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Association Between Serum Lipids and Survival in Patients With Amyotrophic Lateral Sclerosis

A Meta-analysis and Population-Based Study

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Abstract

Background and Objective

To explore the association between lipids, polygenic profile scores (PPS) for biomarkers of lipid metabolism, markers of disease severity, and survival in patients with amyotrophic lateral sclerosis (ALS).

Methods

We meta-analyzed the current literature on the prognostic value of lipids in patients with ALS. Subsequently, we evaluated the relationship between lipid levels at diagnosis, clinical disease stage, and survival in all consecutive patients diagnosed in the Netherlands. We determined the hazard ratio (HR) of each lipid for overall survival, defined as death from any cause. A subset of patients was matched to a previous genome-wide association study; data were used to calculate PPS for biomarkers of lipid metabolism and to determine the association between observed lipid levels at diagnosis and survival.

Results

Meta-analysis of 4 studies indicated that none of the biomarkers of the lipid metabolism were statistically significantly associated with overall survival; there was, however, considerable heterogeneity between study results. Using individual patient data (N = 1,324), we found that increased high-density lipoprotein (HDL) cholesterol was associated with poorer survival (HR of 1.33 (95% CI 1.14–1.55, $p < 0.001$)). The correlation between BMI and HDL cholesterol (Pearson r −0.26, 95% CI −0.32 to −0.20) was negative and between BMI and triglycerides (TG) positive (Pearson r 0.18, 95% CI 0.12–0.24). Serum concentrations of total cholesterol and LDL cholesterol were lower in more advanced clinical stages (both $p < 0.001$). PPS for biomarkers of lipid metabolism explained 1.2%–13.1% of their variance at diagnosis. None of the PPS was significantly associated with survival (all $p > 0.50$).

Discussion

Lipids may contain valuable information about disease severity and prognosis, but their main value may be driven as a consequence of disease progression. Our results underscore that gaining further insight into lipid metabolism and longitudinal data on serum concentrations of the lipid profile could improve the monitoring of patients and potentially further disentangle ALS pathogenesis.

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Glossary

ALS = amyotrophic lateral sclerosis; **ALSFRS** = ALS functional rating scale; **BMI** = body mass index; **EEC** = El Escorial criteria; **ENCALS** = European Network to Cure ALS; **FTD** = frontotemporal dementia; **HDL** = high-density lipoprotein; **HDL-C** = HDL cholesterol; **HR** = hazard ratio; **LDL-C** = low-density lipoprotein cholesterol; **PPS** = polygenic profile score; **TC** = total cholesterol; **TG** = triglycerides.

Lipids act as structural components of neuronal membranes, signaling molecules and energy substrates required for normal functioning of neurons.[1] Although the exact pathophysiologic mechanisms underlying amyotrophic lateral sclerosis (ALS) are unknown,[2] it is likely that the origins of the condition lie in a multistep process,[3] followed by intraneuronal disease propagation, altered neuronal metabolism, and ultimately neuronal death. Dysregulated energy metabolism is a consequence of this process,[4] which also affects biomarkers of the lipid metabolism, such as cholesterol, its carriers (i.e. LDL and HDL cholesterol), and triglycerides (TG). Albeit little is known about changes in the preclinical stage, 2 recent studies comprising a Mendelian randomized study,[5] and a prospective cohort study of over 500,000 people,[6] related premorbid metabolic changes to the risk of ALS.

The association between biomarkers of lipid metabolism, prognosis, and disease progression after disease onset has proven more difficult to characterize. Although high lipid levels have been shown to increase metabolic stress[7-9] and potentially lead to a more aggressive disease course,[2] some studies have suggested that abnormal lipid levels may actually be beneficial to the patient's prognosis.[10-14] Elucidating the interplay between clinical phenotype and lipid metabolism may reveal potential therapeutic interventions and better address the mixed results from dietary interventions obtained thus far.[15,16] In this study, therefore, we aim to summarize the current literature and to explore the relationships between lipids, ALS survival, polygenic profile scores (PPS) for lipid levels, and markers of disease progression in a large population-based study, to address the disparate data in the literature.

Methods

A two-step approach was used. First, we conducted a systematic review to summarize and meta-analyze the current literature on the prognostic value of biomarkers of lipid metabolism in patients with ALS. Second, we assessed the prognostic value of lipids in a large population-based cohort study, explored their relationship with disease severity, and assessed the causal association between PPSs and survival after disease onset. Throughout the text, we define "biomarkers of lipid metabolism" as an umbrella term for total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and TG.

Systematic Review

Search and Study Selection

We conducted the systematic search in 4 literature databases: PubMed, EMBASE, DARE, and the Cochrane Library; the study protocol for the systematic review is presented in the supplementary material (eAppendix 1: Systematic Review Protocol,, links.lww.com/WNL/C516). Additional forms or information, such as data collection forms, can be provided on request. The primary purpose of the meta-analyses was to provide an explanatory summary of the current literature. All databases were last searched in June 2022. Search terms included the MeSH terms: "Amyotrophic Lateral Sclerosis"; "Motor Neuron Disease"; "Cholesterol"; "Cholesterol, LDL"; "Cholesterol, HDL"; "Triglyceride"; "Lipid"; "Prognosis"; "Survival"; "Mortality"; "Kaplan-Meier estimate"; and "Proportional Hazard Models." Studies were selected on the basis of the following inclusion criteria: (1) participants diagnosed with ALS according to the revised El Escorial criteria (EEC)[17]; (2) reporting of at least one of the following measurements: TC, HDL-C, LDL-C, or TG, obtained after symptom onset; (3) reporting of survival time and hazard ratio (HR); and (4) written in English or Dutch. Study eligibility was not based on sample size. All articles were screened independently by 2 reviewers for title and abstract (M.J.v.M. and A.H.). Included and excluded articles were discussed; if no consensus was reached, a third reviewer was consulted (R.P.A.v.E.).

