# Estimation of the presence of small dense lipoprotein cholesterol in acute ischemic stroke

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## Abstract

Small dense low-density lipoprotein (sdLDL) is an established risk factor in ischemic heart disease. However, its clinical significance in acute ischemic stroke (AIS) is uncertain. This study evaluates the prognostic value of the presence of sdLDL in patients with AIS by determining whether it contributes to clinical outcome or not. We studied 530 consecutive patients admitted within the first 48 hours after onset of ischemic stroke and 50 corresponding controls. Serum lipid parameters were measured on admission by standard laboratory methods. The percentage of AIS patients with sdLDL was significantly higher than the one of matched controls with sdLDL. Concerning comparisons between AIS patients with or without sdLDL, the percentages of males and patients with histories of smoking, hypertension, and cardiovascular disease were significantly higher in AIS patients with sdLDL. Concerning the grade of severity, modified Rankin Scale (mRS) on discharge was significantly higher in AIS patients with sdLDL. On logistic regression analysis, age (OR=2.29, P<0.001), male gender (OR=0.49, P<0.01), history of atrial fibrillation (OR=3.46, P<0.001), and the presence of sdLDL (OR=1.59, P<0.05) were significantly associated with poor prognosis (mRS on discharge >3). Our study showed that the presence of sdLDL might be independently associated with a poor prognosis after AIS.

## Introduction

Acute ischemic stroke (AIS) is one of the major causes of mortality and long-term disability in the world.[1] The association of various risk factors and AIS should be investigated in populations with different genetic and environmental backgrounds.[2] In recent years, it has become important to consider novel risk factors in order to improve our understanding of disease pathogenesis and prevention.[3] Atherogenic dyslipidemia is a common disorder of lipoprotein metabolism that is closely associated with increased risk of cardiovascular disease (CVD).[4,5] It is characterized by elevated triglycerides (TG) and normal or slightly elevated low-density lipoprotein (LDL) cholesterol (LDL-C) with a preponderance of small dense LDL (sdLDL) particles. However, the relevance of sdLDL particles for AIS prediction is underappreciated compared to CVD.[6-9] In this study, we evaluated whether the presence of sdLDL was an independent determinant of AIS after adjustment for conventional risk factors or not.

## Materials and Methods

### Subjects

The study group consisted of 530 consecutive patients (305 male and 225 female patients; mean age: male 71.5±11.0 years, female 78.6±11.0; P<0.001) admitted to our hospital within the first 48 hours after the first onset of ischemic stroke between January 2006 and December 2012. Stroke was defined as rapid development of clinical signs of focal or global disturbance of cerebral function lasting 24 h or longer, with no apparent cause beyond that of vascular origin. Experienced neurologists used computed tomography (CT) or magnetic resonance imaging (MRI) to confirm a diagnosis of ischemic stroke and to rule out hemorrhagic stroke. Clinical entities were classified into lacunar, atherothrombotic and cardioembolic infarctions according to the 3rd Edition of the Cerebrovascular Disease Classification by the National Institute of Neurological Disorders and Stroke (NINDS-III).[10] Other types of strokes (transient ischemic attack, subarachnoid hemorrhage, brain hemorrhage, and arteriovenous malformation) and severe systemic diseases [collagenosis, endocrine, and metabolic diseases (except for diabetes mellitus), and inflammation, liver, neoplastic, and renal diseases] were excluded.

On admission, essential patient information, including age, gender, height, weight, blood pressure, and previous medication, was collected. The following vascular risk factors and co-morbidity conditions were also evaluated: smoking habits; hypertension (HT), according to a previous diagnosis; current antihypertensive treatment; measured blood pressure of >140/90 more than once; presence of cardiovascular disease (previous myocardial infarction, or angina pectoris); diabetes mellitus (DM), according to a previous diagnosis, or prescribed medication for diabetes; hyperlipidemia (those who exhibited a total cholesterol level higher than 240 mg/dL or LDL level above 160 mg/dL, or those who had been taking antihyperlipidemic drugs); and degree of severity [the modified Rankin Scale (mRS) was used for this evaluation]. Corresponding controls (n=50) were selected based on the absence of neurological and cardiovascular diseases as well as the same exclusion criteria as described for the cases in clinic patients with non-stroke neurological disorders.

