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Survival predictive model for severe trauma patients using proteases/antiproteases system components

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Abstract

Background: Assessing the traumatic injuries severity, as well as estimating the severe trauma patient's prognosis are the key moments in their management. Predictive models for severe trauma outcome need improvement.
Material and methods: In the clinical study (65 severe trauma patients), proteases, antiproteases and treatment outcome (survival/non-survival) were considered. There were used two statistical instruments – dimension reduction analysis (principal component analysis) to prepare the data for modeling and modeling itself through multivariate logistic regression.
Results: Principal component analysis evidenced 12 "latent" factors grouped in four models. The survival predictive model had the following characteristics: calibration χ^2=1.547, df=7, p=.981; determination – 0.759; discrimination, sensitivity – 90.7%, specificity – 81.8 %, area under ROC curve – 0.95 (95%CI 0.912, 1.000). The model enrolled four "latent" factors (three destructive and one protective), male gender and ARDS development.
Conclusions: In our research, the survival predictive model for severe trauma patients on base of proteases/antiproteases system components after dimension reduction procedure was elaborated. The model showed good characteristics and needs validation to be implemented in daily clinical practice.
Key words: trauma, survival predictive model, proteases, antiproteases.

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Introduction

Trauma remains an important medical problem. A series of scores and algorithms were elaborated to estimate the severity and to predict the treatment results for injured patients, but, actually, there are no any universal scores [1]. Moreover, existing scores applied in different medical systems or different populations with their specific demographic structure showed different ability to predict the outcomes [2, 3]. This, in turn, makes researchers be active in efficient variables/biomarkers identification that will increase the potential models' predictive power.

The potential source for biomarkers in trauma patients is the components of proteases/antiproteases system. These elements are some active immune response participants in trauma and especially in severe trauma, activated by Systemic Inflammatory Response Syndrome (SIRS) development with proteases release by neutrophils in both, injured and non-injured tissues [4, 5]. Thus, the proteases and their antagonists, antiproteases, could be used as predictors in survival predictive models. At the same time, the relationships between proteases/antiproteases system elements are, evidently, complex and this is why it is more rational to use some integrative indicators for analyses. This allows characterizing pathophysiological process/processes instead of individual proteases or antiproteases effect/effectsPrincipal component analysis represents a statistical instrument for dimension reduction. Analyzing dataset, this method extracts the "latent" factors, which are the numerical expression (quantification) of pathophysiological/fundamental factors/processes involved in traumas evolution and outcome, including the appearance of indirect lesions. Each extracted factor is estimated by linear regression technique, having at least two predictors and is used for modeling instead of initial variables [6].

The aim of this research was to elaborate a survival predictive model for severe trauma patients on base of proteases/antiproteases system components after dimension reduction procedure.

Material and methods

Ethical committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Chisinau, the Republic of Moldova) approved the study design (Protocol 33/46 of 16.12.2016).

In a clinical prospective analytical study, 65 severe trauma patients admitted within first 24 hours after trauma in Intensive Care Unit of Emergency Medicine Institute (ICU EMI), Chisinau, The Republic of Moldova, were enrolled. Plasma samples (venous blood) were collected at 3, 6, 12 and 24 hours after traumatic impact, the potential model being

applicable at 24 hours after trauma. For every blood sample, there were estimated 10 proteases/antiproteases system parameters – two antiproteases (α_2-macroglobulin (α_2M), α_1-antitrypsin (α_1AT)) and eight proteases (Cathepsin D activity (CDA), Cathepsin H activity (CHA), Cathepsin L activity (CLA), Cathepsin G activity (CGA), Elastase activity (EA), Trypsin activity (TA), Adenosinedesaminase activity (ASDS) and Adenilatdesaminase activity (ALDS). In addition, gender (binary variable), aging (continuous variable), Acute Respiratory Distress Syndrome (ARDS) development (binary variable) were considered for modeling. The outcome (dependent variable) was trauma patient survival (survived or non-survived). Criteria for severe trauma was NISS more than 15 points [7], ARDS diagnosis being determined considering Berlin definition [8].