Data Collection and Meta-analysis

For each included study, we extracted the following variables: author, publication year, country, number of participants, and statistical analysis parameters (that is, covariates, HR, and 95% CI). We used the Quality in Prognosis Studies tool to determine the quality and risk of bias of the included articles.[18] Studies that provided a HR for at least one, nondichotomized, biomarker of the lipid metabolism were included in the meta-analysis. Standardized HRs (SE) were back-transformed to mmol/L by dividing by the study standard deviation; if studies reported biomarkers of lipid metabolism in mg/dL, data were converted to mmol/L by dividing the HR (SE) by 0.02586 for TC, LDL-C, and HDL-C or by 0.01129 for TG. Meta-analyses were conducted using a Bayesian hierarchical model using a noninformative uniform prior for the log HR and a weakly informative prior for the heterogeneity parameter (half normal with standard deviation of 0.5). As sensitivity analysis, we varied the prior for the heterogeneity parameter using either a standard deviation of 0.25 or 1.0.[19] Funnel plots were used to visually inspect publication bias and study heterogeneity (eFigure 1, links.lww.com/WNL/C516). We estimated the heterogeneity between studies using the I^2 statistic and expressed this as percentage. The meta-analyses provide the pooled HR on

survival across studies for each biomarker of lipid metabolism in mmol/L.

Population-Based Cohort

For the second part of this study, we conducted a prospective analysis of the national registry of the Netherlands ALS Center, selecting all consecutive patients diagnosed in the University Medical Center Utrecht (UMCU), Utrecht, the Netherlands, between January 1, 2012, and December 31, 2017, to ensure sufficient follow-up time for survival. All patients were diagnosed with possible, probable laboratory supported, probable, or definite ALS.[17] The UMCU is a referral center for all patients with ALS across our country. All clinical characteristics were collected at the time of diagnosis. The King's clinical staging system[20] was determined according to the standard operating procedures provided by the European Network to Cure ALS (ENCALS).[21] Patients with more than 30 hexanucleotide repeats in the *C9orf72* gene were considered to be *C9orf72* carriers.[22] We defined survival time as time between date of diagnosis and date of death or date last known to be alive. Survival information was updated at quarterly intervals by cross-referencing with the municipal population register. All patients were administratively censored on 9 July 2020. Data were further supplemented with the revised ALS functional rating scale (ALSFRS-R) collected at time of diagnosis.[15] For a subset of patients, longitudinal data of the ALSFRS-R were available, obtained during either clinical follow-up or previous participation in clinical research.

Blood Sample Collection

Blood samples were collected from patients in a nonfasting state on the day of diagnosis or within one month after diagnosis.[23] We determined TC, LDL cholesterol, HDL cholesterol, and TG with the Beckman Coulter AU5800 clinical chemistry analyzer series. Normal ranges were defined according to the central diagnostic laboratory of the UMCU: TC 3.5–6.5 mmol/L, LDL-C < 3.5 mmol/L, HDL-C > 0.90 mmol/L for male patients, HDL-C > 1.1 mmol/L for female patients, and TG 0.0–2.0 mmol/L.

Statistical Analysis

We performed our statistical analyses using RStudio (version 1.1.4, RStudio: Integrated Development for R, Boston, USA, rstudio.com/). Mean and SD were determined and summarized for continuous variables; for categorical variables, we determined frequency and proportion. The Cox proportional hazard model was applied to assess the association between the risk of death and biomarkers of lipid metabolism at diagnosis. All models were adjusted for the 8 clinical predictors—combined in a linear predictor—from the ENCALS survival model,[24] namely age at onset, diagnostic delay, bulbar onset, definite ALS according to the revised EEC,[17] prediagnostic progression rate (ΔFRS),[25] percentage (%) of predicted forced vital capacity, presence of frontotemporal dementia (FTD), and carrier of the *C9orf72* repeat expansion. For each analysis, the following sensitivity analyses were conducted: (1) adding an interaction term between biomarker level and sex (i.e. is the effect of the biomarker different for male patients vs female patients?) and similarly for age at diagnosis, (2) adding quadratic terms to explore potential nonlinear relationships between the risk of death and the biomarker level, and (3) additional adjustment for body mass index (BMI) and weight loss, factors known to be associated with both the lipid level and survival.[26] Data missing for any variable except the outcome were addressed by creating multiple imputed data sets (n = 100), using predictive mean and bootstrapping, discarding the first 100 iterations (burn-in). In total, 9.2% of all observations were missing and, therefore, imputed. All covariates were included in a stratified imputation model per diagnostic year; survival time was included as cumulative hazard rate (Nelson-Aalen estimator).[27] The results across imputations were pooled using Rubin rules.[28]

We further explored longitudinal trends in disease progression rate by assessing the relationship between lipid levels at diagnosis and decrease in ALSFRS-R since diagnosis using linear mixed-effects models. Models contained a fixed effect for time since diagnosis (in months), lipid level, and the interaction between time and lipid level; the random part contained a random slope for time and intercept per patient. We used a likelihood ratio test to assess the significance of the interaction between lipid level and time (i.e., is the rate of ALSFRS-R progression dependent on lipid level?). In addition, we assessed the cross-sectional association between lipid levels, BMI, and King's Clinical Staging[20] at diagnosis using linear regression models. Sensitivity analyses were conducted by introducing interaction terms for sex to assess potential male-female differences. All analyses of the TG level were performed on the natural logarithm scale because of their right-skewed distribution.

