Plasma neurofilament light predicts mortality in patients with stroke

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Given the heterogeneity of stroke brain injury, there is a clear need for a biomarker that determines the degree of neuroaxonal injury across stroke types. We evaluated whether blood neurofilament light (NFL) would fulfill this purpose for patients with acute cerebral infarction (ACI; N = 227), aneurysmal subarachnoid hemorrhage (aSAH; N = 58), or nontraumatic intracerebral hemorrhage (ICH; N = 29). We additionally validated our findings in two independent cohorts of patients with ICH (N = 96 and N = 54) given the scarcity of blood biomarker studies for this deadliest stroke type. Compared to healthy individuals (N = 79 and N = 48 for the discovery and validation cohorts, respectively), NFL was higher for all stroke types. NFL associated with radiographic markers of brain tissue damage. It correlated with the extent of early ischemic injury in patients with ACI, hemorrhage severity in patients with aSAH, and intracranial hemorrhage volume in patients with ICH. In all patients, NFL independently correlated with scores from the NIH Stroke Scale, the modified Rankin Scale, and the Mini-Mental State Examination at blood draw, which respectively assess neurological, functional, and cognitive status. Furthermore, higher NFL concentrations independently associated with 3- or 6-month functional disability and higher all-cause mortality. These data support NFL as a uniform method to estimate neuroaxonal injury and forecast mortality regardless of stroke mechanism. As a prognostic biomarker, blood NFL has the potential to support the rehabilitation services and improving clinical trial efficiency for stroke therapeutics and devices.

INTRODUCTION

Stroke is a leading cause of death worldwide, but outcomes after a stroke range widely. Some individuals experience temporary, non-disabling symptoms, whereas others develop varying degrees of neurological, physical, and/or cognitive impairment. Clinical scales provide insight into stroke severity, but they are often crude non-continuous measures of the extent of tissue injury. The National Institutes of Health Stroke Scale (NIHSS) correlates only modestly with volume of infarcted tissue (1), and this correlation is modulated by prestroke factors like the degree of leukoaraiosis (2). The intracerebral hemorrhage (ICH) score (3) and the World Federation of Neurosurgical Societies score (4) are useful outcome predictors of ICH and subarachnoid hemorrhage (SAH), respectively, but they are not well validated as neuroaxonal injury markers. There is thus a clear need for biomarkers that provide a continuous measure of the degree of neuroaxonal injury regardless of whether the stroke is ischemic or hemorrhagic. Such biomarkers have the potential to improve stroke trial efficiency and our ability to predict clinically meaningful outcomes like poststroke cognitive impairment and response to rehabilitation.

Neurofilament proteins, major cytoskeletal components of axons, have emerged as promising candidate biomarkers of neuronal injury. They are abundantly and exclusively expressed in neurons, released into extracellular fluids during axonal injury, and show prognostic utility for several neurological disorders (5, 6). Initial studies investigating neurofilament proteins as biomarkers for stroke focused largely on SAH and measured neurofilament light (NFL) (7–9) or phosphorylated neurofilament heavy (pNFH) (10–13) in cerebrospinal fluid (CSF). These studies found elevated CSF neurofilament proteins are to be successfully translated to the clinic. For instance, associations of blood neurofilament with functional outcomes and, in the case of ACI, with infarct volumes were observed in only some of the studies in which such correlations were examined (13–17, 19–26).
Inconsistent findings among reports may be explained, at least in part, by sample size, the fact that neurofilament proteins in CSF (9, 13, 16) and blood (13, 15–18, 20, 21, 26) progressively rise in the days after the stroke, and the stringency of the statistical analyses. In addition to discrepant findings, the relationship between blood neurofilament proteins and poststroke survival has yet to be rigorously evaluated.

Given the critical need for efficient and unbiased biomarkers for stroke, we undertook a comprehensive study to investigate the hypothesis that blood NFL could serve as a uniform means to predict the degree of neuroaxonal injury and all-cause mortality across all major types of ischemic and hemorrhagic stroke. Furthermore, because data on blood NFL in ICH are limited (25), we used two independent validation cohorts to confirm findings from our discovery ICH sample series and evaluated the temporal profile of blood NFL after ICH.

**RESULTS**

**Poststroke trends of plasma NFL over time**

To test our hypothesis, we first measured NFL in plasma from 393 participants enrolled in the Mayo Clinic Florida Familial Cerebrovascular Diseases Registry between the dates of October 2010 and December 2018 (27). This series comprised plasma from 79 healthy individuals and 314 patients with stroke [227 with ACI, 58 with aneurysmal SAH (aSAH), and 29 with ICH] for whom plasma was collected within 20 days of stroke. Baseline characteristics, comorbidities, stroke information, and poststroke outcomes are summarized in table S1 for healthy controls and patients with stroke (overall and according to stroke type). Table S2 provides these same summaries for patients with ACI stratified by subtype using the TOAST classification system: large-artery atherosclerosis (LAA), small-vessel occlusion (SVO), cardioembolism (CE), stroke of undetermined etiology (UDE), and stroke of other determined etiology (ODE) (28).

Although subject-specific longitudinal data on NFL were not available in this cross-sectional series, we visualized poststroke trends of NFL over time. As shown in Fig. 1A, we observed an increase in plasma NFL in the days after the stroke, which peaked about 9 days after stroke. Plasma NFL in blood collected 9 to 20 days after stroke was significantly higher than in blood collected 0 to 8 days after stroke when adjusting for stroke group only ($P < 0.001$) and when additionally adjusting for age at blood draw, sex, body mass index (BMI), current smoking, hypertension, diabetes, and physical activity ($P < 0.001$). Subsequent association analyses were thus stratified by time from stroke to blood draw (0 to 8 or 9 to 20 days) when sample size per-participant was adequate and also adjusted for time from stroke to blood draw as a continuous variable when analyzing NFL in all samples for a given stroke group or NFL concentrations based on timing from stroke to blood draw (0 to 8 or 9 to 20 days) are shown. The black horizontal bars represent median NFL concentrations. **$P < 0.001$ in multivariable analysis comparing plasma NFL between the indicated group and healthy controls after adjusting for age at blood draw, sex, BMI, current smoking, hypertension, diabetes, and physical activity. ***$P < 0.001$ in analysis comparing plasma NFL between the indicated group and patients with aSAH after adjusting for time from stroke to blood draw. See also tables S3 and S5.**

![Fig. 1. Plasma NFL is elevated in patients with ischemic and hemorrhagic stroke from the discovery study.](http://stm.sciencemag.org/)

**Plasma NFL is elevated in patients with ACI, aSAH, and ICH**

In healthy individuals, plasma NFL concentrations strongly correlated with age (Spearman’s $r = 0.81$, $P < 0.001$; fig. S1A). Compared to healthy controls, plasma NFL was significantly elevated in patients with stroke combined and in patients with ACI, aSAH, or ICH, both in unadjusted analysis and when adjusting for the potential confounding variables listed above (all $P < 0.001$; Fig. 1B and table S3). Similarly, plasma NFL was significantly elevated in all studied ACI subtypes in comparison to controls (LAA, SVO, CE, and UDE; all $P < 0.001$; fig. S2 and table S4). Differences in NFL between controls and patients with stroke were most evident for patients with ICH and for patients with a time from stroke to blood draw between 9 and 20 days (Fig. 1B and table S3).

