Extended lipid profile in Romanian ischemic stroke patients in relation to stroke severity and outcome: a path analysis model

Adina Hutanu[1], Mihaela Iancu[2], Minodora Dobreanu[3], Oana Roxana Oprea[4], Stefan Barbu[4], Smaranda Maier[5], Amelia Tero-Vescan[6], Zoltan Bajko[5], Rodica Balasa[5]

[1]CCAMF, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania
[2]3rd Department of Medical Informatics and Biostatistics, University of Medicine and Pharmacy "Iuliu-Ha-tieganu", Cluj-Napoca, Romania
[3]Department of Laboratory Medicine, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania
[4]George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania
[5]Department of Neurology, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania
[6]Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Romania

Corresponding author:
Prof. Minodora V. Dobreanu
University of Medicine, Pharmacy, Science and Technology Tirgu Mures
38 Gh Marinescu, 3rd floor, 107
540136 Tirgu Mures, Romania
Phone: +40744201611
E-mail: minodora.dobreanu@umfst.ro

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Abstract

Introduction: Our aim was to evaluate the extended lipid profile in ischemic stroke patients and the relationship with stroke type, severity and outcome.
Material and methods: We prospectively enrolled 124 ischemic stroke patients and 40 healthy controls; baseline plasma and erythrocyte membrane fatty acids concentrations and common lipid profile were analysed. Stroke severity was evaluated by NIHSS on admission, while the functional outcome was defined by mRS at discharge and after 3 months.
Results: Total cholesterol, triglycerides, HDL-cholesterol, DHA, adrenic, stearic and lauric acid were all lower in patients, taking into account that 87.7% of patients did not receive statins before admission. There was a different pattern in plasma and erythrocyte membrane of fatty acids between patients and controls, also omega-3 index was significantly lower in patients. Patients with poor outcome without statins had significantly lower triglyceride (p = 0.028), while the total cholesterol levels were significantly lower in patients with poor outcome (p = 0.03) but with treatment initiated after admission. Bivariate analysis revealed that patients with poor outcome had significantly lower triglyceride levels regardless the statins use, while the total cholesterol and HDL-cholesterol levels were significantly lower in patients with poor outcome under statin treatment. The long-term outcome were positively influenced by age (β^* = 0.22, p = 0.001), and NIHSS score at admission (β^* = 0.55, p < 0.001), and negatively by cholesterol levels (β^* = –0.17, p = 0.031).
Conclusions: DHA, adrenic, stearic and lauric acid were lower in stroke patients; plasma adrenic acid was consumed during the acute phase. The most important predictors for long-term outcome was NIHSS at admission followed by age and total cholesterol.

Key words: DHA, ischemic stroke, National Institutes of Health and Stroke Scale (NIHSS), stroke outcome, modified Rankin Scale (mRS), adrenic acid.

Introduction

Lipids have a controversial role in ischemic stroke patients; while some studies show an association of low levels of lipids with stroke severity and poor outcome, others do not. It is well known that elevated concentrations of total cholesterol, low-density lipoprotein cholesterol (LDL cholesterol) and triglycerides are considered important risk factors for cardio-cerebrovascular diseases. As secondary prevention of recurrent ischemic stroke, lipid-lowering therapies are generally associated with favorable results and reduced mortality. Contradictory aspects appear during acute ischemic stroke episodes; some studies have shown that lower levels of total cholesterol [1–3] and triglycerides [4–6] are associated with poor outcome and more severe neurological deficit, while others affirm the positive correlation between serum triglyceride levels and stroke severity [7]. Another important lipid class with a close relationship with the serum total cholesterol level is represented by fatty acids (FAs). Classified into saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), the plasma FAs pool reflects dietary intake during a short period of time, while RBC membrane FAs reflect the levels within the last 3 months, as erythrocyte life span is more reliable for evaluating the FAs profile [8]. FAs concentrations is determined both by dietary intake and endogenous synthesis under $\Delta 9$, $\Delta 6$, and $\Delta 5$ desaturase activity [9]. Alpha linolenic acid (ALA) and linoleic acid (LA) are $\omega 3$ and $\omega 6$ essential fatty acids, also precursors of important long-chain metabolites under the activities of elongases and $\Delta 6$ and $\Delta 5$ desaturases [10]. Among them, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) seem to have important anti-inflammatory effects and confer the protection of neurons and the blood-brain barrier (BBB) during ischemia, as revealed by many experimental studies [11–14]. While arachidonic acid (AA) exerts mostly pro-inflammatory effects via pro-inflammatory eicosanoids – prostaglandins and thromboxanes in the presence of cyclooxygenase (COX) – and leukotrienes and lipoxins in the presence of lipoxygenase (LOX) [10], shortly after oxygen cessation, the nervous system will synthesize neuroprotectin D1 (NPD1), a potent anti-inflammatory component derived from DHA that inhibits pro-inflammatory cytokine production [15, 16]. Desaturase activities may vary among individuals, or under various circumstances; thus altered endogenous desaturation leads to an imbalance of both PUFAs and their long-chain metabolites AA, DHA, and EPA. However, $\Delta 6$ and $\Delta 5$ desaturase activities are in relation with the unsaturated fatty acid concentrations, provided from the exogenous intake or from the cell membrane pool, the ALA being preferentially desaturated, followed by LA [17]. The omega-3 index represents the sum of EPA and DHA content expressed as a percentage of the total FAs profile [18].

