Investigation of inflammation and oxidative stress markers in sepsis and burn injury

Ph.D. thesis

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1. Introduction

Besides injury to the skin, severe burn trauma may initiate pathophysiological processes and changes affecting the entire organism. Metabolic processes activated by stress and pain, and neuro-hormonal changes result in hyper-metabolism and consequently, a catabolic metabolism. The activation of the immune system causes a release of a large amount of inflammatory mediators. Pro-inflammatory cytokines play a key role in initiating SIRS. SIRS results in a life-threatening condition by causing multiple organ failure or dysfunction that pose a great challenge in terms of treatment and thus, necessitate special intensive therapy.

Changes in surface markers accompanying leukocyte activation and migration show a characteristic pattern and play an important role in the initiation and development of the inflammatory reaction following burn injury. At present, hundreds of specific CD markers are known. They are known to be involved in immune processes, cellular connections and tissue differentiation. Adhesion molecules CD11a, CD11b, CD18 and CD49d have a significant role in leukocyte attachment and recruitment. CD14 a is a recognising molecule bound to the surface of monocytes and macrophages. Only few human data are available in the literature in connection with CD markers after burn trauma.

Cytokines and growth factors have a significant impact on MMP and TIMP expression and alter the balance of the MMP-TIMP system in the period immediately after the burn injury. Today, this enzyme system represents a new class of biomarkers and with its varied expression patterns, plays a role in the development several pathological conditions. There are no data available regarding time changes within the MMP-TIMP system in severely burnt patients for the period immediately after the injury, and differences between survivors and non-survivors have not been studied to date.

The damaging effect of ROSs released during burn manifest mostly through destroying cell membranes rich in poly-saturated fatty acids i.e. lipid peroxidation. Lipid peroxidation end-products can be detected in tissues damaged by burn and in oedema fluid. Accumulation of free radicals can be observed parallel with marked oedema formation characteristic of the early stages of burn injury. Pathophysiological abnormalities developing as a result of burn injury are partly due to OS, as the resulting ROS causes damage to vital structures including nucleic acids of lipids, amino acids, proteins, cells and mitochondria and also serve as mediators of inflammatory cellular activation and enothelial dysfunction.

Under physiological conditions, p-Tyr is mainly produced in the kidneys, enzymatically from Phe via PAH. Reactive oxygen and nitrogen radicles lead to abnormal amino acid production. As a result of hydroxil radicle overproduction, it transforms into Phe p-Tyr, m-Tyr and o-Tyr. M-Tyr and o-Tyr are only produced in response to free radicals, thus, they are sufficient markers of OS. It is important to be aware of the fact that the enzymatic expression of p-Tyr is 100 times more pronounced than a free-radical reaction. The various Tyr isoforms and their behaviour in the kidneys in burn have not been measured to date, consequently, there are no data available with respect to the kinetics of Tyr isoforms in burn patients.

The immune-suppressive status developing immediately after burn trauma significantly increases patients' predisposition to infection. Early anti-inflammatory overload means a bad prognosis due to more frequent occurrence of sepsis and higher mortality rate. The most common complication and leading cause of death in burn is sepsis, which, according to new approaches, is the response of the organism to the infection. SIRS, developing as result of burn injury, and molecular changes during sepsis have similar consequences, in that they can be characterised by a damage associated molecular pattern (DAMP) and a pathogen associated molecular pattern (PAMP). Criteria for the clinical diagnosis of sepsis in burn patients were defined by the „American Consensus Conference".

Acute kidney failure developing during sepsis is one of the most common complications with ROS playing a role in its development. Inflammation and tissue hypoxia may induce ROS production in patients in a critical condition, while, generally, ROS may influence parenchymal kidney damage by causing microvascular and functional changes. Several studies have dealt with changes in amino acid levels in sepsis. As a result of catabolic metabolism observable in sepsis, the levels of most amino acids decrease, however, this decrease shows different degrees in the case of aromatic and branched-chain amino acids. It is important for the present study, that a significant increase has been described in serum levels of Phe and Tyr in septic patients as well.

2. Aims

2.1 Examination of CD markers in burn

Our aim was to investigate the expression and prognostic function of CD markers in circulating leukocytes at the early stage of burn injury.

- We intended to describe the kinetics of CD markers at the early stage of burn.
- We aimed at finding differences within the expression of CD markers in circulating leukocytes as compared to a control group.
- We wanted to compare CD marker expression in survivor and non-survivor patients.

2.2 Examination of MMP-9 and TIMP-1 in burn

Our aim was to provide important, missing data about changes in the MMP-TIMP system and their potential prognostic function in burn injury.

- We set out to investigate plasma concentrations of MMP-9 and TIMP-1 and MMP-9/TIMP-1 ratio in the early phase of SIRS in burn.
- Our study aimed at finding a correlation between plasma concentrations of MMP-9 and TIMP-1, MMP-9/TIMP-1 and TBSA .
- A further goal was to reveal significant changes upon investigating plasma concentrations of MMP-9 and TIMP-1, and the MMP-9/TIMP-1 ratio compared to healthy individuals.
- Our study also aimed at investigating differences in serum levels of MMP-9, and TIMP-1 and differences in MMP-9/TIMP-1 ratio between survivor and non-survivor patients.

2.3 Examination of tyrosine isoforms in burn

Our aim was to investigate time-related changes, prognostic value and the behaviour of various Tyr isoforms in the kidney in early stages of burn injury.

- Our aim was to investigate changes in p-, m- and o-Tyr values in the serum and in the urine.
- We intended to compare serum levels of p-, m- and o-Tyr with healthy controls.
- A further aim was to compare p-, m- and o-Tyr EX values in the urine with healthy controls.
- In order to describe the behaviour of various Tyr isoforms in the kidney, FE of Tyrs were calculated and compared with healthy controls.
- We wanted to search for correlation between infection markers and Tyr isoforms.
- We aimed at finding correlation between Tyr isoforms and clinical parameters.
- The present study intended to find the prognostic functions of various Tyr isoforms.

2.4 Examination of tyrosine isoforms in sepsis

As the most severe complication of burn injury is sepsis, we aimed at describing the time-related changes and behaviour of various tyrosine isoforms in the kidney in septic patients as well.

- We intended to examine changes in p-, m- and o-Tyr values in the serum and urine in septic patients following ICU admission.
- We intended to compare p-, m- and o-Tyr values in the serum and urine with a healthy control group.
- In order to describe the behaviour of various Tyr isoforms in the kidney, we wanted to calculate the FE of Tyrs and investigate its change with time.
- We intended to find a correlation between Tyr isoforms and infection markers.
- We intended to search for the prognostic role of Tyr isoforms.