When comparing plasma NFL among stroke groups, we found that NFL was significantly higher in patients with ACI or ICH compared to patients with aSAH in analyses adjusting for length of time from blood draw (both $P < 0.001$; Fig. 1B and table S5). Results were similar in analysis stratified by length of time from stroke to blood draw (0 to 8 or 9 to 20 days) and when adjusting for potential

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confounding variables when possible (table S5). No differences in plasma NFL were identified among ACI subtypes after correcting for multiple testing (fig. S2 and table S6).

### Plasma NFL correlates with radiographic markers

To determine the relationship between plasma NFL and brain tissue damage, we evaluated associations between NFL and (i) infarct volume in patients with ACI determined using the Alberta Stroke Program Early Computerized Tomography (CT) Score (ASPECTS), (ii) severity of SAH in patients with aSAH determined using the modified Fisher grading scale, and (iii) the volume of intracranial hemorrhage in patients with ICH determined using the ABC/2 formula. A summary of ASPECTS, modified Fisher scores, and ABC/2 scores is provided in table S7. In all three groups, higher plasma NFL was indicative of worse outcomes (Table 1). Specifically, when adjusting for time from stroke to blood draw, higher NFL associated with lower ASPECTS in patients with ACI (P < 0.001), and this remained significant when additionally adjusting for potential confounding variables when possible (P < 0.001). When further examining this association according to ACI subtype (and adjusting only for time from stroke to blood draw), we observed significant associations for patients with CE (P < 0.001) or UDE (P = 0.006), but not with LAA (P = 0.41; table S8). When adjusting for time from stroke to blood draw only, we additionally observed that higher NFL associated with higher modified Fisher scores in patients with aSAH (P = 0.008), but not with ABC/2 scores in the smaller group of patients with ICH (P = 0.063; Table 1).

### Plasma NFL is associated with neurological, functional, and cognitive status at blood draw

We next examined the relationship between plasma NFL and clinical measures at the time of blood draw. The NIHSS, the modified Rankin Scale (mRS), and the Mini-Mental State Examination (MMSE) were used to respectively assess neurological, functional, and cognitive status. These analyses were performed for all patients with stroke combined (ACI, aSAH, and ICH) and separately for patients with ACI, aSAH, or ICH. In all patients with stroke, higher NFL associated with a worse performance for all three tests. NFL positively correlated with NIHSS and mRS scores (all P < 0.001; Table 2) and negatively correlated with MMSE scores (P < 0.001; table S9) when adjusting for time from stroke to blood draw and also when additionally adjusting for potential confounding variables. Similar findings were observed when stratifying patients by time from stroke to blood draw (0 to 8 or 9 to 20 days) and when separately analyzing ACI and aSAH groups or ACI subtypes (Table 2 and tables S9 to S11). In patients with ICH, NFL positively correlated with NIHSS (P = 0.004; Table 2) and nominally negatively correlated with MMSE scores.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Patient group</th>
<th>Association measure</th>
<th>Estimate (95% CI)</th>
<th>P</th>
<th>Estimate (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPECTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>225</td>
<td>ACI</td>
<td>OR</td>
<td>1.49 (1.28–1.72)</td>
<td>&lt;0.001</td>
<td>1.55 (1.32–1.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0–8 days from stroke to blood draw</td>
<td>195</td>
<td>ACI</td>
<td>OR</td>
<td>1.45 (1.23–1.73)</td>
<td>&lt;0.001</td>
<td>1.54 (1.28–1.87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Modified Fisher score = 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>58</td>
<td>aSAH</td>
<td>OR</td>
<td>1.70 (1.20–2.64)</td>
<td>0.008</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ABC/2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>29</td>
<td>ICH</td>
<td>β coefficient</td>
<td>0.39 (−0.02 to 0.80)</td>
<td>0.063</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

A rigorous multivariable analysis adjusting for variables other than time from stroke to blood draw was only performed for the large ACI groups.† For analysis of ASPECTS, the 9 to 20 days from stroke to blood draw subgroup were not examined because of an insufficient sample size for analysis of this ordered categorical variable. For analysis of modified Fisher score = 4, the 0 to 8 days and 9 to 20 days from stroke to blood draw subgroups were not examined because of the small number of patients with modified Fisher score < 4 in these subgroups. For analysis of ABC/2 scores, the 0 to 8 days and 9 to 20 days from stroke to blood draw subgroups were not examined due to small sample size.
Because of the lack of variation in mRS scores in this small group, associations between NFL and mRS scores were not analyzed for patients with ICH.

Plasma NFL predicts 3-month functional outcome

mRS scores 3 months after stroke were available for 234 patients (74.5%), allowing us to evaluate associations between plasma NFL measured within 20 days of stroke and this 3-month functional outcome for 168 patients with ACI, 50 with aSAH, and 16 with ICH. In all patients combined, and in patients with ACI or aSAH, NFL was significantly associated with a 3-month mRS score of >3 when adjusting for time from stroke to blood draw and when also adjusting for potential confounding variables when possible (P < 0.001; Fig. 2A and table S12). These associations were consistent in analysis stratified by time from stroke to blood draw (0 to 8 or 9 to 20 days; table S12). When assessing associations between NFL and 3-month mRS scores according to ACI subtype, higher NFL was associated with greater mRS scores in patients with CE (P < 0.001) and UDE (P = 0.004), but not with LAA (P = 0.34) in analysis adjusting only for time from stroke to blood draw (table S13).

Table 2. Associations of NFL concentrations with NIHSS and mRS scores at blood draw. β values, 95% CIs, and P values result from linear regression models. ORs, 95% CIs, and P values result from binary logistic regression models. β values are interpreted as the change in mean NIHSS score (on the square root scale) for each doubling in NFL concentrations. ORs are interpreted as the multiplicative increase in the odds of an mRS score of >3 for each doubling in NFL concentrations. P values of <0.0071 (associations with NIHSS at blood draw) and <0.0083 (associations with mRS score of >3 at blood draw) are considered statistically significant after correcting for multiple testing (see also table S10 for association of NFL concentrations with NIHSS and mRS at blood draw by ACI subtype). Information was unavailable regarding NIHSS scores for 5 of the 314 patients. Information was unavailable regarding mRS scores for 6 of the 314 patients. β, regression coefficient.

