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Effective high-density lipoprotein cholesterol is associated with carotid intima-media thickness and vascular events after acute ischemic stroke

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ABSTRACT

Background and aims: Effective high density lipoprotein cholesterol (HDL-C) is a measure of HDL functionality. We evaluated if HDL-C is associated with carotid intima-media thickness (cIMT) and incident major adverse cardiovascular events (MACE) in patients with acute ischemic stroke from two prospective cohort studies.
Methods: In the MARK-STROKE cohort, 299 patients with acute ischemic stroke or TIA were included. Outcome was available in 219 patients during a median follow-up of 294 days. In CIRCULAS, 382 acute ischemic stroke patients were included and a 90-day follow-up was available in 213 patients. HDL-C was calculated based on symmetric dimethylarginine (SDMA) and HDL cholesterol concentrations. Main outcome was incident MACE (death, stroke, and myocardial infarction) and the main measure was cIMT.
Results: In both studies, HDL-C was inversely associated with cIMT in linear regression analysis adjusted for age, sex and creatinine. In MARK-STROKE, the adjusted hazard for MACE was significantly reduced for patients with one unit increase (mg/dL) of HDL-C (hazard ratio 0.95 [95% confidence interval (CI): 0.92, 0.99]). In the CIRCULAS cohort, stroke patients with higher HDL-C had less incident MACE during 90 days of follow-up (odds ratio: 0.97 [95% CI: 0.94, 0.99] for one unit increase). Neither SDMA nor HDL cholesterol predicted outcome.
Conclusions: Our findings imply a protective role of biologically effective HDL after acute cerebral ischemia for secondary events and emphasize the relevance of lipoprotein functionality in these patients.

1. Introduction

High-density lipoproteins (HDL) confer anti-thrombotic, anti-arteriosclerotic and anti-inflammatory effects in the vasculature. Recent studies have emphasized the importance of HDL functionality compared with absolute concentrations. [1] Besides cholesterol efflux, anti-oxidative and anti-inflammatory properties of HDL confer endothelial-protective effects. However, dysfunctional HDL found in patients with diabetes, metabolic syndrome or chronic kidney disease can cause endothelial injury promoting atherosclerotic processes. [2,3] A hallmark of endothelial dysfunction is the impairment of vaso-protective nitric oxide (NO), which can be aggravated by endogenous inhibitors of NO metabolism. [4] Among these endogenous metabolites involved, symmetric dimethylarginine (SDMA) can transform the physiological anti-arteriosclerotic HDL into dysfunctional pro-arteriosclerotic lipoproteins inhibiting endothelial NO production. [3] Based on these studies, an algorithm was proposed, which allowed the calculation of biologically effective HDL cholesterol (HDL-C). [3] More importantly, these findings translate into clinical outcome. Higher HDL-C was associated with a reduced all-cause and cardiovascular mortality in patients with low SDMA levels, whereas mortality was increased with higher HDL-C in patients with high SDMA. [5] Interestingly, calculated HDL-C concentrations revealed a better risk discrimination than HDL cholesterol. [5] Although HDL-C predicted all-cause

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and cardiovascular mortality in two cardiovascular cohorts, i.e. LURIC and MONICA/KORA S3,[5] its role in patients with stroke remains unclear. So far, low HDL cholesterol is known to predict stroke recurrence, [6] but does not reflect HDL functionality appropriately. Here, we hypothesized that reduced HDL-C as parameter of HDL functionality is associated with vascular risk and outcome after stroke in two independent cohorts.