Every individual was informed about the aims of the study and gave written consent before participation. Written informed consent was obtained from a relative in the case of patients with impaired cognition. The whole study was planned and executed following the Ethical Guidelines of the Helsinki Declaration. In accordance with institutional research guidelines, the local institutional review committee approved the whole study protocol.

### Biochemical analyses

Blood samples were collected into evacuated tubes containing EDTA and one serum sample tube after a 12-h fasting period. Plasma and serum were separated by immediate centrifugation at 1500×g for 10 min at 4°C. Aliquots of each sample were stored at −80°C. Plasma and serum samples were thawed immediately before analysis. Serum total cholesterol (TC) and triglyceride (TG) concentration were assayed by routine enzymatic methods. HDL-C was measured using the same enzymatic

method after precipitation of the plasma with phosphotungstic acid in the presence of magnesium ions. LDL cholesterol level was estimated by the equation of Friedewald *et al*.[11] Plasma ApoB level was measured by rocket immunoelectrophoresis. The coefficients of variation for TC, HDL, TG, and ApoB measurements were all <3%. Since over 90 % of plasma ApoB is recovered in the LDL fraction, the plasma ApoB concentration roughly corresponds to the number of LDL particles. Therefore, the LDL-cholesterol concentration divided by plasma ApoB concentration approximately indicates the cholesterol content of a LDL particle. Hirano *et al*. found that the LDL/ApoB ratio was significantly correlated with LDL size.[12] The preponderance of sdLDL could be estimated by the extent to which the LDL/Apo B ratio was less than 1.2.[12-14] The measurement of sdLDL is not influenced by oxidative stress or other factors.[12-14] Therefore, LDL/ApoB values are stable from acute to chronic stage of AIS.

## Statistical analysis

Statistical analysis was conducted using SPSS22.0 (IBM SPSS Inc., Chicago, IL, USA). We employed the $c^2$ test to compare the backgrounds of the patients, and non-parametric Mann-Whitney test to compare the clinical evaluation scales between the two groups. Logistic regression analysis was conducted regarding the association of the risk factors and the odds ratio and 95% confidence interval (CI) were evaluated. P<0.05 was considered significant.

## Results

The general and metabolic characteristics of all the patients are shown in Table 1. As expected, AIS patients had a greater percentage with a history of hypertension, DM, and CVD. Levels of systolic and diastolic blood pressure and TG were significantly different between the groups. The percentage of AIS patients with sdLDL was significantly higher than that of matched controls with sdLDL. To determine whether the plasma level of sdLDL differed among various types of strokes, the 530 stroke patients were divided into four groups, thrombotic stroke (191), lacunar stroke (170), cardioembolic stroke (162), and other types of strokes (7). The percentage of stroke patients with sdLDL was not significantly different between various types of strokes.

Concerning comparisons of AIS patients with or without sdLDL, the percentage of males and percentage of patients with histories of smoking, hypertension, and cardiovascular disease (CVD) were significantly higher in AIS patients with sdLDL. There were no significant differences regarding other risk factors. Concerning the grade of severity, mRS on discharge was significantly higher in AIS patients with sdLDL (Table 2).

To clarify the association of the risk factors with poor prognosis (mRS on discharge >3), logistic regression analysis was conducted. Age (OR=2.29, P<0.001), male gender (OR=0.49, P<0.01), history of atrial fibrillation

(OR=3.46, P<0.001), and the presence of sdLDL (OR=1.59, P<0.05) were significantly associated with poor prognosis. Other factors did not show a significant correlation (Table 3).

## Discussion and Conclusions

Several risk factors have been established for AIS, among them the most important being age, sex, hypertension, and other cardio- and peripheral-vascular co-morbidity conditions.[3-7] In this study, we have noted a higher prevalence of these traditional risk factors in the AIS patient group. However, previous studies concerning the association between AIS and sdLDL were less conclusive.[15-17] Atherogenic dyslipidemia has been confirmed as one of the principal processes underlying CVD, while sdLDL is considered an emerging risk factor for ischemic heart disease. Clinical significance of atherogenic dyslipidemia in CVD development and in the onset of AIS has not been fully elucidated. This study found that the percentage of AIS patients with sdLDL was significantly higher than that of matched controls with sdLDL. The results of this study suggest a significant association between AIS and sdLDL. The presence of sdLDL was an important predictor of AIS onset even when all other traditional AIS risk factors were taken into account. These findings suggest that measuring only LDL might not be sufficient for an