The aim of the study is to investigate the extended lipid profile in ischemic stroke patients in relation to a healthy group and stroke outcome.

Material and methods

In total, 124 consecutive patients with acute ischemic stroke, admitted within 72 h after clinical onset in Tîrgu Mureş regional stroke center during January 2015 – July 2016, along with 40 controls, were prospectively enroled in the study. The diagnosis of ischemic stroke was established by the on-call neurologist, based on the history (acute onset of neurological signs and symptoms), neurological examination and cerebral computed tomography (CT) scan. The study protocol complied with the World Medical Association Declaration of Helsinki and was approved both by the Ethics Committee of the University of Medicine and Pharmacy Tîrgu Mures, and the Emergency Clinical County Hospital Tîrgu Mureş (no. 112/17.11.2014); all participants signed an informed written consent form prior to recruitment in the study. Inclusion criteria were: adults within 72 h after clinical onset of ischemic stroke, without clinical signs of infection or anti-inflammatory treatment (except treatment for secondary stroke prevention), without cerebrovascular events 3 months prior to admission (ischemic or hemorrhagic stroke, traumatic brain injury (TBI)), and without major cardiac, renal, hepatic, autoimmune or malignant diseases, as exclusion criteria. Controls recruited for the study were adults, healthy volunteers (with similar distribution of age and gender as ischemic patients) who met similar admission criteria: no evident clinical signs of infection or inflammation, without history of stroke, TBI, major cardiac, renal, hepatic, autoimmune or malignant diseases. Only eligible patients (n = 114) were included in the study for the baseline lipid profile, while plasma and erythrocyte FA compositions were available only in 65 eligible patients.

Definition of stroke severity and functional outcome

After admission, all recruited patients underwent neurological examination; stroke severity was assessed by the National Institutes of Health and Stroke Scale (NIHSS) [19] on day 1 after admission and before discharge from the hospital. Stroke outcome was assessed by the modified Rankin Scale (mRS) at discharge from the hospital for short-term outcome; good functional outcome was considered mRS in the range 0–2 and poor

functional outcome mRS 3–6 (where 6 means death) [20]. Stroke subtype was classified according to TOAST criteria (Trial of Org 10172 in Acute Stroke Treatment) in large artery atherosclerosis (LAA), cardioembolic (CE), lacunar or small vessel occlusion (SVO) and other causes [21].

Blood sampling and measurement

For laboratory assessment of the lipid profile, the blood was harvested in the first morning after admission between 7 and 9 a.m. into clot accelerator tubes for serum, and sodium heparin tubes for plasma. Serum and heparin plasma aliquots were stored at –80°C until the lipid parameters were evaluated. Erythrocytes (Na heparin tubes) were also kept at –80°C until baseline red blood cells (RBC) membrane fatty acids were extracted and analyzed by liquid chromatography-mass spectrometry (LC/MS) (data were available only for 65 ischemic patients and 40 controls). Serum total cholesterol, triglycerides and HDL cholesterol were measured on an Architect 4000 (Abbott Laboratories Abbott Park, Illinois) while low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald formula.

Because plasma concentration more closely reflects recent dietary intake, for a more representative lipid profile, red cell membrane FAs were evaluated by LC/MS (Agilent 1200 HPLC Series system, Agilent Technologies, Santa Clara, USA), as described elsewhere [8]. Briefly, after membrane phospholipid hydrolysis, extraction in hexane and concentration by evaporation, the fatty acid profile was analysed by comparison with a mixture of standard samples. Plasma and RBC membrane FAs were expressed as concentrations of each lipid fraction; among the SFA class 16:0 palmitic acid, 18:0 stearic acid, and 12:0 lauric acid were quantified; 18:1 oleic acid was reported as ω9-MUFA; 18:2 linoleic acid, 22:4 adrenic acid and 20:4 arachidonic acid represent the ω6-PUFA class, while 22:6 DHA and 20:5 EPA represent the ω3-PUFA class.

