Hypovolemia and oxidative stress in burned patients

Ph.D. thesis

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1. Introduction

Burn is a common injury mainly in the developing countries caused by direct thermal injury, electrical trauma, chemicals or radiation. Thermal injury usually causes superficial injury only which heals without any scar formation. In serious cases airways, oral-cavity or oesophagus can be affected too. The severity of the injury is determined by the depth of burn (it depends on the temperature and time of exposure) and the extent of burned surface. Burn injury affecting more than 20% of the body surface (TBSA) causes a generalised illness. This condition requires special intensive care because not only the skin affected by burn injury or underlying anatomical structures are damaged but pathophysiological changes affecting the whole body can develop.

Burn injury is associated with pain. It can upset the balance in the neuroendocrine system, an overwhelming antiinsulinar hormone production can be observed, hypothalamus-hypophysis-adrenal-glands-axle will be activated. These changes can lead to a catabolic metabolism. The organism is in an immunsuppressed status, the immunoglobuline production is decreased (down-regulation), and it can lead to different kinds of infections. In the kidneys vasoconstriction can develop, glomerular filtration rate is decreased. The disengaged haemoglobin, myoglobin can precipitate in the tubule causing acute renal failure. The adaptive mechanisms for restoration of the circulating blood volume are initiated. The ADH secretion is increased the ANP secretion is decreased. In the stomach and bowels vasoconstriction can develop caused by disengaged Tx-A_2. Due to the decreased circulation of the mesenterial blood vessels the barrier function of the bowel is damaged. It can lead to bacterial and endotoxin translocation. The activated immune system produces a lot of different inflammatory mediators. Macrophage and leukocyte activation can force the production of free radicals and metabolites of arachidon acid metabolism. These factors can play a role in the early oedema formation, cytokine production (TNF α, IL-1, -2, -6). The liberated metabolites play an important role in the systemic inflammatory reactions and inflammatory reactions developing around the wound. The citokine IL-6 takes part in the initiation of inflammatory response moreover it has a prognostic value because its level correlates with the severity of burn injury and mortality rate. The increased vascular permeability is responsible for fluid and protein loss into the interstitial space. On the basis of the above mentioned mechanisms generalised oedema and hypovolemia develops after burn injury affecting more than 20% of the body. Its adequate therapy is crucial in the treatment of burned patients.

2. Aims

Fluid replacement can be guided by schemata, hourly urine output and invasively measured parameters. It shows that the ideal parameter has not been found yet. Schemata can not cover the fluid requirement of each individual patient moreover there are factors (inhalation injury or delayed fluid resuscitation) which can increase the fluid requirement of burned patients whereas the effect of several factors has not been studied on the fluid requirement of burned patients. It has not been proven if fluid resuscitation guided by invasively measured parameters (ITBVI, SVV) is better than common hourly urine-output is used as method of guidance.

Oxidative stress alone and also its ability to lead to a systemic inflammatory response syndrome play important role in decreasing capillary permeability and formation of osmotic active molecules. These changes play an important role in the oedema formation after burn trauma. Moreover it is known that Ischemia-reperfusion may be the underlying cause of oxidative stress response but the correlation between the severity of thermal injury and the developing oxidative stress has not yet been studied satisfactorily. Moreover it has not been cleared how different fluid resuscitation strategies influence the developing oxidative stress reaction.

Finding an answer to of the following questions was aimed at:

1. Hypovolemia following injury can be treated by schemata. The most commonly used Parkland formula underestimates the fluid requirement in 45 - 63% of the cases. In our retrospective study factors with unknown effect on the fluid requirement in burn injury were investigated. We tried to make the Parkland formula more precise.

2. We wanted to compare the efficiency of ITBVI guided fluid replacement to the hourly urine output guided therapy.

3. The early normalisation of $ScvO_2$ could increase the survival rate in septic patients. Due to this fact we studied how the earlier normalisation of $ScvO_2$ using one out of the above mentioned fluid administration protocols influences the organ functions on the 5 consecutive days after injury. The applicability of an early goal directed therapy was also studied.

4. Only a few data exist in the literature regarding the correlation between extent of burn injury and the resulting oxidative stress. We wanted to study the correlation of these parameters and changes of oxidative stress marker levels on the 5 consecutive days after burn trauma.

5. Ischemia-reperfusion is a well known mechanism of free radical formation. After restitution of the circulating blood volume it plays a role in the free radical formation in burn injury. We intended to examine how a more adequate fluid resuscitation protocol could influence the oxidative stress in patients suffering from burn trauma.

3. Methods

3. 1. Patients:

Patients admitted to our intensive ward or to the 1st surgical department were involved in the studies after permission of the regional ethics committee. After obtaining permission from the local ethics committee, informed consent was obtained from the patients or their nearest relative. Inclusion criteria were burn injury affecting more than 15% of the body surface and in hospital fluid resuscitation started within three hours based on our protocol. The extent of burn was calculated using the Lund-Browder chart. Exclusion criteria were documented chronic renal or heart disease, electrical injury, acute kidney failure developed within 72 hours and age under 18 years. By suspected inhalation injury (chest X-ray, smooth in the throat) bronchoscopy was used for verification.

3. 2. Fluid resuscitation protocoll:

Fluid resuscitation was guided by the hourly urine output (HUO) or invasive haemodynamic parameters (Figure 1.)