<table>
<thead>
<tr>
<th>Disease group</th>
<th>Association between NFL concentrations and NIHSS at blood draw</th>
<th>Association between NFL concentrations and mRS score &gt; 3 at blood draw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI) P</td>
<td>β (95% CI) P</td>
</tr>
<tr>
<td>All patients with</td>
<td>Adjusting for time from stroke to blood draw</td>
<td>Adjusting for time from stroke to blood draw, age</td>
</tr>
<tr>
<td>stroke†</td>
<td></td>
<td>at blood draw, sex, BMI, hypertension, diabetes, and physical activity*</td>
</tr>
<tr>
<td>Overall</td>
<td>0.57 (0.49–0.66) &lt;0.001</td>
<td>0.56 (0.47–0.65) &lt;0.001</td>
</tr>
<tr>
<td>0–8 days</td>
<td>0.60 (0.50–0.71) &lt;0.001</td>
<td>0.57 (0.46–0.68) &lt;0.001</td>
</tr>
<tr>
<td>9–20 days</td>
<td>0.60 (0.42–0.78) &lt;0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>ACI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.49 (0.40–0.58) &lt;0.001</td>
<td>0.47 (0.37–0.56) &lt;0.001</td>
</tr>
<tr>
<td>0–8 days</td>
<td>0.50 (0.39–0.61) &lt;0.001</td>
<td>0.46 (0.34–0.58) &lt;0.001</td>
</tr>
<tr>
<td>9–20 days</td>
<td>0.49 (0.27–0.71) &lt;0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>aSAH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.87 (0.61–1.13) &lt;0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>0–8 days</td>
<td>1.08 (0.74–1.42) &lt;0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>9–20 days</td>
<td>0.76 (0.41–1.12) &lt;0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>Nontraumatic ICH‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.53 (0.18–0.88) 0.004</td>
<td>N/A</td>
</tr>
<tr>
<td>0–8 days</td>
<td>0.31 (–0.02 to 0.64) 0.064</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*All models involving all patients with stroke were also adjusted for stroke type (ACI, aSAH, or ICH). A rigorous multivariable analysis adjusting for variables other than time from stroke to blood draw was only performed for the large ACI and all patients with stroke groups. †Patients with ICH were not included in analysis of mRS at blood draw due to their lack of variability for this outcome.
For the two largest groups of all patients with stroke combined, and patients with ACI, we next examined whether these associations of increased NFL with greater 3-month mRS scores were independent of NIHSS scores at stroke or at blood draw. When additionally adjusting the aforementioned multivariable models for NIHSS scores at the time of stroke, associations between NFL and 3-month mRS scores remained significant for all patients with stroke combined and for patients with ACI (all $P \leq 0.001$; table S14). However, when adjusting models for NIHSS scores at the time of blood draw, associations between NFL and mRS scores weakened for all patients with stroke ($P = 0.012$) and for patients with ACI ($P = 0.041$; table S14).

We also examined the ability of NFL to independently predict an mRS score of $>3$ by estimating the area under receiver operating characteristic curve (AUC) with and without NFL included in the multivariable logistic regression model. An AUC equal to 1.0 indicates perfect predictive ability for a given model, whereas an AUC equal to 0.5 represents predictive ability equal to chance. For all patients with stroke, adding NFL into the multivariable models that included NIHSS scores at stroke or at blood draw (in addition to the other aforementioned confounding variables) increased the AUC from 0.908 to 0.925 (when considering NIHSS at stroke) or from 0.940 to 0.947 (when considering NIHSS at blood draw; table S15). Results were similar for patients with ACI (table S15).
Plasma NFL predicts survival
We next investigated whether NFL predicts survival in patients with stroke. With a median length of follow-up of 8.7 months (range, 0 days to 8.2 years), 112 (35.7%) patients died. In analyses adjusting only for time from stroke to blood draw, higher NFL associated with significantly poorer survival for all patients with stroke combined [hazard ratio (HR), 1.55; P < 0.001], patients with ACI (HR, 1.49; P < 0.001), and patients with aSAH (HR, 1.88; P < 0.001; Fig. 2, B to D, and table S16). These results were consistent when also adjusting for age at blood draw, sex, BMI, current smoking, hypertension, diabetes, and physical activity for all patients with stroke combined and for patients with ACI (table S16). Similar results were seen when evaluating associations between NFL and survival according to ACI subtypes (table S17).

For the two largest patient groups (all patients with stroke and patients with ACI), we assessed whether associations of increased NFL with worse survival were independent of NIHSS scores. These associations remained significant when additionally adjusting multivariable models for NIHSS scores at the time of stroke or at blood draw (P ≤ 0.007; table S18). We also examined the ability of NFL to independently predict survival in all patients with stroke by estimating the c-index with and without NFL included in the multivariable model, where a c-index of 1.0 for a given Cox regression model indicates perfect predictive ability, and a value of 0.5 indicates a predictive ability equal to chance. These analyses revealed that adding NFL into multivariable Cox regression models that also included the aforementioned potential confounding variables and either NIHSS scores at stroke or at blood draw respectively increased the c-index from 0.774 to 0.793 or from 0.810 to 0.816 (table S19). Results were similar for patients with ACI alone (table S19).

Plasma NFL is elevated and progressively increases following ICH in patients from the validation cohorts
Given that we found only one study investigating blood NFL as a prognostic biomarker for ICH (25), and given the lack of longitudinal assessments of blood NFL after ICH, we sought to validate and extend our findings above using two independent sample series from patients with ICH. The first corresponded to a cohort study of ICH from Yale University (Yale ICH study) that collected plasma from 96 individuals 72 hours after ICH. The second was a longitudinal cohort from the University of Pennsylvania (UPenn) composed of serum from 54 individuals collected 0 to 12, 12 to 36, and 60 to 84 hours after ICH. For comparison to these two cohorts, NFL was measured in plasma or serum from 48 and 46 healthy controls, respectively. Samples from these individuals, who were distinct from the control group used in our discovery study, were collected at Mayo Clinic Florida or at Massachusetts General Hospital and Washington University through their Dominantly Inherited ALS (DIALS) Network study. Baseline characteristics, comorbidities, stroke information, and poststroke outcomes are summarized in table S20 for individuals in each ICH cohort and for control subjects.

