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Serum Low-Density Lipoprotein Cholesterol Levels are Associated with Relapse in Neuromyelitis Optica Spectrum Disorder

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Background: The relationship between serum low-density lipoprotein cholesterol (LDL-C) and the risk of relapse in neuromyelitis optica spectrum disorder (NMOSD) remains uncertain. We aimed to examine the association between serum LDL-C level and relapse in NMOSD patients.

Methods: We conducted an analysis of the prospective observational NMOSD cohort study with consecutive 184 hospitalized NMOSD patients from department of neurology. Blood samples were collected to measure LDL-C level upon admission. Primary and relapse were evaluated during hospitalization. The relationship between serum LDL-C level and relapse were analyzed by linear curve fitting analyses. Crude and adjusted odds ratios (OR) of LDL-C for relapse with 95% confidence intervals were analyzed using multiple logistic regression models. ROC curve analysis was used to identify the target lipid-lowering value of LDL-C and the probability of relapse was evaluated by the Kaplan–Meier Plot.

Results: Over a mean disease course of 100±87 days, 59.24% (n=109) participants developed relapse with higher LDL-C than the primary group (n=75) (p<0.001). Adjusted smoothed plots suggested that there were linear relationships between serum LDL-C level and relapse (p< 0.001). The OR (95% CI) between serum LDL-C level and relapse were 2.67 (1.76–4.04, p<0.001), and 2.38 (1.48–3.83, p<0.001) respectively in NMOSD patients before and after adjusting for potential confounders. The target LDL-C lowering values were 2.795 mmol/L with potential benefits to prevent relapse in NMOSD.

Conclusion: In this sample of NMOSD patients, we found that the elevated serum LDL-C was independently and positively associated with the relapse, and serum LDL-C should be well-controlled to prevent the relapse of NMOSD.

Keywords: neuromyelitis optica spectrum disorder, NMOSD, low-density lipoprotein cholesterol, relapse, multivariate analysis

Introduction

Neuromyelitis optica (NMO) is an antibody-mediated autoimmune inflammatory disease of the central nervous system (CNS) characterized by optic neuritis (ON) and transverse myelitis (TM). The discovery of specific antibodies against aquaporin-4 (AQP4-IgG) has broadened the clinical spectrum of the disease, then the 2015 International Panel for NMO Diagnosis defined a unified diagnostic criteria for NMO spectrum disorder (NMOSD).[1] The disease has a high recurrence and disability. Epidemiological researches have shown that the prevalence peaked in middle-aged adults with 60% relapsed within 1 year and 90% relapsed within 3 years.[2,3] Most patients have a poor recovery with severe visual impairment, limb dysfunction, and urinary dysfunction.[4] Therefore, identifying risk factors is important for preventing disease recurrence.

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Currently, the risk factors and the underlying mechanism for relapse in NMOSD patients are not clear. Previous studies have suggested that female, middle age, vaccination, infection, free thyroxine (FT4) level, positive serum AQP4-IgG, high serum cholesterol and glycerol level may be related to relapse.[5–7] Dyslipidemia with high TG and low HDL-C may be associated with disability and disease activity in NMOSD patients.[8] Meanwhile, TG level may be positively correlated with poor recovery.[9] There is few research on the recurrence of NMOSD and cholesterol (CHO) metabolites. In clinical practice, we found that the serum lipid level of recurrent NMOSD patients was higher than that of the initial ones, especially in low-density lipoprotein cholesterol (LDL-C). While LDL-C is a critical component of the cellular plasma membrane and is essential for normal neuronal function. Previous studies showed that LDL-C is involved in the oxidation of some CNS inflammation diseases.[10] Therefore, whether LDL-C plays a role in relapse of NMOSD need to be study.

Based on current evidence, it needs to be further investigated whether elevated LDL-C levels are an important risk factor for relapse in patients with NMOSD. Considering that elevated LDL-C has the function of inflammatory mediators in the central nervous system, we hypothesized that there is a correlation between LDL-C and risk of relapse in NMOSD patients, and the specific risk linear curve is not yet clear. Thus, the purpose of this prospective observational study was to assess the relationship between LDL-C level and relapse in NMOSD patients, further to detective a target value of LDL-C lowering therapy and potential benefits to prevent relapse.

Subjects and Methods

Ethics

This study was performed according to the principles of the Declaration of Helsinki, and was approved by the ethics committee of the Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. We obtained informed consent from patients prior to sample collection.

Design

This was an analysis of the prospective observational NMOSD cohort study, and the NMOSD database has been previously described in articles before.[11–13] Participants were scheduled for follow-up every 3 months. At each follow-up visit, EDSS was measured, medication use, and possible endpoint events were documented by trained research staff and physicians. Our present study was designed to explore the correlation between LDL-C level and relapse in NMOSD patients. Consecutive patients were included from Ren Ji Hospital in China from January 2016 to May 2024, and patients' data were collected based on electronic medical records in hospital.

Study Subjects

Diagnosis Criteria

Patients were diagnosed of NMOSD according to the criteria defined by the 2015 International consensus diagnostic criteria for NMOSD,[1] core clinical characteristics including the following: (1) Optic neuritis; (2) Acute myelitis; (3) Area postrema syndrome: episode of otherwise unexplained hiccups or nausea and vomiting; (4) Acute brainstem syndrome; (5) Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic lesions on Magnetic Resonance Imaging (MRI); (6) Symptomatic cerebral syndrome with NMOSD-typical brain lesions. There are two types of criteria: (1) NMOSD with AQP4-IgG:1) At least 1 core clinical characteristic; 2) Positive test for AQP4-IgG using best available detection method (cell-based assay strongly recommended). (2) NMOSD without AQP4-IgG or NMOSD with unknown AQP4-IgG status:1) at least 2 core clinical characteristics occurring as a result of one or more clinical attacks and meeting all of the following requirements: a. at least 1 core clinical characteristic must be optic neuritis, acute myelitis with longitudinally extensive transverse myelitis (LETM), or area postrema syndrome; b. dissemination in space (2 or more different core clinical characteristics) c. fulfillment of additional MRI requirements, as applicable; 2) negative tests for AQP4-IgG using best available detection method or testing unavailable.

First relapse was defined as the onset of patient-reported or objectively observed events and imaging lesion related to new symptoms, and worsening original symptoms, with a duration of more than 24 hours, in the absence of other recognized causes such as fever and infection.[14]

Inclusion Criteria

This study includes patients with the following criteria: 1) diagnosis of NMOSD; 2) time from admission-to-lumber puncture ≤24 hours; 3) Both cerebrospinal fluid protein (CSF) and serum samples were collected before administration of any corticosteroids or other immunosuppressive therapy; and 4) integrity of clinical data (age, gender, inducing factors, clinical symptom, and CSF data at baseline).

Exclusion Criteria

Exclusion of alternative diagnoses following: 1) patients diagnosed with cerebral infarction, hyperlipidemia, atherosclerotic plaque, continuous use of statin and other lipid-lowering treatment >4 weeks; 2) renal insufficiency or renal failure; and 3) data were not available for review, including incomplete clinical and laboratory data.

Clinical and Laboratory Data

The data for demographic characters [sex, age, systolic blood pressure (SBP), body mass index (BMI)], onset season, inducing factors (respiratory infection, gastrointestinal infection) and clinical features [disease duration, autoimmune diseases (systemic lupus erythematosus, Sjögren's syndrome, rheumatic arthritis and Hashimoto thyroiditis), expanded disability status scale (EDSS) score, vision impaired, muscle weakness, numbness symptoms and muscular sphincter dysfunction] were assessed after admission. The EDSS score, assessed within 24 hours after entry, was evaluated independently by two neurologists who were certified for competency in EDSS scoring.