Statistical analysis

All statistical data were analysed by SPSS software, using appropriate tests for continuous variables, *t*-test for parametric distribution data and Mann-Whitney test for nonparametric distribution. The continuous variables were expressed as mean (± SD) for parametric distribution and medians and IQR (interquartile range) for nonparametric data distribution. The χ^2 or Fisher's exact test was used for testing bivariate associations between the categorical variables. Stroke severity evaluated by NIHSS and functional outcome expressed by mRS were dichotomized into mild stroke (NIHSS ≤ 4) vs. moderate/severe (NIHSS > 4), and good outcome (mRS < 3) vs. poor outcome (mRS ≥ 3). Spearman rank correlation was performed in order to determine the correlation between lipid profile, stroke severity and functional outcome.

Path analysis was used to test a model with at least one dependent variable, the model involving the relationships between lipid profile (HDL cholesterol, total cholesterol, triglycerides), age, gender, NIHSS at admission and short-term outcome (mRS scores). Because the fatty acids were determined for a small sample of patients, we did not include the significant fatty acids from bivariate analysis in the tested model. The model depicted in the results contained one-way arrows showing the direction of the path from exogenous variables to endogenous variables and two-way arrows indicating the correlations between the two variables contained in the path. The path diagram showed the standardized path coefficient of the regressing exogenous variable on the endogenous variable. Because there were variables with deviation from Gaussian distribution (TG, NIHSS at admission), the model parameters were estimated using maximum likelihood estimation with robust standard errors (MLR) that was considered robust to non-normality. The goodness of fit of the tested model was described by the following criteria: a non-significant χ^2 test, root mean square error of approximation (RMSEA) < 0.05, non-significant test for RMSEA (*p*-value for RMSEA ≤ 0.05 should be > 0.05), standardized root mean square residuals (SRMR) < 0.08, comparative fit index (CFI) and Tucker-Lewis fit index (TLI) ≥ 0.90 [22].

Path analysis was conducted using the lavaan (0.5–23) package [23] in R software, version 3.4.0.

Statistical significance for all statistical tests including the obtained path model were achieved if the $p < 0.05$.

Results

Comparative analysis of lipid profile between patients and controls

The mean age ± standard deviation was 71.68 ±10.22 years for the patient group and 67.90 ±13.95 years for the control group, while gender distribution was 57% female vs. 43% male for the patient group and 57.5% female vs. 42.5% male for the control group. No significant difference in age ($p = 0.070$) or gender distributions ($p = 0.985$) was found between the two studied groups.

There were no differences in the frequency of statin use prior to admission, the groups being homogeneous (patients vs. controls: 10 (8.8%) vs. 4 (10.0%)).

Table I. Baseline serum lipid levels compared between patients and controls

Lipid parameters [mg/dl]	Stroke patients (n = 114)	Controls (n = 40)	P-value
Total cholesterol	184.1 ±45.7	214.7 ±42.8	0.0005
Triglycerides	103.6 (79.0–135.5)	118.8 (94.2–151.5)	0.038
HDL cholesterol	47.0 ±11.2	52.3 ±12.3	0.016
LDL cholesterol	113.6 ±39.9	133.9 ±38.3	0.006

Data are described as mean ± standard deviation or median (25% percentile – 75% percentile); p-values obtained from Student-t test for independent samples with equal variances or Mann-Whitney exact test.

Comparative analysis of the lipid profile between patients and healthy controls is summarised in Table I; there were significantly lower levels of all serum lipids: total cholesterol (p = 0.0005), triglycerides (p = 0.04), HDL cholesterol (p = 0.02) and LDL cholesterol (p = 0.004) in study subjects vs. controls. We did not find any differences in the total cholesterol, TG and HDL cholesterol averages in patients with pre-admission statin treatment, compared to those without prior treatment (p > 0.05).