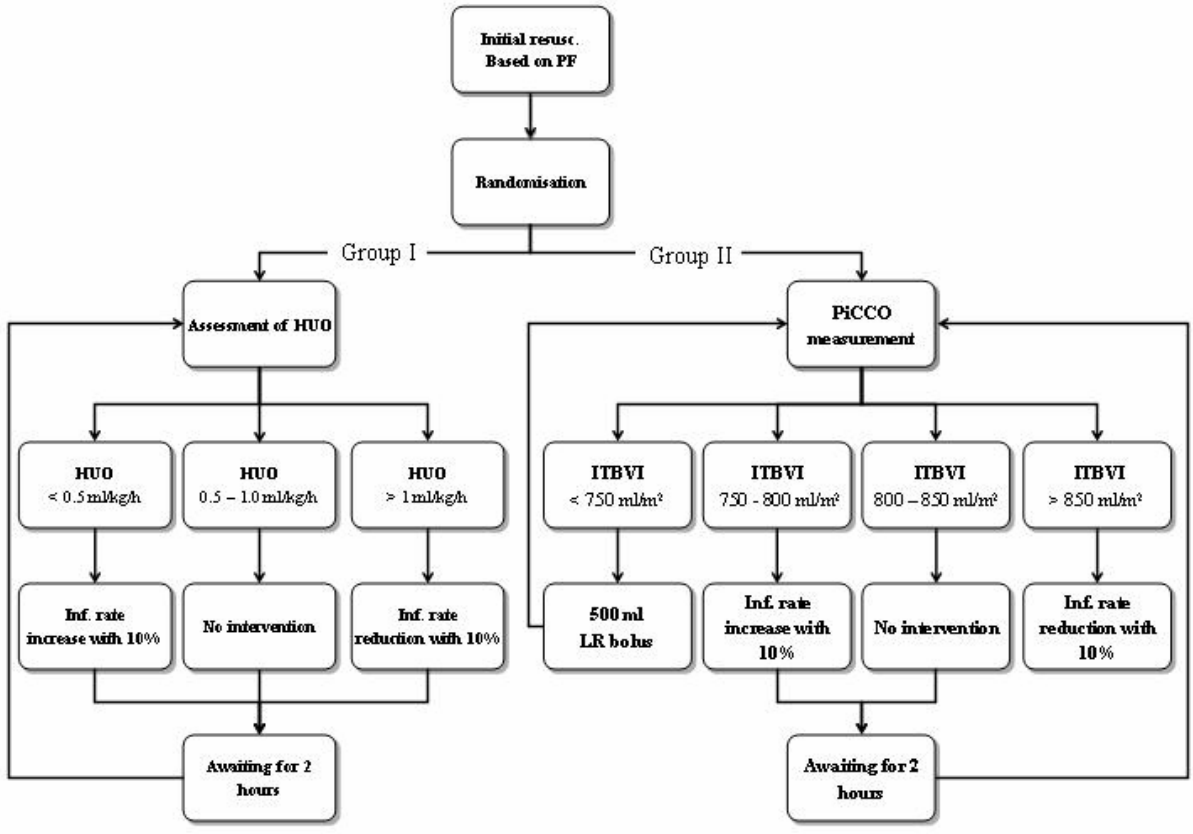

Figure 1 *Flowchart of fluid administration in both groups. PF = Parkland formula, PiCCO = transpulmonary haemodynamic measurement, ITBVI = Intrathoracic blood volume index, HUO = hourly urine output,*

3. 3. Measurement-techniques:

Measurement of malondialdehyde with Ohakawa method

Plasma MDA is one of the derivatives originating from oxidative damage of the poly-unsaturated fatty acids, thus it indirectly shows intensity of lipidperoxidation due to oxidative stress. We attained plasma from EDTA anticoagulated blood - centrifuged at 4000 rpm for 10 minutes - and mixed with sodium-dodecyl sulphate, acid buffer and EDTA. Thiobarbiturate solution was added to the mixture and incubated for an hour at 90 ^{o}C. After cooling, adding butanol and repeating centrifugation, the supernatant was measured with spectophotometry at 532 nm. Tetrametoxipropane was used as a standard and MDA was expressed in μmol l^{-1}. The value of control population was 0.24$\pm$0.05 μmol l^{-1}.

Measurement of reduced gluthation in whole blood.

Reduced gluthation is a basic endogenous antioxidant, the level of which is reduced due to oxidative stress of different origin. A sample of 0.2 ml EDTA anticoagulated blood was haemolysed with 0.8 ml of distilled water, mixed with 4 ml trichlore acetic acid (TCA) of 10% concentration. After centrifugation the supernatant was mixed with 4 ml TRIS buffer of pH 8.7. A colour reaction was induced with 100 μl of 10 mM 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) followed by photometry at 412 nm. Using a standard GSH series for calibration, values were expressed in μmol l^{-1}. The value of control population was 806$\pm$55 μmol l^{-1}.

Measurement of plasma protein sulphydril groups level with Ellman's reagent.

Plasma SH groups originate predominantly from plasma proteins and participate in the defence against oxidative stress. To determine SH groups 100 μl plasma, 100 μl Ellman's reagent (1 mM DTNB in methanol) and 800 μl EDTA containing TRIS buffer were mixed and photometry was performed at 412 nm. GSH standard series were used for calibration. The PSH amount was expressed in μmol l^{-1}. The value of control population was 51.8$\pm$1.2 μmol l^{-1}.

Determination of superoxide dismutase enzyme activity in whole blood.

Superoxide dismutase is an enzymatic endogenous antioxidant which catalyzes the dismutation of the superoxide free radical to H_2O_2. To determine SOD activity 100 µl of EDTA anticoagulated blood was haemolysed with 900 µl distilled water and a mixture of ethanol and chloroform (2:1) was used to remove haemoglobin. Determination of the enzyme activity was based on the inhibition of the spontaneous oxidation of adrenaline to adrenochrome. Spectrophotometric measurements were performed at 480 nm against sodium carbonate buffer (pH 10.2) blind at 37 ºC. The values of SOD enzyme activity were expressed in U ml^{-1}. The value of control population was 735$\pm$48 U ml^{-1}.

Determination of catalase enzyme activity in whole blood.

CAT metabolizes H_2O_2 by reducing it to water and oxygen. This prevents the secondary generation of toxic intermediates. This reaction is particularly important when hydrogen peroxide levels are elevated. To determine CAT activity 100 µl of EDTA anticoagulated blood was washed with 900 µl saline solution. Following centrifugation washing procedure was repeated, and changes in extinction at 240 nm was measured at 37 ºC in phosphate buffer containing 10 µl of red blood cells and 30 mM H_2O_2 solution. The value of control population was 1931$\pm$72 BU ml^{-1}.

Determination of reactive oxygen species production in whole blood.

Activated leukocytes, mainly neutrophils, are potential sources of reactive oxygen species during inflammation. Free radical generating capacity of circulating

leukocytes was assessed by measuring the amount of reactive oxygen species in whole blood, with chemiluminescense method based upon the reaction of luminol with free radicals. To sum up, 20 µl EDTA anticoagulated blood was diluted in 1400 µl Dulbecco's modified Eagle's medium (DMEM) nutrient mixture of 37ºC. 30 µl of 3-aminophtalhydrazide was added and the cuvette, which was immediately placed to Whole Blood Lumi-aggregometer (Chrono-Log, Model 560, USA). The mixture was stirred and incubated at 37 ºC during measurement. After determining the spontaneous radical production, 50 µl phorbol-12 myristate-13 acetate (PMA) was injected into the cuvette and the resulting light output was recorded on a chart recorder (Chrono-Log, Model 707, USA). The peak value of free radical production, the maximal rate of radical production were calculated from the recorded curve, and the results were related to the white blood cell counts. The lag phase between PMA stimulation and the start of steep elevation in radical production was also determined. These values in control population were: peak 34.0±5.5 AU, slope 0.048±0.007 AU, lag phase 215±30 sec.

3. 4. Statistical analysis:

Statistical Program for Social Sciences 11.5 for Windows (SPSS®, Chicago, Ill., USA) was used for statistical analyses. The distribution of our date was examined at first. If our data does not show normal distribution Spearman test was used for proving the correlation between parameters. Changes over time were analyzed with multi-measure ANOVA the distributions between groups were studied with Chi-square test. Differences between groups were compared with Mann-Whitney U test. Data are given as median and interquartile range. In case of normal distribution one sample t-test and ANOVA were used. Data were expressed as mean and standard deviation. Statistical significance was ascribed to a p value < 0.05. For assessment of the necessary case numbers power analysis was performed.

