010: advancement or stagnation of German trauma management? *World J Surg.* 2012;36:2125–2130.
15. Kleber C, Lefering R, Kleber AJ, Buschmann CT, Bail HJ, Schaser KD, Haas NP. [Rescue time and survival of severely injured patients in Germany] [in German]. *Unfallchirurg.* 2012 March 16 [Epub ahead of print].
16. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med.* 1985;13:818–829.
17. Kunes P, Holubcova Z, Kolackova M, Krejsek J. Pentraxin 3 (PTX 3): an endogenous modulator of the inflammatory response. *Mediators Inflamm.* 2012;2012:920517.
18. Lefering R. Development and validation of the Revised Injury Severity Classification (RISC) score for severely injured patients. *Eur J Trauma Emerg Surg.* 2009;35:437–447.
19. 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;270:2957–2963.
20. Mantovani A, Garlanda C, Doni A, Bottazzi B. Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. *J Clin Immunol.* 2008;28:1–13.
21. Matzinger P. The danger model: a renewed sense of self. *Science.* 2002;296:301–305.
22. Mees ST, Toellner S, Marx K, Faendrich F, Kallen KJ, Schroeder J, Haier J, Kahlke V. Inhibition of interleukin-6-transsignaling via gp130-Fc in hemorrhagic shock and sepsis. *J Surg Res.* 2009;157:235–242.
23. Moore F, Moore E. Evolving concepts in the pathogenesis of postinjury multiple organ failure. *Surg Clin North Am.* 1995;75:257–277.
24. Pape HC, Giannoudis P, Krettek C. The timing of fracture treatment in polytrauma patients: relevance of damage control orthopedic surgery. *Am J Surg.* 2002;183:622–629.
25. Pape HC, Griensven MV, Hildebrand FF, Tzioupis CT, Sommer KL, Krettek CC, Giannoudis PV. Systemic inflammatory response after extremity or truncal fracture operations. *J Trauma.* 2008;65:1379–1384.
26. Regel G, Pape HC, Pohlemann T, Seekamp A, Bosch U, Tschern H. [Scores as decision aids] [in German]. *Unfallchirurg.* 1994;97:211–216.
27. Rose-John S, Scheller J, Elson G, Jones SA. Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J Leukoc Biol.* 2006;80:227–236.
28. Rotstein OD. Modeling the two-hit hypothesis for evaluating strategies to prevent organ injury after shock/resuscitation. *J Trauma.* 2003;54:S203–S206.
29. Souza DG, Soares AC, Pinho V, Torloni H, Reis LF, Teixeira MM, Dias AA. Increased mortality and inflammation in tumor necrosis factor-stimulated gene-14 transgenic mice after ischemia and reperfusion injury. *Am J Pathol.* 2002;160:1755–1765.
30. Strecker W, Gebhard F, Rager J, Bruckner UB, Steinbach G, Kinzl L. Early biochemical characterization of soft-tissue trauma and fracture trauma. *J Trauma.* 1999;47:358–364.
31. Stubig T, Mommsen P, Krettek C, Probst C, Frink M, Zeckey C, Andruszkow H, Hildebrand F. [Comparison of early total care (ETC) and damage control orthopedics (DCO) in the treatment of multiple trauma with femoral shaft fractures: benefit and costs] [in German]. *Unfallchirurg.* 2010;113:923–930.