To achieve the aim of study, two statistical instruments – dimension reduction (principal component analysis (PCA, varimax rotation) and multivariate logistic regression were used. Through dimension reduction analysis were extracted as named "latent" factors, maximal three for every PCA model. Also, taking into account the relatively small patients sample Kaiser-Meyer-Olkin (KMO) (measures of how suited your data is for factor analysis) and Bartlett (if the number of extracted factors was appropriate) tests were applied. If KMO was more than .5 and Bartlet test was significant (Bonferroni correction for multiple comparisons) the model was considered for further analysis. In addition, the PCA models were optimized by variable elimination if correlation coefficient was less than .6 or if a parameter was associated with two extracted factors and difference between the absolute correlation values was less than .3.

Extracted "latent" factors, being estimated quantitatively (continuous variable), together with other potential predictors mentioned above were considered for modeling through multivariate logistic regression (backward conditional method). To estimate the model's predictive potential, were considered the following characteristics: determination (Nagelkerke R Square), calibration (Hosmer–Lemeshow test and classification plots) and discrimination (specificity, sensibility, area under ROC curve and sensibility/specificity optimization by cut-off changing). In addition, were appreciated model's stability (resampling by bootstrapping) and multicollinearity analysis.

Results

According to PCA results (tab. 1), there were evidenced 4 models, corresponding to 4 blood samples, collected at 3, 6, 12 and 24 hours after trauma. Each model had 3 "latent" (extracted) factors. Every factor, in turn, enrolled between 2 and 5 proteases/antiproteases system components. In addition, taking into account the parameters that estimated the factors, it was possible to identify, at least presumably, the potential factors roles, divided into potentially destructive or potentially protective.

Within multiple regression analysis for outcome prediction, the data collected at 24 hours after the trauma were considered (ARDS diagnosis, Age, Gender and "latent" factors estimated before). The *null hypothesis* – the model's covariates are not able to predict the survival probability better than a model with constant only. The a*lternative hypothesis* – at least one covariate is able to predict the survival probability better than a model with constant only. The *null hypothesis* was rejected by *Omnibus Test of Model Coefficients* ($\chi^2 = 51.569$, df=6, p<.001, significance level (α) being .05.

Determination coefficient (Nagelkerke R Square), showed the value .759 (75.9%) – more than 75% from survival probability dispersion was explained by the elaborated model's covariates.

The calibration (Hosmer–Lemeshow test) had closed to ideal value of $\chi^2 = 1.547$, df=7, p=.981, being nonsignificant, and confirmed the results fidelity.

The discrimination properties (cut-off was increased up to .55 for accuracy increasing) had the optimal values for specificity 81.8% (18 out of 22), sensibility 90.7% (39 out of

Table 1

The principal component analysis output (varimax rotation)

Sample timing, hours after trauma	Extracted factors	Components	Potential effect
3 hours	1st Factor	α_2M_3, CHA_3, TA_3	Protective
	2nd factor	$ASDS_3$, $ALDS_3$	Destructive
	3rd factor	EA_3, CDA_3	Destructive
6 hours	1st Factor	$ASDS_6$, $ALDS_6$, α_2M_6 (negative correlation)	Destructive
	2nd factor	CHA_6, TA_6, α_2M_6	Protective
	3rd factor	EA_6, CGA_6, CDA_6	Destructive
12 hours	1st Factor	α_1AT_{12} (negative correlation), TA_{12}, $ASDS_{12}$, EA_{12} (negative correlation), α_2M_{12}	Protective
	2nd factor	$ALDS_{12}$, CDA_{12}, CLA_{12}	Destructive
	3rd factor	CHA_{12}, CGA_{12}	Destructive
24 hours	1st Factor	EA_{24}, α_1AT_{24}	Destructive
	2nd factor	TA_{24}, α_2M_{24}, CGA_{24}	Protective
	3rd factor	$ASDS_{24}$, $ALDS_{24}$	Destructive

α_2-macroglobulin (α_2M), α_1-antitrypsin (α_1AT), Cathepsin D activity (CDA), Cathepsin H activity (CHA), Cathepsin L activity (CLA), Cathepsin G activity (CGA), Elastase activity (EA), Trypsin activity (TA), Adenosinedesaminase activity (ASDS) and Adenilatdesaminase activity (ALDS).

