Investigation of conventionally used and new inflammatory markers in polytraumatized and burn patients

Ph.D. thesis

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1. Introduction

Despite the dynamic development of perihospital care, multiple or polytrauma as the „epidemic of the 21st Century" has remained the leading cause of death in the population under the age of 40. Based on the so-called 'Berlin definition' from 2012, polytrauma is the combination of trauma where the complications of each injury – shock, coagulation disorders, multiple organ failure, etc.- expand, and the features of the injuries make them impossible to treat every single injury at the same time. As life-saving is the prior goal of therapy care givers are forced to make compromises. Life saving procedures are the therapy's main direction of impact, which forces the caretakers to make compromises. Traumatic injuries affect the body as a whole – due to pro- and anti-inflammatory responses, neuroendocrine effects, coagulopathy, etc. - which result in damage to non-primary organs. The complex, cascading defense mechanisms of the human body are activated by primary and secondary injuries, this is the so-called two hit theory. Local tissue damage, the severity of primary tissue and organ injuries, and the consequent pro-inflammatory responses are influenced by the size of the traumatic impact (such as the so-called '*first hit*') and the perfusion conditions. In addition, endogenous and exogenous factors also play a crucial role in initiating post-traumatic processes and influence the severity of complications. A *second 'hit'* caused by antigen loading may include, for example, respiratory distress syndrome in hypoxia; instability of the caridovascular system; metabolic acidosis, ischemia / reperfusion injury, tissue death or infection. Elements of the '*second hit'* may be improperly treated or unrecognized injuries, surgical procedures involving tissue damage, massive transfusion, or the so-called *lethal tirad*: hypothermia, coagulopathy, acidosis. Besides elements of the so-called *'first hit'* tissue damage associated with polytraumatization may be affected by the general condition of the injured organism, genetic factors, antigen loading, and the induction of inflammatory cytokines and phospholipids in proportion to local and systemic release. As a result of tissue injury, the so-called damage associated molecular pattern (DAMP) develops, cytokines and phospholipids may be expressed on the cell surface or in the extracellular space as a result of trauma to the tissue. In doing so, for example, they are involved in mediating inflammatory processes. Through the priming effect, inflammatory cytokines stimulate the recruitment and phagocytotic activity of polymorphonuclear leukocytes (PMNL) in the hours following injury. This type of activation of immune cells is the body's primary line of defense, during which PMNLs produce proteases and oxygen free radicals, resulting in a respiratory explosion and an

oxidative stress response. To counteract the pro-inflammatory response following injury, anti-inflammatory mediators are formed depending on the severity and time course of the traumatic impact. The enhanced anti-inflammatory (hypo-inflammatory) response is responsible for the immunosuppressed condition following polytrauma, which means a high susceptibility to infections and consequent septic complications. The phenomenon serves the restoration of the immunological status of the organism and is called compensatory anti-inflammatory response syndrome (CARS). The aim of the body's defense mechanisms seems to be to balance the pro- and anti-inflammatory processes following injury, on the one hand by inducing reparative mechanisms and inhibiting the spread of pathogens, and on the other hand by preventing autoaggressive processes and conditions involving secondary tissue damage and infection. These mechanisms are called elements of mixed antagonist response syndrome (MARS).

One of the main characteristics of burns is that the organs and organ systems that are primarily not damaged actually, suffer damage as well. As with polytrauma, if the extent of the burn injury affects more than 20% of the body surface, pathophysiological changes caused by cytokines and other inflammatory mediators released at the site of the injury lead to the development of a burn injury affecting the entire body. If the burn damage exceeds one-third of the total body surface area, a condition called burn shock develops with the consequent development of multiorgan failure, the treatment of which is a serious challenge for the intensive care units from several aspects.

As a result of burn trauma, complex processes take place at the level of both the systemic circulation and the microcirculation which are difficult or impossible to restore by fluid resuscitation administered during therapy. Severe burn injury results in significant tissue damage and consequent hypovolemic shock leading to the formation and release of a number of local and systemic mediators. Similar to the processes following polytrauma, the level of cytokines produced by PMNLs increases as a result of the pro-inflammatory response activated by severe burn injury. Parallel with the development of a pro-inflammatory response following severe burn trauma, the body also strives for a state of equilibrium (CARS and MARS, respectively).

The most common, often fatal, complication of polytraumatic and severe burn injury is sepsis, which, according to the current approach, is the body's impaired response to infection. The body responds to traumatic tissue injuries and infectious damage in a similar way. This is probably due to the fact that the genetic structure of the

mitochondria released during injury and the pathogens that attack the body are very similar. The response which is similar to that generated by tissue damage is called pathogen associated molecular pattern (PAMP). The immature immune system recognizes molecules that cause both DAMP and PAMP, resulting in a fairly similar response. Since 2016, the so-called Sepsis 3 definitions have been used in the diagnosis and treatment of sepsis. In burn patients, the criteria for sepsis were defined by the American Burn Association (ABA) based on the pathophysiological and clinical characteristics of burn injury.

