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The diagnostic value of early cytokine response in patients after major trauma – preliminary report

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Abstract

This study was aimed to give a better understanding of the mechanisms of early immune response to trauma by assessing the concentration of cytokines in peripheral blood. The study group comprised 32 patients admitted to the Emergency Department due to injury. Depending on the magnitude of the Injury Severity Score (ISS) trauma patients were divided into two groups. In group A (ISS ≥ 20), 13 patients had complications, and five died, while in group B (ISS < 20) only three patients had complications (e.g. respiratory failure and infections). Depending on the extent of the injury, significant differences were observed in the concentrations of cytokines in the treatment groups. The highest levels of IL-6 and IL-1Ra in both groups were recorded in the third hour of hospitalisation and were considerably higher in group A compared to the concentration of these cytokines in group B ($p = 0.001$). In patients with complications, IL-6 and IL-1Ra concentrations were significantly higher compared to those without complications. Spearman's rho-correlation showed a statistically significant positive correlation between baseline concentrations of IL-6 ($r = 0.64$, $p < 0.001$) and IL-1Ra ($r = 0.37$, $p = 0.042$) and the values of the ISS. A high diagnostic sensitivity calculated from ROC curves was found for IL-6 concentrations. In summary, our findings suggest that elevated levels of the cytokines tested, determined in the peripheral blood shortly after injury, may be significantly associated with the occurrence of severe complications, which in some patients can lead to death. Monitoring the levels of these cytokines in patients with a high risk of serious complications should be used routinely.

Key words: *inflammation, IL-6, trauma, sepsis, MODS, IL-1Ra.*

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Introduction

According to the World Health Organisation, traumas are still one of the main causes of severe disability and death [1, 2]. Severe infections and multiple organ dysfunction syndrome (MODS) are among the most severe traumatic complications burdened with high mortality [3]. The risk of severe infection and MODS is increased by immune disorders accompanying trauma, the mechanisms of which are still poorly understood. There are a number of hypotheses explaining the mechanisms of organ dysfunction that occurs after trauma, regardless of the cause of the injury. One of these hypotheses points to the increased pro- and anti-inflammatory response in the first hours after major trauma, but does not precisely explain the difference between physiological and pathological immune response to injury [4]. A key role in the pathological response to major trauma or infection is played by mediators of systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response syndrome (CARS), which are produced in "excess" and can quickly lead to the damage of organs not affected by the trauma, and the patient's death [5]. On the other hand, it was found that patients without septic and organ complications, after extensive trauma, can also demonstrate increased production of pro- and anti-inflammatory mediators [6]. It has been found that the early innate immune response to trauma such as SIRS or CARS can be not only part of the physiological but also the pathological reaction [7-10].

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It has been observed that the inflammatory reaction has two stages, which are not precisely spaced in time. A simplified model of immune system reaction to injury assumes that the first stage is the proinflammatory phase, in which inflammatory cytokines are the primary mediators (e.g. TNF-α, IL-1, IL-6). The second stage is an anti-inflammatory phase, wherein the increased activity of the inflammatory cytokines stimulates the synthesis of anti-inflammatory cytokines (e.g. IL-4, IL-10, TGF-β) with a strong immunosuppressive effect [7-10]. Most recent genetic studies suggest that pro- and anti-inflammatory reactions can proceed in parallel, which is another factor complicating the discrimination between physiological and pathological response [11, 12]. From a clinical point of view, this problem is essential for improving the results of treatment because early detection of pathological response to trauma and infection may be important in predicting the course of disease, and starting proper diagnostics and early treatment. Persistent infection in the wound, infected tissue necrosis, or the presence of another undiagnosed source of infection, which is found only during autopsy (e.g. in the respiratory tracts), exacerbate local and systemic immune disorders, which may lead to the development of severe sepsis and multiple organ dysfunction syndrome [7-12]. In the classical model of the inflammatory response to injury, CARS reaction is supposed to lead to a reduction in the severity of SIRS proinflammatory response in order to achieve a balance between pro- and anti-inflammatory response, but in fact in some patients it leads to an increase of cellular immune disorders and severity of immunosuppression [11]. Determining the quantitative relationship between excessive inflammatory response and increasing immunosuppression remains an unresolved problem [12]. It still has not been established whether an early, excessive inflammatory response or the parallel intensification of immunosuppression of immune cells is a more important factor leading to severe organ complications. The solution to this problem may be important for the efficacy of an appropriate therapy (e.g. early immunomodulatory biological therapy), the aim of which, on the one hand, should be to limit the excessive inflammatory response, and on the other to stimulate the cells regulating the immune response to trauma and to reduce immunosuppression. Efforts to stimulate cellular immunity in critically ill patients with infections are promising [13], while attempts to reduce the inflammatory response to trauma are still not very effective [14].