3. Methods

3.1 The research ethical background of our investigations

The study protocol was designed in accordance with the ethical guidelines of the 2003 and 2008 Declaration of Helsinki. The measurements were performed at three different times (2484/2005, 2913/2007, 4282/2011, 4422/2012, 4422/2012). After receiving permission from the local ethics committee, informed consent was obtained from the patients or their closest relatives.

3.2. Patient

Our studies were performed for 5 and 6 days following the patient's intake, This study interval has been chosen because we had presumed from our earlier studies that this period would open a wide time window that could be enough for detecting both the ascending and descending oxidative stress responses

3.2.1 Examination in case of burn patients

In the case of burn injury at different times the following tests were performed: examination of CD markers (n = 35), examination of MMP-9 and TIMP-1 system (n = 31), tyrosine isoforms (n = 15). In our prospective descriptive studies our patients included on the basis of the following criteria were divided into two subgroups, survivors and nonsurvivors according to outcome. As a control group 18 persons were tested for CD markers, 10 person for the MMP-TIMP system and 15 person for healthy volunteers for tyrosine isoforms. Control groups did not show any difference in age and gender compared to the patient groups.

- Inclusion criteria in case of burn patients:

- burn injury affecting more than 15 % of the body surface
- the patient has been admitted to our ward within 3 hours after burning

- Exclusion criteria in case of burn patients:

- electrical injury
- age less than 18 years
- extreme burn severity (TBSA>80%)
- presence of any obvious bacterial infection on admission

- presence of any malignant disease
- documented previous medication affecting the inflammatory response of the body to burns (e.g.chronic use of corticosteroid)
- previously documented chronic left heart or renal insufficiency

3.2.2. Examination in case of septic patients

In order to establish the diagnosis of sepsis the second International Consensus Guidelines for sepsis were followed and several severity point systems (SAPS II, APACHE II, SOFA, MODS) were calculated. The patients included in the study (n = 20) were divided into two subgroups, survivors and non-survivors.

- Inclusion criteria in case of septic patients:

- Severe sepsis or septic shock detected at the time of admission
- Positive microbiological results on admission

- Exclusion criteria in case of septic patients:

- age less than 18 years
- presence of any malignant disease
- documented treatment that affects the immune response, or immunosuppression status
- oliguria on admission

3.3. Measurement methods

3.3.1 Sample collection

The control groups consisted of healthy volunteers from whom a peripheral blood sample was taken once. Blood samples from our patients were also taken without pain from the arterial cannula needed for their treatment. On admission and during the investigation period in parallel with the morning blood sampling, in the case of burned patients samples were taken before painful interventions. Incase of Tyr examination, urine was collected for 24 hours in both burned, septic and control groups. We measured both serum and urine creatinine levels. To standardize results, urinary levels of the assessed substances were corrected for urinary creatinine concentration, and fractional excretion (FE) was calculated.

3.3.2 Measurement of CD markers

All blood samples were transferred in a cooler on 4ºC and processed in 6 hours after takeoff in the Department of Surgical Research and Techniques. Flow cytometry was used to analyze the adhesion molecule (CD11a, CD11b, CD18, CD49d), LPS receptor CD14, and leukocyte activation marker CD97 expression on leukocytes. 200 µl of EDTA anticoagulated whole blood was mixed with 10 µl of mouse anti-human monoclonal antibodies CD11a, CD11b, CD18, CD49d, CD14, and CD97 (BD Pharmingen; San Diego, CA) conjugated with FITC or phycoerythrin were used for immunofluorescence staining of leukocytes for 15 min in dark at room temperature. Erythrocytes were haemolysed with diluted Becton Dickinson fluorescence activated cell sorter (BD FACS) Lysing Solution for 12 min. The leukocytes were washed twice in phosphate buffer solution (PBS), and finally resuspended in CellFIX solution. Cell immunofluorescence and light scatter data were acquired on a FACSCalibur (Becton Dickinson, USA) flow cytometer and analyzed by Cellquest software. Mouse isotype controls (BD Pharmingen San Diego, CA) were used to determine the non-specific background fluorescence. Binding of antibodies to leukocytes was quantified as the mean channel fluorescence in arbitrary units that exceeded non-specific background fluorescence. The measurements were made at the Department of Biophysics, University of Pécs, Hungary.

3.3.3 Measurement of serum MMP-9 /TIMP-1

Plasma was isolated from heparin anticoagulated blood samples by low speed centrifugation at 4 °C, and stored at -80 °C until analyzed in a single batch. MMP-9 and TIMP-1 were determined by the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) techniques according to the manufacturer's instructions (R&D Systems Inc., Minneapolis, MN, USA). In comparison with standard MMP and TIMP curves, the concentrations of MMP and TIMP in plasma were determined spectrophotometrically (Multiskan Ascent microplate photometer, Type: 354, Thermo Electron Corporation, Waltham, MA USA) by reading the absorbance at 450 nm, and were expressed as entire amounts in the plasma (ng/ml). The assays were executed at the Department of Surgical Research and Techniques, University of Pécs, Hungary.

3.3.4 Measurement of tyrosine isoforms

Serum and urine samples were stored at -80 °C until further examinations. Trichloro-acetic acid (TCA; 125 µl) was added to 500 µl serum or urine and samples were then incubated on ice for 30 min. Precipitate was separated by centrifugation. The supernatant was filtered by a syringe filter (0.2 µm) (Millipore, Billerica, MA, USA). Finally, serum and urinary levels of meta-, ortho-, para-tyrosine and phenylalanine were determined using reverse phase-HPLC (Shimadzu USA Manufacturing INC) (C18 silica column, 250x4 mm) with fluorescence detection (λEX=275 nm; λEM=305 nm for the tyrosines and λEX=258 nm; λEM=288 nm for phenylalanine) as described earlier. Concentrations were calculated using an external standard. The measurements were made at the 2nd Department of Medicine and Nephrological Center.

3.3.5 Measurement of inflammatory and renal function markers

Daily serum PCT, CRP, creatinine and urinary creatinine measurements were part of the routine monitoring of burned patients and they were carried out at the Institute of Laboratory Medicine, University of Pécs. The average hourly noradrenaline requirement was calculated on each day.

3.4 Statistical analysis

Statistical Package for the Social Sciences (SPSS) Statistics software, version 20.0 (IBM Corporation, USA) was used for statistical analysis. Data were expressed as median and inter-quartile range (IQR (standard 25^{th}-75^{th} percentile) as distribution was not normal according to Kolmogorov-Smirnov test. Kruskal-Wallis test followed by Mann Whitney U test was used for interday analysis. Patients were compared with healthy controls using Mann Whitney U test. Jonckheere-Terpstra test was used to detect significant trends across the study period. Correlations between variables were assessed using Spearman's test. Values of $p<0.05$ were considered significant

4. Results

4.1 Changes in CD markers in burn

4.1.1 Kinetics of CD markers

Expression of granulocytes CD11a, CD49d and CD97 was significantly higher compared to values measured upon admission on days presented on Figures 7.a, 7.c and 7.d similarly to values of lymphocyte CD97 and monocyte CD49d (Figures 3.a, 4.a), that showed significantly increasing tendencies. Monocyte CD14 expression (Figure 4.c) compared to the value measured on admission showed a significantly decreased value on days presented of the figure besides a significantly decreasing tendency.

