Proteases/Antiproteases System in ALI/ARDS Caused by Polytrauma (an experimental study)

Oleg ARNAUT

State Medical and Pharmaceutical University "NicolaeTestemitanu"
Department of Human Physiology and Biophysics
National Centre of Emergency Medicine, Chisinau, Republic of Moldova
arnautoleg1@gmail.com, arnaut@abv.bg

***Abstract* — Trauma is considered as an epidemic and remains a major medical and socio-economical problem despite of major successes in pre- and intrahospital management.ALI/ARDS is one of the most important and frequent complication in intensive care unit, being a predictor of serious systemic disorders. Incidence and mortality of polytrauma patients complicated by ALI/ARDS (Acute Lung Injury/Acute Respiratory Distress Syndrome) are not well studied. For multiple trauma in different studies these vary from 12-39% and 25-40% respectively. The importance of proteases/antiproteases system in ALI/ARDS caused by polytrauma is poor investigated. We analyzed a1antitrypsin,ά2macroglobulin, elastase, cathepsin G values for experimental ALI/ARDS polytrauma model.**

***Index Terms* — proteases, antiproteases, polytrauma, ALI/ARDS.**

I. BACKGROUND

Trauma is considered as an epidemic and remains a major medical and socio-economical problem despite of major successes in pre- and intrahospital management. In the USA in general structure of mortality the rate of trauma is about 6%, being the first cause of death for children, adolescents and adults up to 44 years. In the USA in general structure of mortality the rate of trauma is about 6%, being the first cause of death for children, adolescents and adults up to 44 years [1, 2, 3, 4].The most unexplored category of injuries is polytrauma injury. There is no common definition for polytrauma. A literature search for the time period January 1950 − August 2008 allowed to give the following definition. Monotrauma is an injury to one body region. Severe monotrauma could be considered if ISS (Injury Severity Score) > 15, or ISS < 15 with significant acute physiological deterioration (cardiovascular or respiratory or neurological). Multitrauma is an injury to more than one body region (not exceeding AIS (Abbreviated injury scale) ≥ 3 in two regions) without SIRS (Systemic inflammatory response syndrome). Severe multitrauma could be considered if ISS > 15, or ISS < 15 with significant acute physiological deterioration (cardiovascular or respiratory or neurological). Polytrauma is an injury to at least two body regions with AIS ≥ 3 and with the presence of SIRS on at least one day during the first 72 hours [5]. ALI/ARDS is one of the most important and frequent complication in intensive care unit, being a predictor of serious systemic disorders (Multiple Organ Dysfunction Syndrome -MODS). Clinically ALI/ARDS is manifested by acute hypoxemia, morphologically by immunocompetent cells (neutrophils) infiltration, interstitial and alveolar edema, venous congestion and intraalveolar hemorrhage. Incidence and mortality of polytrauma patients complicated by ALI/ARDS are not well studied. For multiple trauma in different studies these vary from 12-39% and 25-40% respectively [6, 7, 8, 9, 10].Proteases, situated in neutrophils granules, which under pathological conditions have ability to destroy normal tissue (especially lung and liver). The most important proteases involved in ALI/ARDS occurring are elastase, cathepsin G, proteainase 3, collagenase, gelatinase. Antiproteases, secteted by the liver, epithelium and some immunocompetent cells. The most important plasma antiproteases are a1antitrypsin (a1AT) and ά2macroglobulin (a2M). [11, 12].The importance of proteases/antiproteases system in ALI/ARDS caused by polytrauma is poor investigated.

Table 1. Test statistics. a1antitrypsin.

	control vs 2 h	control vs 5 h	control vs 24h	2 h vs 5 h	2 h vs 24 h	5 h vs 24 h
Z	-1,504	-4,123	-4,055	-2,711	-1,709	-3,053
Asymp. Sig. (2-tailed)	,133	,000	,000	,007	,088	,002

II. MATERIAL AND METHODS

The study is approved by the Ethical Research Committee of State Medical University "NicolaeTestemitanu". 30 male rabbits aged 3 months with a mean weight of 2913±100g were included for the study. Arterial (central ear artery) and venous catheters (marginal ear vein) (G20) were installed. The trauma was performed at an adequate level of anesthesia. The trauma represented bilateral fracture of the tibia (2 injuries with AIS ≥ 3) just below the knee in a sterile environment, using stapler and induced blood loss by aspiration (central ear artery) of 25% of the blood volume. 4 samples (before the trauma, in 2, 5, 24 hours) were collected for gas and biochemical analysis (elastase, carhepsin G activity and a1AT, a2M concentration). In 24 hour following trauma procedure rabbits were sacrificed. Lung, kidney, spleen,

heart and liver of each animal were taken for histopathological examinations. The diagnosis of ALI/ARDS caused by polytrauma was considered if histopathological examination determined inflammatory changes at least in 2 organs and gasometrical criteria (PaO2/FiO2 < 300) had been performed. Statistical analysis was performed by SPSS 20.

Table 2. paired samples test. α2macroglobulin.