This study was aimed to better understand the mechanisms of early immune response to trauma. On the basis of the measured parameters (assessing the concentration of cytokines in peripheral blood), an attempt was made to determine immune markers, which, depending on the size of the trauma, may have practical significance in the early diagnosis of severe post-traumatic complications. The selection of cytokines was made on the basis of previous studies assessing the impact of surgery on the immune system [15].

Material and methods

The study group comprised 32 patients, including 23 men and nine women, age range 18-80 years, average 42.74 ±18.41 years, admitted to the Emergency Department (ED) of the Bielański and Praski hospitals due to trauma. The largest group consisted of patients after traffic accidents with multi-organ injuries. The characteristics of the studied groups are presented in Table 1.

The Injury Severity Score (ISS), which is most commonly used in the international classification of trauma severity, was used to assess the severity of the patients' status [16]. The entire group of patients, depending on the severity of the condition and the extent of injury, was divided into two subgroups, where group A ($n = 20$) included patients with ISS ≥ 20 points and group B ($n = 12$) patients with ISS < 20 points. This division was adopted due to the specificity of trauma of the studied groups to better assess the impact of the extent of the injury on the investigated immune parameters. ISS is the sum of the squares of the three highest scores in the Abbreviated Injury Scale (AIS) for the values of three body areas. According to this scale, severe body injury in most publications is defined as > 15 points on the ISS. In patients with ISS < 15 points, the mortality is less than 5%, and for the values of ISS in the range of 16-24 points, the mortality is at the level of 11-14%, while for the ISS > 25 points, the mortality rate is over 36% [17, 18].

All patients were assessed for the concentrations of selected cytokines (IL-6, IL-1Ra) at the time of admission to the ED (0 h) and at 3, 6, 12, and 24 hours of hospitalisation. The control group for the analysis of cytokine concentrations consisted of 20 healthy volunteers in similar age range and gender. Routine laboratory tests were performed in all patients. Clinical monitoring was focused on the following parameters: type of injury and treatment, including the number of operations/reoperations, type and number of complications, length of hospital stay and duration of respiratory treatment, and mortality.

The study did not qualify patients over 80 years of age, persons after chemo- or radiotherapy, patients with pre-existing chronic liver disease (cirrhosis), kidney diseases, diabetes mellitus, chronic inflammatory diseases, and patients chronically treated before admission to the hospital with steroids or non-steroidal anti-inflammatory drugs.

Cytokine measurement

The analysis of cytokine concentrations in peripheral blood plasma was performed on the basis of commercially available kits for ELISA enzymatic immunoassay, in accordance with the instructions provided by the manufacturer (Quantikine Immunoassay human IL-1Ra and human IL-6, R & D Systems Europe, Minneapolis, USA). The sample of venous blood was centrifuged at a speed of 3200/min for 15 minutes, then plasma for further tests was

Table 1. The characteristics of the studied groups of patients

Parameters		**The studied group of patients**	
		A, *n* = 20	**B, *n* = 12**
Age [years]		43.31 ±18.71	41.83 ±17.89
Sex [M : F]		16 : 4	7 : 5
Type of injury	Polytrauma	15	1
	Head damage	2	3
	Chest trauma	–	1
	Abdominal injury	–	–
	Trauma of the upper limbs	–	–
	Trauma of the lower extremities	1	5
	Burns	2	–
	Trauma manifold	1	1
Treatment (ad hoc procedure)	Surgery:		
	Laparotomy	2	–
	Splenectomy	1	–
	sewing kidney	1	–
	Orthopaedic surgery (repositions, fixation, stabilisation, prosthetics)	8	7
	Drainage of pneumothorax	3	–
	Respirator	6	
	Transfusions	7	1
	Other	17	5
Complications	MODS	3	–
	Respiratory failure	5	–
	Infection (general, local)	4	4
	Internal bleeding	4	–
	Pulmonary (embolism/stroke)	2	–
	Poorly healing wounds	3	1
	Other (fever, post-traumatic epilepsy)	9	1
Hospitalisation time	< 24 h	5	5
	< 1-7 days	3	4
	< 8-14 days	5	2
	> 15 days	7	1
Death		5	