Being both sources and target cells for cytokines leukocytes are also involved in inflammatory processes. Activation of leukocytes and changes in the physical characteristics of the cells play an important role in initiating and developing a pro-inflammatory response following injury. Following traumatic injury, pro-inflammatory cytokine production, as the body's primary line of defense, activates and stimulates the phagocytic activity of PMNLs and the production of reactive oxygen species (ROS).

In addition to the "classic" functions of platelets in the process of blood coagulation, during their activation, they also secrete pro-inflammatory molecules, cyto- / chemokines, vasoactive amines, eicosanoids which mainly attack leukocytes and endothelial cells. Interactions between platelets and leukocytes are essential for the proper functioning of pro-inflammatory processes following traumatic insults in the body. Pro-inflammatory mediators released from platelets activate elements of the inflammatory cascade, coagulation, and complement systems. Platelets play a role not only in inflammatory but also in infectious processes. Coagulopathy and thrombocytopenia as complications of trauma to the body are associated with increased morbidity and mortality.

The first step in the cellular response to traumatic tissue injury is a nonspecific immune response in which leukocytes become activated, nonetheless, functional changes can best be examined by sophisticated methods.

Previous studies have shown that the gravitation sedimentation of whole blood from healthy individuals, which is inhibited in coagulation, contains (up to 10%) more white blood cells in the upper half of the blood column than the original concentration. If their specific gravity is lower than at rest due to water uptake associated with activation, the proportion of ascending, antiseduced white blood cells will increase and the cell concentration in the upper half of the blood column will be more than 15% of the original. This ratio is called the leukocyte anti-sedimentation rate (LAR), which can be examined

after one-hour blood sedimentation. Based on this concept, the platelet anti-sedimentation rate (PAR) can also be determined and measured.

The local and systemic release of pro-inflammatory cytokines following tissue injury initiate so-called acute phase reactions in the liver, ensuring the protection of tissues and the body's defense against pathogens. One of the acute phase proteins is the so-called C-reactive protein. As a result of a pro-inflammatory response or infectious noxa following tissue injury, CRP production is increased in liver cells over hours. Although the diagnostic value of CRP has been questioned in the past, given that its production is part of a non-specific acute-phase response, CRP is in fact considered a valuable marker of acute and chronic inflammatory processes. However, changes in CRP kinetics can only be assessed with knowledge of the clinical picture.

Physiologically, thyroid glands produce procalcitonin (PCT), a protein which has a low plasma concentration (pg/ml), among healthy individuals. Its induction, formation and biological function are not fully known. PCT values are independent from daily fluctuation, and in most cases, are not elevated in case of local infections. PCT induction is not observed in autoimmun diseases, after minimal invasive procedures, upon transplanted organ rejection or in viral infections. It is a suitable marker in the evaluation of antimicrobial therapy or the prediction of the prognosis: decreasing tendency correlates with positive outcome and with the successful elimination of the infection source, while elevated serum levels or an inceasing tendency may indicate poor outcome, persisting inflammation or infection.

2. Aims and objectives

2.1 Investigation of LAR among polytrauma and burn victims

- Our aim was to investigate the role and kinetics of LAR after severe mechanical injury and at the initial stage of severe burn injury.
- We aimed to describe the temporal profile of LAR after severe injury to the body (polytrauma, burn injury) and at its' early stages.
- We looked for a difference in LAR values compared to members of the control group.
- We wanted to investigate the kinetics of LAR in the survivor and the deceased patient groups.
- We looked for a difference in LAR values inbetween members of the survivor and the deceased groups compared to the members of the control group.

2.2 Investigation of laboratory markers (CRP, PCT) conventionally used in inflammatory and infectious processes in polytraumatized and burn patients

- The goal of our study was to investigate the changes and the possible prognostic role of laboratory markers (CRP, PCT) conventionally used in inflammatory and infectious processes during polytrauma and burn injury.
- We aimed to describe the kinetics of CRP and PCT immediately after and at early stages of severe injury (polytrauma, burns).
- We wanted to examine the evolution and behaviour of CRP and PCT levels among members of the survivor and non-survivor groups.

2.3 Investigation of LAR and laboratory markers (CRP, PCT) conventionally used in inflammatory and infectious processes in polytraumatized and burn patients in the period around the development of sepsis

- As the most serious complication of polytrauma and severe burn is sepsis, our aim was to investigate temporal changes and potential prognostic significance of LAR and laboratory markers (CRP, PCT) conventionally used in inflammatory and infectious processes among septic patients 3 days before and 3 days after the onset of sepsis.
- We aimed to investigate changes in LAR and laboratory markers (CRP, PCT) conventionally used in inflammatory and infectious processes in the period around the development of sepsis.
- We wanted to describe the role of LAR and laboratory markers (CRP, PCT) conventionally used in inflammatory and infectious processes in the prediction of septic complications.