4.1.2 Changes in serum levels of CD markers as compared with the control group

Expression of granulocytes CD11a (Figure 1.a) and CD18 (Figure 1.b) was significantly lower in burn patients on days shown on the figures, similarly to expression of lymphocytes CD11a (Figure 2.a), CD11b (Figure 2.b), CD18 (Figure 2.c), CD49d (Figure 2.d), CD97 (Figure 9.a), and monocytes CD11a (Figure 3.b), CD11b (Figure 3.c), CD18 (Figure 3.d), CD49d (Figure 4.a), CD97 (Figure 4.b) and monocyte CD14 (Figure 4.c). Expression of granulocyte CD49d (Figure 1.c) was significantly higher at the end of the study period. Expression of granulocyte CD97 (Figure 1.d) was significantly lower in burn patients during the first two days, while significantly higher values were found on days five and six compared to controls.

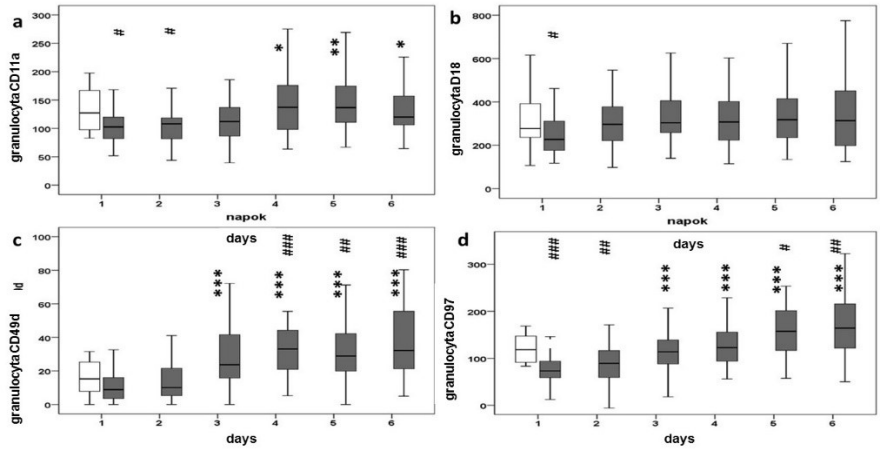

Figure 1. Granulocyte CD11a (a), CD18 (b), CD49d (c) and CD97 (d) expression in the control and burned groups. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the patient group; white boxes show healthy control results. The "#" symbols show significant differences (p <0.05) compared with controls (# p <0.05; ## p <0.01; ### p <0.001). Asterisks indicate statistical differences in the patient group compared with day 1(* p<0.05; ** p<0.01; *** p<0.001).

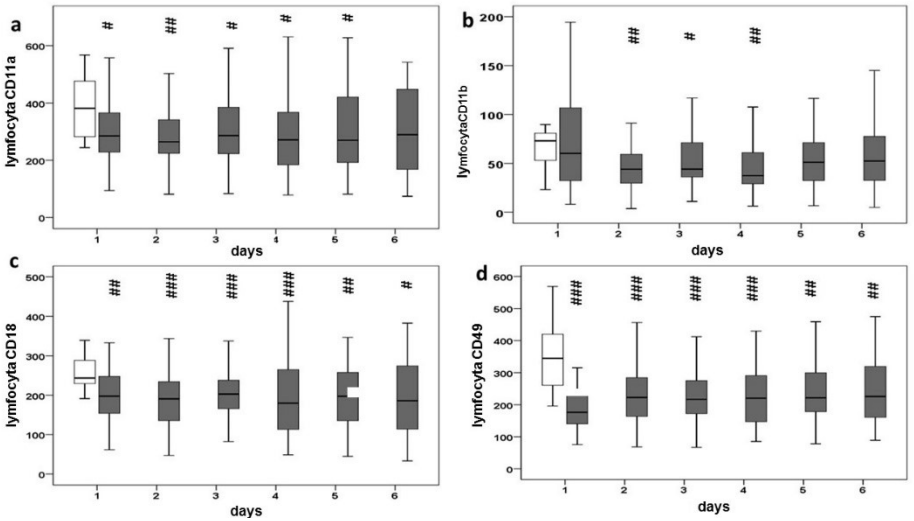

Figure 2. Lymfocyte CD11a (a), CD11b (b), CD18 (c) and CD49d (d) expression in the control and burned groups. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the patient group; white boxes show healthy control results. The "#" symbols show significant differences (p <0.05) compared with controls (# p <0.05; ## p <0.01; ### p <0.001). Asterisks indicate statistical differences in the patient group compared with day 1(* p<0.05; ** p<0.01; *** p<0.001).

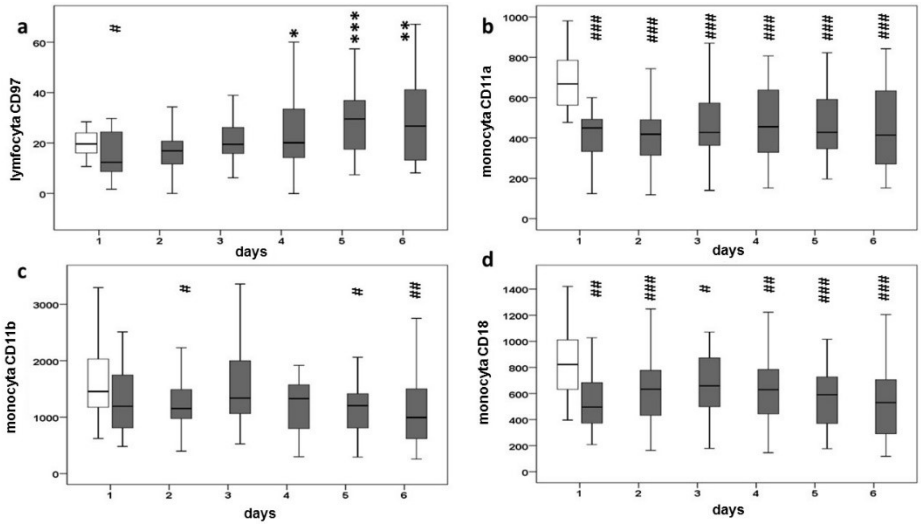

Figure 3. Lymfocyte CD97 (a), monocyte CD11a (b), CD11b (c) and CD18 (d)) expression in the control and burned groups. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the patient group; white boxes show healthy control results. The "#" symbols show significant differences (p <0.05) compared with controls (# p <0.05; ## p <0.01; ### p <0.001). Asterisks indicate statistical differences in the patient group compared with day 1(* p<0.05; ** p<0.01; *** p<0.001).

