Serum neurofilament light levels are predictive of all-cause mortality in late middle-aged individuals

Andrew D. Nguyen,[a,b,c*] Theodore K. Malmstrom,[c,d] Geetika Aggarwal,[a,b,c] Douglas K. Miller,[e] Bruno Vellas,[f,g] and John E. Morley[a]

[a]Division of Geriatric Medicine, Department of Internal Medicine, Saint Louis University School of Medicine, St. Louis, MO, USA
[b]Department of Pharmacology and Physiology, Saint Louis University School of Medicine, St. Louis, MO, USA
[c]Henry and Amelia Nasrallah Center for Neuroscience, Saint Louis University, St. Louis, MO, USA
[d]Department of Psychiatry and Behavioral Neuroscience, Saint Louis University School of Medicine, St. Louis, MO, USA
[e]Regenstrief Institute, Inc., Indianapolis, IN, USA
[f]Gérontopôle de Toulouse, Institut du Vieillissement, Centre Hospitalo-Universitaire de Toulouse, Toulouse, France
[g]UMR 1295 INSERM, University of Toulouse III, Toulouse, France

eBioMedicine 2022;82: 104146
Published online xxx
https://doi.org/10.1016/j.ebiom.2022.104146

Summary

Background Blood biomarkers can offer valuable and easily accessible indicators of normal biological processes, pathogenic conditions, and responses to therapeutic interventions. Recent studies found that levels of neurofilament light chain (NfL) in the blood are associated with mortality in three European cohorts of older adults (median ages 73, 93, and 100 years). Whether similar associations exist in younger adults and in other ethnic groups is currently not known.

Methods We utilized a cohort study that included 294 African Americans (baseline ages 49–65). Serum NfL levels were measured using a Meso Scale Discovery-based assay. Vital status was determined by matching through the National Death Index.

Findings Seventy-two participants (24.5%) died during the 14–15 years of follow up (2000–2014). Baseline serum NfL levels were significantly higher in the decedent group (86.1±65.7 pg/ml vs. 50.1±28.0 pg/ml, $p < 0{\cdot}001$). In binomial logistic regression models adjusted for age, gender, education, baseline smoking status, BMI, and total comorbidities (0–11), serum NfL levels remained a strong predictor of all-cause mortality, and sensitivity analyses employing multiple additional covariates did not substantively change the relationship. Further, Kaplan-Meier curves based on serum NfL quartiles showed reduced survival in groups with higher serum NfL levels.

Interpretation This study found a positive association between serum NfL levels and mortality in late middle-aged and older individuals. While our findings support that serum NfL levels may be a useful biomarker for all-cause mortality, further studies are needed to understand the biological mechanisms underlying this association.

Funding National Institute on Aging, Saint Louis University.

Keywords: Aging; Neurofilament light chain; Mortality; Biomarker

Introduction

Blood biomarkers can offer valuable and easily accessible indicators of normal biological processes, pathogenic conditions, and responses to therapeutic interventions. A number of studies have explored blood-based biomarkers in the context of aging[1,2] and mortality.[3–5] Among them, two recent studies in European cohorts found that levels of neurofilament light chain (NfL) in the blood are associated with mortality.[4,5] NfL is a cytoskeletal protein in neurons that is released upon neuronal injury or death. It is detectable in cerebrospinal fluid (CSF) as well as in blood, and NfL levels are elevated in several neurodegenerative diseases, stroke, and traumatic brain injury.[6,7]

Kaeser and colleagues[4] reported that increased plasma NfL levels are associated with mortality in centenarians (median age 100 years, n=135), as well as a

*Corresponding author at: Division of Geriatric Medicine, Department of Internal Medicine, Saint Louis University School of Medicine, 1402 S. Grand Blvd, St. Louis, MO 63104, USA.
E-mail address: andy.d.nguyen@health.slu.edu (A.D. Nguyen).