2.4 Investigation of PAR and LAR in burns

- Given that the early phase of burn is associated with a decrease in platelet count and leukocyte activation, we aimed to describe the role and the temporal profile of PAR and LAR in burn.
- We aimed to describe changes in PAR and LAR in a homogeneous population of burn patients.
- We wanted to investigate the kinetics of PAR and LAR in the survivor and non-survivor groups.
- We aimed to compare the behaviour of PAR and LAR between survivor and non-survivor group members.
- A further aim was to study the kinetics of PAR and LAR in non-septic and septic burn patients.
- We wanted to compare the behaviour of PAR and LAR between non-septic and septic patients.

3. Patients and methods

3.1 The research ethical background of the investigations

The protocol of our studies was designed in accordance with the ethical guidelines of the 2003 and 2008 Helsinki Declarations, and the measurements were performed at two different times according to the protocol approved by the Regional Research Ethics Committee of the University of Pécs (number of licenses: 4422/2012 and 6635/2020, respectively). Patients, or in the event of their impediment, their next of kin, were informed in detail about the course of the study, after which they gave their written consent.

3.2 Patients

The kinetics of the parameters were examined for five days after intensive care unit (ICU) admission of the patients. Within 5 days, in uncomplicated cases, the inflammatory response accompanying the injury peaks and begins to decline. The minimum number of patients was determined based on our previous studies. Sampling was continued until the patient left the ICU.

3.2.1 Examinations in the mixed group of polytraumatized and burn patients

Our prospective, descriptive study was performed on polytraumatized and burn patients admitted to the 10-bed intensive care unit of the Department of Anaesthesiology and Critical Care Akác utca, Clinical Centre,University of Pécs, between March 2013 and September 2015. They were treated uniformly, according to the valid ATLS and ABLS guidelines. Patients enrolled in the study were divided into survivors and non-survivors according to outcome. The control group in the LAR study included 10 healthy volunteers who did not differ in age and gender compared to members of the patient group.

3.2.1.1 Inclusion criteria for the mixed group of polytraumatized and burn patients

- Injury severity score (ISS) value: minimum 16
- Total burnt surface area (TBSA): at least 15% of the total body surface area affected
- the patient was admitted to the ICU in the first 3 hours after trauma

3.2.1.2 Exclusion criteria for the mixed group of polytraumatized and burn patients

- Age: under 18 years old
- Mechanical traumal or burn that are incompatible with life
- Known underlying tumor disease
- Chronic steroid use or immunosuppressive therapy that affects the normal immune response (eg. radiotherapy, chemotherapy)
- Chronic, severe organ disease before ICU admission (eg NYHA stage 4 heart failure, chronic hemodialysis, cirrhosis of the liver, etc.)

3.2.2 Examinations in the group of polytraumatized and burn patients who become septic

In polytraumatized and burn patients who become septic, the kinetics of the studied parameters were analyzed 3 days before and 3 days after the clinical diagnosis of sepsis. The day on which sepsis was diagnosed based on clinical signs and samples were taken for microbiological inoculation and antimicrobial treatment was initiated was considered to be day zero. In order to establish the diagnosis of sepsis, the so-called Sepsis 2 definition and criteria formulated by the ABA were used. Patients were treated according to the current sepsis treatment guideline.

3.2.3 Examinations in the homogeneous group of burn patients

Our prospective, descriptive study was performed on burn patients admitted to the central 16-bed intensive care unit of the Department of Anaesthesiology and Critical Care of the Clinical Center, University of Pécs, between March 2016 and July 2018. They were treated uniformly, according to ABLS guidelines. Patients enrolled in the study were divided into survivors and deceased, and non-septic and septic groups, respectively. The inclusion and exclusion criteria were the same as for the burn patients listed in sections 3.2.1.1 and 3.2.1.2. As a control group, PAR and LAR groups included 10 healthy volunteers each who did not differ in age and gender compared to members of the given patient group.

3.3 Methods of measurements

3.3.1 Method and frequency of sampling

Blood samples were taken from our patients painlessly from the arterial cannula required for their intensive care. Blood samples were taken at the time of ICU admission and susequently, durning morning blood sampling throughout the study period. Control groups consisted of healthy volunteers from whom a total of one blood sample was taken. In these cases, blood was also drawn from an upper limb artery (radial or brachial artery).

3.3.2 The measurement of LAR

LAR was determined from an arterial blood sample in a Westergreen tube containing sodium citrate (5.2 mL, sodium citrate 0.105M; Vacutainer, Becton Dickinson, Meylan, France). Using a ruler attached to the side of the test tube from the outside, the length of the whole blood column was measured, and then a clearly visible line was drawn on the wall of the test tube with an alcohol felt-tip pen at half the length, marking the bisector line.

After a one-hour sedimentation time, blood above and below the halfway line of the blood was injected into a “blood count” test tube (3ml, K2 EDTA; Vacutainer, Becton Dickson, Meylan, France) (**Figure 1**).