PPS

As an exploratory analysis, we estimated PPS for biomarkers of lipid metabolism.[29] The PPS estimates the sum of additive genetic effects across all alleles that affect the biomarkers of lipid metabolism at the patient level. We used the PPS to explore a potential genetic link between lipid metabolism, ALS, and survival time by assessing (1) how much of the variance in biomarker levels at diagnosis can be explained by genetic profile scores and (2) whether the genetic profile score itself is associated with overall survival time. Because PPS does not change over time,[30] a statistical association between the genetic profile score and survival may be evidence of abnormal lipid levels caused by genetic variation or hold potential for therapeutic interventions.[30] Moreover, their time invariance allowed us to estimate the link between the genetic profile score and overall survival time, defined as time between symptom onset and death.

For all individuals who were enrolled in both our population-based registry and our latest genome-wide association study (GWAS),[5] we calculated the PPS. PPS was based on summary statistics from a GWAS on biomarker levels of lipid metabolism in the UK Biobank.[31] For each

Table 1 Overview of Included Studies

Author	Year	Survival time from	No. of patients	Lipids	Multivariable analysis	Conclusion	Risk of bias[a]
Nakamura et al.[32]	2022	symptom onset	78	TC, LDL-C, HDL-C, TG	Age, sex, ALSFRS-R slope, BMI slope, bulbar onset, VC	HDL-C levels are an independent predictor of worse survival	Low
Ingre et al.[10]	2020	date of diagnosis	99	TC, LDL-C, HDL-C, TG, Ratio	Age, sex, symptom duration, site of onset, BMI, ALSFRS-R score, ΔFRS	High TC, LDL-C, or ratio levels are predictors of better survival	Low[b]
Barone et al.[48]	2019	date of PEG placement	47	TC, Ratio	Age, BMI	No effect of cholesterol levels on survival	Moderate
Ahmed et al.[12]	2018	symptom onset	96	TC, LDL-C, HDL-C, TG, Ratio	Age, symptom duration, cognitive, and/or behavioral involvement	High TC levels are a predictor of better survival	Low[b]
Huang et al.[13]	2015	symptom onset	413	TC, LDL-C, HDL-C, TG	Age, sex, symptom duration, ALSFRS-R score	High TG levels are a predictor of better survival	Low[b]
Rafiq et al.[45]	2015	study inclusion	512	TC, LDL-C, HDL-C, TG, Ratio	Age, sex, symptom duration, weight, site of onset, VC	No effect of cholesterol levels on survival	Moderate[b]
Sutedja et al.[49]	2011	study inclusion	303	TC, LDL, HDL, Ratio	Age, site of onset, VC	No effect of cholesterol levels on survival	Moderate
Dorst et al.[11]	2011	symptom onset	488	TC, LDL, TG, Ratio	Age, sex, ALSFRS-R score, BMI, glucose serum level	No effect of cholesterol levels on survival	Moderate
Dupuis et al.[14]	2008	symptom onset	369	TC, LDL, HDL, TG, Ratio	None	High ratio levels are a predictor of worse survival	Moderate

Abbreviations: ALSFRS-R = ALS functional rating scale; ΔFRS = (48 − ALSFRS-R score)/symptom duration, PEG = percutaneous endoscopic gastrostomy; ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia; LDL = low-density lipoprotein; HDL = high-density lipoprotein; TC = total cholesterol; TG = triglycerides; Ratio = LDL-C/HDL-C; BMI = body mass index; VC = vital capacity; QUIPS = Quality in Prognosis Studies.
Table summarizes the baseline demographics of the included studies.
[a] Risk of Bias based on QUIPS tool (eFigure 3, links.lww.com/WNL/C516).[18]
[b] Included in meta-analysis.

single-nucleotide polymorphism, we calculated a weight for each biomarker using the summary-BavesR module in the Genome-Wide Complex Trait Bayesian analysis toolkit (default parameters)[29] and a linkage-disequilibrium matrix originating from 50,000 unrelated individuals of inferred European ancestries included in the UK Biobank. Because the genotype data originated from several different cohorts in the ALS GWAS, we scaled the PPS per GWAS cohort to a mean of zero and a standard deviation of 1. Linear regression models were used to calculate how much of the variance in the biomarker level was explained by their PPS (expressed as adjusted R^2); 95% confidence intervals were obtained by means of bootstrapping. Simple univariable Cox models for overall survival time (i.e., from onset to death) were used to estimate HRs.

Standard Protocol Approvals, Registrations, and Patient Consents

The medical ethics committee and institutional review board of the University Medical Center Utrecht (METC NedMec) approved this study (Study Registration Number: METC 19–190). Written consent was obtained from all study participants before this study.

Data Availability

All protocol, analyses, and anonymized data will be shared on request. We take full responsibility for data, analyses and interpretation, and conduct of the research.

