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SIGNIFICANCE OF DETERMINING LEVELS OF APOLIPOPROTEINS A-I AND B IN THE DIAGNOSTICS AND ASSESSMENT OF LIPID-RELATED ATHEROGENIC RISK IN HYPERALPHA-LIPOPROTEINEMIA, HYPOCHOLESTEROLEMIA AND HYPO-HDL-CHOLESTEROLEMIA

ZNAČAJ ODREĐIVANJA NIVOA APOLIPOPROTEINA A-I I B U DIJAGNOSTICI I PROCENI ATEROGENOG RIZIKA LIPIDSKOG POREKLA U HIPERALFA-LIPOPROTEINEMIJI, HIPOHOLESTEROLEMIJI I HIPO-HDL-HOLESTEROLEMIJI

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Summary: The significance of determining apolipoproteins apoB and apoA-I and their correlation with lipid status parameters were tested in hyperalpha-lipoproteinemia (30 women), hypocholesterolemia (10 men) and hypo-HDL-cholesterolemia (15 women and 21 men). Control groups were 20 normolipidemic men and women, each. ApoA-I showed positive correlation with HDL-cholesterol in hyperalpha-lipoproteinemia, with total and HDL-cholesterol in hypocholesterolemia, and with total and LDL-cholesterol in females with hypo-HDL-cholesterolemia, and negative correlation with cholesterol ratios only in hypocholesterolemia. ApoB showed a positive correlation with total and LDL-cholesterol in all groups, and with cholesterol ratios in hyperalpha-lipoproteinemia and hypo-HDL-cholesterolemia. The apoB/apoA-I ratio, correlating with the majority of lipid parameters, and with the highest percentage of pathological values in all tested groups, was singled out as the most sensitive parameter for the evaluation of lipid-related atherogenic risks.

Keywords: apolipoproteins, hyperalpha-lipoproteinemia, hypocholesterolemia, hypo-HDL-cholesterolemia, prognostic value

Kratak sadržaj: Značaj određivanja apolipoproteina apoA-I i apoB, i njihova korelacija s parametrima lipidskog statusa, procenjivani su u hiperalfa-lipoproteinemiji (30 žena), hipoholesterolemiji (10 muškaraca) i hipo-HDL-holesterolemiji (15 žena i 21 muškarac). Kontrolne grupe činilo je po 20 normolipidemičnih muškaraca i žena. ApoA-I pozitivno korelira s HDL-holesterolom u hiperalfa-lipoproteinemiji, ukupnim i HDL-holesterolom u hipoholesterolemiji, i ukupnim i LDL-holesterolom kod žena s hipo-HDL-holesterolemijom, a negativno s holesterolskim odnosima samo u hipoholesterolemiji. ApoB pozitivno korelira s ukupnim i LDL-holesterolom u svim grupama, a s holesterolskim odnosima u hiperalfa-lipoproteinemiji i hipo-HDL-holesterolemiji. Odnos apoB/apoA-I je u korelaciji s najvećim brojem lipidskih parametara, uz najveći procenat patoloških vrednosti u svim grupama ispitanika, te se izdvaja kao najsenzitivniji parametar za procenu aterogenog rizika lipidskog porekla.

Ključne reči: apolipoproteini, hiperalfa-lipoproteinemija, hipoholesterolemija, hipo-HDL-holesterolemija, prognostička vrednost

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Introduction

Majority of currently known recommendations point out lipid parameters, first of all LDL-cholesterol (LDL-C), as basic risk factors for the occurrence of vascular diseases and the main therapeutic target. However, some more recent studies suggested that the values of apolipoprotein B (apoB) and apolipoprotein A-I (apoA-I), that is the apoB/apoA-I ratio, repre-

sent a more sensitive indicator of the risk of vascular diseases and a more potent predictor of the future vascular events in patients on hypolipoproteinemic therapy (1–5).