4. Studies

4. 1. Factors affecting fluid requirement on the first day after severe burn trauma

4. 1. 1. Aim of the study:

The object of the study was to asses the effect on the fluid requirement of the following parameters in the first 24 hours after injury: age, gender, BBS, BMI and presence of deep burn injury. Our data were compared to the calculated fluid requirement based on the Parkland formula.

4. 1. 2. Results:

Fifty five patients met the inclusion criteria, 8 patients were excluded due to insufficient data of pre-hospital fluid administration. Renal failure was not detected in the first 3 days therefore, no exclusion was applied on this basis. The study population consisted of 47 patients (7 females, 40 males), with the median age of 48 years (IQR 39-62), median BBS was 35.1% (IQR 25.2-51.4). Full thickness burn was present in 15 cases.

The median VIWB(24h) was 4.66 ml kg^{-1} $\%^{-1}$ (IQR 4.15-5.13) in the first 24 hours. The fluid requirement of each patient is shown in figure 2.

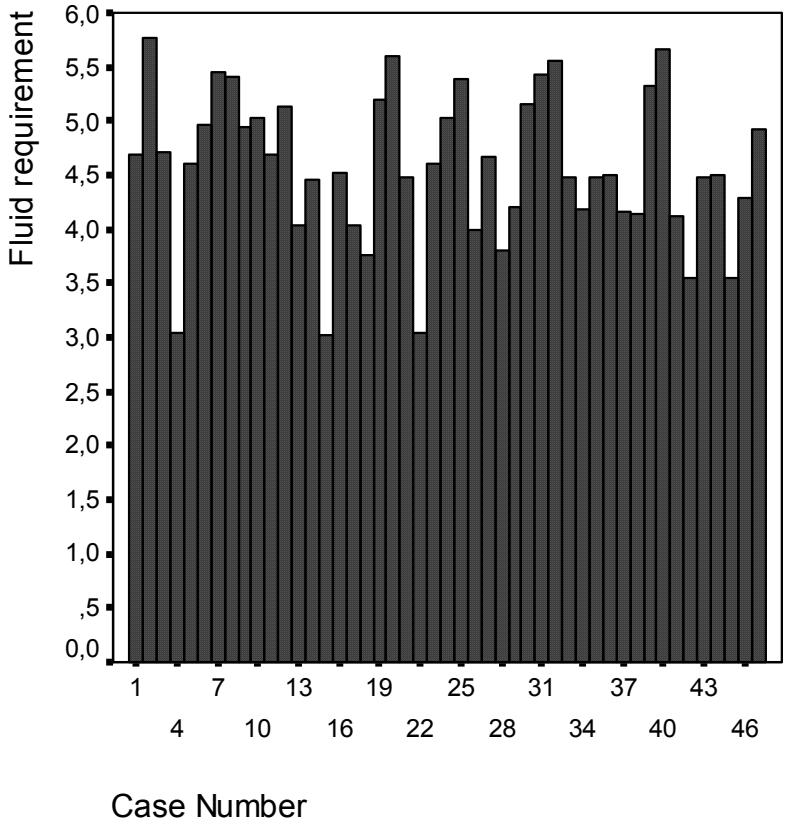

Figure 2. *Graph shows fluid requirement of each individual patient in the first 24 hours time expressed as ml* kg^{-1} $\%^{-1}$.

Patients received significantly higher median total volume of intravenous infusion than calculated by the PF: 14,500 ml day^{-1} (IQR 8,550-18,640) versus 9,800 ml day^{-1} (IQR 7,500-14,000), respectively ($p < 0.05$). Negative linear correlation was found between VIWB(24h) and BMI ($r = -0.570$, $p < 0.01$) (Figure 3.) as well as between VIWB(24h) and BBS ($r = -0.553$, $p < 0.01$) (Figure 4.)

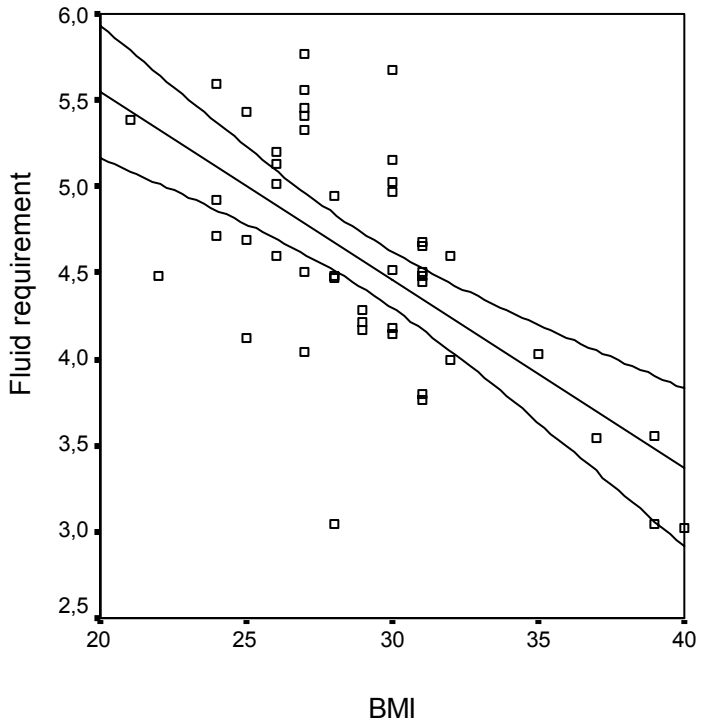

Figure 3. *Negative correlation was found between infused volume related to body weight and burned surface area and body mass index (BMI, r = -0.570, p < 0.01). Fluid requirement is expressed as ml kg^{-1} %$^{-1}$, BMI as kg m^{-2}.*

