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Variants of plasma lipoprotein profile in persons with maladaptation

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ABSTRACT

Introduction and aim. Atherogenic lipoprotein profile of plasma is an important risk factor for coronary artery disease. It is characterized by high ratio of logarithm of triglycerides (TG) to high-density lipoprotein cholesterol (HDL-Ch) called as "atherogenic index of plasma" (AIP). AIP reflect the actual composition of the lipoprotein spectrum and thus predict both the cardiovascular risk and effectiveness of therapy. This article initiates the project "Relationships between plasma lipoprotein profile and neuroendocrine-immune complex parameters and the influence on them of the factors of the Truskavets' spa". The first goal is the formation of homogeneous cluster groups.

Material and methods. The object of observation were 41 volunteers: 20 women aged 30-76 years and 21 men aged 24-69 years without clinical diagnose but with dysfunction of neuroendocrine-immune complex and metabolism, characteristic for maladaptation. We estimated lipoprotein profile of plasma: total cholesterol level and its content in composition of HDL, VLDL and LDL. Based on them, two AIPs were calculated: TG/HDL-Ch named as Dobiásová&Frohlich as well as previously widely used Klimov's AIP as ratio (VLDLCh + LDLCh)/HDLCh.

Results. Using the method of cluster analysis, the sample was divided into 5 homogeneous groups, different from each other. It was found that 11 members of the V cluster, the oldest in the sample, exclusively women, had the maximum for sampling increased total Cholesterol, HDL-Ch and Triglycerides levels. At the opposite pole localized 11 members of I cluster, the youngest in the sample, in whom the levels of listed variables as well as LDLCh and Dobiásová&Frohlich AIP was decreased and gender representation was almost the same. The intermediate positions of the members of the other three clusters reflect, as a rule, the intermediate levels of the listed variables. A characteristic feature of 7 members of IV cluster was the maximally increased levels of both Dobiásová&Frohlich's and Klimov's AIP. The age and sex together determines Dobiásová&Frohlich's AIP on 34%. Overall classification accuracy by 6 discriminant variables is 96,3%.

Conclusion. The plasma lipoprotein profile of persons with maladaptation is characterized by a wide range of values, from increased to decreased. This should be kept in mind during the next study of its connections with the parameters of the neuroendocrine-immune complex.

Keywords: lipoprotein profile of plasma, atherogenic indexes, males, females, cluster analysis.

Introduction

Atherogenic lipoprotein profile of plasma is an important risk factor for coronary artery disease. It is characterized by high ratio of low-density lipoprotein cholesterol (LDL-Ch) to high-density lipoprotein cholesterol (HDL-Ch) and increased level of triglycerides (TG). Predominance in plasma of small dense LDL and small HDL particles is associated with an increased risk of coronary artery disease while large HDL particles are associated with decreased risk. Thus, to estimate the risk of atherosclerosis more accurately the measurement of particle size distribution in LDL by gradient gel electrophoresis has been recommended. Dobiásová & Frohlich [2] revealed a strong positive correlation (r=0.803) between *fractional esterification rate of cholesterol in plasma depleted of apoB containing lipoproteins* (FER_{HDL}) and Log(TG/HDL-Ch). This parameter, which authors propose to call "atherogenic index of plasma" (AIP) directly related to the risk of atherosclerosis in the above cohorts. Authors also confirmed in a cohort of 35 normal subjects a significant inverse correlation of LDL size with FER_{HDL} (r=-0.818) and AIP (r=-0.776). In another study by the same authors, it was shown that treatment with simvastatin&niacin decreased both AIP and FER_{HDL} while both placebo and

antioxidants was ineffective. Authors concluded that AIP reflect the actual composition of the lipoprotein spectrum and thus predict both the cardiovascular risk and effectiveness of therapy [3].

This article initiates the project "Relationships between plasma lipoprotein profile and neuroendocrine-immune complex parameters and the influence on them of the factors of the Truskavets' spa". Our previous experience shows the wide variability of the lipoprotein profile parameters of Truskavets' spa patients: from low to high, as well as the multidirectional effects of its therapeutic factors on the parameters of humans and rats [6,7,11-16,19-22]. Therefore, the first goal of the research is the formation of homogeneous cluster groups.

Material and research methods

The object of observation were 41 volunteers: 20 women aged 30-76 years and 21 men aged 24-69 years without clinical diagnose but with dysfunction of neuro-endocrine-immune complex and metabolism, characteristic for maladaptation and premorbid (intermediate between health and illness) state [15,19,20].