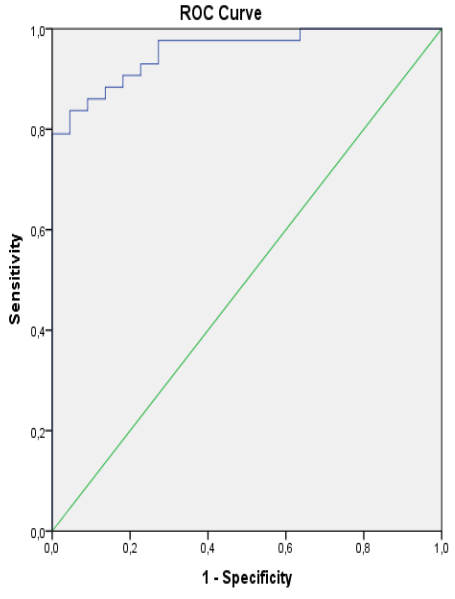

Fig. 1. Area under ROC curve for survival predictive model based on data collected within first 24 hours after trauma

43) and overall percentage (87.7%), all these parameters being higher than target value of 80%. Area under ROC curve for proposed model was estimated at level of .956 (95%CI 0.912, 1) and represented significance in relation to the value of .5 ($p<0.001$) (fig. 1).

The final model included the constant (B=7.816), ARDS diagnosis (B=-4.731), male gender (-.333), the values of factor $2_{\text{model 2}}$ (B=4.038), factor $3_{\text{model 1}}$ (-2.752), factor $2_{\text{model 3}}$ (-2.623) and factor $2_{\text{model 4}}$ (-2.623) (tab. 2). The variable Age and other extracted factors did not show significance, the proposed model being represented as follows:

$$p = \frac{1}{1+e^{-b}} \text{ (formula 1), where}$$

p – 3ability to survive

b = 7.816 + 4.038 x factor 2_{model2} *- 2.752 x factor* 3_{model1} *- 2.623 x factor* 2_{model3} *- 1.504 x factor* 2_{model4} *- 3.333 x male - 4.731 x ARDSe (exponent) – constant equal to 2.71828*

The resampling procedure by bootstrapping (999 samples) showed the significance of potential predictors evidenced in elaborated models, without signs inversion, 95% CI for coefficients being relatively wide (tab. 2b).

Discussion

The alternative approach, used in this research, especially "preparing" the data for modeling by PCA allowed taking into account proteases/antiproteases components in complex, as physiopathological elements instead of individual role of separated elements. Finally, four significant extracted factors took part from elaborated predictive model (tab. 1 and 2).

Table 2

Coefficients for elaborated model equation (a) and bootstrapping for variables in the equation (b)

a. Final model coefficients								
	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
ARDS diagnosis	-4.731	1.739	7.397	1	.007	.009	.000	.267
factor $3_{\text{model 1}}$	-2.752	.883	9.723	1	.002	.064	.011	.360
factor $2_{\text{model 2}}$	4.038	1.292	9.767	1	.002	56.693	4.506	713.222
factor $2_{\text{model 3}}$	-2.623	.950	7.617	1	.006	.073	.011	.468
Male gender	-3.333	1.513	4.851	1	.028	.036	.002	.693
factor $2_{\text{model 4}}$	-1.504	.751	4.011	1	.045	.222	.051	.968
Constant	7.816	2.555	9.362	1	.002	2480.270		

