RELATIONSHIP BETWEEN ADMINISTRATION OF STATINS AND BLOOD SERUM LEVELS OF SELECTED BIOCHEMICAL PARAMETERS

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Abstract: Results of biochemical tests of 172 patient data (among them 84 men data and 88 women data, resp.) before and after administration of statins were studied. All monitored patients are characterized by lipoprotein metabolism failure or other kind of lipidaemia. The calculations were performed using several chemometrical methods pursuing visualization of most important biochemical parameters and classification of the patient blood samples by means of up-to-date software packages. The dependences of the content of most common biochemical parameters upon the treatment of patients by statins were elucidated in detail.

Key words: coronary artery diseases, statins, biochemical parameters, multivariate data analysis.

1. Introduction

Coronary heart disease remains the leading cause of mortality in the civilized countries. In medicine it is now an accepted principle that cholesterol lowering is of major importance in diminishing the risk of a developing coronary artery disease. Most patients with coronary artery disease require intensive drug therapy to achieve target lipid levels (KASTELEIN, 1999; SHIMOKATA *et al.*, 2004).

The introduction of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (statins) in 1987 was a major advance in prevention and treatment of cardiovascular diseases. Several clinical trials have demonstrated the benefit of lipid lowering caused by statins for the primary and secondary prevention of coronary heart disease (LIAO, 2002; ISTVAN and DEISENHOFER, 2001).

The clinical practise of cardiovascular medicine has been innovated by the advent of the statins. The statins have a wide range of beneficial biologic effects in addition to lipid lowering, a phenomenon commonly termed a pleiotropic effect (FUJITA *et al.*, 2008; GOUNARI *et al.*, 2009). Statins act by inhibiting 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase and thereby reducing cholesterol synthesis (GARCIA *et al.*, 2008).

2. Material and methods

2.1 Description of data

Data from the out-patient department in Prešov were obtained in cooperation with the specialist internal (GROMANOVA, 2008). In this study, biochemical data steming

from 172 probands were analyzed by several chemometrical methods in order to reveal relationships between administration of statins and the level of biochemical parameters. The investigated patients were characterized by lipoprotein metabolism failure and other kind of lipidaemia.

Two data tables (for *men* - 84 samples, and for *women* – 88 samples) containing the sample origin (patients) in rows and investigated variables in columns (10 biochemical parameters plus 1 combined parameter and age of the patient) were assembled. The following biochemical variables (often called as parameters) were selected: total cholesterol (tCHOL), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), triacylglycerols (TG), aterogenic index (AI) - the combined parameter, creatinine (CREA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine kinase (CK), and gamma-glutamyl transferase (GGT). The parameter values were measured before administration of statins and one year after the statin treatment. The patient samples objects were therefore divided into two groups: class 1 – parameter values before the treatment by statins and class 2 – parameter values one year after the treatment.

2.2 Multidimensional data analysis

The results of biochemical tests were subjected to multidimensional data analysis using four software packages. Statgraphics Plus 5.1 was utilized to perform *cluster analysis* (VANDEGINSTE *et al.*, 1997) as well as *principal component analysis* (MASSART *et al.*, 1997)*;* SPSS 15 and SAS packages enabled to accomplish *discriminant analysis* and *logistic regression* (OTTO, 1999; SHARMA, 1996). *Correlation analysis* (MELOUN and MILITKY, 2002) was also made in addition to the mentioned more sophisticated statistical techniques using software JMP 6.0.

3. Results and discussion

3.1 Correlation analysis

Correlation analysis was performed in common for the patients' results obtained before and after the statins administration. It is represented by the correlation table containing the calculated pair (Pearson) correlation coefficients, which indicate the strength of correlation between all pairs of variables - biochemical parameters for *men* and *women*, distinctively. In order to see the measure of their importance, the correlation coefficient values were compared to the critical value of the sample correlation coefficient, $r = 0.215$ for *men* dta set and 0.210 for *women* data set, which depends on the number of degrees of freedom (the sample number minus two) and the selected probability (usually 0.05). Consequently, all values in the correlation tables (Table 1, Table 2) indicating significant correlations are marked in bold. It can be seen from the observed values that many of the observed significant correlations (marked bold) are very well understandable, as e.g. between tCHOL a LDLc (strongest correlation for *men* and *women*), tCHOL and its component parts (LDLc and HDLc),