Plasma and RBC membrane FA concentrations in patients and controls are summarised in Table II. There was higher plasma concentration of palmitic acid in the patient group compared to controls, but it was not significant (p = 0.083), while stearic and lauric acids were significantly lower in patients vs. controls (p < 0.05). Oleic acid was found to be significantly higher in ischemic patients vs. controls (p = 0.009), while among ω6-PUFA, linoleic acid and adrenic acid were lower in ischemic stroke patients than in controls (p < 0.001). There was no significant difference in the plasma concentration of AA (p = 0.942), but the ratio AA/DHA + EPA was significantly higher in patients compared to controls (p < 0.001), DHA being significantly lower in stroke patients than in healthy controls (p < 0.001). For SFAs, a similar pattern was observed in RBC membrane; only stearic and lauric acid values were statistically significant: p = 0.038 for stearic acid and p = 0.027 for lauric acid. The membrane DHA level was lower in patients vs. controls (p = 0.029) and the AA/DHA + EPA ratio followed the same trend in the RBC membrane as in plasma, being higher in patients compared to controls (p < 0.001). Omega-3 index was found to be very low, with a median 1.35 (IQR: 0.73–2.30) for stroke patients and 1.97 (IQR: 1.67–2.51) for controls. Despite the low omega-3 index values in both study groups, there was a significant difference between patients and healthy controls (p = 0.002).

The relation between lipid profile and stroke type

According to the TOAST subtype classification, AT stroke (n = 67) represents 58.8% of all patients, CE stroke (n = 43) represents 37.1% and SVO stroke (n = 4) represents only 3.4% of total stroke patients. In relation to stroke subtype, cholesterol, triglycerides and LDL cholesterol levels were higher in AT compared to CE + SVO stroke (p = 0.002 for cholesterol, p = 0.015 for triglycerides and p = 0.001 for LDL cholesterol), with no statistical difference regarding HDL cholesterol (p = 0.398). There was a significant difference regarding AA plasma level (p = 0.011) and DHA (p = 0.037), higher values being observed in CE + SVO stroke patients compared to AT stroke: 37.11 (IQR: 18.59–58.16) vs. 20.68 (IQR: 13.75–32.99) for AA plasma concentration and 3.78 (IQR: 2.89–6.25) vs. 2.69 (IQR: 1.99–5.61) for DHA. There were non-significant differences regarding plasma concentrations of lauric, linoleic and adrenic acids (p = 0.051, p = 0.083 and p = 0.067), higher values being observed in CE + SVO stroke patients compared to AT stroke: 3.10 (IQR: 1.39–4.03) vs. 1.84 (IQR: 0.95–2.95) for plasma concentration of lauric acid, 43.43 (IQR: 31.03–48.01) vs. 31.83 (IQR: 25.65–43.90) for linoleic acid and 0.65 (IQR: 0.17–1.02) vs. 0.30 (IQR: 0.12–0.84) for adrenic acid.

The association between lipid profile and stroke severity

In relation to stroke severity, total cholesterol and LDL cholesterol were significantly lower in patients with NIHSS > 4 compared to NIHSS ≤ 4 (p = 0.011 and p = 0.046). For HDL cholesterol, the difference was not statistically significant (p = 0.248) while triglyceride levels were lower in moderate/severe stroke compared to mild stroke (p = 0.066). There was no difference regarding plasma or red blood cell membrane FA composition between mild and severe stroke (p > 0.05).

The association between lipid profile and short-term stroke outcome

Because of the association between total cholesterol, HDL cholesterol, TG and use of statins after hospital admission (p < 0.05), in order to highlight the relation between classic lipid profile and mRS at discharge, statistical analysis was done by stratifying statin use after admission. 87.7%

Table II. Baseline plasma and red blood cells (RBC) membrane fatty acid (FA) profile between patients and controls

