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RESEARCH ARTICLE

PROTEASES AND ANTIPROTEASES NEW POTENTIAL BIOMARKERS/VARIABLES FOR POLYTRAUMA SURVIVAL MODELING? A PILOT RESEARCH

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ABSTRACT

Background: Despite big progresses in early management of trauma patients, traumas still represent an actual subject at international scale being the main cause of death for persons younger than 44 years. Polytrauma is the most unexplored and unsearched category of traumas. There is no international consensus according the most efficient scale, many of them returning different results in estimating the patient's condition complications and patient's mortality risk in case of trauma. The described situation makes us to search some solutions inclusively new factors with a higher predictive power in estimating the polytrauma patient's outcomes. We supposed that such an instrument could be different protease/antiprotease system's components. **Objectives:** The aim of this research was to estimate the predictive potential of proteases and antiproteases by polytrauma population survival rate modeling. **Methods:** In a prospective pilot study, 65 polytrauma patients admitted in acute period of trauma were analyzed. Plasma samples were collected at 3, 6, 12 and 24 hours after traumatic impact. We measured the values of two antiproteases concentration and enzymatic activity of six proteases. In order to identify the potential biomarkers for survival rate, we have compared proteases/antiproteases system components between survived and non-survived patients. The evidenced potential biomarkers were used for regression analysis modeling, discrimination, determination and calibration characteristics being estimated. In addition, the resampling procedure for model's stability estimation was applied. **Results:** The comparative evaluation among evidenced molecular phenotypes in survived and non-survived patients allows to consider a seria of primary outcome potential biomarkers/predictors. The outcome modelling by regression analysis used these potential predictors. Finally, five parameters, especially α_2M_3, $CDEA_3$, ARDS, α_2M_6, $CHEA_6$ EEA_3 and $CGEA_{12}$, were the components (efficient variables) from models that predict the survival rate using their values at 3, 6 and 12 hours after the trauma, results being adjusted to age, gender and ARDS diagnosis. **Conclusions:** In our research, we estimated the predictive potential of different protease/antiprotease system's components for polytrauma population. Using this data, three predictive models were obtained. Without any doubts, they can be used in clinical practice after validation and improvement by including more variables in equation. The identified survival prediction biomarkers could be used as base stones of potential therapeutic strategies

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INTRODUCTION

In our days, despite big progresses in shortening hospital admission period, improved diagnostic tools, early management and others, traumas with all its variants still represent an actual subject at international scale. In general lethality structure, they are on the third place after cardiovascular diseases and cancers being the main cause of death or disabilities that could be prevented among persons younger than 44 years (McCullough, 2014; Deng, 2016). In addition, while the death rate from oncological and cardiac diseases has favorable trend, the death rate from trauma is rising faster than the parallel population increase. This is a sign that, in the long perspectives, probably, the trauma wills prevalate in general lethality structure (Kunitake *et al*., 2018). To identify the patients with different injury severities and mortality risk or different scenario of unfavorable evolution, there are used several terms like "severe trauma", "major trauma" and "polytrauma".

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The analysis of entries/documents in Web of Science database shows 24441, 19471 and 2813 entries for these notions, respectively. The terms "severe trauma" and "major trauma" are very close, almost synonymic, but the criteria are not very well defined and fixed. Thus, the limit value of ISS (Injury Severity Score) or NISS (New Injury Severity Score) varies in different studies at the threshold of 16-17 points (McCullough, 2014; Femling *et al*., 2014; Winfield, 2010) Compared to the first two, the polytrauma is the most unexplored and unsearched category of traumas and its notion still remains a subject of disputes. In most of the related articles, criteria for polytrauma is taken the anatomical scale ISS value over 15 points. At the same time, there are other authors that consider different threshold values. According different sources, this value varies from 15 up to 26 and more. (Hsieh, 2018; Rau, 2017; Butcher, 2013). According to the New Berlin Definition, validated following the results of relative high evidence studies, the polytrauma is defined as a severe lesion of at least 2 body regions, scored by AIS (Abbreviated Injury Scale) with ≥ 3 points, being present at least one of 5 altered physiological parameters (systolic blood pressure ≤ 90 mmHg, GCS ≤ 8, acidosis, coagulopathy and age ≥ 70 years) (Pape, 2014).

