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PROGNOSTIC MARKERS IN ACUTE PANCREATITIS

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Abstract

Background. We have studied R122H-polymorphism of the PRSS1 gene and N34S-polymorphism of the SPINK1 gene in patients with various forms of acute pancreatitis. On this basis, new methods for prognosticating the course of AP have been developed.
Methods. In the examination of patients clinical, laboratory and instrumental methods of investigation were used in accordance with the protocol for the provision of medical care to patients with acute pancreatitis. In addition, a genetic analysis of R122H-polymorphism of the PRSS1 gene and N34S-polymorphism of the SPINK1 gene was performed.
Results. It was found that in the examined patients with AP, carriers of the favorable R-allele of R122N-polymorphism of the PRSS1 gene are more common (RR- and RH-genotype – 27.27% and 64.77% respectively), with a lower number of pathological

Résumé

Marqueurs pronostiques dans la pancréatite aigue

Introduction. Nous avons étudié le polymorphisme des gènes R122H et PRSS1 et le polymorphisme du gène SPINK1 chez les patients présentant diverses formes de pancréatite aiguë (PA). Sur cette base, ont été développées de nouvelles méthodes de prévision de AP.
Méthodes. Dans l'examination des patients ont été appliquées des méthodes cliniques, de laboratoire et instrumentales conformément aux protocoles de soins de la pancréatite aiguë. Également, une analyse génétique du polymorphisme R122H du gène PRSS1 et du polymorphisme N34S du gène SPINK1 a été conduite.
Résultats. On a trouvé que chez les patients examinés avec de la PA les porteurs du polymorphisme du gène R-allèle R122N-PRSS1 sont plus communs (RR- et

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NN-homozygotes (7.96 % of individuals), as well as the favorable N-allele of N34S-polymorphism of the SPINK1 gene (NN genotype 42.05% and NS-genotype 54.55%) with less pathological SS-homozygotes (3.40%). The informativeness of the proposed methods for predicting the severity of acute pancreatitis and the development of pancreatic necrosis was 90% and 96.6% respectively.

Conclusions. The working out approaches to the prognostication of the AP course enable an integrated analysis of its clinical, laboratory and instrumental features, with an assessment of the probable negative impact of genetically determined disorders and inactivation of trypsin on the nature of the disease development. The implementation of this approach can significantly increase the reliability of prognosticating the severity of AP course and the development of pancreatic necrosis.

Abbreviations: AP – acute pancreatitis, PRSS1 – cationic trypsinogen, SPINK1 – serine protease inhibitor of Kazal type 1.

Key words: acute pancreatitis, prognostication, mutation, PRSS1, SPINK1.

RH-génotype – 27,27% et 64,77% des sujets, respectivement), avec un nombre réduit de SS-homozygotes moins anormales (7,96% des sujets) ainsi que d'un polymorphisme favorable N-N34S du gène SPINK1 (NN-génotype- 42,05% et NS-génotype – 54,55%), avec des quantités plus faibles de SS-homozygotes pathologiques (3,40%). Le manque des méthodes d'information proposées de prédire la gravité de la pancréatite aiguë et du développement de la nécrose du pancréas était de 90,0% et de 96,6% respectivement.

Conclusions. De nouvelles approches de prévision de la pancréatite aiguë permettent de réaliser une analyse intégrée de ses caractéristiques cliniques, de laboratoire et instrumentales avec l'évaluation d'un éventuel impact négatif des désordres déterminés génétiquement et de l'inactivité de la trypsine sur le caractère de la maladie. L'application de cette approche peut augmenter de manière significative la probabilité de prédire la gravité de la PA et le développement de la nécrose pancréatique.

Abréviations: PA – pancréatite aiguë, PRSS1 – trypsinogène cationique, SPINK1 – sérine inhibitrice de protéase de type Kazal 1.

Mots clés: pancréatite aiguë, la prévision, la mutation, PRSS1, SPINK1.

Introduction

Despite of more than twenty years of existence of integrated systems for assessing the severity of the patient's condition and the steady increase in their numbers, the problem of the reliable prognostication of AP course and development of its complications remains far from its final solution[1-6]. This is due to the fact that the existing prognostic scales today are characterized by a number of significant disadvantages. In particular, the low discriminating ability to prognosticate the mortality of a disease for a particular patient with a relatively accurate mortality prognostication for a group of patients. It is also, low prognostic sensitivity with rather high specificity. This allows to predict the likelihood of death of the patient, but it does not allow the reliable detection of patients who are supposed to recover. Such features of integrated scales allow stratification of patients for generalized scientific research, but practically make it impossible to use them to determine the tactics for a particular patient [1-5].