	Paired Differences					t	df	Sig. 2-tailed
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
control vs 2 h	,0764	,1122	,0212	,0329	,1199	3,605	27	,001
control vs 5 h	,0429	,1529	,0289	-,0164	,1022	1,483	27	,150
control vs 24h	-,0779	,2142	,0405	-,1609	,0052	-1,923	27	,065
2 h vs 5 h	-,0336	,0672	,0127	-,0596	-,0075	-2,644	27	,013
2 h vs 24 h	-,1543	,1346	,0255	-,2065	-,1021	-6,063	27	,000
5 h vs 24 h	-,1207	,1145	,0216	-,1651	-,0763	-5,577	27	,000

III. RESULTS

A1AT (a1antitrypsin) plasma values after polytrauma represented abnormal distribution. The difference between groups was determined by Friedman test ($p < 0.0001$) and Wilcoxon test. Value of a1AT in 2 hours (47.27 ± 17.26) after trauma had tendency to decrease versus control (56.97 ± 15, $p = 0.133$) and after 24 hours (40.17 ± 8.2, $p = 0.088$), was higher in comparison with value in 5 hours (34.83 ± 7.05, $p = 0.007$). Value in 5 hours decreased compared to control ($p < 0.001$) and in 24 hours ($p = 0.002$). Value in 24 hours rises versus control ($p < 0,001$) (Table 1).

Table 3. Paired Samples Test. Cathepsin G activity.

	Paired Differences					t	df	Sig. (2-tailed)
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
control vs 2 h	5,53	7,62	1,44	2,57	8,48	3,83	27	,001
control vs 5 h	6,57	8,72	1,65	3,18	9,95	3,98	27	,000
control vs 24h	-9,09	12,64	2,39	-13,99	-4,19	-3,81	27	,001
2 h vs 5 h	1,04	7,54	1,42	-1,88	3,96	,73	27	,471
2h vs 24 h	-14,62	11,42	2,16	-19,05	-10,19	-6,77	27	,000
5 hvs 24 h	-15,66	7,18	1,36	-18,44	-12,87	-11,53	27	,000

α2macroglobulin values had normal distribution. The difference between groups was determined by the General Lineal Model ($p < 0.0001$) and Paired Samples Test (Table 2). Controls value (0.49 ± 0.15) was higher than after 2 hours (0.41 ± 0.07, $p = 0.001$), with a tendency to increase versus 5 hours (0.45 ± 0.06, $p=0.15$), to decrease in comparison with 24 hours (0.57 ± 0.09, $p = 0.065$). In 2 hours value was lower compared to 5 ($p=0.013$) and 24 ($p < 0,001$) hours, in 5 hours lower than in 24 hours ($p < 0.001$).

Cathepsin G activity had normal distribution. The difference between groups was determined by the General Lineal Model ($p < 0.0001$) and Paired Samples Test (Table 3). Cathepsin G value at 2 hours (23 ± 7.43) was lower compared to controls (28.52 ± 9.99, $p = 0.001$) and at to 24 (37.61 ± 6.09, $p < 0.001$) with a tendency to rise versus 5 hours (21.96 ± 4.87, $p = 0.471$). In 5 hours it decreased in comparison with control and 24 hours ($p < 0.001$ and $p < 0.001$, respectively). In 24 hours value was higher than control ($p = 0.001$).

Elastase activity value had normal distribution. The difference between groups was determined by Paired Samples Test (Table 4). Elastase activity at 2 hours (75.75 ± 26.13) tended to increase compared to controls (73.51 ± 24.28). After 5 (84.91 ± 36.83) it increased compared to controls ($p = 0.025$).In 24 hours it had a tendency to return to control value (78.17 ± 34.66, $p = 0.457$).

Table 4. Paired Samples Test. Elastase activity

	Paired Differences					t	df	Sig. (2-tailed)
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
control vs 2 h	-2,239	30,86	5,833	-14,20660	9,728	-,384	27	,704
control vs 5 h	-11,40	25,40	4,799	-21,25048	-1,555	-2,38	27	,025
control vs 24h	-4,658	32	6,048	-17,06744	7,752	-,770	27	,448
2 h vs 5 h	-9,164	37,38	7,065	-23,65912	5,332	-1,297	27	,206
2 h vs 24 h	-2,419	36,32	6,864	-16,50236	11,665	-,352	27	,727
5 h vs 24 h	6,745	47,30	8,940	-11,59744	25,087	,755	27	,457

After analysis were determined following correlations:

1. Hystological Score correlates to cathepsin G before the trauma (0.629, $p<0.001$) and after 2 hours (0.671, $p<0.001$); to a1AT before the trauma (-0.603, $p=0.001$), after (0.559, $p=0.02$) and 24 hours (-0.539, $p=0.03$) after the trauma.
2. Cathepsin G activity correlates to elastase activity before (-0.414, $p=0.028$) and in 5 hours (-0.439, $p=0.019$); correlates to ά2macroglobulin values in 2 (0.556, $p=0.002$) and in 24 (0.396, $p=0.037$) hours after the trauma.
3. Elastase activity correlates to ά2macroglobulin values after 2 hours (0.473, $p=0.011$).

IV. CONCLUSION

1. The most sensitive plasma predictor/indicator of the ALI/ARDS in polytrauma patients in 2 hours after trauma is a2M, in 5 hours is a1AT, in 24 hours is cathepsin G activity.
2. The positive (cat G, before the trauma and in 2 hours) and negative (a1AT, before the trauma, in 24 hours) correlation with final histological examination evidence predisposition for ALI/ARDS and importance of cathepsin G/a1AT in ALI/ARDS developing after polytrauma.
3. The negative correlation between cathepsin G and elastase before the trauma and in 5 hour after trauma evidences of competition between these enzymes for a2M, and, probably, has impact for quant duration – a possible mechanism of ALI/ARDS in polytrauma.
4. Proceeding from the arguments examined components of proteases/antiproteases system are recommended for predictors/indicators as well as active participants in this process. This allows optimization of existing and to create new prophylaxis and treatments to reduce the incidence and mortality, as well as the optimal use of financial resources for ALI/ARDS caused by polytrauma..

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