We estimated lipoprotein profile of plasma: total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack) and content of it in composition of HDL (by the enzyme method by Hiller [5] after precipitation of VLD&LD Ls); VLDL (calculated by the level of triglycerides, estimated by meta-periodate method, as ratio TG/2.1834); LDL (calculated by a difference between a total cholesterol and cholesterol in composition HD and VLD lipoproteins) according to instructions [4] with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) and corresponding sets of reagents. In addition, paying tribute to tradition, the level of β-LP by Burstein & Samai was determined.

Reference values of variables, taking into account sex and age, are borrowed from the handbook [8].

Based on them, two AIPs were calculated: TG/HDL-Ch named as Dobiásová&Frohlich as well as previously widely used Klimov's AIP as ratio (VLDLCh + LDLCh)/HDLCh [10].

Each volunteer was tested twice with a 4-day interval.

Results processed using the software package "Statistica 5.5".

Results and discussion

As expected, the lipoprotein profile parameters ranged from low to high. Therefore, in the second phase, Cluster analysis was conducted. Clustering cohort of persons was realized by iterative k-means metod. In this method, the object belongs to the class Euclidean distance to which is minimal. The main principle of the structural approach to the allocation of uniform groups consists in the fact that objects of same class are close but different classes are distant. In other words, a cluster (the image) is an accumulation of points in n-dimensional geometric space in which average distance between points is less than the average distance from the data points to the rest points [1,17].

We have identified 5 clusters (Table 1 in Appendix). The data in Tables 1 and 2 confirm the correctness of the clustering of the sample.

Table 2. Euclidean Distances between Clusters

Cluster Number	Distances below diagonal Squared distances above diagonal				
	No. 1	No. 2	No. 3	No. 4	No. 5
No. 1	0,00	0,34	1,03	1,38	2,70
No. 2	0,58	0,00	0,19	0,44	1,15
No. 3	1,01	0,44	0,00	0,16	0,40
No. 4	1,17	0,67	0,40	0,00	0,43
No. 5	1,64	1,07	0,63	0,66	0,00

In the next stage carried Analysis of Variance and ranking variables for coefficient η^2:

$\eta^2=Sb^2/(Sb^2+Sw^2)$; $R=\eta$; $F=[Sb^2(n-k)]/[Sw^2(k-1)]$, where

Sb^2 is Between Variance; Sw^2 is Within Variance; n is number of sample (82); k is number of groups-clusters (5).

It was found (Table 3) that the most characteristic feature of clusters are the levels of total Cholesterol as well as Triglycerides/VLDLP-Ch. HDLP-Ch make much smaller contribution to the distribution of the sample into clusters. Instead, the contribution of Klimov's AIP is minimally.

Table 3. Analysis of Variance

Variables	Between SS	Within SS	η^2	R	F	signif. p
Cholesterol total, mM/L	64.38	8.516	0.883	0.940	144	10^{-6}
Triglycerides, mM/L	33.83	5.920	0.851	0.923	109	10^{-6}
VLDLP Cholesterol, mM/L	7.039	1.243	0.850	0.922	108	10^{-6}
HDLP Cholesterol, mM/L	8.332	2.179	0.793	0.890	72.7	10^{-6}
Dobiásová's & Frohlich's AIP	6.178	2.609	0.703	0.839	45.0	10^{-6}
LDLP Cholesterol, mM/L	7.828	4.487	0.636	0.797	33.1	10^{-6}
Klimov's Atherogenic Index	5.354	4.883	0.523	0.723	20.8	10^{-6}

For the purpose of correct comparison, registered variables (V) expressed as Z-scores calculated by formula:

$Z=(V/N - 1)/Cv$, where

N is Mean of Normal (reference) Variable, Cv is Coefficient its variation.

Further, the registered variables were condensed into 5 patterns (Fig. 1).