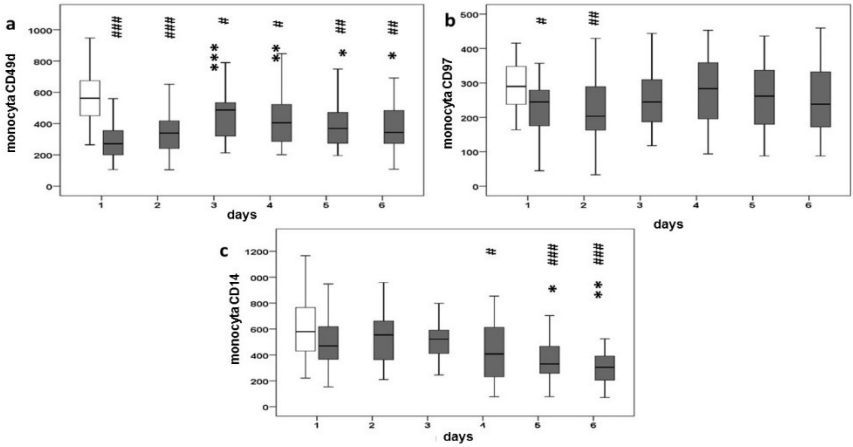

Figure 4. Monocyte CD49d (a), CD97 (b) and CD14 (c) expression in the control and in the burned groups. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the burned group; white boxes show healthy control results. The "#" symbols show significant differences (p <0.05; ## p <0.01; ### p <0.001). Asterisks indicate statistical differences in the patient group compared with day 1(* p<0.05; ** p<0.01; *** p<0.001).

4.1.3 Serum levels of CD markers in survivors and non-survivors

Expression of granulocyte CD11a (Figure 5.a), lymphocytes CD11a (Figure 5.b), CD11b (Figure 5.c), CD18 (Figure 5.d) and monocyte CD97 (Figure 5.e) was significantly higher in survivors than in non-survivors on days shown on the figure.

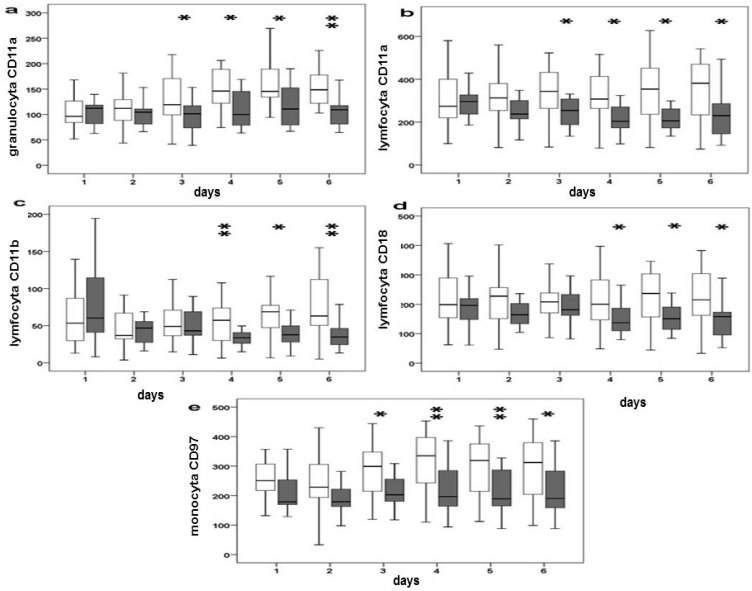

Figure 5. Granulocyte CD11a (a), lymfocyte CD11a (b), CD11b (c), CD18 (d) and monocyte CD97 (e) expression in the survivor and non-survivor groups. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the non-survivor group, white boxes show the suvivor group. Asterisks indicate statistical differences within the burned group between survivors and non-survivors (* p<0,05; ** p<0,01; *** p<0,001).

4.2 Changes in MMP-9 and TIMP-1 in burn

4.2.1 Kinetics of MMP-9, TIMP-1 and MMP-9/TIMP-1 ratio

MMP-9 showed a significantly decreasing tendency compared to the first day; the level was significantly lower on days shown on Figure 6.a, similar to MMP-9/TIMP-1 ratio (Figure 6.c). TIMP-1 was significantly elevated; it was significantly higher on days shown on Figure b. compared to the first day.

4.2.2 Changes in serum levels of MMP-9 and TIMP-1 as compared to the control group

MMP-9 level on admission and on day two was significantly higher, on days 4-6 it was significantly lower in burn patients (Figure 6.a). In the case of TIMP-1, we found no significant difference on admission, however, its value was significantly higher in burn patients on days shown on Figure 6.b. MMP-9/TIMP-1 ratio on admission was significantly higher in burn patients, but it was significantly lower on days on Figure 6.c.

4.2.3 Correlation between MMP-9, TIMP-1 and MMP-9/ TIMP-1 ration with extent of burn

MMP-9 (Figure 6.d) and MMP-9/TIMP-1 on admission showed a weak positive significant correlation with TBSA. TIMP-1 showed no correlation with extent of burn.

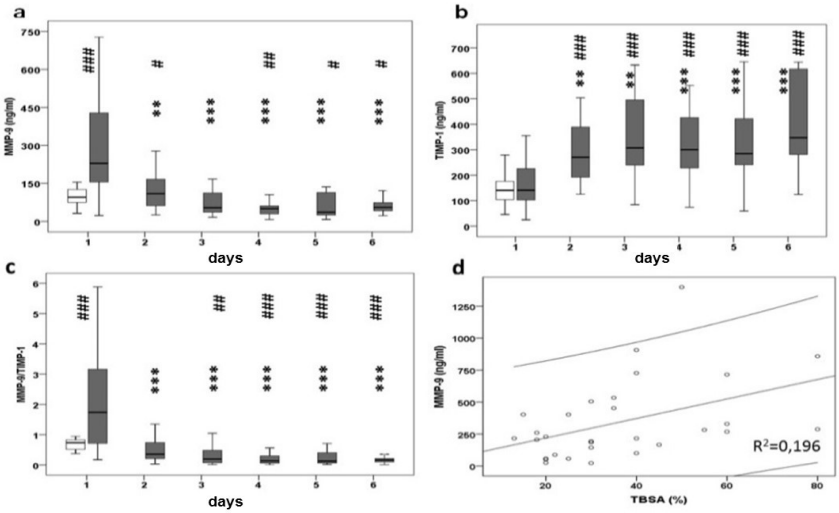

Figure 6. Serum level of MMP-9 (a), TIMP-1 (b) MMP-9/TIMP-1 ratio (c) in the control and patient groups and MMP-9 correlation with burned body surface (d). Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the burned group; white boxes show healthy control results. The "#" symbols show significant differences ($p < 0.05$) compared with controls (# $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$). Asterisks indicate statistical differences within the burned group compared with day 1 (* $p<0.05$; ** $p<0.01$; *** $p<0.001$). Part **d** shows the weak correlation between TBSA and MMP-9 on admission. TBSA indicates burned body surface.