There are increasingly more studies indicating that apoB (6–10) is a better marker of the risk factor and guideline in statin therapy than is LDL-C, or any of the cholesterol parameters in general (1, 3). It has been shown that apoB is superior in predicting subclinical atherosclerosis, determined by the presence of extracoronary plaques and coronary calcium deposits (2). The advantage of apoB is seen in the fact that each lipoprotein particle contains one molecule of apoB ($apoB_{100}$ in VLDL, IDL, LDL and Lp(a), and $apoB_{48}$ in chylomicrons and their remnants) and that total serum apoB, determined by commercial sets which detect both $apoB_{100}$ and apo B_{48}, thus reflects the total number of atherogenic particles (11), of which LDL, certainly, make the great majority. However, as LDL is a heterogeneous group consisting of more subpopulations of particles that differ in the cholesterol content, LDL-C, in contrast to apoB, cannot be an equivalent to the number of LDL particles (12, 13). This discord is critical in the case of the presence of small dense LDL particles, which are poor in cholesterol, but very atherogenic (8,14–16).

In view of the well-documented inverse correlation between the level of HDL-cholesterol (HDL-C) and risk of atherosclerotic cardiovascular diseases (11,17–19), HDL-C has first been considered a protective, antiatherogenic lipid fraction. However, with the knowledge that human HDLs represent a heterogeneous population of particles, having different physical and chemical properties (18, 20), there appeared a need for differentiating them, especially in the sense of recognition of HDL_C (or HDL_1) particles of high atherogenic potential, which are saturated with cholesterol, and which, because of the content of apoE, are removed via LDL receptors, thus reducing significantly their capacity to take up LDL particles from the circulation. Besides, in the frame of both HDL_2 and HDL_3 subfractions, there are two main populations of particles in view of their apoprotein content: those that contain only apoA-I [Lp(A-I)] and those that also contain apoA-II [Lp(A-I, A-II)] (21–23). This fact has pointed out the need for determining apoA-I as a main carrier of the protective action of HDL particles. Namely, apoA-I is of crucial importance in taking up the excess of cellular cholesterol from peripheral tissues and its esterification, which allows accumulation of this cholesterol in HDL particles and its reverse transport towards the liver. In contrast to this, there are opinions that apoA-II hinders this process (19, 24, 25).

The role of apolipoproteins in the assessment of lipid-related atherogenic risks is considered increasingly more important. Besides, the determination of apolipoproteins, which is carried out in the second stage of the stepwise laboratory diagnostics of lipid disorders, is indispensable for diagnosing normolipidemic dyslipoproteinemias, which are rather widespread in the general population (26). These discrete disorders, which are characterized by the disorders in particular lipoprotein species, their subfractions or apolipoproteins, at quite normal levels of total cholesterol (TC) and triglycerides (TG), are also associated with an increased risk for the development of early atherosclerosis.

The aim of this investigation was to examine the significance of determining apoA-I and apoB, as well as the apoB/apoA-I ratio, in the diagnostics and assessment of atherogenic risk in hyperalpha-lipoproteinemia, hypocholesterolemia and hypo-HDL-cholesterolemia.

Material and Methods

The investigation encompassed 76 subjects of both genders (31 males and 45 females), singled out in the frame of a three-month routine work of the Lipid Group of the Institute of Laboratory Medicine of the Clinical Center – Novi Sad, part of which were also the Station for Atherosclerosis Prevention and the Laboratory for Lipid Studies.

The criteria for including a subject into the study were as follows: HDL-C value above 2.00 mmol/L and TG below 2.00 mmol/L (group of subjects with hyperalpha-lipoproteinemia); TC below 4.00 mmol/L and TG below 1.70 mmol/L (group of subjects with hypocholesterolemia); and HDL-C equal to or less than 1.00 mmol/L, regardless of the values of other investigated parameters of lipid status (group of subjects with hypo-HDL-cholesterolemia). Control group consisted of 40 healthy normolipidemic subjects, 20 of each gender.

After the statistical treatment of results in the control group and establishing significant differences in the values of apoB and apoB/apoA-I ratio in respect of gender, four gender-uniform groups were formed, namely, the group with hyperalpha-lipoproteinemia (30 females), group with hypocholesterolemia (10 males) and two groups with hypo-HDL-cholesterolemia [female group (n=15) and male group (n=21)]. Values of the investigated parameters were compared with those of the control groups of healthy normolipidemic males (n=20) and females (n=20).