As in our discovery study, plasma NFL concentrations strongly correlated with age in healthy controls (Spearman’s r = 0.77, P < 0.001; fig. S1B), as did serum NFL (Spearman’s r = 0.76, P < 0.001; fig. S1C). As anticipated, plasma and serum NFL concentrations from 46 matched controls were also strongly correlated (Spearman’s r = 0.96, P < 0.001; fig. S1D). Compared to healthy controls, NFL was significantly elevated in patients from the Yale ICH study, and for all three stroke–to–blood draw time periods (0 to 12 hours, 12 to 36 hours, and 60 to 84 hours) in the longitudinal UPenn cohort, both in unadjusted analysis and when adjusting for age at blood draw and sex (all P < 0.001; Fig. 3, A and B, and tables S21 and S22).

We evaluated the temporal profile of NFL in the longitudinal serum series through pairwise comparisons of NFL among the three stroke–to–blood draw time periods. There was no increase in NFL from 0 to 12 hours to 12 to 36 hours after stroke. However, NFL in serum collected 60 to 84 hours after stroke was significantly elevated in comparison to concentrations at both earlier time periods (P ≤ 0.002; Fig. 3 and table S23).

Plasma NFL correlates with hemorrhage volume in patients with ICH in the validation cohorts
In the Yale ICH study, we observed a strong positive association between NFL measured 72 hours after ICH and hemorrhage volume, determined using the ABC/2 formula, in both unadjusted analysis and multivariable models adjusted for age, sex, hypertension, diabetes, and BMI (P < 0.001; Table 3). For the longitudinal UPenn series, no such association was seen when NFL was measured in plasma collected 0 to 12 hours after ICH in either unadjusted analysis or multivariable models adjusted for age, sex, hypertension, and diabetes (Table 3). However, positive associations between hemorrhage volume and NFL were detected when NFL was measured 12 to 36 hours (P = 0.009) or 60 to 84 hours (P = 0.026) after ICH, although findings did weaken for the smaller 60- to 84-hour group (N = 29) in multivariable analysis (Table 3).

Plasma NFL is associated with neurological status at initial evaluation or at blood draw in the validation cohorts
In the Yale ICH study, we observed a strong positive association of NFL with NIHSS at initial evaluation and at blood draw both in unadjusted analysis and when adjusting for age, sex, hypertension,
diabetes, and BMI (P < 0.001; Table 4). Serum NFL similarly associated with NIHSS at initial patient evaluation in the longitudinal UPenn cohort in unadjusted analysis and when adjusting models for age, sex, hypertension, and BMI, and this was most evident when NFL was measured 12 to 36 and 60 to 84 hours after ICH (Table 4).

Plasma NFL predicts 3- and 6-month functional outcomes in patients with ICH
The functional status of patients with ICH in the Yale ICH study was evaluated 3 and 6 months after stroke. In both unadjusted analysis and analysis adjusted for age, sex, hypertension, diabetes, and BMI for the Yale ICH study series and for age, sex, hypertension, and diabetes for the longitudinal UPenn ICH series. P values of <0.0125 are considered statistically significant after correcting for multiple testing.

The multivariable logistic regression models that included NIHSS scores at initial evaluation and other previously mentioned confounding variables. In the Yale ICH study, adding NFL into the multivariable model examining associations with 3-month mRS scores increased the AUC from 0.749 to 0.754 (table S25). An even greater increase in the AUC was observed when NFL was included into the multivariable model examining associations with 6-month mRS scores (from 0.780 to 0.819; table S25). In the longitudinal cohort, adding NFL to the multivariable models examining associations with 3-month mRS scores increased the AUC from 0.929 to 0.941 when NFL was measured 0 to 12 hours from stroke and from 0.891 to 0.934 when NFL was measured 12 to 36 hours from stroke (table S25).

DISCUSSION
Stroke severity can vary drastically from temporarily symptomatic and non-disabling to disabling and ultimately fatal. This heterogeneity underscores the need for robust measures of clinical outcomes. Reliable prognosis not only is important to the patient, family, and caregivers but also informs treatment and rehabilitation decisions and can improve multiple aspects of clinical trial design. Despite encouraging data supporting blood NFL as an indicator of poststroke neuronal injury, it has yet to be translated to the clinic as a stroke biomarker. This is partly due to the lack of independent validation studies among multiple centers and insufficient rigorous investigations across stroke types. Toward overcoming these barriers, we evaluated the prognostic utility of blood NFL in ischemic and hemorrhagic stroke.

In our discovery study, we noted elevated plasma NFL in patients with ACI, aSAH, or ICH compared to controls, with differences most evident for patients with ICH, which has a high morbidity and mortality rate (30). Blood NFL was also higher in patients with ICH in our two validation cohorts compared to healthy controls. For patients with ischemic stroke, a rise in blood NFL has been observed within 24 hours of stroke to admission to hospital (14, 16, 17), and longitudinal studies consistently show a progressive increase in its concentration, which is estimated to peak between ~7 and 21 days but can remain elevated for several months (16–18, 20). Longitudinal measures of blood NFL in patients with aSAH and ICH have not been reported, but analysis of our cross-sectional discovery cohorts suggests that NFL progressively rises after aSAH and ICH. This was confirmed in our longitudinal cohort of patients with ICH showing that serum NFL was higher 60 to 84 hours after stroke in comparison to concentrations measured within 36 hours of ictus. The gradual increase in NFL after stroke may reflect axonal degeneration secondary to the primary insult. This may partly explain why the differences in NFL that we observed between healthy controls and patients with stroke were especially evident when blood was drawn 9 to 20 days (versus 0 to 8 days) after the stroke in our discovery cohort or 60 to 84 hours (versus 0 to 36 hours) after ICH in our longitudinal validation cohort.

To test the prognostic utility of NFL across stroke types, we investigated the relationship between blood NFL and widely used outcome measures. We found that NFL associated with radiographic indicators of brain tissue damage. In patients with ACI, NFL associated with ASPECTS, a 10-point topographic grading system used to estimate ischemic changes on CT scans (31). These data are in agreement with a study showing that serum NFL correlates with infarct volume when measured 3 or 7 days following ACI (17). However, no
association between infarct volume and serum NFL measured within 24 hours of admission was observed (14, 17, 22). We also noted that plasma NFL associated with radiologic severity in patients with aSAH. The latter was assessed using the modified Fisher grading scale, which grades SAH secondary to intracranial aneurysm rupture (32). We found that high plasma NFL increased the odds of a worse score. In contrast to our finding, it was reported that NFL measured in plasma collected from patients at admission to hospital

Table 4. Associations of NFL concentrations with NIHSS at initial evaluation and at blood draw in patients with ICH from the validation cohorts. \( \beta \) values, 95% CIs, and \( P \) values result from linear regression models. \( \beta \) values are interpreted as the change in mean NIHSS (on the square root scale) for each doubling in NFL concentration.