**Table 1. Biochemical characteristics of controls and acute ischemic stroke patients with various types of stroke.**

| | Controls (n=50) | AIS patients | | | | |
|---|---|---|---|---|---|---|
| | | Total (n=530) | Thrombotic stroke (n=191) | Lacunar (n=170) | Cardioembolic (n=162) | Others (n=7) |
| Age (years) | 72.1±8.8 | 74.5±11.6 | 74.4±11.7 | 72.1±11.6 | 77.5±10.6 | 67.4±11.4 |
| Gender, male (%) | 60.0 | 57.5 | 59.7 | 61.8 | 50.6 | 57.1 |
| Smokers (%) | 10.0 | 20.8* | 25.1* | 23.5* | 13.6 | 0 |
| Hypertension (%) | 20.0 | 66.6* | 67.5* | 68.2* | 63.6* | 71.4* |
| Diabetes mellitus (%) | 6.0 | 23.8* | 29.3* | 25.3* | 15.4 | 28.6* |
| Hypercholesterolemia (%) | 18.0 | 31.3** | 33** | 32.4** | 27.8** | 42.9** |
| Cardiovascular disease (%) | 4.0 | 12.5* | 15.7* | 5.9 | 16* | 0 |
| BMI (Kg/m3) | 22.0±3.5 | 22.8±5.0 | 23.4±6.2 | 23.0±3.7 | 21.8±4.5 | 24.5±1.9 |
| SBP (mmHg) | 122.5±12.7 | 155.1±25.2** | 154.5±25.6** | 161.5±24.9** | 148.4±23.4** | 165.3±29.0** |
| DBP (mmHg) | 74.3±10.8 | 83.9±17.4** | 80.1±15.2** | 86.9±17.0** | 85.0±19.5** | 86.6±16.5** |
| TC (mg/dL) | 183.5±30.4 | 185.1±40.3 | 185.6±35.9 | 193.0±41.7 | 174.7±41.2 | 215.3±44.5 |
| LDL-C (mg/dL) | 113.9±24.6 | 116.6±33.4 | 117.1±30.0 | 122.1±34.3 | 109.5±35.2 | 136.3±24.1 |
| HDL-C (mg/dL) | 49.1±12.1 | 46.8±12.9 | 46.3±12.3 | 44.6±13.2 | 46.4±13.4 | 51.9±14.0 |
| TG (mg/dL) | 87.1±23.5 | 108.0±55.9* | 111.4±61.2* | 116.6±60.4* | 94.0±38.5 | 135.1±73.1* |
| ApoB (mg/dL) | 90.0±18.4 | 96.2±24.3 | 97.3±23.3 | 99.3±25.3 | 91.1±23.5 | 99.3±25.4 |
| LDL/ApoB | 1.30±0.10 | 1.21±0.15** | 1.21±0.14** | 1.23±0.14** | 1.20±0.18** | 1.22±0.09** |
| sdLDL (%) | 32.0 | 59.1** | 59.2** | 54.1** | 63.6** | 71.4** |

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; sdLDL, small dense LDL. Data are expressed as means or means ± standard deviation. *P<0.05 *versus* control; **P<0.001 *versus* control.

accurate assessment of AIS risk.

Particles of sdLDL have been suggested to be more atherogenic than large buoyant LDL due to several characteristics, including higher penetration into the arterial wall, lower binding affinity for the LDL receptor, prolonged plasma half-life, and lower resistance to oxidative stress.[12-14] Recent studies have found that plasma sdLDL is influenced by such risk factors as gender, age, glucose level, body mass index, and conventional lipid parameters.[18] In this study, concerning comparisons of AIS patients with or without sdLDL, the percentage of males and percentages of patients with histories of smoking, hypertension, and CVD were significantly higher in AIS patients with sdLDL. AIS patients with sdLDL had a tendency toward accelerated atherogenicity. Concerning the grade of severity, mRS on discharge was significantly higher in AIS patients with sdLDL, though there was no significant difference regarding NIHSS on admission. On logistic regression analysis, age, female gender, history of atrial fibrillation, sdLDL, and NIHSS on admission were significantly associated with poor prognosis, and they were independent outcome predictors for poor prognosis (mRS on discharge >3). We found that the presence of sdLDL might be an independent predictor for the severity of AIS and proved its advantages for this prediction.