stored at –80°C. The lower limit of assay sensitivity was 0.70 pg/ml and 22 pg/ml for IL-6 and IL-1Ra, respectively. Concentration readings were carried out using a DIALAB ELX 808 spectrophotometer and Gen 51.10 software. The concentration of cytokines was evaluated by comparing the absorbance values to a standard curve prepared by measuring the absorbance of samples of known concentration at a wavelength of $\lambda = 450$ nm. The results are presented as median (range) pg/ml. According to the results in the control group, the assumed normal range for IL-6 was on average 17.61 ±11.01 pg/ml, and 1098.08 ±446.29 pg/ml for IL-1Ra.

Ethics

The study acquired the consent of the Bioethics Committee of the Medical University of Warsaw. Each patient gave written or oral consent to perform the research and access their disease history. All procedures were in accordance with the Declaration of Helsinki.

Statistical analysis

Comparisons of concentration values of the tested parameters with the norm and between groups were performed using the Mann-Whitney *U* test. The significance

of changes in parameter concentrations was tested using the Wilcoxon test. The relationship between the change in concentration over time and the occurrence of complications was evaluated using the chi-square test. The influence of age or sex on the concentration of the tested cytokines was assessed based on the analysis using the χ^2 independence test and the Mann-Whitney U test. The relationship between cytokine levels and the extent of the injury was checked by the rho-Spearman correlation test. A series of Receiver Operating Characteristic (ROC) curve analysis was carried out to assess whether the likelihood of serious complications, including death, can be assessed based on the cytokine levels and the ISS. The level of significance was assumed at $p < 0.05$. The analyses were performed using SPSS 13.0 for Windows.

Results

Clinical part

In the presented material, the main causes of multiple organ injury were traffic accidents and falls from height, found in 53% of patients. Of the 32 patients, 22 (68.75%) required hospitalisation for more than 24 hours, including five patients treated in the intensive care unit (ICU). Seven patients underwent emergency surgeries (splenectomy, exploratory laparotomy, repositions and bone fixation, chest tube insertion), including two patients who required reoperation due to internal bleeding. Moreover, eight patients required further deferred surgical interventions (repositions or bone fixation). Of the entire group of 32 patients in which cytokines were measured, 16 patients (50%) had complications. The highest number of complications was observed in group A (n = 13/20, ISS ≥ 20 points), while in group B (ISS < 20 points) they occurred in 3/12 patients (Table 1). In the group of the most seriously ill, two patients died within 12 h of admission to the ED, and the other three on the fifth day of treatment in a hospital ward. Patients with lighter injuries, after relevant medical procedures, were discharged home or transported to other units within 12 h.

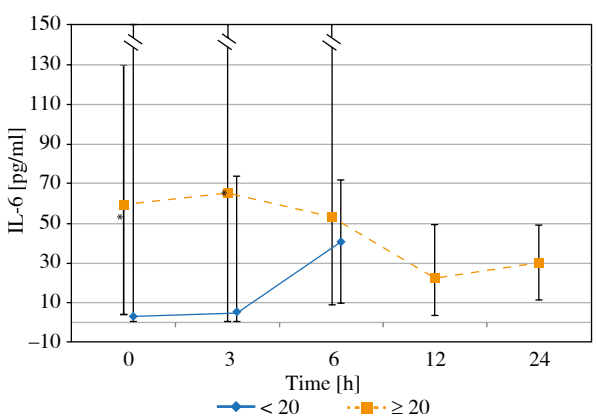

Fig. 1. IL-6 concentration (pg/ml) in relation to the extent of the injury (according to the Injury Severity Score [ISS] score). *$p < 0.05$; group A ISS ≥ 20; group B ISS < 20