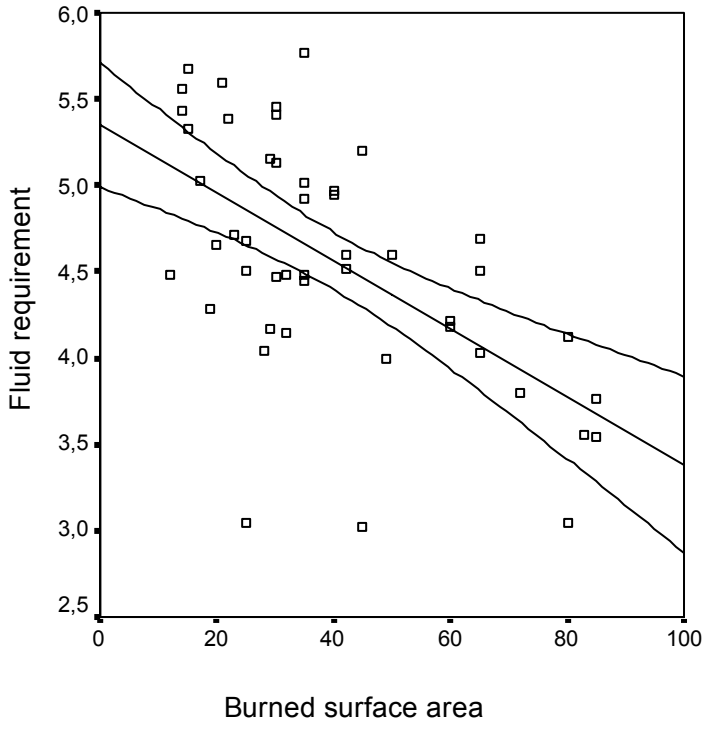

Figure 4. *Negative correlation was found between infused volume related to body weight and burned surface area (r = -0.553, p < 0.01). Fluid requirement is expressed as ml kg^{-1} %$^{-1}$, burned surface area as percentage.*

According to these observations, correction factors can be applied in order to improve the predictive accuracy of Parkland formula (Table I.and II.).

BMI	Correction factor
20-25 kg m^{-2}	1.30
25-30 kg m^{-2}	1.18
30-35 kg m^{-2}	1.05
35-40 kg m^{-2}	0.90

Table I. Correction factors of the Parkland formula for sections of body mass index (BMI).

BBS	Correction factor
< 20%	1.3
20-40%	1.2
40-60%	1.1
60-80%	1.0
80-100%	0.9

Table II*. Correction factors of the Parkland formula for sections of burned body surface area (BBS).*

The rate of fluid administration in patients with full thickness burn injury was significantly higher than in patients having only superficial injury: 4.95 ml kg^{-1} %$^{-1}$ (4.60 to 5.38) and 4.47 ml kg^{-1} %$^{-1}$ (4.03 to 4.90), respectively (p < 0.01) (Figure 5.).

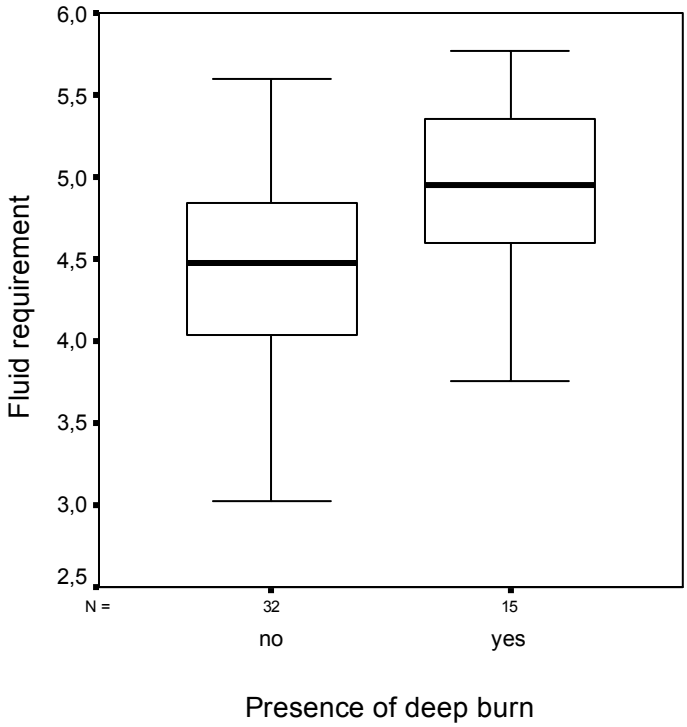

Figure 5. *The box represents the 25th-75th percentiles, the dark line is the median and the extended bars represent the minimum and maximum values. No = patients having superficial burn injury only, yes = patients having deep burn injury (p < 0.01). Fluid requirement is expressed as ml kg^{-1} %$^{-1}$.*

4. 2. The use of arterial thermodilution to monitor fluid resuscitation in burns suggests a more rapid fluid administration protocol that leads to a concomitant increase in central venous oxygenation.

4. 2. 1. Aim of the study:

The object of the study was to assess the effect of fluid resuscitation guided by invasively measured parameters on the saturation of central venous haemoglobin ($ScvO_2$) and the different organ functions characterised by multiple organ dysfunction score (MODS).

4. 2. 2. Results:

The study population consisted of 24 patients (2 females, 22 males), median age 46 years (IQR 39-63), and median BBS 43% (IQR 30-63). Each patient was mechanically ventilated during the study period.

Significantly more fluid was administered in the ITBVI-Group than the HUO-Group on the first day after injury (5.7 ml BBS^{-1} kg^{-1}, IQR 4.5 - 6.5; 4.7 ml BBS^{-1} kg^{-1}, 4.4 - 5.2, respectively, (p=0.019). Moreover, 58 % of the extra fluid was administered in the first eight hours, 28% in the second eight hours and only 14% in the last eight hours.

$ScvO_2$ was significantly lower in HUO-Group than in ITBVI-Group for the first 24 hours but no significant differences were detected in the second and third days. The MODS was significantly higher in the HUO-Group than the ITBVI-Group at 48 and 72 hours after injury. However, no significant differences were present between groups in the first 24 hours after injury. No significant differences were detected in lactate level, PaO_2, PaO_2/FiO_2 ratios and EVLWI between the HUO and ITBVI-Groups, on the first, second and third days after injury (Table III).