Results

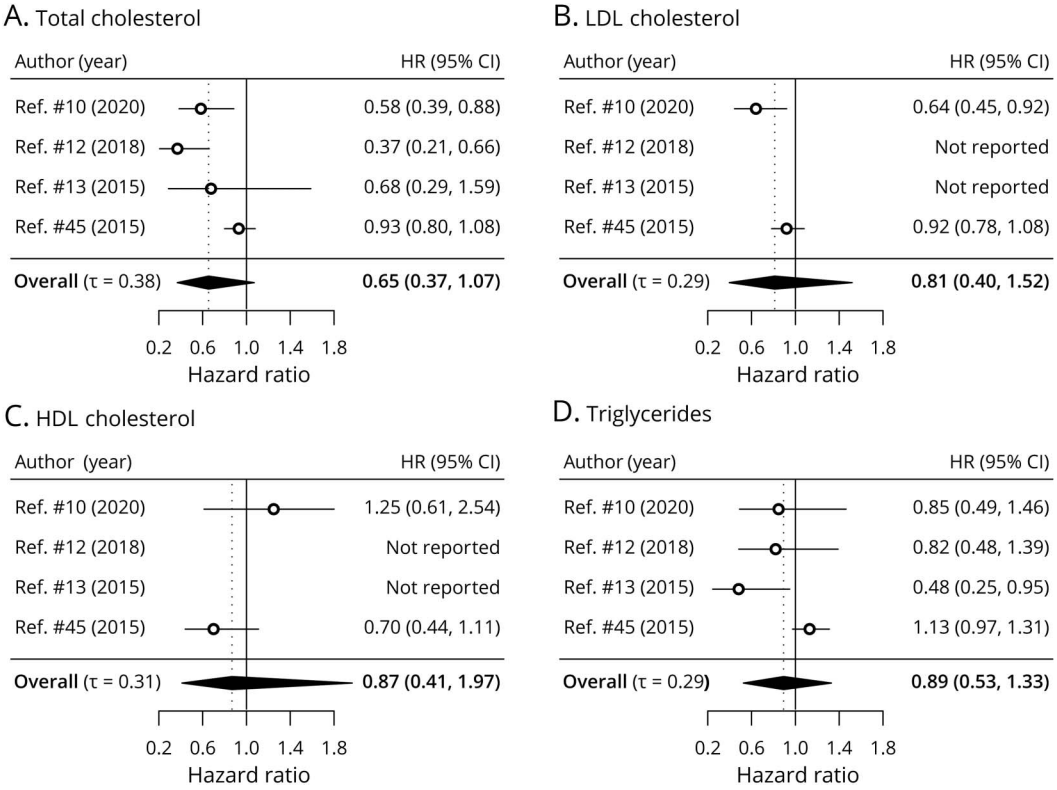
Systematic Review and Meta-analysis

Of the 624 citations screened, 9 articles were included (eFigure 2, links.lww.com/WNL/C516), 5 of which found a significant association between survival time and serum levels of TC, LDL/HDL ratio, HDL cholesterol, or TG; their characteristics are summarized in Table 1. Studies included different prognosticators in their multivariable model; none adjusted for all known prognosticators in patients with ALS.[24] 4 studies reported a nondichotomized HR and were included in the meta-analysis, resulting in a total sample size of 1,120 patients (Figure 1). The risk of bias assessment of the individual studies is presented in eFigure 3. None of the biomarkers of the lipid metabolism reached statistical significance (Figure 1), although the 95% credible intervals included clinically relevant effect sizes. There was, however, considerable heterogeneity between study results, reflected as τ, indicating possible differences in methodology. Changing the prior assumptions resulted in similar findings (not shown). In eFigure 1, we provide the funnel plot to explore publication bias; it should be noted that, given the small number of studies, their interpretation is limited.

Population-Based Cohort

In total, 1,324 patients with ALS were enrolled in our population-based registry. At the time of administrative censoring (July 2020), 1,185 deaths (89.5% of enrolled population) had occurred during 2,370 person-years of follow-up. The median survival

Figure 1 Forest Plot of the Included Studies for Biomarkers of Lipid Metabolism



Meta-analysis of the reported HRs in the literature. HR of each lipid for survival defined as the time in months from study enrollment to death from any cause or administrative censoring. The overall HR reflects the pooled HR across studies in mmol/L. Abbreviations: HR = hazard ratio; CI = credible interval.

since diagnosis was 16.5 months (95% CI 15.7–17.5). Baseline characteristics of the cohort are listed in Table 2; 688 patients (52%) had been enrolled in our latest GWAS study and were included in the PPS analysis. Overall, 20.1% of the patients had elevated TC, 42.0% elevated LDL-C, 4.9% reduced HDL-C, and 19.2% elevated TG levels on the day of diagnosis.

After adjustment for age, site of onset, diagnostic delay, prediagnostic progression rate (ΔFRS), vital capacity, presence of FTD, *C9orf72* repeated expansion, and El Escorial classification,[24] a 1 mmol/L increase of HDL-C was found to be associated with a higher risk of death and shorter survival time after ALS diagnosis, HR of 1.33 (95% CI 1.14–1.55, $p < 0.001$, Table 3). This effect was larger for male patients than for female patients: HR (male patients) 1.48 vs HR (female patients) 1.13, although not statistically significantly different (interaction term $p = 0.094$). The effect was similar for different ages at diagnosis (HR-interaction 1.00; 95% CI 0.98–1.02, $p = 0.97$). Introduction of a nonlinear term did not result in a significant model improvement ($p = 0.84$). Additional adjustment for weight loss (HR of 1.37, 95% CI 1.17–1.61) or body mass index (HR of 1.28, (95% CI 1.09–1.50) did not alter our results.