Changes in cytokine concentration

Depending on the extent of the injury, significant differences were observed in the concentrations of cytokines in the treatment groups (A vs. B). In group A, in patients with severe injury (ISS ≥ 20 points), baseline concentrations of IL-6 at the time of admission to the ED were significantly higher compared with the standard and on average amounted to 59.80 (3.87-130.07) pg/ml (p = 0.003) and remained significantly elevated until the sixth hour of observation, while in the 12th hour they returned to the baseline and reached normal level within 24 hours of observation. In group B, baseline levels of this cytokine were similar to the standard value but were not significantly increased and averaged 3.53 (0.45-192.21) pg/ml ($p > 0.05$). Baseline levels of IL-6 in group A were significantly higher than in group B (p = 0.001). Further observation of the dynamics of changes of the response to trauma and concomitant infection and complications showed that the highest level of IL-6 in group A were recorded in the third hour of hospitalisation and were considerably higher compared to the concentration of this cytokine in group B measured at the same time point (A vs. B: 65.10 [0.04-1192.07] pg/ml vs. 5.12 [0.04-73.99] pg/ml, p = 0.001). Differences of IL-6 concentrations between the groups at six hours of monitoring were not significant (p = 0.093). In group B, in patients with lighter injuries, the value of IL-6 levels in all measurement time points did not differ significantly from the norm. The highest concentration in this group was recorded at six hours of hospitalisation (Fig. 1).

Baseline concentrations of IL-1Ra in both groups were significantly elevated compared with the control group and on average in group A amounted to 1273.97 (430.02-13031.49) pg/ml, and 740.731 (264.21-14074.14) pg/ml in group B ($p > 0.05$ for both groups). Baseline levels of IL-1Ra in group A were also significantly higher than in group B (p = 0.015). Further observation of the dynamics of response to injury and complications demonstrated that the highest concentrations of this cytokine in both groups of patients was recorded at three hours of hospitalisation (A vs. B: 6813.84 [87.87-16800] pg/ml vs. 1358.42 [215.93-8886.03] pg/ml, p = 0.053) (Fig. 2). In group B (ISS < 20 points) the concentration of IL-1Ra at 6 h was in the normal range and lower than the baseline. In group A, concentration values were at a similar slightly lower level as the value at 3 h of observation. They returned to the baseline only at 12 h of observation and reached the normal status as in group B at 24 h of observation.

Statistical analysis performed in the entire group showed that in patients with post-traumatic complications (C$^+$), IL-6 concentration was significantly higher

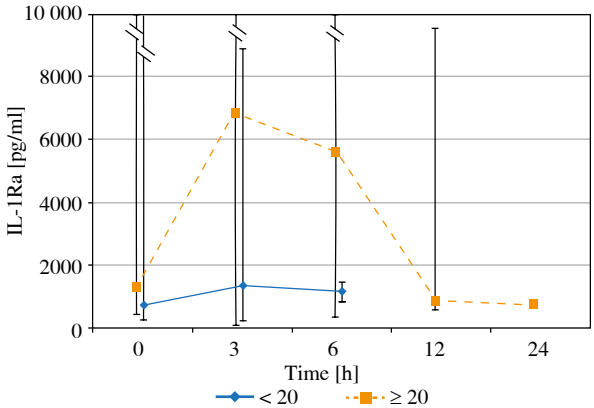

Fig. 2. IL-1Ra concentration (pg/ml) in relation to the extent of the injury (according to the Injury Severity Score [ISS] score). *$p < 0.05$, group A ISS ≥ 20; group B ISS < 20

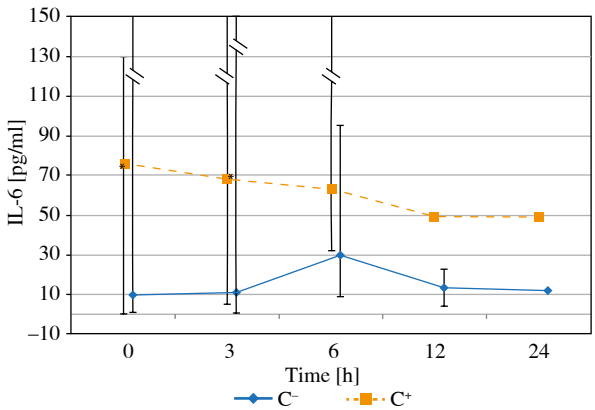

Fig. 3. IL-6 concentration [pg/ml] in relation to the complications. *$p < 0.05$, post-traumatic complications (C^+), without complications ($C^–$)