Research in Context

Evidence before this study

Biological aging is a complex process, and the identification of blood biomarkers for aging and mortality has been challenging. Two recent studies found that neurofilament light chain (NfL) levels in the blood are associated with mortality in three European cohorts of older adults (median ages 73, 93, and 100 years). Whether similar associations exist in younger adults and in other ethnic groups was not known.

Added value of this study

Our study investigated the association between circulating NfL levels and mortality in late middle-aged and older African American individuals. We found that serum NfL levels were a robust and consistent predictor of all-cause mortality. Therefore, this study extends the scope of this association with regard to age, race, and geography.

Implications of all the available evidence

Together, the available evidence support that circulating NfL levels may be a broadly applicable biomarker for all-cause mortality and may be a useful measurement for studies testing the effectiveness of interventions on lifespan.

slightly younger study population (median age 93 years, *n*=180). These individuals were in the Danish 1915 West birth cohort and the Danish 1905 birth cohort, respectively. Similarly, Rübsamen and colleagues[5] reported that serum NfL levels are associated with mortality in older adults without predefined neurological conditions (median age 73 years, *n*=385). These individuals were participants in the Memory and Morbidity in Augsburg Elderly (MEMO) study based in Augsburg, Germany. Together, these studies highlight the possibility that circulating NfL levels may be predictive of mortality.

However, the generalizability of these findings is unclear. Specifically, whether similar associations exist in younger individuals and in other ethnic groups is currently not known. In this study, we investigated whether serum NfL levels are associated with mortality in late middle-aged individuals in the African American Health (AAH) cohort.[8,9]

Methods

Study participants

AAH study has been described in previous reports.[8,9] In summary, AAH study is a well-characterized population-based cohort that includes 998 self-identified African American individuals ages 49–65 years at baseline in 2000–2001 in the St. Louis, Missouri metropolitan area. Inclusion criteria have been previously reported,[8,10] and includes: self-reported Black or African American race, birth dates between January 1, 1936 and December 31, 1950, standardized Mini-Mental Status Examination (MMSE) scores ≥ 16, and willingness to sign informed consent. Recruitment proportion (participants/enumerated eligible persons) was 76%, and participants completed in-home interviews and assessments at baseline, year 3 follow-up, and year 9 follow-up. To minimize the potential for self-reporting bias, the research team utilized validated and reliable criteria for collecting self-reported data. This includes the use of the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36), version 2.0, which has improved wording to minimize ambiguity and replaces a number of dichotomous response sets with five levels to enhance internal consistency.[11] As another example, objective income was measured by response to an unfolding income question based on eight categories. A subset of participants agreed to donate blood for the study at baseline, and serum samples were stored at −80 °C until laboratory analyses were performed. The final analytic sample for this study includes 294 participants with baseline serum NfL measurements and follow up vital status.

Ethics

All study procedures were approved by the Institutional Review Board at Saint Louis University (IRB #9657) and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants.

Measures

Serum NfL levels were measured using the R-PLEX human neurofilament L antibody set (Meso Scale Discovery, F217X) in 96-well plate format. Samples were diluted 2-fold in diluent buffer and tested in duplicate according to the manufacturer's instructions. Samples from decedents and survivors were randomized across four batches in a blinded manner. The mean coefficient of variation of duplicate NfL measurements was 3.8% (0–27.5%). Vital status was determined through a National Death Index from 1/1/2000 through 12/31/2014. Deaths are coded as 1. Disease measures were based on self-report of physician-based diagnoses and included: hypertension, diabetes, cancer, COPD, heart attack, congestive heart failure, angina, asthma, arthritis, stroke, and kidney disease.