The solution of this problem in individuals with hereditary predisposition to the unfavorable course of AP is particularly relevant. For instance, in cases where the development of the disease occurs against the background of genetically determined disorders of intra-acinar inactivation of trypsin. At present, the main causes of such disorders are considered to be: R122H-mutation of PRSS1 – cationic trypsinogen[7-11] and N34S-mutation of SPINK1 – serine protease inhibitor Kazal type 1[12-18]. These genetic transversals can significantly affect the nature of the AP course and the development of its complications[19-22]. It determines the appropriateness to assess the prognostic value of the genetically determined disorders that may influence the nature of the AP development.

Material and methods

The study involved 88 patients with various forms of AP who were treated at the surgical department of the Chernivtsi regional clinical hospital from 2012 to 2015. All the patients underwent a standard comprehensive laboratory and instrumental examination. In addition, R122H-polymorphism of the PRSS1 gene and N34S-polymorphism of the SPINK1gene were established.

The alleles of the polymorphic regions of the PRSS1 gene third exon were studied by means of the Polymerase Chain Reaction (PCR) on the programmable amplifier „Amply-4L" (Russia) with an

individual temperature program for specific primers: sense (5'-GGTCCTGGGTCTCATACCTT-3 '), antisense (5'- GGGTAGGAGGCTTCACACTT-3 '). In order to discriminate the mutational H122-allele of the PRSS1 gene, the *Afl*III restriction endonuclease was used according to the manual (Fermentas®, Germany).

Studying the N34S-polymorphism of the SPINK I gene third exon was performed using the restriction endonuclease *Pst*I and specific primers: sense 5'-CAATCACAGTTATTCCCCAGAG-3, antisense5'-GTTTGCTTTTCTCGGGGTGAG-3'.

Comparison of qualitative parameters was carried out using Fisher's exact test. The statistical dependence between the values was verified using Pearson correlation coefficient for normally distributed samples and Spearman's rank correlation coefficient was used for the samples, the distribution of which differed from the normal, including the conformity of the distribution of the genotypes with the Hardy-Weinberg equilibrium.

Results and discussion

Studying the distribution of the R122H-polymorphism genotypes of the PRSS1 gene established patients with AP are carriers of a favorable R-allele (RR- and RH-genotypes more frequently by 27,27% and 64,77%, respectively) in the case of a lower number of pathological NN –homozygotes (7.96% of people). At the same time, the number of heterozygous carriers of the mutational RH genotype 64.77% (57) individuals was reliably more than the number of RR- and HH-homozygotes 27.27% (24) and 7.96% (7) people, respectively (p <0 , 05).

Studying the distribution of the N34S-polymorphism genotypes of the SPINK1 gene we found a favorable wild-type of N-allele („wild-type" (D. Whitcomb, 2013), Wt) in most patients – 69.32% (61), while the pathological „mutant" S-variant was identified in 30.68% (27) people. There were 42.05% (37) homozygous carriers of the „wild" NN genotype (N34), 54.55% (48) people with NS-heterozygotes (N34S) and 3.40% (3) homozygous carriers of the „mutant" S allele (SS genotype , 34S).

Table 1. The clustering division of patients with different forms of acute pancreatitis according to R122H polymorphism the PRSS1 gene and N34S polymorphism the SPINK1 gene, n=88

Index	Cluster	
	the 1st cluster R112R-genotype, N34N- genotype, N34S- genotype (n=28)	**the 2st cluster R122H- genotype, H122H- genotype, S34S- genotype (n=60)**
The hospitalization time (hours)	112.84	35.92
Leukocytes (10^9/l)	11.08	12.04
Glucose (mm/l)	5.63	8.05
Calcium (mm/l)	2.05	1.94
Procalcitonin (ng/ml)	0.52	1.45
Blood Amylase ($^{mg}/_{c*l}$)	12.89	21.15
Blood Lipase (U/l)	88.39	201.14
Blood Trypsin (IU)	11.73	14.98
α_1-Antitrypsin ($^{micromole}/_{hour*l}$)	24.65	15.59
α_2-Macroglobulini (g/l)	3.40	2.35
Hematocrit (%)	39.85	46.03
Urine Amylase ($^{mg}/_{c*l}$)	85.92	171.24
Body temperature (C°)	37.63	37.81
Body Mass Index (kg/m^2)	25.53	26.41
APACHE II (point)	13.26	15.08
SAPS (point)	12.12	15.94
MODS (point)	11.63	17.59
SOFA (point)	7.24	13.15
Balthazar Index (point)	1.29	3.27

The distribution of genotypes according to the polymorphic variants R122H of the PRSS1 gene and N34S of the SPINK1 gene among the patients examined for AP corresponded to the Hardy-Weinberg equilibrium.