Parameter	Group	Day 1	Day 2	Day 3
$ScvO_2$ (%)	HUO	68 (64-71)	72 (61-77) †††,	76 (71-78) †††,**
	ITBVI	74 (71-78)	74 (69 – 79)	77 (73-79) ††,**
		p=0.024	NS	NS
MODS	HUO	4.0 (2.0 – 5.0)	5.0 (4.3-5.8) †	5.0 (4.3 – 6.0) †
	ITBVI	3.5 (3.0 – 5.0)	4.0 (3.0-4.3)	3.0, (3.3 – 3.8) *
		NS	p=0.024	p=0.014
ITBVI (ml m^{-2})	HUO	723 (590-844)	802 (701-959) †††	860 (785-965) †††,*
	ITBVI	851 (753-970)	873 (817-920)	970 (903-1020) ††,**
		p=0.009	p=0.043	p=0.003
CVP (cmH_2O)	HUO	6.0 (4.0 – 11.5)	8.0 (4.0 – 12.0)	11.5 (5.0 – 12.8) †
	ITBVI	8.0 (4.8 – 12.0)	10.0 (6.8 – 18.3)	12.0 (7.0 – 15.8) †
		NS	NS	NS
CI (l m^{-2})	HUO	3.0 (2.4-3.5)	3.9 (3.0-5.0) ††	3.8 (2.9-4.7) ††
	ITBVI	3.5 (3.3-3.8)	4.2 (3.4-5.2) *	4.3 (3.1-5.0) **
		p=0.013	NS	NS
Serum lactate	HUO	2.4 (1.7 – 3.6	2.0 (1.6 – 3.1) †	1.7 (1.3-2.8) ††,*
(mmol l^{-1})	ITBVI	2.3 (1.3 – 4.7)	2.2 (1.6 - 3.2)	2.0 (1.4 – 2.3)
		NS	NS	NS
PaO_2/FiO_2 ratio	HUO	43.7 (32.8 – 49.6)	34.5 (29.2 – 37.5)††	32.6 (28.6-34.9) ††,*
(kPa)	ITBVI	44.5 (33.7 – 55.1)	29.7 (26.6 – 32.9)††	30.5 (27.2-33.9) ††
		NS	NS	NS
EVLWI (ml kg^{-1})	HUO	6.0 (5.0 – 7.0)	7.0 (6.0 -9.0)	7.0 (6.5 – 7.0)
	ITBVI	6.0 (5.0 – 7.0)	6.0 (5.5 – 7.8)	6.5 (6.0 – 7.8)
		NS	NS	NS
EVLWI/ITBVI	HUO	0.0082 (0.0068 – 0.0098)	0.0085 (0.0078 – 0.0103)	0.0081 (0.0068 – 0.0098)
(m^2 kg^{-1})	ITBVI	0.0070 (0.0059 – 0.0088)	0.0072 (0.0055 – 0.0082)	0.0069 (0.0054 – 0.0086)
		p=0.025	p=0.008	p=0.023
Urine output	HUO	0.8 (0.6-1.0)	0.9 (0.8-1.0) †	0.8 (0.7-1.0) *
(ml kg^{-1} h^{-1})	ITBVI	1.1 (0.9-1.4)	1.0 (0.7-1.1)	1.0 (0.9-1.2)
		p=0.0008	NS	p=0.0012
Haemoglobin (g/l)	HUO	125.0 (110.0-138.8)	120.0 (102.5-130.0)	112.3 (101.3–120.3)†††,**
	ITBVI	112.5 (100.3-120.5)	110.3 (100.3-115.5)	100.3 (90.5–110.3)†††,**
		p=0.002	p=0.007	p=0.009

Table III. *Summary of measured and calculated parameters of the patients during the study period (72 h). Data are expressed as median and interquartile range. Median values were calculated taking all measurements of all patients of a group on the same day. $ScvO_2$ = oxygen saturation of the central venous haemoglobin, MODS = multiple organ dysfunction score, ITBVI = intrathoracic blood volume index, CVP = central venous pressure, CI = cardiac index, EVLWI = extravascular lung water index. Symbols indicate within group differences, † = p<0.05, †† = p<0.01, ††† = p<0.001 compared to day 1, * = p<0.05, ** = p<0.01, *** = p<0.001 compared to day 2, p values and NS indicate intergroup statistical differences.*

The two main outcome parameters, i.e. MODS calculated 48 and 72 hours after injury, were in a significant negative linear correlation with $ScvO_2$ measured on day 1 (r=-0.684, p= 0.004, r=-0.677, p=0.003). A significant linear correlation was found between ITBVI and $ScvO_2$ (Figure 6). Strong correlation was found between ITBVI and CI.

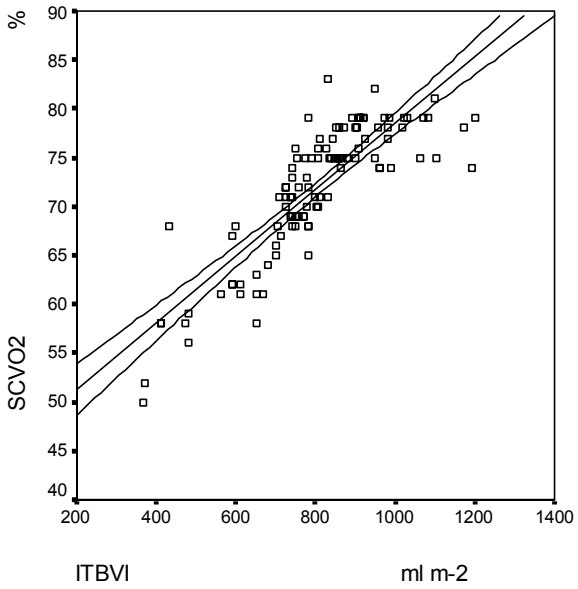

Figure 6: *Correlation between ScvO2 and ITBVI on the first three days. (r=0,855, p=0,008).*

Similarly, there were correlations between $ScvO_2$ as the first outcome parameter and CI. There was a significant negative correlation between SVV as a measure of preload and $ScvO_2$.
HUO and CVP did not correlate with $ScvO_2$, nor any other haemodynamic parameters. No correlation was found between serum lactate level and MODS measured on days 1-3, $ScvO_2$ measured on days 1-3, ITBVI, HUO, and CVP values (Table IV).

Parameters	Correlation factors	p value
ITBVI - $ScvO_2$	r=0.855	p=0.0008
ITBVI - CI	r = 0.491	p = 0.0008
$ScvO_2$ - CI	r = 0.412	p = 0.0009
SVV - $ScvO_2$	r = -0.329	p = 0.001
$ScvO_2$ - HUO	r = 0.114	NS
$ScvO_2$ - CVP	r = 0.087	NS
$ScvO_2$ - lactate	r = -0.218	NS
Lactate - ITBVI	r = -0.241	NS
Lactate - HUO	r = -0.018	NS
Lactate - CVP	r = -0.248	NS

Table IV*: Correlation coefficients between parameters on the first day ITBVI = intrathoracic blood volume index, CI = cardiac index, $ScvO_2$ = saturation of the central venous haemoglobin, SVV = stroke volume variability, HUO = hourly urine output, CVP = central venous pressure, NS = non significant*

4. 3. Assessment of the correlation between oxidative stress marker levels and extent of burn injury

4. 3. 1. Aim:

Data in the literature are under debate regarding the correlation between oxidative stress reaction developing after burn trauma and the extent of injury. The object of this study was the assessment of this question.

4. 3. 2. Results:

White blood cell count was elevated in both groups on admission. It was significantly higher in the high-extent-group ($p<0.05$). It decreased in both groups on the following days. (Fig. 7a). The relative number of the granulocytes showed a decreasing tendency on the first three days. From day 4 the relative number of the granulocytes was significantly higher in the high-extent-group ($p<0.05$) (Fig. 7b). In contrast to the leukocytes lymhocytes showed an increasing tendency. Significant difference could be observed between groups from day 4. (Fig. 7c). The relative number of monocytes differed significantly only on day 4. (Fig. 7d).