b. Bootstrap for Variables in the Equation						
	B	Bias	S.E.	Sig.	95% Confidence Interval for B	
					Lower	Upper
ARDS diagnosis	-4.731	-89.957	715.811	.002	-615.579	-2.512
factor $3_{\text{model 1}}$	-2.752	-45.593	305.169	.003	-389.184	-1.728
factor $2_{\text{model 2}}$	4.038	86.037	528.509	.002	2.488	671.286
factor $2_{\text{model 3}}$	-3.333	-62.414	372.735	.006	-562.397	-.793
Male gender	-2.623	-53.494	413.554	.001	-381.894	-1.756
factor $2_{\text{model 4}}$	-1.504	-22.276	133.442	.004	-143.446	-.403
Constant	7.816	140.631	1045.749	.001	5.189	1136.440

Constant – equation constant's value, B – B coefficients, S.E. – standard errors, Wald – Wald statistics, df – degrees of freedom, Sig. – significance threshold, Exp (B) – odds ratio values, 95% C.I. for EXP(B) – 95% confidence interval for odds ratio.

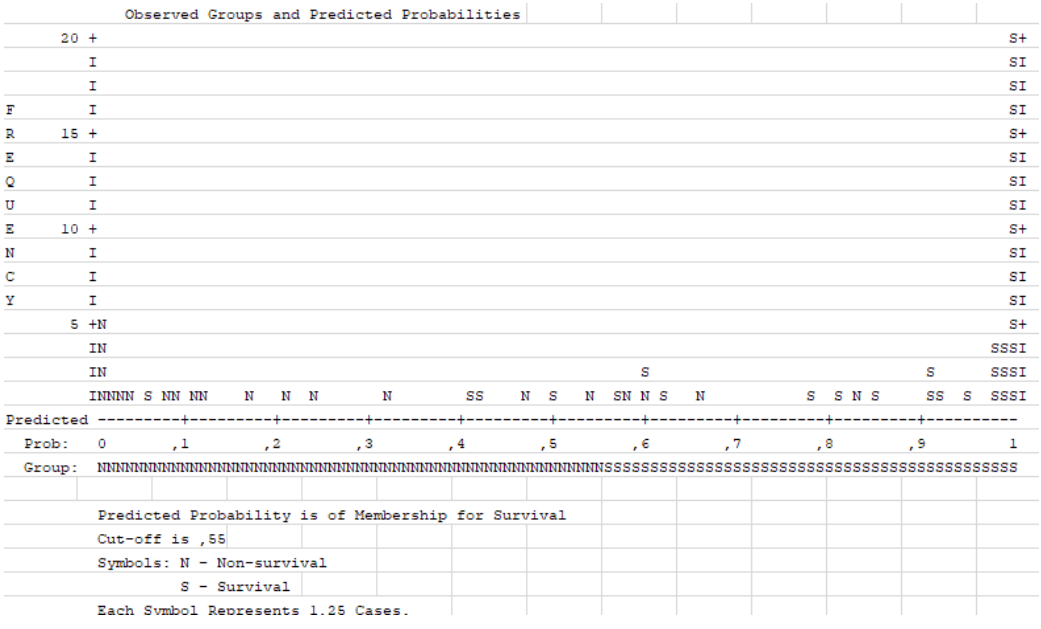

Fig. 2. Classification plot for survival predictive model in severe trauma based on data collected within first 24 hours after trauma

The third extracted factor (PCA model at 3 hours) was in positive association with two proteases – AE_3 and ACD_3, the factor was estimated as destructive one (tab 1). In accordance with literature the elastase represents the key substance in both, direct and indirect lung lesions [9], having multiple destructive effects as degradation the elastin fibers, ciliar apparatus lesions, apoptosis amplification etc. [10]. Moreover, the elastase represents multiple proinflammatory effects [11]. Cathepsin D, in turn, was demonstrated to have ability for enzyme, hormones, antigen and neuropeptide processing, having a function to activate Caspase 8, an important element in apoptosis cascade. In addition, Cathepsin D showed complement activation and increasing for trauma patients [12].

