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Znaczenie frakcji cholesterolu HDL w udarze niedokrwiennym mózgu o nieznanej etiologii (ESUS)

The meaning of HDL subfractions in ischemic stroke of undertermined etiology (ESUS)

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Abstract

Background. Approximately 1.1 million inhabitants of Europe suffer from a stroke each year, and ischemic stroke accounts for approximately 80% of all cases. A substantial proportion of stroke risk remains unexplained. Identification of the prognostic factors is essential in order to prepare an adequate preventive strategy. ***The Aim.*** The study aimed to assess high density cholesterol (HDL) subfractions in patients with ischemic with stroke of undetermined etiology (ESUS). ***Material and methods***. We prospectively investigated 520 patients with confirmed ischemic stroke. Out of them we included 65 patients to ESUS group [age 54 (47-58) years, 42% males]. 36 patients without stroke were control group [age 53 (47-58) years, 61% males]. In all patients HDL subfraction distribution was measured using Lipoprint (Quantimetrix). ***Results.*** The level of HDL cholesterol was significantly lower in ESUS group than in controls [1,1 mmol/L, (0,95-1,46) vs 1,37 (1,19-1,6) p=0,02]. The levels of small subfractions of HDL cholesterol were significantly higher in patients with stroke (p=0,004). Patients in stroke group had lower levels of large HDL [13,51 mg/dl (±6,21) vs 18,12 (±7,29); p=0,002]. In the multivariate analysis the only HDL subfraction significantly associated with the occurrence of ESUS was low level of HDL -1 (odds ratio [OR] 5,6 95%Cl 2,074-15,176; *p*=0,001). ***Conclusion.*** The level of high large HDL –C especially HDL 1 was inversely associated with ESUS stroke risk. When looking for causes of ischemic strokes, attention should be paid on HDL level and also HDL subfractions. Understanding the mechanism of stroke formation is extremely important to choose better therapy that will reduce the negative effects of stroke and reduce the risk of a possible stroke recurrence. *Geriatria 2019; 13: 5-11.*

Keywords: stroke, high-density lipoprotein cholesterol, lipoprint

Streszczenie

Wstęp. Każdego roku około 1,1 miliona mieszkańców Europy cierpi na udar mózgu, z czego udar niedokrwienny stanowi około 80% wszystkich przypadków. U znacznej część udarów przyczyna pozostaje niewyjaśniona. Identyfikacja czynników prognostycznych jest niezbędna do przygotowania odpowiedniej strategii profilaktycznej. ***Cel.*** Badanie miało na celu ocenę subfrakcji cholesterolu o wysokiej gęstości (HDL) u pacjentów z udarem niedokrwiennym o nieokreślonej etiologii (ESUS). ***Materiał i metody.*** Badaliśmy prospektywnie 520 pacjentów z potwierdzonym udarem niedokrwiennym. Wśród nich było 65 pacjentów zakwalifikowana jako ESUS [wiek 54 (47-58) lat, 42% to mężczyźni]. 36 pacjentów bez udaru było grupą kontrolną [wiek 53 (47-58) lat, 61% to mężczyźni]. U wszystkich pacjentów mierzono rozkład subfrakcji HDL za pomocą urządzenia Lipoprint (Quantimetrix). ***Wyniki.*** Poziom cholesterolu HDL był znacznie niższy w grupie ESUS niż w grupie kontrolnej [1,1 mmol / L, (0,95-1,46) vs 1,37 (1,19-1,6) p = 0,02]. Poziom małych subfrakcji cholesterolu HDL był istotnie wyższy u pacjentów z udarem (p = 0,004). Pacjenci w grupie z udarem mieli niższe poziomy dużych subfrakcji HDL [13,51