tCHOL and TG (for *men* only), as well as the negative correlation between index of aterogenity AI and so called "good" cholesterol HDLc (high values of HDLc correspond to low values of AI). Similarly, significant correlations between the pairs AST – ALT and GMT – ALT are expectable. What is also interesting are the negative correlations for *men* between Age and any of the variables TG, and AI, which is pronounced after the statins intake. Strong positive correlations of TG with tCHOL and with AI as well as strong negative correlation of TG with HDLc, all observed only after the statins, may also be remarkable.

Table 1. Summary of correlation coefficients expressing mutual correlation between the pairs of investigated biochemical parameters for *men* data set.

Variable	**Age**	**CREA**	**AST**	**ALT**	**GMT**	**ALP**
Age	1.000					
CREA	0.170	1.000				
AST	-0.081	-0.128	1.000			
ALT	**-0.287**	-0.090	**0.889**	1.000		
GMT	-0.170	0.137	0.168	**0.358**	1.000	
ALP	**0.290**	-0.042	-0.039	-0.048	0.021	1.000
CK	0.021	0.048	0.110	0.039	0.036	**-0.298**
tCHOL	-0.111	0.040	-0.033	0.054	-0.035	0.001
HDLc	0.171	-0.128	0.094	0.135	0.022	-0.130
LDLc	-0.020	0.002	-0.034	0.025	-0.102	0.098
TG	**-0.352**	0.181	-0.073	0.006	0.122	-0.143
AI	**-0.281**	0.156	-0.092	-0.053	-0.072	0.129
continuation	**CK**	**tCHOL**	**HDLc**	**LDLc**	**TG**	**AI**
CK	1.000					
tCHOL	-0.084	1.000				
HDLc	0.123	**0.346**	1.000			
LDLc	-0.094	**0.927**	0.240	1.000		
TG	-0.099	**0.403**	-0.205	0.099	1.000	
AI	-0.176	**0.588**	**-0.526**	**0.608**	**0.528**	1.000

3.2 Cluster analysis

Cluster analysis was performed distinctively for the *men* data set and the *women* data set. The cluster analysis results are most frequently represented by a dendrogram. Figure 1 depicts the dendrogram, which was calculated using squared Euclidean distance, commonly used in practice. It shows three significant clusters. The first significant cluster is created by tCHOL, LDLc, TG and AI, all representing high cardiovascular risk. The second significant cluster is formed by AST, ALT (characterizing liver enzymes) and third cluster is created by HDLc with CK, CREA with GMT, to which ALP and Age variables are added at larger distance value (Figure 1), which means that these variables are less similar compared to the four ones mentioned. The results for women data set (Figure 2) are similar except the observed clustering of Age, this time with CREA instead of ALP. However, with regard to the most important variables indicating lipidaemia, the situation among *men* and *women* is similar.

Table 2. Summary of correlation coefficients expressing mutual correlation between the pairs of investigated biochemical parameters for wo*men* data set.

Variable	**Age**	**CREA**	**AST**	**ALT**	**GMT**	**ALP**
Age	1.000					
CREA	**0.389**	1.000				
AST	0.079	0.105	1.000			
ALT	-0.257	-0.113	**0.680**	1.000		
GMT	-0.103	0.163	0.188	**0.279**	1.000	
ALP	0.207	-0.017	0.055	-0.003	0.028	1.000
CK	0.085	0.036	**0.455**	0.253	-0.039	0.105
tCHOL	0.120	0.082	0.130	0.095	0.172	0.156
HDLc	**0.329**	0.124	**0.418**	0.133	0.004	-0.186
LDLc	0.037	-0.016	-0.031	-0.003	0.100	0.164
TG	-0.090	0.065	-0.210	0.040	**0.253**	**0.341**
AI	-0.197	0.023	**-0.375**	-0.119	0.101	**0.328**
continuation	**CK**	**tCHOL**	**HDLc**	**LDLc**	**TG**	**AI**
CK	1.000					
tCHOL	-0.110	1.000				
HDLc	0.007	**0.368**	**1.000**			
LDLc	-0.143	**0.859**	0.122	1.000		
TG	-0.068	0.168	**-0.477**	0.074	**1.000**	
AI	-0.084	**0.320**	**-0.670**	**0.458**	**0.642**	1.000