2. Patients and methods

2.1. Study design, ethical approval and patient consent in MARK-STROKE

The bioMARKers in STROKE (MARK-STROKE) cohort is an on-going prospective observational single-center study at the University Medical Center Hamburg-Eppendorf as previously described. [7] The inclusion criteria were age ≥18 years and diagnosis of stroke or transient ischemic attack at discharge. For this exploratory cohort study, patients recruited between November 2017 and December 2019 without available HDL cholesterol or SDMA values were excluded. Furthermore, the algorithm to calculate biologically effective HDL cholesterol is only valid for SDMA concentrations ≥0.48 μmol/l. Therefore, all patients with SDMA levels <0.48 μmol/l were excluded and cross-sectional analyses were calculated for 299 patients (Supplemental Fig. 1). From December 2019 until March 2020, patients were followed up by phone or mail. For 219 patients, we recorded major cardiovascular events (i.e. death, myocardial infarction, stroke). The study protocol was approved by the Ethics Committee of the Hamburg Board of Physicians (PV4715). The investigation was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

2.2. Study design, ethical approval and patient consent in CIRCULAS

The inclusion criteria for this exploratory cohort study were age ≥18 years and diagnosis of stroke at discharge. Patients without available HDL cholesterol or SDMA values and with SDMA levels <0.48 μmol/l were excluded. Three-month follow-up was conducted in person or by phone. Written informed consent was obtained from all subjects. The investigation was conducted in accordance with the Declaration of Helsinki.

2.3. Clinical assessment

Neurological deficits were assessed by the National Institute of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS) at admission. Demographic parameters, past medical history, including comorbidities (arterial hypertension, hyperlipidemia, diabetes mellitus, atrial fibrillation, prior stroke, prior myocardial infarction), laboratory (creatine kinase (CK), creatinine, C-reactive protein (CRP), triglycerides, total cholesterol, low density lipoprotein (LDL) cholesterol and HDL cholesterol and imaging data (infarct type, internal carotid artery (ICA) stenosis, and carotid intima-media thickness (cIMT)) were obtained from medical records.

2.4. Carotid intima-media thickness

In MARK-STROKE, the greatest cIMT measured in the right and left common carotid artery was used to define individual cIMT, as described previously. [8,9] In CIRCULAS, the average cIMT was measured in the right and left common carotid artery, as described elsewhere. [10]

2.5. Laboratory measurements

Laboratory measurements were performed from serum and EDTA plasma samples collected at admission as described elsewhere. [11,12] SDMA was quantified by liquid chromatography-tandem mass spectrometry from EDTA plasma samples as previously described. [13] In brief, 25 μL of EDTA plasma was diluted in methanol with stable isotope labeled internal standard on 96-well plates. After protein precipitation, analytes were converted into butyl esters derivatives and analysed with calibrators and quality controls (QC) by liquid chromatography-tandem mass spectrometry. SDMA concentrations were calculated using calibration curves based on four levels in triplicates. Plate wise QC were run in two levels by triplicates. Coefficients of variation and bias of QC were below 15%. HDL-C was calculated using the following equation: HDL-C = (1.869 Ln(SDMA)+(0.227–1.054· Ln (SDMA))· $\sqrt{}$HDL cholesterol +1.372) [2].

2.6. Statistical analysis

Relationships between HDL cholesterol, SDMA and HDL-C with continuous variables were assessed with Spearman's correlation or linear regression analyses (beta coefficient and 95% confidence interval, CI). For regression analysis, we calculated beta coefficients or odds ratios (ORs) for different models: unadjusted (model 1), adjusted for age, sex and GFR (model 2), and adjusted for age, sex, GFR, LDL cholesterol, prevalent arterial hypertension, prevalent diabetes, prevalent hypercholesterolemia (model 3). Time-to-event analysis for HDL-C and MACE was assessed with Kaplan-Meier survival analyses and log rank test. The associations between one unit increase in HDL cholesterol (mg/dL), SDMA (μmol/L) and HDL-C (mg/dL) or biomarker levels above the median with incident MACE were determined by Cox regression analyses with results presented as hazard ratio (HR) with corresponding 95% CI. For Cox regression, we calculated HRs for different models: unadjusted (model 1), adjusted for age, sex, GFR (model 2), and adjusted for age, sex, GFR, LDL cholesterol, prevalent arterial hypertension, prevalent diabetes, and prevalent hypercholesterolemia (model 3). A p value < 0.05 was considered statistically significant. Due to the exploratory design of our study, we did not adjust for multiple comparisons. Statistical analysis was performed with IBM SPSS Statistics (version 27, IBM Corp., Armonk, NY, USA) and GraphPad Prism (version 9 for Windows, La Jolla, USA).