Plasma FAs [µg/ml]; median (IQR)	**Ischemic patients (*n* = 65)**	**Controls (*n* = 40)**	***P*-value**
Saturated FAs:			
Palmitic acid	282.59 (220.04–395.16)	248.84 (226.82–281.22)	0.083
Stearic acid	137.22 (83.41–187.94)	181.37 (155.96–205.84)	< 0.001
Lauric acid	2.12 (0.99–3.55)	4.20 (1.32–6.46)	< 0.001
ω9-MUFA:			
Oleic acid	736.86 (603.72–1047.41)	633.36 (535.01–727.15)	0.009
ω6-PUFA:			
Linoleic acid	35.45 (27.21–46.22)	49.11 (46.47–59.83)	< 0.001
Adrenic acid	0.47 (0.16–0.96)	1.35 (0.99–1.80)	< 0.001
Arachidonic acid	26.43 (15.46–43.62)	24.58 (17.99–35.63)	0.942
ω3-PUFA:			
EPA	0.037 (0.020–0.060)	0.043 (0.030–0.087)	0.051
DHA	3.10 (2.28–5.92)	7.86 (5.75–9.29)	< 0.001
AA/DHA + PA	9.09 (4.85–12.85)	3.52 (2.07–5.03)	< 0.001
Omega-3 index	0.26 (0.15–0.51)	0.66 (0.49–0.81)	< 0.001
RBC membrane FAs [µg/ml]; median (IQR)	**Ischemic patients (*n* = 65)**	**Controls (*n* = 40)**	***P*-value**
Saturated FAs:			
Palmitic acid	124.12 (86.41–206.25)	136.65 (115.73–189.29)	0.362
Stearic acid	1.82 (1.22–2.88)	2.24 (1.67–3.08)	0.038
Lauric acid	0.23 (0.17–0.33)	0.31 (0.20–0.49)	0.027
ω9-MUFA:			
Oleic acid	3.18 (1.90–5.75)	1.50 (1.13–2.19)	< 0.001
ω6-PUFA:			
Linoleic acid	3.27 (1.98–6.90)	2.47 (1.64–3.77)	0.076
Adrenic acid	6.44 (2.24–12.18)	4.49 (2.96–7.30)	0.200
Arachidonic acid	111.89 (43.75–199.38)	102.51 (59.70–138.69)	0.597
ω3-PUFA:			
EPA	0.46 (0.17–1.07)	0.51 (0.20–0.86)	0.888
DHA	2.71 (1.03–9.28)	4.62 (3.19–6.19)	0.029
AA/DHA + EPA	31.44 (22.07–44.02)	19.9 (14.17–23.93)	< 0.001
Omega-3 index	1.35 (0.73–2.30)	1.97 (1.67–2.51)	0.002

Data were described as median (IQR: 25% percentile – 75% percentile); p-values obtained from Mann-Whitney exact test.

(*n* = 100) of patients used statins after hospitalization and 12.3% (*n* = 14) did not. We noted that patients with a poor outcome (mRS ≥ 3) and without statin use had a significantly lower triglyceride level ($p = 0.028$) while the total cholesterol levels were significantly lower in patients with a poor outcome but treated with statin ($p = 0.03$) (Table III). HDL cholesterol values were lower in poor outcome patients compared to good outcome for both treatment groups, but the statistical significance level was not reached ($p = 0.090$ for patients without statin use after admission).

Table III. Bivariate analysis of the relationship between lipid profile and functional outcome expressed by mRS at discharge: stratified analysis by statin treatment after hospitalization

Parameter		mRS at discharge		*P*-value
	Statin use	< 3 points	≥ 3 points	
Total cholesterol, mean ± SD [mg/dl]	Yes[a]	195.7 ±45.1	176.5 ±35.1	0.030
	No[b]	157.4 ±66.1	145.4 ±41.9	0.704
HDL cholesterol, mean ± SD [mg/dl]	Yes[a]	48.9 ±11.2	45.2 ±11.9	0.126
	No[b]	45.4 ±6.3	39.3 ±5.6	0.090
LDL cholesterol, mean ± SD [mg/dl]	Yes[a]	121.4 ±39.8	109.7 ±31.8	0.137
	No[b]	87.9 ±60.4	90.3 ±36.7	0.935
Triglycerides, median, IQR [mg/dl]	Yes[a]	112.2 (82.6–138.0)	98.6 (72.0–128.2)	0.170
	No[b]	99.7 (86.5–161.4)	84.2 (76.0–84.7)	0.028

SD – standard deviation; p-values obtained from Student t-test for independent samples with equal variances and Mann-Whitney exact test ; [a]mRS < 3 vs. mRS ≥ 3 group with statin use: $n_1 = 63$; $n_2 = 36$; [b]mRS < 3 vs. mRS ≥ 3 group without statin use: $n_1 = 8$; $n_2 = 6$.

There was no difference in the RBC membrane concentration of fatty acids between the two groups ($p > 0.05$) and regarding plasma fatty acids only stearic acid had a significant difference in distribution values ($p = 0.021$), with higher values in poor outcome patients.

The association between lipid profile and long-term stroke outcome

In order to highlight the relation between classic lipid profile and mRS at 3 months, we performed stratified analysis by statin use after hospitalization. We noted that patients with poor outcome (mRS ≥ 3) had significantly lower triglyceride regardless of statin use, while the total cholesterol levels and HDL cholesterol were significantly lower in patients with poor outcome under statin treatment (Table IV).

There was not enough evidence for significant differences in the RBC membrane concentration of fatty acids and mRS at 3 months ($p > 0.05$) or plasma fatty acids and mRS at 3 months ($p > 0.05$); higher values for plasma concentrations of fatty acids were observed in poor outcome patients.

Path analysis

For the path analysis a Spearman rank correlation matrix was estimated from data described in Table V. It highlighted that the correlations between the mRS scores at discharge and at 3 months and all variables included in the structural model were statistically significant.