Longitudinal ALSFRS-R data, that is, 2 or more measurements, were available for 419 of the 1,324 patients (31.6%). Average progression rate after diagnosis was 0.79 points per month (95% CI 0.73–0.85). With each mmol/L increase in HDL-C, the monthly ALSFRS-R progression rate increased by 0.10 points per month (95% CI −0.07 to 0.26, $p = 0.21$), indicating a similar directional effect as observed on survival, albeit not statistically significant. None of the other biomarkers of the lipid metabolism was significantly associated with the monthly progression rate (all $p > 0.15$).

Figure 2 and Figure 3 present the standardized distributions of the biomarkers of lipid metabolism stratified by BMI category and King's clinical stage at diagnosis, respectively. Both HDL-C (Pearson r −0.26, 95% CI −0.32 to −0.20) and TG (Pearson r 0.18, 95% CI 0.12–0.24) were associated with BMI at diagnosis (both $p < 0.001$); these relationships were similar for male patients and female patients (both interaction terms $p > 0.40$). Similarly, TC and LDL-C depended on King's clinical staging and showed a declining trend for more advanced disease stages (both $p < 0.001$); again, these associations were similar for male patients and female patients (both interaction terms $p > 0.75$). The results were similar when categorizing the ALSFRS-R into 4 equal categories (*results not shown*).

Analysis of PPSs

Finally, in Table 4, we summarize how much of the variance in lipid levels observed at diagnosis can be attributed to the

Table 2 Baseline Demographics and Clinical Characteristics on the Day of Diagnosis

Characteristic	All patients (N = 1,324)	PPS cohort[b] (N = 688)
Age at diagnosis, y	66 (11)	66 (10)
Sex, male	748 (56%)	393 (57%)
Site of symptom onset, bulbar	449 (34%)	229 (33%)
Diagnostic delay,[a] mo	9 (9)	9 (8)
ALSFRS-R total score	38 (6)	39 (6)
ΔFRS,[a] points per month	−0.83 (1.24)	−0.74 (1.07)
Forced vital capacity, % predicted	87 (22)	91 (22)
Body mass index, kg/m^2	25 (3)	25 (3)
Presence of frontotemporal dementia	111 (8%)	47 (7%)
Presence of *C9orf72* repeat expansion	95 (7%)	54 (8%)
Prognostic risk profile	−3.86 (1.67)	−3.97 (1.63)
Biomarkers of lipid metabolism		
Total cholesterol, mmol/L	5.58 (1.18)	5.54 (1.15)
LDL-C, mmol/L	3.38 (1.00)	3.36 (0.97)
HDL-C, mmol/L	1.48 (0.38)	1.48 (0.37)
Triglycerides, mmol/L	1.57 (0.88)	1.56 (0.86)

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSFRS-R = revised ALS functional rating scale; ΔFRS = (48 − ALSFRS-R total score)/diagnostic delay[25]; Prognostic Risk Profile = Linear predictor of ENCALS survival model[24]; ENCALS = European Network to Cure ALS; HDL-C = high-density lipoprotein cholesterol; GWAS = genome-wide association study; LDL-C = low-density lipoprotein cholesterol.
Data are expressed as mean (SD) or n (%).
[a] Data are expressed as median (IQR).
[b] Patients who had GWAS data available for analysis of their polygenic profile score (PPS).

respective PPS, expressed as adjusted R^2, and how the PPS relate to overall survival since symptom onset. Each PPS was significantly correlated with the respective lipid level (Pearson r_{TC} 0.11, $p = 0.002$; Pearson $r_{LDL\text{-}C}$ 0.23, $p < 0.001$; Pearson $r_{HDL\text{-}C}$ 0.36, $p < 0.001$; Pearson $r_{log\text{-}TG}$ 0.33, $p < 0.001$), with the explained variance at diagnosis ranging from 1.2% to 13.1%. None of the PPS was, however, significantly associated with overall survival time (all $p > 0.50$).

Table 3 Hazard Ratios for Biomarkers of Lipid Metabolism in Population-Based Cohort