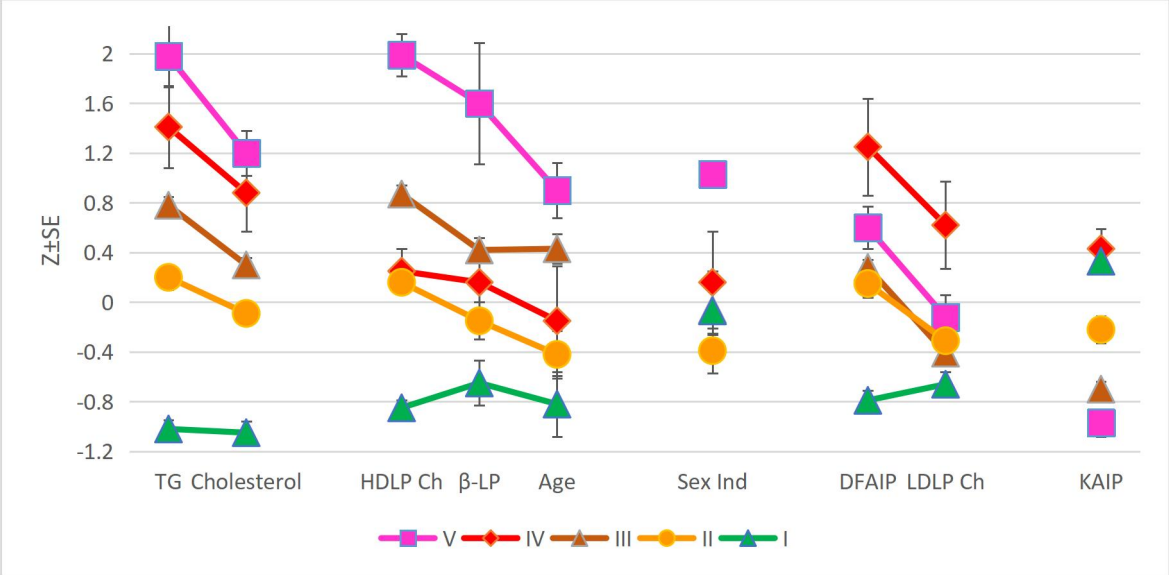


Fig. 1. Profiles of registered parameters of clusters members

In order to identify among the registered parameters, those for which the clusters differ from each other, a discriminant analysis was performed [9]. The program forward stepwise included in the discriminant model 6 parameters, including age and sex. The rest of the variables were left out of the model (Tables 4 and 5).

Table 4. Discriminant Function Analysis Summary for Variables, their actual levels for Clusters as well as Reference levels and Coefficients of Variability
Step 6, N of vars in model: 6; Grouping: 5 grps; Wilks' Λ: 0,0081; approx. $F_{(24)}$=31,3; $p<10^{-6}$

	Clusters (n)					**Parameters of Wilk's Statistics**						
Variables currently in the model	**V (11)**	**IV (7)**	**III (26)**	**II (27)**	**I (11)**	Wilks Λ	Partial Λ	F-remove (4.71)	p-value	Tolerancy	Reference (41)	Cv
Cholesterol total, mM/L	7.11 0.13	6.12 0.16	5.87 0.05	5.06 0.06	4.01 0.11	0,024	0,332	36,2	10^{-6}	0,373	5.37 0.16	0.192
Klimov's Atherogenic Index	2.26 0.07	3.21 0.14	2.40 0.03	2.52 0.06	2.81 0.09	0,023	0,349	33,6	10^{-6}	0,310	2.89 0,13	0.298
Dobiásová's & Frohlich's AIP	1.21 0.07	1.34 0.11	1.03 0.02	0.81 0.04	0.37 0.03	0,034	0,238	57,6	10^{-6}	0,053	0.59 0.05	0.587
Triglycerides, mM/L	2.62 0.12	1.93 0.09	1.79 0.05	1.16 0.06	0.39 0.04	0,026	0,306	40,9	10^{-6}	0,064	1.13 0.10	0.587
Age, years	57.6 2.7	44.7 5.4	51.8 1.5	41.4 2.3	36.5 3.1	0,009	0,878	2,50	0,050	0,968	46.5 1.9	0.264
Sex Index (M=1; F=2)	2.00 0.00	1.57 0.20	1.46 0.10	1.30 0.09	1.45 0.16	0,009	0,895	2,11	0,088	0,969	1.49 0.07	0.333
Variables currently not in the model	**V (11)**	**IV (7)**	**III (26)**	**II (27)**	**I (11)**	Wilks Λ	Partial Λ	F to enter	p-value	Tolerancy	Reference (41)	Cv
HDLP Cholesterol, mM/L	2.20 0.08	1.47 0.08	1.73 0.03	1.45 0.03	1.06 0.03	0,008	0,965	0,65	0,627	0,057	1.39 0.06	0.298
LDLP Cholesterol, mM/L	3.72 0.07	3.77 0.13	3.32 0.04	3.07 0.04	2.76 0.08	0,008	0,959	0,75	0,559	0,027	3.46 0.10	0.192
β-LP by Burstein & Samai, units	61.5 5.1	46.4 2.2	49.1 1.0	43.1 1.6	37.9 1.9	0,008	0,960	0,74	0,560	0,028	44.7 1.6	0.234