Using path analysis we tested a hypothetical model involving relationships of associations and dependencies between lipid profile and mRS at 3 months. Because of multicollinearity between

Table IV. Bivariate analysis of the relationship between lipid profile and functional outcome expressed by mRS at 3 months: stratified analysis by statin treatment after hospitalization

Parameter		mRS at 3 months		*P*-value
	Statin use	< 3 points	≥ 3 points	
Total cholesterol, mean ± SD [mg/dl]	Yes[a]	199.5 ±42.9	161.2 ±35.3	0.001
	No[b]	157.4 ±66.0	136.1 ±39.3	0.530
HDL cholesterol, mean ± SD [mg/dl]	Yes[a]	49.4 ±11.1	45.6 ±10.9	0.204
	No[b]	45.4 ±6.3	38.0 ±5.1	0.052
LDL cholesterol, mean ± SD [mg/dl]	Yes[a]	124.3 ±39.1	99.1 ±33.3	0.016
	No[b]	88.0 ±60.4	82.2 ±34.5	0.851
Triglycerides, median, IQR [mg/dl]	Yes[a]	114.8 (85.0–137.0)	74.7 (69.0–99.5)	< 0.0001
	No[b]	99.7 (86.5–161.4)	84.4 (84.0–84.7)	0.045

SD – standard deviation; p-values obtained from Student t-test for independent samples with equal variances and Mann-Whitney exact test; [a]mRS < 3 vs. mRS ≥ 3 group with statin use: $n_1 = 58$; $n_2 = 18$; [b]mRS < 3 vs. mRS ≥ 3 group without statin use: $n_1 = 8$; $n_2 = 5$.

Table V. Spearman correlation matrix between all studied variables

Parameter	1	2	3	4	5	6	7	8
Age	1.00							
Gender	0.05	1.00						
NIHSS at 1 day after admission	0.26*	0.09	1.00					
TG at 1 day after admission	–0.24*	–0.09	–0.20*	1.00				
HDL at 1 day after admission	–0.08	0.08	–0.13	–0.24*	1.00			
Total cholesterol at 1 day after admission	–0.25*	–0.10	–0.25*	0.45*	0.26*	1.00		
mRS at discharge	0.33*	0.21*	0.69*	–0.24*	–0.20*	–0.24*	1.00	
mRS at 3 months	0.46*	0.11	0.66*	–0.40*	–0.16	–0.34*	0.92*	1.00

**Significant correlation coefficients ($p < 0.05$).*

mRS at discharge and mRS at 3 months, we did not include in the tested model mRS at discharge. The model was tested only for patients who used statin after hospital admission, due to the relatively small number of patients without established treatment.

The proposed model described in Figure 1 had a good fit to data (statistics $\chi^2 = 6.94$, d$f = 5$, $\chi^2/df = 1.39 < 2$, Satorra-Bentler scaling factor = 0.86, $p = 0.225$), robust RMSEA = 0.071, $p = 0.325$ for the null hypothesis that RMSEA < 0.05, standardized root mean square residual SRMR = 0.045, comparative fit index (CFI) = 0.980, and Tucker-Lewis Index (TLI) = 0.941.

There was a significant linear association between the total cholesterol level and mRS at 3 months; lower cholesterol values were significantly correlated with higher mRS scores at 3 months ($\beta = –0.17$, $p = 0.031$). The long-term outcome (mRS at 3 months) was positively influenced by age ($\beta = 0.22$, $p = 0.001$), and NIHSS score at admission ($\beta = 0.55$, $p < 0.001$), higher values for these variables being associated with higher mRS at 3-month scores (poor outcome). The model explained 48% of the variance of mRS at 3 months (Figure 1).

Discussion

Our study revealed that routine serum lipid concentrations (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) in Romanian stroke patients were lower than in healthy controls. Moreover, patients with a poor long-term outcome had lower triglyceride and total cholesterol levels than patients with a good outcome, regardless of the statin therapy after admission. Although the general hypothesis is that elevated lipid concentrations are involved in the pathogenesis of cardio-cerebrovascular diseases, in the literature, there have been some studies with similar results. Simundic *et al.* found significantly lower levels of triglycerides and HDL cholesterol in ischemic patients than in controls, but across ischemic patients, the study found higher serum triglyceride levels in patients with more severe stroke [7]. In 2013, the triglyceride paradox in ischemic stroke was described by Jain *et al.* after

