Effect of N-acetylcysteine treatment on oxidative stress and inflammation after burn injury

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Abstract

Oxidative stress and inflammation generate edema in burns. The aim of our study was to assess effect of N-acetylcysteine (NAC) on oxidative stress, inflammation, fluid requirement, multiple organ dysfunction (MOD) score and vasoactive drug requirement.

In this study fifteen patients were on standard therapy, whereas for other 15 patients NAC was supplemented. Blood samples were taken on admission and on the next five consecutive mornings. Levels of malondialdehyde, protein sulfhydril (PSH) groups, reduced gluthation (GSH), activity of myeloperoxidase, catalase and superoxide dismutase enzymes and induced free radical generating capacity were measured as well as concentrations of TNF-α, IL-6, IL-8, and IL-10. MOD score, use of vasopressor agents and fluid requirements were recorded daily.

NAC treatment increased GSH level on days 4-5 ($p<0.05$) and PSH level on days 2-6 ($p<0.05$) compared to controls. Plasma IL-6 was lower on days 4–5 ($p<0.05$), IL-8 on days 4–6 ($p<0.05$) and IL-10 on days 4–6 ($p<0.05$) in NAC group. NAC group required less catecholamines than controls ($p<0.01$) from day 4 without significant differences in MOD score.

NAC treatment is associated with a diminished oxidative stress reflected in preserved antioxidant levels, lower inflammation mirrored in lower interleukin levels and less vasopressor requirement.

Key words: burn injury, oxidative stress, Interleukin-6, Interleukin-8, Interleukin-10, N-acetylcysteine

Introduction

Burn injury induces systemic inflammatory response (SIRS) in the early phase playing an important role in edema formation resulting in severe hypovolemia [1]. Elevated levels of circulating cytokines produced by T and B lymphocytes, monocytes, macrophages and keratinocytes have been observed suggesting their important role in the pathophysiological responses following burn injury [2-4]. Fluid resuscitation management can influence inflammatory response after burn injury [5].

Oxidative stress has also been involved in burn injury induced pathophysiological changes [6]. Free radicals damage cell functions, and as mediators play an important role in the activation of cells responsible for the inflammatory reaction and endothelial dysfunction. This may lead to multiple organ failure (MOF) [7]. The protective effect of antioxidants after thermal injury is proven in animal models. Antioxidant vitamin A, C, and E therapy in burn trauma provides cardioprotection by interrupting cardiac inflammatory cytokine secretion [8]. High dose vitamin C infusion maintains hemodynamic stability in the presence of a reduced resuscitation fluid volume [9]. N-acetylcysteine (NAC) may influence both the inflammatory response and oxidative stress causing edema after burn injury. Konukoglu and associates showed that thermal injury is associated with increased pulmonary lipid peroxidation, and NAC treatment preserved the organ function. Reduced gluthatione level (GSH) was higher in NAC treated rats [10]. Use of NAC resulted in the significant improvement of burn induced immunosuppression, as reflected in contact hypersensitivity response in rats and it was able to significantly restore cell-mediated immunity [11]. Treatment of rats with NAC significantly elevated GSH, while decreased malondialdehyde (MDA) level and myeloperoxidase (MPO) activity [12].

Only few data exists regarding the effect of the antioxidant therapy in patients suffering from burn injury. Adjuvant administration of high-dose vitamin C during the first 24 hours after thermal injury significantly reduced resuscitation fluid volume requirements, body weight gain, and wound edema in humans [13]. Reduction in the severity of respiratory dysfunction was also apparent in these patients. Comparing patients treated with NAC to the patients receiving standard care suffering from inhalation injury, the NAC treatment significantly improved the lung injury score (LIS),

lung resistance and hypoxia scores [14]. High-dose of NAC is a well-tolerated and safe medication for a prolonged therapy of patients with cystic fibrosis [15].

We hypothesized that antioxidant therapy with NAC administration might beneficially modulate the oxidative stress, inflammatory response and edema formation after burn injury. We aimed to compare the level of oxidative stress markers, pro- and anti-inflammatory fluid requirement of NAC treated patients with patients receiving standard care without NAC supplementation. The aim of selection of the studied parameters was to cover inflammation and oxidative stress. TNF-α, IL-6 and IL-8 were chosen to assess pro-inflammatory whereas IL-10 to investigate anti-inflammatory processes. MDA levels, MPO activity, peak value and rate of free radical generating capacity represent the pro-oxidant activity in the blood. GSH, plasma sulfhydryl (PSH) levels are the non-enzymatic, superoxide dismutase (SOD) and catalase (CAT) activity are the enzymatic compounds of anti-oxidant processes.

Patients and methods

Subjects and study design

This prospective, randomised study was performed on patients admitted to our 16 bed intensive ward. The study protocol was completed in accordance with the ethical guidelines of the 2003 Declaration of Helsinki. After receiving permission from the local ethics committee the patients or nearest relative provided a written, informed consent, and they were informed clearly about the details of the study and blood sampling.

Inclusion criteria were burn injury affecting more than 20% of the body surface, necessity of immediate intensive care and admission to our ward within 3 hours after injury. Exclusion criteria were extreme burn severity Baux index > 120, chronic left heart or renal insufficiency and hematological disease documented in past medical history, age less than 18 years or documented previous medication affecting the inflammatory response of the body to burn injury (e.g. chronic use of steroids, cytostatic treatment in the last 30 days) and absence of consent to the study.

Patient treatment protocol

Patients were treated in a uniform way and practice patterns were not changed during the study period.

Fluid resuscitation was guided by intrathoracic blood volume index (ITBVI). Only Ringer's Lactate (LR) solution was used for intravenous fluid replacement. While the normal range for ITBVI is between 850 and 1000 ml m^{-2} the goal of our resuscitation was to maintain ITBVI between 800 and 850 ml m^{-2}. By targeting the lower limit, we tried to avoid intravenous fluid overload. Comparing this type of fluid resuscitation to the conventional, hourly urine output guided therapy, ITBVI guided resuscitation is associated with earlier normalization of saturation of central venous haemoglobin and lower multiple organ dysfunction score [16] moreover, it reduces oxidative stress [17] and it decreases the shift towards anti-inflammatory processes and has beneficial effect on the expression of leukocyte cell surface markers [5].