4.2.4 Serum levels of MMP-9 and TIMP-1 in survivor and non-survivor burn patients.

There was no significant difference between MMP-9 and MMP-9/TIMP-1 values between survivors and non-survivors (Figures 7.a and 7.c). TIMP-1 showed a different tendency in survivors and non-survivors from day four, its values were significantly higher on days five and six in the non-survivor group (Figure 7.b).

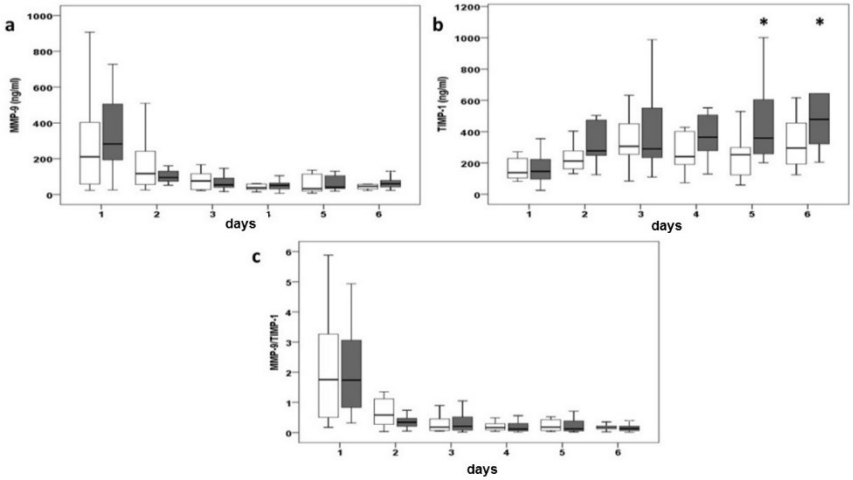

Figure 7. Serum level of MMP-9 (a), TIMP-1 (b) and MMP-9/TIMP-1 ratio (c) in the survivor and non-survivor groups. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the non-survivor group, white boxes show the suvivor group. Asterisks indicate statistical differences within the burned group between survivors and non-survivors (* p<0,05; ** p<0,01; *** p<0,001).

4.3 Changes of tyrosine isoforms in burn

4.3.1 Kinetics of tyrosine isoforms in burn

Serum p-Tyr showed a significantly decreasing tendency, its values were significantly lower on days showed on Figure 8.a compared to day one. Serum m-Tyr showed a non-significant, while serum o-Tyr showed a significantly increasing tendency. They showed significantly higher values compared to day one, on days shown on Figures 8.b and 8.c. Regarding serum Phe levels, neither a significant tendency nor a significant difference was revealed. (Figure 8.d).

4.3.2 Changes of tyrosine isoforms in burn patients as compared to the control group

On admission, tyrosine isoforms showed no significant difference compared to the control group, however, serum levels of p-Tyr were significantly lower on days shown on Figure 8.a. In the case of serum m-Tyr and o-Tyr levels, significantly elevated values

were measured on days shown on Figure 8.b and 8.c. Serum level of Phe on day two was significantly higher compared to the control group (Figure 8.d).

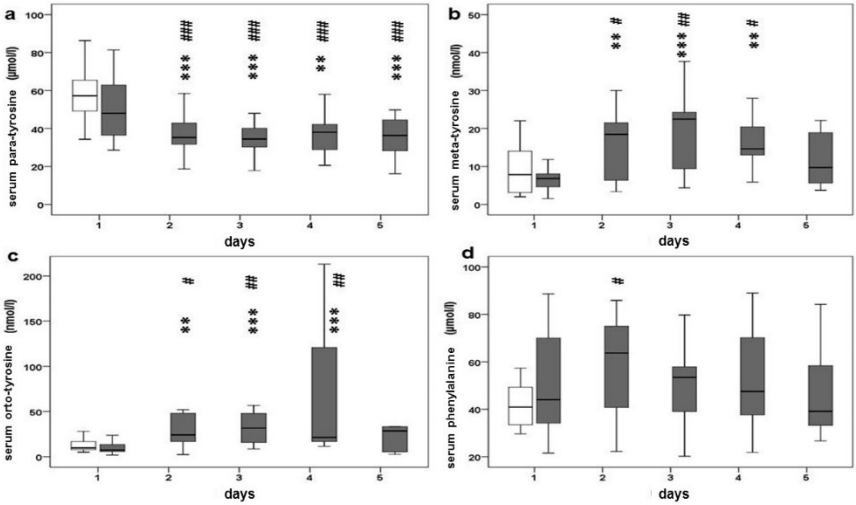

Figure 8. Serum level of p-Tyr (a), m-Tyr (b), o-Tyr (c) and Phe (d) in burned group. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the burned group; white boxes show healthy control results. The "#" symbols show significant differences ($p < 0.05$) compared with controls (# $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$). Asterisks indicate statistical differences within the burned group compared with day 1 (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

4.3.3 Changes of tyrosine isoform excretion in burn

$EX_{p\text{-}Tyr}$ in the urine showed a significantly increasing tendency. Significantly elevated values were found both as compared to day one and to the control group on days indicated on Figure 9.a. $FE_{m\text{-}Tyr}$ showed no significant tendency compared to values measured on admission. However, significantly higher values were measured on day five (Figure 9.b). Neither a significant tendency, nor a significant difference was revealed in connection with $EX_{o\text{-}Tyr}$. (Figure 9.c).

4.3.4 Changes of the fractional excretion of tyrosine isoforms in burn

$FE_{p\text{-}Tyr}$ showed a significantly increasing tendency. Significantly increased values were measured compared both to day one and to the control group on days shown on Figure 10.a. $FE_{m\text{-}Tyr}$ showed neither a significant tendency, nor a significant difference (Figure 10.b). No significant tendency was seen in connection to $FE_{o\text{-}Tyr}$, and although we found no significant differences compared to day one, results of measurements were significantly higher compared to those of the control group on days shown on Figure 10.c.

