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Brief Report

Impaired antioxidative activity of high-density lipoprotein is associated with more severe acute ischemic stroke

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ABSTRACT

Background/aims: High-density lipoprotein (HDL) has important anti-atherogenic functions, including antioxidant effects. However, it is unclear whether the antioxidative activity of HDL is associated with the severity and outcome of acute ischemic stroke. We aimed to evaluate this association.
Methods: We prospectively studied 199 consecutive patients admitted with acute ischemic stroke and followed them up until discharge. We measured HDL antioxidant capacity, HDL-associated paraoxonase-1 (PON1) activity and HDL-associated myeloperoxidase (MPO) levels. Severe stroke was defined as National Institutes of Health Stroke Scale (NIHSS) at admission ≥5. Dependency was defined as modified Rankin scale at discharge between 2 and 5.
Results: Patients with severe stroke had lower HDL antioxidant capacity, higher MPO levels and higher MPO/PON1 ratio. Independent risk factors for severe stroke were female gender (RR 2.80, 95% CI 1.37–5.70, $p = 0.005$), glucose levels (RR 1.01, 95% CI 1.0–1.02, $p < 0.01$) and HDL antioxidant capacity (RR 1.03, 95% CI 1.01–1.06, $p < 0.05$). Patients who were dependent at discharge had lower HDL antioxidant capacity, higher MPO levels and higher MPO/PON1 ratio. Independent predictors of dependency at discharge were lack of lipid-lowering treatment (RR 6.86, 95% CI 1.83–25.67, $p < 0.005$) and NIHSS (RR 1.56, 95% CI 1.29–1.88, $p < 0.0001$). The HDL antioxidant capacity did not differ between patients who died during hospitalization and those who were discharged. The only independent predictor of in-hospital mortality was NIHSS (RR 1.16, 95% CI 1.06–1.27, $p < 0.005$).
Conclusions: Impaired antioxidative activity of HDL is associated with more severe acute ischemic stroke and might also predict a worse functional outcome in these patients.

1. Introduction

Classical epidemiological studies showed an inverse relationship between high density lipoprotein-cholesterol (HDL-C) levels and the risk for cardiovascular events, including ischemic stroke [1]. However, previous studies reported conflicting results regarding the association between HDL-C levels and ischemic stroke severity or functional outcome [2]. Moreover, HDL-C levels do not appear to predict mortality in patients with acute ischemic stroke [2].

In recent years, the relationship between HDL-C levels and cardiovascular risk has been challenged by genetic and clinical studies [3]. HDL possesses various atheroprotective functions and accumulating evidence supports that HDL functionality is more important than HDL-C levels for cardiovascular risk prediction [3]. The atheroprotective functions of HDL include the ability to promote cholesterol efflux from arterial wall macrophages, antioxidative and antiinflammatory effects and protective effects on the endothelium [4].

The pleiotropic functions of HDL have been attributed to the HDL components that include proteins, lipids, metabolites and small RNA molecules [4]. Perturbations in HDL composition under various pathologic conditions could affect HDL functions [4–6]. Among the proteins that associate with HDL and play a role in its atheroprotective functions is paraoxonase-1 (PON1), which contributes to the antioxidative and antiinflammatory effects of HDL [7] and myeloperoxidase (MPO), which affects oxidative stress and atherosclerosis [8]. The ratio of MPO levels/PON1 activity has been proposed as an indicator of dysfunctional HDL [9].

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Previous studies have reported an association between ischemic stroke severity and markers of oxidative stress, such as nitrite/nitrate metabolites [10]. Furthermore, patients with stroke were shown to have dysfunctional HDL, manifested by impaired composition in proteins linked to oxidative stress, as compared to controls [11,12], but it is unclear whether this is associated with stroke severity and outcome.

The aim of the present study was to evaluate the association between the antioxidative activity of HDL and the severity and outcome of acute ischemic stroke.

2. Materials and methods

We prospectively studied 199 consecutive patients who were admitted with acute ischemic stroke (42.7% males, age 78.6 ± 6.5 years, range, 54–92 years) and followed them up until discharge. We measured HDL antioxidant capacity, HDL-associated PON1 activity and HDL-associated MPO levels. Severe stroke was defined as National Institutes of Health Stroke Scale (NIHSS) at admission≥5. Dependency was defined as modified Rankin scale at discharge between 2 and 5. Details on Materials and Methods are available in the online-only Supplementary material.