The determination of the parameters of lipid status, as well as of apoA-I and apoB, was carried out from the blood sample after a fasting period of 12–14 hours. Concentrations of serum TC, cholesterol in HDL fraction and TG were determined by standard enzymatic procedure (on a Technicon RA-XT instrument, using BioMerieux reagents). Isolation of cholesterol from the HDL fraction was carried out by a standard precipitation method after Burstein et

al. (using Randox reagents). Values of LDL-C (Friedewald et al.) as well as the ratios LDL/HDL-C and TC/HDL-C were obtained by calculation. Concentrations of apoA-I and apoB were determined by an immunoturbidimetric method (Olympus reagents and Olympus AU 400 apparatus), whereas the apoB/apoA-I ratio was obtained by calculation.

Statistical treatment of the results encompassed the calculations of mean values and standard deviations of tested parameters and the assessment of statistical significance of their relationship (linear correlation). Significance of the obtained differences between the values of the test and corresponding control groups was assessed by Student's t-test.

Results were treated using Microsoft Excel 2000 program for statistical data treatment.

Results

Analysis of tested parameters in the control group of healthy normolipidemic persons showed that female subjects had significantly lower values of apoB ($p<0.05$) and apoB/apoA-I ratio ($p<0.05$) compared to males, whereas the values of apoA-I were not significantly different (1.44 vs. 1.39 g/L). Because of the observed differences, gender-dependent reference values of these parameters were formed (*Table I*). In *Table II* are shown the values of tested parameters of lipid status in gender-uniform groups of subjects.

In the group of female subjects with hyperalphalipoproteinemia, significantly higher values of apoA-I ($p<0.001$) and apoB ($p<0.001$) were found compared to the female control group. On the other hand, male subjects with hypocholesterolemia had significantly lower values of apoA-I ($p<0.001$) and apoB ($p<0.001$) than the male control group. However, the increase of the apoB/apoA ratio in either of the two groups was not significant (0.65 vs. 0.60 and 0.72 vs. 0.70, for women and men respectively) (*Figure 1*).

Among the subjects with hypo-HDL-cholesterolemia similar differences in the level of apolipoproteins were observed for men and women compared to the corresponding control group (*Figure 1*). Namely, significantly lower values of apoA-I ($p<0.001$) and significantly higher values of apoB/apoA-I ratio ($p<0.001$) were found. Concentrations of apoB were also higher in both groups of subjects, the difference being statistically significant only in females ($p<0.001$).

Analysis of the relation between apoA concentrations and values of tested lipid-status parameters (*Figure 2*) showed the existence of significant positive correlation with the levels of HDL-C ($p<0.001$) in both the group of subjects with hyperalpha-lipoproteinemia and those with hypocholesterolemia. In the latter, positive correlation with TC ($p<0.05$), and negative correlation with the ratios LDL/HDL-C ($p<0.05$) and TC/HDL-C ($p<0.05$) attained statistical significance. In the group of subjects with hypo-

Table I Values of apoA-I, apoB and apoB/apoA-I ratio in control subjects.