<table>
<thead>
<tr>
<th>Group</th>
<th>NIHSS at initial evaluation</th>
<th>NIHSS at blood draw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( N )</td>
<td>Unadjusted analysis</td>
</tr>
<tr>
<td>Yale ICH study series</td>
<td>96</td>
<td>0.51 (0.28–0.73)</td>
</tr>
<tr>
<td>UPenn ICH study series</td>
<td>36</td>
<td>0.34 (0.05–0.63)</td>
</tr>
<tr>
<td>0–12 hours from stroke to blood draw</td>
<td>40</td>
<td>0.42 (0.20–0.64)</td>
</tr>
<tr>
<td>12–36 hours from stroke to blood draw</td>
<td>29</td>
<td>0.57 (0.24–0.91)</td>
</tr>
</tbody>
</table>

*Multivariable models were adjusted for age, sex, hypertension, diabetes, and BMI for the Yale ICH study series and for age, sex, hypertension, and diabetes for the longitudinal UPenn ICH series. \( P \) values of <0.0125 are considered statistically significant after correcting for multiple testing. Information was unavailable regarding NIHSS at initial evaluation (24 from the Yale ICH study series) and NIHSS at blood draw (41 from the Yale ICH study series).

Table 5. Associations of NFL concentrations with mRS at 3 and 6 months after stroke in patients with ICH from the validation cohorts. ORs, 95% CIs, and \( P \) values result from binary logistic regression models. ORs are interpreted as the multiplicative increase in the odds of mRS > 3 for each doubling in NFL concentration.

<table>
<thead>
<tr>
<th>Group</th>
<th>mRS &gt; 3 at 3 months after stroke</th>
<th>mRS &gt; 3 at 6 months after stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( N )</td>
<td>Unadjusted analysis</td>
</tr>
<tr>
<td>Yale ICH study series</td>
<td>96</td>
<td>1.45 (1.07–2.02)</td>
</tr>
<tr>
<td>UPenn ICH study series</td>
<td>36</td>
<td>1.96 (1.15–4.14)</td>
</tr>
<tr>
<td>0–12 hours from stroke to blood draw</td>
<td>40</td>
<td>3.32 (1.68–9.14)</td>
</tr>
<tr>
<td>60–84 hours from stroke to blood draw</td>
<td>29</td>
<td>4.23 (1.61–19.22)</td>
</tr>
</tbody>
</table>

*Multivariable models were adjusted for age, sex, hypertension, diabetes, and BMI for the Yale ICH study series and for age and sex for the longitudinal ICH series (note that multivariable analysis was not possible for the 60 to 84 hours from stroke to blood draw group due to its small sample size). \( P \) values of <0.0125 are considered statistically significant after correcting for multiple testing. Information was unavailable regarding mRS at 6 months (4 from the Yale ICH study series).
for aSAH does not correlate with Fisher scores (23). We also observed that higher NFL associated with intracranial hemorrhage volume in both ICH validation cohorts. In the longitudinal cohort, this association was apparent for NFL measured 12 to 36 and 60 to 84 hours after ICH, but not during the earliest time period (0 to 12 hours). As a whole, these findings strongly support blood NFL as a predictor of radiologic outcomes and highlight that the timing of NFL measurements must be considered to maximize their predictive value.

We additionally examined associations between NFL and indicators of neurological, functional, and cognitive status at the time of blood draw or at first evaluation. In our Mayo Clinic sample series, higher NFL associated with worse outcomes based on the NIHSS, mRS, and MMSE at blood draw in all patients with stroke combined and separately for patients with ACI or aSAH. For patients with ICH in the discovery cohort, plasma NFL correlated with NIHSS scores and nominally correlated with MMSE scores. Similarly, NFL correlated with NIHSS scores at time of blood draw and/or at initial evaluation in our two validation cohorts of patients with ICH. Previous studies evaluating the relationship between blood NFL and clinical scales at the time of blood draw are sparse, but Traenka et al. (26) reported that serum NFL correlates with NIHSS scores at the time of blood sampling in patients with cerebral artery dissection. Although associations between blood neurofilament and MMSE scores in patients with stroke have not, to our knowledge, been examined, blood NFL does correlate with MMSE scores in other neurodegenerative diseases (33, 34).

The ability of NFL to predict the clinical status of patients at blood draw is expected to inform their prognosis. Our data demonstrating the relationship between NFL and future outcomes, namely, 3- and 6-month mRS scores, support this notion. Our findings that blood NFL in patients with ischemic stroke correlate with 3-month mRS are consistent with those of previous studies. The Dichgans group (17) found that NFL in serum collected 7 days after admission to hospital for ACI independently correlated with 3-month mRS. Similarly, Pedersen et al. (20) observed that serum NFL measured within 14 days of ACI or 3 months after ictus associated with 3-month mRS. However, these associations were lost when analyses were limited to NFL measured between 6 and 8 days or within 24 hours of stroke (20). Because only 24 patients had blood sampled within 24 hours, the analysis of early NFL measures with 3-month mRS scores was likely underpowered in this study (20). Nevertheless, discrepant findings exist among studies evaluating early NFL concentrations and functional outcomes of ischemic stroke. Whereas De Marchis et al. (14) failed to detect associations of blood NFL measured 24 hours after stroke and 3-month mRS, Uphaus et al. (22) reported that serum NFL measured 24 hours after admission did predict unfavorable 3-month mRS scores.

As observed in patients with ischemic stroke, we found blood NFL to associate with 3-month mRS scores in patients with aSAH and with 3- and 6-month mRS scores in patients with ICH. In our longitudinal study of patients with ICH, 3-month mRS scores correlated with NFL measured 12 to 36 hours, but not 0 to 12 hours, after stroke. In a previous study, plasma NFL measured in patients with aSAH at admission reportedly correlated with 1-month mRS scores despite NFL concentrations not being higher than in control individuals (23). In patients with ICH, plasma NFL measured neither at admission nor 24 hours after ictus associated with 1-month mRS scores (25).

Last, given that stroke is a leading cause of death worldwide, and given the absence of a blood biomarker for all-cause mortality, we evaluated whether plasma NFL would fulfill this purpose. Both in all patients with stroke combined and in patients with ACI or aSAH alone, NFL correlated with survival after blood draw.