Table 5. Summary of Stepwise Analysis for Hemodynamics Variables, ranked by criterion Lambda

Variables currently in the model	F to enter	p-value	Λ	F-value	p-value
Cholesterol total, mM/L	141,2	10^{-6}	0,120	141,2	10^{-6}
Klimov's Atherogenity Index	17,98	10^{-6}	0,062	57,51	10^{-6}
Dobiásová's & Frohlich's AGI	14,47	10^{-6}	0,035	42,36	10^{-6}
Triglycerides, mM/L	43,93	10^{-6}	0,010	49,16	10^{-6}
Age, years	2,562	0,045	0,009	38,07	10^{-6}
Sex Index (M=1; F=2)	2,109	0,088	0,008	31,31	10^{-6}

Next, the 6-dimensional space of discriminant variables transforms into 4-dimensional space of a canonical roots, which are a linear combination of discriminant variables. The differentiating ability of the root characterizes the canonical correlation coefficient (r*) as a measure of connection, the degree of dependence between groups (clusters) and a discriminant function. It is for Root 1 0,962 (Wilks' Λ=0,0081; $\chi^2_{(24)}$=364; $p<10^{-6}$), for Root 2 0,874 (Wilks' Λ=0,1092; $\chi^2_{(15)}$=167; $p<10^{-6}$), for Root 3 0,718 (Wilks' Λ=0,4608; $\chi^2_{(8)}$=58; $p<10^{-6}$) and for Root 4 0,221 (Wilks' Λ=0,9510; $\chi^2_{(3)}$=3.8; p=0,285). The first root contains 74,2% of discriminative opportunities, the second 19,2%, the third 6,3%, and the last 0,3% only.

Table 6 presents raw (actual) and standardized (normalized) coefficients for discriminant variables. The raw coefficient gives information on the absolute contribution of this variable to the value of the discriminative function, whereas standardized coefficients represent the relative contribution of a variable independent of the unit of measurement. They make it possible to identify those variables that make the largest contribution to the discriminatory function value.

Table 6. Standardized and Raw Coefficients and Constants for Variables

Coefficients	**Standardized**			**Raw**		
Variables currently in the model	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Cholesterol total, mM/L	-1,124	0,886	-0,200	-3,336	2,628	-0,594
Klimov's Atherogenity Index	0,630	-1,166	-1,158	2,502	-4,632	-4,600
Dobiásová's & Frohlich's AIP	-1,724	3,870	0,407	-9,345	20,98	2,208
Triglycerides, mM/L	1,011	-3,589	-0,267	3,609	-12,81	-0,952
Age, years	-0,108	-0,240	0,246	-0,011	-0,024	0,024
Sex Index (M=1; F=2)	-0,059	-0,291	-0,275	-0,129	-0,627	-0,594
			Constants	15,94	-0,604	14,17
			Eigenvalues	12,49	3,221	1,064
			Cumulative proportions	0,742	0,934	0,997

The calculation of the discriminant root values for each person as the sum of the products of raw coefficients to the individual values of discriminant variables together with the constant enables the visualization of each patient in the information space of the roots (Fig. 2).

Table 7 presents the full structural coefficients, that is, the coefficients of correlation between the discriminant root and variables. The structural coefficient shows how closely variable and discriminant functions are related, that is, what is the portion of information about the discriminant function (root) contained in this variable. There are also average values (centroids) of Roots and Z-scores of Variables.

