Preparing for CADASIL therapy

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Chapter 5

Serum Neurofilament Light correlates with CADASIL disease severity and survival

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Abstract

Objective

To validate whether serum Neurofilament Light-chain (NfL) levels correlate with disease severity in CADASIL, and to determine whether serum NfL predicts disease progression and survival.

Methods

Fourty-one (pre-)manifest individuals with CADASIL causing *NOTCH3* mutations and 22 healthy controls were recruited from CADASIL families. At baseline, MRI-lesion load and clinical severity was determined and serum was stored. Disease progression was measured in 30/41 patients at 7-year follow-up, and survival of all individuals was determined at 17-year follow-up. Serum NfL levels were quantified using an ultra-sensitive molecule array. Generalized estimated equation regression (GEE) was used to analyse association between serum NfL, MRI-lesion load, disease severity and disease progression. With GEE-based Cox regression, survival was analysed.

Results

At baseline, serum NfL levels correlated with MRI-lesion load [lacune count (s=0.64, P=0.002), brain atrophy (r=-0.50, P=0.001), and microbleed count (s=0.48, P=0.044)], cognition [CAMCOG (s=-0.45, P=0.010), MMSE (r=-0.61, P=0.003), GIT (r=-0.61, P<0.001), TMT-A (r=0.70, P<0.001)) and disability (mRS (r=0.70, P=0.002)]. Baseline serum NfL predicted 7-year changes in disability (B=0.34, P<0.001) and cognition (CAMCOG B=-4.94, P=0.032), as well as 17-year survival. Higher NfL levels were associated with increased mortality (HR=1.8 per 2-fold increase in NfL levels, P=0.006).

Interpretation

Serum NfL levels correlate with disease severity, disease progression and 17-year survival in CADASIL patients. Serum NfL is a promising biomarker to monitor and predict disease course in CADASIL, as well as potentially assessing therapeutic response in future clinical trials.

Introduction

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is the most prevalent hereditary form of cerebral small vessel disease (SVD), and is caused by mutations in the *NOTCH3* gene.[1] CADASIL patients suffer from recurrent ischemic strokes with a mean age at onset of 45-50 years, although age at first stroke is highly variable.[1] Cognitive decline is a main but also variable feature, initially manifesting as executive dysfunction and progressing to global impairment in all cognitive domains and complete care dependency.[2] Migraine with aura affects up to two thirds of patients.[3] In CADASIL, mutant NOTCH3 proteins aggregate in the tunica media of small arteries,[4–6] directly or indirectly compromising cerebrovascular reactivity.[7,8] On MRI, white matter hyperintensities can be observed in pre-manifest patients from the third decade, while lacunes, microbleeds and brain atrophy usually appear later in the disease course.[1,9,10]

Lacunes and brain parenchymal fraction (BPF) have been shown to be predictors of disease progression in CADASIL patients.[11–17] However, a good fluid biomarker would have advantages above the more cumbersome neuroimaging markers and could ideally also be used as a marker for therapeutic response in future clinical trials. Various potential fluid biomarkers have been investigated for CADASIL,[18,19] but most do not seem to be feasible. Recently, serum Neurofilament light-chain levels were shown to correlate with disease severity in CADASIL.[20]

Neurofilament light-chain protein (NfL) levels in serum have made a recent surge in the field of biomarkers for brain disease.[21–27] NfL is a component of the neurofilament complex, which has a scaffolding role in neuronal axons, and is released into the cerebrospinal fluid (CSF) and blood upon neuronal damage,[21] in some studies remaining elevated in blood for several months.[22,23] NfL levels have been shown to correlate with disease severity and progression in Huntington's disease,[24] Frontotemporal Dementia[25,26] and Amyotrophic Lateral Sclerosis.[27] In Multiple Sclerosis, NfL levels in blood have even been shown to be responsive to treatment, showing its potential as a therapeutic response marker.[28,29] In sporadic SVD and CADASIL, serum NfL levels were shown to be associated cross-sectionally with neuroimaging markers, processing speed and disability, but the associations between serum NfL and disease progression and survival were not investigated.[20,22]

Here, we explored whether serum NfL, measured using the ultra-sensitive single molecule array, may be a feasible fluid biomarker for monitoring CADASIL disease severity, predicting disease progression and predicting long-term survival. We used a well characterized cohort of CADASIL patients and controls, which has been uniquely followed-up for 17 years.

Methods

Cohort

At baseline, in 2000, 41 patients and 22 controls, from a total of 15 CADASIL families of which 10 families had ≥2 participants, were enrolled in the study. Thirty-two patients had a *NOTCH3* mutation in exon 4 and nine patients had a mutation in exon 8, 11, 19 or 20. A full medical history, MRI, serum withdrawal, neuropsychological testing and genetic *NOTCH3* testing was performed, as described earlier.[30] The average age at baseline was 46 years in patients and 39 years in healthy relatives (Table 5.1). Seven years later this same cohort was invited to participate in a follow-up study. Thirty patients consented to participate. Eleven participants were lost-to-follow-up due to death (6 patients) or severe disease (5 patients). Five patients did not consent to MRI in the follow-up study.[15] Seventeen years after baseline, survival data from all participants of the baseline study was obtained from the Dutch population registry. These studies were approved by the medical ethics committee of the Leiden University Medical Center (P80/98) and each participant gave informed consent.