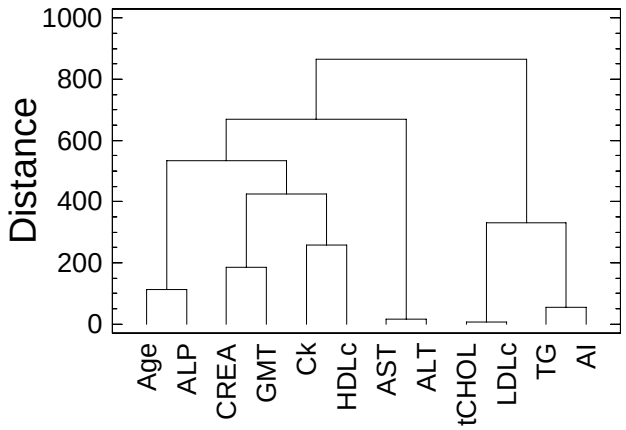

Fig. 1. Dendrogram of cluster analysis of 12 variables for 84 objects (*men*). Ward`s clustering method, Squared Euclidean distance. Software Statgraphics Plus 5.1.

3.3 Principal component analysis

The obtained principal component analysis results are visualized in the biplot form (Figure 3). Biplot simultaneously represents the samples together with twelve selected

variables, depicted by the rays, which start from the origin and end at the point determining the variable position in the plane of principal components.

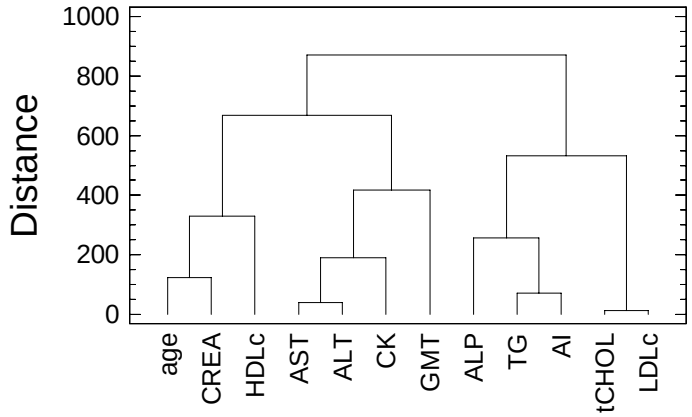

Fig. 2. Dendrogram of cluster analysis of 12 variables for 88 objects (wo*men*). Ward`s clustering method, Squared Euclidean distance. Software Statgraphics Plus 5.1.

A close position of tCHOL, AI, LDLc and TGT at the shown PCA biplot confirms their mutual dependence found also by correlation analysis and it helps to understanding the PC1 axis as that expressing the cardiovascular risk. Almost opposite position of HDLc is in correspondence with the previous statement. The variables AST, ALT and GMT as well as their opponents ALP and Age are in the perpendicular position (located along the PC2 axis) to the cholesterol variables, which mean their independence with respect to them. This is also in accordance with the results of correlation analysis.

3.4 Discriminant analysis

Discriminant analysis makes a linear combination of the originally selected variables in the way that the discrimination between the classes is mostly pronounced. Table 3 shows a summary of the results of linear discriminant analysis (LDA), quadratic discriminant analysis (QDA) and logistic regression (LR) for the *men* data set. All these methods are used for classification of the patient samples into two classes – before (class 1) and after (class 2) treatment by statins. The results for training set (used for calculating the classification model) and leave-one-out method validation (one object is left out from the training set in a stepwise mode until all objects are interchanged) are represented in per cents. Success in validation step, where the samples independent of the calculations performed in the training process, is more important for overall assessment of the classification method. It is obvious that the predictive ability is better for LDA than the QDA. However, the most promising are the results achieved by logistic regression.