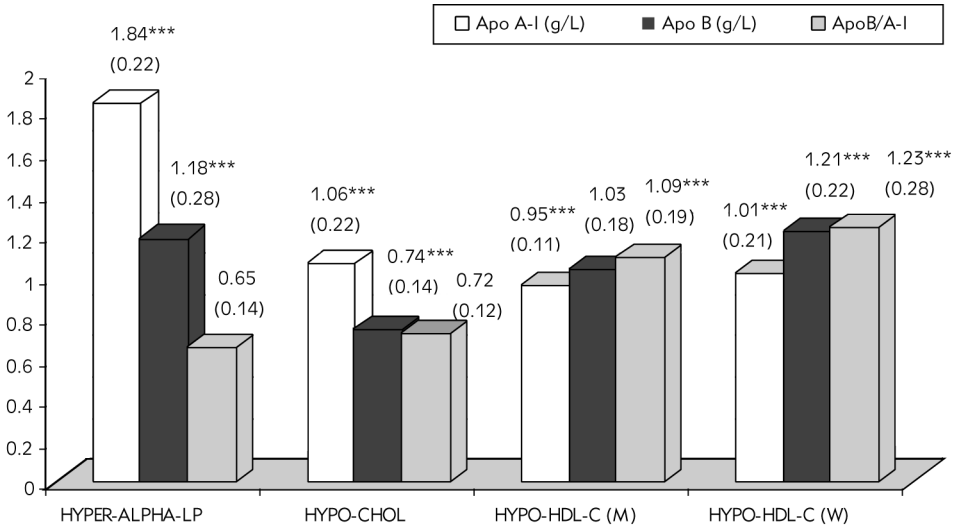
	apoA-I (g/L)			apoB (g/L)			apoB/apoA-I		
	$\bar{x}$	SD	ref. val.	$\bar{x}$	SD	ref. val.	$\bar{x}$	SD	ref. val.
Women	1.44	0.15	1.29–1.60	0.86*	0.11	0.75–0.97	0.60*	0.09	0.51–0.69
Men	1.39	0.13	1.27–1.52	0.97	0.10	0.87–1.06	0.70	0.09	0.61–0.79

Legend:
apo – apolipoprotein; $\bar{x}$ – mean value; SD – standard deviation; ref. val. – reference value
* – $p<0.05$ with respect to men

Table II Values of investigated parameters of lipid status in tested groups.

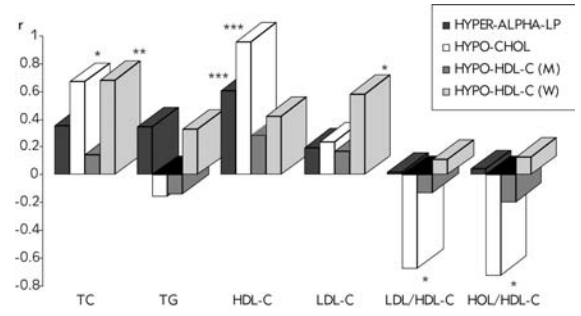
Parameter mmol/L	HYPER-ALPHA-LP (n=30)		HYPO-CHOL (n=10)		HYPO-HDL-C (M) (n=21)		HYPO-HDL-C (W) (n=15)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
TC	6.60	1.27	3.32	0.54	4.74	0.74	5.58	0.97
TG	1.04	0.41	1.06	0.33	1.96	0.94	2.36	0.90
HDL-C	2.27	0.24	1.00	0.32	0.77	0.17	0.82	0.15
LDL-C	3.86	1.21	1.83	0.39	3.08	0.67	3.68	0.79
LDL/HDL-C	1.72	0.56	2.03	0.86	4.08	1.34	4.64	1.32
TC/HDL-C	2.93	0.57	3.59	1.11	6.50	1.97	6.99	1.62

Legend:
HYPER-ALPHA-LP – hyperalpha-lipoproteinemia; HYPO-CHOL – hypocholesterolemia; HYPO-HDL-C (M) – hypo-HDL-cholesterolemia (men); HYPO-HDL-C (W) – hypo-HDL-cholesterolemia (women)
TC – total cholesterol; TG – triglycerides; HDL-C – HDL-cholesterol; LDL-C – LDL-cholesterol; $\bar{x}$ – mean value; SD – standard deviation



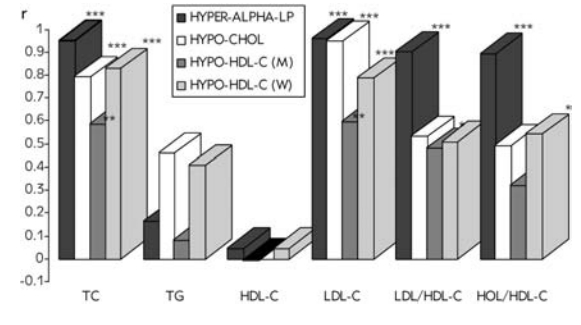
Legend:
HYPER-ALPHA-LP – hyperalpha-lipoproteinemia; HYPO-CHOL – hypocholesterolemia; HYPO-HDL-C (M) – hypo-HDL-cholesterolemia (men); HYPO-HDL-C (W) – hypo-HDL-cholesterolemia (women)
*** – $p<0.001$ with respect to control group of the same gender

Figure 1 Values of apoA-I, apoB and apoB/apoA-I ratio for particular groups of subjects.