Taking into account that the mutation of R122H is autosomal dominant [7,9,21], and the mutation of N34S is inherited by the autosomal recessive type [12,14,21], the patients with the AP were divided into 2 groups according to the criterion of the presence or absence of genetically determined disorders of intra-acinar inactivation of trypsin. The control group included 28 people with favorable RR-, NN- and NS-genotypes. The main group

Table 2. The quantitative grade of the informational content of the prognostic criterions of the difficulty of acute pancreatitis using Kulbak functional

Index	Informational content	Points	Index	Informational content	Points
Time	**11.3**		**Trypsin**	**5.1**	
	<61	-12		<11	6
	61-90	-2		11-14	4
	91-120	12		>14	-12
	>120	15	**Calcium**	**3.6**	
SOFA	**10.8**			<1.95	-12
	<9	12		1.95-2.10	1
	9-11	5		>2.10	12
	>12	-12	**Amylase blood**	**3.5**	
MODS	**10.6**			<=10	12
	<13	16		>10	-3
	13-15	1	**APACHE II**	**3.3**	
	>15	-12		<14	5
Lipase	**9.8**			14-15	-1
	<76	12		>15	-12
	76-100	11	α1-antitrypsin	**2.9**	
	101-125	4		>=10	3
	>125	-12		<10,0	-6
Baltazar	**8.6**		**Glucose**	**2.5**	
	1	12		<7.1	3
	2	2		7.1-9.5	-4
	3	-10		>9.5	-12
	4	-12			
Procalcitonin	**7.6**		**Optical Density Venous Blood**	**1.8**	
	<0.41			0.53-0.63	7
	0.41-0.8	12		>0.63	3
	>0.8	7		<0.53	-12
		-12			
Urine Amylase	**6.4**		**Temperature**	**1.4**	
	<61	11		<37.1	4
	61-150	4		37.1-38.0	1
	>150	-12		>38.0	-6
SAPS	**5.3**		α2-Macroglobulini	**1.1**	
	<11	7		>=1.10	3
	11-15	4		<1.10	-3
	>15	-12			

included 60 people with mutational RH-, HH- and SS-genotypes.

To prognosticate the severity of the AP course a statistical analysis of its 19 most informative laboratory-instrumental criteria was carried out (Table 1).

The discovered differences formed the basis for a cluster analysis, which was carried out by two methods – the k-means algorithm with the calculation of the Euclidean metric and calculating the distance between the classes by the method of mean relation using the Voronoi metric. While using both methods the same results were obtained.

When checking the results, it was found that 8 out of 9 patients with favorable R122R-, N34N- and N34S-polymorphisms were correctly allocated to the 1st cluster and the 1 person was incorrectly assigned to the 2nd cluster. 8 out of 10 people with mutation R122H-, H122N-, and S34S-polymorphisms were correctly assigned to the 2nd cluster and 2 persons were incorrectly distributed to the 1st cluster. Therefore, the first type error (the risk of hyperdiagnosis) was α = 0.2, and the second type error (hypodiagnosis risk) – β = 0.125. Thus, the power of the criterion was 0.875.

The informativeness of the laboratory-instrumental criteria of AP was assessed using the Kullback–Leibler divergence, on the basis of which the diagnostic coefficients are given in table 2.

To divide the patients into groups depending on the severity of the disease course, a sequential Wald method was used: starting with the values that have the most informative content, points were added until they reached the prognostic threshold. If we take α = 0.01 and β = 0.01, the prognostic threshold to assign patients to the group where a mild clinical course of AP is predicted to develop will be 20 points, and to assign patients to the group with risk of developing a severe clinical course of the disease it will be under 20 points. It means that in each individual patient the points given in table 2 are added until its number reaches > 20 (mild course of the disease) or <- 20 (severe course).

When checking the proposed method, we found the following: all 10 patients with severe course were assigned to the right groups. 2 out of 10 patients with mild course, were distributed incorrectly, that is, according to the results of the distribution they belonged to individuals at risk of development of the AP severe course. Thus, the first type error (the risk of hyperdiagnosis) was α = 0.2, and the second type error (hypodiagnosis risk) – β = 0. Therefore, the power of the criterion according to the results of the verification was 1.