2.7. Validation in the circulating biomarkers in acute stroke (CIRCULAS) study

All measurements and analyses were performed similarly compared with the MARK-stroke cohort. Of note, in the CIRCULAS study, plasma samples from acute stroke patients were collected upon admission within 24 h of symptom onset and a follow-up was available at 90 days. Likewise, beta coefficients and odds ratios (ORs at 90 days) were calculated for different models: unadjusted (model 1), adjusted for age and sex (model 2), and adjusted for age, sex, (NIHSS at admission for outcome), LDL cholesterol, creatinine, prevalent arterial hypertension, prevalent diabetes, and prevalent hypercholesterolemia (model 3). Time-to-event analyses were not possible due to a fixed time-point for follow-up (90 days). All analyses for the validation cohort were performed in R, version 3.5.0.

2.8. Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

3. Results

3.1. SDMA, effective HDL cholesterol and cIMT

Baseline characteristics of both cohorts including anthropometric measures and clinical presentation are given in Table 1. Biologically effective HDL cholesterol (HDL-C, calculated according to the previously proposed algorithm) median levels were 30.1 [21.2, 42.0] mg/dl and

Table 1
Baseline characteristics.

Characteristics	MARK- STROKE (n = 299)	CIRCULAS (n = 382)
Demographic parameters		
Age, years	70.1 ± 12.0	75.1 ± 11.2
Male	196 (66)	207 (54)
Smoking	129 (43)	122 (48)
Hypertension	226 (76)	307 (82)
Hyperlipidemia	97 (32)	134 (36)
Diabetes	51 (17)	67 (18)
Atrial fibrillation	74 (25)	103 (28)
Prior myocardial infarct	36 (12)	38 (10)
Prior stroke	52 (17)	88 (24)
BMI, kg/m^2	26.2 ± 4.3	26.3 ± 5.0
Laboratory parameters		
Symptom to blood draw, h	44.0 [24.5, 89.4]	3.1 [1.4, 5.2]
Cholesterol, mg/dL	183 [152, 215]	176 [146, 208]
Triglycerides, mg/dl	122 [90.5, 168]	101 [77, 136]
LDL cholesterol, mg/dL	103 [75, 134]	108 [83, 136]
HDL cholesterol, mg/dL	48.5 [39.0, 59.8]	48 [40, 58]
SDMA, µmol/L	0.61 [0.55, 0.71]	0.70 [0.59, 0.87]
Effective HDL cholesterol, mg/dL	30.1 [21.2, 42.0]	23.1 [13.3, 34.6]
CRP, mg/dL	1.5 [0.9, 3.5]	0.4 [0.2, 0.9]
Creatinine, mg/dL	0.96 [0.82, 1.20]	1.00 [0.90, 1.20]
Medication		
Blood-thinning	256 (86)	182 (49)
Lipid-lowering	214 (72)	127 (35)
Antihypertensive	225 (75)	254 (69)
Neurological parameters		
TIA	89 (30)	0 (0)
NIHSS, points	1 [0, 4]	5 [2, 12]
mRS, stage	1 [0, 2]	3 [2, 4]
Intima-media-thickness (mm)	1.3 [1.1, 1.6]	0.8 [0.7, 0.9]
Symptomatic ICA stenosis	46 (15)	34 (9)

Data are mean ± SD or median [IQR], as appropriate. Categorical variables are given as numbers (percentages) of participants. LDL, low-density lipoprotein; HDL, high-density lipoprotein; CK, creatine kinase; SDMA, symmetric dimethylarginine; CRP, C-reactive protein; TIA, transient ischemic attack; NIHSS, National Institute of Health Stroke Scale; mRS, modified Rankin Scale; ICA, internal carotid artery.

