Application of Cluster Analysis for Determination of the Oxidative Stress Decompensation Criteria

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Abstract. This case-control study was conducted on 73 male patients with combined thoracic injuries. The main goal of the current study was to investigate oxidative damage of proteins and lipids in patients with the severe combined thoracic trauma on the first days after injury. The second aim was to evaluate the possibility of outcome prediction through oxidative stress markers determination. Concentrations of malonic dialdehyde and proteins carbonyl groups were determined according to spectrophotometric methods. As there were no differences between survival and nonsurvival groups of patients for oxidative stress markers, cluster analysis was performed for stratification of patients' population by both oxidative stress markers simultaneously. It was found that oxidative stress develops from 1-2-nd day after the severe combined thoracic trauma and generally its level can be reliably estimated through determination of relative concentrations of both malonic dialdehyde and proteins carbonyl groups. The degree of oxidative stress is proportional to the severity of injury and patients' state on admission (traumatic shock) and does not depends on patients' age and concomitant alcohol exposure. Also its progression is not linear as the result of severe drop of the oxidative stress markers` concentration in premorbid phase of wound dystrophy. Cluster analysis is useful tool for analyzing medical and biological data from investigations when synergistic multifactorial relations are present between pathophysiological processes that are determined by interactions with compensatory and adaptive mechanisms directed at homeostasis saving during critical states.

Keywords: Combined Thoracic Trauma, Oxidative Stress, Malonic Dialdehyde, Carbonyl Groups of Proteins, Outcome Prediction, Cluster Analysis.

1 Introduction

Thoracic injuries are associated with 30 % – 40 % mortality level and trauma associated fatalities of 20 % – 25 %, also 50 % – 75 % of deceased patients with polytrauma have a thoracic injury [1,2].

Severe trauma not only undermines the local tissue but also leads to shock, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome or even death [3]. Most recently, the role of free radicals has been a largely debated and reported topic. Once produced in excess, free radicals are responsible for inducing oxidative stress [4] that involves the modification of cellular macromolecules by reactive oxygen species, often leading to cell death [5]. It can be a reason of severe complication development during pathophysiologic response after combined thoracic injury called wound dystrophy. Tissue injury results into local and systemic release of proinflammatory cytokines and phospholipids proportionally to the severity of polytrauma. Activated neutrophils are able to induce secondary tissue and organ damage by formation of reactive oxygen species [5,6]. As a result of this, the presence of thoracic injuries in a polytraumatized patient significantly increases the risk of systemic complications and lethal outcome [7]. However, diagnostic value of the oxidative stress markers for metabolic monitoring is not investigated at all yet.

There are no ideal statistical methods, which can be used for analyzing medical and biological data. Formal statistical tests exist to examine whether a set of data are Normal or whether two variances are equal, although results from these should always be interpreted in the context of the sample size and associated statistical power in the usual way [8]. Nonparametric methods are geared toward hypothesis testing rather than estimation of effects. It is often possible to obtain nonparametric estimates and associated confidence intervals, but this is not generally straightforward [9].

2 Aim

The main goal of the current study was to investigate oxidative damage of proteins and lipids in patients with the severe combined thoracic trauma on the first days after injury. The second aim was to evaluate the possibility of outcome prediction through oxidative stress markers determination.

3 Materials and methods

3.1 Patients

This case-control study was conducted on 73 male patients with combined thoracic injuries treated in anesthesiology and intensive care department for patients with combined trauma of Kharkiv Municipal Clinical Emergency Hospital named by prof. O.I. Meshchaninov. Primary inclusion criteria were ISS > 16, two or more injured body regions, severe blunt thoracic injuries (AIS 3 and more). Presence of concomitant chronic disease in subcompensation or decompensation phase was set as excluding criteria. Examination was performed on 1-2-nd day after trauma (10.75 – 33.5 hours). The cohort

was divided into groups according to outcome – survival (n = 42) and nonsurvival (n = 31). 15 male healthy volunteers at the same age were comprised into control group.

3.2 Oxidative stress markers

Patients' plasma was assayed for oxidative stress markers using spectrophotometric methods in Biochemistry department of Kharkiv National Medical University. The concentration of malonic dialdehyde was determined according to TBA-activity of deproteined plasma [10]. Proteins carbonyl groups level was determined with the help of dinitrophenylhydrazine reaction with plasma proteins, extracted from blood [11]. In order to avoid influence of infusion therapy on concentrations of oxidative stress markers, its levels were divided on total protein concentration determined according to biuret reaction [12].