mg / dl (± 6,21) vs 18,12 (± 7,29); p = 0,002]. W analizie wieloczynnikowej jedyną subfrakcją HDL istotnie związaną z występowaniem ESUS był niski poziom HDL -1 (iloraz szans [OR] 5,6 95% Cl 2,0745,176; p = 0,001). ***Wnioski.*** Wysoki poziom dużych HDL-C, zwłaszcza HDL 1, był odwrotnie proporcjonalnie związany z ryzykiem udaru ESUS. Szukając przyczyn udarów niedokrwiennych, należy zwrócić uwagę na poziom HDL, a także na subfrakcje HDL. Zrozumienie mechanizmu powstawania udaru jest niezwykle ważne, aby wybrać lepszą terapię, która zmniejszy negatywne skutki udaru i zmniejszy ryzyko ewentualnego nawrotu udaru. *Geriatria 2019; 13: 5-11.*

Słowa kluczowe: udar, lipoproteiny o dużej gęstości, lipoprint

Introduction

In 2014, this term *Embolic stroke of undetermined source* (ESUS) was coined by the CS/ESUS international working group [1]. Up to a third of strokes are rendered cryptogenic or of undetermined etiology. This number is specifically higher in younger patients. At times, inadequate diagnostic workups, multiple causes, or an under-recognized etiology contributes to this statistic. ESUS, a new clinical entity particularly refers to patients with embolic stroke for whom the etiology of embolism remains unidentified despite through investigations ruling out established cardiac and vascular sources [2]. In the ASCO classification, cause is completely unknown when stroke subtyping does not confer to atherosclerosis (A), small vessel disease (S), cardiac disease (C), or other cause (O) [1]. Dyslipidemia is an important risk factor for coronary artery disease and stroke [3]. Lipid abnormalities, including high levels of low-density lipoprotein cholesterol (LDL-C), elevated triglycerides and low levels of high-density lipoprotein cholesterol (HDL-C) are confirmed risk factors of CV events [4]. Lipoproteins are major mediators of atherosclerosis, which remains the leading cause of death in the world. Despite lowering LDL-C has convincingly been shown to reduce major adverse cardiovascular events (MACEs), residual cardiovascular risk remains high in a significant number of patients [5].

Human plasma HDLs are a highly heterogeneous lipoprotein family consisting of several subclasses differing in density, size, shape and lipid and protein composition [6].

HDL is separated into 10 subfractions: HDL 1-3 are defined as large HDL; HDL 4-7 are defined as intermediate HDL; HDL 8-10 represents small HDL. The beneficial effect of HDL is largely attributable to its key role in reverse cholesterol transport, whereby excess cholesterol in the peripheral tissues is transported to the liver, reducing the atherosclerotic burden. However, mounting evidence indicates that HDL also has pleiotropic properties, such as anti-inflammatory, anti-oxidative, and vasodilatory properties, which may contribute in reducing the incidence of cardiovascular events. [7] In light of the epidemiologic evidence that low HDL-C was inversely related to coronary outcomes, raising HDL-C has been proposed as a potential therapeutic target. However, after the failure of several HDL-C raising drugs including torcetrapib, dalcetrapib, and niacin, attention is focusing on specific HDL subfractions as possible cardiovascular protectors or negative risk factors [5]. The aim of this study was to assess HDL subfractions in patients with ESUS.

Material and methods

Study population

We prospectively investigated 520 patients with confirmed ischemic stroke. Out of them we included 65 patients to ESUS group [age 54 (47-58) years, 42% males]. 36 patients without stroke were control group [age 53 (47-58) years, 61% males]. In all patients HDL subfractions distribution were measured using Lipoprint (Quantimetrix). The exclusion criteria were as follows: unstable hypertension, atrial fibrillation, hyperthyroidism, pregnancy and breastfeeding, dialysis, cancer, autoimmunological disease, reception of cytostatic, immunosuppressive drugs, glycocorticosteroids, antiretroviral drugs, transplant and treating of hematogenous preparation during last 6 months, active infection, alcoholism, addiction from medicines, infection of HBV (hepatitis B virus), HCV (hepatitis C virus), HIV (human immunodeficiency virus), surgical intervention or serious injury during last 1 month, vaccination during last 3 months, incapable of giving agreement.