compared to those without complications ($C^–$) (at three hours of observation C^+ vs. $C^–$: 68.69 [5.121-1192.068] pg/ml vs. 10.63 [0.035-392.716] pg/ml, $p = 0.001$), whereas the baseline values were not significantly different (Fig. 3). The highest concentrations were observed in patients who died (in the third hour of observation 83.26 [59.01-1192.07] pg/ml). Concentration curves were similar in both groups. In patients who died, a sharp increase was recorded in the third hour of the study and then a sharp decline in the concentration of this cytokine to the values observed in the group without complications.

The concentration of IL-1Ra in patients with complications was significantly higher compared to the group without complications (at three hours of monitoring C^+ vs. $C^–$: 8403.9 [676.87-16800] pg/ml vs. 905.7 [87.87-16800] pg/ml, $p = 0.002$), while significantly higher levels in group C^+ were recorded already during baseline examination (Fig. 4). Similarly as for IL-6, the highest concentrations of IL-1Ra were observed in deceased patients in the third hour of the study (4421.99 [1825.07-16559.1] pg/ml, $p = 0.002$). However, the curves in both groups were different. In the group with complications, the highest increase was recorded at three hours of hospitalisation, while in the group without complications at six hours of monitoring. At six hours of observation, concentrations of IL-1Ra in both groups were not at similar levels and averaged 5580.7 (513.47-16800) pg/ml for C^+ and 1227.40 (341.74-16800) pg/ml for $C^–$ ($p = 0.846$).

The analysis of the Mann-Whitney *U* test showed statistically significant differences of IL-6 and IL-1Ra concentrations in patients who died compared with those who stayed alive. The studied groups of patients (A vs. B and C^+ vs. $C^–$) did not demonstrate statistically significant differences in relation to the age and gender of patients. There were no statistically significant changes in concentrations of biochemical and morphological parameters during observation. Changes in selected parameters were at very

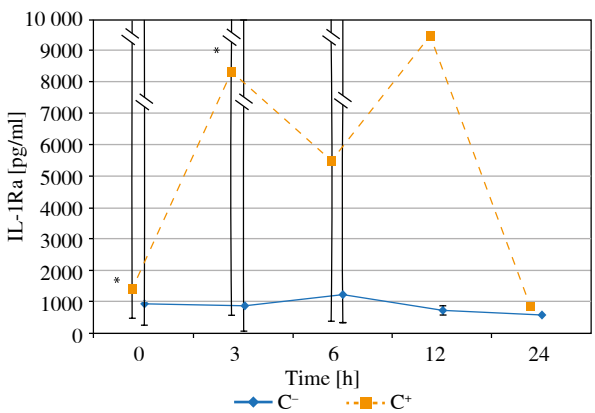

Fig. 4. IL-1Ra concentration [pg/ml] in relation to the complications. *$p < 0.05$ post-traumatic complications (C^+), without complications ($C^–$)

similar levels in different time intervals, regardless of the study group.

Subsequently, the correlation was investigated between cytokine levels and the values of the assessment of patient severity status according to the ISS score. Spearman's rho-correlation showed a statistically significant positive correlation between baseline concentrations of IL-6 ($r = 0.71$, $p < 0.001$) and IL-1Ra ($r = 0.49$, $p = 0.004$) and the values of the ISS score (Figs. 5 and 6).

In all patients, ROC curve analysis was performed to assess the risk of severe complications and death on the basis of cytokine concentrations (Fig. 7). The high diagnostic sensitivity calculated from ROC curves was found for IL-6 concentrations. The sensitivity for this cytokine was 0.8 and specificity 0.72. It was found that an IL-6 level equal to 64.17 pg/ml correctly classified serious complications and mortality in 80% of cases. The sensitivity for IL-1Ra was 0.80 and specificity 0.84. With respect to the levels of this cytokine, the cut-off point was 7717.15 pg/ml, which meant