The second extracted factor (PCA model at 6 hours) was, probably, protective because of positive association with trypsin and α_2-macroglobulin. The last one represents the antiprotease substance, that is able to inactivate almost all proteases [13]. Trypsin, unexpectedly, in different research showed a series of positive effects as anti-inflammatory, antiedematic, antioxidant, first two effects demonstrated for trauma patients [14, 15]. The Cathepsin H positive association could be explained by incomplete determination coefficient abilities in linear regression equation for extracted factors in PCA.

The PCA model at 12 hours evidenced 3 factors, second one being significant for final predictive model. This factor was estimated as destructive because of positive association with three proteases – AAMP, ACD and ACL. Cathepsin L, in accordance with literature data, represented a proteolytic activity for almost all proteins, including enzymes and receptors [16]. It is associated with Cathepsin D, controlled apoptosis and neovascularization, enrolled in immune response [17].

The second extracted factor from the fourth PCA model was explained by trypsin, α_2-macroglobulin and cathepsin G values, all components having positive association. Taking into account this, especially first two, the "latent" factor was interpreted as protective one. However, the equation for "latent" factor quantitative estimation included cathepsin G, which represents a series of potential negative effects. It is able to activate coagulation cascade, immune response, to destroy the vascular matrices and to generate edema [18-21]. This, in turn, probably, determined the destructive nature of this parameter, trypsin, α_2-macroglobulin being just associated with severity of potential lesions without direct implication.

Regarding the final predictive model, the main result of this study, it is important to mention good characteristics despite of relatively small sample size (one of the limitations). At the same time, resampling showed the reproductibility of the experiment. The model had relatively high determinant coefficient, the value being close to 80%. Calibration and discrimination showed good model fit and good sensibility/specificity abilities. The most important covariate was factor $3_{\text{model 1}}$ (AE and ACD as predictors). It determined 23% of dependent variable dispersion. Speculating, AE and AED inhibition within first 3 hours after trauma could be a perspective direction for further researches. On the second place was factor $2_{\text{model 2}}$ (17.1%), followed by ARDS diagnosis (12.7%), male gender (10.3%), factor $2_{\text{model 3}}$ (7.3%) and factor $2_{\text{model 4}}$ (5.5%). These data suggest idea about the antiprotease treatment optimization in dependence on the admission timing, that, was not taken into consideration before – possible reason for antiproteases treatment failure [22, 23].

In accordance with elaborated model's results, ARDS, factor $3_{\text{model 1}}$, factor $2_{\text{model 3}}$, factor $2_{\text{model 4}}$ and male gender were the parameters that decrease survival probability (OR=.009, 95%CI .000, .267; OR=.064 95%CI .011, .360; OR=.073, 95%CI .011, .468; OR=.222, 95%CI .051, .968 and OR=.036, CI95% .002, .693, respectively). Factor $2_{\text{model 2}}$, had a protec-

tive effect, OR being 56.693 (CI 95% 4.506, 713.222) (Table 2a). Resampling (bootstrapping, 999 samples) showed wide confidence intervals for model's covariates with significance and no changing signs in front of parameters. In addition,it is important to mention no multicollinearity between predictors included in the final model.

Conclusions

In our research, the survival predictive model for severe trauma patients on base of proteases/antiproteases system components after dimension reduction procedure was elaborated. The model showed good characteristics and needs validation to be implemented in daily clinical practice.

Dimension reduction analysis with "latent" factor extraction has a perspective in research with numerous potential biochemical parameters, taking into account their complex interactions, estimated factors, probably, being quantitatively estimated for different pathophysiological mechanisms.

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Authors' contribution
OA conceptualized the idea, conducted literature review, wrote the manuscript, revised and approved the final text; GI and OA interpreted the data.

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Ethics approval and consent to participate
The research project was approved by Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 46, 16.12.2016).

Conflict of Interests
No competing interests were disclosed.