23.1 [13.3, 34.6] mg/dl in MARK-STROKE and CIRCULAS, respectively (Table 1). HDL-C inversely correlated with age and creatinine in both studies (Supplementary Table 1). HDL-C was associated with cIMT in unadjusted and models adjusted for age, sex, and GFR in MARK-STROKE and CIRCULAS (Table 2). This association remained significant after additional adjustment for LDL cholesterol, prevalent arterial hypertension, diabetes and hypercholesterolemia only in MARK-STROKE and showed a trend in CIRCULAS (Table 2).

Table 2
Regression analysis of HDL-C levels with carotid intima-media thickness.

	Model	β coefficient (95% CI)	*p* value
MARK-STROKE	1	−0.007 (−0.010, −0.003)	0.001**
	2	−0.006 (−0.010, −0.001)	0.010*
	3	−0.006 (−0.011, −0.001)	0.010*
CIRCULAS	1	−0.002 (−0.004, −0.001)	0.003**
	2	−0.002 (−0.003, −0.000)	0.043*
	3	−0.002 (−0.003, −0.000)	0.079

Linear regression analysis with beta coefficients (95% confidence interval) (model 1: unadjusted; model 2: adjusted for age, sex and GFR; model 3: adjusted for age, sex, GFR, prevalent arterial hypertension, prevalent diabetes and hypercholesterolemia) (MARK-STROKE: n = 299, CIRCULAS: n = 382). Models were calculated for one unit increase in effective high-density lipoprotein cholesterol (HDL-C′ in mg/dL) and one unit increase in carotid intima-media thickness (mm). *$p < 0.05$, **$p < 0.01$.

3.2. SDMA, effective HDL cholesterol and incident MACE

During follow-up (median 294 [IQR: 204, 439] days), we registered 24 incident MACE in 219 MARK-STROKE patients. Patients with HDL-C levels above the median had a longer disease-free survival and lower risk of incident MACE (Fig. 1A and Table 3, HR 0.15 [95% CI: 0.05, 0.47], model 3). In continuous Cox regression analysis, increasing HDL-C concentrations were incrementally associated with lower MACE