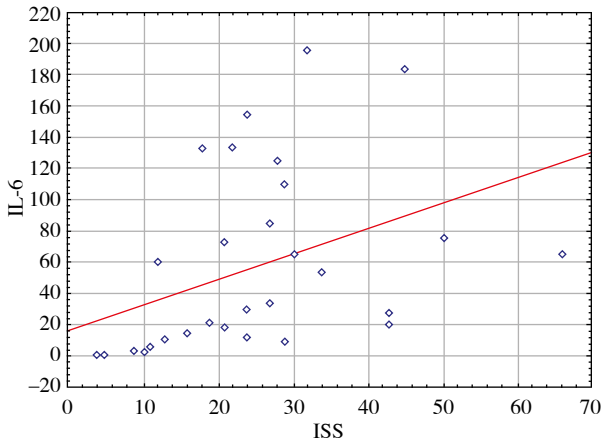

Fig. 5. Correlation plot between baseline levels of IL-6 and the Injury Severity Score (ISS) scores

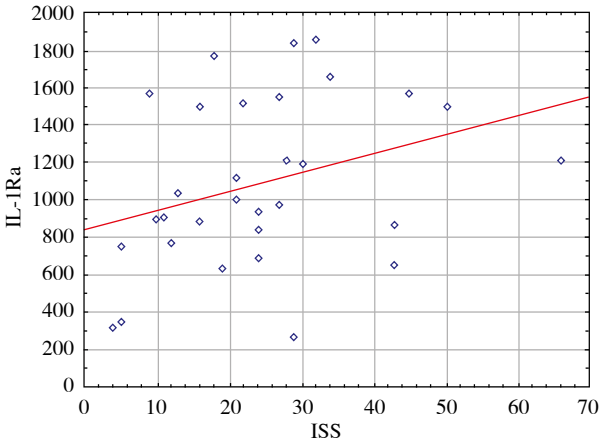

Fig. 6. Correlation plot between baseline levels of IL-1Ra and the Injury Severity Score (ISS) scores

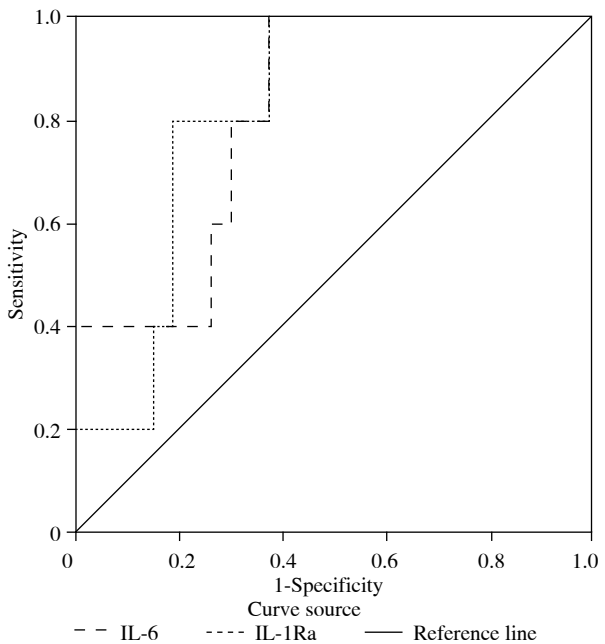

Fig. 7. Prognostic value for serum IL-6 and IL-1Ra levels in the whole group of patients after trauma (ROC curve)

that the level of this cytokine classified mortality in 80% of cases. This indicates that IL-1Ra is a similar prognostic indicator.

Discussion

According to the World Health Organisation, post-traumatic mortality rates are still very high and show an increasing tendency. Disorders of innate immune response that may increase the risk of serious complications play a key role in the immune response to trauma and infection. The mechanism of these disorders is multifactorial and is still poorly understood. Significant differences between post-traumatic patients hinder risk assessment of the occurrence of serious complications, leading to the death of some patients. The diversity of patients treated in the ED is caused primarily by the type and extent of the injury, which affects the applied therapeutic procedures (e.g. the need to perform emergency surgery, blood transfusion). Scales assessing the severity of the patient's condition, the extent of the injury, and routine laboratory tests have limited sensitivity and specificity in evaluating the risk of severe post-traumatic complications and death. In order to better assess the risk of severe complications in patients with trauma, there is a need for better understanding of the early immune response mechanisms to injury, which may determine the further course of the disease. Important parameters of the innate immune response are humoural mediators of cellular immunity to injury, the assessment of which may be helpful in differentiating physiological and pathological response, burdened with a high rate of severe complications [4].