Approval from the Bioethics Commission of the Medical University of Lodz (No. RNN/272/16/KE) was obtained. All methods in this study were performed

in accordance with the guidelines and regulations approved by the Bioethics Commission of the Medical University of Lodz. Written informed consent was obtained from all the patients.

HDL-subfractions analysis

The LipoPrint© test (Quantimetrix Corporation, Redondo Beach, CA, USA) utilizes non-denaturing, linear polyacrylamide gel electrophoresis to separate and measure lipoprotein subfractions. The electrophoresed gels were scanned to determine the relative area of each lipoprotein subfractions, which was multiplied by the total cholesterol of the sample in order to calculate the amount of cholesterol in each subfraction. Additional advantage was that this method requires only 25 µl of serum or plasma. The LipopPrint© test procedure consisted of 4 basic steps: a) electrophoresis; b) scanning; c) analysis of the results; d) report generation. 25 µl of sample was mixed with 300 µl of Lipoprint Loading Gel and placed upon the upper part of the 3% polyacrylamide gel. After 30 min of photopolymerisation at room temperature, electrophoresis was performed for 50 min with 3 mA for each gel tube. Each electrophoresis chamber involves two quality controls (provided by the manufacturer). For quantification, scanning was performed with a digital scanner and a Mac personal computer (Apple Computer Inc, USA). After scanning, electrophoretic mobility and the area under the curve were calculated qualitatively and quantitatively with the Lipoprint system Template and the Lipoware software (property of Quantimetrix Co, Redondo Beach, CA, USA), respectively. HDL subfractions were distributed as ten bands (HDL-1 to HDL-10, respectively). HDL-1, HDL-2 and HDL-3 are defined as large HDL; HDL-4, HDL-5, HDL-6 and HDL-7 are defined as intermediate HDL; HDL-8, HDL-9 and HDL-10 comprise the small HDL portion. The cholesterol concentration of each HDL subfraction was determined by multiplying the relative area under the curve of each subfraction by the HDL cholesterol concentration of the sample.

Other Laboratory tests

Blood samples for standard laboratory tests were collected from patients assigned to either group in a hospital setting. The blood for correct lipidogram evaluation was taken from patients who were fasting, that is, refraining from eating meals for at least 12 hours. Triglycerides, total, and HDL-C levels were evaluated using the AU680 device (Beckman Coulter Poland, Warsaw, Poland). TC was measured enzymatically with standardized calibrators: cholesterol esterase and oxidase, respectively according to the manufacturer's specifications. HDL-C was measured enzymatically with lipoproteins immune complex with standardized calibrators: cholesterol esterase and oxidase. LDL-C concentrations was calculated by Friedewald's formula: LDL-C (mmol/L) = TC - HDL-C - TG/2.2. TG was measured enzymatically with glycerol phosphate oxidase and H202 determination in the presence of peroxidase.

Statistical analysis

The STATISTICA 13.1 software package (StatSoft, Poland) was used for analysis. Results were be considered significant if $p<0,05$. The Shapiro-Wilk test was used to assess the normality of distribution. Data were presented as mean and standard deviation or median and interquartile range (25%-75%), depending data scale and distribution. To compare two groups, Student's t-test for continuous variables with normal distribution and with homogeneity of variance was used. For data with normal distribution but with failing homogeneity of variance, the Welsh test was conducted. Mann-Whitney U test for non-normally distributed variables was used.

The dichotomy data was analyzed by the chi squere test or chi squere with Yeates correction. Variables significant in univariate analysis (significance level $p < 0.05$) were used for the construction of a multivariate logistic regression model. The quality of the models and the usefulness of the HDL subfractions were evaluated using receiver operating characteristic (ROC) curves.