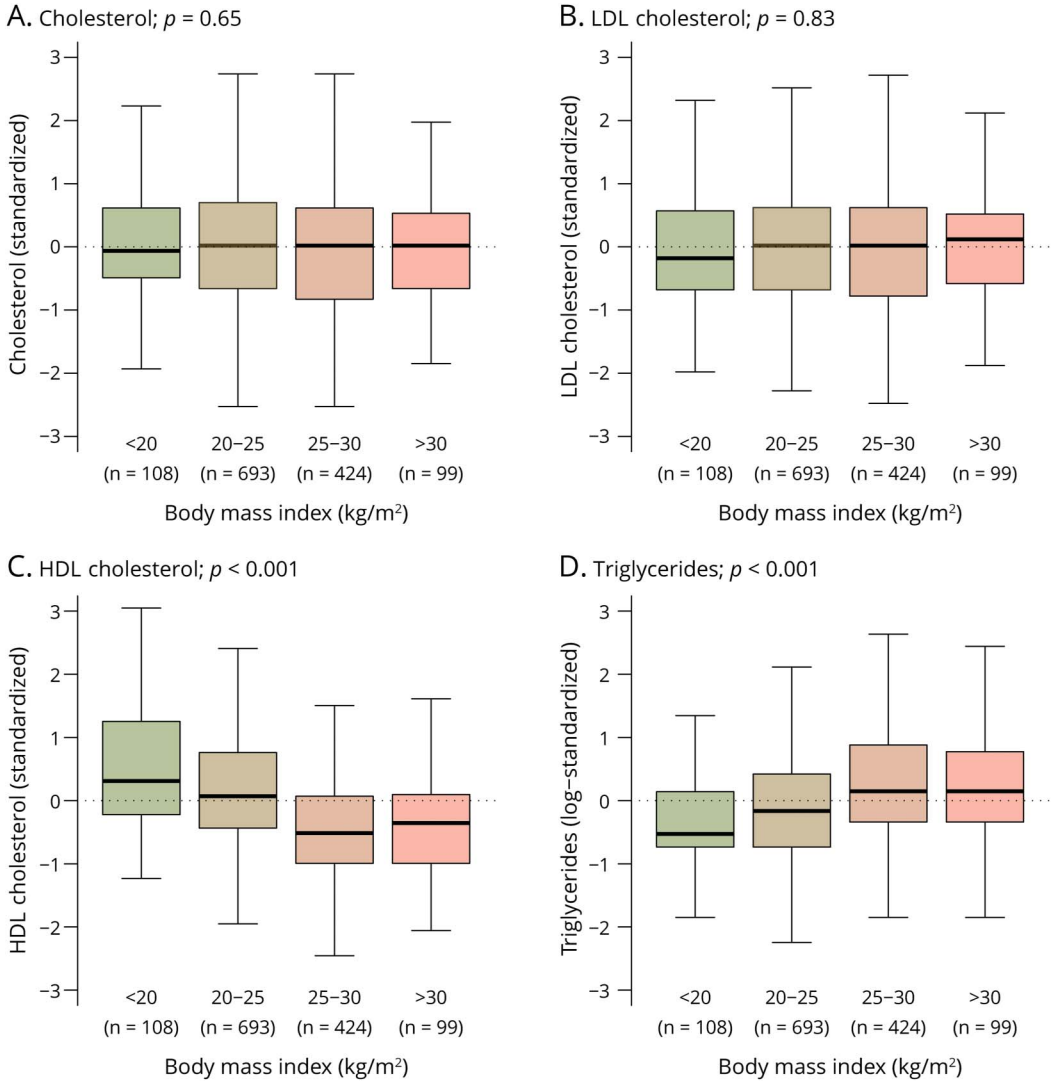
Lipid	Hazard ratio	95% CI	*p* Value
Total cholesterol (mmol/L)	1.04	0.99–1.09	0.087
LDL-C (mmol/L)	1.03	0.98–1.09	0.25
HDL-C (mmol/L)	1.33	1.14–1.55	<0.001
Triglyceride (log-mmol/L[a])	0.91	0.80–1.03	0.153

Hazard ratio of each lipid for survival defined as the time in months from study enrollment to death from any cause or administrative censoring.
Abbreviations: HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.
Hazard ratios are determined with a Cox proportional hazards model adjusted for age, site of onset, diagnostic delay, prediagnostic progression rate (ΔFRS), vital capacity, presence of FTD, *C9orf72* repeat expansion, and El Escorial classification.[24] A HR larger than 1 reflects a poorer survival outcome.
[a] Analysis was performed on the natural logarithm scale because of a right-skewed distribution.

Discussion

In this study, we have shown the extensive variability in the literature regarding the prognostic value of the lipid profile. The study heterogeneity is mainly driven by differences in study design, statistical models, sample size, and the patient population enrolled. In the second part of our study, only HDL cholesterol had additional prognostic value for predicting survival after diagnosis in patients with ALS in a prospective, population-based registry. Changes in components of the lipid profile were primarily related to disease severity. We found no immediate associations, however, between lipid-based polygenic scores and overall survival, yet another indication that changes in the lipid profile may be primarily a consequence of disease. Our results underscore that obtaining greater insight into lipid metabolism and longitudinal data on serum concentrations of the lipid profile could improve the monitoring of patients and potentially further disentangle ALS pathogenesis.

Figure 2 Biomarkers of the Lipid Metabolism Stratified by BMI Category at Diagnosis