Strengths of our study include (i) the evaluation of three types of stroke, as well as ACI subtypes, in patients recruited concurrently from the same center and assessed using the same assay, thus allowing direct comparisons among groups; (ii) the inclusion of two independent validation cohorts to confirm our findings in ICH; (iii) the longitudinal assessment of serum NFL in patients with ICH; (iv) the use of a highly sensitive and well-validated assay to quantify NFL (35); and (v) the rigor of our statistical analyses.

Our study also has limitations. First, as this was a retrospective study, our discovery sample series was composed of plasma drawn from patients at various times from the day of stroke up to 20 days after stroke. Although one advantage to this design is that it allowed us to explore when, after a stroke, NFL rises above normal, the heterogeneity in the timing of blood sampling has potential to influence associations of interest. To address this possibility, our association analyses were both stratified by time from stroke to blood draw and adjusted for time from stroke to blood draw. Second, the sizes of the discovery aSAH and ICH cohorts were relatively small, which decreased the power of some analyses and/or our ability to examine certain associations. The fact that our findings in patients with ACI were congruent with those of other studies (17, 18, 20), and that our findings in patients with ICH were validated across three independent groups, nonetheless gives us confidence in the soundness of our results.

Findings from the present study have important implications. Although our data, in aggregate with those of others, suggest less utility for NFL measured within 24 hours of stroke in guiding acute patient care decisions, they do provide compelling evidence supporting blood NFL as a promising prognostic biomarker for patients with ACI, aSAH, and ICH. At present, age and stroke severity are among the strongest predictors of outcome. Stroke severity is commonly estimated on the basis of the degree of neurologic impairment determined by scales like the NIHSS (36–38). Functional, cognitive, and radiographic scales also provide insights into stroke severity. Yet, like the NIHSS, these scales are often noncontinuous measures of the extent of tissue injury, and some scales are specific to a stroke type. Furthermore, many clinical circumstances compromise their validity as a marker of new injury specific for an acute stroke. For instance, anesthetic and paralytic agents as well as medications given for pain, agitation, or depression can influence the accuracy of the NIHSS and other scales. These circumstances would not affect measures of blood NFL.

In addition to its prognostic value, blood NFL can positively affect multiple facets of ischemic and hemorrhagic stroke research. Subacute NFL measures would be of great value to clinical trials seeking to reduce stroke complications or to reduce final neuroaxonal injury. They also have potential to substitute for repeated head imaging. Although head imaging is generally tolerated, it can expose patients to ionizing radiation (39) and the risks of acute adverse reactions to contrast (40) and claustrophobia (41), as well as add to the cost of care (42). NFL measures not only would be more cost-effective than protocol-specified brain magnetic resonance imaging (MRI) but also could be applied to all patients, even those who are not suitable MRI candidates (due to claustrophobia, ferromagnetic devices, or medical instability). For example, NFL would be useful for clinical trials comparing pre-perfusion/recanalization strategies for treating acute ischemic stroke, which often rely on final

infarct volume as a surrogate endpoint (43). Moreover, postoperative NFL concentrations could be used as a biomarker in lieu of brain imaging to detect symptomatic ischemic stroke and silent brain infarcts.

Our data highlight areas in need of additional investigation if they are to be applied to the clinic. Among the key steps in translating our findings from bench to bedside are delineating the earliest optimal window at which blood NFL concentrations correlate with long-term functional outcomes and establishing normative NFL values by age in healthy individuals. Furthermore, including blood NFL as a prognostic biomarker in randomized clinical trials focusing on recovery would be particularly informative. Should blood NFL correlate with meaningful outcomes (disability, function, and cognition) in this setting, its incorporation into the care of patients in the clinic would be justified.

Overall, on the basis of our data indicating that plasma NFL provides a uniform method to estimate neuroaxonal injury and forecast outcomes, including all-cause mortality, across stroke mechanisms, it has the potential to assist in planning supportive and rehabilitation services and with improving the efficiency of trials of acute therapeutic and recovery-enhancing interventions.

MATERIALS AND METHODS

Study design

The goal of this study was to investigate blood NFL as a predictor of neuroaxonal injury and all-cause mortality across major types of ischemic and hemorrhagic stroke. NFL in plasma or serum was measured using the NF-light digital immunoassay from Quanterix (see Supplementary Materials and Methods). Our primary analyses were to determine whether blood NFL is elevated after stroke and whether NFL associates with (i) radiographic markers; (ii) neurological, functional, and cognitive status at blood draw/initial evaluation or 3 months after stroke; and (iii) all-cause mortality. We first completed a retrospective study involving 393 individuals enrolled in the Mayo Clinic Florida Familial Cerebrovascular Diseases Registry (314 patients with ACI, aSAH, or ICH and 79 control individuals) (27). We included all patients (no randomization) for whom plasma was available and collected within 20 days from stroke. Our study also included two independent sample series from patients with ICH: (i) plasma collected from 96 patients 72 hours after ICH through the Yale ICH study and (ii) serum collected longitudinally from 54 patients at UPenn. NFL was measured in a blinded manner. Sample sizes for all series were based on what was available when the study was initiated and not on sample size calculations. Written informed consent was obtained from all participants or their legally authorized surrogate if they were unable to give written consent, and biological samples were obtained with ethics committee approval. Additional information on our sample series is provided in Supplementary Materials and Methods.

Statistical analysis: Mayo Clinic discovery series

All statistical tests for the discovery and validation series were two-sided and performed using R Statistical Software (version 3.6.2; R Foundation for Statistical Computing, Vienna, Austria).

NFL was compared according to time from stroke to blood draw in patients with stroke (0 to 8 days versus 9 to 20 days) using linear regression models that were adjusted for stroke group only and also additionally adjusted for age at blood draw, sex, BMI, current smoking, hypertension, diabetes, and physical activity. Because of its skewed distribution, NFL concentrations were examined on the base 2 logarithm scale in this and all subsequently described regression analysis.

In addition to performing statistical analyses irrespective of length of time from stroke to blood draw and adjusting regression models involving solely patients with stroke for this time length (as a continuous variable), we also performed analyses (sample size permitting) in the separate patient subgroups with a length of time from stroke to blood draw of 0 to 8 days or 9 to 20 days (while still adjusting for time from stroke to blood draw).