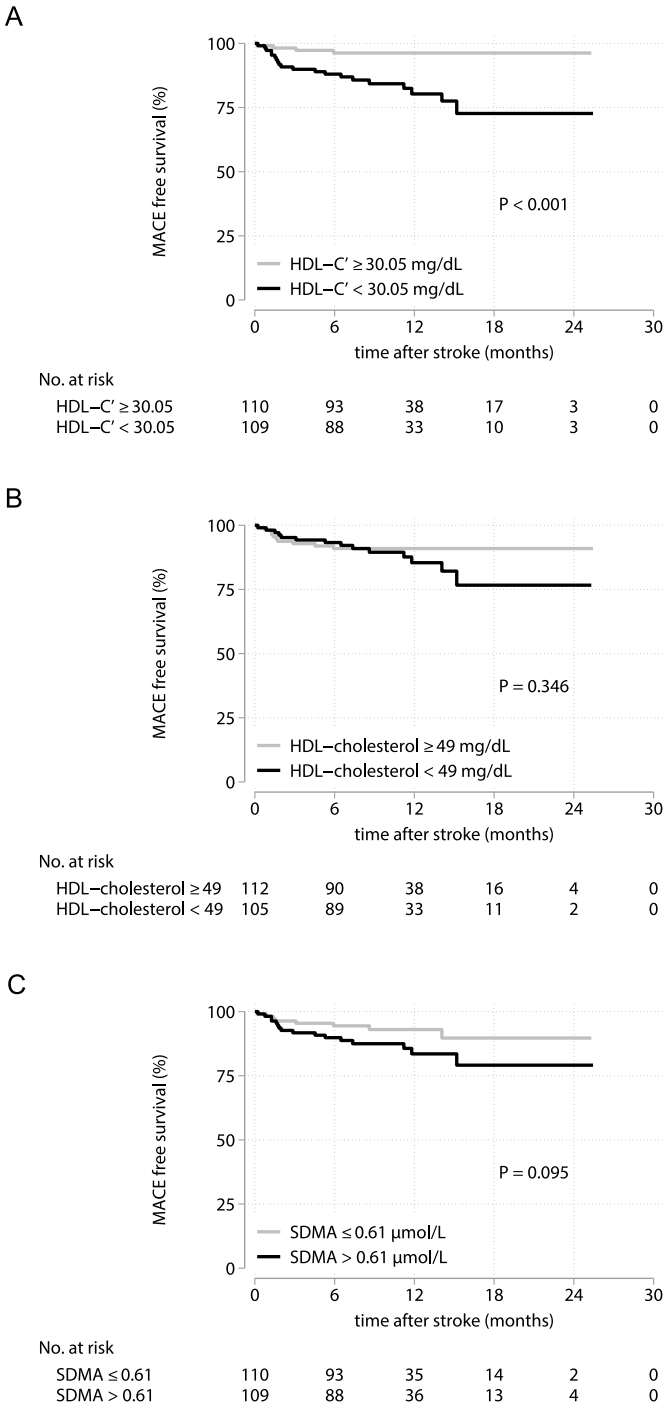

Fig. 1. Incident MACE after acute ischemic stroke or TIA.
Kaplan-Meier curves for HDL-C (A), SDMA (B) and HDL cholesterol (C) with MACE (death, myocardial infarction, stroke) during follow-up after stroke or TIA in MARK-STROKE (n = 219). HDL, high-density lipoprotein; HDL-C, effective high-density lipoprotein cholesterol; MACE, major adverse cardiovascular events; SDMA, symmetric dimethylarginine.

Table 3
Cox regression analyses of HDL-C, SDMA and HDL cholesterol with incident MACE in MARK-STROKE.

	Model	Hazard Ratio [95% CI]	*p* value
HDL-C	1	0.18 [0.06, 0.54]	0.002**
	2	0.17 [0.05, 0.51]	0.002**
	3	0.15 [0.05, 0.47]	0.001**
SDMA	1	2.00 [0.86, 4.68]	0.109
	2	2.09 (0.78, 5.57]	0.142
	3	2.26 [0.84, 6.07]	0.107
HDL cholesterol	1	0.67 [0.30, 1.51]	0.331
	2	0.71 [0.30, 1.69]	0.441
	3	0.71 [0.30, 1.71]	0.446

Cox regression analysis of biomarker levels above the median with hazard ratios (95% confidence interval, CI) during median follow-up of 294 days. Model 1: unadjusted, model 2: adjusted for age, sex and GFR, model 3: adjusted for age, sex, GFR, LDL cholesterol, smoking, prevalent arterial hypertension, prevalent diabetes and hypercholesterolemia; n = 219. *$p < 0.05$, **$p < 0.01$. HDL, high-density lipoprotein; HDL-C′, effective high-density lipoprotein cholesterol; LDL, low-density lipoprotein; MACE, major adverse cardiovascular events; SDMA, symmetric dimethylarginine.

incidence (HR 0.95 [95% CI: 0.92, 0.99] for one unit increase in mg/dL, model 3). Neither HDL cholesterol nor SDMA at baseline was associated with MACE in this cohort (Fig. 1B and C and Table 3). We validated these findings in the CIRCULAS cohort. We recorded 53 incident MACE in 213 stroke patients. During a follow-up of 90 days, HDL-C levels above the median were associated with fewer incident MACE, whereas HDL cholesterol and SDMA alone did not (Table 4, model 3). In addition, increasing HDL-C concentrations were incrementally associated with lower MACE incidence in Cox regression analysis in the CIRCULAS cohort (OR: 0.97 [95% CI: 0.94, 0.99] for one unit increase in mg/dL, model 3).