3. Results

3.1. Antioxidant capacity of HDL and stroke severity

Patients' characteristics at admission are shown in Supplementary Table 1. At admission, 51.2% of the patients had severe stroke. HDL samples (used at equal HDL-C concentration) were assessed for their antioxidant properties using the DCF assay, where an increase in fluorescence signal indicates reduced antioxidant potential. The fluorescence signal correlated positively with NIHSS at admission ($r = 0.2554$, $p = 0.0006$). Furthermore, patients with severe stroke had higher fluorescence signal (i.e. lower HDL antioxidant capacity) than patients with mild stroke (Fig. 1A).

The fluorescence signal did not correlate with HDL-associated PON1 activity, but correlated with HDL-associated MPO levels ($r = 0.3315$, $p < 0.0001$) and MPO/PON1 ratio ($r = 0.3300$, $p < 0.0001$). The NIHSS scale did not correlate with HDL-associated PON1 activity, but correlated with HDL-associated MPO levels ($r = 0.2412$, $p = 0.0015$) and MPO/PON1 ratio ($r = 0.2257$, $p = 0.0031$). Patients with severe stroke had similar HDL-associated PON1 activity (Fig. 1B), higher HDL-associated MPO levels and higher MPO/PON1 ratio than patients with mild stroke (Fig. 1C, D).

In addition, patients with severe stroke were more frequently women and had higher serum glucose levels and lower alcohol consumption than patients with mild stroke (Supplementary Table 2). Independent predictors of severe stroke were female gender (relative risk (RR) 2.80, 95% confidence interval (CI) 1.37–5.70, $p = 0.005$), increased glucose levels (RR 1.01, 95% CI 1.0–1.02, $p < 0.01$) and reduced antioxidant capacity (RR 1.03, 95% CI 1.0–1.06, $p < 0.05$).

3.2. Antioxidant Capacity of HDL and Dependency at Discharge

The median duration of hospitalization was 6 days (range, 1–26 days). At discharge, 53.8% of the patients were dependent. Patients who were dependent at discharge had higher fluorescence signal (i.e. lower HDL antioxidant capacity), similar HDL-associated PON1 activity, higher HDL-associated MPO levels and higher MPO/PON1 ratio than patients who were independent (Fig. 2A–D).

In addition, patients who were dependent at discharge were older, less likely to be treated with lipid-lowering agents prior to admission and had higher serum glucose levels, higher heart rate, lower triglyceride levels, lower alcohol consumption and higher NIHSS at admission than patients who were independent (Supplementary Table 3). Independent predictors of dependency at discharge were the lack of treatment with