Statistical approach

Data were analyzed using IBM SPSS Statistics version 28.0 (IBM Corp., Somers, NY). Descriptive statistics are

reported as means ± standard deviations (SD) or percentages. Mann-Whitney U test was used to compare differences in NfL levels by gender, survival status, and MMSE worse quintile. Analysis of co-variance adjusted for age and gender was used to compare baseline serum NfL levels for survivors and decedents. Logistic regression (LR) was used to investigate the association of serum NfL with 15-year all-cause mortality adjusted for age, gender, years of education, smoking status (never, former, current), BMI, and total comorbidities (0–11). A sensitivity analysis was done in which additional variables were added to the LR model (income, clinically-relevant levels of depressive symptom, social support, cognition (MMSE), falls in past year, hospitalized in past year (y/n), Medicaid insurance (y/n), and BMI. Adjusted odds ratios and 95% confidence intervals (CIs) are reported for LR analyses. A time-to-event (Kaplan-Meier) analysis with a log rank test for linear trend of factor levels was also done to investigate the association of NfL (quartiles) with mortality (years).

Role of the funding sources

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Baseline characteristics of the study population are provided in Table 1. Of the 294 participants included in the study, 67.0% were women, and the mean age at baseline was 56.4 ± 4.3 years. Baseline serum NfL levels (58.9±43.3 pg/mL; interquartile range 29.78; skewness 3.1; kurtosis 12.2) are within the range of previous studies.[12,13]

Serum NfL levels were positively correlated with age ($r = 0.33$, $p < 0.001$), which is consistent with previous reports.[4,5,12,14] Levels in men and women were 64.8 pg/mL and 56.1 pg/mL, respectively, and showed a trend toward a significant difference by Mann-Whitney U test ($p = 0.068$). When dichotomized into groups by age, we found significant effects of age ($p < 0.001$) and gender ($p = 0.042$) on serum NfL levels by two-way ANOVA, but not of the age/gender interaction ($p = 0.828$). The higher NfL levels observed in men is consistent with several previous reports that examined NfL levels in plasma and CSF.[14–17] The study population included persons with a number of self-reported comorbidities, including diabetes (24.8%), heart attack (7.8%), and kidney disease (4.1%).

A total of 72 participants (24.5%) died during the 14–15 years of follow up (2000–2014). Decedents were ages 58.31±4.2 (median age 59; age range 50–64; 25th percentile age 55, 50th percentile age 59, 75th percentile age 62) at baseline and survivors were ages 55·83±4.3 (median age 56; age range 49–65; 25th percentile age 52, 50th percentile age 56, 75th percentile age 59) at baseline. Baseline serum NfL levels were significantly higher among decedents versus survivors (86.1± 65.7 pg/mL vs. 50.1±28.0 pg/mL, $p < 0.001$ by Mann-Whitney U test). A LR analysis adjusted only for age found a significant association between serum NfL and mortality (odds ratio [OR] = 1.02, 95% confidence interval [CI] = 1.01–1.02). In follow-up multivariable LR models with additional baseline covariates, serum NfL levels remained a robust predictor of all-cause mortality. As shown in Table 2, higher age, baseline smoking status, higher comorbidities, and higher serum NfL levels are associated with higher mortality; higher BMI is associated with lower mortality. Moreover, when additional covariates, including income (below 20K; OR = 0.87, 95% CI = 0.42–1.79), depression (OR = 0.85, 95% = CI 0.40–1.82), social support (worst quintile; OR = 1.12, 95% CI = 0.49–2.55), MMSE score (OR = 0.95, 95% CI = 0.83–1.08), falls (past year; OR = 0.82, 95% CI = 0.39–1.73), hospitalization (past year; OR = 1.12, 95% CI = 0.50–2.50), health insurance (OR = 0.66, 95% CI = 0.29–1.47), and BMI (OR = 0.94, 95% CI = 0.89–1.00), were tested in a LR model, the positive association of serum NfL (OR = 1.01, 95% CI = 1.01–1.02) with mortality was not significantly changed.