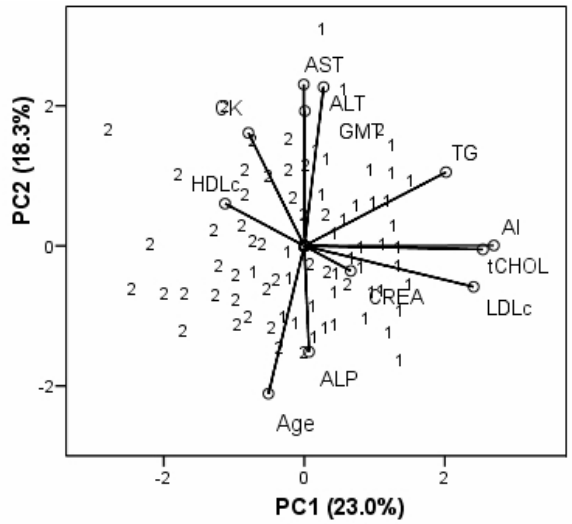

Fig. 3. Biplot PC1 vs. PC2 (*men*), 12 variables (with the symbols specified in part 2), 84 patient samples. Software Statgraphics Plus 5.1.

Table 3. Results of discriminant analysis (LDA, QDA) and LR for two software packages (SPSS, SAS) – classification of the *men* samples into two categories: before and after statin treatment.

Method	Results	SPSS		SAS	
		Training set	Leave-one-out	Training set	Leave-one-out
LDA	true/all	73/84	67/84	73/84	67/84
	% true	86.9	**79.8**	86.9	**79.8**
QDA	true/all	73/84	n.a.	70/84	55/84
	% true	86.9	n.a.	83.3	**65.5**
LR	true/all	75/84	n.a.	75/84	72/84
	% true	89.3	n.a.	89.3	**85.7**

n.a. – not accessed.

Tabable 4.: Results of discriminant analysis (LDA, QDA) and LR for two software packages (SPSS, SAS) – classification of the *women* samples into two categories: before and after statin treatment.

Method	Results	SPSS		SAS	
		Training set	Leave-one-out	Training set	Leave-one-out
LDA	true/all	81/88	74/88	81/88	74/88
	% true	92.0	**84.1**	92.0	**84.1**
QDA	true/all	81/88	n.a.	76/88	61/88
	% true	92.0	n.a.	86.4	**69.3**
LR	true/all	79/88	n.a.	79/88	77/88
	% true	89.8	n.a.	89.8	**87.5**

n.a. – not accessed.

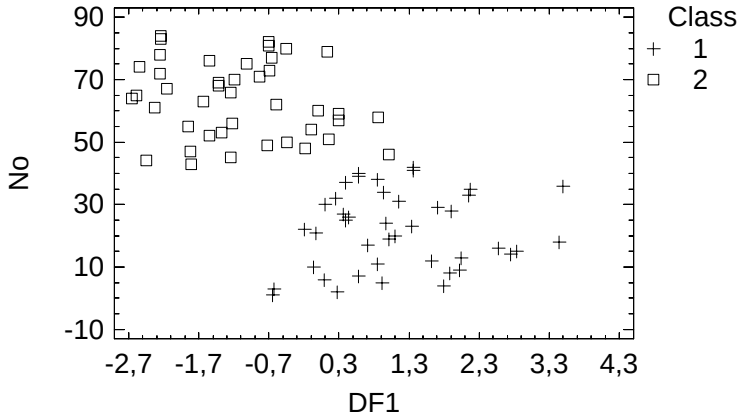

Fig. 4. LDA Scatterplot of 84 patient samples (*men*) marked by number (No) calculated from 12 variables. DF1 is the only discriminant function. Class 1- *before*, class 2 – *after* treatment by statins. Software Statgraphics Plus 5.1.

A good separation between the investigated two categories of the patient samples is confirmed also in Figure 4, calculated also for the *men* data set. The results for *women* data set are collected in Table 4. All results referring to the *training set* are close to or over 90% of the correct results, the best outcome in validation by *leave-one-out method* was obtained for LR.

4. Conclusions

Treatment of patients with the risk of a developing coronary artery disease can be successfully monitored using the visualization and classification methods of multidimensional statistical analysis. The interrelation of eleven biochemical laboratory parameters as well as the patient's age and gender was investigated in detail. Achieved results enable to evaluate the treatment by statins in each individual case. They may also help in estimation of the pertaining cardiovascular risk after the statin drug administration and in monitoring the dynamic progression of the disease.

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