Results

General characteristics of patients

There were no differences in blood pressure values as well as in body mass index (BMI) in both groups. Among patients with ESUS 52% had stable hypertension, 9% had coronary artery disease, 38% were smoking. In control group 43% patients had hypertension, also 9% had coronary artery disease and 13% were smoking. There were no significant differences in the way of treatment between groups. Basic characteristic of patients is presented in Table I.

Table I. Basic characteristics of patients in both groups

Parameter	Patients with ESUS	Controls	P
Number of patients	65	36	0,89
Median age (years)	54 (47-58)	53 (47-58)	0,001
Gender (male) (%)	42	61	0,059
BMI (kg/ m^2)	26 (22,4-28,7)	25 (21,8-28,1)	0,49
Hypertension (%)	52	43	0,36
CAD (%)	9	9	0,76
Smoking (%)	38	13	0,02
SBP (mmHg)	136 (±18,7)	128 (±19,7)	0,053
DBP (mmHg)	83 (±9,9)	82 (±9,0)	0,72
Creatinine (μm/l)	1,58* (1,11-2,0)	1,3* (0,86-1,7)	0,21

ABBREVIATIONS: BMI, body mass index; CAD, coronary artery disease; DBP; diastolic blood pressure; SBP; systolic blood pressure

Lipids assessment in stroke patients

There were no differences between stroke and control group in the levels of LDL C, total cholesterol, triglycerides. The level of HDL-C was significantly lower in ESUS group than in controls [1,19 mmol/L, (0,95-1,46) vs 1.37 (1,19-1,6) p=0,02] (Table II).

HDL-C subfractions assessment in both groups

Patients in stroke group had lower levels of large HDL [13,5 mg/dl (±6,21) vs 18,1 (±7,29); p=0,002] (Table III). In our study mainly low level of HDL-1 (large density) was associated with an elevated stroke risk [3 mg/dl (1-7) vs 6 (5-9); p=0,002; 6,75% (3,2-11,5) vs 11,2 (7,4-15,7) p=0,006] (Table IV and V). Patient in ESUS group had higher level of small HDL like HDL-10 and HDL-8 compared to controls [11,8% (7,1-24,1) vs 6,10 (1,8-12,2) p=0,003; 7,9% (6,4-9,5) vs 6,4 (4,9-8,8) p=0,04; respectively] (Table V).

Table II. Evaluation of biomarkers in both groups

Parameter	Patients with ESUS (mediana)	Controls (mediana)	P
LDL cholesterol (mmol/l)	2,83 (2,07-4,0)	3,02 (2,66-3,67)	0,37
Triglycerides (mmol/l)	1,6 (1,13-2,02)	1,36 (0,86-1,76)	0,257
HDL cholesterol (mmol/l)	1,19 (0,95-1,46)	1,38 (1,21-1,6)	*0,011*

ABBREVIATIONS: HDL, high density lipoprotein; LDL, low density lipoprotein

Table III. Evaluation of HDL subfraction dividing into large, intermediate and small in both groups

Evaluation of HDL subfraction in both groups			
Parameter	**Patient with ESUS (mediana)**	**Controls (mediana)**	**P**
Large [%]	30,50 (23,2-35,5)	35,90 (26,4-40,5)	*0,03*
Intermediate [%]	45,20 (28,2-50,9)	45,00 (41,6-51,3)	0,39
Small [%]	27,65 (17,4-42,8)	18,50 (11,7-29,5)	*0,004*
Large [mg/dl]	13,51±6,21*	18,12±7,29*	*0,002*
Intermediate [mg/dl]	20,31±9,62*	24,70±9,42*	0,05
Small [mg/dl]	13,94±6,5*	11,54±8,18*	0,13

For the parameters with non-normal distribution there are given median values (lower and higher values).
For the parameters with normal distribution there are given mean values ± standard deviation (SD), [1] p t Student, [2] p Levenea.
ABBREVIATIONS: HDL high density lipoprotein cholesterol, (*) SD Standard deviation