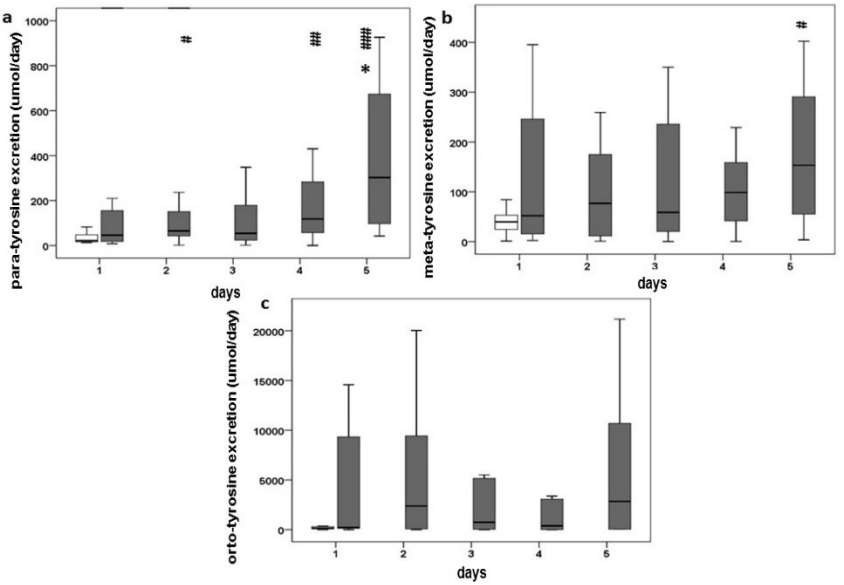

Figure 9. Changes of p-Tyr (a), m-Tyr (b) and o-Tyr (c) excretion in burned group. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the burned group; white boxes show healthy control results. The "#" symbols show significant differences (p <0.05) compared with controls (# p <0.05; ## p <0.01; ### p <0.001). Asterisks indicate statistical differences within the burned group compared with day 1 (* p<0.05; ** p<0.01; *** p<0.001).

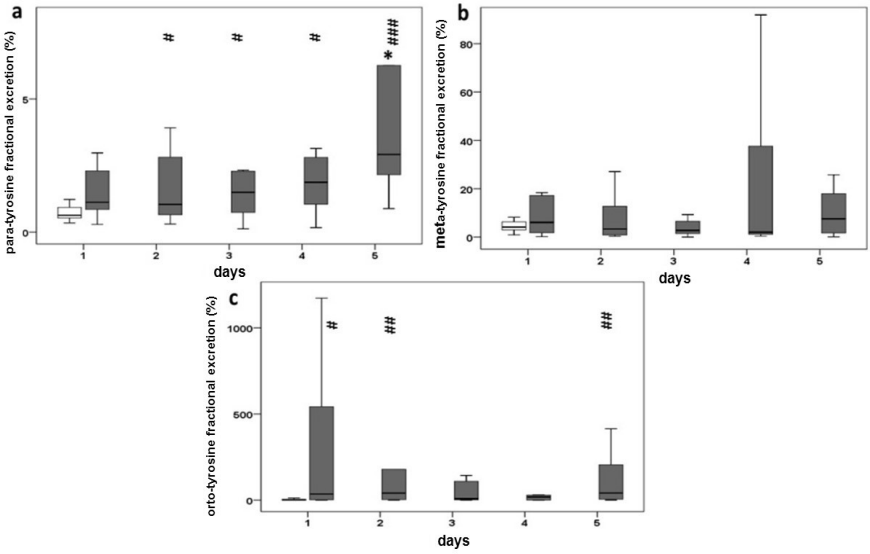

Figure 10. Changes of p-Tyr (a), m-Tyr (b) and o-Tyr (c) fractional exkretion in burned group. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the burned group; white boxes show healthy control results. The "#" symbols show significant differences (p <0.05) compared with controls (# p <0.05; ## p <0.01; ### p <0.001). Asterisks indicate statistical differences within the burned group compared with day 1 (* p<0.05; ** p<0.01; *** p<0.001).

4.3.5 Serum levels of tyrosine isoforms in survivor and non-survivor burn patients

Serum p-Tyr was higher in survivors throughout the entire study but it was only significant on admission (Figure 11.a). Serum-Tyr (Figure 11.b) and o-Tyr levels were lower in survivors during the present study. However, this difference was only significant on days shown on Figure 11. c. We found no significant differences in serum levels of Phe (Figure 11. d) and in the EX and FE of tyrosine isoforms.

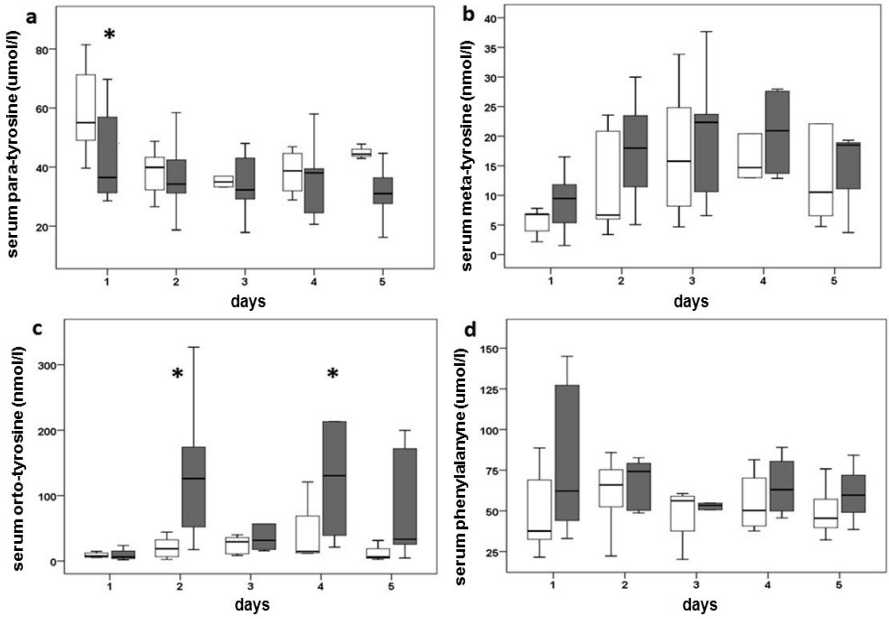

Figure 11. Serum level of p-Tyr (a), m-Tyr (b), o-Tyr (c) and Phe (d) in survivore and non-survivor groups.
Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the non-survivor group, white boxes show the suvivor group. Asterisks indicate statistical differences within the burned group between survivors and non-survivors (* p<0,05; ** p<0,01; *** p<0,001).