The next task of our work was to simulate a system for prognostication of the development of pancreatic necrosis in individuals with different variants of R122H-polymorphism of the PRSS1 gene and N34S-polymorphism of the SPINK1 gene.

We considered 19 values, which are shown in table 1, as the main prognostic markers of pancreatic necrosis. The most reliable of them were: time before hospitalization, blood lipase and urine amylase. According to these criteria the patients were divided into two clusters. The main group consisted of n1 = 20 people with a favorable genotype and n2 = 50 people with a mutation genotype. The control group included 8 people with the RR-, NN- and N34S-genotype and 10 people with the RH-, HH-, and SS-genotypes. The errors were taken to be equal to α = 0.01 and β = 0.01.

According to the results of the cluster distribution of patients in the main group for blood lipase and amylase urine, it was found that 1 out of 20 patients with a healthy genotype was assigned to the 2nd cluster, and 4 out of 50 patients with a

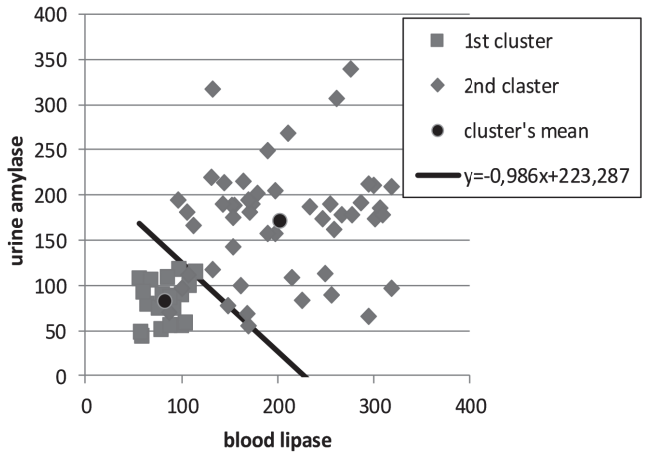

Figure 1. The cluster's divisions of the main group patients according to blood lipase and urine amylase with an isolated line

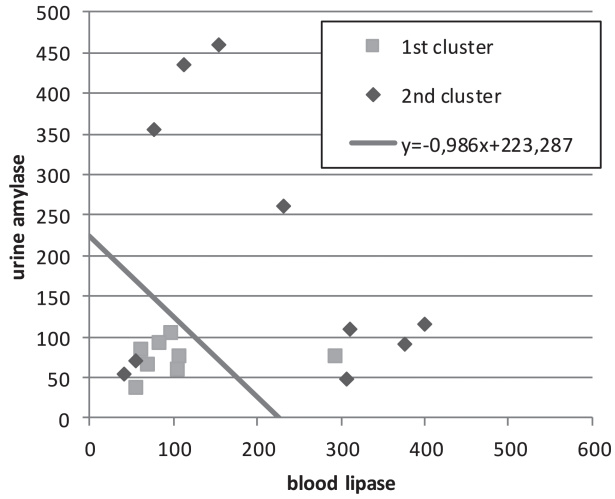

Figure 2. The cluster's divisions of the control group patients according to blood lipase and urine amylase with an isolated line

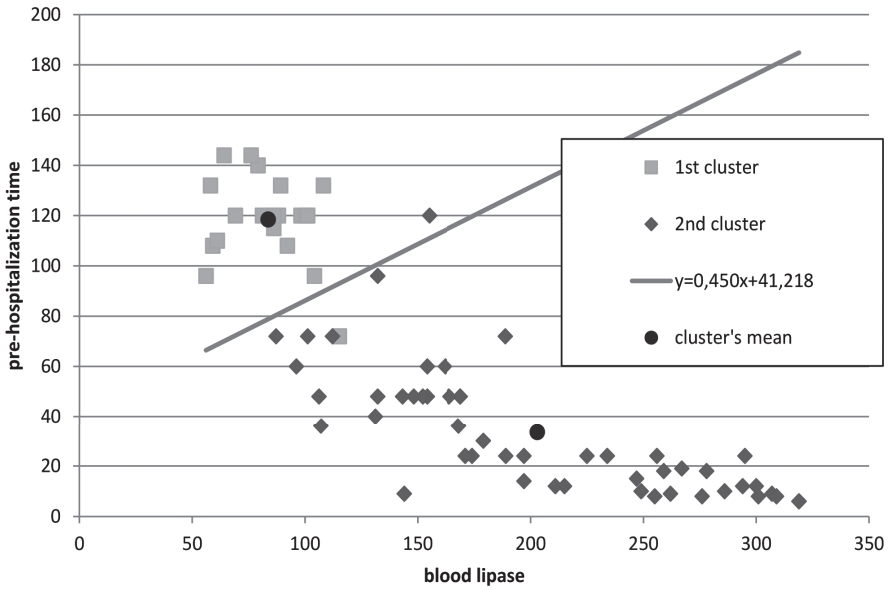

Figure 3. The cluster's divisions of the main group patients according to blood lipase and pre-hospitalization time with an isolated line