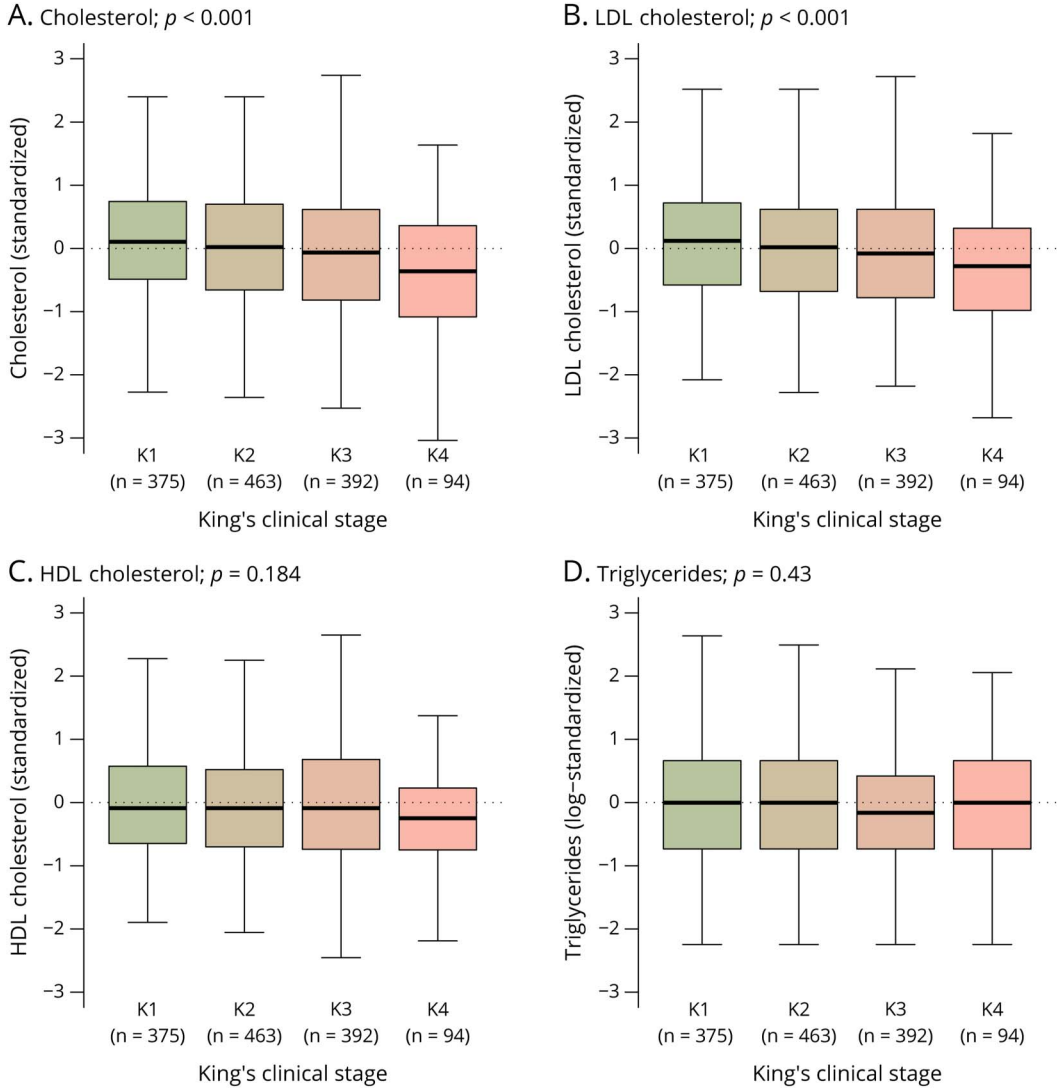


Boxplots summarizing the cross-sectional concentrations of the lipids linked to body mass index (BMI) at diagnosis. Scales are standardized to provide a direct comparison between lipids; interpretation is straightforward, where the scale reflects the number of standard deviations above or below the mean lipid level as presented in Table 2. Abbreviations: HDL = high-density lipoprotein; LDL = low-density lipoprotein. *p* values are based on the likelihood ratio test.

First, our literature search into the relationship between survival and lipid profile showed that the results of these studies are mixed.[10-14,32] The included studies analyzed lipids either continuously or as binary factor (e.g., high vs low). Binary categorization of the lipid levels into normal or abnormal may lead to spurious associations and be too limited to describe the gradual associations with prognosis. When pooling results across studies in a meta-analysis, none of the lipids were statistically significantly associated with survival, but individual study results varied considerably. The variation may be explained by (1) differences in the disease stage and the phenotype of the population enrolled and (2) differences in study methodology (e.g., follow-up time, statistical approach, and sample size).

Second, our analysis of a population-based registry confirmed the nonprognostic value of most lipids; HDL-C was, however, found to be predictive of overall survival since diagnosis. This finding was recently confirmed in both Japanese[32] and Swedish[10] patients, although insignificantly in the latter. We were not able to show the association between HDL-C and disease progression determined by the ALSFRS-R, as follow-up data were limited. The prognostic value of HDL-C could be the result of a surrogate association with disease progression. Respiratory insufficiency or symptoms of dyspnea have been associated with the lipid profile,[33] while dietary changes alter lipid concentrations.[34] Weight loss is observed in up to 60% of patients with ALS,[26] and changes in BMI have a direct impact on the lipid profile.[35,36] This impact was also found in our study population; there was a strong association between HDL-C and BMI, where HDL-C increases as BMI decreases. However, adjusting for BMI or other markers of disease severity minimally affected the association between HDL-C and survival.

Figure 3 Biomarkers of the Lipid Metabolism Stratified by King's Clinical Staging at Diagnosis



Boxplots summarizing the cross-sectional concentrations of the lipids linked to the 4 King's clinical stages. Scales are standardized to provide a direct comparison between lipids; interpretation is straightforward, where the scale reflects the number of standard deviations above or below the mean lipid level as presented in Table 2. Abbreviations: HDL = high-density lipoprotein; LDL = low-density lipoprotein. *p* values are based on the likelihood ratio test.