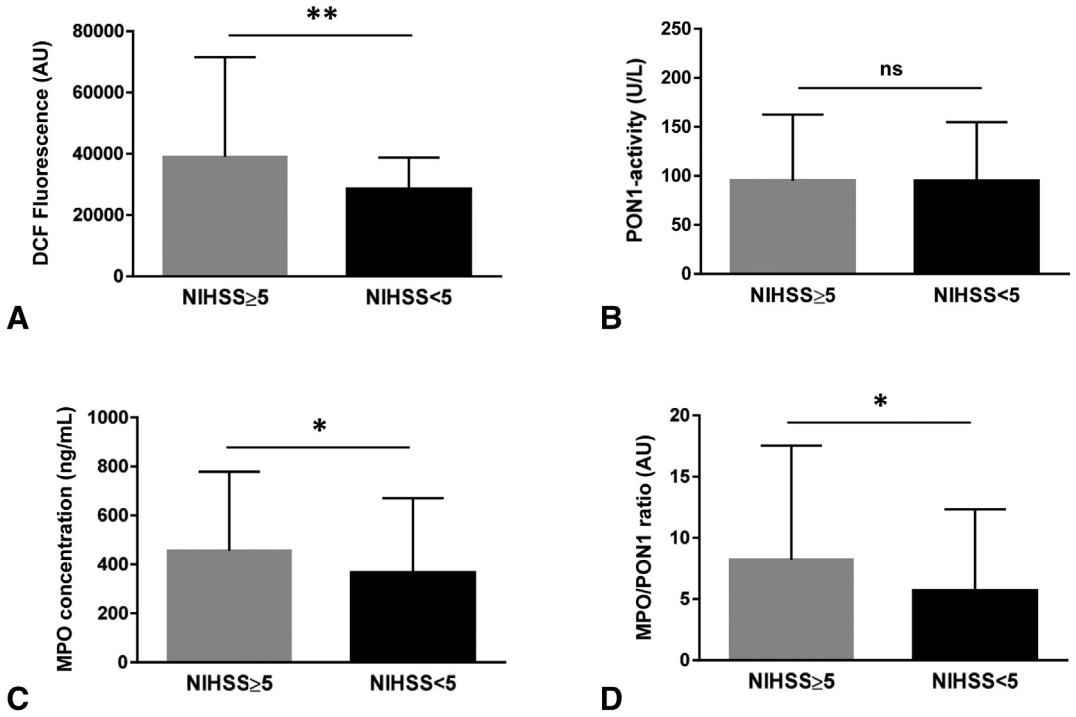

Fig. 1. HDL antioxidant capacity, HDL-associated PON1 activity, HDL-associated MPO levels and MPO/PON1 ratio in patients with severe (NIHSS ≥ 5) or mild stroke (NIHSS < 5). (A) Fluorescence intensity resulting from oxidation of DCFH by HDL in the presence of oxLDL. (B) HDL-associated PON1 activity, expressed in U per L of HDL. (C) HDL-associated MPO levels, expressed in ng MPO per mL of HDL. (D) Value of HDL-associated MPO levels divided by value of HDL-associated PON1 activity. p was calculated by non-parametric Mann-Whitney *U* test; $^{**}p < 0.005$; $^{*}p < 0.05$; AU: arbitrary units; ns: non-significant.

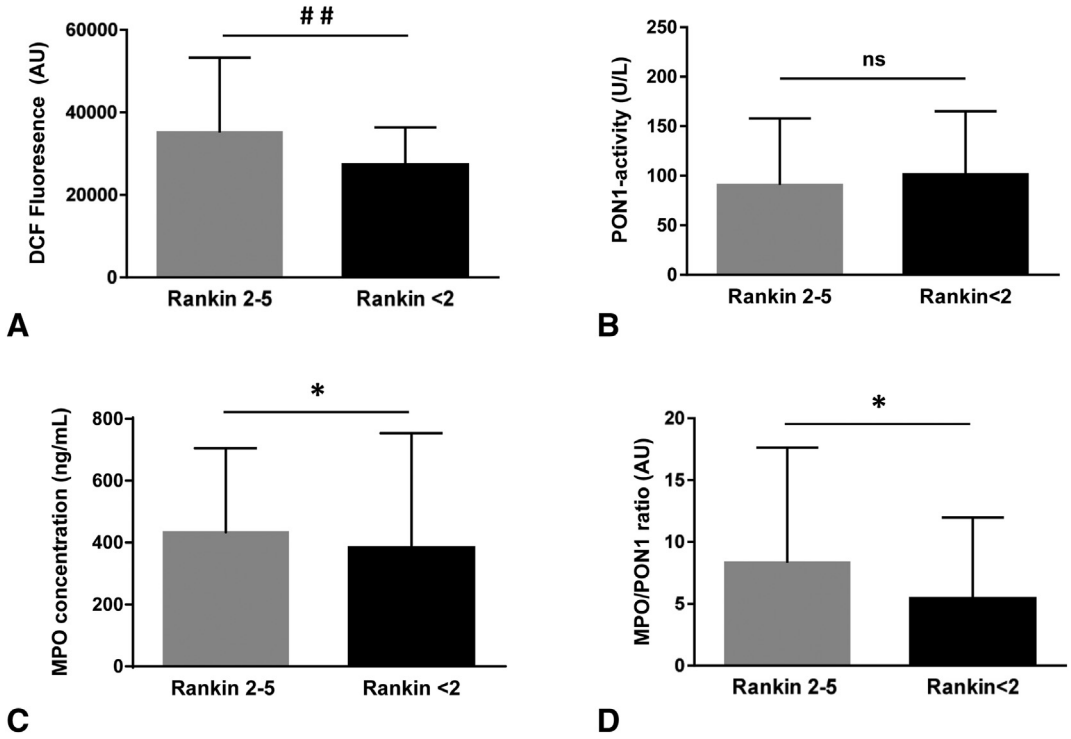

Fig. 2. HDL antioxidant capacity, HDL-associated PON1 activity, HDL-associated MPO levels and MPO/PON1 ratio in patients who were dependent (Rankin 2–5) or independent (Rankin ≪ 2) at discharge. (A) Fluorescence intensity resulting from oxidation of DCFH by HDL in the presence of oxLDL. (B) HDL-associated PON1 activity, expressed in U per L of HDL. (C) HDL-associated MPO levels, expressed in ng MPO per mL of HDL. (D) Value of HDL-associated MPO levels divided by value of HDL-associated PON1 activity. p was calculated by non-parametric Mann-Whitney *U* test; $^{\#\#}p < 0.001$; $^{*}p < 0.05$; AU: arbitrary units; ns: non-significant.

lipid-lowering agents (RR 6.86, 95% CI 1.83–25.67, $p < 0.005$) and NIHSS at admission (RR 1.56, 95% CI 1.29–1.88, $p < 0.0001$).