Fasting venous blood samples were collected upon admission and prior to drug administration, such as glucocorticoid or lipid lowering drugs in the inpatient department. Blood specimens were collected using coagulation-promoting vacuum tubes to detect the level of LDL-C, which were measured with commercially available quantitative test kit obtained from Biotechnology Co., Ltd (Shanghai, China). Intra- and interassay coefficients of variation were 5% and 10%, respectively. The detection values ranged from 0.05 to 11.60 mmol/L for LDL-C. The LDL-C reference value in our laboratory was <3.12 mmol/L. Due to the dynamic changes in LDL-C levels in NMOSD patients during disease course, the LDL-C levels that closest to the patient's disease status over time were included in study. Thus, the LDL-C levels on first-time relapse in recurrent patients and those on the last follow-up time in non-relapse patients were used in analysis.

Blood samples were also collected to measure serum data [high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), alanine transaminase (ALT), glucose, blood urine nitrogen (BUN), uric acid (UA), serum creatinine (SCR), blood white blood cell (WBC), serum AQP4-IgG, and serum MOG-IgG]. Lumbar puncture examination was completed within 24 hours after admission, and cerebrospinal fluid was collected to detect CSF data [protein, albumin quotient (QALB), IgG, IgG Index, oligoclonal band (OB)]. MRI enhancement was performed during hospitalization to observe lesion distribution (optic nerve, spinal and brainstem). All the above determinations were performed in the hospital's laboratory by individuals blinded to the clinical data.

On admission, the treatment (glucocorticoid, IVIG, cyclophosphamide, azathioprine, plasma exchange, antihypertensive drugs, antidiabetic drugs and lipid lowering drugs) were recorded. Some primary and relapse NMOSD patients have previously been diagnosed with optic neuritis and myelitis, therefore, the above therapeutic drugs could be used and should be included in this study. All data were documented by trained research staff and physicians.

Endpoint Events and Follow Up Time

The primary endpoint event of this study was the first relapse. The designed follow-up period is up to 18 months to investigate the prevalence of first relapse according to literature related to course and prevalence of relapse.[2,3] The follow-up time (disease course) was defined as during the time of diagnosis as relapse or to the most recent follow-up if no relapse occurred.

Groups

In the baseline characteristics analysis, groups were organized by LDL-C level. Normal range of LDL-C level in both male and female population were 0 to 3.12 mmol/L, thus included patients were categorized into normal (<3.12 mmol/L) and high (≥3.12 mmol/L) LDL-C level groups. NMOSD patients were also divided into primary group and relapse group according to the recurrence of disease. In multivariate logistic regression for the effects of LDL-C level on relapse, included patients were further subdivided into 3 tertiles (first, second, and third) based on serum LDL-C levels. Seropositive AQP4-IgG was identified when its value was higher than 5.0 RSR U/mL. Thus, the included patients were categorized into AQP4-IgG seropositive and seronegative groups.

Statistical Analysis

Baseline characteristics of participants are presented by LDL-C level. Categorical variables were presented as counts and percentages and analyzed by Fisher's exact test or the Chi-square test. Continuous variables were reported as mean ± standard deviation (SD) (normal distribution) and analyzed by the *t* test, or reported as median and interquartile range (partial distribution) and analyzed by the Mann–Whitney *U*-test. The association between serum LDL-C level and relapse was assessed by linear curve fitting analyses (generalize additive models) and multivariate logistic regression analysis. Baseline variables that were considered relevant to LDL-C and relapse or that showed a univariate relationship with relapse were entered into a multivariate logistic regression model ($p<0.2$). Variables for inclusion were carefully chosen, given the number of events available, to ensure parsimony of the final model. Both non-adjusted and multivariate adjusted models were applied, and interaction and stratified analyses were conducted. Receiver operating characteristic (ROC) curve analysis was used to identify the optimal cut-off value of LDL-C to distinguish relapse from primary groups, further to detect a target value of LDL-C lowering in patients with NMOSD. The area under curve (AUC) was calculated to summarize the diagnostic accuracy of each ROC curve. An AUC value <0.7 indicates a low diagnostic accuracy, 0.7–0.9 indicates moderate accuracy, and >0.9 indicates a high accuracy. Risk of relapse related to LDL-C grouped by target value was estimated by the Kaplan–Meier method and the difference of relapse was calculated by the Log rank test. Statistical analyses were performed using Statistical Package of the Social Sciences Software version 25.0 (SPSS, Chicago, IL, USA) and R (version 3.4), and statistical graphics were generated using GraphPad PRISM 6 (Graph Pad Software Inc., San Diego, CA, USA), setting the level of significance at a two-tailed *p*-value of <0.05.

Results

Baseline Characteristics

A total of 221 consecutive candidates were included for the study at the time of the final survey in May 2024. Among these candidates, those who had met the exclusion criteria were excluded (n=28), those who had missing data related to serum LDL-C, CSF data, and EDSS score were excluded from the eligible candidates for this study (n=8). An unreliable value of serum LDL-C (<0.50 mmol/L) (n=1) was also excluded from the pool of eligible candidates for this study. As a result, a total of 184 NMOSD patients with complete and valid data were eligible for this study. A flowchart of the study is shown in Figure 1.

Among 184 study subjects, females accounted for 70.65% (n=130) and males for 29.35% (n=54). The age of the enrolled subjects ranged from 13 to 81 years (female, 15–81 years; male, 13–75 years) with a mean age of 46.25±15.94 years (female, 46.77±15.59 years; male, 45.02±16.84 years). Among the included subjects, relapse patients accounted for 59.24% (n=109) and primary patients for 40.76% (n=75). The demographic and clinical parameters of participants are presented in Table 1 and Supplement Table 1.

The LDL-C value of female ranged from 1.06–5.36 mmol/L with a mean LDL-C of 2.91±0.88 mmol/L, and that of male ranged from 1.05–4.22 mmol/L with a mean LDL-C of 2.44±0.74 mmol/L. The mean (SD) of LDL-C levels in primary and relapse groups were 2.40 ± 0.77 mmol/L and 3.03 ± 0.83 mmol/L, respectively, and the LDL-C level was significantly higher in relapse group than that in primary group in all patients ($p<0.001$) (Figure 2A). Similar results were observed in both female (3.12 ± 0.84 mmol/L vs 2.54 ± 0.83 mmol/L, $p<0.001$), and male (2.75 ± 0.75 mmol/L vs 2.16 ± 0.62 mmol/L, $p<0.001$) (Figure 2B). Between the relapse and primary groups, there was significant difference in gender

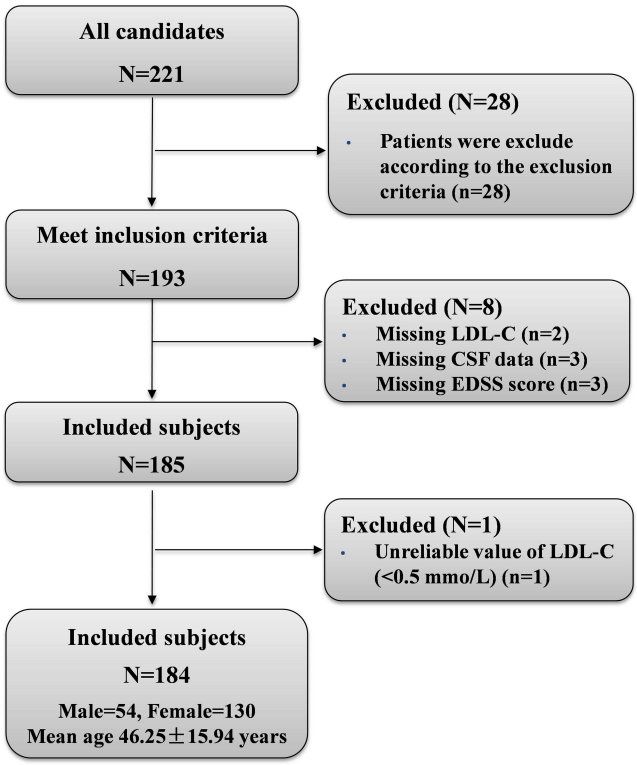

Figure 1 A flowchart of the study.