4.4.1 Kinetics of tyrosine isoforms in septic patients

P-Tyr showed a significantly increasing tendency; significantly elevated values were measure compared to day one on days shown on Figure 19.a. Serum m-Tyr (Figure 12.b), o-Tyr (Figure 12.c) and Phe (Figure12.) showed neither a significant tendency, nor a significant difference compared to day one.

4.4.2 Changes in serum levels of tyrosine isoforms in septic patients as compared with the control group

Serum levels of p-Tyr was significantly lower on admission and on day two as compared with those of the control group, by day five, this difference had disappeared (Figure 12.a). Serum m-Tyr was significantly higher in the sepsis group on days shown on Figure 12.b, while in the case of serum o-Tyr there were no significant changes detected (Figure 12.c). Serum levels of Phe were significantly higher compared to the control group during the entire study (Figure 12.d).

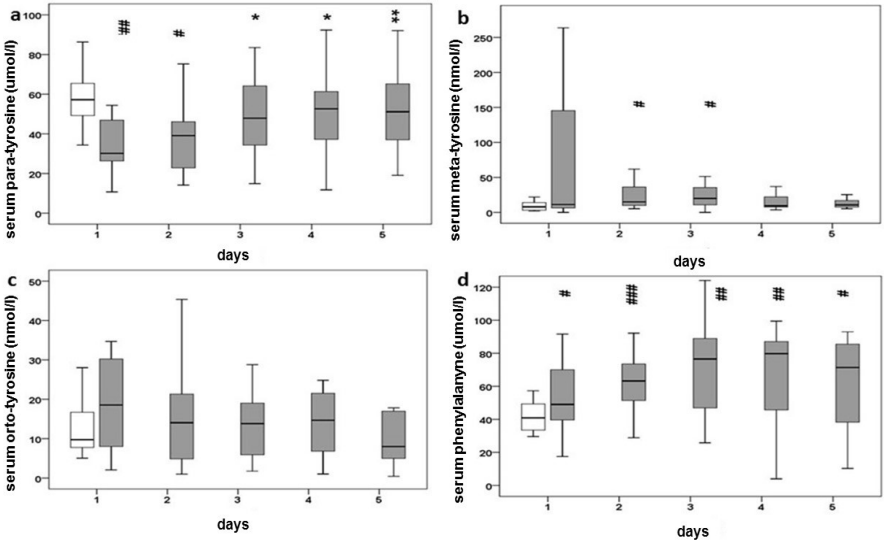

Figure 12. Serum level of p-Tyr (a), m-Tyr (b), orto-Tyr (c) and Phe (d) in septic group. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the septic group; white boxes show healthy control results. The "#" symbols show significant differences (p <0.05) compared with controls (# p <0.05; ## p <0.01; ### p <0.001). Asterisks indicate statistical differences within the septic group compared with day 1 (* p<0.05; ** p<0.01; *** p<0.001).

4.4.3 Changes of tyrosine isoform excretion in septic patients

$EX_{p\text{-}Tyr}$ in the urine showed a significantly elevating tendency. Significantly elevating values were found both compared to day one, and compared to the control group on days shown on Figure 13.a. $EX_{m\text{-}Tyr}$ showed a significantly decreasing tendency. Compared to day one, significantly lower values and compared to the control group, significantly higher values were measured on days indicated on Figure 13.b. $EX_{o\text{-}Tyr}$ levels showed no significant tendency. Compared to day one, significantly decreased values, compared to the control group, significantly higher values were measured on days on Figure 13.c.

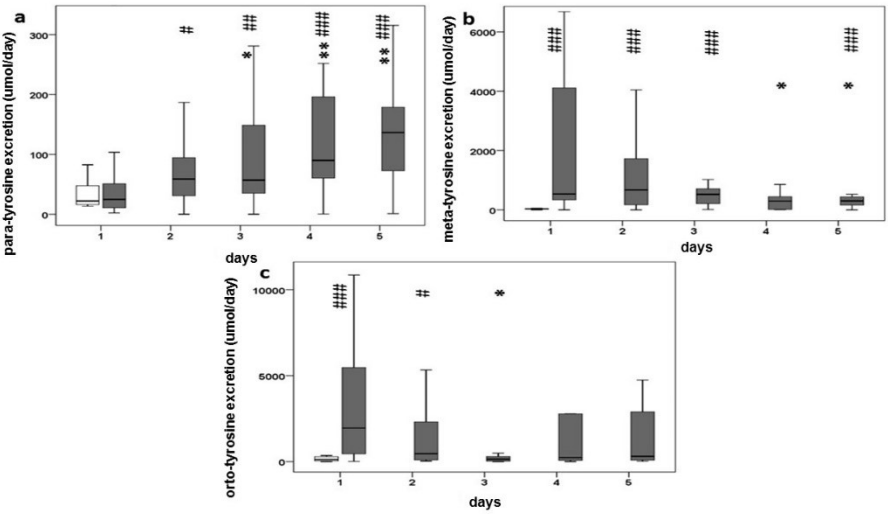

Figure 13. Changes of p-Tyr (a), m-Tyr (b) and o-Tyr (c) exkretion in szeptic group. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the septic group; white boxes show healthy control results. The "#" symbols show significant differences (p <0.05) compared with controls (# p <0.05; ## p <0.01; ### p <0.001). Asterisks indicate statistical differences within the septic group compared with day 1 (* p<0.05; ** p<0.01; *** p<0.001).

4.4.4 Changes of fractional excretion of tyrosine isoforms in septic patients

FEp-Tyr did not present a significant tendency but, compared to day one, on day four, a significantly higher value was measured compared to the control group during the entire study (Figure 14. a). FEm-Tyr showed a significantly decreasing tendency. Compared to day one, significantly lower values were measured on day five. Compared to the control group, significantly higher values were found on days shown on Figure 14. b. $FE_{o\text{-}Tyr}$ did not show a significant tendency and even when compared to day one, no significant difference was revealed. However, compared to the control group its values were significantly higher throughout the entire study period (Figure 14. c).

4.4.5 Comparison tyrosine isoforms and clinical parameters in survivor and non-survivor septic patients

Upon comparing survivor and non-survivor groups, significant differences were found in the amount of excreted urine on admission and in FEm-Tyr levels on the first and second day (Figure 15.). Regarding other parameters examined, no significant changes were found between survivors and non-survivors.