3.3. Antioxidant capacity of HDL and in-hospital mortality

During hospitalization, 7.5% of the patients died ($n = 15$). A trend for higher fluorescence signal (i.e. lower HDL antioxidant capacity) was observed for patients who died during hospitalization compared with those who were discharged, but it did not reach significance (Supplementary Fig. 1A). The former had increased HDL-associated PON1 activity and MPO levels compared to the latter (Supplementary Fig. 1B, C). The MPO/PON1 ratio did not differ between the two groups (Supplementary Fig. 1D).

Furthermore, patients who died during hospitalization had more frequently atrial fibrillation, higher heart rate, lower body mass index and higher NIHSS at admission than patients who were discharged (Supplementary Table 4). The only independent predictor of in-hospital mortality was NIHSS at admission (RR 1.16, 95% CI 1.06–1.27, $p < 0.005$).

4. Discussion

In the current study, we showed for the first time that the HDL antioxidant capacity in patients with acute ischemic stroke correlates negatively with NIHSS at admission and represents an independent predictor of severe stroke. The HDL antioxidant capacity was measured following normalization for HDL-C levels and therefore the observed differences in HDL antioxidant capacity of patients reflect differences in HDL functionality and not in HDL-C levels.

Several studies showed that, under pathologic conditions, HDL loses its atheroprotective functions and might become atherogenic because of changes in its composition [4]. Changes in proteomic composition can affect the levels and activity of PON1 and MPO [6,13], which have both been linked to cardiovascular risk [7,14]. In addition, the MPO/PON1 ratio appears to represent an important indicator of coronary heart disease risk [14]. Here, we observed a negative correlation between HDL antioxidant capacity and HDL-associated MPO levels, while the HDL-associated MPO levels and the HDL-MPO/HDL-PON1 ratio correlated positively with NIHSS at admission. Furthermore, patients who were dependent at discharge had lower HDL antioxidant capacity and higher HDL-MPO levels as well as higher HDL-MPO/HDL-PON1 ratio than patients who were independent. There was no correlation between DCF fluorescence signal and PON1 activity. Such lack of association was also reported in other populations, including patients with systemic lupus erythematosus and ankylosing spondylitis [15,16]. Furthermore, patients with metabolic syndrome were shown to have more proinflammatory HDL than dyslipidemic patients, even with comparable levels of PON1 activity [17]. Collectively, our findings indicate that the levels of HDL-associated MPO could affect the antioxidant properties of HDL in patients with acute ischemic stroke in association with the severity of stroke. Furthermore, our results suggest that, during acute ischemic stroke, HDL particles display disturbed atheroprotective capacity and possibly disturbed ability to fully protect the blood brain barrier that correlates with the severity of stoke. However, the molecular mechanisms leading to MPO increase and reduced antioxidant capacity of HDL in patients with acute ischemic stroke and their relationship with stroke occurrence, severity or outcome need to be further examined in future studies.

One limitation of the study could be that we used apoB-depleted serum, which contains the HDL fraction, and not HDL isolated by gradient density ultracentrifugation. Nevertheless, the latter technique is suitable only for a limited number of samples. In several other studies, apoB-depleted serum has been used for the evaluation of HDL atheroprotective properties, including its antioxidant properties by the DCF assay [18,19]. Another limitation could be the sample size and it is possible that our study was not powered to identify other predictors of the outcomes of interest, particularly in-hospital mortality. Indeed, only 15 patients died during hospitalization. However, our study was prospective and included consecutive patients, reducing the risk of

bias. Moreover, to the best of our knowledge, this is the first study that evaluated the association between the antioxidant capacity of HDL and the severity and outcome of acute ischemic stroke.

Overall, our study shows that the impaired antioxidant activity of HDL is associated with more severe acute ischemic stroke and might also predict a worse functional outcome in these patients. The functional integrity of HDL may be an important, still unexplored marker to evaluate the risk for stroke or its outcome.

Author contributions

KT and AC designed the study and wrote the paper. KK performed all analyses with the help of CG. KK also analyzed the results with the help of KT, CG and AC. MP, KC, SMA and AS participated in the collection of data. CS and AIH participated in the design of the study. All authors approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2019.06.004.

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