Table IV. Evaluation of HDL subfraction [1-10] (mg/dl) in both groups

Evaluation of HDL subfraction 1-10 [mg/dl] in both groups.			
Parameter	**Patient with ESUS (mediana)**	**Controls (mediana)**	**P**
HDL-1 [mg/dl]	3,00 (1,0-7,0)	6,00 (5,0-9,0)	*0,002*
HDL-2 [mg/dl]	5,00 (3,0-8,0)	7,00 (4,0-10)	0,04
HDL-3 [mg/dl]	4,00 (2,0-5,0)	4,00 (3,0-7,0)	0,23
HDL-4 [mg/dl]	4,00 (1,0-6,0)	5,00 (4,0-8,0)	*0,009*
HDL-5 [mg/dl]	5,00 (3,0-6,0)	5,00 (4,0-7,0)	0,32
HDL-6 [mg/dl]	8,77±4,15*	10,22±4,48*	0,13
HDL-7 [mg/dl]	4,00 (3,0-5,0)	4,00 (3,0-4,0)	0,63
HDL-8 [mg/dl]	4,00 (3,0-5,0)	3,00 (2,0-5,0)	0,20
HDL-9 [mg/dl]	3,00 (3,0-4,0)	2,00 (2,0-4,0)	0,18
HDL-10 [mg/dl]	6,00 (3,0-10,0)	3,00 (1,0-8,0)	*0,02*

For the parameters with non-normal distribution there are given median values (lower and higher values).
For the parameters with normal distribution there are given mean values ± standard deviation (SD),
[1] p t Student, [2] p Levenea.
ABBREVIATIONS: HDL high density lipoprotein cholesterol (subfractions 1-10), (*) SD Standard deviation

Table V. Evaluation of HDL subfraction [1-10] (%) in both groups

Evaluation of HDL subfraction 1-10 [mg/dl] in both groups.			
Parameter	**Patient with ESUS (mediana)**	**Controls (mediana)**	**P**
HDL-1 [%]	6,75 (3,2-11,5)	11,20 (7,4-15,7)	*0,006*
HDL-2 [%]	10,75 (7,8-14)	13,00 (8,1-17,2)	0,11
HDL-3 [%]	7,65 (5-9,7)	9,40 (5,5-11,5)	0,32
HDL-4 [%]	8,60 (1,1-11,1)	11,30 (6,1-12,8)	0,01
HDL-5 [%]	9,20 (6,4-11,5)	9,70 (7,8-11,3)	0,69
HDL-6 [%]	18,15 (15,2-22,2)	17,50 (15,8-21,5)	0,82
HDL-7 [%]	7,25 (6,4-8,6)	7,20 (5,5-8,6)	0,49
HDL-8 [%]	7,90 (6,4-9,5)	6,40 (4,9-8,8)	*0,04*
HDL-9 [%]	7,07±3,16*	5,74±3,32*	0,06
HDL-10 [%]	11,80 (7,1-24,1)	6,10 (1,8-12,2)	*0,003*

For the parameters with non-normal distribution there are given median values (lower and higher values).
For the parameters with normal distribution there are given mean values ± standard deviation (SD),
[1] p t Student, [2] p Levenea.
ABBREVIATIONS: HDL high density lipoprotein cholesterol (subfractions 1-10), (*) SD Standard deviation

Multivariate logistic regression analysis

In the multivariate analysis the only HDL subfraction significantly associated with the occurrence of ESUS was low level of HDL -1 (odds ratio [OR] 5,6 95%Cl 2,074-15,176; *p*=0,001) (Table 6 and Figure 1).