Trauma management problem, especially because of poor outcomes, obviously, raised the idea of some predictive scores' conception. Thus, a series of scores and algorithms were created to assess the severity of traumas and possible patient condition evolution in the period following the trauma. The elaborated mathematical models were based on different criteria (anatomical, physiological or mixed scores) (Arnaut, 2020). In the same time there is no international consensus in the articles found on PubMed/Medline, Web of Science, and EBSCO databases according the most efficient scale, many of them returning different results in estimating the patient's condition complications and patient's mortality risk in case of trauma (Butcher *et al*., 2013; Pape, 2014; Butcher, 2009). This situation may be related to economic factors and differences in the medical systems, particularities of demographic structure or other aspects of different countries (Rutledge, 1996). Because of that, at the patient's evaluation there are disagreements on the predicted outcomes, different scores often estimating completely different results. The described situation makes us to search some solutions inclusively new factors with a higher predictive power in estimating the patient's outcomes. One of these factors seem to be some substances from protease/antiprotease system. This hypothesis comes from the presumption that the late manifestations of the immune response to the trauma will increase the mortality risk of the trauma patients, proteases and antiproteases being a part of him. Thus, a well-recognized phenomenon characterized by changes in vital signs and laboratory indicators in adult trauma populations is the Systemic inflammatory response syndrome (SIRS). This syndrome often follows the "initial hit" of the traumatic event, being a "second hit" sometimes more dangerous than the trauma itself, often resulting in different organs' injury, along with deterioration of the patient's clinical condition (Al-Mahdi, 2017). Responsible for this "remote" injuries are granulocytes that play a key role during the course of various infectious and inflammatory diseases. While the prompt activation of PMN leukocytes is crucial for a successful elimination of infections, overwhelming activation of PMN leukocytes may have deleterious effects for the host because of proteases releasing in both, trauma affected and unaffected tissues.

A thorough understanding of the delicate balance of this two-sided Janus-face of leukocytes may open the way for new therapeutic strategies in the treatment of infectious and inflammatory diseases and, more important for our discussion, their complications (Nussler, 1999). In addition, proteases and antiproteases, reflecting the host immune response, could be used as survival rate predictor. The aim of this research was to estimate the predictive potential of different protease/antiprotease system's components for polytrauma population survival rate modeling.

METHODS

Research project was approved by the Ethics Committee of SUMF "Nicolae Testemițanu". In Emergency Medicine Institute from Chisinau, Republic of Moldova, in a prospective study, 65 polytrauma patients admitted in acute period of trauma were analyzed, criteria for polytrauma patients being Berlin definition (Pape, 2014). Plasma samples (venous blood collection and centrifugation) were collected at 3, 6, 12 and 24 hours after traumatic impact. We measured the values of two antiproteases (α_2M and α_2AT) concentration and enzymatic activity of six proteases (EEA, CDEA, CHEA, CLEA, CGEA, TEA), totally 8 proteases/antiproteases system components, the values being determined by spectrophotometric analysis. The statistical analysis was performed using SPSS 21 (License No. 20130626-3). The continuous data were represented using the mean and median, the dispersion parameters being standard deviation and interquartile range. To describe the dichotomous data have been used the frequencies and proportions. In order to identify the potential biomarkers for survival rate, we have compared proteases/antiproteases system components between survived and nonsurvived patients using nonparametric tests (taking in account the data type and their distribution) without multiple comparison correction. Moreover, for the following analysis we have used the parameters with p value $\leq .1$, because of potential adjustment effects in multivariate analysis. Taking in account the dichotomous nature of outcome (death/survive) and complex relationship inside the proteases/antiproteases system, the survival modeling was performed using the multivariate logistic regression. In addition to potential biomarkers we have considered gender, age and ARDS diagnosis according to Berlin definition as eventual efficient variables. In order to match the regression analysis conditions, the data was tested for multicollinearity. The potential model was characterized using determination (*Nagelkerke R Square*), calibration (*Hosmer – Lemeshow test*) and discrimination (*sensibility, specificity, mean validation, ROC curve and classification graph, cut-off* modification) parameters. In addition, the proposed model's stability analysis was performed (resampling using *bootstrapping*). Statistical modeling tests were adjusted for all proposed models using the Bonferroni correction.