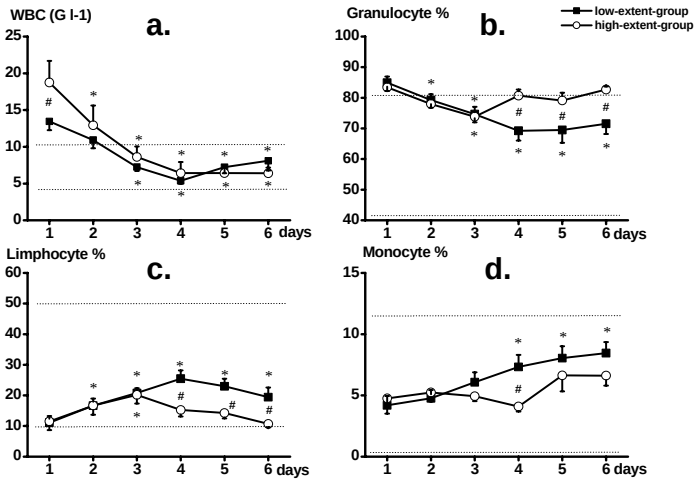

Figure 7: *White blood cell count (a), relative number of circulating granulocytes (b), lymphocytes (c), monocytes (d) in the two groups of patients.*
** = p<0.05 vs. day 1., # = p<0.05 low-extent-group vs. high-extent-group.*
Squares represent the result of low-extent-group circles show the results of high-extent-group.

The malondialdehyd (MDA) concentration in the plasma (Fig. 8a) was significantly higher compared to healthy volunteers but significant difference could not be observed between groups. The PMA stimulated free radical production in the whole blood (Fig. 8b) showed a marked ($p<0.05$) elevation from day 3 in both groups and reached the maximum value on day 5 in both groups. Significant difference could not be observed between groups. Maximum rate of stimulated free radical production (Fig. 8c) was significantly higher in the low-extent-group on day 4. The activity of mieloperoxidase (MPO) (Fig.8d) does not show significant difference between groups.

The level of plasma sulphydril groups (PSH) (Fig. 9a) was significantly higher in both groups compared to healthy volunteers from day 1 but significant difference could not be observed between groups. The concentration of hydrogenated glutathione (GSH) was higher in both groups on admission than in healthy population. The GSH concentration showed a decrease in the high-extent-group till day 4 in the low-extent-group during the whole observation period compared to the level measured on admission. (Fig. 9b). The activity of superoxide dismutase (SOD) (Fig. 9c) was lower in both groups than in healthy volunteers. Significant difference could be observed between groups on days 3 and 6. The activity of catalase (KAT) (Fig. 9d) was significantly higher compared to the healthy population.

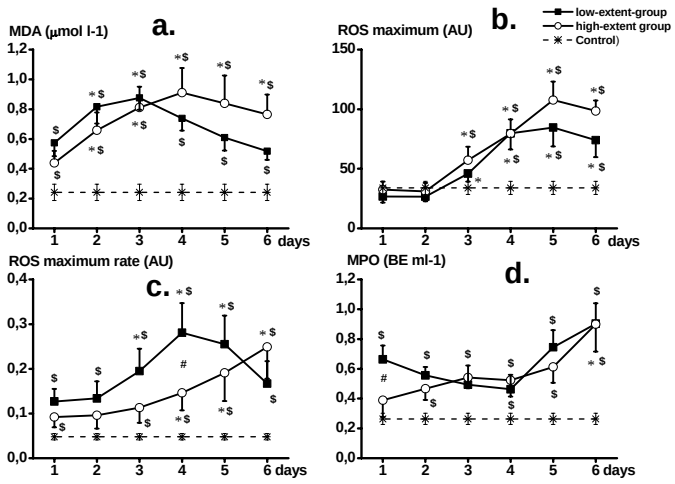

Figure 8*: Plasma concentration of malondialdehyd (a), maximum value of PMA stimulated free radical production in whole blood (b) maximum rate of radical production (c), activity of myeloperoxidase enzyme (d) in the two groups of patients. Squares represent the results of low-extent-group circles show the results of high-extent-group. * = p<0.05 vs. day 1., # = p<0. 05 low-extent-group vs. high-extent-group, $ = patient- group vs. control population.*

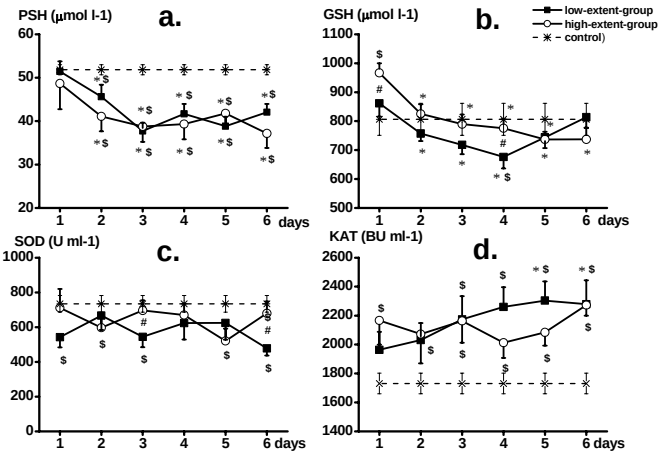

Figure 9*: Concentration of plasma sulphydril groups (a), concentration of reduced glutathione (b), activity of superoxide dismutase enzyme (c) and activity of catalase enzyme (d) in the whole blood in the two groups of patients.*
*Squares represent the results of low-extent-group, circles show the results of high-extent-group. * = p<0.05 vs. day 1., # = p<0. 05 low-extent-group vs. high-extent-group, $ = patient- group vs. control population*

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4. 4. Effects of types of fluid resuscitation on the oxidative stress markers following burn injury.

4. 4. 1. Aim:

The object of the study was to assess the effects of types of fluid resuscitation (ITBVI or HUO guided) on the oxidative stress reaction developing after burn trauma.

4. 4. 2. Results:

16 patients were involved in the study (3 females and 13 males) the mean age was in the HOU-group 54±20 years in the ITBVI-group 59±13 years, BBS were 38.0±13.0 % and 37.0±21.0% respectively.

4. 4. 2. 1. Blood parameters:

WBC count was elevated at the time of hospital admission in both groups and gradually decreased thereafter. There were no differences in WBC count changes between the HUO- and ITBVI-group (Fig.10a). At the time of hospital admission marked granulocytosis and lymphocytopenia were observed (Fig.10b, Fig.10c). Significant differences could be found in the relative number of granulocytes and lymphocytes on day 4 and 5 between HUO-group and ITBVI-group ($p<0.05$) The relative number of monocytes stabilized in the HUO-group during the whole observation period, but tended to increase moderately in the ITBVI-group (Fig.10d).