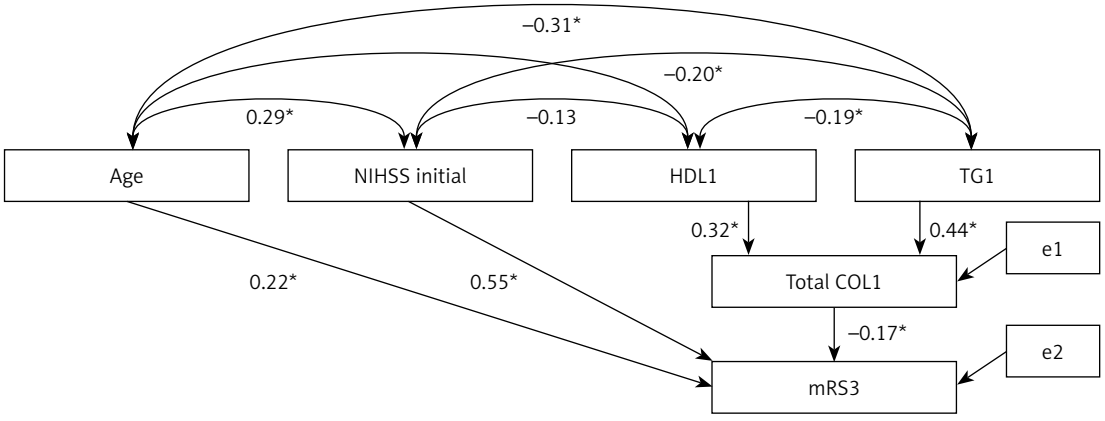

Figure 1. Path diagram of lipid profile and long-term outcome for patients who used statin after hospital admission. Model described the standardized path coefficients (β) and residual variances (ei) for dependent variables

a study on 334 patients with ischemic stroke. The results of this study revealed that low triglyceride levels at admission are associated with stroke severity and poor outcome at discharge, but after adjustment for age, gender and NIHSS, triglyceride level was no longer a predictor of functional outcome [6]. Two other studies regarding triglyceride levels in ischemic stroke patients suggest an inverse association between triglyceride levels at admission with infarct volume [5] and stroke severity [4]. Choi *et al.* found a J-shaped association between triglyceride levels and outcome defined by neurological deterioration/improvement by 4 points within a week after symptoms onset, suggesting that both low and elevated triglyceride levels could be potential risk factors for poor short-term outcome in ischemic stroke [24]. In our study, serum triglyceride levels were significantly lower in patients versus controls ($p = 0.038$), with lower values in poor compared to good long-term outcome ($p < 0.05$), regardless of the statin therapy. Total cholesterol and HDL cholesterol levels were significantly lower in patients versus healthy controls ($p < 0.0005$ for total cholesterol and $p = 0.016$ for HDL cholesterol), in line with a similar study [7]. Lipids are major membrane and brain constituents, and the observation that lipid levels are lower in poor outcome patients has been explained by poor nutritional status or nutritional deficiency [25]. High cholesterol levels associated with good outcome could be explained by several hypotheses: reducing the neurotoxic effects by increasing gamma-glutamyltransferase activity [26], antioxidant protection by neutralising free radicals [27], or increasing tolerance to anoxia [28, 29]. A study on hospitalised adults revealed that high cholesterol levels predict better recovery from disability [30], less severe stroke and lower all-cause mortality [3], while a very recent large sample size study underlines the low rate mortality after ischemic stroke in patients with hyperlipidemia, independent of statin use [31], and a negative association between cholesterol and long-term outcome [32]. In our study, the results of path analysis testing the relationships of associations and dependencies between lipid profile and mRS at 3 months revealed a negative linear association between the total cholesterol level and mRS at 3 months ($\beta = -0.17$, $p = 0.031$); mRS was positively influenced by age ($\beta = 0.22$, $p = 0.001$) and NIHSS score at admission ($\beta = 0.55$, $p < 0.001$), higher values for these variables being associated with a higher disability score. The most important predictor for mRS at 3 months was stroke severity (NIHSS at admission), followed by age and total cholesterol.