Characteristic	Total (*n* = 294)	
Age (years), mean ± SD	294	56.4 ± 4.4
Female	294	67.0%
Education (years), mean ± SD	293	12.8 ± 3.0
Current smoker	294	23.1%
Body mass index (kg/m^2)	291	30.6 ± 6.6
Total Comorbidities (0–11)[a], mean ± SD	294	1.96 ± 1.4
Serum NfL (pg/mL), mean ± SD	294	58.9 ±43.3
Deceased in 14–15 year follow up	294	24.5%

***Table 1*: Baseline characteristics for AAH participants.**
[a] Comorbidities include hypertension, diabetes, cancer, COPD, heart attack, congestive heart failure, angina, asthma, arthritis, stroke, kidney disease.

Characteristic	Odds Ratio (95% CI)	*p* value[a]
Age (years)	1.10 (1.02–1.18)	0.014
Female	0.71 (0.36–1.39)	0.322
Education (years)	0.90 (0.81–1.00)	0.051
Previous Smoker (vs. Non-Smoker)	1.76 (0.83–3.72)	0.138
Current Smoker (vs. Non-Smoker)	2.80 (1.22–6.44)	0.015
BMI (kg/m^2)	0.94 (0.89–0.99)	0.027
Total Comorbidities (0-11)	1.36 (1.07–1.72)	0.011
Serum NfL (pg/mL)	1.01 (1.01–1.02)	0.002

***Table 2*: All-Cause Mortality and NfL in AAH cohort.**
[a] Multivariable binary logistic regression model (decedents coded 1, survivors coded 0).

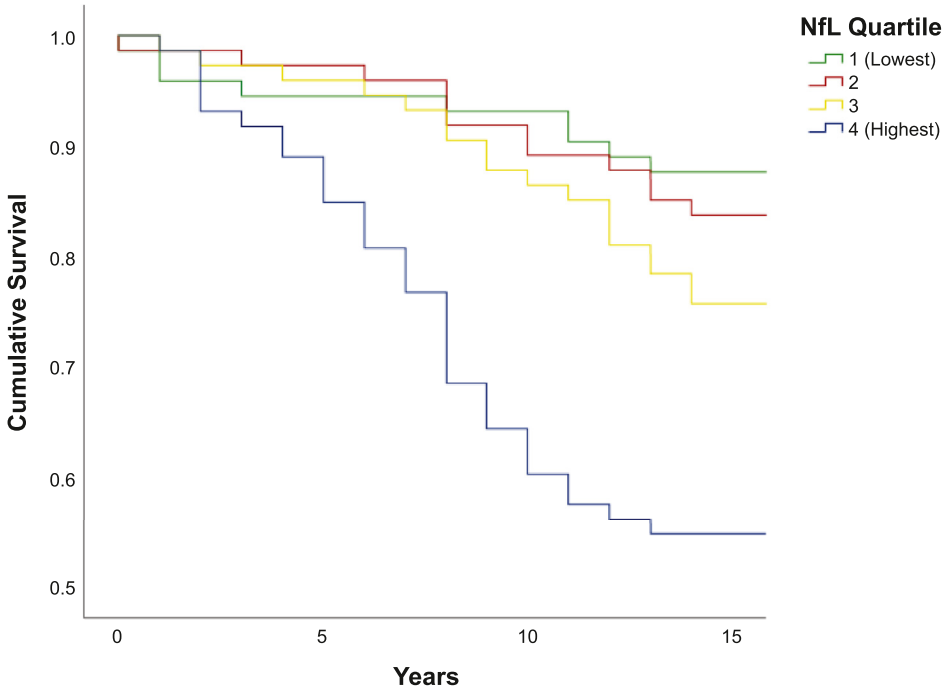

Figure 1. Kaplan-Meier curves based on serum NfL quartiles. Quartile 1, *n*=73, NfL 28.45±4.9, min 17.58, max 35.30; quartile 2, *n*=74, NfL 40.78±3·1, min 35.47, max 46.03; quartile 3, *n*=74, NfL 53.97±5.5, min 46.14, max 64.18; quartile 4, *n*=73, NfL 112.89±57.5, min 64.32, max 338.95.