4. Discussion

Here, we show that low HDL-C is associated with increased cIMT and incident MACE after stroke. Previous studies have shown opposite associations of HDL cholesterol and SDMA with carotid arteriopathy. A meta-analysis showed a robust inverse correlation of total HDL cholesterol with carotid IMT [14], whereas carotid IMT was positively associated with SDMA concentrations. [15] In this study, HDL-C was independently associated with cIMT after adjustment. The anti-arteriosclerotic effects of HDL have been attributed to reverse cholesterol transport. In line with this mechanism, higher cholesterol efflux capacity was associated with cIMT, progression of carotid plaques, incident cardiovascular events and coronary disease status. [16–18] In these studies, cholesterol efflux capacity was minimally influenced by traditional risk factors and HDL cholesterol itself. [16,17] In contrast, SDMA strongly and dose-dependently reduced cholesterol efflux capacity. [5] SDMA is a predictor of all-cause mortality after ischemic stroke but the overall value of SDMA as a biomarker of cardiovascular disease is unclear. [19,20] In two cardiovascular cohorts, higher HDL cholesterol levels were associated with lower mortality only in patients with low SDMA levels, whereas this association was completely lost if SDMA was high. [5] Moreover, high SDMA can transform HDL not only into a dysfunctional, but "toxic" lipoprotein, which impairs endothelial repair and triggers pro-inflammatory cascades. [3] Mechanistically, SDMA can trigger micro- and macrovascular inflammation and can activate store-operated Ca^{2+}-channels increasing intracellular Ca^{2+} in monocytes. [20,21] In line with these findings, HDL-C predicted incident MACE after stroke in two independent stroke cohorts, whereas absolute HDL cholesterol and SDMA did not. The association of HDL-C with outcome was independent of traditional risk factors. Similar to HDL-C, cholesterol efflux capacity is minimally affected by these factors, whereas HDL cholesterol concentrations are strongly associated with multiple metabolic variables and cardiovascular risk factors. [17] Our data suggest that HDL-C has the discriminatory potential to improve individual risk stratification and to facilitate a more personalized treatment of stroke patients. Our findings sketch potential treatment regimes, which are not based on absolute lipid concentrations, but functionality. In this case, patients with cerebrovascular disease and normal absolute lipid levels might benefit from statin therapy if biologically effective HDL levels are low.

Limitations of our study are the small sample sizes in both cohorts and the restriction of analyses to SDMA $\geq$0.48 μmol/L. Furthermore, the observational design of our study does not allow causal relationships.

In conclusion, our findings imply a detrimental role of low HDL-C concentrations in acute stroke independent of traditional risk factors and underline the importance of lipoprotein functionality.

Table 4
Logistic regression analysis of effective HDL-C, SDMA and HDL cholesterol with incident MACE in CIRCULAS.

	Model	Odds Ratio [95% CI]	*p* value
HDL-C'	1	0.50 [0.35, 0.72]	<0.001***
	2	0.57 [0.38, 0.86]	0.008**
	3	0.60 [0.38, 0.94]	0.025*
SDMA	1	1.22 [0.90, 1.64]	0.195
	2	1.05 [0.78, 1.42]	0.754
	3	0.82 [0.36, 1.85]	0.630
HDL cholesterol	1	0.68 [0.48, 0.96]	0.030*
	2	0.67 [0.46, 0.99]	0.044*
	3	0.48 [0.23, 1.01]	0.054

Logistic regression analysis of biomarker levels above the median with odds ratios (95% confidence interval, CI). Model 1: unadjusted; model 2: adjusted for age, sex, and GFR; model 3: adjusted for age, sex, GFR, LDL cholesterol, smoking, prevalent arterial hypertension, prevalent diabetes and hypercholesterolemia; n = 213. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. HDL, high-density lipoprotein; HDL-C′, effective high-density lipoprotein cholesterol; LDL, low-density lipoprotein; MACE, major adverse cardiovascular events; SDMA, symmetric dimethylarginine.

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CRediT authorship contribution statement

Edzard Schwedhelm: Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Steffen Tiedt:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **Susanne Lezius:** Methodology, Formal analysis, Writing – review & editing. **Teresa Allegra Wölfer:** Investigation, Data curation, Writing – review & editing. **Märit Jensen:** Investigation, Data curation, Writing – review & editing. **Robert Schulz:** Investigation, Data curation, Writing – review & editing. **Rainer Böger:** Investigation, Data curation, Writing – review & editing. **Christian Gerloff:** Investigation, Data curation, Writing – review & editing. **Götz Thomalla:** Investigation, Data curation, Writing – review & editing. **Chi-un Choe:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2022.08.001.

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