Legend:
HYPER-ALPHA-LP – hyperalpha-lipoproteinemia; HYPO-CHOL – hypocholesterolemia; HYPO-HDL-C (M) – hypo-HDL-cholesterolemia (men); HYPO-HDL-C (W) – hypo-HDL-cholesterolemia (women)
TC – total cholesterol; TG – triglycerides; HDL-C – HDL-cholesterol; LDL-C – LDL-cholesterol
* – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$

Figure 2 Correlation between apoA-I values and investigated lipid status parameters.



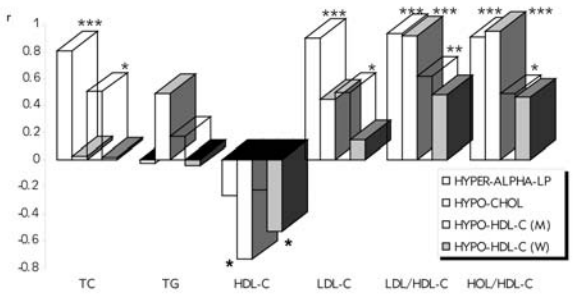
Legend:
HYPER-ALPHA-LP – hyperalpha-lipoproteinemia; HYPO-CHOL – hypocholesterolemia; HYPO-HDL-C (M) – hypo-HDL-cholesterolemia (men); HYPO-HDL-C (W) – hypo-HDL-cholesterolemia (women)
TC – total cholesterol; TG – triglycerides; HDL-C – HDL-cholesterol; LDL-C – LDL-cholesterol
* – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$

Figure 3 Correlation between apoB and investigated lipid status parameters.

HDL-cholesterolemia, a significant positive correlation was found between the apoA-I concentration and TC levels ($p<0.01$), but also with serum LDL-C ($p<0.05$) in the female group.

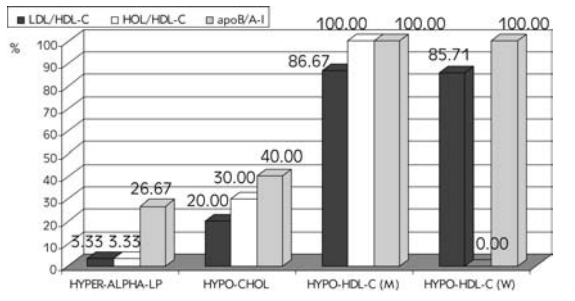
Concentrations of apoB showed a significant positive correlation with the values of TC and LDL-C in all groups of subjects ($p<0.001$ for both parameters except for the male group with hypo-HDL-cholesterolemia, where $p<0.01$ for both parameters) (*Figure 3*). Highly significant positive correlation was also found with the ratios LDL/HDL-C and TC/HDL-C ($p<0.001$) in the female group with hyperalpha-lipoproteinemia. Somewhat weaker positive correlation was observed with the ratio LDL/HDL-C ($p<0.05$) in males and with the ratio TC/HDL-C ($p<0.05$) in females with hypo-HDL-cholesterolemia.

The apoB/apoA-I ratio showed a high significance of the positive correlation with both TC and



Legend:
HYPER-ALPHA-LP – hyperalpha-lipoproteinemia; HYPO-CHOL – hypocholesterolemia; HYPO-HDL-C (M) – hypo-HDL-cholesterolemia (men); HYPO-HDL-C (W) – hypo-HDL-cholesterolemia (women)
TC – total cholesterol; TG – triglycerides; HDL-C – HDL-cholesterol; LDL-C – LDL-cholesterol
* – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$

Figure 4 Correlation between the apoB/apoA-I ratio and investigated lipid status parameters.