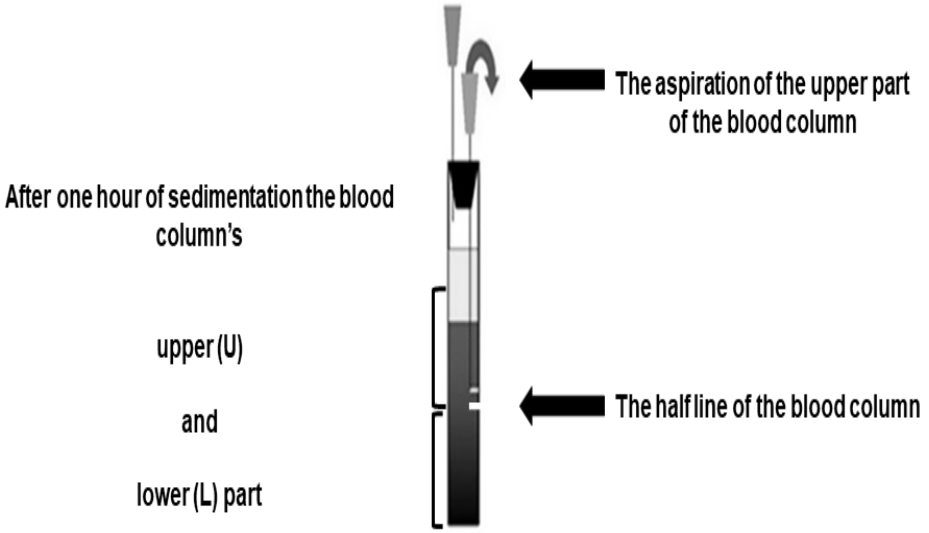

Figure 1. Measurement principle of LAR and PAR

(After one hour of gravity sedimentation in a single-use Westergreen tube, aspiration of the upper blood sample half.)

The tubes were then delivered to the Department of Laboratory Medicine, University of Pécs, Clinical Center in order to determine the qualitative blood count. During this procedure , the number of leukocytes in each blood column - upper (U) and lower (L) - was determined. Subsequently, the LAR = 100 (U-L) / (U+L) formula was used for counting,which gives the number of leukocytes as a percentage of the original leukocyte count that crossed the halfway line of the blood sample in the Westergreen tube during one hour of sedimentation. Although the sampling was done manually, from the point of view of the obtained values, the development of some kind of automated method for the halving of the blood column and the extraction of the upper and lower blood sample parts would definitely be promising in the future.

3.3.3 The measurement of PAR

PAR was determined from an arterial blood sample (5.2 ml, sodium citrate 0.105M; Vacutainer, Becton Dickinson, Meylan, France) collected in a Westergreen tube containing sodium citrate, similar to LAR. Sampling and processing of the tubes was performed in the same way as described in Section 3.3.2 (**Figure 1**).

During this procedure, the number of platelets was determined in each blood column - upper (U) and lower (L) . Subsequently, the PAR = 100 (U-L) / (U+L) formula was used for calculation which gives the number of platelets that crossed the halfway line of the blood sample in the Westergeen tube during the one-hour sedimentation as a percentage of the original platelet count.

3.3.4 Measurement of the conventional laboratory markers (CRP, PCT) used in inflammatory and infectious processes

CRP and PCT levels were measured as part of routine daily laboratory monitoring of the ICU patients with burn and polytrauma. The measurements were performed at the Department of Laboratory Medicine, Clinical Centre,University of Pécs.

3.4 Statistical analysis

IBM SPSS Software v22 (SPSS, IBM Corporation, Chicago, IL, USA) was used for statistical analysis. As our data did not show a normal distribution, the results were expressed as median within a 25–75% intrequartile range, respectively, and plotted as 95% confidence intervals. The Mann-Whitney U test was used to analyze the daily kinetics of the studied parameters and to determine the differences between the groups.

Changes within each group were described using the Kruskall-Wallis test, the trend of significance levels during the study period was examined using the Jonckheere-Terpstra test. The probability value of $p < 0.05$ was considered as statistically significant in all cases.

4. Results

4.1 Examinations in the mixed group of polytraumatized and burn patients

The study included 16 patients with burn injury (ABSI: 7 [IQR: 5-8]) and 20 patients with polytrauma (ISS: 29 [IQR: 22-34]) who were divided into survivors and non-survivors despite intensive care. 10 patients developed septic complications. We found significant differences between survivors and non-survivors in terms of age ($p < 0.05$) and in the extent of burned area in patients with burn injury ($p < 0.05$) as well. However, in polytraumatized patients, ISS did not differ between survivors and non-survivors. We also found a significant difference in age between burned and polytraumatized patients ($p < 0.05$) and in the heterogeneous group of patients with trauma in terms of gender ($p < 0.01$).

4.1.1 Changes of LAR in polytraumatized and burned patients compared to the control group

The results of burn and polytraumatized patients were compared to the gender- and age-matched control group of 10 healthy volunteers. During the study, LAR values showed a significantly increasing tendecy ($p < 0.001$). On the first day, we found no significant difference between the healthy controls and all the patients that had suffered trauma. The difference became significant on the second ($p < 0.05$), third ($p < 0.05$), fourth ($p < 0.001$), and fifth ($p < 0.001$) days (**Figure 2**).