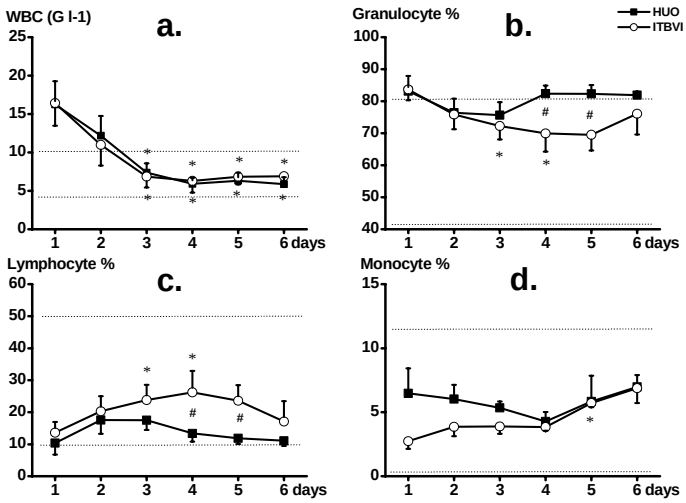

Figure 10*: White blood cell count (a), relative number of circulating granulocytes (b), lymphocytes (c), monocytes (d) in the two groups of patients.*
*Circles represent HUO = hourly urine output group, squares represent ITBVI = intrathoracic blood volume index group, * = p<0.05 vs. day 1., # = p<0.05 HUO-group vs. ITBVI- group.*

The haemoglobin level of patients was within the normal range on hospital admission, and in comparison to this value decreased significantly ($p<0.05$) (Fig.11a). The platelet count was at the lower range of normal at the time of hospital admission, and a further significant decrease was observed in both Groups on day 3, 4 and 5 ($p<0.05$). A significant difference ($p=0.05$) could be observed between the two groups on day 5-6 (Fig.11b). The fibrinogen level was in the physiological range at the time of hospital admission in both groups, and rose continuously above the normal range in both groups (Fig.11c). Statistically significant differences could not be found between groups.

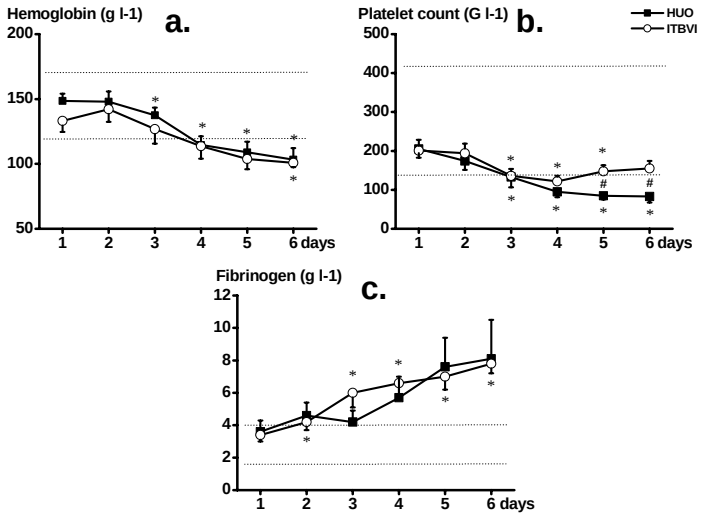

Figure 11*: Haemoglobin concentration (a), platelet count (b), fibrinogen (c) level in the two groups of patients.*
*Circles represent HUO = hourly urine output group, squares represent ITBVI = intrathoracic blood volume index group, * = p<0.05 vs. day 1., # = p<0.05 HUO-group vs. ITBVI-group.*

4. 4 2. 2. Oxidative stress parameters:

Plasma MDA concentration was significantly higher ($p<0.05$) in both groups than in healthy volunteers during the whole study period (Fig.12a). The PMA stimulated free radical production in the whole blood was around the normal range on admission and the day thereafter. From day 3 it started to increase, significantly exceeding the values of healthy volunteers in both groups reached a peak value on day 5 ($p=0.05$) (Fig.12b). The lag phase between the PMA stimulation and the start of rapid increase in radical production exceeded the control values in both groups. It was significantly longer in ITBVI- than in HUO-group on days 4, 5 and 6 ($p=0.05$) (Fig.12c). The rate of radical production was higher in HUO-group, and accelerated radical production was observed from day 4 that significantly differed from the ITBVI-group values at day 6 ($p<0.05$) (Fig12d).

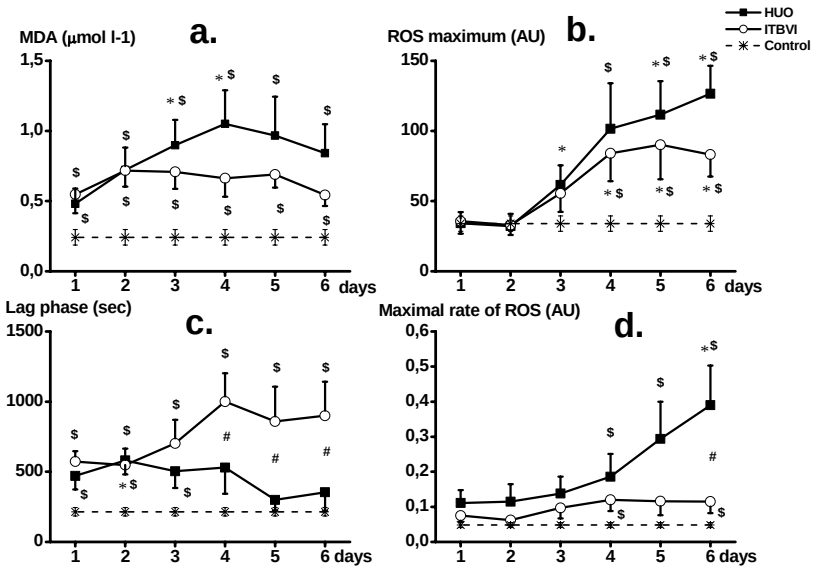

Figure 12*: Plasma concentration of malondialdehyd (a), maximum value of PMA stimulated free radical production in whole blood (b), lag phase between the PMA stimulation and the start of steep elevation in radical production (c), maximum rate of radical production (d) in the two groups of patients. Circles represent HUO = hourly urine output group, squares represent ITBVI = intrathoracic blood volume index group. Control = control population, * = p<0.05 vs. day 1., # = p<0.05 HUO-group vs. ITBVI-group, $ = patient group vs. control population.*

The level of plasma sulphydril in both groups was below the value of control healthy population during the observation period and it showed a significant decrease (p=0.05) (Fig.13a). Statistically significant differences were not observed between groups. The starting level of reduced glutathione concentration in whole blood significantly exceeded the mean value of the control healthy population on admission, and a significant decline (p<0.05) could be observed (on days 2-6) later in both groups (Fig.13b). The SOD enzyme activity in whole blood was lower in both groups than in healthy volunteers during almost the whole observation period (Fig.13c), but this difference was not significant. Catalase enzyme activity in whole blood was above the normal range in both groups than in healthy volunteers during almost the whole observation period. This increase of CAT activity was significant in the HUO-group during the whole observation period and in ITBVI-group except day 3 (Fig.13d).