mutation genotype were assigned to the 1st cluster. At the same time, 3 of them were geometrically located among the points of the 1st cluster (Fig. 1). Equation of the straight line dividing two clusters: y = – 0.9856x + 223.87. When the result was checked in the control group it was found that 1 patient with normal genotype was allocated to the 2nd cluster, and 2 patients with the mutation genotype were assigned to the 1st cluster (Fig. 2). It means that the risk of hypodiagnosis was 12.5%, and the risk of hyperdiagnosis – 20%.

In the cluster distribution of patients in the main group according to the time before hospitalization and blood lipase, it was found that 1 person with a favorable genotype was allocated to the 2nd cluster, and 1 person with a mutation genotype to the 1st cluster (Fig. 3). The straight line separating the clusters looks like: y = 0.450x + 41.218. For the control group the risk of hypodiagnosis was 12.5%, and that of hyperdiagnosis 10% (Fig. 4).

As a result of the cluster distribution of patients in the main group for urine amylase and the time before hospitalization, it was found that 1 patient with a favorable genotype was incorrectly distributed to the 2nd cluster, and all patients with a mutation genotype were correctly included to the 2nd cluster (Fig. 5) . The straight line separating the clusters looks like: y = 0.457x + 45.121. In the control group there were no cases of hypodiagnosis, and the risk of hyperdiagnosis was 10% (Fig. 6).

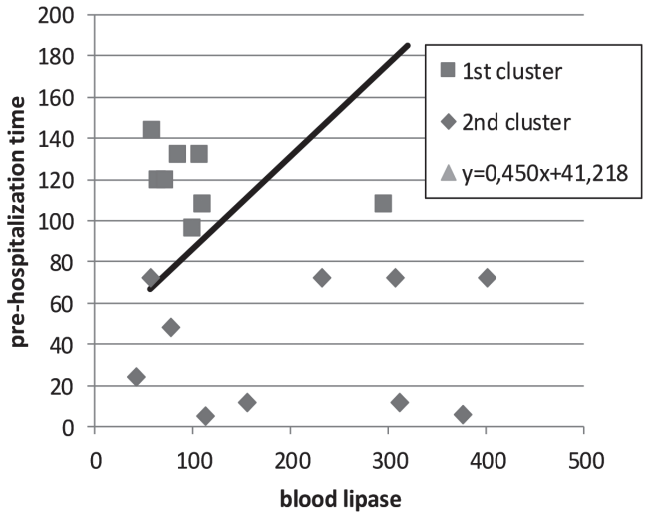

Figure 4. The cluster's divisions of the control group patients according to blood lipase and pre-hospitalization time with an isolated line

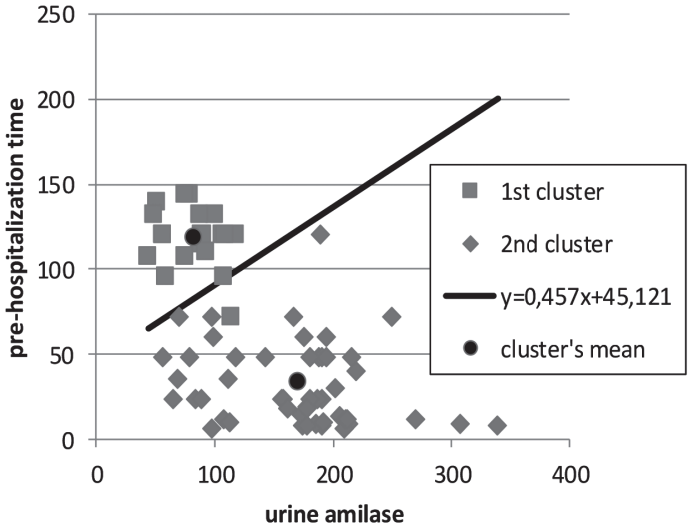

Figure 5. The cluster's divisions of the main group patients according to urine amylase and pre-hospitalization time with an isolated line

On the basis of a comprehensive statistical analysis of the results of the research we have developed the following rule of prognosticating the development of pancreatic necrosis. The patient is expected to develop pancreatic necrosis if at least two of the following inequalities are present:

1) urine amylase > –0,9856 × blood lipase +223,287;
2) time before hospitalization < 0,450 × blood lipase + 41,218;
3) time before hospitalization < 0,457 × urine amylase + 45,121.