(76.15% female vs 62.67% female, p=0.048), disease duration [80 (112) vs 60 (60) days, p=0.002], gastrointestinal infection (0.92% vs 6.67%,p=0.031), vision impaired (54.13% vs 38.67%, p=0.039), LDL-C (3.03 ± 0.83 mmol/L vs 2.40 ± 0.77 mmol/L, p<0.001), HDL-C (1.44 ± 0.39 mmol/L vs 1.30 ± 0.44 mmol/L, p=0.027), ALT [23.00 (17.00) U/L vs 19.00 (13.50) U/L, p=0.029], UA (0.30 ± 0.11 mmol/L vs 0.26 ± 0.09mmol/L, p=0.047), SCR (54.95 ± 14.77 μmol/L vs 59.69 ± 16.85 μmol/L, p=0.045), and AQP4-IgG positive (57.80% vs 38.67%, p=0.011), but there was no significant difference in age, SBP, BMI, onset season, inducing factors (respiratory infection), autoimmune diseases, EDSS score, muscle weakness, numbness symptoms, muscular sphincter dysfunction, TG, glucose, BUN, WBC, MOG-IgG, CSF data, imaging lesion distribution and treatment (all, p>0.05) (Supplement Table 1).

Table 1 Baseline Characteristics of Participants by LDL-C Level

Characteristics	Normal LDL-C Group (n=113)	High LDL-C Group (n=71)	*P* value
Demographic characters			
Female (%)	72 (63.72)	58 (81.69)	0.009
Age (years)	45.60 ± 17.57	47.30 ± 12.98	0.484
SBP (mmHg)	112.74 ± 15.78	112.32 ± 16.65	0.864
BMI (kg/m^2)	21.72 ± 3.23	21.01 ± 3.06	0.145
Disease course (days)	60 (66)	90 (108)	0.182
Onset season			
Spring and winter (%)	58 (51.33)	35 (49.30)	0.788
Inducing factors			
Respiratory infection (%)	19 (16.81)	16 (22.54)	0.336
Gastrointestinal infection (%)	5 (4.42)	1 (1.41)	0.408

(*Continued*)

Table 1 (Continued).

Characteristics	Normal LDL-C Group (n=113)	High LDL-C Group (n=71)	*P* value
Clinical features			
Autoimmune diseases (%)	47 (41.59)	26 (36.62)	0.502
EDSS score	4.85 ± 2.25	4.92 ± 2.13	0.837
Vision impaired (%)	47 (41.59)	41 (57.75)	0.033
Muscle weakness (%)	72 (63.72)	52 (73.24)	0.1801
Numbness symptoms (%)	78 (69.03)	56 (78.87)	0.144
Muscular sphincter dysfunction (%)	39 (34.51)	31 (43.66)	0.213
Laboratory data			
LDL-C (mmol/L)	2.20 ± 0.48	3.69 ± 0.44	<0.001
LDL-C of female (mmol/L)	2.25 ± 0.50	3.73 ± 0.45	<0.001
LDL-C of male (mmol/L)	2.11 ± 0.44	3.51 ± 0.38	<0.001
HDL-C (mmol/L)	1.27 ± 0.38	1.56 ± 0.41	<0.001
TG (mmol/L)	1.28 ± 0.59	1.29 ± 0.60	0.880
ALT (U/L)	21.00 (14.00)	22.00 (18.00)	0.724
Glu (mmol/L)	5.41 ± 1.72	6.04 ± 1.62	0.014
BUN (mmol/L)	5.25 ± 1.62	5.21 ± 1.68	0.890
UA (mmol/L)	0.29 ± 0.11	0.27 ± 0.10	0.195
SCR (μmol/L)	58.10 ± 16.75	54.96 ± 13.99	0.190
Blood WBC ($\times 10^9$/L)	8.60 ± 2.55	7.29 ± 2.99	0.213
AQP4-IgG positive (%)	54 (47.79)	38 (53.52)	0.449
MOG-IgG positive (%)	104 (92.04)	65 (91.55)	0.907
CSF data			
Protein (g/L)	0.39 (0.24)	0.39 (0.21)	0.494
QALB ($\times 10^{-3}$)	6.27 (3.57)	6.54 (2.32)	0.436
IgG (mg/dL)	41.70 (43.62)	38.60 (27.37)	0.719
IgG index	0.55 ± 0.16	0.52 ± 0.19	0.197
OB positive (%)	6 (7.23)	4 (7.27)	0.992
Imaging lesion			
Optic nerve (%)	27 (23.89)	25 (35.21)	0.097
Spinal (%)	67 (59.29)	41 (57.75)	0.836
Brainstem (%)	15 (13.27)	2 (2.82)	0.017
Treatment			
Glucocorticoid (%)	96 (84.96)	58 (81.69)	0.559
IVIG (%)	13 (11.50)	5 (7.04)	0.321
Cyclophosphamide (%)	7 (6.19)	6 (8.45)	0.561
Azathioprine (%)	9 (7.96)	11 (15.49)	0.110
Plasma exchange (%)	18 (15.93)	11 (15.49)	0.937
Antihypertensive drugs (%)	10 (8.85)	10 (14.08)	0.267
Antidiabetic drugs (%)	15 (13.27)	11 (15.49)	0.674
Lipid lowering drugs (%)	8 (7.08)	13 (18.31)	0.020
Endpoint events			
Relapse of total (%)	53 (46.90)	56 (78.87)	<0.001
Relapse of female (%)	37 (51.39)	46 (79.31)	<0.001
Relapse of male (%)	16 (39.02)	10 (76.92)	0.017

Abbreviations: ALT, Alanine transaminase; AQP4, aquaporin-4; BMI, body mass index; BUN, blood urine nitrogen; CSF, cerebrospinal fluid protein; EDSS, Expanded Disability Status Scale; HDL-C, High density lipoprotein cholesterol; IgG, immunoglobulin G; IVIG, Intravenous immunoglobulin; LDL-C, Low-density lipoprotein cholesterol; OB, Oligoclonal Bands; QALB, cerebrospinal fluid albumin / serum albumin ratio; SBP, systolic blood pressure; SCR, serum creatinine; TG, Triglyceride; UA, uric acid; WBC, white blood cell.