3.3 Data analysis

Data are represented as Median (95% confidence interval) and were collected in a Microsoft Excel 2010 spreadsheet before transfer to GraphPad Prism 5.03 which was used for statistical analysis. Mann-Whitney test was used to assess differences between two groups and Kruskal-Wallis test – to compare three and more groups. Two-sided Fisher's exact test and Chi-square test were performed to consider differences in nominal data. Cluster analysis was performed with the help of STATGRAPHICS Plus 5.0. Clustering was performed according to centroid method with squared Euclidean distance metric. The significance level was specified as $p < 0.05$.

4 Results

4.1 Demographics

Clinical characteristics of patients groups are detailed in Table 1. The survival and nonsurvival groups had a similar age, admission time, number of patients with concomitant alcohol exposure ($p > 0.05$). Nonsurvivors had significantly higher points of Injury Severity Score (ISS) as well as lover points of Revised Trauma Score (RTS).

Table 1. Characteristics of the patients` groups.

	Survivors	Nonsurvivors	p
Number of patients	42	31	
Patients age, years	41 (38.21 – 44.89)	42 (36.7 – 46.46)	1
Trauma severity according ISS	24.5 (22.73 – 28.22)	34 (30.38 – 38.53)	0.0006
Trauma severity according RTS	7.84 (7.05 – 7.68)	6.17 (5.35 – 6.46)	<0.0001
Prehospital time, hours	1 (0.854 – 1.97)	1 (0.435 – 3.29)	0.8434
Number of patients with concomitant alcohol exposure	23 (54.7 %)	15 (48.4 %)	0.6407

4.2 Changes of oxidative stress markers concentrations

Figure 1 represents changes of malonic dialdehyde and proteins carbonyl groups relative concentrations in plasma from patients with combined thoracic injuries on the 1-2-nd day after trauma.

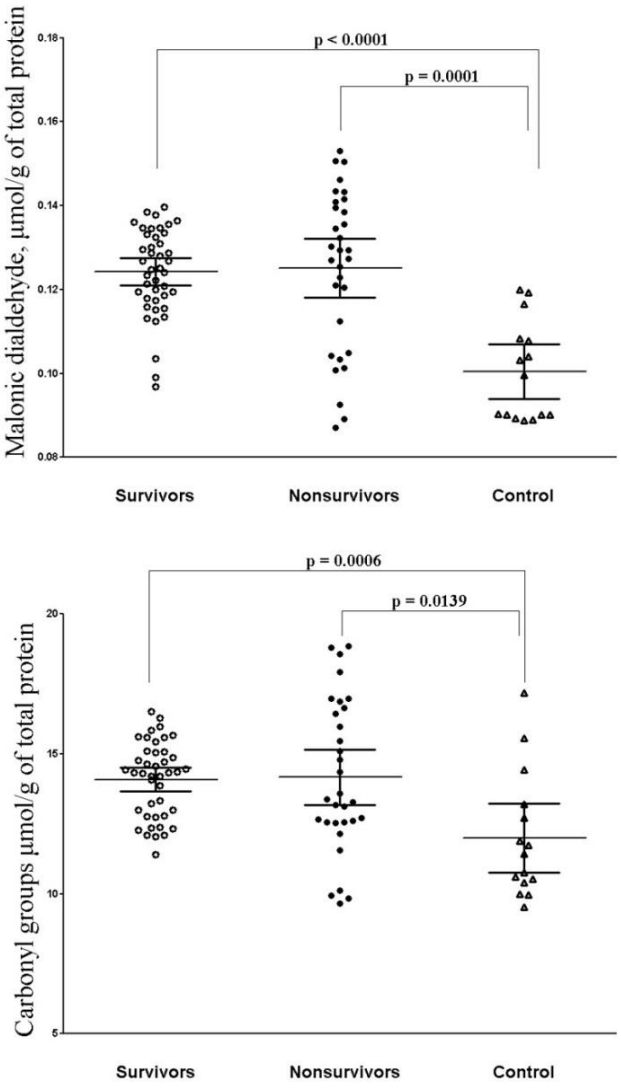

Fig. 1. Changes of oxidative stress markers` concentrations in blood plasma from patients with combined thoracic injuries on the 1-2-nd day after trauma. Horizontal line indicates Mean and swabs – 95 % confidence interval.