RESULTS

To identify the potential biomarkers for modelling we have compared the value of examined parameters for survive and non-survive patients (Table 1). According to the analysis, crosstabulation (continuity correction) shown the significance ($\chi^2 = 4.556$, df=1, p=.033) and medium effect size (.07) for ARDS diagnosis, established for 18 from 22 (81.8%) non-survival cases vs 51.2% (22 cases from 43) in survival group, gender being non-significant ($\chi^2 = .275$, df=1, p=.600).

Table 1. Comparison between survival and non-survival patients

		Non-survive		Survive		
		Mean (SD)/ Count (%)	Median (IR)	Mean (SD)/ Count (%)	Median (IR)	Crosstabulation/ Mann-Whitney Test
ARDS	No	4 (18.2)		21 (48.8)		p = .033
	Yes	18 (81.8)		22 (51.2)		
Gender	Female	6 (27.3)		16 (37.2)		p = .600
	Male	16 (72.7)		27 (62.8)		
Age, years		36.7 (15.2)	34 (25)	39.6 (18.2)	33 (33)	p = .393
TEA_3, nM/s • l		121.8 (60.7)	105 (70)	177.7 (111.9)	160 (120)	p = .027
TEA_6, nM/s • l		135.0 (61.9)	130 (80)	144.9 (71.0)	140 (100)	p = .642
TEA_{12}, nM/s • l		115.5 (39.4)	105 (30)	160.9 (68.2)	150 (110)	p = .006
TEA_{24}, nM/s • l		130.9 (50.6)	110 (90)	155.4 (76.0)	160 (90)	p = .226
α_1AT_3, µM/l		23.50 (9.7)	21.10 (13.2)	25.6 (13.5)	26.4 (17.3)	p = .584
α_1AT_6, µM/l		25.9 (13.1)	24.6 (20.6)	28.6 (17.6)	25.5 (22.4)	p = .628
α_1AT_{12}, µM/l		26.9 (12.1)	27.18 (16.5)	21.8 (13.0)	18.0 (17.3)	p = .109
α_1AT_{24}, µM/l		30.2 (11.0)	32 (15.8)	22.4 (11.8)	21.8 (16.1)	p = .009
α_2M_3, µM/l		0.54 (0.24)	0.44 (0.25)	0.96 (0.58)	0.80 (0.78)	p = .001
α_2M_6, µM/l		0.50 (0.17)	0.46 (0.14)	0.94 (0.57)	0.82 (0.68)	p = .000
α_2M_{12}, µM/l		0.62 (0.24)	0.55 (0.30)	0.89 (0.40)	0.79 (0.54)	p = .005
α_2M_{24}, µM/l		0.66 (0.53)	0.62 (0.44)	0.89 (0.53)	0.71 (0.62)	p = .140
$CDEA_3$, ng/s • l		16.6 (13.9)	13.26 (16.6)	9.3 (5.0)	8.3 (5.1)	p = .010
$CDEA_6$, ng/s • l		14.5 (12.1)	11.67 (12.3)	10.5 (3.4)	9.7 (5.4)	p = .335
$CDEA_{12}$, ng/s • l		16.65 (7.8)	16.68 (12.9)	13.2 (7.8)	11.1 (8.9)	p = .067
$CDEA_{24}$, ng/s • l		13.7 (7.5)	11.1 (11.9)	14.2 (11.8)	11.1 (14.0)	p = .713
$CGEA_3$, nM/s • l		23.1 (10.4)	22.0 (17.4)	31.5 (20.3)	32.1 (25.7)	p = .050
$CGEA_6$, nM/s • l		21.0 (6.72)	21.5 (19.4)	36.3 (21.6)	32.6 (31.4)	p = .002
$CGEA_{12}$, nM/s • l		38.9 (31.3)	34.0 (23.4)	28.7 (19.4)	24.5 (16.4)	p = .089
$CGEA_{24}$, nM/s • l		22.5 (16.8)	19.8 (13.6)	20.2 (14.6)	19.3 (22.5)	p = .824
$CHEA_3$, nM/s • l		46.9 (16.6)	49.68 (29.4)	54.0 (25.2)	52.9 (31.5)	p =.193
$CHEA_6$, nM/s • l		39.0 (8.3)	39.6 (10.5)	87.8 (94.7)	66.7 (141.4)	p =.022
$CHEA_{12}$, nM/s • l		56.1 (32.2)	58.6 (45.4)	49.6 (28.8)	42.5 (24.7)	p =.250
$CHEA_{24}$, nM/s • l		51.7 (33.5)	39.8 (30.7)	45.3 (30.6)	36.8 (32.2)	p =.295
$CLEA_3$, µg/s • l		58.79 (6.6)	58.22 (8.9)	55.1 (7.0)	55.1 (6.9)	p =.023
$CLEA_6$, µg/s • l		54.15 (8.2)	54.63 (12.3)	55.5 (7.6)	54.5 (11.2)	p =.565
$CLEA_{12}$, µg/s • l		58.7 (8.2)	59.6 (12.6)	54.0 (9.0)	51.9 (13.0)	p =.031
$CLEA_{24}$, µg/s • l		55.7 (6.9)	54.3 (8.9)	53.6 (7.7)	52.9 (6.5)	p =.102
EEA_3, nM/s • l		352.4 (102.2)	357.2 (108.9)	264.7 (118.6)	251.5 (144.0)	p =.001
EEA_6, nM/s • l		350.4 (117.2)	351.5 (108.9)	258.3 (115.4)	266.6 (110.1)	p =.005
EEA_{12}, nM/s • l		349.1 (143.3)	339.8 (200.3)	276.0 (128.1)	277.8 (199.0)	p =.053
EEA_{24}, nM/s • l		381.9 (160.4)	383.3 (165.2)	289.4 (128.3)	288.5 (187.7)	p =.033