The results of the present study suggest that the assessment of concentrations of selected pro- and anti-inflammatory cytokines (IL-6, IL-1Ra) in the serum of posttraumatic patients can be a useful indicator facilitating the separation of patients burdened with increased risk of complications. Significantly higher cytokine concentrations were recorded in patients with severe trauma, and they correlated with the extent of the injury. With the use of the examined cytokines in the entire group of patients admitted to the ED, it was possible to extract a group of patients at high risk of complications just three hours after admission; the values of the immune markers were significantly elevated compared with patients with no post-traumatic complications. It should be emphasised that the baseline concentrations of IL-6, although higher than normal, were similar in both

groups (C^+, C^-), indicating the need to monitor changes in the concentration of IL-6, because a single test performed after the admission to the ED had limited diagnostic value. In contrast, IL-1Ra concentration at the time of the first examination was two-fold higher in patients with later complications compared with the group with no complications, which may indicate a higher usefulness of IL-1Ra assessment in comparison to IL-6 in the initial period after injury. The analysis of cytokines in patients with traffic multi-organ injuries, performed in other centres, showed that the assessment of IL-6 and IL-1Ra concentrations can be helpful in the early diagnostics of post-traumatic complications [19-22, 25]. Raymondos *et al*. [19] obtained similar results assessing the risk of Acute Respiratory Distress Syndrome (ARDS) in patients with multiorgan injury by analysing the levels of several cytokines in serum, including IL-6 and IL-1Ra. The authors of the latter study suggested that the new diagnostic approach and an attempt to modulate the early post-traumatic inflammatory response can have a positive impact on treatment outcomes. The study was conducted in a small group of patients treated in the Intensive Care Unit (ICU), assessed according to the ISS (ISS > 23 points), who were admitted to hospital within an hour of the onset of trauma and survived for at least 24 hours. Despite a variety of injuries, the occurrence of complications was dependent on the levels of cytokines, which was also confirmed by our research. In addition, the authors of that study found that the risk of pulmonary complications was dependent on the concentration of IL-8 in bronchoalveolar lavage (BAL). In contrast to our study, that work did not monitor the changes in cytokine concentrations during the first hours of treatment.

In our study, the highest values of IL-6 and IL-1Ra were recorded three hours after admission in patients with later complications compared with the group without complications. The diagnostic value of the examined cytokines was also confirmed by another of our own research projects, which did not include the division of patients according to the ISS. Similarly, at three hours of monitoring the inflammatory response, based on the analysis of the levels of cytokines, one could identify a group of patients at high risk of subsequent complications and death. As shown by studies of other authors, concentrations of IL-6 and IL-1Ra can play an important prognostic role in the assessment of mortality in patients with extensive burns [20]. The analysis was carried out in patients with burns (thermal, electrical, and chemical) of more than 20% of body surface. The cytokine profile was determined on days 1 and 3 after the burn injury. In comparison with the results of our study, early cytokine response was not studied, different screening techniques were applied (Bio Arrary Plex), and a control group in a different age group was used in comparison to the test group, which eventually did not substantially affect the study results. The diagnostic value of the cytokines studied by us was also confirmed by the assessment of the baseline correlations of IL-6 and IL-1Ra concentrations with the ISS score.

The results of the current study also confirmed the works of other authors, who used other immunological techniques. Kirchoff *et al*. [21] examined the cytokine profile produced by peripheral blood monocytes using flow cytometry and found that persisting high levels of IL-6 in patients with multiorgan injury were associated with tissue damage and correlated with subsequent development of complications. A relationship was observed between clinical MODS symptoms and cytokine response. The activity of monocytes was significantly decreased, reaching the lowest level 24 hours after the injury. The study was conducted in a small group of patients with ISS > 30 points and multiple organ dysfunction syndrome score > 2 points (according to the Marshall score) at admission, and 24 and 72 hours after admission.

A study based on a different group of patients, after knee joint fracture (average ISS = 5.5 points), demonstrated that elevated levels of cytokines in fluid collected from the knee joint had important diagnostic value in the assessment of the long-term effects of the injury (chronic inflammation of bone and joints, development of post-traumatic degenerative changes in the joints) [22].