Legend:
HYPER-ALPHA-LP – hyperalpha-lipoproteinemia; HYPO-CHOL – hypocholesterolemia; HYPO-HDL-C (M) – hypo-HDL-cholesterolemia (men); HYPO-HDL-C (W) – hypo-HDL-cholesterolemia (women)

Figure 5 Percentages of pathological states of tested ratios LDL/HDL-C, TC/HDL-C and apoB/apoA-I for particular groups of subjects.

LDL-C, and with tested ratios LDL/HDL-C and TC/HDL-C ($p<0.001$ for all parameters) in the group with hyperalpha-lipoproteinemia (*Figure 4*). Positive correlation with TC and LDL-C ($p<0.05$ for both parameters) was also observed in male subjects with hypo-HDL-cholesterolemia, whereas the ratios LDL/HDL-C and TC/HDL-C correlated with the ratio apoB/apoA-I in both the group with hypocholesterolemia ($p<0.001$) and the male group with hypo-HDL-cholesterolemia ($p<0.01$ and $p<0.05$, respectively). Negative correlation with HDL-C was observed in male subjects with hypocholesterolemia ($p<0.05$) and women with hypo-HDL-cholesterolemia ($p<0.05$).

With the aim of evaluating sensitivity of particular tested parameters in the assessment of lipid-related atherogenic risk, percentages of their pathological values were determined within particular test groups (*Figure 5*). In the group with hyperalpha-lipoproteinemia, pathological values of the LDL/HDL-C and TC/HDL-C ratios were observed with 3.33% of subjects, whereas 26.67% had the value of the ratio apoB/apoA-I outside the reference span. In the group of male subjects with hypocholesterolemia, pathological values of the ratio LDL/HDL-C were found with 20.00%, the ratio TC/HDL-C with 30.00%, and apoB/apoA-I with 40.00% of subjects. The highest percentage of pathological values was observed in subjects with hypo-HDL-cholesterolemia: the LDL/HDL-C ratio was pathological in 86.67% males and 85.71% females, TC/HDL-C ratio with all males (100.00%), but in none of the females, whereas the ratio apoB/apoA-I was pathological with all subjects regardless of the gender.

Discussion

Analysis of the control group of healthy normolipidemic subjects showed that significantly lower values of apoB ($p<0.05$) and apoB/apoA-I ratio ($p<0.05$) were present with women compared to men, whereas the concentration of apoA-I was not significantly higher (*Table I*). The obtained results are in agreement with the previous reports (17, 27–30), which indicated the differences in distribution of apoB and apoA-I, both in respect of gender and age. A study carried out on a control sample of the population of Northern Italy (31) showed that the levels of apoA-I and apoB increased with the age of subjects, and, in each age category, concentrations of apoA-I were higher with women than with men, exhibiting a high significance ($p<0.001$). However, covariance analysis indicated a significant effect of gender on apoB level, its values being higher with males aged up to 50, and after that age higher values being found in women. The age of our subjects varied in a wide span, from 30 to 65, but we could not form age categories within the test groups. In accordance with the observed gender differences, we thought it justified to form reference values of these parameters for males and females separately (*Table I*), as well as for gender-uniform groups (*Table II*).

Several studies showed that apoA-I can be a better parameter in the assessment of the risk of coronary heart disease than HDL-C (11, 32–34). The AMORIS Study (7) showed that apoA-I was a significant risk factor of fatal myocardial infarction with men, which also retained its predictive role after including TC and TG in the multivariate model. However, its negative correlation with the incidence of coronary events was not statistically significant after including subjects' age in the assessment of this relationship. Some other studies, such as the ARIC Study (35), the Physicians' Health Study (36), Quebec Cardiovascular Study (8) and Northwich Park Health Study (9), showed also that, although apoA-I was a

potent predictor of cardiovascular risk when considered individually, statistical significance of the correlation disappeared in the multivariate analysis. Besides, the MONICA/KORA Augsburg cohort study (6), carried out on middle-aged men and women without previous coronary event, showed that elevated levels of HDL-C were associated with significantly lower risk of the occurrence of coronary event in both genders, whereas this relation with apoA-I, although present, was not statistically significant.