In this study, we also investigated the difference in plasma and RBC membrane fatty acid composition between ischemic stroke patients and healthy controls, as well between different functional outcome groups. Plasma levels of stearic and lauric acids were significantly lower in patients compared to controls ($p < 0.001$), while palmitic acid was found to be higher, though not statistically significantly, in the patient versus control group ($p = 0.083$). Oleic acid is a member of ω9 fatty acids, abundant in olive oil, canola oil and sunflower oil; it is considered to exert an anti-inflammatory effect through PPARγ activation, along with the inhibition of reactive oxygen species (ROS) [33]. In the present study, oleic acid was found to be the only FA higher in ischemic patients compared to healthy controls ($p = 0.009$), with no difference between patient subgroups. The plasma level of adrenic acid was significantly lower in ischemic stroke patients versus healthy controls ($p < 0.001$), without a significant difference in RBC membrane. Adrenic acid is an ω6 PUFA abundant in adrenal glands but also in cerebral tissue, which may be converted into arachidonic acid by β-oxidation. An experimental study on bovine adrenal cortical arteries suggests that adrenic acid mediates vascular arterial tone, by concentration-dependent relaxation, regulating the blood flow. The results may suggest that this effect may act in multiple vascular regions where adrenic acid is abundant [34]. The lower level of adrenic acid in plasma but not RBC membranes of ischemic stroke patients compared to healthy controls could be explained by the hypothesis that adrenic acid serves as a substrate for synthesis of arachidonic acid or vasoactive metabolites such as F2-dihomo-IsoPs, a new potential marker of oxidative stress [35, 36]. Low plasma adrenic acid may explain the lack of vascular relaxation in the ischemic brain region, but further studies are needed to investigate the role of adrenic acid in the brain and the mechanism of action at this site. Linoleic acid (ω9 PUFA) in our study was significantly lower in patients versus controls ($p < 0.001$). A nested case-control study regarding linoleic acid in stroke pathogenesis in the Japanese population revealed that linoleic acid may protect against ischemic stroke, with several mechanisms being involved (lowering systolic and diastolic blood pressure, lowering total cholesterol levels and BMI) and being inversely associated with the risk of stroke [37]. ω3 PUFAs are considered to have the most protective actions upon neuronal tissue; in our study, plasma and RBC membrane DHA levels were found to be lower in patients versus controls ($p < 0.001$ for plasma and $p = 0.029$ for RBC membrane), with a similar concentration in patients with good and poor outcome. The reason for the failure to find a correlation of ω3 PUFA concentrations with stroke severity and outcome

was probably the small number of patients and low-grade severity (median NIHSS on admission was 4.0 IQR: 2.0–7.5). Regarding the plasma EPA concentration between patients and healthy controls, the analysis revealed a lower level in ischemic patients compared to controls, at the limit of statistical significance (p = 0.051). A study in the Japanese population found that EPA plasma concentration is lower in ischemic stroke patients (CE and AT) compared to controls but with no difference regarding the DHA concentration [38]. Overall, both plasma and RBC membrane omega-3 index values are significantly lower in ischemic patients, especially due to the low level of DHA.

Patients with a poor outcome at discharge had a higher plasma level of stearic acid, compared to those with a good outcome. In the literature there is a lack of data about the influence of stearic acid in stroke outcome. Didisheim and Mibashan proposed as a possible mechanism the activation of factor XII by stearic acid [39]. In addition to initiation of the coagulation cascade, factor XIIa is also involved in activation of inflammation via the activated kallikrein-kinin system [40, 41].

One limitation of the study was the small size of the sample (especially of the very severe cases) and the reduced number of patients with FA plasma composition evaluated, which did not allow the testing of a path model including fatty acids.

Lipid profile and plasma/RBC fatty acid levels were quantified only a short time after admission, not in a dynamic way to investigate the potential correlations between inflammatory markers and lipid profile. Also, we were not able to evaluate the FA profile in cerebrospinal fluid, a place where the biological parameters are first modified after ischemic stroke. The results of the present study revealed the direct relationship between the lipid profile and ischemic stroke outcome (measured by mRS). Although in the multivariate statistical analysis only some demographic and clinical data were included, such as age and stroke severity on admission, there are many potential confounding factors (considered as a study limitation) which may influence the outcome: body mass index (BMI), hypertension or antihypertensive medication, smoking status, or physical activity. Because the path analysis requires a much larger number of subjects compared to other multivariate analysis techniques, further large sample studies are required to demonstrate the stability of estimates in the recursive model of this study or to test other mediation models.

In conclusion, the effect of extended lipid profile on stroke outcome is controversial and has not been extensively investigated in large patient cohorts. In our study plasma and RBC membrane DHA levels were lower in ischemic stroke patients compared to controls; adrenic acid was significantly lower only in patients' plasma. Patients with a poor short-term outcome have significantly lower total cholesterol level and higher stearic acid plasma level, compared to those with good evolution at discharge. After adjusting for age and gender in the path analysis, the most important predictors for functional long-term outcome measured by mRS at 3 months were stroke severity (NIHSS at admission) followed by age and total cholesterol level.

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Conflict of interest

The authors declare no conflict of interest.

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