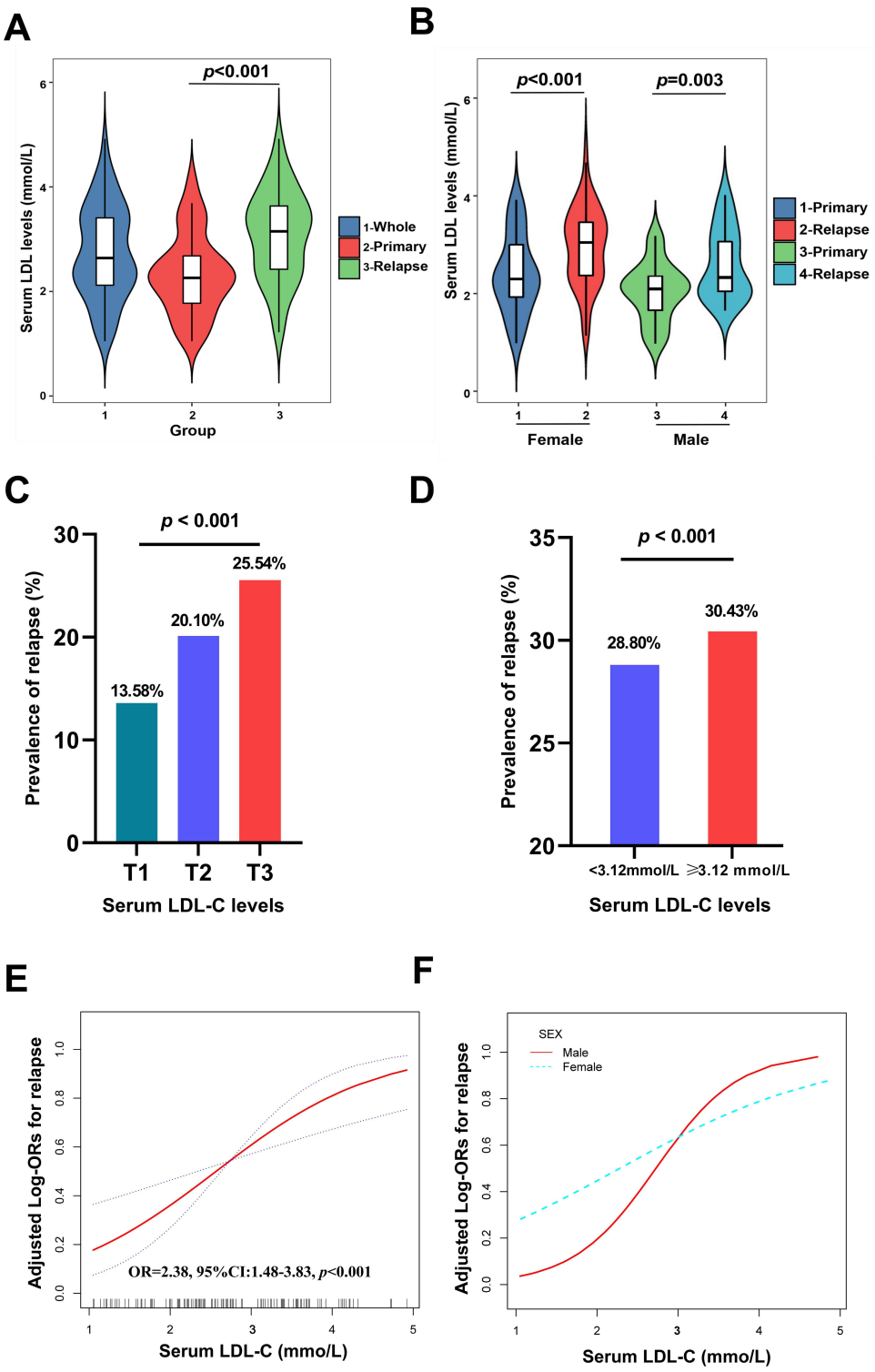

Figure 2 Association between serum LDL-C level and risk of relapse. (**A**) The LDL-C level was significantly higher in relapse group than that in primary group in all patients (p<0.001). (**B**) Similar results were observed in both female and male (p<0.001 and p=0.003). (**C**) The prevalence of relapse showed grade increase according to the increased LDL-C tertile's levels in all patients (p<0.001). (**D**) The prevalence of relapse in the high LDL-C group was significantly higher than that in the normal LDL-C group in all patients (p<0.001). (**E**) Linear relationships between the LDL-C level and relapse after adjusted for confounder factors. Solid lines represent the fitting curve and dotted lines represent the corresponding 95% CI. (**F**) Hierarchical analysis by sex also showed there are linear relationships between LDL-C level and relapse in women and men.

Comparison of Relapse According to the Serum LDL-C Level

This current analysis is to observe whether the prevalence of relapse increased with LDL-C levels. The mean LDL-C levels were 1.85±0.39 mmol/L, 2.68±0.28 mmol/L, and 3.76±0.43 mmol/L in the first, second, and third LDL-C tertiles for all patients ($p<0.001$). Prevalence of relapse showed grade increase according to the LDL-C tertile's levels. Prevalence of relapse were 25/184 (13.58%), 37/184 (20.10%), and 47/184 (25.54%) in the first, second, and third LDL-C tertile in all patients. The prevalence of relapse in the third tertile was more than that in the first tertile in all patients (25.54% vs 13.58%, $p<0.001$) (Figure 2C), in female patients (27.69% vs 14.61%, $p=0.001$), and in male patients (22.22% vs 11.11%, $p=0.012$) (Supplement Table 2). Prevalence of relapse also showed statistically significant grade increase in high LDL-C level group than that in the normal LDL-C level group according to the clinic value of LDL-C (Figure 2D) (Supplement Table 3).

Linear Curve Fitting of the Relationship Between LDL-C Level and Relapse

This current analysis is to observe the linear relationship between LDL-C levels and probability of relapse. Adjusted smoothed plots suggest that there were linear relationships between the LDL-C level and relapse after adjusting for sex, age, onset season, gastrointestinal infection, vision impaired, HDL-C, ALT, UA, SCR, AQP4-IgG, and IVIG (Figure 2E). Hierarchical analysis by sex also showed there are linear relationships between LDL-C level and relapse in women and men (Figure 2F). Spearman's correlation coefficients (95%) for the relationship between LDL-C level and relapse was 0.317 (0.148–0.468, $p< 0.001$) in female, 0.305 (0.032–0.535, $p=0.025$) in male, and 0.352 (0.214–0.476, $p< 0.001$) in all patients.

Association Between Serum LDL-C Level and Risk of Relapse

The current analysis is to evaluate the risk of LDL-C levels for relapse. Univariate analysis showed that sex, age, onset season, gastrointestinal infection, vision impaired, HDL-C, ALT, UA, SCR, AQP4-IgG, and IVIG were regarded as confounding factors related to relapse ($p<0.2$, Supplement Table 4). In multiple logistic regression analysis, the crude odds ratios (OR) (95% CI) for the relationship between LDL-C level and relapse was 2.67 (1.76–4.04, $p<0.001$) in all patients. When multivariate analysis was performed after adjusting for sex, age, onset season, gastrointestinal infection, vision impaired, HDL-C, ALT, UA, SCR, AQP4-IgG, and IVIG, the OR (95% CI) was 2.38 (1.48–3.83, $p<0.001$) in all patients, which showed that the association between LDL-C level and relapse was statistically significant in all patients. After subdividing subjects according to the LDL-C tertile, the crude OR (95% CI) were 1.0 (reference), 1.94 (0.94–3.98, $p=0.071$), and 4.80 (2.19–10.53, $p<0.001$), and 1.0 (reference), 1.50 (0.67–3.36, $p=0.223$), and 3.88 (1.55–9.72, $p=0.003$) in all patients after adjusting for sex, age, onset season, gastrointestinal infection, vision impaired, HDL-C, ALT, UA, SCR, AQP4-IgG, and IVIG, which showed that the statistical significance was maintained. The association between LDL-C level and relapse was statistically significant in all patients after subdividing subjects according to the clinic value of LDL-C (Table 2). Both sensitivity analysis and hierarchical analysis (according to sex, age, onset season, vision impaired, HDL-C, ALT, UA, SCR, AQP4-IgG, and IVIG) also showed that the association between LDL-C level and relapse was statistically significant (Figure 3) (Supplement Table 5 and 6).