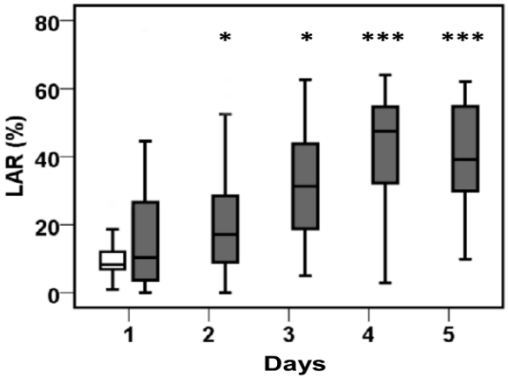

Figure 2 Changes of LAR in polytraumatized and burnt patients compared to the control group

The **white column** indicates the control and the **dark columns** indicate all polytraumatized and burnt patient groups.
The * symbol indicates a significant difference between the control and the heterogeneous group of patients who suffered trauma.
(* $p < 0.05$; *** $p < 0.001$).
Data are given as median, interquartile range, and 95% confidence interval.

4.1.2 Changes in LAR in surviving and non-surviving polytraumatized and burn patients compared with the control group

The LAR value increased continuously from the ICU admission in the survivor group ($p < 0.05$) reaching its' peak value on day 4. An increase in LAR ($p < 0.05$) was observed in the non-survivor group from the third day, the peak value was also observed on the 4th day. In survivors, LAR was significantly elevated on the day of admission compared to both the non-survivor ($p < 0.01$) and the control ($p < 0.05$) groups. This significant difference remained on day 2 compared to both the non-survivor ($p < 0.01$) and the control ($p < 0.001$) groups. There was no significant difference in LAR between the survivor and the non-survivor group from day three. Compared to the values measured in the control group, the LAR in survivors remained significantly higher on days 3 ($p < 0.001$), 4 ($p < 0.001$), and 5 ($p < 0.001$). In the non-survivor group, the increase reached the significant level ($p < 0.05$) on days 3 ($p < 0.05$), 4 ($p < 0.05$) and 5 ($p < 0.05$) (**Figure 3**).

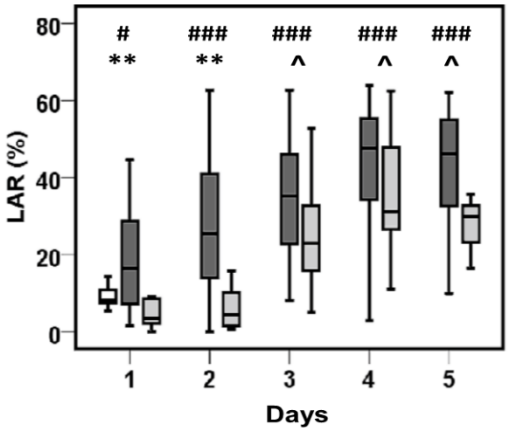

Figure 3 Changes of LAR in survivor and non-survivor polytraumatized and burnt patient groups compared to the control group

The **white column** represents the control group, the **dark gray columns** represent the survivor group, and the **light gray columns** represent the non-survivor polytraumatized and burnt patient group.
The * symbol indicates a significant difference between the survivor and the non-survivor patient groups (** $p < 0.01$).
The # symbol indicates a significant difference between the survivor group and the control group (# $p < 0.05$; ### $p < 0.001$).
The symbol ^ indicates a significant difference between the non-survivor group and the control group (^ $p < 0.05$).
Data are given as median, interquartile range, and 95% confidence interval.

4.2 Investigation of CRP, PCT levels in polytraumatized and burn patients

The conventional laboratory marker used in the diagnosis of inflammatory processes, serum CRP levels (Department of Laboratory Medicine, University of Pécs, Clinical Center reference value: 5 mg / l) also showed an increasing tendency similar to LAR ($p < 0.001$). From day 2, the 5% confidence interval was also higher than the upper limit of the normal laboratory value throughout. (Data not shown). The parameter used in the diagnosis of sepsis did not show clear kinetics of serum PCT levels (Department of Laboratory Medicine, Clinical Center, University of Pécs, 0.5 ng / ml) and did not substantially exceed the upper limit of normal as determined by the laboratory. (Data not shown).

4.2.1 Changes in CRP, PCT levels in survivor and non-survivor polytraumatized and burn patients

Serum CRP was found to be significantly higher in the survivor group compared to non-surviving patients ($p < 0.05$) already on the day of ICU admission compared to the reference value (5 mg / l) determined by the Department of Laboratory Medicine Clinical Cente, University of Pécs. In the non-surviving group, CRP started to increase on the second day. On days 2 and 3, we found no significant difference between survivors and no-survivors. In survivors, CRP began to decrease on day 4, when CRP levels were significantly elevated ($p < 0.05$) among non-survivors compared to survivors. In non-survivors, a decrease in CRP was observed on day 5, by that time the significant difference between the two groups had disappeared (**Figure 4a**). Serum PCT was higher on the first day compared to the reference value (0.5 ng / ml) determined by the Department of Laboratory Medicine, Clinical Center, University of Pécs. In the survivor group, median values did not exceed the upper limit of normal, and PCT levels in the survivor and non-survivor groups did not differ significantly. We found no significant difference in PCT levels between survivor and non-survivor patients during the remaining period of the study.