Generally, these results do not support the opinion that the determination of apoA-I could provide more information for the assessment of the risk of coronary heart disease than the HDL-C level. However, it should be borne in mind that these studies were carried out on a smaller number of subjects than was the case with the AMORIS Study, so that this could be the reason for the lack of statistical significance in the assessment of predictive ability of apoA-I (6). Besides, the determination of apoA-I, as the main carrier of the protective lipoprotein potential, would be of special significance in hypolipoproteinemias, as well as in the states with lowered HDL-C values. Namely, it was observed that accelerated catabolism and low levels of HDL-C were not always associated with early atherosclerosis (6), and neither were increased HDL-C levels obligatorily protective (18). This may be the reason why highly significant positive correlation between apoA-I and HDL-C ($p<0.001$) existed in our subjects with hypoalpha-lipoproteinemia and hypocholesterolemia, but lacked in hypo-HDL-cholesterolemia (*Figure 2*). There was also a positive correlation between apoA-I and TC in the group with hypocholesterolemia ($p<0.05$) and in women with hypo-HDL-cholesterolemia ($p<0.01$). Negative correlation with lipid ratios ($p<0.05$ for LDL/HDL-C and TC/HDL-C), observed in the subjects with hypocholesterolemia, and a highly significant ($p<0.001$) deviation of apoA-I concentration from the control values in all tested groups of subjects (*Figure 1*), also point out the significance of this parameter in the assessment of lipid-related atherogenic risk.

Significance of apoB for the assessment of cardiovascular risk has been undoubtedly recognized; however, the advantages of its determination compared to cholesterol indices are still subject of research (37). Although there exist results that negate its advantage (35, 36), the majority of studies have pointed out that apoB is superior in this assessment. AMORIS Study (7), designed to compare potentials of LDL-C and apoB for predicting risk of coronary events, showed that apoB was a highly significant predictor at any concentration of TC and TG, in the subjects of both genders, and in all age groups, which was not shown for LDL-C. Similarly, in the multivariate analysis of the Quebec Cardiovascular Study (8), apoB showed the strongest correlation with the risk of occurrence of a coronary event. In the MONICA/KORA Augsburg cohort study (6), strong correlation was found between this parameter and sudden coronary events in both genders, even in the multivariate analysis involving conventional risk factors. A study that was concerned with the connection of thrombogenic factors with recurrent coronary events (10) also pointed out the advantage of apoB compared to LDL-C, retaining also significant association with the rate of coronary event in multivariate analysis. Researchers involved in the Northwich Park Health Study (9) reported that apoB was a better predictor of cardiovascular risks compared to TC or LDL-C and that, together with hypertriglyceridemia, increased the risk of cardiovascular diseases.

Hyperalpha-lipoproteinemia, so-called »longevity syndrome«, is characterized by increased concentrations of apoA-I, the values of apoB and apoB/apoA-I being within reference values. In our female subjects with hyperalpha-lipoproteinemia, singled out only on the basis of the HDL-C values exceeding 2.00 mmol/L, we found, however, a significantly higher value of apoB ($p<0.001$) compared to control (*Figure 1*), and its significant positive correlation with TC and LDL-C, as well as with the ratios LDL/HDL-C and TC/HDL-C ($p<0.001$, for both parameters) (*Figure 3*). This suggests a potential atherogenic effect which might be a consequence of increased values of both HDL-C and LDL-C in this group of subjects. Hence, at higher HDL-C levels, in addition to apoA-I, it would be necessary to determine the levels of apoB, too, and, first of all, of apoE, which in these states would allow differentiating familial hyperalpha-lipoproteinemia (true »longevity syndrome« with an evident protective role) from hyper-HDL_C-cholesterolemia associated with high atherogenic risk. In the group with hypocholesterolemia we found significant lowering of apoB ($p<0.001$) with a positive correlation with TC and LDL-C ($p<0.001$, for both parameters). Bearing also in mind the significant decrease of apoA-I in this group of subjects, it may be concluded that hypocholesterolemia is most probably a consequence of generally enhanced catabolism or a lower synthetic capability of the organism, and not of hypobeta-lipoproteinemia. In the subjects with hypo-HDL-cholesterolemia, higher values of apoB were found compared to control, the difference being statistically significant only with women ($p<0.001$). The significance of the positive correlation with TC and LDL-C levels was also higher with female subjects ($p<0.001$ vs. $p<0.01$ for both parameters), the correlation with the ratio LDL/HDL-C in men ($p<0.05$) and TC/HDL-C with women ($p<0.05$). The obtained results suggest the need for determining apoA-I and apoB in the states with a low HDL-C level, to assess the atherogenic potential of HDL particles, bearing in mind the fact that only some forms of hypoalpha-lipoproteinemia are associated with early atherosclerosis.