ROC Curve of LDL-C Level to Identify Target Value of LDL-C Lowering Therapy

The current analysis is to calculate a target value of LDL-C lowering therapy. The ROC curve for the LDL-C level used to identify the optimal cut-off value of LDL-C is presented in Table 3. In all patients, the cut-off value of LDL-C level was 2.795 mmol/L, which gave a sensitivity of 57.80% and a specificity of 78.67%, as well as an area under the curve (AUC) of 0.706 (95% CI: 0.631–0.783). These results suggest that the target value of 2.795 mmol/L has a good performance in discriminating relapse from primary patients. After subdividing subjects according to the sex, the target value of LDL-C level was 2.790 mmol/L in female and 2.695 mmol/L in male, also showed that both the AUC were approach to 0.70 with a certain accuracy to discriminating relapse from primary patients. The ROC curves for the LDL-C level to identify the optimal cut-off value of LDL-C by gender is showed in Figure 4.

Table 2 Multivariate Logistic Regression for Effects of LDL-C on Relapse

Patients, N	Model 1 (Unadjusted)		Model 2 (Adjusted)	
	Incidence, n (%)	OR (95% CI) *P*	N	OR (95% CI) *P*
LDL-C (continuous) mmol/L, 184	109 (59.24)	2.67 (1.76, 4.04) < 0.001	184	2.38 (1.48, 3.83) < 0.001
LDL-C (categorical)				
Normal <3.12 mmol/L, 113	53 (46.90)	1.0 (reference)	113	1.0 (reference)
High ≥3.12 mmol/L, 71	56 (78.87)	4.23 (2.14, 8.34) < 0.001	71	3.92 (1.97, 7.83) < 0.001
LDL-C (categorical)				
T 1 (1.05–2.31) mmol/L, 61	25 (40.98)	1.0 (reference)	61	1.0 (reference)
T 2 (2.32–3.21) mmol/L, 61	37 (60.65)	1.94 (0.94, 3.98) 0.071	61	1.50 (0.67, 3.36) 0.223
T 3 (3.23–5.36) mmol/L, 62	47 (75.81)	4.80 (2.19, 10.53) <0.001	62	3.88 (1.55, 9.72) 0.003
p for trend		2.54 (1.59, 4.06) < 0.001		2.26 (1.31, 3.90) 0.003

Notes: Model 1: unadjusted, Model 2: adjusted for sex, age, onset season, gastrointestinal infection, vision impaired, HDL-C, ALT, UA, SCR, AQP4-IgG, and IVIG.

Difference of Relapse Grouped by LDL-C Lowering Target Value

To valid the potential benefits of LDL-C lowering target value, prevalence of relapse was compared and probability of relapse was evaluated by the Kaplan–Meier Plot between the high and low LDL-C patients grouped by target value.

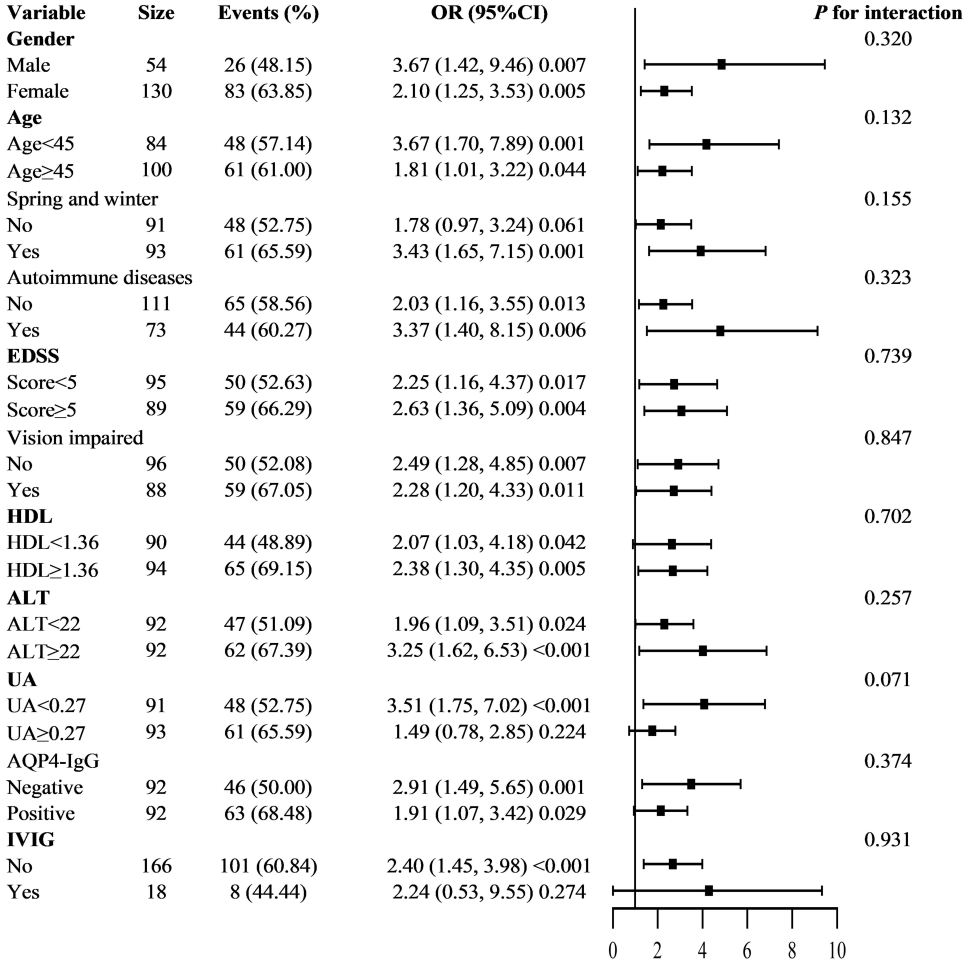

Figure 3 Hierarchical analysis on relationship of LDL-C and relapse. Each stratification adjusted for all the factors (sex, age, onset season, gastrointestinal infection, vision impaired, HDL-C, ALT, UA, SCR, AQP4-IgG, and IVIG) except the stratification factor itself.

Table 3 ROC Analyses of LDL-C Level to Identify Target Value in LDL-C Lowering

Variable	Cut-off (Target value)	AUC (95% CI)	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	ACC (%)
Whole	2.795	0.706 (0.631, 0.783)	57.80	78.67	56.19	79.75	66.30
Female	2.790	0.690 (0.594, 0.787)	62.65	72.34	52.31	80.00	66.15
Male	2.695	0.675 (0.531, 0.821)	46.15	85.71	75.00	63.16	66.67

Abbreviations: ACC, accuracy; AUC, area under curve; NPV, negative predictive value; PPV, positive predictive value; ROC, Receiver operating characteristic.

Prevalence of relapse were 46/105 (43.81%) and 63/79 (79.75%) in the low and high LDL-C groups by 2.795 mmol/L in all patients ($p<0.001$). The prevalence of relapse by 2.790 mmol/L in female and 2.695 mol/L in male also showed significantly difference respectively ($p<0.001$ and $p=0.010$) (Supplement Table 7). Kaplan–Meier Plot showed the probability of relapse was higher in high LDL-C level patients than those in low LDL-C level patients categorized by target value, and the hazard ratio (HR) for relapse was 1.51 (95% CI=1.03–2.19, $p= 0.027$) overall (Figure 5).