The association between NFL and age in controls was examined using Spearman’s test of correlation. Comparisons of NFL concentrations between controls and patients with stroke (overall and according to stroke type and ACI subtype) were made using linear regression models. β coefficients and 95% confidence intervals (CIs) were estimated and are interpreted as the difference in mean NFL concentration (on the base 2 logarithm scale) between the two groups of interest. Models were initially adjusted for length of time from stroke to blood draw as a linear term and a quadratic term to allow for a nonlinear relationship between this variable and NFL concentrations. Subsequently, linear regression models were also adjusted for a predefined group of potential confounding variables, which included age at blood draw, sex, BMI, current smoking, hypertension, diabetes, and physical activity, when the sample size allowed for such a multivariable analysis (29). To adjust for multiple testing, we used a Bonferroni correction separately for each group of similar tests, after which P values of <0.0063 were considered as significant when making comparisons of NFL among controls and patients with stroke (eight overall pairwise comparisons), and P values of <0.0056 were considered as significant when comparing NFL among different stroke subtypes (nine overall pairwise comparisons).

Associations of NFL with radiographic markers (ASPECTS, modified Fisher score, and ABC/2) were examined using regression models appropriate for the given radiographic measure. In patients with ACI (overall and separately for ACI subtypes), the association between NFL and ASPECTS was assessed using proportional odds logistic regression models that were adjusted for time from stroke to blood draw, where ASPECTS between 0 and 6 were collapsed into one category due to their rare frequencies. Odds ratios (ORs) and 95% CIs were estimated and are interpreted as the multiplicative increase in the odds of a lower ASPECTS for each doubling in NFL concentration. Additional multivariable adjustment for the aforementioned predefined confounding variables was also examined in the larger overall ACI group only. In patients with aSAH, the association between NFL and modified Fisher score was examined using a binary logistic regression model that was adjusted for length of time from stroke to blood draw, where modified Fisher score was dichotomized as <4 or 4; an OR and 95% CI were estimated and correspond to each doubling in NFL concentration. Last, in patients with ICH, the association between NFL and ABC/2 scores was evaluated using a linear regression model that was adjusted for length of time between stroke and blood draw, where ABC/2 scores were examined on the square root scale owing to their skewed distribution. A β coefficient and 95% CI were estimated and are interpreted as the change in the mean ABC/2 score (on the square root scale) for each doubling in NF concentration. P values of <0.05 were considered as statistically significant for tests of association of NFL with the separate ABC/2 and modified Fisher score outcomes, which were each examined in only one patient group. As associations
between NFL and ASPECTS were examined in four different groups of patients with ACI, \( P \) values of <0.0125 were considered as statistically significant in those analyses after applying a Bonferroni correction for multiple testing.

In patients with stroke (overall and according to stroke type and ACI subtype), associations of NFL with measures obtained at blood draw (NIHSS, mRS, and MMSE) and post-blood draw outcomes (3-month mRS and survival) were evaluated using regression models that are appropriate for the nature of the given outcome measure. Specifically, associations between NFL and NIHSS were examined on the square root scale due to its skewed distribution. \( \beta \) coefficients and 95% CIs were estimated and are interpreted as the change in mean NIHSS score (on the square root scale) for each doubling of NFL concentration. Associations of NFL with mRS scores were evaluated using binary logistic regression models, where mRS was dichotomized as \( \leq 3 \) or >3. ORs and 95% CIs were estimated. Associations between NFL and MMSE scores were examined using binary logistic regression models, where MMSE scores were dichotomized on the basis of the sample median (<median, \( \geq \)median) in the given patient group being examined owing to its bimodal distribution. ORs and 95% CIs were estimated. Last, associations between NFL concentration and survival were evaluated using Cox proportional hazards regression models. The baseline time point for survival was the date of blood draw, and censoring occurred on the date of last follow-up. HRs and 95% CIs were estimated. All HR and OR estimates correspond to a doubling in NFL concentration. All regression models (Cox, linear, logistic) were initially adjusted for length of time from stroke to blood draw (as a linear term only). Subsequently, for the two largest groups of patients with ACI and all patients with stroke, Cox, linear, and logistic regression models were also adjusted for age at blood draw, sex, BMI, current smoking, hypertension, diabetes, and physical activity. We used a Bonferroni correction to account for multiple testing separately for each outcome measure, after which \( P \) values of <0.0071 (or <0.0083 for tests involving mRS and survival) were considered as statistically significant (six or seven overall tests of association were performed for each outcome).

We further evaluated the ability of NFL to independently predict 3-month mRS and survival in two ways. First, the aforementioned logistic regression (for 3-month mRS) and Cox regression (for survival) models were additionally adjusted for NIHSS (separately for NIHSS scores at stroke and NIHSS scores at blood draw). Second, we assessed the predictive ability of the aforementioned multivariable logistic and Cox regression models (including NIHSS scores) with and without NFL in the model. Specifically, when examining 3-month mRS (which was dichotomized as \( \leq 3 \) or >3), AUC was estimated both with and without NFL in the multivariable model. When evaluating survival, we estimated c-index with and without NFL in the multivariable model.

**Statistical analysis: Validation series**

Similar to the Mayo Clinic discovery series, NFL concentrations were examined on the base 2 logarithm scale in all analysis of the validation series due to their skewed distribution. All analyses were performed separately for the Yale ICH study and the three separate stroke—to–blood draw time periods (0 to 12, 12 to 36, and 60 to 84 hours) for the longitudinal ICH series.

The association between NFL and age in controls was examined using Spearman’s test of correlation. Comparisons of NFL concentrations between controls and patients with ICH were made using linear regression models. \( \beta \) coefficients and 95% CIs were estimated and are interpreted as the difference in mean NFL concentrations (on the base 2 logarithm scale) between the two groups of interest. Unadjusted models were first examined, and these were followed by multivariable models that were adjusted for age at blood draw and sex. We applied a Bonferroni correction to adjust for the four different comparisons versus controls that were made, after which \( P \) values of <0.0125 were considered as significant.

For the longitudinal ICH series, comparisons of NFL concentrations between the 0- to 12-hour, 12- to 36-hour, and 60- to 84-hour stroke–to–blood draw time periods were made using paired Wilcoxon signed rank tests. Only patients with ICH with an NFL measurement for both of the time periods involved in a given pairwise comparison were included for analysis of that comparison. \( P \) values of <0.0167 were considered as statistically significant after using a Bonferroni correction to adjust for the three pairwise comparisons between groups that were made.

Associations of NFL with ICH volume and NIHSS at initial evaluation and blood draw were examined in patients using unadjusted and multivariable linear regression models. Multivariable models were adjusted for age, sex, hypertension, diabetes, and BMI for the Yale ICH study series and for age, sex, hypertension, and diabetes for the longitudinal ICH series from UPenn. ICH volume and NIHSS were examined on the square root scale owing to their skewed distribution. \( \beta \) coefficients and 95% CIs were estimated and are interpreted as the change in the mean outcome measure (on the square root scale) for each doubling in NFL concentration. Multivariable models were adjusted for age, sex, hypertension, diabetes, and BMI for the Yale ICH study series and for age and sex in the longitudinal ICH series (adjustment for additional variables in the ICH series was not possible for this binary outcome where ability to adjust for covarlates is more limited). \( P \) values of <0.0125 were considered as statistically significant after applying a Bonferroni correction for multiple testing separately for each outcome measure, as association tests were performed for four separate patient groups.