After reaching the goal of ITBVI, norepinephrine was administered if mean arterial pressure was lower than 60 mmHg; and dobutamine was administered in case central venous haemoglobin saturation was lower than 70%. If inhalation injury was suspected (facial burn, soot in the throat), bronchoscopy was carried out for verification. Excision and grafting were started within 72 hours. 20-30 % burnt surface was excised and grafted in one sitting. Operations were repeated in every 3-4 days. Enteral feeding was commenced on the first day after injury when hemodynamic stability was reached.

Randomization, taking study samples, scoring systems

After assessment for eligibility patients were randomly assigned into NAC or standard group. In the NAC group the standard treatment was supplemented with administration of NAC as a bolus of 150 mg kg^{-1} followed by a continuous administration of 12 mg kg^{-1} h^{-1} [18-20] for 5 days. Blood samples were taken on admission (day 1) and on the mornings of the next five consecutive days (days 2-6). Leukocyte count of the patients, the percentage of granulocytes, lymphocytes and monocytes, PCT, oxidative stress marker MDA, PSH, GSH levels, SOD, and CAT enzyme activities in hemolysate and phorbol 12-myristate 13-acetate (PMA) induced free radical generating capacity in whole blood as well as pro- and anti-inflammatory citokine levels (TNF-α, IL-6, IL-8, IL-10) were measured daily. Inotrop and vasopressor administration were assessed, multiple organ dysfunction (MOD) [21]

and sequential organ failure assessment (SOFA) [22] scores were calculated in each patient daily after admission, and during the entire stay in intensive care unit (ICU).

For assessment of inotrop and vasopressor administration a scoring system has been developed by our group. The inotrop/vasopressor requirement of the patients was assessed hourly during the study period. Patients were assigned into no drug, low dose or high dose subgroups. The daily score was calculated by summing all hourly values. The cut off value between low and high dose groups regarding norepinephrine was 0.2 $\mu g\ kg^{-1}\ min^{-1}$, while regarding dobutamine, it was 15 $\mu g\ kg^{-1}\ min^{-1}$ (Table 1).

Measurement of oxidative stress markers

Most of the biochemical assays have been described earlier [23]. Briefly, the plasma MDA was determined with Ohkawa method [24]. MDA is one of the derivatives originating from oxidative damage of the poly-unsaturated fatty acids, thus indirectly shows intensity of lipidperoxidation due to oxidative stress. GSH is a basic endogenous antioxidant, the level of which is reduced due to oxidative stress. GSH was measured according to Sedlak and Lindsay [25]. PSH level was determined similarly with Ellman's reagent. PSH groups originate predominantly from plasma proteins and participate in the defence against oxidative stress. SOD enzyme activity in washed erythrocyte hemolysate (mainly Cu/Zn-SOD) was determined by the method of Misra and Fridovich [26]. SOD is an enzymatic endogenous antioxidant which catalyzes the dismutation of the superoxide free radical to H_2O_2. CAT enzyme activity in hemolysate was determined by the Aebi method [27]. CAT metabolizes H_2O_2 by reducing it to water and oxygen. This prevents the second generation of toxic intermediates. Activated leukocytes, mainly neutrophils, are potential sources of ROS during inflammation. The free radical generating capacity in whole blood was measured with a Whole Blood Lumi-aggregometer (Chrono-Log, Model 560, USA), according to the manufacturer's instructions. This chemiluminescense method is based upon the reaction of luminol with free radicals, and PMA was used to induce radical production. The peak value of free radical production and the maximal rate of radical production were calculated from the recorded curve, and the results were related to the leukocyte counts.

Cytokine measurements:

Blood samples were taken according to standard procedures in tubes containing anticoagulant EDTA, and were centrifugated for 10 min at 2000 g to obtain plasma fraction. Plasma samples from non-stimulated blood were stored at −80 °C and analyzed in a single batch at the end of the study. The frozen plasma samples were thawed just before assaying. The patient management was independent of these results. Concentrations of TNF-α, IL-6, IL-8 and IL-10 were measured by the Cytometric Bead Array Human Inflammation Kit (BD Biosciences, USA) according to the manufacturer's instructions. FACS Calibur (BD Biosciences, USA) flow cytometer was used for acquisition of data. The results were analyzed by Cellquest software (BD Biosciences).

Serum PCT was measured by immunoluminometric assay (LUMItest, Brahms Diagnostika, Berlin, Germany).

Statistics:

The sample size was calculated by power analysis based on our earlier results [17]. Fifteen patients were required in each group. Values of $p<0.05$ were considered significant. Data were expressed as median and 10^{th}-90^{th} and 25^{th}-75^{th} percentile. Mann-Whitney test was used to compare the values of the two groups. Kruskal-Wallis rank sum test was used for intergroup analyses and testing differences between groups at different time points. Differences between groups regarding clinical risk ratios were analyzed with Fischer's exact test.

Results

Demographic and initial data

30 patients were involved in this prospective randomised study (Fig. 1) There were no significant differences between groups regarding age, burned surface area, extent of deep burn injury, and occurrence of inhalation injury, mechanism of burn

injury and in calculated organ function scores. Demographic and initial clinical properties in the NAC and standard groups are summarized in Table 2.

Leukocytes

Leukocyte count was markedly elevated in both patient groups, and decreased during the study period reaching the level of significance from day 3 without any differences between groups (Fig. 2a). Burn trauma induced acute severe granulocytosis and lymphocytopenia. The relative number of granulocytes decreased, the relative number of lymphocytes increased from day 2, and granulocyte ratio was lowest (Fig. 2b) and that of the lymphocyte ratio was highest (Fig. 2c) on days 3-4 in both groups without significant differences between groups. Opposite to NAC treatment, in patients with standard therapy an increasing tendency in granulocyte ratio, and a decreasing tendency in lymphocyte ratio was observed from day 3. The relative number of monocytes increased significantly on day 5 (Fig. 2d) in both groups.