Discussion

This study found a positive correlation between LDL-C level and relapse, and the prevalence of relapse was increased with the graded LDL-C levels in NMOSD patients, furthermore, the association between LDL-C level and relapse was independent after adjustment for confounding risk factors and hierarchical analysis. Therefore, these results demonstrated that serum LDL-C should be well-controlled to prevent the relapse of NMOSD.

In physiological conditions, Lipids, especially lipoproteins, are components of the central neuron and is important for neuronal function.[15,16] Brain cholesterol metabolism is produced mainly by astrocytes, which is the location that merged with AQP4 cause the occurrence of NMOSD.[17,18] LDL-C has the function of transporting human plasma cholesterol and lipid substances cross the blood-brain barrier to participate in the synthesis of myelin oligodendrocytes, astrocytes, and neuron membrane structures in the CNS.[19,20] Peroxidation of lipid membrane and cholesterol metabolism have been involved in the physiopathology of brain diseases, and neurotoxicity was proved to be related to increased brain lipid peroxidation together with hypercholesterolemia.[21] Clinical studies also have shown that the esterification of free cholesterol is a feature of demyelination in white matter of brain and spinal cord.[22] In 2013, Weinstock Guttman and colleagues carried out a follow-up study, which found that high levels of LDL-C may predict the severity of demyelination in CNS, and its mechanism may be related to that the increased LDL-C promote the inflammatory activity.[23] In addition, cholesterol metabolites in the CNS can release into CSF, blood and urine. In 2017, Crick PJ and colleagues found that elevated concentrations of 25-hydroxycholesterol in CSF suggests cholesterol metabolism of demyelination or

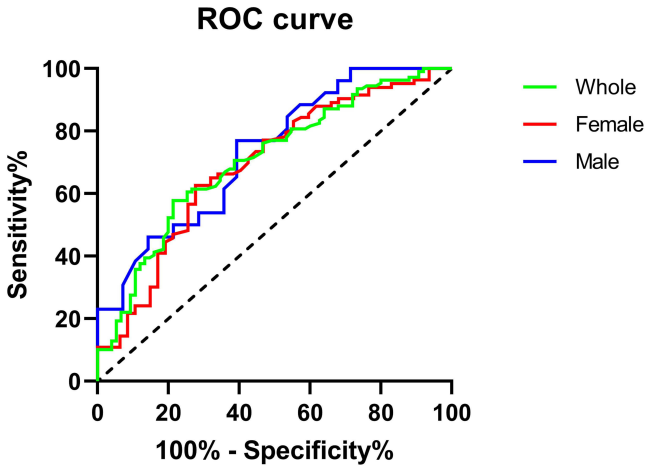

Figure 4 ROC curve of LDL-C level to identify target value of lowering LDL-C. The target value of lipid lowering in NMOSD patients was determined by the optimal cut-off value of LDL-C using ROC curves. These results suggest that the target LDL-C value of 2.795 mmol/L, 2.790 mmol/L, and 2.695 mmol/L have good performance in discriminating relapse from primary patients in whole, female and male patients, respectively. An AUC value <0.7 indicates low diagnostic accuracy, 0.7–0.9 indicates moderate accuracy, and >0.9 indicates high accuracy.

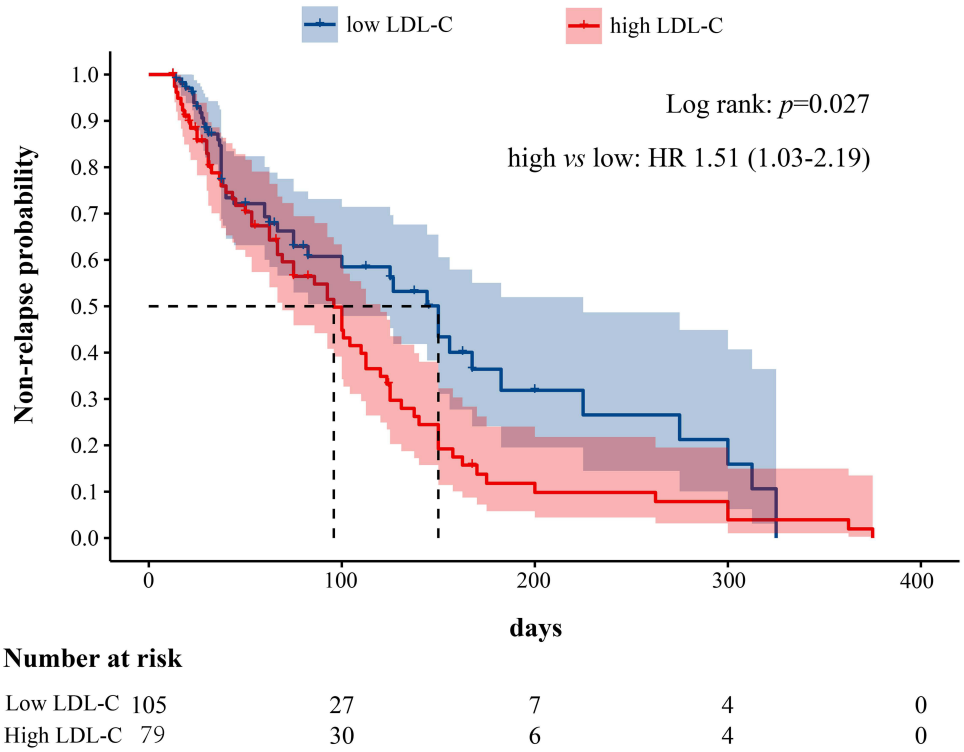

Figure 5 Kaplan-Meier Plot of relapse grouped by target value of lowering LDL-C. Patients with high level of LDL-C exhibited significantly shorter time for relapse (median survival = 98 days) than those with low LDL-C level (median survival = 180 days) (HR= 1.51, 95% CI=1.03–2.19, *p*= 0.027, Kaplan-Meier plot and Log rank test).

neuronal death.[24] Therefore, the damage of myelin phospholipids in CNS can be assessed by the level of LDL-C in peripheral blood circulation. Thus, the role of serum LDL-C level in NMOSD patients is further investigated in our study.

Cholesterol levels are associated with the severity of NMO. Studies have found that the values of LDL-C, CHO, TG and CHO/HDL-C in NMO patients were significantly higher than those in normal subjects.[9] Furthermore, another study suggested that serum apolipoprotein B (Apo B) level might indicate severer disability of NMO disease, while Apo B is the main component of LDL-C so as to be a biomarker of LDL-C.[25] Latest study found that serum LDL was positively correlated with neurological damage in NMOSD patients and might promote microglial activation and exacerbate myelin damage in NMOSD.[26] It further indicates that LDL-C level may associate with the severity of NMO, which supports the results in our study. Previous studies have revealed that there is a close relationship between dyslipidemia and CNS immune inflammation. A retrospective study was performed in a large sample of 8983 patients from the North American Multiple Sclerosis Research Council, which showed that dyslipidemia is associated with increased risk of disability progression in MS patients.[27] Clinical studies revealed that serum lipid profile variables particularly LDL-C level is associated with inflammatory MRI activity measures and worsening disability in CNS demyelinating disease.[23] Low HDL-C and high TG is correlated with disability and disease activity in NMOSD.[8] Animal studies have the same conclusion. A rat model study observed the elevated levels of serum LDL-C and CHO concentration in the experimental autoimmune encephalomyelitis.[28] The above study supports the conclusion that the high level of LDL-C is closely related to the inflammation and demyelination disease of CNS. Therefore, the high level of LDL-C may lead to the attack of NMOSD, which supports the results of our study.