We performed additional examination of the ability of NFL to independently predict 3- and 6-month mRS in two ways, similar to the analysis of the Mayo Clinic discovery series. First, the aforementioned multivariable binary logistic regression models were additionally adjusted for NIHSS at initial evaluation (NIHSS at blood draw was not examined here due to missing data in the Yale ICH study series and lack of any data in the longitudinal ICH series). Next, we assessed the predictive ability (as measured by AUC) of the aforementioned multivariable logistic regression models (including NIHSS at initial evaluation) with and without NFL in the model.

**SUPPLEMENTARY MATERIALS**

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**Materials and Methods**

Fig. S1. Blood neurofilament concentrations in healthy controls correlated with age.

Fig. S2. Plasma NFL was elevated in all studied ischemic stroke subtypes.

Table S1. Subject characteristics at baseline for controls, all patients with stroke, and according to stroke type.

Table S2. Patient characteristics according to ACI subtype.
Table S3. Comparisons of NFL concentrations between controls and patients with stroke according to stroke type.
Table S4. Comparisons of NFL concentrations between controls and patients with ACI according to subtype.
Table S5. Comparisons of NFL concentrations among patients with ACI, aSAH, or ICH.
Table S6. Comparisons of NFL concentrations among ACI subtypes.
Table S7. Summary of scores from ASPECTS (patients with ACI), the modified Fisher grading scale (patients with aSAH), and ABC/2 (patients with ICH).
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Table S9. Associations of NFL concentrations with MMSE scores at blood draw by stroke type.
Table S10. Associations of NFL concentrations with NIHSS and mRS scores at blood draw by ACI subtype.
Table S11. Associations of NFL concentrations with MMSE scores at blood draw by ACI subtype.
Table S12. Associations of NFL concentrations with 3-month mRS scores. Table S13. Associations of NFL concentrations with 3-month mRS scores by ACI subtype. Table S14. Assessment of whether associations between increased NFL concentrations and poorer 3-month mRS scores are independent of NIHSS.
Table S15. Examination of the ability of NFL concentrations to independently predict 3-month mRS scores.
Table S16. Associations between NFL concentrations and survival after stroke.
Table S17. Associations between NFL concentrations and survival after stroke according to ACI subtype.
Table S18. Assessment of whether the association between increased NFL concentrations and poorer survival is independent of NIHSS.
Table S19. Examination of the ability of NFL concentrations to independently predict survival.
Table S20. Subject characteristics of the validation series.
Table S21. Comparisons of plasma NFL concentrations between controls and patients in the Yale-ICH study validation series.
Table S22. Comparisons of serum NFL concentrations between controls and patients in the UPenn validation series.
Table S23. Comparison of NFL concentrations between the 0- to 12-hour, 12- to 36-hour, and 60- to 84-hour time periods in the UPenn ICH validation series.
Table S24. Assessment of whether associations between increased NFL concentrations and poorer 3- and 6-month mRS scores are independent of NIHSS at initial evaluation in the validation series.
Table S25. Examination of the ability of NFL concentrations to independently predict 3- and 6-month mRS scores in the validation series.

References (44–51)

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References and Notes

Acknowledgments: We are grateful to all individuals who donated samples and would like to acknowledge R.H. Al-Shaik for assistance in sample collection. Funding: This work was supported by the NIH (R35NS090723 to L.P., P01NS084974 to L.P., P01NS099114 to T.F.G. and T.F.G., R56AG059992 to G.J.F., RO3NS112859 to G.J.F. and K.N.S., P30AG12342 to G.J.F., R01NS10721 to K.N.S., R01NS075209 to K.N.S., U01NS13445 to K.N.S., U01NS106513 to K.N.S., R01NR01833 to K.N.S., U24NS107215 to K.N.S., and U24NS107136 to K.N.S.), the Earl & Nyaundra Swanson Neuroscience Research Fund to J.F.M., the Harley N and Rebecca N Hotchkiss Endowed Fund in Neuroscience Research, Honoring Ken and Marietta to J.F.M., the Donald G and Jodi P Heeringa Family, and the Haworth Family Professorship in Neurodegenerative Diseases funds to Z.K.W. The Mayo Clinic Florida Familial Cerebrovascular Diseases Registry received funds from the Mayo Foundation for Medical Education and Research. Author contributions: L.P., J.F.M., T.F.G., and M.K.B. contributed to the conception and/or design of the study. J.F.M., B.L.C., G.J.F., K.N.S., L.H.S., O.A.R., M.K.B., G.K.V., Z.K.W., B.E.O., T.M.M., J.I.B., K.A.N., and R.L.W. contributed through the collection of patient samples and/or clinical data. T.F.G., K.R.J.-W., and A.R.B. assisted with, or measured, plasma or serum NFL concentrations. Statistical analyses were performed by M.G.H. and P.W.J. The manuscript was written by T.F.G., M.G.H., J.F.M., and T.G.B., and all authors read it and provided comments and revisions.

Competing interests: L.P. is a consultant for Expansion Therapeutics. T.M.M. is an advisory board member and received support for clinical studies from Biogen, has a licensing agreement and received material support from Ionis, has a licensing agreement with C2N, and has consulted for Cytokinetics and Disarm Therapeutics.

Data and materials availability: All requests for data or materials should be made to L.P. and J.F.M. They will be made available through appropriate administrative channels (material transfer agreements).

Submitted 31 May 2019
Resubmitted 10 April 2020
Accepted 29 July 2020
Published 11 November 2020
10.1126/scitranslmed.aay1913

Plasma neurofilament light predicts mortality in patients with stroke


Sci Transl Med 12, eaay1913.
DOI: 10.1126/scitranslmed.aay1913

Circulating marker of brain damage
The outcome of a stroke varies greatly between patients from temporary mild symptoms to permanent disability and death. Clinical scale for measuring disease severity shows poor correlation with brain tissue damage. Identification of better markers of tissue damage could improve the ability to predict outcome and promote the development of better therapies. Now, Gendron et al. showed that the expression of circulating neurofilament light (NFL) was increased in multiple cohorts of patients with stroke compared to controls and correlated with brain tissue damage. In addition, blood concentration of NFL correlated with functional outcome and mortality. The results suggest that blood NFL might be used as prognostic marker after stroke.