Table 7. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables

Variables currently in the model	**Correlations Variables-Roots**			**V (11)**	**IV (7)**	**III (26)**	**II (27)**	**I (11)**
Root 1 (74,2%)	**R 1**	**R 2**	**R 3**	**-4,85**	-2,70	-1,57	+1,33	**+7,00**
Cholesterol total	**-0,751**	-0,297	-0,096	**1.20**	0.88	0.30	-0.09	**-1.05**
Triglycerides	**-0,657**	-0,235	-0,050	**1.98**	1.41	0.78	0.20	**-1.02**
β-LP by Burstein&Samai				**1.60**	0.16	0.42	-0.15	**-0.65**
HDLP Cholesterol				**1.99**	0.25	0.87	0.16	**-0.85**
Age	**-0,174**	-0,156	0,121	**0.90**	-0.15	0,43	-0,42	**-0,82**
Sex Index (M=1; F=2)	**-0,084**	-0,204	-0,124	**1.03**	0.16	-0.06	-0.39	-0.07
Root 2 (19,2%)	**R 1**	**R 2**	**R 3**	-3.20	**1.98**	0.31	1.39	-2.21
Dobiásová's&Frohlich's AIP	-0,420	**0,134**	-0,292	0.60	**1.25**	0.28	0.15	-0.79
Klimov's Atherogenic Index	0,117	**0,168**	-0,883	-0.97	**0.43**	-0.70	-0.22	0.33
LDLP Cholesterol				-0.12	**0.62**	-0.41	-0.31	-0.66

The localization along the first root axis in the extreme left (negative) zone (Fig. 2) of the members of V cluster reflects maximum for sampling total Cholesterol and Triglycerides levels as well as Age and Sex Index (an all-female cluster). Also worthy of attention are β-LP by Burstein&Samai (significantly correlated with TG) and HDLP-Ch, which are not included in the model, apparently due to duplication of information such as the child's age and his school class.

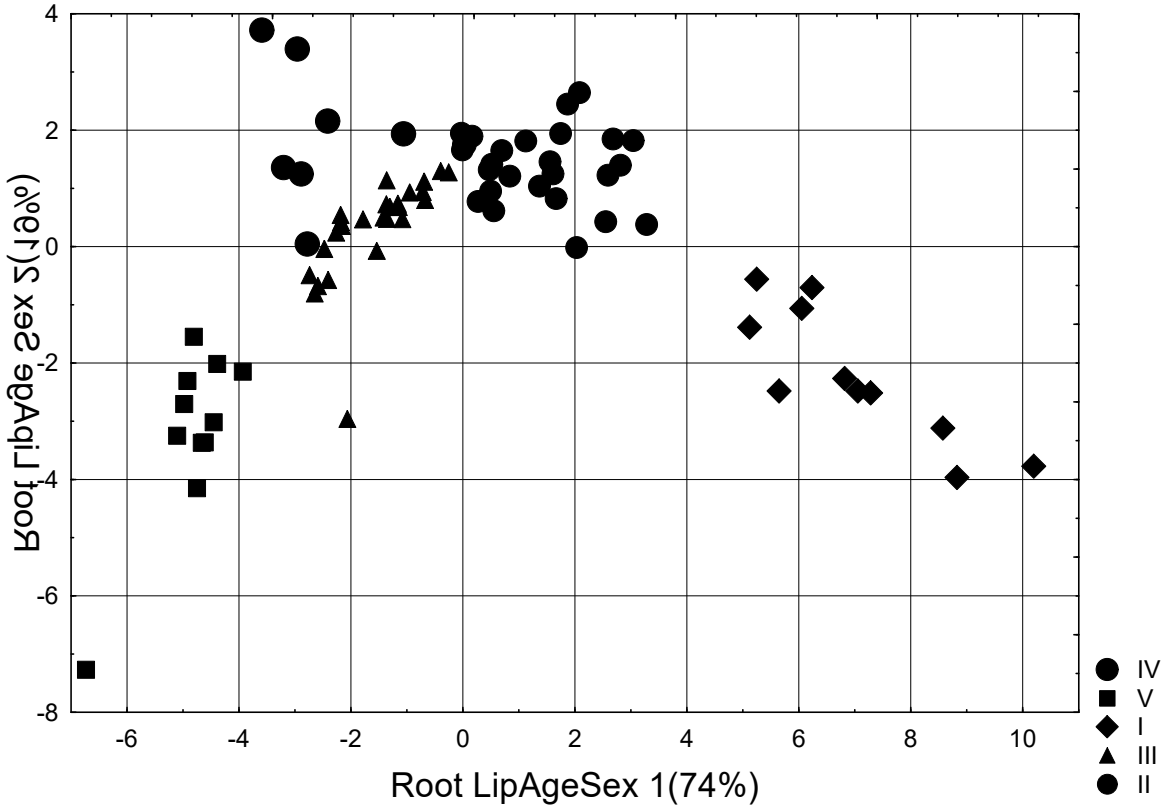


Fig. 1. Scattering of individual values of the first and second discriminant roots of patients of different lipids clusters