SD - Standard Deviation, IR - Interquartile Range

Table 2. The proposed models characteristics

	Model 1	Model 2	Model 3
Timing (hours after trauma)	3	6	12
Components	α_2M_3 $CDEA_3$ ARDS	α_2M_6 $CHEA_6$ EEA_3	α_2M_6 $CHEA_6$ EEA_3 $CGEA_{12}$
H_0 vs H_1 (Omnibus Test of Model Coefficients)	χ^2 = 27.843, df=3, p<.001	χ^2 = 35.137, df=3, p<.001	χ^2 = 41.195, df=3, p<.001
Calibration Hosmer – Lemeshow test	χ^2 = 8.975, df=7, p=.254	$\chi2$ = 7.584, df=7, p=.371	$\chi2$ = 3.209, df=7, p=.865
Determination Nagelkerke R Square	0.483	0.578	0.650
Discrimination			
Sensibility, %	90.7	83.7	83.7
Specificity, %	63.6	86.4	86.4
Overall percentage, %	81.5	84.6	84.6
Cut-off	.54	.50	.67
Area under ROC curve (95% CI)	0.866 (0.778, 0.953)	0.895 (0.818, 0.973)	0.922 (0.861, 0.983)

Continuous variables (Age and proteases/antiproteases system components) were compared using Mann-Whitney Test (Table 1). Finally, for modelling were considered dichotomous variable ARDS and continuous variables with $p < .1$ (Table 1). All these parameters were introduced in the model in order to elaborate a survival probability estimation instrument for polytrauma patients using the biomarkers collected at 3, 6, 12 and 24 hours after the traumatic event. Thus, we had obtained three models as follows (Table 2 and Table 3).