Oxidative stress markers

Pro-oxidant markers

Plasma MDA level increased significantly on day 2 in both groups, but without any significant differences between groups (Fig. 3a). Plasma MPO activity increased on days 5-6 in both groups comparing to day 1 but the elevation was not significant. No significant differences were found between groups (Fig. 3b). Peak value and rate (Fig. 3c, 3d) of free radical generating capacity showed significant elevation in both groups from day 4 compared to the day 1 values without any significant differences between groups (Fig. 3c, 3d).

Endogenous antioxidants

GSH in hemolysate decreased significantly in both groups from day 3. GSH level was significantly higher in NAC treated group compared to standard therapy on days 4-6 (Fig. 4a). PSH level decreased moderately in NAC group showing significant differences from day 4, whereas it showed a marked decrease in standard group from day 2. Differences in PSH levels were significant between groups from day 2 (Fig. 4b). SOD activity in hemolysate was unchanged during study period

without any significant differences between groups (Fig. 4c). Catalase activity in hemolysate was markedly increased in standard group and showed a slight elevation in NAC group, without any significant differences between groups or comparing to levels on admission (Fig. 4d).

Cytokine levels

The average values of TNF-α concentrations were close to the detection limit of the assay (3.7 pg ml^{-1}). IL-6 showed a significant elevation from day 2 in standard group and in NAC treated patients compared to day 1. Significant differences could be found between groups on days 4-5 (Fig. 5a). Serum IL-8 levels were elevated on days 2-6 in standard group and on days 3-4 in NAC group. The groups differed significantly on days 4-6 (Fig. 5b). IL-10 Showed only a slight decreasing tendency in standard group and a more pronounced decrease in NAC treated group reaching the level of significance from day 3. Significant differences could be found between groups on days 4-6 (Fig. 5c).

Clinical outcome parameters

Although the study is underpowered regarding clinical outcome parameters the inotrop and vasopressor requirements in patients of standard group were significantly higher on days 4-6 (Fig. 6). There were no significant differences in fluid requirement, in preload of patients reflected in ITBVI and in base excess, lactate levels and saturation of central venous hemoglobin at the end of fluid resuscitation (Table 3). Average hourly urine output did not differ between groups during the 1st 24 hours. Significant differences could not be observed in days on respirator and in ICU length of stay (data not shown). In standard group 4 patients died of MOF caused by sepsis, 1 died of left heart insufficiency, 1 died due to gas exchange failure. In NAC group 2 deaths were caused by septic MOF, 1 by gas exchange insufficiency and 1 by cardiogenic shock, but this difference was not statistically significant.

NAC treatment was well tolerated. Adverse effects, apart from more pronounced sputum production not affecting the ventilator requirement were not observed during the study period.

Discussion

In this study, we found that NAC treatment increased the level of endogenous antioxidants and diminished interleukin production in the acute phase of burn trauma. The need for inotropic and vasopressor drug administration significantly decreased in NAC treated patients, but other advantages in clinical outcome parameters could not be proven.

Oxidative stress can be evaluated by either measuring the end products of lipid peroxidation, or the antioxidant capacity and the activity of antioxidant enzymes. Burn injury induces severe oxidative stress in humans. Similarly to our results, Jutkiewicz-Sypniewska and co-workers have found decreased antioxidant capacity, and elevated TBARS (MDA) level in burned children [28]. Bertin-Maghit and co-workers have also found decreased levels of antioxidants, and elevated TBARS in burned patients [29]. Pintaudi and co-workers have found significantly elevated MDA level on admission following burn injury [30].

Burn injury induced oxidative stress gives a good rationale of antioxidant therapy of patients, but only few data exists regarding the effect of the antioxidant therapy in patients suffering from burn injury. N-acetylcysteine is not an endogenous antioxidant, but, its use is based on a convincing rationale [31]. In animal model administration of NAC increased GSH and decreased MDA levels in the lung 1 hours and 1 day post burn injury [32]. In rat pulmonary contusion model administration of NAC significantly diminished MDA levels in lung compared to controls [33]. NAC treatment prevented the decrease in lung glutathione and significantly lowered serum isoprostane levels, neutrophil infiltration and cytokine levels in the broncho-alveolar lavage during high tidal volume ventilation [34]. Treatment of rats with NAC significantly elevated the reduced GSH levels while decreasing MDA levels and MPO activity after burn injury. The data of Ocal and associates suggested that NAC has a crucial cytoprotective effect on intestinal mucosal barrier and prevents burn injury-induced bacterial translocation [35]. In a rat two hit trauma model administration of NAC increased tissue GSH levels while decreased MDA levels, but it did not influence survival [36]. In humans NAC administration increased serum glutathione level in patients undergoing IL-2 induced lymphokine-activated killer cell treatment [37]. Similarly to the above cited experimental and clinical data GSH levels were

significantly higher in our NAC treated patients from day 2, but in contrast to earlier results NAC treatment did not alter plasma MDA level.

The close correlation between reactive oxygen species (ROS) production and inflammation can explain the effect of NAC on cytokine production. Curbo and associates provided evidence for a novel redox dependent pathway for regulation of cytokine activity by extracellular reduction of intramolecular disulfides at the cell surface by members of the thioredoxin enzyme family [38]. The anti-inflammatory effect of NAC can be based on regulatory effect of ROS on translocation of transcription factor NF-κB to cell nuclei [39]. Moreover, IL-8 production is regulated by mitogen-activated protein kinases, but ROS can alter IL-8 production too. In human gastric carcinoma cells NAC could decrease IL-8 production [40].

In the acute phase of burn injury monocytes are main sources of circulating interleukins. Toumpanakis and associates found that antioxidant supplementation with NAC decreased interleukin production of monocytes at rest and exercise in humans [41]. Radomska-Lesniewska and associates concluded that NAC is an effective inhibitor of TNF-α, IL-1β and IL-8 release in endothelial and epithelial cells [42]. NAC administration could decrease tissue infiltration of neutrophils in the lungs, ileum and colon in a rat acute pancreatitis model as well as IL-6 levels [43]. In porcine ischemia-reperfusion model administration of NAC could decrease the inflammatory cytokine and thio-barbituric acid reactive substances levels in broncho-alveolar lavage [44]. Our data are in concordance with these results showing a less pronounced cytokine production in NAC treated patients, moreover they support the results of our earlier work in which a diminished inflammatory reaction reflected in lessened leukocyte cell surface marker expression was observed after NAC treatment [45]. Because TNF-α levels were close to the detection limit of our assay we were not able to compare NAC and standard groups in this regard.