At the opposite pole are localized patients of I cluster, the youngest in the sample, in whom the levels of listed variables are minimal for the sample and gender representation is almost the same. The intermediate positions of the members of the other three clusters reflect, as a rule, the intermediate levels of the listed variables. Additional demarcation of members of IV cluster occurs along the axis of

the second root. Their top position reflects the maximally increased levels of both Dobiásová&Frohlich's and Klimov's AIP. In general, all clusters are quite clearly delineated on the planes of two roots even, which is documented by calculating the Mahalanobis distances (Table 8).

Table 8. Squared Mahalanobis Distances between Lipids Clusters and F-values (df=6,7; for all $p<10^{-6}$)

Lipids Clusters	IV (7)	V (11)	I (11)	III (26)	II (27)
IV (7)	0	39,4	117	16,9	28,4
V (11)	26,6	0	142	23,8	59,5
I (11)	78,0	122	0	81,6	46,5
III (26)	14,5	28,6	98,2	0	9,9
II (27)	24,6	72,5	56,6	20,4	0

The same discriminant parameters can be used to identify the belonging of one or another person to one or another cluster. This purpose of discriminant analysis is realized with the help of classifying functions (Table 9). These functions are special linear combinations that maximize differences between groups and minimize dispersion within groups. An object belongs to a group with the maximum value of a function calculated by summing the products of the values of the variables by the coefficients of the classifying functions plus the constant.

Table 9. Coefficients and Constants for Classification Functions for Lipids Clusters

Lipids Clusters	IV	V	I	III	II
Variables currently in the model	p=,085	p=,134	p=,134	p=,317	p=,330
Cholesterol total, mM/L	96,17	88,16	51,39	85,76	79,26
Klimov's Atherogenic Index	19,09	24,26	52,26	13,33	16,03
Dobiásová's & Frohlich's AIP	202,3	120,0	28,83	164,8	159,6
Triglycerides, mM/L	-116,5	-60,65	-30,00	-94,49	-97,56
Age, years	0,132	0,323	0,189	0,267	0,162
Sex Index (M=1; F=2)	11,17	13,08	11,17	9,868	9,094
Constants	-362,5	-358,5	-189,6	-283,5	-239,3

In this case, we can retrospectively recognize members of V, I and II lipids clusters **unmistakably**, the members of IV cluster are classified with one mistake, and III cluster with two errors. Overall classification accuracy is 96,3% (Table 10).

Table 10. Classification Matrix for Lipids Clusters

	Rows: Observed classifications Columns: Predicted classifications					
Group	Percent Correct	IV p=,08537	V p=,13415	I p=,13415	III p=,31707	II p=,32927
IV	85,7	6	0	0	1	0
V	100,0	0	11	0	0	0
I	100,0	0	0	11	0	0
III	92,3	0	0	0	24	2
II	100,0	0	0	0	0	27
Total	96,3	6	11	11	25	29

The inclusion of age and gender in the discriminant model suggests their relationship with the parameters of the plasma lipoprotein profile. And indeed, the level of triglycerides naturally increases with age (Fig. 2), as does Dobiásová&Frohlich's AIP. Both of these parameters are determined by age by 29% (Table 11).

