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High-density lipoprotein profile in newly-diagnosed lower extremity artery disease in Slovak population without diabetes mellitus

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Abstract

OBJECTIVE: To assess the relationship of high density lipoprotein subfractions to newly-diagnosed lower extremity artery disease (LEAD) in individuals without diabetes mellitus and without hypolipidemic therapy.

METHODS: This cross-sectional study involves 106 subjects: 51 had newly diagnosed LEAD and no diabetes anamnesis and were not on hypolipidemic therapy; and 55 controls were without clinical presentation of LEAD and were normolipidemic. Analysis of HDL subclasses was performed by an innovative electrophoresis method on polyacrylamide gel (PAG), the Lipoprint HDL System.

RESULTS: In LEAD subjects, total HDL-C levels as well as HDL2 (intermediate-to-large particles) subfraction levels were decreased ($p<0.0001$ and $p<0.019$ respectively). Interestingly the HDL3 (small particles) subfraction was significantly higher and lost its proportional relationship within the HDL cholesterol fraction ($p<0.025$, $p<0.01$ respectively).

CONCLUSION: These findings pointed out that:

- the reduction of HDL-C and especially HDL2 subpopulation opposite to the increase of small HDL3 subclass may be considered as important predictors of cardiovascular diseases.
- there are undisputable advantages of using Lipoprint HDL to identify HDL subfractions; the presence of high concentration of small HDL in patients with PAD/LEAD emphasizes that the potentially proatherogenic subclass of HDL family is linked to small HDL.

Abbreviations:

ABI	- ankle-brachial index
BMI	- body mass index
DM	- diabetes mellitus
F	- female
HDL	- high density lipoprotein
LEAD	- lower extremity artery disease
LDL	- low density lipoprotein
M	- male
nm	- nanometer
ns	- non significant
p	- level of significance
PAD	- peripheral artery disease
PAG	- polyacrylamide gel
SD	- standard deviation
VLDL	- very low density lipoprotein

ORIGINAL ARTICLE

INTRODUCTION

Lipoproteins are composed of a heterogeneous mixture of particles with specific size, density and buoyancy.

The high density lipoprotein (HDL) family forms a protective part of plasma lipoproteins (Gordon *et al.* 1989; Gordon *et al.* 1977). It consists of large HDL, intermediate HDL, and small HDL subclasses (Kostner & Laggner 1989). Recent studies pointed out that the quality of HDL cholesterol is as important as the quantity in terms of atherogenesis. The large HDL and intermediate HDL subclasses are widely considered as anti-atherogenic parts of the HDL family (Alabakovska *et al.* 2002). The possible involvement of the small HDL subclass in atherogenesis is currently the subject of wide interest.

The association of possible HDL cholesterol (HDL-C) abnormality with peripheral arterial disease has not been investigated yet. In this study HDL subclasses were identified in patients with LEAD. Patients with peripheral artery disease (PAD) in the meaning of LEAD as a consequence of atherosclerosis may be often affected by lower extremity pain, worsen ambulation and in addition mutilating wounds. Furthermore patients with LEAD are not rarely affected by normal-to-high HDL atherogenic dyslipidemia. Thus, they tend to have significantly higher risk of cardiovascular morbidity and mortality (Oravec *et al.* 2012; Oravec *et al.* 2011a,b,c,d).

According to particle size it is possible to differentiate three major HDL cholesterol subclasses: large HDL (8.8–13 nm), intermediate HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm). By density range HDL cholesterol is divided into HDL2 (1.063–1.125 g/mL) (intermediate-to-large particles) and HDL3 (1.125–1.21 g/mL) (small particles) subspecies (Asztalos *et al.* 2004; Rainwater *et al.* 1997; Otvos *et al.* 1992; Albers & Aladjem 1971).

Large and intermediate HDL cholesterol levels have been inversely associated with atherogenic index of plasma and BMI, positively with adiponectin, which acts against insulin resistance, atherogenesis and inflammation. On the contrary, small HDL particles seem to be positively associated with atherogenic index of plasma, BMI and metabolic dyslipidemia (Tsuzaki *et al.* 2012).

It has been suggested that large and intermediate HDL particles are carrying anti-atherogenic properties, on the other hand there exists a relationship between small HDL and cardiovascular risk factors (Cooney *et al.* 2009).

The apo A-I HDL particles form HDL2 and the apo A-I and A-II particles form HDL3. There is a strong association between the apo A-I HDL particles and the cholesterol mobilization effect from periphery. Nevertheless HDL particles containing apo A-I and apo A-II show lower mobilizing effect of cholesterol from periphery (Kralova Lesna *et al.* 2012; Huang *et al.* 1995). We have an evidence that apo A-II is associated with visceral fat accumulation and impairment of the metabolism of large VLDL, which is rich in triglycerides (Despres 2007).