In a porcine model NAC did not influence the early post-traumatic organ injury, and initiation of inflammatory responses significantly, or endotoxin tolerance. In vitro, NAC significantly reduced pro-inflammatory cytokine release in normal blood only [46].

In human studies NAC treatment did not influence the survival [18-20]. Our data in concordance with the above mentioned results do not prove a beneficial effect of NAC on mortality rate. Only vasopressor requirement was less in NAC

treated group. It can be a consequence of less pronounced inflammation in NAC treated patients. The late onset of the effect of NAC treatment (after edema formation was complete) can explain the lack of difference in fluid requirements between groups.

Limitations of the study

More sensitive tests would have been needed to measure changes in TNF-α production. Our study is underpowered for drawing conclusion on mortality and clinical outcome parameters. The inotrope and vasopressor requirements are operator dependent variables and differences in these requirements between the groups may not entirely be related to the effects of NAC.

The study has to be continued until enough patients can be involved for drawing conclusions regarding clinical outcome parameters.

Figure 1.

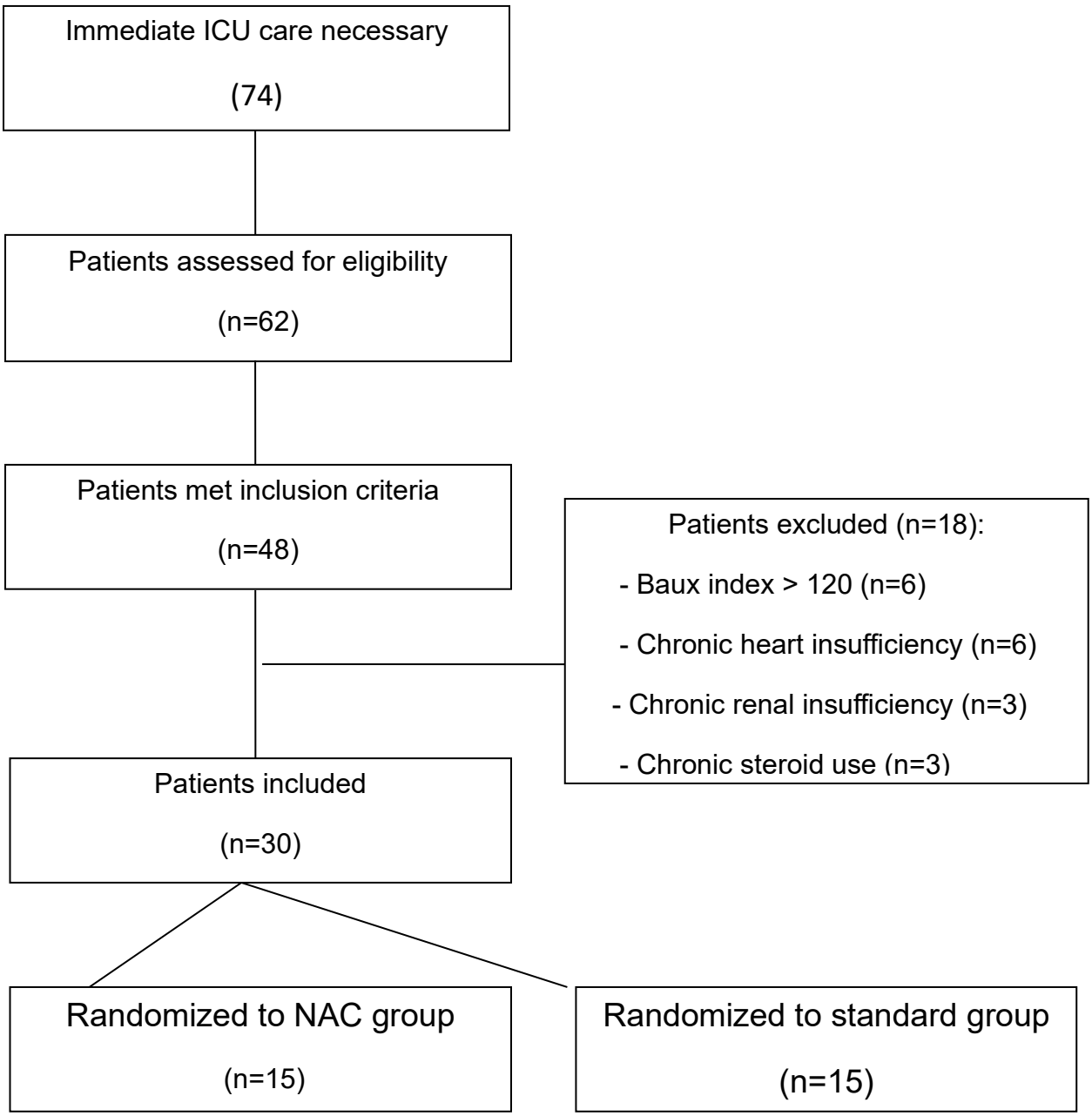

Immediate ICU care necessary
(74)
Patients assessed for eligibility
(n=62)
Patients met inclusion criteria
(n=48)
Patients excluded (n=18):
- Baux index > 120 (n=6)
- Chronic heart insufficiency (n=6)
- Chronic renal insufficiency (n=3)
- Chronic steroid use (n=3)
Patients included
(n=30)
Randomized to NAC group
(n=15)
Randomized to standard group
(n=15)