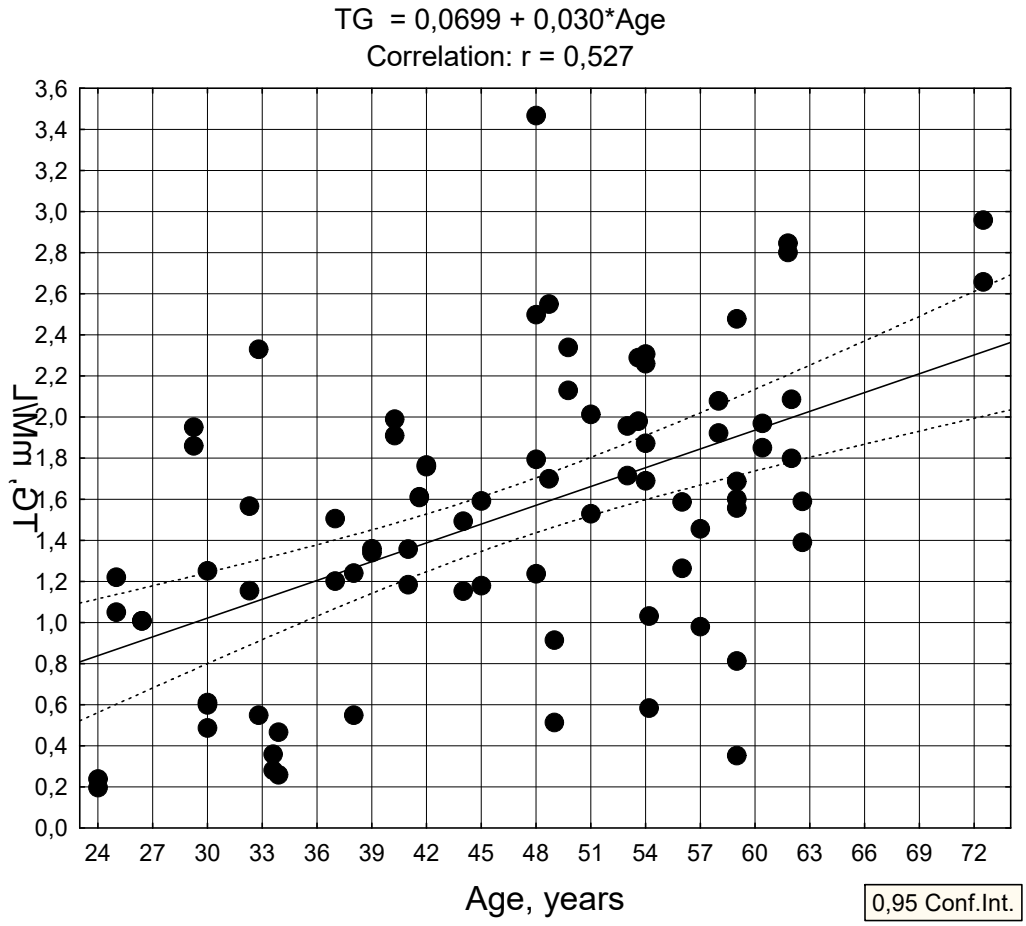


Fig. 2. Scatterplot of correlation between Age (X-line) and plasma Triglycerides level (Y-line)

Table 11. Regression Summary for Age
R=0.535; R^2=0.286; Adjusted R^2=0.268; $F_{(2.8)}$=15.8; $p<10^{-6}$

N=82		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(79)}$	p-level
Variables	r		Intercpt	34.5	3.5	9.75	10^{-6}
Triglycerides, mM/L	**0,527**	0,707	0,212	12,23	3,676	3,33	0,001
Dobiásová's & Frohlich's AIP	**0,431**	-0,201	0,213	-7,414	7,840	-0,95	0,347

Gender, or rather Sex Index, also upregulates lipid parameters, but to a lesser extent (Table 12).

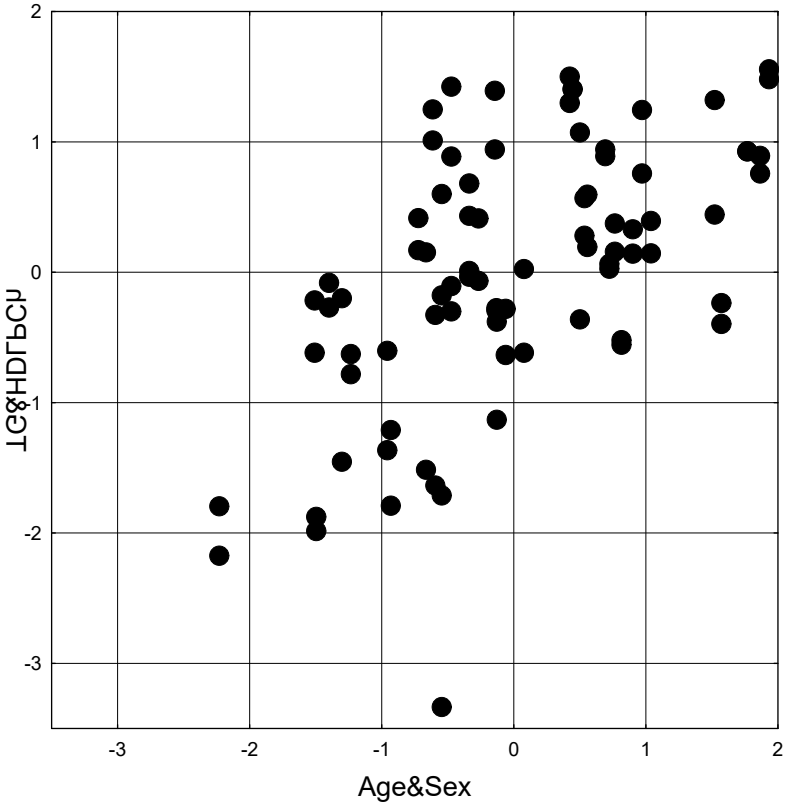
Table 12. Regression Summary for Sex Index
R=0.330; R^2=0.109; Adjusted R^2=0.086; $F_{(2.8)}$=4.8; p=0.011