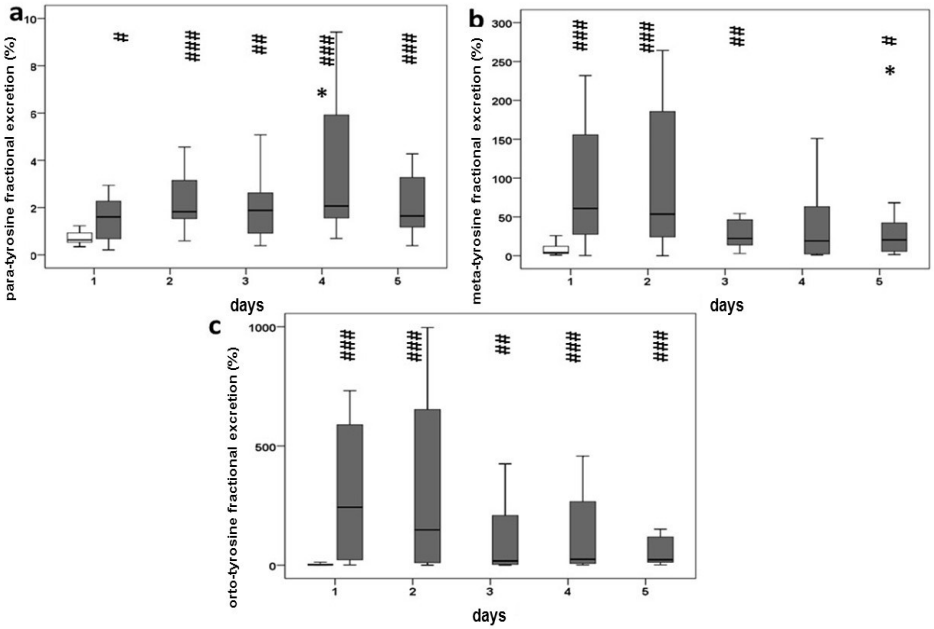

Figure 14. Changes of p-Tyr (a), m-Tyr (b) and o-Tyr (c) fractional exkretion in szeptic group. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the septic group; white boxes show healthy control results. The "#" symbols show significant differences (p <0.05) compared with controls (# p <0.05; ## p <0.01; ### p <0.001). Asterisks indicate statistical differences within the septic group compared with day 1 (* p<0.05; ** p<0.01; *** p<0.001).

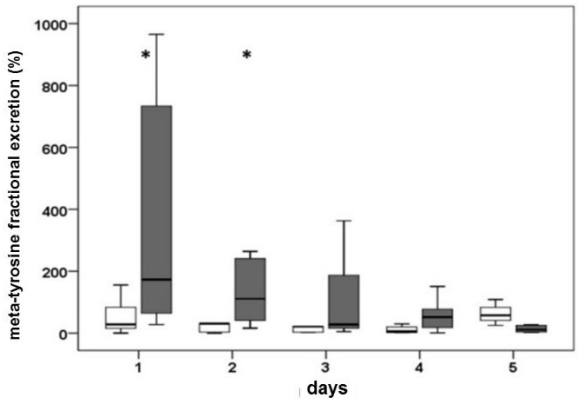

Figure 15. FEm-Tyr in survivore and non-survivor groups. Data are expressed as median and IQR (standard 25th75th percentile and 5th and 95th confidence interval). Shaded boxes represent the non-survivor group, white boxes show the suvivor group. Asterisks indicate statistical differences within the septic group between survivors and non-survivors (* p<0,05; ** p<0,01; *** p<0,001).

5. Summary

Our study investigated inflammatory and OS parameters subsequent to burn trauma and in sepsis . Sepsis is the most common complication and the leading cause of mortality in burn. In line with previous data in the literature, we found that burn was accompanied by significant inflammatory response and OS. From among inflammatory markers, we investigated CD marker expression of leukocytes that both play a role in inflammatory reactions and cell adhesion. Our study proved that an adequate inflammatory reaction is necessary for survival. This is supported by the fact, that surface marker expression was lower in burn patients compared with the control group and that in the case of certain markers, this decrease was more pronounced in non-survivor patients. Our results correlate with earlier findings of our research group, namely, that excessive anti-inflammatory reaction (elevated IL 10 level) may be a predictor of an adverse outcome. The MMP-TIMP system plays a role in wound healing and tissue remodelling. This is supported by the correlation observed between MMP-9, and the MMP-9/TIMP-1 ratio and the extent of burn. Furthermore, to our current knowledge, the MMP/TIMP system is also involved in the modulation of inflammatory processes and cytokines released during the inflammatory process and are able to influence the production of these chemicals. Accordingly, both MMP-9 and TIMP-1 levels were elevated during our investigation and this elevation was more marked in the case of an inhibitor chemical that manifested in the significant decrease in the MMP-9/TIMP-1 ratio in the first phase of our study. In our study, higher inhibitory TIMP-1 levels were found in patients who did not survive, which underlines the pivotal role of the need for an adequate inflammatory reaction for survival.

Our third study investigated changes of m-Tyr and o-Tyr produced by the body under normal conditions in response to p-Tyr and OS in burn and septic patients. In both patient groups p-Tyr levels decreased, in the background of which, damage to the co-factor of the PAH enzyme caused by OS may be suspected, that could verify the presence of OS in both pathological processes. Our supposition was supported by the elevation observed in serum levels of m-Tyr and o-Tyr. Under normal circumstances, the kidneys aim at a complete resorption of p-Tyr. Increasing FEp-tyr indicates deterioration of this function. This may be due to subclinical renal damage, as it can also be observed in patients with normal creatinine levels. Kidney damage may result from inflammation,

OS or hypovolaemia. Our study did not include investigation of sensitive markers of glomelular and tubular damage. A study is currently under way to provide missing data. Initial results support the above theory. Increased FE of tyrosine isoforms produced in response to OS refers to intra-renal synthesis and thereby, further supports the role of OS.

6. Novel findings

- Our study was the first to describe the kinetics of granulocytes, lymphocytes and monocytes CD11, CD18, CD49d and CD97 on a human patient population subsequent to burn injury. The expression of granulocyte CD11a, lymphocytes CD11a, CD11b, and CD18 and monocyte CD97 was significantly lower in burn patients who did not survive.
- A weak, but significant positive correlation was observed between extent of burnt body surface and MMP-9 levels.
- TIMP-1 level showed a significantly higher increase in non-survivor burn patients.
- Our study was the first to examine the kinetics of various tyrosine isoforms in burn and septic patients. Although serum p-Tyr was decreased in both burn and septic patients, due to the different kinetics of the disease a decreasing tendency could be observed in burn patients and an increasing trend was found in patients with sepsis.
- In burn patients, serum levels of m-Tyr and o-Tyr were both elevated.
- In septic patients, only m-Tyr was significantly increased.
- FEp-Tyr was elevated compared to controls in both groups.
- In septic patients, FEm-Tyr was higher and FEo-Tyr was elevated compared to the control group; in burn patients only FEo-Tyr was elevated.
- Serum p-Tyr was significantly lower and o-Tyr was significantly higher in non-survivor burn patients on admission.
- FEm-Tyr in the urine was significantly higher among non-survivor septic patients.