Lower extremity artery disease is a serious organovascular arterial disease and is a powerful predictor of cardiovascular, cerebrovascular, renovascular and other organovascular diseases (Gašpar *et al.* 2011; Gavorník 2010). Patients with LEAD exhibit normal-to-high atherogenic dyslipidemia, including HDL spectrum presumably. Probably small HDL may likely make up the atherogenic part of the HDL family and in this way build up proatherogenic phenotype which places people at high risk for LEAD. Exclusion of patients with diabetes mellitus was performed because there is a complex modification of physiologic lipoprotein metabolism especially by glycation (HDL loses its anti-inflammatory property and even induces more inflammation if previously exposed to AGE-albumin) (Okuda *et al.* 2011; Austin *et al.* 1990). The aim of this pilot study was to explore major representative HDL subclasses of the HDL family in patients with LEAD.

PATIENTS AND METHODS

This study was carried out on Slovak individuals recruited from the pool of outpatients from the department of internal medicine. LEAD was diagnosed according to the ESC Guidelines on the diagnosis and treatment of peripheral artery diseases, including ultrasound methods like the ankle-brachial index (ABI) (Gavornik *et al.* 2010).

A group of newly diagnosed LEAD individuals consisted of 30 male (average age 56±8) and 21 female (average age 61±7) in claudication stage – C2 according to the Angiologic Section of Slovak Medical Chamber (Gavorník 2010); Fontaine stage II, of combined atherosclerotic (E1) and arteriolosclerotic (E2) genesis, mixed proximal and microvascular type (AAP and AAMV) in second pathophysiological stage of vascular damage (P2).

They were all non-smokers who were not receiving any hypolipidemic pharmacotherapy and did not have diabetes mellitus.

The control group consisted of 55 normolipidemic probands, all non-smokers, who had no clinically apparent impairment, or laboratory signs of cardiovascular disease. There were 29 male (average age 51±14) and 26 female (average age 55±13) probands in the control group.

All subjects gave written, informed consent, and the study was approved by the local ethics committee. A blood sample from a cubital vein was collected in the morning after a 12 hour fasting period. EDTA-K2 plasma was obtained for the analysis of HDL subfractions, including large HDL, intermediate HDL and small HDL. The separation and measurement was performed by the Lipoprint HDL system (Quantimetrix Corp., CA, USA) (Morais *et al.* 2005a,b; Morais *et al.* 2003) using an innovative electrophoresis method on polyacrylamide

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gel (PAG), the Lipoprint LDL&HDL system (Rainwater *et al.* 1997). A colour coded profile was generated where each HDL subfraction was clearly seen.

Statistical evaluation used data expressed as means ± standard deviation for continuous variables and proportions for categorical variables. Distribution was tested for normality by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Subjects were matched by age and sex. The differences between groups were analyzed by Student´s T-Test. Pearson correlation analysis was used to evaluate the relationship between total HDL cholesterol and its subfractions. The level of significance was set at $p<0.05$.

RESULTS

Individuals diagnosed with LEAD and the control group were matched for age and gender (Table 1).

Table 2 shows quantitative composition of the HDL subpopulations obtained in 51 examined subjects with LEAD compared to the control group. Patients who developed LEAD had lower total HDL cholesterol concentrations ($p<0.0001$). The analysis of the HDL subclasses of the patients with LEAD showed a significantly lower proportion of intermediate HDL ($p<0.019$) and moreover significantly higher levels of small HDL (HDL3) ($p<0.025$). Figure 1 represents subjects with LEAD, meanwhile Figure 2 healthy individuals. The concentrations of large HDL were not significantly changed.

There was strong correlation between total HDL and their subfractions in controls but not in LEAD affected subjects where the correlation was disturbed in relation to small HDL (asterisk – significant at the level 0.01) (Table 3).

DISCUSSION

The new diagnostic method, Lipoprint system, quantifies not only the well-known atherogenic lipoproteins like small LDL (Hoefner *et al.* 2001), but can separate certain HDL subpopulations with the likely proatherogenic effect (Van *et al.* 2007). By using this methodological diagnostic novelty it has been found out that LEAD patients seem to have an atherogenic lipoprotein profile in meaning of low large-to-intermediate HDL subfraction and high small HDL subfraction. Additionally it has to be stressed that LEAD subjects had disturbed constellation of HDL subfractions, malfunctioning small HDL subfraction, pointed out by lost correlation with other HDL subpopulations. Selecting subjects without high-modifying factors like DM, hypolipidemic therapy and smoking made this experiment more reliable. The number of participants and lack of anthropometric parameters can be considered as limitation.

Based on these findings, it can be assumed that HDL cholesterol subfractions, especially small HDL play a much more important role in atherogenesis than it used to be generally accepted. Does it always stand about cholesterol that less is more? This frequently asked medical question about cholesterol can be answered: Yes, when we talk about particle size and functionality.

Until now, the main pathogenetic factor associated with vascular atherosclerotic injury was hypercholesterolemia, particularly high levels of small LDL. Extended view on atherogenesis and consequent cardiovascular events comprise residual risk that is accompanied with HDL disturbance (Dukát 2008). This work can contribute to the clue towards the complexity of HDL subpopulations involving the ischemic and sclerotic processes in vascular wall (like arteriolosclerosis, mediocalcinosis) and in this manner to better evaluate the global cardiovascular risk.