N=82		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(79)}$	p-level
Variables	r		Intercpt	0.845	0.265	3.19	0,002
HDLP Cholesterol, M/L	**0,324**	0,240	0,173	0,333	0,240	1.39	0,169
Triglycerides, mM/L	**0,295**	0,106	0,173	0.076	0.124	0.61	0,542

Taken together, age and gender determine the level of Dobiásová&Frohlich's AIP as well as its components by 34% (Table 13 and Fig. 3).

Table 13. Factor structure of Roots

Left set	R
Age	-0.914
Sex Index	-0.581
Rigt set	R
Triglycerides, mM/L	-0.963
HDLP Cholesterol, M/L	-0.894
Dobiásová's & Frohlich's AIP	-0.741



R=0.582; R^2=0.339; $\chi^2_{(6)}$=34; p<10^{-5}; Λ Prime=0.648

Fig. 3. Scatterplot of canonical correlation between Age&Sex (X-line) and TG&HDLP Ch (Y-line)

Conclusion

The plasma lipoprotein profile of persons with maladaptation is characterized by a wide range of values, from increased to decreased. This should be kept in mind during the next study of its connections with the parameters of the neuroendocrine-immune complex.

Acknowledgment

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Accordance to ethics standards

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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Appendix

Table 1. Members of Clusters and Distances from Respective Cluster Center

	Members of Cluster Number 5 and Distances from Respective Cluster Center Cluster contains 11 cases
Case No.	Distance
C 1	0,542
C 3	0,162
C 5	0,231
C 22	0,132
C 45	0,245
C 49	0,236
C 55	0,110
C 59	0,206
C 67	0,143
C 69	0,371
C 79	0,373

	Members of Cluster Number 1 and Distances from Respective Cluster Center Cluster contains 11 cases
Case No.	Distance
C 13	0,116
C 27	0,170
C 32	0,074
C 34	0,359
C 36	0,191
C 42	0,086
C 44	0,031
C 48	0,193
C 51	0,470
C 54	0,121
C 58	0,148

Case No.	Members of Cluster Number 2 and Distances from Respective Cluster Center Cluster contains 27 cases Distance
C 6	0,293
C 10	0,211
C 11	0,060
C 15	0,227
C 16	0,200
C 17	0,210
C 21	0,179
C 23	0,164
C 30	0,213
C 31	0,217
C 35	0,106
C 37	0,030
C 38	0,199
C 39	0,103
C 40	0,062
C 43	0,235
C 47	0,185
C 50	0,232
C 52	0,236
C 53	0,035
C 60	0,235
C 61	0,275
C 63	0,191
C 68	0,499
C 70	0,459
C 80	0,377

Case No.	Members of Cluster Number 4 and Distances from Respective Cluster Center Cluster contains 7 cases Distance
C 66	0,281
C 73	0,249
C 76	0,045
C 77	0,344
C 78	0,399
C 81	0,302
C 82	0,143

	Members of Cluster Number 3 and Distances from Respective Cluster Center Cluster contains 26 cases
Case No.	Distance
C 2	0,171
C 4	0,130
C 7	0,048
C 8	0,118
C 9	0,214
C 12	0,121
C 14	0,180
C 18	0,211
C 19	0,147
C 20	0,129
C 24	0,240
C 25	0,158
C 26	0,107
C 28	0,053
C 29	0,114
C 33	0,151
C 41	0,037
C 46	0,227
C 56	0,162
C 57	0,226
C 64	0,256
C 65	0,136
C 71	0,448
C 72	0,175
C 74	0,220
C 75	0,186