As another way to examine the relationship between serum NfL levels and mortality, we separated individuals into quartiles based on serum NfL levels and examined survival in these groups. As shown in Figure 1, the Kaplan-Meier curves show that there was a trend for groups with higher serum NfL levels to have reduced survival (Log Rank [Mantel-Cox] Chi-Square = 24.17, $p < 0.001$).

Discussion

In this study, we explored the relationship between circulating NfL levels and mortality in late middle-aged individuals in the AAH study.[8,9] We found increased baseline serum NfL levels in participants who died during the 14–15 years of follow up. In LR analyses adjusted for multiple covariates, serum NfL levels were a robust and consistent predictor of all-cause mortality, and a time-to-event analysis confirmed the positive association.

Our findings are in agreement with two recent studies that found associations between circulating NfL levels and mortality.[4,5] It is worth noting that these studies focused on older European individuals (with median ages of 100 years, 93 years, and 73 years in three separate cohorts). In contrast, the mean age in the current study was 56.4 years. Our study also extends these previous findings in that they replicated the association between circulating NfL levels and mortality in individuals of a different racial background. Specifically, participants were predominantly late middle-aged and older African Americans in a community-dwelling population in St. Louis, Missouri. Thus, our study expands the scope of this association with regard to age, race, and geography.

The reason for the association between circulating NfL levels and mortality is unclear. A recent study that found a similar association suggested that the association reflects the contribution of deterioration of the nervous system to late-life mortality.[4] In our study, we were unable to determine if the increased NfL levels are due to neurological diseases.[6,7] While serum NfL levels were higher in the quintile with lowest Mini-Mental State Exam (MMSE) scores (75.5±67.5 pg/mL vs. 54.9±34.6 pg/mL, $p = 0.043$ by Mann-Whitney U test), this relationship is not significant after adjusting for age and education. Notably, the AAH study group is a population-representative sample, in which the number of neurological diseases is likely to be low. We did not have proper evaluations to determine who had neurological diseases, as these evaluations were beyond the scope of the study. We therefore cannot rule out the possible contribution of neurological diseases on increased serum NfL levels in this study. Moreover, it is not possible to determine whether the source of the increased serum NfL in the mortality group was from the central

nervous system or the periphery. This issue is complicated by the fact that circulating NfL levels have been reported to be increased in individuals with peripheral neuropathy.[18,19] In future studies, measuring NfL levels in both CSF and blood will help address this issue.

The strengths of this study are the relatively long follow-up period and the inclusion of a late middle-age group with a different racial background and geographic location than previous studies. Nevertheless, this study has limitations. First, since the participants were predominantly late middle-aged and older African American individuals living in a restricted geographic area, these results may not generalize to other populations. Second, this study only investigated a single biomarker; future studies should incorporate measurements of additional biomarkers.

In conclusion, this study found a positive association between serum NfL levels and mortality in late middle-aged and older individuals. Our findings support that circulating NfL levels may be a broadly applicable biomarker for all-cause mortality and may be a useful measurement for studies testing the effectiveness of interventions on lifespan. Further studies are needed to understand the biological mechanisms underlying this association.

Contributors

ADN, TKM, DKM, BV, and JEM co-designed the study. ADN, TKM, GA, DKM, and JEM collected and analyzed the data. AND, TKM, and GA verified the underlying data. ADN and TKM wrote the manuscript. All authors contributed to the interpretation of the results and critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

Data sharing statement

A de-identified data set of the outcomes presented in this manuscript will be made available 6 months after publication on a case-by-case basis on reasonable request by email to the corresponding author for research purposes.

Declaration of interests

None declared.

Acknowledgments

This research was supported by an NIH grant to D. K. Miller (AG010436) and by institutional funds from Saint Louis University. We are grateful to the study volunteers for their participation.