There was significant increase of malonic dialdehyde relative concentration in blood patients' plasma in the survival group on the 1-2-nd day after trauma on 25.47 % in comparison to the control group. The same level of significant increase was observed in the nonsurvival group – on 24.87 % in comparison to the control group. Analogous dynamics was observed for proteins carbonyl groups relative concentration. There were significant increases on 18.92 % in blood plasma of patients in the survival group and on 17.51 % in the nonsurvival group, in comparison to the control group. Significant differences for neither malonic dialdehyde nor proteins` carbonyl groups relative concentrations were found between patients groups.

4.3 Cluster analysis

Cluster analysis was used for the stratification of the patients' population by malonic dialdehyde and proteins carbonyl groups relative concentrations simultaneously in relationship to outcome. Figure 2 represents the dendrogram as the result of cluster analysis with denoted clusters.

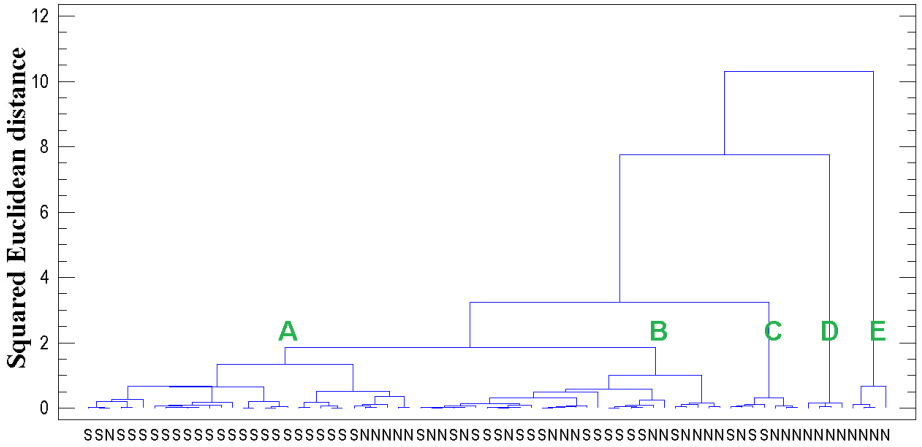

Fig. 2. The Dendrogram of cluster analysis according to centroid method with squared Euclidean distance metric performed by malonic dialdehyde and proteins carbonyl groups relative concentrations simultaneously in relationship to outcome. S – survival group, N – nonsurvival group, A, B, C, D, E – names of clusters.

The mortality level in cluster A was 20 %, in clusters B – 46 %, C – 57 % and in clusters D and E – both 100 %. Characteristics of discovered clusters are detailed in Table 2. Patients' age and number of patients with concomitant alcohol exposure were almost the same in all clusters, as there were p values 0.6779 and 0.6948, respectively. There were significant differences of mortality, ISS and RTS scales, and relative concentrations of malonic dialdehyde and proteins carbonyl groups between observed clusters of patients with combined thoracic injuries.

Table 2. Characteristics of the clusters.

	A	B	C	D	E	P
Number of patients	30	28	7	4	4	
Mortality	6 (20 %)	13 (46 %)	4 (57 %)	4 (100 %)	4 (100 %)	0.0013
Age, years	43 (38.3 – 46.6)	40 (36.5 – 45.2)	35 (23.7 – 49.2)	50 (19.6 – 79.4)	42 (23.5 – 59)	0.6779
ISS, score	22 (21.2 – 25.5)	33.5 (25.2 – 34)	34 (30.9 – 43.6)	49 (30.9 – 63)	41 (35.8 – 43.7)	<0.0001
RTS, score	7.84 (7.114 – 7.686)	6.965 (5.944 – 7.140)	6.613 (4.078 – 7.915)	6.638 (5.479 – 8.059)	4.25 (2.973 – 6.219)	0.0032
Number of patients with concomitant alcohol exposure	18 (60 %)	14 (50 %)	3 (42.8 %)	1 (75 %)	2 (50 %)	0.6948
Malonic dialdehyde, μmol/g of protein	0.1277 (0.124 – 0.13)	0.1287 (0.125 – 0.132)	0.1034 (0.09 – 0.105)	0.1506 (0.146 – 0.155)	0.0909 (0.08 – 0.102)	<0.0001
Proteins carbonyl groups, μmol/g of protein	15.3 (15 – 15.7)	12.6 (12.4 – 12.8)	14.4 (14 – 15.1)	18.7 (17.9 – 19.2)	9.8 (9.5 – 10.2)	<0.0001

5 Discussion

The decision of dividing concentrations of the oxidative stress markers on concentration of total protein was made in to account that in case of massive infusion/transfusion therapy absolute concentrations of the oxidative stress markers cannot truly reflect real state of free radical homeostasis, as was previously shown [13].