The role of LDL-C in the relapse pathogenesis of NMOSD is unclear. The possible mechanism may be related to the oxidative stress and inflammation response. Elevated serum LDL-C is associated with increased level of oxidized LDL, which aggravates the reaction of oxidative stress and release of inflammatory mediators and cytokines in the peripheral circulation, including monocyte chemotactic protein 1, interleukin 6, interleukin 10 and tumor necrosis factor α.[29–32] High level of 27-hydroxycholesterol, which is the endogenous oxidized cholesterol in the blood circulation, could cause blood-brain barrier damage, subsequently, LDL-C in the blood circulation invades the CNS through the blood-brain barrier.[33–35] LDL-C would be oxidized under stress state in the CNS, as a proinflammatory factor, oxidized LDL-C could

activate a variety of inflammatory mediators (iNOS, COX-2, IL-1, IL-6) and induce the expression of apoptosis genes with result of neuronal damage.[36,37] The metabolic components of LDL-C and proinflammatory cytokines secreted by immune cells play a neurotoxic role, and a large number of immune cells were gathered, this leads to astrocytes damage.[19] It can also promote the production of 24-hydroxycholesterol and lipid peroxidation products, which circulation aggravates nerve cell damage in CNS.[38–40]

In addition, vascular comorbidity, such as hypercholesterolemia, is associated with disability progression of inflammatory demyelinating disease.[27] Studies also showed that statins treatment may be beneficial in MS and NMOSD,[41] which supports the results in our study. Statins inhibit ApoB (main component of LDL) and attenuate oxidation of cholesterol can reduce the pathogenic effect of AQP4-IgG.[42] Attenuation of cholesterol results in displacement of AQP4 from lipid rafts to non-rafts fraction of the membrane. Changes in the location of AQP4 on the membrane significantly reduced the complement dependent cytotoxic effect of AQP4-IgG, which is related to the pathogenesis of NMOSD.[43] Therefore, the elevated serum LDL-C may be associated with the risk of relapse in NMOSD patients. The theoretical target value for lipid-lowering management showed in our study may be benefit to prevent the relapse in NMOSD patients. All above mechanism comes from studies of CNS demyelination related diseases. Of course, the specific mechanism still needs to be further studied.

Several limitations should be considered in the interpretation of our results. First, this was a prospective observational study, and although we did multiple logistic regression analysis, establishing a causal link needs further prospective randomized lipid-lowering clinical trial study with a large sample. Second, the study analyzed Chinese NMOSD patients, who belong to Asian people, and whether the relapse of NMOSD is related to the genes of Asian people needs further study. It also needs more warrants investigation in other populations to confirm the generalizability of our results. Third, the retest results of LDL-C during follow-up were not included in this study. Further investigation is needed.

Conclusions

In conclusion, this study demonstrates that elevated serum LDL-C was independently and positively associated with the relapse in NMOSD patients. This study also provides a theoretical target value for lipid-lowering management in NMOSD. Further studies are required a randomized controlled trial study with a large sample to identify the value of LDL-C level in relapse in NMOSD patients.

Data Sharing Statement

The datasets used and/or analyzed are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The authors confirm that this study was performed in accordance with relevant guidelines and regulations.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing financial interests.