Figure 2

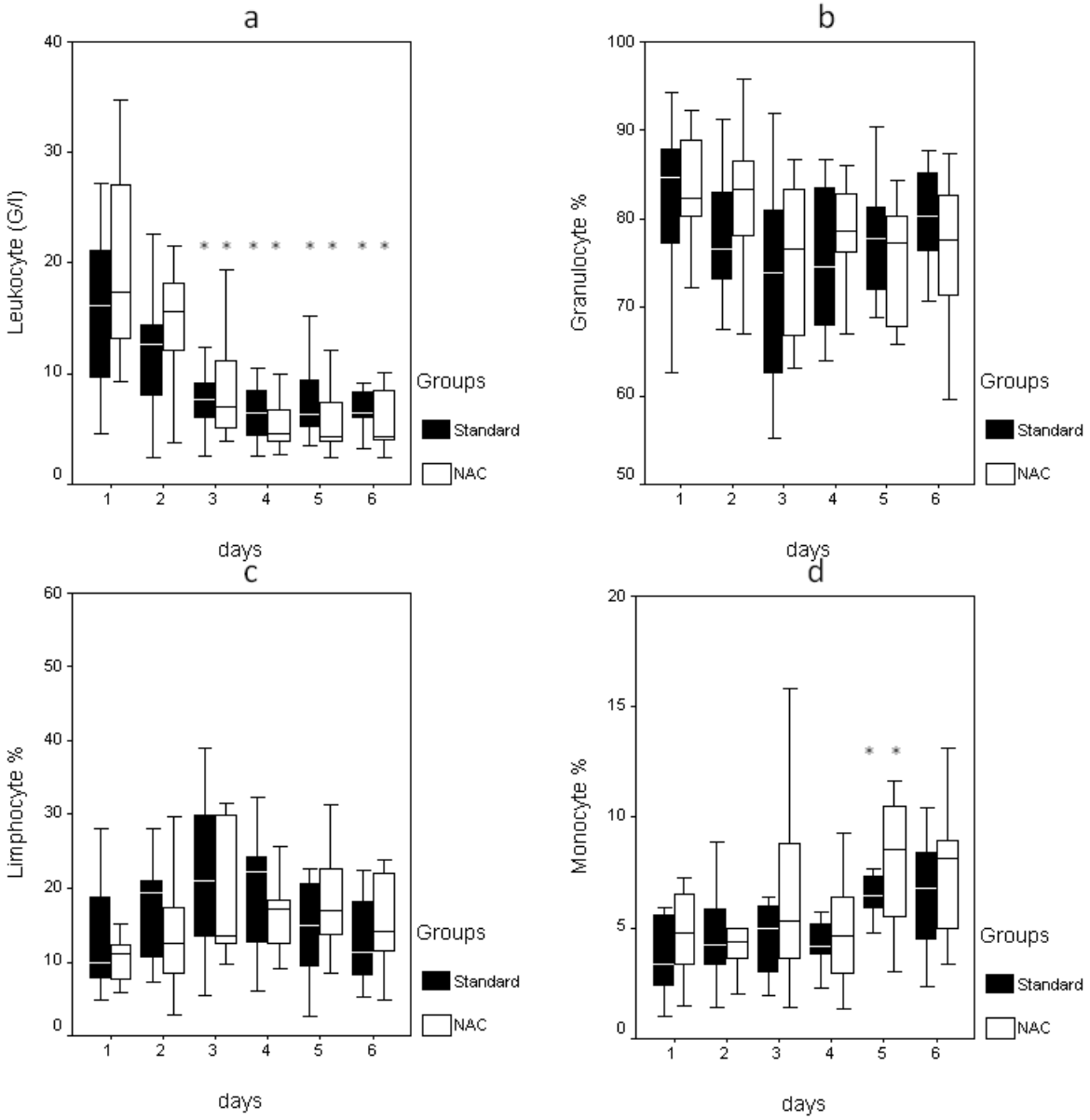

a
Leukocyte (G/l)
days
Groups
Standard
NAC
b
Granulocyte %
days
Groups
Standard
NAC
c
Limphocyte %
days
Groups
Standard
NAC
d
Monocyte %
days
Groups
Standard
NAC