Table VI. The multivariate analysis - stepwise logistic regression

Variable	OR	95% CI for OR		p-value
		Lower limit	Upper limit	
HDL 1	5,6	2,074	15,176	0,001

ABBREVIATIONS: HDL High Density Lipoprotein; OR odds ratio

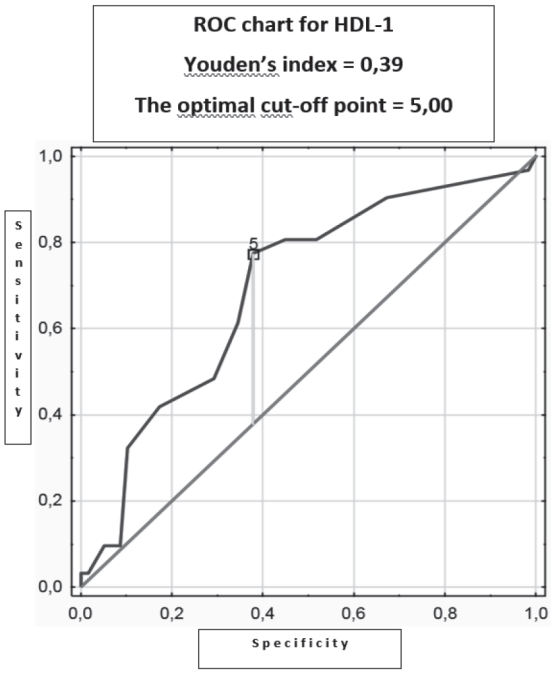

Figure 1. ROC chart for HDL 1.

Discussion

In this study we presented the influence of individual subfractions of HDL-C on ischemic stroke of undetermined etiology. We assessed that in our group of patients with ESUS the level of HDL-C was significantly lower compared to controls what is consistent with the results of the studies indicating low HDL –C as risk factor of cardio-vascular diseases. [8]

Patients in stroke group had lower levels of large HDL (HDL-8 and 9 and10) and higher level of small HDL (HDL-1and 2 and 3) compared to control group. In the multivariate analysis the only HDL subfraction significantly associated with the occurrence of ESUS was low level of HDL -1. Previous studies have shown reduced cardiovascular (CV) risk with increasing HDL-C levels.

A metaanalysis of 4 large prospective studies revealed that every 1 mg/dL decrease in HDL-C was related to a 2-3% increase in cardiovascular events .[9][10]

On the other hand in the study of Allard-Ratick et al. elevated HDL-C levels were paradoxically associated with an increased risk of adverse CV events in an at-risk population, suggesting dysfunctional HDL and impaired atheroprotection [11]. HDL particles are known to be heterogeneous - depending on the size and density of molecules, so-called subfractions are distinguished: in HDL ten particles (HDL 1-10). Heterogeneity HDL particles is also associated with their different biological activity - it is known that the subfraction of larger HDL molecules is responsible for the clinically beneficial effect with which HDL-C was commonly associated, while all other smaller subpopulations of HDL molecules - so-called medium and small HDL, may have adverse effects and do not inhibit the inflammatory process.[12]

The results of our study are confirm these reports. In the study of Li JJ et al. high large HDL-C was associated with lower MACEs risk independent of potential confounders. The authors concluded that higher large HDL-C but not medium, small, or total HDL-C is associated with lower cardiovascular risk, highlighting the potential beneficial of HDL subfractionation. [5]

In our study we observed higher level of large HDL-C in patients without stroke what may suggest their potentially protective function.

Žitňanová I et al. found that small HDL subfractions positively correlated with lipoperoxide levels and negatively with trolox equivalent antioxidant capacity in plasma suggesting a negative role of these subfractions what confirmed the hypothesis of atherogenic properties of small HDL subfractions. [13]

In our stroke group there was a higher percent of small HDL what may suggest potential connection of atherogenic properties of small HDL subfractions with ESUS.

Summary

Higher level of large HDL-C is associated with lower risk of ESUS, highlighting the potential beneficial of HDL subfractionation. When looking for causes of ischemic strokes, particular attention should be paid to HDL subfractions. Understanding the mechanism of stroke formation is extremely important to choose better therapy that will reduce the negative effects of stroke and reduce the risk of a possible stroke recurrence.

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Conflict of interest
None

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