References

1. Wingerchuk DM, Banwell B, Bennett JL, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology*. 2015;85(2):177–189. doi:10.1212/WNL.0000000000001729
2. Houzen H, Kondo K, Niino M, et al. Prevalence and clinical features of neuromyelitis optica spectrum disorders in northern Japan. *NEUROLOGY*. 2017;89(19):1995–2001. doi:10.1212/WNL.0000000000004611
3. Viswanathan S, Wah LM. A nationwide epidemiological study on the prevalence of multiple sclerosis and neuromyelitis optica spectrum disorder with important multi-ethnic differences in Malaysia. *Mult Scler*. 2019;25(11):1452–1461. doi:10.1177/1352458518792430
4. Jarius S, Paul F, Weinshenker BG, et al. Neuromyelitis optica[J]. *Nat Rev Dis Primers*. 2020;6(1):85. doi:10.1038/s41572-020-0214-9
5. Rechtman A, Zveik O, Haham N, Freidman-Korn T, Vaknin-Dembinsky A. Thyroid hormone dysfunction in MOGAD and other demyelinating diseases. *J Neurol Sci*. 2024;15(457):122866. doi:10.1016/j.jns.2024.122866
6. Ma X, Kermode AG, Hu X, Qiu W. Risk of relapse in patients with neuromyelitis optica spectrum disorder: recognition and preventive strategy. *Mult Scler Relat Disord*. 2020;46:102522. doi:10.1016/j.msard.2020.102522
7. Yin HX, Wang YJ, Liu MG, et al. Aquaporin-4 antibody dynamics and relapse risk in seropositive neuromyelitis optica spectrum disorder treated with immunosuppressants. *Ann Neurol*. 2023;93(6):1069–1081. doi:10.1002/ana.26623
8. Cho EB, Cho HJ, Choi M, et al. Low high-density lipoprotein cholesterol and high triglycerides lipid profile in neuromyelitis optica spectrum disorder: associations with disease activity and disability. *Mult Scler Relat Disord*. 2020;40:101981. doi:10.1016/j.msard.2020.101981
9. Wu K, Wen L, Duan R, et al. Triglyceride level is an independent risk factor in first-attacked neuromyelitis optica spectrum disorders patients. *Front Neurol*. 2019;10:1230. doi:10.3389/fneur.2019.01230
10. Brizzi P, Tonolo G, Carusillo F, Malaguarnera M, Maioli M, Musumeci S. Plasma lipid composition and LDL oxidation. *CLIN CHEM LAB MED*. 2003;41(1):56–60. doi:10.1515/CCLM.2003.010
11. Xiang W, Wu Y, Li H, et al. Serum albumin level can predict immunotherapy response of neuromyelitis optica spectrum disorders in the acute phase. *J INFLAMM RES*. 2024;17:909–917. doi:10.2147/JIR.S442532
12. Zhu DS, Yu L, Li M, et al. High serum creatinine is associated with reduction of vision impaired in patients with NMOSD. *J NEUROIMMUNOL*. 2017;310:32–37. doi:10.1016/j.jneuroim.2017.06.003
13. Ding J, Zhu DS, Hong RH, et al. The differential expression of natural killer cells in NMOSD and MS. *J CLIN NEUROSCI*. 2020;71:9–14. doi:10.1016/j.jocn.2019.11.022
14. Fujihara K. Neuromyelitis optica spectrum disorders: still evolving and broadening. *CURR OPIN NEUROL*. 2019;32(3):385–394. doi:10.1097/WCO.0000000000000694
15. Birolini G, Verlengia G, Talpo F, et al. SREBP2 gene therapy targeting striatal astrocytes ameliorates Huntington's disease phenotypes. *BRAIN*. 2021;144(10):3175–3190. doi:10.1093/brain/awab186
16. Montani L. Lipids in regulating oligodendrocyte structure and function. *SEMIN CELL DEV BIOL*. 2021;112:114–122. doi:10.1016/j.semcdb.2020.07.016
17. Trager J, Widder K, Kerth A, Harauz G, Hinderberger D. *Effect of Cholesterol and Myelin Basic Protein (MBP) Content on Lipid Monolayers Mimicking the Cytoplasmic Membrane of Myelin*. CELLS-BASEL; 2020:9.
18. Winkler A, Wrzos C, Haberl M, et al. Blood-brain barrier resealing in neuromyelitis optica occurs independently of astrocyte regeneration. *J CLIN INVEST*. 2021;131.
19. Werkman IL, Kovilein J, de Jonge JC, Baron W. Impairing committed cholesterol biosynthesis in white matter astrocytes, but not grey matter astrocytes, enhances in vitro myelination. *J NEUROCHEM*. 2021;156(5):624–641. doi:10.1111/jnc.15113
20. Wang H, Kulas JA, Wang C, Holtzman DM, Ferris HA, Hansen SB. Regulation of beta-amyloid production in neurons by astrocyte-derived cholesterol. *Proc Natl Acad Sci U S A*. 2021;118: e2102191118.
21. Belaid-Nouira Y, Bakhta H, Bouaziz M, Flehi-Slim I, Haouas Z, Ben CH. Study of lipid profile and parieto-temporal lipid peroxidation in AlCl(3) mediated neurotoxicity. Modulatory effect of fenugreek seeds. *Lipids Health Dis*. 2012;11(1):16. doi:10.1186/1476-511X-11-16
22. Grassi S, Giussani P, Mauri L, Prioni S, Sonnino S, Prinetti A. Lipid rafts and neurodegeneration: structural and functional roles in physiologic aging and neurodegenerative diseases. *J LIPID RES*. 2020;61(5):636–654. doi:10.1194/jlr.TR119000427
23. Weinstock-Guttman B, Zivadinov R, Horakova D, et al. Lipid profiles are associated with lesion formation over 24 months in interferon-beta treated patients following the first demyelinating event. *J Neurol Neurosurg Psychiatry*. 2013;84(11):1186–1191. doi:10.1136/jnnp-2012-304740
24. Crick PJ, Griffiths WJ, Zhang J, et al. Reduced plasma levels of 25-hydroxycholesterol and increased cerebrospinal fluid levels of bile acid precursors in multiple sclerosis patients. *Mol Neurobiol*. 2017;54(10):8009–8020. doi:10.1007/s12035-016-0281-9
25. Li Y, Wang H, Hu X, Peng F, Yang Y. Serum lipoprotein levels in patients with neuromyelitis optica elevated but had little correlation with clinical presentations. *Clin Neurol Neurosurg*. 2010;112(6):478–481. doi:10.1016/j.clineuro.2010.03.017
26. Chen M, Chu YH, Yu WX, et al. Serum LDL promotes microglial activation and exacerbates demyelinating injury in neuromyelitis optica spectrum disorder. *Neurosci Bull*. 2024;40(8):1104–1114. doi:10.1007/s12264-023-01166-y
27. Marrie RA, Rudick R, Horwitz R, et al. Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. *NEUROLOGY*. 2010;74(13):1041–1047. doi:10.1212/WNL.0b013e3181d6b125
28. Shore VG, Smith ME, Perret V, Laskaris MA. Alterations in plasma lipoproteins and apolipoproteins in experimental allergic encephalomyelitis. *J LIPID RES*. 1987;28(2):119–129. doi:10.1016/S0022-2275(20)38718-6
29. Estruch M, Sanchez-Quesada JL, Ordonez LJ, Benitez S. Electronegative LDL: a circulating modified LDL with a role in inflammation. *Mediators Inflamm*. 2013;2013:181324. doi:10.1155/2013/181324
30. Tekin IO, Orem A, Shiri-Sverdlov R. Oxidized LDL in inflammation: from bench to bedside. *Mediators Inflamm*. 2013;2013:762759. doi:10.1155/2013/762759
31. Lara-Guzman OJ, Gil-Izquierdo A, Medina S, et al. Oxidized LDL triggers changes in oxidative stress and inflammatory biomarkers in human macrophages. *REDOX BIOL*. 2018;15:1–11. doi:10.1016/j.redox.2017.11.017
32. Cimmino G, Cirillo P, Conte S, et al. Oxidized low-density lipoproteins induce tissue factor expression in T-lymphocytes via activation of lectin-like oxidized low-density lipoprotein receptor-1. *CARDIOVASC RES*. 2020;116(6):1125–1135. doi:10.1093/cvr/cvz230

33. Cicolari S, Pavanello C, Olmastroni E, et al. Interactions of oxysterols with atherosclerosis biomarkers in subjects with moderate hypercholesterolemia and effects of a nutraceutical combination (bifidobacterium longum BB536, red yeast rice extract) (randomized, double-blind, placebo-controlled study). *NUTRIENTS*. 2021;14(1):13. doi:10.3390/nu14010013
34. Ran RJ, Zheng XY, Du LP, Zhang XD, Chen XL, Zhu SY. Upregulated inflammatory associated factors and blood-retinal barrier changes in the retina of type 2 diabetes mellitus model. *Int J Ophthalmol*. 2016;9(11):1591–1597. doi:10.18240/ijo.2016.11.09
35. Griffiths H, Irundika D, Lip G, Spickett C, Polidori C. Oxidised LDL lipids, statins and a blood-brain barrier. *Free Radic Biol Med*. 2014;75(Suppl 1):S15–S16. doi:10.1016/j.freeradbiomed.2014.10.591
36. Alrafiah A, Neff LS, Broughton P, Bradshaw AD, DeLeon-Pennell KY. Thymoquinone protects neurons in the cerebellum of rats through mitigating oxidative stress and inflammation following high-fat diet supplementation. *Biomolecules*. 2021;12(1):11. doi:10.3390/biom12010011
37. Jung YS, Park JH, Kim H, et al. Probucol inhibits LPS-induced microglia activation and ameliorates brain ischemic injury in normal and hyperlipidemic mice. *ACTA PHARMACOL SIN*. 2016;37(8):1031–1044. doi:10.1038/aps.2016.51
38. Sodero AO. 24S-hydroxycholesterol: cellular effects and variations in brain diseases. *J NEUROCHEM*. 2021;157(4):899–918. doi:10.1111/jnc.15228
39. Cigliano L, Spagnuolo MS, Napolitano G, et al. 24S-hydroxycholesterol affects redox homeostasis in human glial U-87MG cells. *MOL CELL ENDOCRINOL*. 2019;486:25–33. doi:10.1016/j.mce.2019.02.013
40. Bjorkhem I, Leoni V, Svenningsson P. On the fluxes of side-chain oxidized oxysterols across blood-brain and blood-CSF barriers and origin of these steroids in CSF (Review). *J Steroid Biochem Mol Biol*. 2019;188:86–89. doi:10.1016/j.jsbmb.2018.12.009
41. Tradtrantip L, Duan T, Yeaman MR, Verkman AS. CD55 upregulation in astrocytes by statins as potential therapy for AQP4-IgG seropositive neuromyelitis optica. *J Neuroinflammation*. 2019;16(1):57. doi:10.1186/s12974-019-1448-x
42. Zhornitsky S, McKay KA, Metz LM, Teunissen CE, Rangachari M. Cholesterol and markers of cholesterol turnover in multiple sclerosis: relationship with disease outcomes. *Mult Scler Relat Disord*. 2016;5:53–65. doi:10.1016/j.msard.2015.10.005
43. Asakura K, Ueda A, Shima S, et al. Targeting of aquaporin 4 into lipid rafts and its biological significance. *BRAIN RES*. 2014;1583:237–244. doi:10.1016/j.brainres.2014.08.014