Figure 3

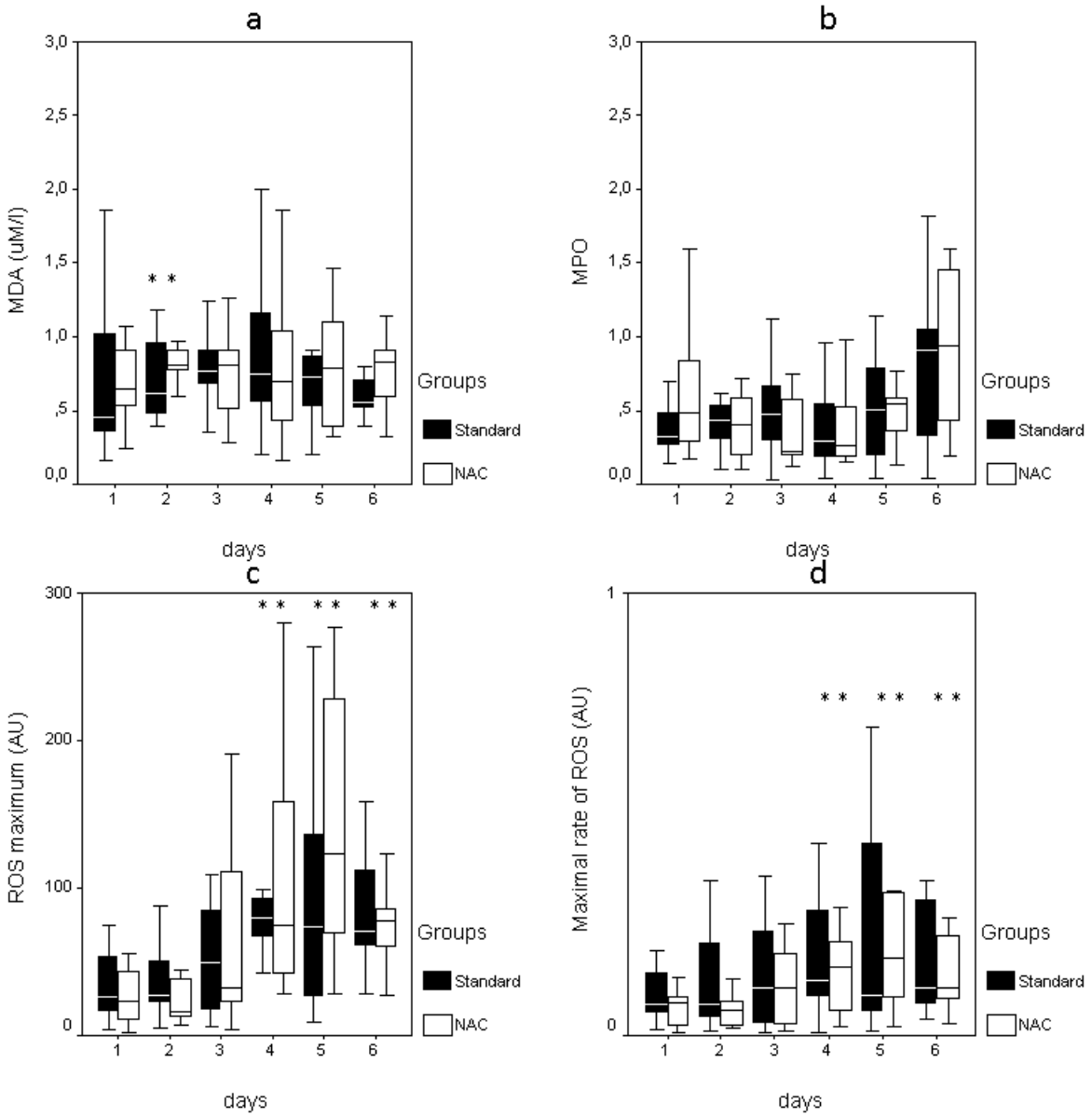

a
MDA (uM/l)
3,0
2,5
2,0
1,5
1,0
,5
0,0
1
2
3
4
5
6
days
Groups
Standard
NAC
b
MPO
3,0
2,5
2,0
1,5
1,0
,5
0,0
1
2
3
4
5
6
days
Groups
Standard
NAC
c
ROS maximum (AU)
300
200
100
0
1
2
3
4
5
6
days
Groups
Standard
NAC
d
Maximal rate of ROS (AU)
1
0
1
2
3
4
5
6
days
Groups
Standard
NAC

Figure 4

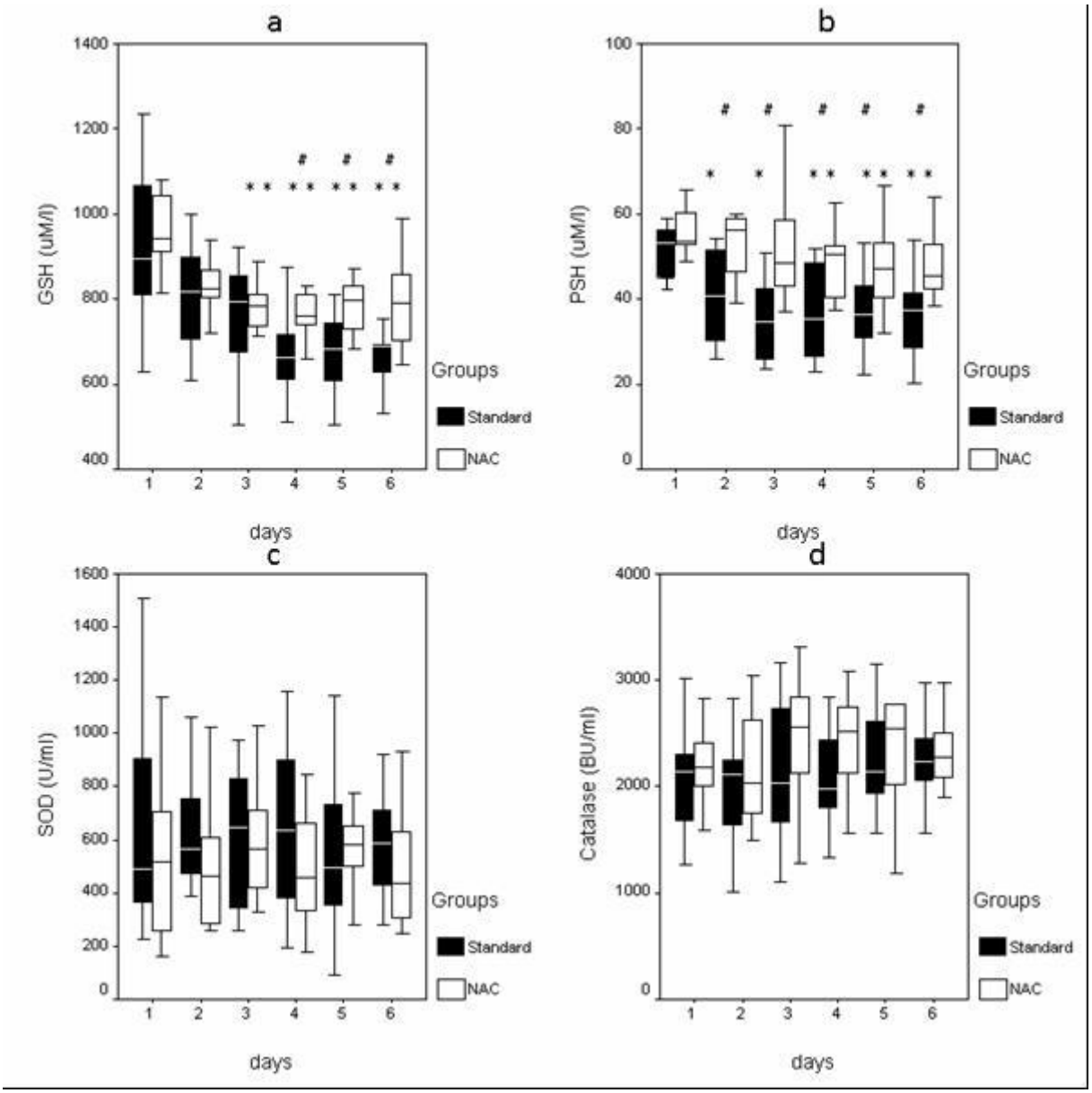

a
GSH (uM/l)
1400
1200
1000
800
600
400
1 2 3 4 5 6
days
Groups
Standard
NAC
b
PSH (uM/l)
100
80
60
40
20
0
1 2 3 4 5 6
days
Groups
Standard
NAC
c
SOD (U/ml)
1600
1400
1200
1000
800
600
400
200
0
1 2 3 4 5 6
days
Groups
Standard
NAC
d
Catalase (BU/ml)
4000
3000
2000
1000
0
1 2 3 4 5 6
days
Groups
Standard
NAC