PCT levels showed no increasing or decreasing trend in any of the patient groups (**Figure 4b**).

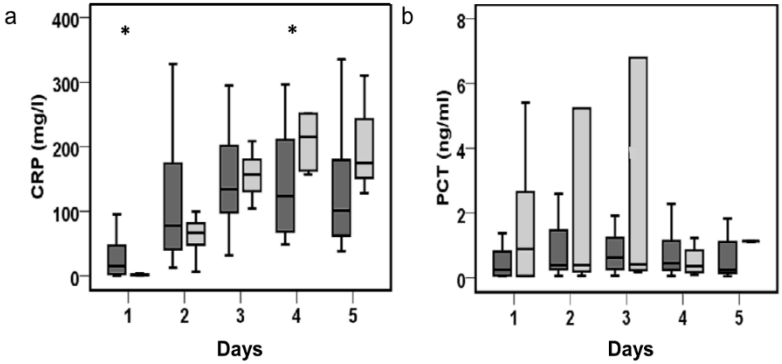

Figure 4 Changes of CRP and PCT between the survivor and the non-survivor polytraumatized and burnt patient groups

The **dark gray columns** represent the survivor group, and the **light gray columns** represent the non-survivor polytraumatized and burnt patient group.
The * symbol indicates a significant difference between the survivor and the non-survivor patient groups (* p <0.05).
Data are given as median, interquartile range, and 95% confidence interval.

4.3 Changes in LAR, CRP, PCT levels in polytraumatized and burn patients that became septic on the days around the onset of sepsis

Of the 36 burned and polytraumatized patients enrolled in the study, 11 (6 with burn, 5 with polytrauma) developed septic complications during the first 2 weeks. In these patients, the kinetics of LAR, CRP, and PCT were analyzed 3 (-3 - -1) days before and 3 (1–3) days after the onset of sepsis.

4.3.1 Kinetics of LAR values in polytraumatized and burn patients that became septic on the days around the onset of sepsis

Comparing the three markers examined, LAR levels (**Figure 5a**) showed a decrease before sepsis developed. The decrease was significant ($p < 0.05$) upon comparing the day prior to the onset of sepsis with day 3 prior to the development of sepsis.

4.3.2 Kinetics of CRP, PCT levels in polytraumatized and burn patients that became septic on the days around the onset of sepsis

CRP levels (**Figure 5b**), similar to the LAR values, also showed a decreasing tendency before the onset of sepsis. This was also significant ($p < 0.05$) for CRP levels prior to the onset of sepsis compared to day -3. PCT levels (**Figure 5c**) showed an increasing tendency from the day of clinical diagnosis of sepsis only. Compared to the days before the onset of sepsis, significant differences ($p < 0.05$) were found only from the onset of infectious complications.

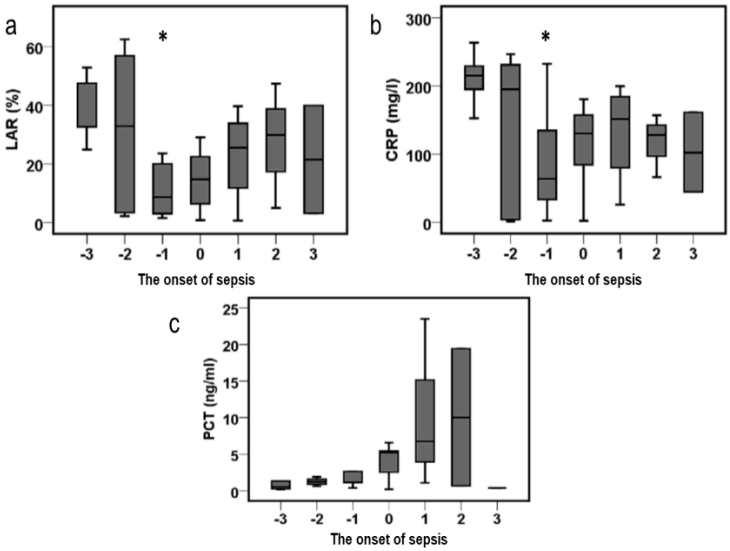

Figure 5 Changes of LAR, CRP, and PCT leves in polytraumatized and burnt patients who became septic in the period around the onset of sepsis

The **dark gray columns** represent the polytraumatized and burnt patients who became septic.
The * symbol indicates a significant difference compared to the 3rd day (day -3) before the onset of sepsis (* $p < 0.05$).
Data are given as median, interquartile range, and 95% confidence interval.

4.4 Investigations of PAR and LAR in burn

Of the 23 patients enrolled and treated at the ICU in this prospective, descriptive study, 15 were male and 8 were female, with a median age of 66 (IQR: 49-80) years. Among the median values of their condition severity indices, ABSI was 7 (IQR: 5 - 8) and TBSA 30 (IQR: 25 - 40)%. Of the patients, 16 survived while 7 died despite the intensive care. 10 patients developed septic complications. At the time of ICU admission, five of the 23 patients were taking anti-platelet medications. There was a significant difference ($p < 0.05$) between the number of burn patients who survived ICU treatment and those who did not.

