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

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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 < 0.001). 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.

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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 and mortality. Among them, two recent studies in European cohorts found that levels of neurofilament light chain (NfL) in the blood are associated with mortality. 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.

Kaeser and colleagues reported that increased plasma NfL levels are associated with mortality in centenarians (median age 100 years, n=135), as well as a
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, Rubsamen and colleagues5 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 reported8,10 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.

Statistical approach

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

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.
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 29.7; 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

Table 1: Baseline characteristics for AAH participants.

| Characteristic                        | Total (n = 294) |
|---------------------------------------|----------------|
| Age (years), mean ± SD                | 56.4 ± 4.4     |
| Female                                | 294            |
| Education (years), mean ± SD          | 12.8 ± 3.0     |
| Current smoker                        | 294            |
| Body mass index (kg/m²)               | 30.6 ± 6.6     |
| Total Comorbidities (0–11)a, mean ± SD| 1.96 ± 1.4     |
| Serum NFL (pg/mL), mean ± SD         | 58.9 ± 43.3    |
| Deceased in 14–15 year follow up      | 245            |

As shown in Table 2, higher age, baseline smoking status, higher comorbidities, and higher serum NFL levels are associated with higher 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 = 0.49–2.53), MMSE score (OR = 0.91, 95% CI = 0.81–1.00), 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.

Table 2: All-Cause Mortality and NFL in AAH cohort.

| Characteristic                        | Odds Ratio (95% CI) | p valuea |
|---------------------------------------|---------------------|----------|
| 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/m²)                            | 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    |

a Multivariable binary logistic regression model (decedents coded 1, survivors coded 0).
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 in late middle-aged individuals in the AAH study.\(^4\)\(^,\)\(^5\) 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.

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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. 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.

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