Aboyans *et al.* (2011) investigated other than usually accepted risk factors for lower extremity artery disease (smoking, hypertension, traditional conception of dyslipidemia, diabetes mellitus) with conclusion that in subjects without traditional risk factors is LEAD often common. In our study potentially new proatherogenic lipoprotein detected in subjects with LEAD – small HDL particles – has been described. Their presence clarifies the problem of atherogenic dyslipidemia.

These results can be taken as an additional idea for a new approach to prevention and treatment of cardiovascular diseases in future clinical practice. Only

Tab. 1. Demographic characteristics of individuals with LEAD and controls.

	LEAD subjects (51)	Controls (55)	*p*-value
Age	58±8	53±14	ns
Sex ratio (M/F)	30/21	29/26	ns

Tab. 2. Lipoprotein concentrations of HDL subfractions.

	HDL total	HDL large	HDL intermediate	HDL small
		Mean(mmol/l) ± SD		
LEAD subjects (n=51)	1.09±0.19	0.48±0.15	0.52±0.10	0.23±0.10
Control (n=55)	1.28±0.27	0.50±0.21	0.57±0.10	0.19±0.06
p-value	<0.0001	ns	0.019	0.025

Tab. 3. Assessment of correlation between total HDL lipoprotein and HDL subclasses.

		HDL large	HDL intermediate	HDL small
LEAD	HDL total	0.613*	0.610*	0.092
Controls	HDL total	0.861*	0.662*	0.348*

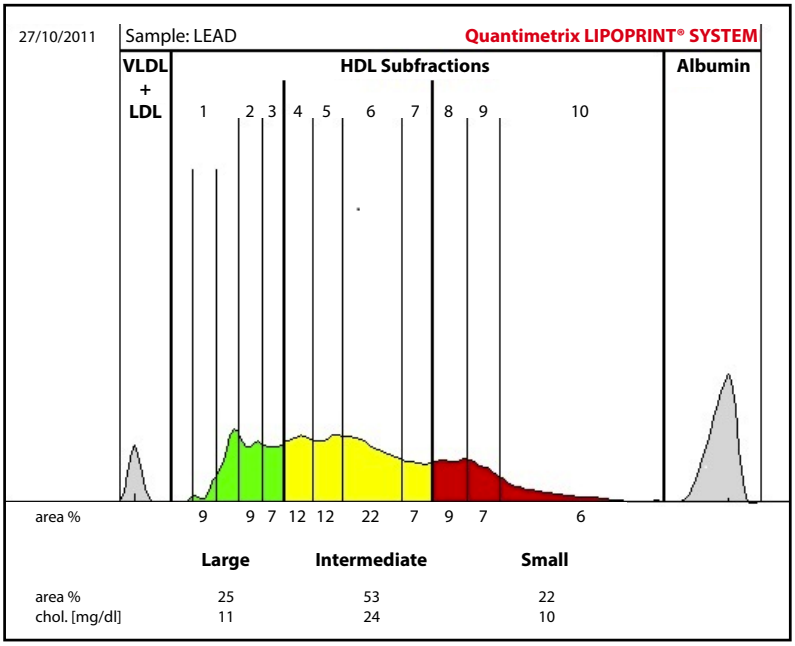

Fig. 1. Lower extremity artery disease, HDL subfractions.

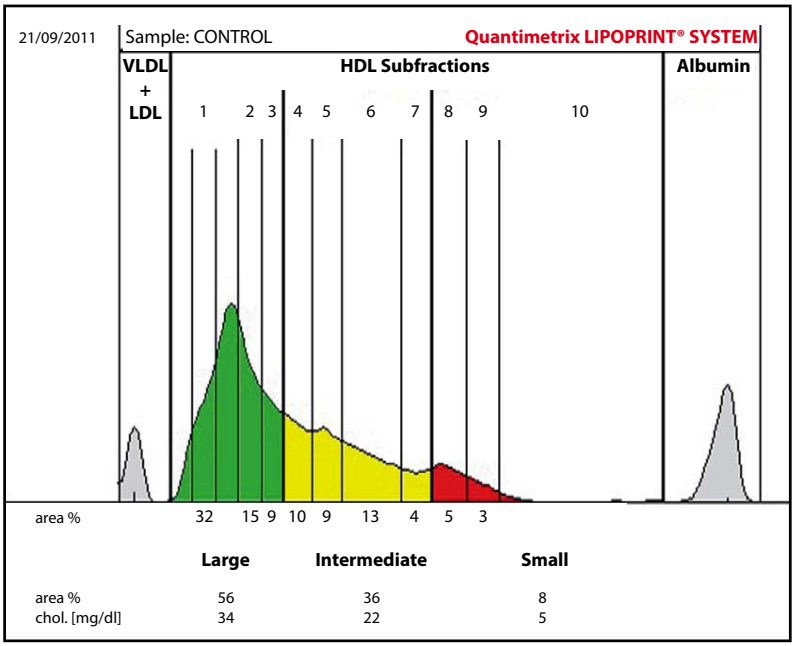

Fig. 2. Control, HDL subfractions.

complex analysis of major lipoprotein classes can provide effective assessment of overall cardiovascular risk profile.

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