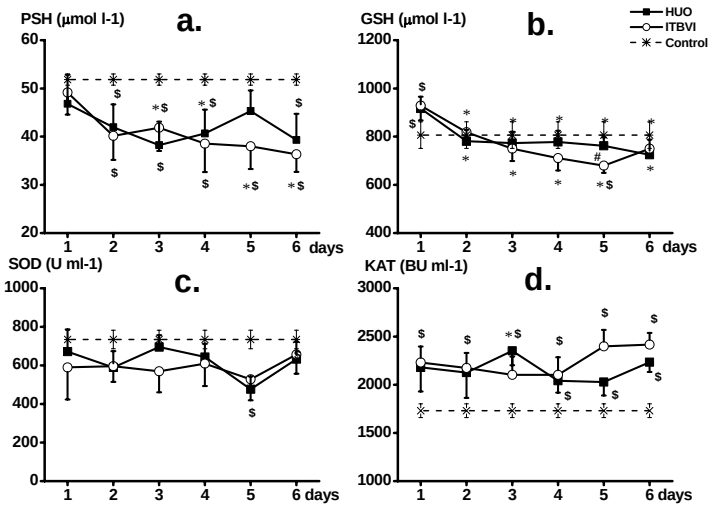

Figure *13:* *Concentration of plasma sulphydril groups (a), concentration of reduced glutathione (b), activity of superoxide dismutase enzyme (c) and activity of catalase enzyme (d) in the whole blood in the two groups of patients.*
*Circles represent HUO = hourly urine output group, squares represent ITBVI = intrathoracic blood volume index group. Control = control population, * = p<0.05 vs. day 1., # = p<0.05 HUO-group vs. ITBVI-group, $ = patient group vs. control population.*

5. Discussion

Free radicals play an important role in the marked oedema formation following burn injury. The hypovolemia caused by the developing oedema is one out of the most characteristic signs in burn injury. The adequate fluid administration is a key factor in the treatment of burn injury, because hypovolemia can upset the balance between oxygen supply and demand and it can result in organ dysfunction, in the most serious cases multiple organ failure can develop. Moreover hypoxemia, hypovolemia, and microcirculation disturbances play a role in free radical formation causing a vicious circle in the body.

Hypovolemia has to be treated as quickly as possible. It can be treated by schemata which can be made more adequate by use of specific correction factors (mentioned in the present study) but each factor influencing the fluid requirement can not be taken into account. The schemata are easy to use. Using correction factors the advantage of the schemata will be lost. The use of schemata with correction factors can not ensure an individually tailored fluid therapy. Fluid administration guided by hourly urine output or ITBVI may be another approach. The most important disadvantage of the first method is that only reduced diuresis can be taken into account because several factors for example presence of osmotically active materials can increase diuresis. Moreover, the underlying cause of the decreased hourly urine output is the reduced renal perfusion which is only a late sign of the resulting hypovolemia. For this reason it would be meaningful to inhibit the development of the hypovolemia. It can be done with ITBVI guided fluid resuscitation. Although Holm and

associates could not prove beneficial effects of this method on the mortality rate according to the present study keeping ITBVI between 800 - 850 ml m^{-2} was associated with $ScvO_2$ levels above 70%. In patients whose fluid resuscitation was guided by HUO the $ScvO_2$ level did not reach the critical 70% value. It may be the underlying cause of data in the published literature suggesting that despite maintaining hourly urine output between 0.5 – 1.0 ml kg^{-1} h^{-1} important base deficit can develop. Based on our data maintaining $ScvO_2$ above 70% after injury can improve organ functions. It emphasyses the importance of an early goal directed therapy in burned patients.

Several studies proved that burn injury is associated with marked oxidative stress and it plays a role in the development of burn oedema. Till now only a few data exist regarding correlation of oxidative stress response and extent of burn injury. Results of human studies are scarce. According to our data the extent of burned surface does not influence significantly the oxidative stress reaction. It means that burn injury affecting more than 15% of body surface is associated with marked oxidative stress reaction. The results demonstrating changes in the pro and antioxidant system were similar in other publications.

The developing hypovolemia after burn injury causes ischemia and ischemia-reperfusion. Both reactions play an important role in the free radical production so it can be supposed that the earlier normalisation of $ScvO_2$, fluid resuscitation guided by ITBVI may have a beneficial effect on the oxidative stress associated with burn injury. Our data proved that ITBVI guided fluid resuscitation via the earlier normalisation of $ScvO_2$ has a beneficial effect on the prooxidant status but does not influence the antioxidant system.

Our data and several publications suggest that decrease of oxidative stress may be beneficial for patients suffering from burn injury. Not only the adequate fluid resuscitation but antioxidants for example N-acetyl-cistein or statins may play an important role in the reduction of oxidative stress. In the future we are going to examine the effects of these compounds on oxidative stress.

6. Conclusions:

1. According to our data the fluid requirement of the patients per body weight and burned surface area is influenced by the extent of burn and body mass index. Significant negative correlation could be found between fluid requirement pro body weight and burned surface and body mass index and extent of burn injury.
2. Using our results on the basis of the body mass index and extent of burn injury correction factors can be developed making the Parkland formula more adequate.
3. Our data proved that the presence of deep burn injury increases the fluid requirement significantly.
4. It has been proven that the measurement of ITBVI makes possible the early and adequate treatment of hypovolemia in burned patients reflected in higher $ScvO_2$ value. As ITBVI shows significant correlation with $ScvO_2$ ITBVI guided fluid administration may be a key point of the early goal oriented therapy of burned patients, which could increase the survival rate.
5. SVV may be appropriate for monitoring fluid resuscitation of burned patients but the applicability of this parameter is restricted to patients who are fully ventilated and in sinus rhythm.
6. At first we performed biochemical monitoring in burned patients in the first six days after injury especially on the oxidative stress markers. A marked oxidative stress has been proven after burn injury.
7. Initially we proved at first that types of fluid resuscitation mainly influence the prooxidant status. The levels of the markers of prooxidant status were lower if fluid resuscitation was guided by ITBVI than by the hourly urine output.
8. According to our data in case of burn injury affecting more than 15 % of body surface no correlation can be found between burned body surface and the developing oxidative stress.
9. It has been proven that the type of fluid resuscitation markedly influences the relative number of different leukocytes. The relative number of granulocytes was lower when fluid resuscitation was guided by ITBVI compared to the hourly urine output guided therapy.