In view of the significance of the determination of apoA-I for assessing the protective role of HDL particles, and since apoB is an indicator of the number of atherogenic cholesterol particles in blood, the apoB/apoA-I ratio could be considered an indicator of the balance between proatherogenic and antiatherogenic lipoproteins in the circulation (4, 11). Potential superiority of this ratio over cholesterol ratios has been the subject of numerous studies. The INTERHEART study (38), in which the significance of main risk factors for a coronary event was assessed in 52 countries worldwide, showed that the apoB/apoA-I ratio is the most significant independent factor of coronary risk, irrespective of either gender, age or ethnicity, and that it is a good identifier of the persons with a risk of either fatal or non-fatal myocardial infarction. In the large prospective AMORIS Study (7), the apoB/apoA-I ratio was shown to be a better predictor of cardiovascular risk than any cholesterol ratio in subjects of both genders. Additionally, in the subgroup with the LDL-C values of less than 3.6 mmol/L, this ratio, in contrast to all investigated cholesterol ratios which in a multivariate analysis lost their predictive values, in this analysis remained highly predictive for coronary event (11). In a prospective study carried out on a group of middle-aged males (9) the apoB/apoA-I ratio was associated with the strongest effect on the cardiovascular risk. In contrast to this, the MONICA/KORA Augsburg cohort study (6) showed that its ability to predict a sudden coronary event was practically identical to that of the TC/HDL-C ratio. The remarkable importance of this apolipoprotein ratio can also be seen in the fact that large studies, such as AMORIS Study (7), AFCAPS/TexCAPS (39) and LIPID Study (40), have promoted apoB and, especially, the ratio apoB/apoA-I as the best indicators in assessment of the residual coronary risk in patients on statin therapy, and hence, best guides in judging the adequacy of statin therapy (1, 3, 4, 11, 41–43).

Although the increase in the apoB/apoA-I ratio was significant only in our subjects with hypo-HDL-cholesterolemia ($p<0.001$) (*Figure 1*), this ratio showed significant positive correlation with cholesterol ratios in all groups of subjects except for women with hypo-HDL-cholesterolemia (*Figure 4*). Positive correlation with TC and LDL-C was also significant in subjects with hyperalpha-lipoproteinemia ($p<0.001$ for both parameters) and in males with hypo-HDL-cholesterolemia ($p<0.05$ for both parameters). Negative correlation with the HDL-C level was observed in males with hypocholesterolemia ($p<0.05$) and in females with hypo-HDL-cholesterolemia ($p<0.05$). Compared to apoB and apoA-I, the ratio apoB/apoA-I correlated with the largest number of lipid parameters, which may indicate its versatility in the assessment of lipid-related risks. In view of the superiority of the apoB/apoA-I ratio over cholesterol ratios in subjects having normal or low LDL-C levels (11), the determination of this ratio would be of special significance in both hypoalpha-lipoproteinemic and hypocholesterolemic states.

By analyzing the incidence of pathological values of the measured ratios (*Figure 5*), it can be seen that largest deviations from reference values were found for the apoB/apoA-I ratio in all groups of subjects, which may indicate its highest sensitivity in detecting lipid-related atherogenic risk.

Our results suggest that the determination of apoA-I and apoB, and calculation of the apoB/apoA-I ratio, would be of extreme significance in laboratory diagnostics and assessment of atherogenic risk in the states with elevated and lowered values of HDL-cholesterol, as well as in hypocholesterolemia. The highest percentage of pathological values found for the apoB/apoA-I ratio in all groups of subjects suggests that this ratio is a most sensitive parameter for the assessment of lipid-related atherogenic risk.

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