Model 1 (outcome prediction using the parameters collected at 3 hours after the trauma) considered ARDS diagnosis, Age, Gender and six proteases/antiproteases system components (TEA_3, α_2M_3, $CDEA_3$, $CGEA_3$, $CLEA_3$ and EEA_3) according to previous analysis results (Table 1). The null hypothesis was rejected (Method forward stepwise (Wald), Omnibus Test of Model Coefficients (χ^2 = 27.843, df=3, p<.001, significance level after the Bonferroni correction being .05/4=.0125 because of four models expected in correspondence with samples intervals), model having the following characteristics

(Table 2). Determination coefficient (Nagelkerke R Square), shown the value closed to 50%. The calibration (Hosmer–Lemeshow test) has the acceptable values, being nonsignificant, and confirmed the results fidelity. The discrimination properties (cut-off was increased up to .54 for accuracy increasing) had the optimal values for sensibility (90.7%, 39 from 43 cases) and overall percentage (81.5%), being less than target value of 80% (14 from 22, 63.6%) for specificity. Area under ROC curve for proposed model was estimated at level of .866 (95%CI 0.778, 0.953) and represented significance in relation with the value of .5 ($p<0.001$) (Fig. 1, blue color curve). The final model included the constant (B = 1.416), ARDS diagnosis (B = 1.886), $CDEA_3$ and α_2M_3 values (B = -.121 and B = 2.841, respectively) (Table 3a). Gender, Age, TEA_3, $CGEA_3$, $CLEA_3$ and EEA_3 did not show the significance, the proposed model being be represented as follows:

$$p = \frac{e^{1.416-1.886*ARDS-.121*CDEA3+2.841*\alpha2M3)}}{1+e^{1.416-1.886*ARDS-.121*CDEA3+2.841*\alpha2M3)}} \text{ (formula 1),}$$

where

p – probability to survive

e (exponent) – constant equal to 2.71828

The resampling using bootstrapping (1000 samples) shown the significance of potential predictors, 95%CI for coefficients being relative wide (Table 3b). Model 2 in comparison with previous model is able to predict the outcome using the biomarkers values at 3 and 6 hours after the trauma. Besides the predictors used before this model took in account the α_2M_6, $CGEA_6$, $CHEA_6$ and EEA_6 (Table 1). In similar way (Method forward stepwise Wald) the null hypothesis was rejected (Omnibus Test of Model Coefficients (χ^2 = 35.137, df=3, p<.001). Determination coefficient increased the value up to 58%. The calibration had acceptable values. The discrimination properties (cut-off was .50) achieved the optimal values (over 80%) for sensibility (83.7%, 36 from 43 cases), specificity (86.4%, 19 from 22) and overall percentage (84.6%) (Table 2). The second model's area under ROC curve for was estimated at level of .895 (95%CI 0.818, 0.973) and represented significance in comparison to the value of .5 ($p<0.001$) (Fig. 1, green curve). The second model had in componence the constant (B = .814), α_2M_6 (B = 4.024), $CHEA_6$ and EEA_3 values (B = .018 and B = -.012, respectively) (Table 3c). Gender, Age, ARDS, TEA_3, α_2M_3, $CDEA_3$, $CGEA_3$, $CGEA_6$, $CLEA_3$, and EEA_6 did not show the significance, the model being represented as follows:

$$p = \frac{e^{.814+4.024*\alpha2M6+.018*CHEA6-.012*EEA3)}}{1+e^{814+4.024*\alpha2M6+.018*CHEA6-.012*EEA3)}} \text{ (formula 2),}$$

where

p – probability to survive

e (exponent) – constant equal to 2.71828

The resampling procedure by bootstrapping (1000 samples) was similar to the previous model's result, potential predictors being significant with relative wide 95%CI (Table 3d). The 3rd model - outcome prediction using the parameters values at 3, 6 and 12 hours after the trauma. In addition to the parameters from previous analysis the TEA_{12}, α_2M_{12}, $CDEA_{12}$, $CGEA_{12}$, $CLEA_{12}$ and EEA_{12} were considered for modeling (Table 1). The null hypothesis was rejected (Method forward stepwise (Wald), Omnibus Test of Model Coefficients (χ^2 = 41.195, df= 3, p<.001). Determination coefficient, the same as the calibration ability, had the maximal value among proposed models, discrimination properties (cut-off was .67) being similar to the second one (Table 2). Area under ROC curve for proposed model was maximal as absolute value (.922, Fig. 1, yellow curve), all models confidence intervals crossing each other. This model, finally, was completed by $CGEA_{12}$ (B = -.044), the coefficient for other predictors being corrected (constant (B = 2.057), α_2M_6 (B = 5.296), $CHEA_6$ (B = .021), and EEA_3 values (B = -.014), Table 3e). Gender, Age, ARDS diagnosis, TEA_3, α_2M_3, $CDEA_3$, $CGEA_3$, $CGEA_6$, $CLEA_3$, EEA_6, TEA_{12}, α_2M_{12}, $CDEA_{12}$, $CLEA_{12}$ and EEA_{12} did not show the significance, model having the below formula:

$$p = \frac{e^{2.057+5.296*\alpha2M6+.021*CHEA6-.014*EEA3-.044*CGEA12)}}{1+e^{2.057+5.296*\alpha2M6+.021*CHEA6-.014*EEA3-.044*CGEA12)}} \text{ (formula 3),}$$

where

p – probability to survive

e (exponent) – constant equal to 2.71828

The resampling results had no difference in comparison with model 1 and model 2 (Table 3f). A potential model estimating the survival probability using the biomarkers values at 24 hours in addition to previous intervals measurements at 3, 6 and 12 hours did not return any important results, the 3rd model remaining unimproved.

DISCUSSION

In this study, the proteases/antiproteases system components were analyzed in order to identify their predictive potential for polytrauma population survival rate modeling. The comparative evaluation among evidenced molecular phenotypes in survived and nonsurvived patients allows to consider a seria of potential biomarkers/predictors of primary outcome (Table 1). These results, without any doubts, are precious for polytrauma's physiopathology understanding and complete the knowledge about the host injury response. In the same time is evident that polytrauma evolution and outcome are the results of complex and systemic relationships between different factors, as well as sophisticated interaction among proteases and antiproteases (Keel, 2005). The modelling of outcome used the potential predictors evidenced in previous stage, the proteases and antiproteases values being adjusted for each other and additionally to age, gender and ARDS diagnosis.

Finally, five components measured at different intervals, especially α_2M_3, $CDEA_3$, ARDS, α_2M_6, $CHEA_6$, EEA_3 and $CGEA_{12}$, were the components (efficient variables) from proposed models (Table 2, Table 3, Fig. 1). Enrollment of α_2M, secreted by hepatocytes (Wang, 2015), an macromolecular antiprotease, is obvious. It regulates intercellular responses by inhibiting almost all human and exogenous proteases, being an ARDS biomarker (Arnaut, 2018). Our research data confirmed the protective effect of this substance, coefficients in regression analysis being positive and odds ration being more than 1 (Table 3). The affirmation related to α_2M are valid for all, $CDEA_3$, EEA_3 and $CGEA_{12}$ proteases, differences being in fact that they represent the destructive elements (Arnaut, 2020; Gao, 2018; Donnelly, 1995), data confirmed by the logistic regression coefficients (odds ratio less than 1) (Table 3). In the same time, $CHEA_6$ was significant and manifested a paradoxal positive coefficient sign instead of expected negative one as it could be suggested by the presumed destructive proteases effects (Farges, 2002; Gu, 2015). The results could be argumented by relative reduced determination coefficient for the second model (Table 2). Probably, the coefficient for $CHEA_6$ can change the sign being adjusted to another potential variables or, may be, cathepsin H has a protective effect as, for example, trypsin (Miller, 1970). Generally, elaborated models have good or acceptable characteristics (Table 2) with some limits.