There were no differences between groups of injured patients for oxidative stress markers (Fig. 1). These data are controversial to statements about the development of

oxidative stress during early phase of wound dystrophy. Shock, regardless of etiology, is characterized by decreased delivery of oxygen and nutrients to the tissues. Therapeutic interventions are directed toward reversing the cellular ischemia and preventing its consequences [14]. Reperfusion injury starts with the simple reoxygenation of tissues after ischemic insult during traumatic shock [15]. The excess production of reactive oxygen and nitrogen species in this phase cause oxidative stress, which in turn result in bond cleavage and lipid and protein molecular breakdown, whose final products become substrates in cases of extreme need [16]. For 1-2-nd day of the treatment pathophysiological processes associated with the phenomenon of ischemia / reperfusion mostly develops. Patients receive large amounts of infusions and transfusions for volume resuscitation of blood loss. Treatment of respiratory insufficiency requires controlled mechanical ventilation with high level of inspired fraction of oxygen. A massive explosion of free oxygen radicals (oxidative burst) occurs resulting from recovery of oxygenated blood delivery to ischemic tissue [5,16]. Hypotension correction requires adrenomimetic use that increases free radical production through Catechol-O-methyltransferase.

The detailed view of the oxidative stress markers distribution on Fig. 1 shows not normal distributions and presence of subgroups in both patients groups. That's why cluster analysis was used for stratification of whole population of the patients. Its well-known, that free radical oxidation of lipids and proteins cannot proceed separately from each other, but are parts of one process – oxidative stress. So cluster analysis was performed through these two oxidative stress markers simultaneously. Interestingly, patients in cluster D had the highest concentrations of oxidative stress markers and patients in cluster E had the lowest ones, but mortality level was the same (100 %) in both clusters. Also patients in clusters D and E had the most severe combined thoracic injuries according to ISS scale, but patients in cluster E had the lowest level of RTS points indicating the most critical patients' state on admission (traumatic shock). All patients from cluster E were in premorbid state and lethal outcomes were occurred within hours after blood sampling for determination of the oxidative stress markers concentrations. These data indicates that's in case of super severe combined thoracic injuries major violations of the oxygen consumption and utilization processes` occurs, that results in to the severe drop of free radical metabolism intensity.

Normal concentrations of the oxidative stress markers, like in control group, are for malonic dialdehyde – 0.1005 μmol/g of protein ± 0.003 μmol/g of protein and for proteins carbonyl groups – 11.998 μmol/g of protein ± 0.5793 μmol/g of protein. Interestingly, that in case of the severe combined thoracic trauma these levels are common with concentrations of the oxidative stress markers in clusters B and C with suspected mortality level 50 % and cannot be interpreted as satisfactory. Good outcome (20 % mortality level prediction) can be suspected in case of slightly elevated relative concentrations of malonic dialdehyde and proteins carbonyl groups, in comparison to normal values. These data confirms statements about vital role of free radical reactions in processes of signal transduction [17]. In case of premorbid pathophysiologic states like severe combined thoracic trauma these thin mechanism of cell signaling became crucial for survival of the injured human macroorganism [18].

It is possible to formulate criteria that can predict mortality on the 1-2-nd day after traumatic event for patients with the severe combined thoracic trauma. Favorable outcome (20% expected mortality) in patients with the malonic dialdehyde level from 0.1004 to 0.1423 μmol/g of protein can be expected in combination with concentrations of the proteins carbonyl groups from 14 to 17.29 μmol/g of protein, and doubtful outcome (50% expected lethality) – from 10.78 to 14 μmol/g of protein. Very high and very low concentrations of the proteins' (less than 10.78 and more than 17.29 μmol/g of protein) and lipids' (less than 0.1004 and more than 0.1423 μmol/g of protein) oxidative stress markers both predicts poor outcome.

Conclusions

This study has found that oxidative stress develops from 1-2-nd day after the severe combined thoracic trauma and generally its level can be reliably estimated through determination of relative concentrations of both malonic dialdehyde and proteins carbonyl groups. The degree of oxidative stress is proportional to the severity of injury and patients` state on admission (traumatic shock) and does not depends on patients' age and concomitant alcohol exposure. Also its progression is not linear as the result of severe drop of the oxidative stress markers' concentration in premorbid phase of wound dystrophy. Cluster analysis is useful tool for analyzing medical and biological data from investigations when synergistic multifactorial relations are present between pathophysiological processes that are determined by interactions with compensatory and adaptive mechanisms directed at homeostasis saving during critical states.

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