4.4.1 Changes in PAR and LAR levels in the total burn population

Both PAR and LAR values showed an increasing tendency during the study period. Peak values were reached on day 5 for PAR and day 4 for LAR. (Data not shown).

4.4.2 Changes in PAR and LAR levels between the survivor and the non-survivor burn patient groups

In the group surviving ICU treatment, PAR values showed a significant increase from day 2 ($p < 0.05$), the peak value was reached on day 5. A statistically significant increase in PAR ($p < 0.05$) was observed in the non-suvivor group from day 3, the peak value was observed on day 5. Members of the survivor group had significantly higher PAR values than those who died on day 2 ($p < 0.05$), 4 ($p < 0.05$), and 5 ($p < 0.05$) (**Figure 6a**). In the survivor group, LAR values showed a significant ($p < 0.05$) increase from day 2, with a peak on day 4. The LAR values of the patients who did not survive showed this type of significant increase ($p < 0.05$) from day 3 only, the peak value was reached on day 5. In the survivor group, LAR values were significantly higher on days 1 ($p < 0.01$), 2 and 3 ($p < 0.05$), and 4 ($p < 0.01$) compared to the non-survivor group. On day 5, there was no statistically significant difference in LAR ($p = 0.211$) (**Figure 6b**).

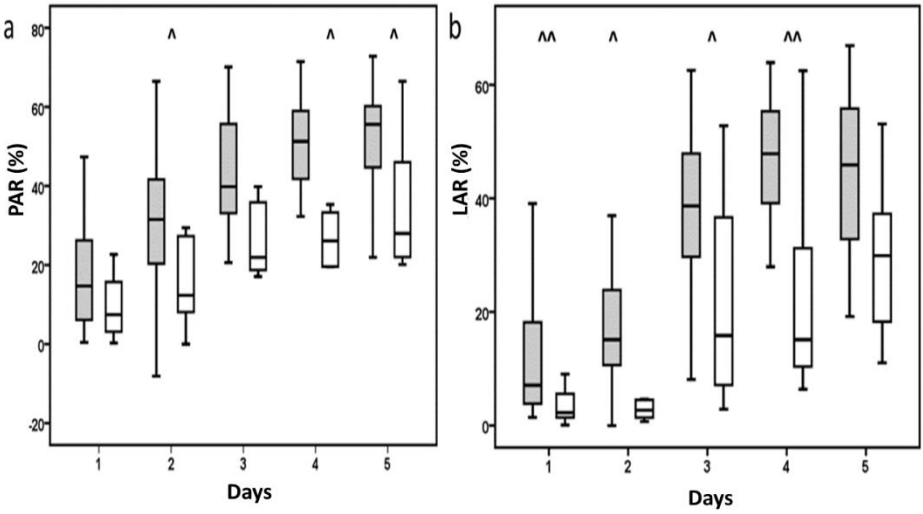

Figure 6 The kinetics of PAR and LAR in the survivor and the non-survivor patients with burns

The **gray columns** represent are the survivors and the **white columns** represent the group of non-survivior patients with burns. The ^ symbol indicates a significant difference between the survivor and the non-survivor group (^ $p < 0.05$; ^ ^ $p < 0.01$). Data are given as median, interquartile range, and 95% confidence interval.

4.4.3 Changes in PAR and LAR levels between non-septic and septic burn patient groups

In the group of patients that did not become septic, PAR values showed a significant increase from day 2 ($p < 0.05$), reaching a maximum value on day 5. In patients that became septic, PAR values showed a significant increase from day 3 only ($p < 0.05$), the maximum value was reached only on day 5. Comparing non-septic and septic patient groups, we have previously found significantly higher ($p < 0.01$) PAR values (**Fig. 7a**) on day 5. In non-septic patients, LAR values showed a significant increase from day 3 ($p < 0.05$), reaching their maximum value on day 5. In the group of patients that became septic, LAR values showed a significant increase only from day 4 ($p < 0.05$), reaching the maximum value on day 5. Comparing non-septic and septic groups, we found a decrease in LAR in patients that became septic on days 1 and 2, however, these values were not found to be statistically significant compared to patients that did not become septic. Regarding LAR values, we found no statistically significant difference between the non-septic and the septic group on days 3, 4, and 5. (**Figure 7.b**).