Table 3. The proposed models' variables coefficients and stability analysis

• Model 1 (at 3 hours after trauma)								
	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
α_2M_3	2.841	1.024	7.699	1	.006	17.132	2.303	127.450
$CDEA_3$	-.121	.051	5.780	1	.016	.886	.802	.978
ARDS	-1.886	.804	5.499	1	.019	.152	.031	.734
Constant	1.416	1.002	1.998	1	.158	4.119		
b. Model 1 equation variable bootstrapping								
	B	Bias	S.E.	Sig.	95% Confidence Interval for B			
					Lower		Upper	
ARDS	-1.886	-.689	2.933	.014	-7.761		-.419	
α_2M_3	2.841	.506	1.355	.001	1.367		6.526	
$CDEA_3$	-.121	-.013	.056	.003	-.259		-.048	
Constant	1.416	.481	2.728	.088	-.637		6.541	
• Model 2 (at 6 hours after trauma)								
	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
EEA_3	-.012	.004	11.501	1	.001	.988	.981	.995
α_2M_6	4.024	1.454	7.657	1	.006	55.927	3.234	967.153
$CHEA_6$	.018	.008	5.543	1	.019	1.019	1.003	1.034
Constant	.814	1.068	.581	1	.446	2.257		
• Model 2 equation variables bootstrapping								
	B	Bias	S.E.	Sig.	95% Confidence Interval for B			
					Lower		Upper	
α_2M_6	4.024	1.682	10.417	.002	2.360		12.134	
$CHEA_6$	.018	.008	.025	.010	.007		.068	
EEA_3	-.012	-.003	.026	.002	-.033		-.006	
Constant	.814	-.297	2.573	.554	-3.247		3.884	
• Model 3 (at 12 hours after trauma)								
	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
EEA_3	-.014	.004	11.570	1	.001	.986	.978	.994
α_2M_6	5.296	1.823	8.440	1	.004	199.555	5.602	7109.178
$CHEA_6$	.021	.009	5.478	1	.019	1.021	1.003	1.039
$CGEA_{12}$	-.044	.020	5.024	1	.025	.957	.921	.994
Constant	2.057	1.246	2.724	1	.099	7.822		
• Model 3 equation variables bootstrapping								
	B	Bias	S.E.	Sig.	95% Confidence Interval for B			
					Lower		Upper	
α_2M_6	5.296	5.096	41.196	.001	3.926		18.178	
$CHEA_6$	.021	.023	.229	.007	.007		.094	
EEA_3	-.014	-.010	.089	.001	-.042		-.010	
$CGEA_{12}$	-.044	-.028	.236	.009	-.161		-.010	
Constant	2.057	.143	8.628	.136	-2.150		6.977	

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.— confidence interval for odds ratio

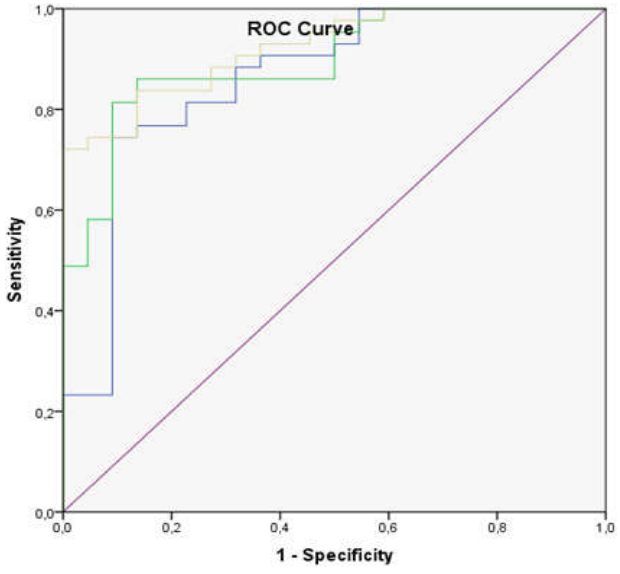

Figure 1. ROC curves for first (blue color), second (green color) and third (yellow color) elaborated models

The first limitation – relatively small number of patients and as a result decreased accuracy and large confidence intervals, normal situation for a pilot study. Second, taking in account the possible combinations variety of trauma a question appears – can we extrapolate the obtained results on all polytrauma patients. Third, using the cut-offs changing we generate the over fitting bias risk.

Conclusion

In our research, we estimated the predictive potential of different protease/antiprotease system's components for polytrauma population. Using this data, three predictive models were obtained. Without any doubts, they can be used in clinical practice after validation and improvement by including more variables in equation. The identified survival prediction biomarkers could be used as base stones of potential therapeutic strategies.

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Abbreviations

ARDS - Acute Respiratory Distress Syndrome
CDEA - Cathepsin D activity
CHEA - Cathepsin H activity
CLEA - Cathepsin L activity
CGEA - Cathepsin G activity
95%CI - 95% confidence interval
EEA - Elastase activity
PMN leukocytes - polymorphonuclear leukocytes
TEA - Trypsin activity
α_2M - α_2-macroglobulin
α_1AT - α_1-antitrypsin

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