Recent findings from the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) have indicated that statins reduce the incidence of stroke regardless of baseline LDL-C and other lipid parameters.[19] Statins also exhibit potent effects on LDL subclasses; in fact, a significant reduction in sdLDL has been noted.[20] We can assume that the assessment of sdLDL may represent a useful tool for improved management of dyslipidemia in AIS patients.

There are some limitations to this study. First, sdLDL was evaluated by semiquantitative estimation, not directly measured. However, this method is applicable to routine clinical use and allows the rapid measurement of a large number of samples. Second, the prognosis was measured by mRS scores on discharge; thus a follow-up period of 3 or 6 months is necessary. Third, female patients were significantly older than male patients. It might have influence on female poor prognosis. A prospective study is needed to clarify whether the presence of sdLDL increases the risk of AIS.

In conclusion, the present study showed that the presence of sdLDL might be independ-

**Table 2. Comparisons of acute ischemic stroke patients with or without small dense low-density lipoprotein.**

| | AIS with sdLDL (n=313) | AIS without sdLDL (n=217) | P value |
|---|---|---|---|
| Age (years) | 73.9±11.3 | 75.4±11.8 | NS |
| Gender, male (%) | 63.9 | 48.4 | <0.001 |
| Smokers (%) | 24 | 16.1 | <0.05 |
| Hypertension (%) | 70.3 | 61.3 | <0.05 |
| Hypercholesterolemia (%) | 32.6 | 29.5 | NS |
| Diabetes mellitus (%) | 23.3 | 24.4 | NS |
| Atrial fibrillation (%) | 21.4 | 18.4 | NS |
| Cardiovascular disease (%) | 14.7 | 9.2 | <0.05 |
| Cardioembolic stroke | 27.2 | 32.9 | NS |
| BMI (Kg/m$^3$) | 23.3±5.6 | 22.1±3.9 | NS |
| SBP (mmHg) | 154.8±26.5 | 155.5±23.3 | NS |
| DBP (mmHg) | 84.1±17.8 | 83.5±16.9 | NS |
| TC (mg/dL) | 176.7±36.4 | 197.1±42.6 | <0.001 |
| LDL-C (mg/dL) | 108.0±29.1 | 129.0±35.3 | <0.001 |
| HDL-C (mg/dL) | 43.9±11.9 | 51.1±13.2 | <0.001 |
| TG (mg/dL) | 124.1±61.8 | 84.9±35.2 | <0.001 |
| ApoB (mg/dL) | 96.2±23.2 | 96.3±25.8 | NS |
| LDL/ApoB | 1.12±0.12 | 1.34±0.10 | <0.001 |
| NIHSS on admission (mean±SD) | 6.18±5.97 | 5.16±5.46 | NS |
| mRS on discharge (mean±SD) | 2.13±1.88 | 1.77±1.75 | <0.05 |
| Adminstration of rt-PA (%) | 9.6 | 9.7 | NS |
| Mortality (%) | 2.9 | 1.8 | NS |

AIS, acute ischemic stroke; NS, not significant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; sdLDL, small dense LDL; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; rt-PA, recombinant tissue plasminogen activator; SD, standard deviation.

**Table 3. Odds ratio for an association of risk factors with poor prognosis.**

| | OR | 95%CI | P value |
|---|---|---|---|
| Age (>75) | 2.29 | 1.48~3.55 | <0.001 |
| Male | 0.49 | 0.32~0.76 | <0.01 |
| Hypertension | 1.35 | 0.87~2.09 | 0.18 |
| Diabetes mellitus | 1.17 | 0.72~1.90 | 0.52 |
| Hypercholesterolemia | 0.88 | 0.56~1.44 | 0.56 |
| Atrial fibrillation | 3.46 | 2.18~5.5.0 | <0.001 |
| Cardiovascular disease | 0.79 | 0.43~1.44 | 0.44 |
| The presence of sdLDL | 1.59 | 1.05~2.41 | <0.05 |

OR, odds ratio; CI, confidence interval; sdLDL, small dense low-density lipoprotein.

ently associated with AIS, suggesting it may be one of the factors indicating a poor prognosis for AIS. In addition, this study highlighted the presence of sdLDL as an independent predictor of the short-term functional prognosis in AIS patients. Further prospective studies are needed to assess the long-term predictive value of sdLDL after AIS.

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