Albeit speculative, one could also hypothesize that the prognostic association might partially reflect a premanifest or prodromal sign of ALS. For example, production of oxidized derivatives of excess cholesterol might be caused by deficiencies in cholesterol metabolism,[7] which in turn may induce neuronal damage leading to muscle function loss.[7,37] Deficiencies in cholesterol metabolism may also lead to dysregulated transport of cholesterol and result in toxicity in the brain.[38]

In an attempt to disentangle this potential causality between lipids and survival, we estimated PPS for biomarkers of lipid metabolism to explore genetic links with lipid metabolism and ALS survival time. As PPS does not change over time,[30] any association between PPS and survival may be an indication that premorbid changes in lipids result in a more aggressive disease as expressed in overall survival time.[29] Our results highlight the predictive value and utility of PPS in patients with ALS as surrogate for actual lipid levels but also underscore that over 80% of the variance in the actual lipid levels were not captured by the PPS. Taking into account, the absence of a large effect between PPS and survival time and the results from other studies in which PPSs were more predictive for actual lipid levels,[39] these observations may support reverse causality, where lipid levels change as a consequence of the disease rather than vice versa.

The clinical relevance of these observations depends on the setting and the intended use of the PPS. Despite the large sample

Table 4 Relationship Between Polygenic Profile Score, Biomarkers Level, and Survival

	Explained variance at diagnosis		Relationship with survival since symptom onset		
Lipid	**Adjusted R^2**	**95% CI**	**Hazard ratio**	**95% CI**	***p* Value**
Total cholesterol	0.012	0.000–0.035	1.02	0.95–1.11	0.54
LDL-C	0.058	0.028–0.096	1.01	0.93–1.09	0.81
HDL-C	0.131	0.087–0.179	0.98	0.90–1.06	0.57
Triglyceride	0.107	0.066–0.156	1.02	0.94–1.10	0.64

Abbreviations: HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.
Confidence intervals around the adjusted R^2 were obtained by means of bootstrapping (n = 10,000) and pooled across imputations.
Hazard ratio of each lipid for survival defined as the time in months from symptom onset to death from any cause or administrative censoring.

size of our cohort, we were primarily powered to detect HRs of 1.1 or greater. An HR of 1.1 would translate to a 46.4% difference in hazard when comparing a patient with -2SD (~2.5th percentile) vs a patient with +2SD (~97.5th percentile). Smaller effect sizes, therefore, could still be deemed relevant, although detecting, for example, an HR of 1.05 or greater with 90% power would require approximately 4,500 survival events. Larger GWASs that link overall survival time to PPS may, therefore, be needed to further investigate potential causal or etiological relationships.[30] Moreover, determining whether a change in the lipid level precedes a change in clinical progression requires longitudinal observations with repeated blood samples to provide more definite evidence.[40] In such studies, it would be key to carefully collect other parameters that influence lipids, which were not collected in our study, such as smoking,[41] diet or the use of cholesterol-lowering drugs (CLD),[42] and preferably assess serum concentration in a fasting state to minimize variability.[43,44] Finally, 42.0% of our patient population had elevated serum concentrations of LDL-C; the mean serum HDL-C was comparable with that of the general Dutch population.[43] Studies that enrolled patients with ALS have reported similar serum concentrations.[10,45] HDL-C values were more or less the same as those found in the general population; however, an elevated LDL-C can be found in approximately 50%–60% of people of similar age in the Netherlands.[46,47] Patients with ALS, therefore, may have lower levels of LDL-C compared with the general population,[46] supporting our finding of decreasing levels in more advanced disease stages. Enrollment of a more geographically and culturally diverse population may improve generalizability of the exact association between lipids and overall survival in ALS, but dedicated case-control studies are needed to confirm true differences in lipid levels between patients with ALS and the general population. Moreover, although our study indicates a relationship with cross-sectional clinical stages, determining whether a change in the lipid level precedes a change in clinical progression requires longitudinal observations with repeated blood samples to provide more definite evidence.

In conclusion, lipids may contain valuable information about disease severity and prognosis because serum concentrations seem to be dependent on disease severity. Our results underscore that gaining further insight into lipid metabolism and longitudinal data on serum concentrations of the lipid profile could improve the monitoring of patients. Because our results are not in line with previous studies on a causal effect of the lipid profile on ALS disease progression, we believe that this new information may contribute to ongoing efforts to disentangle ALS pathogenesis.

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Appendix Authors

Name	Location	Contribution
Mark R. Janse van Mantgem, MD	UMCU	Design of the study; analyzed and interpreted the data; involved in the process of the systematic review; drafted and revised the manuscript for intellectual content
Wouter van Rheenen, MD, PhD	UMCU	Acquisition of GWAS data; statistical support during genetic analysis; revised the manuscript for intellectual content.
Anemone V. Hackeng, BSc	UMCU	Involved in the process of the systematic review; revised the manuscript for intellectual content
Michael A. van Es, MD, PhD	UMCU	Acquisition of the data; revised the manuscript for intellectual content.
Jan H. Veldink, MD, PhD	UMCU	Acquisition of the data; revised the manuscript for intellectual content.
Leonard H. van den Berg, MD, PhD	UMCU	Principal investigator; acquisition of the data; revised the manuscript for intellectual content.
Ruben *p*.A. van Eijk, MD, PhD	UMCU	Design of the study; analyzed and interpreted the data; drafted and revised the manuscript for intellectual content

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Association Between Serum Lipids and Survival in Patients With Amyotrophic Lateral Sclerosis: A Meta-analysis and Population-Based Study

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