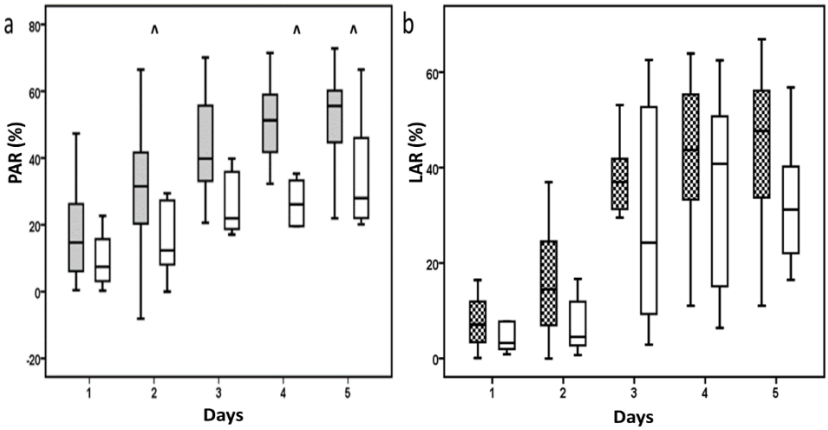

Figure 7 The kinetics of PAR and LAR in the non-septic and the septic patients with burns

The **gray** and the **chess board pattern columns** represent are the non-septic and the **white columns** represent the group of septic patients with burns.
The ^ symbol indicates a significant difference between the non-septic and the septic group (^ $p < 0.05$;).
Data are given as median, interquartile range, and 95% confidence interval.

5. Summary

In our study, we investigated new and conventionally used laboratory markers in inflammatory conditions after polytrauma and severe burn injury, as well as in patients that developed sepsis. The latter condition is one of the most common complications of polytrauma and burns and has been the leading cause of death despite intensive care. Supported by previous research data from the literature , we have demonstarted that severe mechanical and burn injury is accompanied by a significant pro-inflammatory response. We found leukocyte activation among heterogeneous trauma patients resulting from trauma (either mechanical or thermal injury), which is important in the pro-inflammatory response process following tissue injury. Our study demonstrated that an adequate inflammatory response is required for survival. This is confirmed by our results which showed significantly increased LAR values among the members of the survivor group on both the day of admission and the following day compared to members of both the deceased and control groups. A later increase in LAR was found in the deceased patient group.

Both CRP and PCT are used as conventional laboratory markers in the everyday clinical practice to distinguish inflammatory and infectious conditions, respectively. Both proteins play an important role in acute phase reaction. In contrast to the survivors of the heterogeneous group of patients with traumatic injury, CRP kinetics in the non-survivor group began to decline later, suggesting that the adequate acute phase response required for survival and pro-inflammatory processes were initiated later subsequent to tissue injury. In the heterogeneous group of patients with traumatic injury, we examined these parameters as well as the behavior of LAR in patients that became septic in the period around the onset of sepsis. On the day before the clinical diagnosis of sepsis and on the day of developing sepsis, both LAR and CRP showed a significant decrease compared to day -3 before sepsis onset. In this regard, LAR, as a non-conventional laboratory parameter, may have a predictive value indicating infectious processes. The importance of this observation is underlined by the fact that PCT kinetics did not show a similar difference before the onset of sepsis.

The last part of our study examined the behaviour of PAR and LAR after severe burn injury. We hypothesized that while leukocyte activation can be desribed by LAR, platelet activation could possibly be characterized by PAR. As a previous increase in PAR was found in patients surviving severe burn injury, it has been hypothesized that this may reflect previous activation of the innate immune system and a more effective

pro-inflammatory response to thermal injury-induced tissue damage. The phenomena described in connection with the PAR and LAR kinetics of the surviving and deceased groups may be due to reduced immune response or an excessive inflammatory response due to burn injury which is in line with our previous findings. In the homogeneous burn patient population, we found significantly higher PAR values on day 5 among the patients that did not develop septic complications compared to the septic patient population. This phenomenon is thought to be an adaptive response to increased “de novo” platelet production. The limitation of study is the low number of cases, in order to state a conclusive evidence, it is necessary to involve additional patients which is not an easy task in the light of number of ICU admissions and treatment after severe burn trauma.

6. Novel findings

- We were the first to describe the behaviour and the kinetics of LAR in heterogeneous (polytraumatized and burned) patients after traumatic injury. In the deceased group, LAR started to increase later and was significantly lower in the first two days similar to the survivor group.

- Significantly elevated LAR values were observed in the survivor group throughout the study period compared to members of the control group. In non-survivors LAR values were found to be significantly elevated only rom day 3 .

- Our study was the first to describe the kinetics of LAR, CRP, and PCT in the period around sepsis in heterogeneous (polytraumatized and burnt) patients after traumatic injury that developed septic complications. In the period before the onset of sepsis, LAR and CRP levels dropped. Significantly lower LAR and CRP levels were described on the day before and after the onset of sepsis compared to 3 days before the onset of sepsis.

- In polytraumatized and burned patients that became septic, PCT levels did not show similar kinetics in the pre-septic period. A significant increase in PCT values compared to day 3 prior to the onset of sepsis was observed only in the days following the onset of sepsis.

- We were the first to examine the behaviour and kinetics of PAR in burn patients. Following burn trauma, increasing kinetics of PAR were observed. In the survivor group, the increase became significant from the second day, while in the deceased the increase became significant from the third day. Significantly higher PAR values were found in surviving burn patients on days 2, 3, and 5 compared to those that died.

- In non-septic burn patients, PAR values were previously elevated compared to members of the septic burn group. Significantly higher PAR values were described among burn patients that did not develop sepsis on day 5.