When checking the reliability of the elaborated rule we found that 1 out of 70 patients in the main group (1.4%) was assigned to the wrong group. 2 out of 18 patients in the control group (11.1%) were distributed into wrong groups. Thus, the prognostic informativeness of the developed rule was 96.6%.

Conclusions

Summarizing the results of the research, we can conclude that the proposed approaches to prognosticate the course of AP enable an integrated analysis of its clinical, laboratory and instrumental features, with an assessment of the likely impact on the nature of the disease of R122H-polymorphism of the PRSS1gene and N34S-polymorphism of the SPINK1 gene. This allows to increase the reliability of prognosticating the severity of AP course up to 90%, and the development of pancreatic necrosis up to 96,6%.

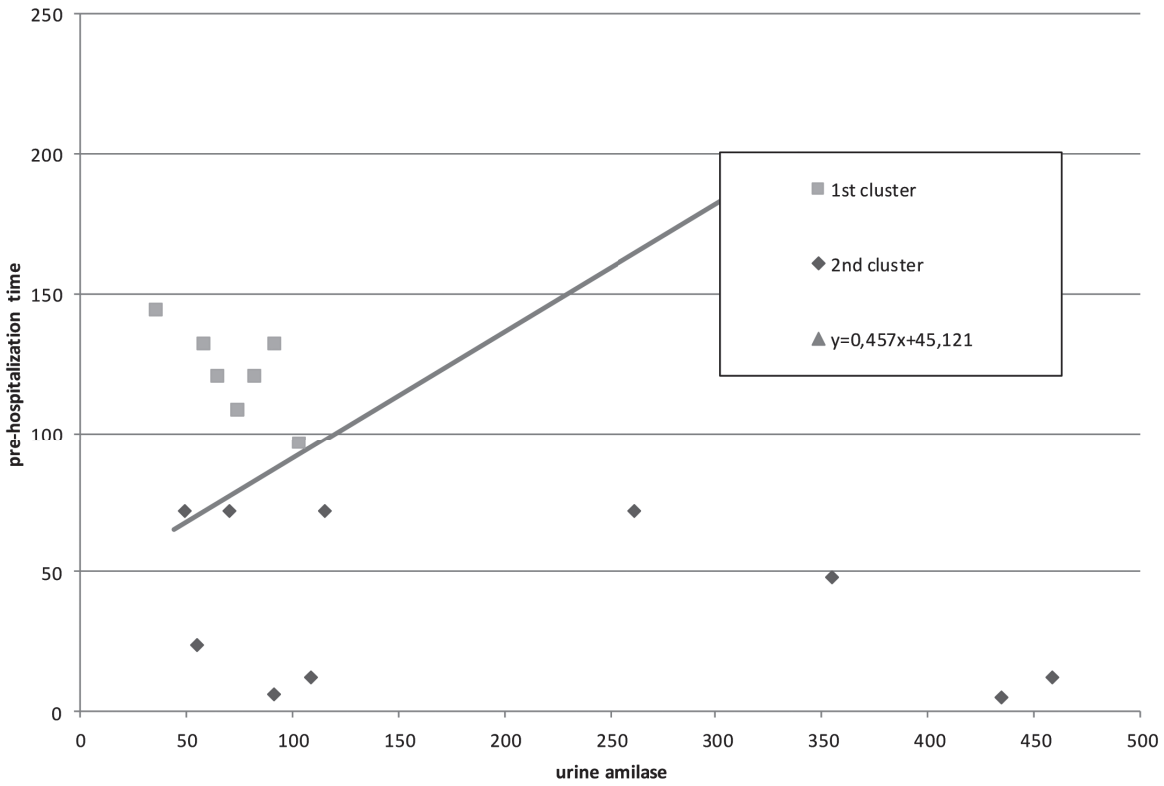

Figure 6. The cluster's divisions of the control group patients according to urine amylase and pre-hospitalization time with an isolated line

Such high values of prognostic information suggest that the application of the developed approaches is a plausible basis to optimize the treatment tactics in patients with acute pancreatitis.

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