Figure 5

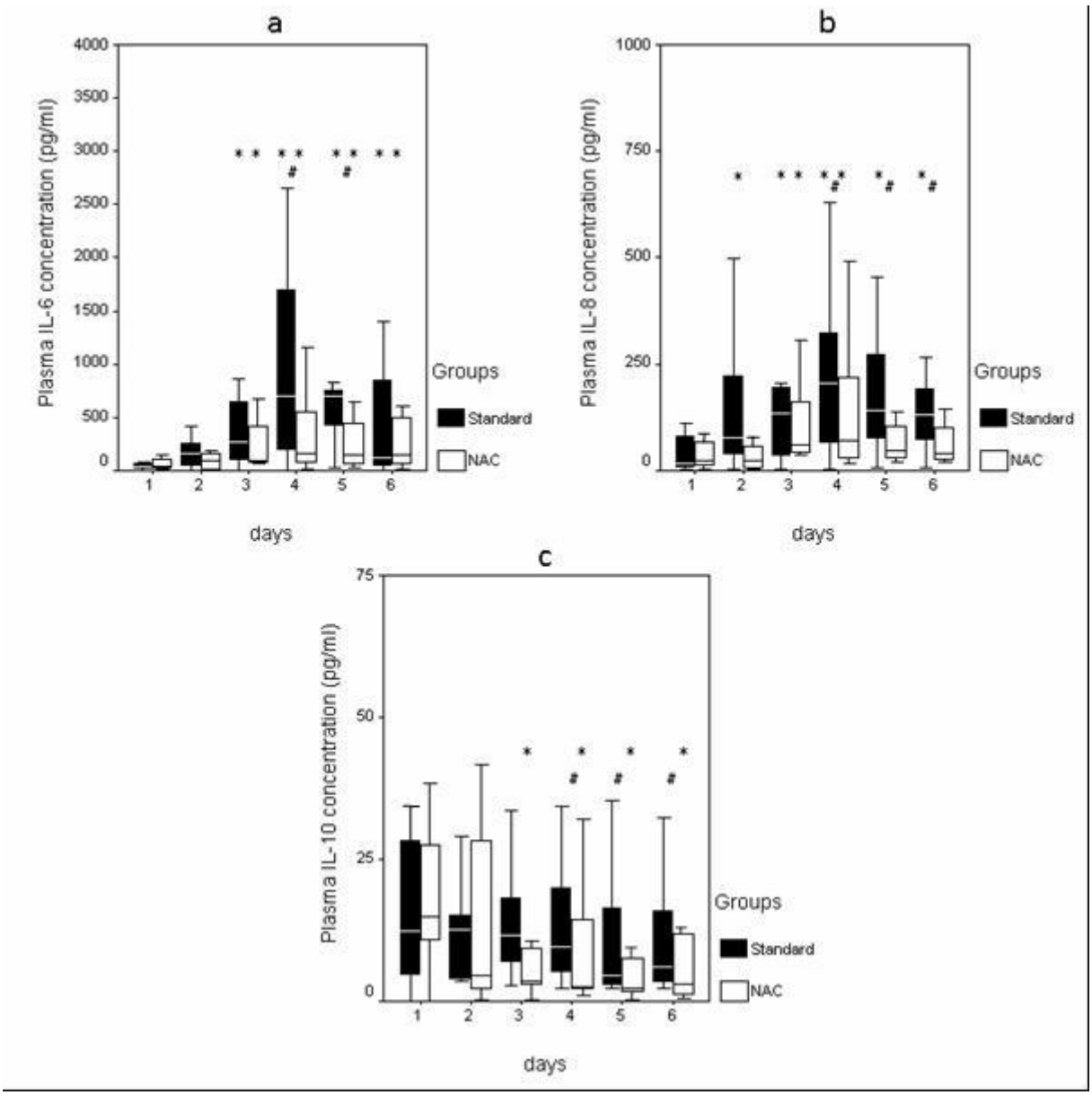
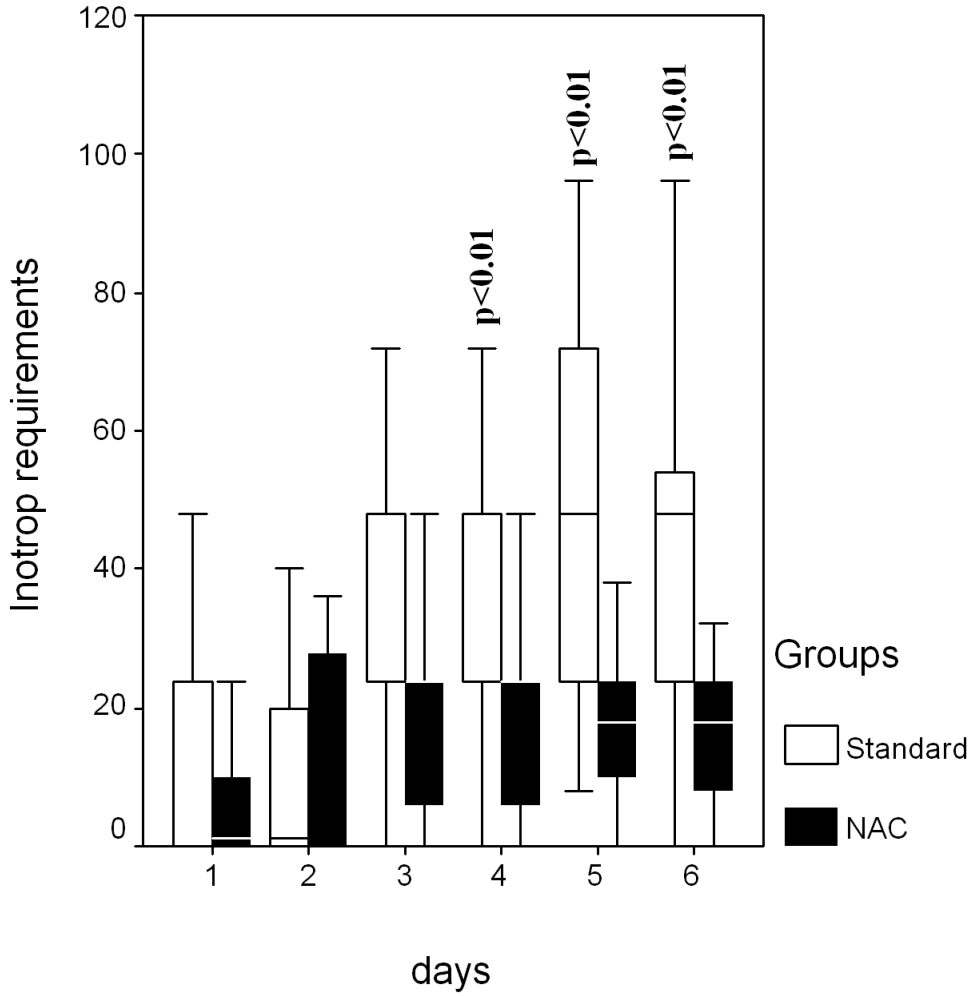