Discussing the results of this study, one should take into account that the local and systemic immune response to trauma and infection can be either physiological or pathological. Increased and prolonged pro- and anti-inflammatory response is a pathological response [4], which can lead to multiple organ failure, but distinguishing these reactions in early immune diagnostic period in patients after major trauma is still very problematic, which has essential diagnostic and therapeutic importance. The analysis of our own results in this respect indicated arapid increase in the concentration of cytokines after the injury (the highest in the third hour of the study), but only in the group after severe trauma, which confirmed the occurrence of systemic inflammatory response syndrome (SIRS) in this group of patients. A physiological response to injury was confirmed by studies in patients without complications, but the differences were significant only in the third hour of observation, indicating that monitoring of cytokines at short time intervals was helpful in distinguishing SIRS systemic pathological response from physiological response. Therefore, the "golden diagnostic window" was short, and in the presented tests it "closed" as early as within six hours of admission to the ED. As shown by other authors, IL-6 concentrations were increasing within 60 minutes of the onset of the injury and remained at a high level for up to seven days [23, 24]. In our study, six hours after the injury, concentrations of IL-6 and IL-1Ra were at a similar level independent of the ISS score and the group of patients with or without complications, which may indicate a "transient" balance between the production of cytokines tested. It can therefore be assumed that the high concentration of IL-6

and IL-1Ra in patients after trauma can be a positive prognostic indicator, but only in the early hours after the injury.

From the pathophysiological point of view, the main mechanisms that can cause severe complications should be elucidated. A study of other authors evaluated the physiological response to injury in patients without complications, scheduled for total hip replacement, who were monitored for changes in cytokine concentration at the end of the procedure, six hours, 24 hours, and six days after the surgery, as compared to preoperative concentration values of these cytokines. A marked increase of IL-6 was observed in the course of the procedure, whereas the concentration of IL-1Ra at the same time point was reduced. Minor differences in IL-1Ra concentration could result mainly from the group selection (planned injury, low blood loss, lower infection, no pain stimulus) [25] and different measurement time points of cytokines tested.

Tests performed in the following phase of the study (ROC curve assessment) showed that the analysed immunological indices may also be promising prognostic markers. Based on the ROC curve analysis, it was found that the threshold values for the tested cytokine differed significantly from the norm for IL-6, and in the case of IL-1Ra they could be in the normal range. The sensitivity and specificity of the indicators examined was high (0.8/0.75 for IL-6; 0.8/0.83 for IL-1Ra), indicating that the tested cytokines may serve as markers of serious complications, including death. They can be determined using readily available commercial enzyme immunoassays. Bedside tests make it possible to monitor selected cytokines in posttraumatic patients. The results of these tests correlate with the level of procalcitonin, but monitoring of interleukin levels seems to be a more sensitive test because the measured interleukins stimulate the synthesis of CRP, procalcitonin, and other markers used in the diagnostics of MODS or sepsis [26, 27]. However, confirmation of the clinical usefulness of measuring the concentration of these markers (IL-6 and IL-1Ra) requires further studies in a larger group of patients treated in the ED. The presented results demonstrate that the cytokine response to trauma varies and depends on many factors, such as the mechanism of injury, type and extent of injury, blood loss, infectious agent, and pain stimulus. The practical application of these examinations can be limited in interpretation of results of those surveys through the variety of material. The diversity of the trauma group is due to the type of trauma and its size, metabolic disorders that may affect the immune system, and the treatment (transfusions, ad hoc operations).

In summary, our findings suggest that elevated levels of the tested cytokines, determined in the peripheral blood shortly after the injury, may be significantly associated with the occurrence of later complications, which in some patients can lead to death. Monitoring the levels of these cytokines in selected patients with a high risk of serious complications should be used routinely. The presented diagnostics of early inflammatory response to injury should have an impact on treatment. Without early immunological diagnostics, attempts to modulate the inflammatory response in specific clinical situations will not be effective because concentrations of IL-6 and IL-1Ra change depending on the time since the injury, its extent, and associated complications. In the case of patients treated in the ED, burdened with the highest risk of death, in addition to the primary treatment, early cytokine response (within the first three hours of hospitalisation) should be considered as a potential target for therapeutic intervention.

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