References

1 Sebastiani P, Thyagarajan B, Sun F, et al. Biomarker signatures of aging. *Aging Cell*. 2017;16(2):329–338.
2 Justice JN, Ferrucci L, Newman AB, et al. A framework for selection of blood-based biomarkers for geroscience-guided clinical trials: report from the TAME biomarkers workgroup. *Geroscience*. 2018;40(5-6):419–436.
3 Barron E, Lara J, White M, Mathers JC. Blood-borne biomarkers of mortality risk: systematic review of cohort studies. *PLoS One*. 2015;10(6):e0127550.
4 Kaeser SA, Lehallier B, Thinggaard M, et al. A neuronal blood marker is associated with mortality in old age. *Nat Aging*. 2021;1(2):218–225.
5 Rübsamen N, Maceski A, Leppert D, et al. Serum neurofilament light and tau as prognostic markers for all-cause mortality in the elderly general population-an analysis from the MEMO study. *BMC Med*. 2021;19(1):38.
6 Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. *J Neurol Neurosurg Psychiatry*. 2019;90(8):870–881.
7 Gendron TF, Badi MK, Heckman MG, et al. Plasma neurofilament light predicts mortality in patients with stroke. *Sci Transl Med*. 2020;12(569):eaay1913.
8 Miller DK, Malmstrom TK, Joshi S, Andresen EM, Morley JE, Wolinsky FD. Clinically relevant levels of depressive symptoms in community-dwelling middle-aged African Americans. *J Am Geriatr Soc*. 2004;52(5):741–748.
9 Miller DK, Wolinsky FD, Malmstrom TK, Andresen EM, Miller JP. Inner city, middle-aged African Americans have excess frank and subclinical disability. *J Gerontol A Biol Sci Med Sci*. 2005;60(2):207–212.
10 Wolinsky FD, Miller DK, Andresen EM, Malmstrom TK, Miller JP. Health-related quality of life in middle-aged African Americans. *J Gerontol B Psychol Sci Soc Sci*. 2004;59(2):S118–S123.
11 Ware J, Kosinski M, Dewey J. *How to Score Version 2 of the SF-36 Health Survey*. Boston: Quality Metric; 2000.
12 Chatterjee P, Goozee K, Sohrabi HR, et al. Association of plasma neurofilament light chain with neocortical amyloid-β load and cognitive performance in cognitively normal elderly participants. *J Alzheimers Dis*. 2018;63(2):479–487.
13 Mattsson N, Cullen NC, Andreasson U, Zetterberg H, Blennow K. Association between longitudinal plasma neurofilament light and neurodegeneration in patients with Alzheimer disease. *JAMA Neurol*. 2019;76(7):791–799.
14 Bridel C, van Wieringen WN, Zetterberg H, et al. Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: a systematic review and meta-analysis. *JAMA Neurol*. 2019;76(9):1035–1048.
15 Lin YS, Lee WJ, Wang SJ, Fuh JL. Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. *Sci Rep*. 2018;8(1):17368.
16 Mattsson N, Insel PS, Palmqvist S, et al. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. *EMBO Mol Med*. 2016;8(10):1184–1196.
17 Mielke MM, Syrjanen JA, Blennow K, et al. Comparison of variables associated with cerebrospinal fluid neurofilament, total-tau, and neurogranin. *Alzheimers Dement*. 2019;15(11):1437–1447.
18 Sandelius Å, Zetterberg H, Blennow K, et al. Plasma neurofilament light chain concentration in the inherited peripheral neuropathies. *Neurology*. 2018;90(6):e518–e524.
19 Mariotto S, Farinazzo A, Magliozzi R, Alberti D, Monaco S, Ferrari S. Serum and cerebrospinal neurofilament light chain levels in patients with acquired peripheral neuropathies. *J Peripher Nerv Syst*. 2018;23(3):174–177.