a
Plasma IL-6 concentration (pg/ml)
4000
3500
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
days
Groups
Standard
NAC
b
Plasma IL-8 concentration (pg/ml)
1000
750
500
250
0
1
2
3
4
5
6
days
Groups
Standard
NAC
c
Plasma IL-10 concentration (pg/ml)
75
50
25
0
1
2
3
4
5
6
days
Groups
Standard
NAC

Figure 6


120
100
80
60
40
20
0
Inotrop requirements
p<0.01
p<0.01
p<0.01
1
2
3
4
5
6
days
Groups
Standard
NAC

Legends for figures

Figure 1: Flow chart of the study

Figure 2: Changes in leukocyte count (a), relative number of granulocytes (b), lymphocytes (c), and monocytes (d) in patients with standard therapy and NAC supplementation. Data are expressed as minimum, maximum, median and interquartile range. NAC = N-acetylcysteine, *Symbols indicate intra-group differences comparing to day 1, * = $p<0.05$.

Figure 3: Changes in plasma malondialdehyde concentration (a), plasma myeloperoxidase enzyme activity (b), peak value (c) and maximal rate (d) of ROS production in whole blood of patients with standard therapy and NAC supplementation. Data are expressed as minimum, maximum, median and interquartile range. NAC = N-acetylcysteine, *Symbols indicate intra-group differences comparing to day 1, * = $p<0.05$.

Figure 4: Changes in GSH concentration in hemolysate (a), concentration of plasma SH groups (b), superoxide dismutase (c), and catalase (d) enzyme activity in hemolysate of patients with standard therapy and NAC supplementation. Data are expressed as minimum, maximum, median and interquartile range. NAC = N-acetylcysteine, *Symbols indicate intra-group differences comparing to day 1, * = $p<0.05$. $^{\#}$Symbols indicate differences between NAC and standard group, # = $p<0.05$.

Figure 5: Changes in plasma IL-6 (a), IL-8 (b), and IL-10 (c) concentration in patients with standard therapy and NAC supplementation, NAC = N-acetylcysteine. *Symbols indicate intra-group differences comparing to day 1, * = $p<0.05$. $^{\#}$Symbols indicate differences between NAC and standard group, # = $p<0.05$.

Figure 6: Inotropic and vasopressor drug requirements in the two groups of patients. NAC = N-acetylcysteine.

Table 1

Scoring system for inotropic and vasopressor drug administration. The cut off value between low and high dose groups regarding norepinephrine was 0.2 μg kg^{-1} min^{-1}, while regarding dobutamine, it was 15 μg kg^{-1} min^{-1} .

drug	no-drug	low dose	high dose
norepinephrine	0	1	2
dobutamine	0	1	2

Table 2

Demographic and clinical data of patients on admission presented as median and 25^{th}-75^{th} percentile. PCT = procalcitonin, SOFA = sequential organ failure assessment, MOD = multiple organ dysfunction, ITBVI = intrathoracic blood volume index.

	NAC (n = 15)	standard (n = 15)	p value
Age (years)	54(43-74)	49(33-61)	NS
Male/female	13/2	11/4	NS
Burned surface area (%)	50(43-56)	46(40-53)	NS
Deep burn injury (%)	35(30-40)	33(30-40)	NS
Inhalation injury (n)	12	10	NS
Flame burn injury (n)	6	7	NS
Blast burn injury (n)	8	6	NS
Scald burn injury (n)	1	2	NS
PCT (ng/ml)	0.3(0.1-1.8)	0.4(0.1-3.5)	NS
SOFA	4(1-9)	5(4-7)	NS
MOD score	4(2-9)	5(4-8)	NS
ITBVI (ml m^{-2})	780 (690–830)	800 (720–820)	NS
Lactate (mmol l^{-1})	2,1(1,3-3,3)	3,0 (1,8-5-5)	NS
BE (mEq l^{-1})	-4,7 ((-7.5)-(-3.4))	-4,3 ((-8,6)-(-2,5))	NS

Table 3

Clinical parameters of the fluid resuscitation. HUO = average hourly urine output during the 1st 24 hours. ITBVI = Intrathoracic blood volume index, $ScvO_2$ = saturation of the central venous hemoglobin, BE = base excess. ITBVI, $ScvO_2$, Lactate and BE were measured at the end of the resuscitation. NS = non significant. Data are presented as median and 25th-75th percentile

	NAC (n = 15)	Standard (n = 15)	p value
Fluid requirement (ml kg^{-1} BBS^{-1})	3.7 (3.3-5.8)	4.2 (3.3-5.1)	NS
HUO (ml kg^{-1} h^{-1})	0.86 (0.68-1.08)	0.88 (0.7-1.5)	NS
ITBVI (ml m^{-2})	824 (695-851)	815 (796-920)	NS
$ScvO_2$ (%)	71 (70-76)	73 (71-79)	NS
Lactate (mmol l^{-1})	1.7 (1.2-2.8)	2.1 (1.3-3.8)	NS
BE (mEq l^{-1})	-1.9 ((-3.4)-(-1.1))	-1.4 ((-3.3)-(-0.9))	NS

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