Neuroimaging and neuropsychological testing

MRI was performed on a 1.5 T MR system at baseline[9] and at 7 year follow-up.[15,16] Lacune count, microbleed count, parenchymal volume, intracranial volume and white matter hyperintensity volume were previously quantified.[15,16,31] Parenchymal volume was normalized to intracranial volume to acquire the brain parenchymal fraction (BPF), as a measure for brain atrophy. White matter hyperintensity volume was normalized to parenchymal volume (WMH_V). The neuropsychological test battery included the Cambridge Cognitive Examination (CAMCOG), Mini–Mental State Examination (MMSE), the Groningen Intelligence Test (GIT), and the Trail Making Test part A (TMT-A) and part B (TMT-B).[32–35] Disability was assessed using the modified Rankin scale (mRS).

Serum NfL measurement

At baseline, blood was withdrawn via standard vena puncture and centrifuged at 2750*g* for 20 minutes at room temperature. The supernatant was aliquoted and stored at -80°C freezer until analysis. Serum NfL levels were quantified using an in-house developed Simoa assay, as previously described.[36] The assay was validated prior to use according to standardized international protocols.[37] The lower level of quantification (LLOQ) was 0.77 pg/mL (10 times the standard deviation of 16 negative samples). Three of the 41 patients (age 22, 30, 39 years) and one of the 22 controls (age 24 years) had a serum NfL below the LLOQ and were excluded from the study. A sensitivity analysis, including these individuals and using half of the LLOQ as the measurement for these individuals, showed similar results.

Statistics

Non-normally distributed data was transformed to obtain plausible normal distributions for further analyses: serum NfL was natural log (ln) transformed and to facilitate interpretation also $\log_2$ transformed for Cox-regression; lacune count, WMH_V and CAMCOG were square root transformed; TMT-A was $\log_{10}$ transformed; and microbleed count was $\log_{10}(1+x)$ transformed. To determine the association between NfL levels and brain MRI lesion load and clinical outcomes at baseline, generalized estimated equation multivariable regression models (GEE), using an independent correlation structure and robust variance estimators, were used to enable correction for correlation within families, since 10 families had ≥2 participants in the study. Sex and age were included in all linear regression GEE analyses. To determine the association between age and NfL in both patients and controls, the GEE model also included mutation status, and the age-mutation status interaction. To determine how NfL compared with MRI markers in predicting disease severity, forward and backward regular linear regression was performed with NfL, lacune count, brain atrophy, WMH_V and microbleed count as potential predictors. Potential predictors were added only if they significantly contributed to the model.

To determine whether NfL levels were associated with 7-year changes in MRI lesion load and clinical deterioration, GEE models were used with either NfL and sex; or NfL, sex and baseline measurement of the tested outcome. For clarity, the correlations in the figures are presented using Pearson (*r*) or Spearman (*s*) correlation coefficients, together with p-values obtained from GEE regression models. Hazard ratios for the relation of baseline NfL with survival at 17-year follow-up were calculated using a multivariate GEE-based Cox regression analysis, including age and the covariate of interest. Area under the curve (AUC) and confidence intervals were calculated and compared using DeLong tests in R package pROC. We did not formally prove equivalence in the prognostic value of NfL versus the neuroimaging markers, as formal equivalence testing would be under-powered in the current data set. GEE-based logistic regression analyses were performed with an independent correlation structure and were corrected for sex. All other statistical analyses were performed in IBM SPSS Statistics version 23.0.0.2. All statistical tests were two-sided and the threshold for statistical significance was 0.05.

Table 5.1: Cohort characteristics at baseline and follow-up

	Cross-sectional study at baseline		7-year follow-up study in patients	
	Controls	Patients	Baseline	Follow-up
	Mean (SD), median (range) or n (%)			
N	22	41	30	30
Age, y	39.8 (12.5)	45.8 (10.4)	44.0 (9.8)	51.1 (9.8)
Female	12 (55%)	22 (54%)	17 (57%)	17 (57%)
Prior stroke/TIA	0 (0%)	23 (56%)	16 (53%)	-
Time since last stroke	-	3.3 (0.1 – 17.7)	3.5 (0.1 – 14.2)	-
Hypertension	6 (27%)	3 (7%)	2 (7%)	-
Diabetes Mellitus	0 (0%)	3 (7%)	3 (10%)	-
Hyperlipidaemia	4 (18%)	15 (37%)	10 (33%)	-
Current or past smoking	14 (64%)	26 (63%)	19 (63%)	-
Serum NfL (pg/mL)	1.66 (0.77 – 9.7)	6.31 (1.22 – 107.7)	5.21 (1.22 – 31.6)	-
MRI data available (n)[a]	**21**	**40**	**25**	**25**
Lacunes on MRI				
0 lacunes	21 (100%)	8 (20%)	6 (24%)	5 (20%)
1-10 lacunes		15 (38%)	14 (56%)	12 (48%)
>10 lacunes		17 (42%)	5 (20%)	8 (32%)
Brain atrophy (BPF)		82.198 (2.977)	82.648 (2.919)	81.836 (3.065)
WMH_V	0.003 (0 – 0.075)	7.297 (0.009 – 19.209)	4.303 (0.009 – 13.939)	7.865 (0.005 – 19.806)
Microbleeds				
0 microbleeds	21 (100%)	29 (73%)	22 (88%)	18 (72%)
1-10 microbleeds		9 (22%)	2 (8%)	5 (20%)
>10 microbleeds		2 (5.0%)	1 (4%)	2 (8%)
Clinical score available (n)[a]	**22**	**41**	**30**	**30**
mRS				
score 0	22 (100%)	13 (32%)	12 (40%)	9 (30%)[b]
score 1-2		17 (42%)	15 (50%)	10 (33%)[b]
score ≥3		11 (27%)	3 (10%)	10 (33%)[b]
CAMCOG	94.2 (4.9)	88.3 (15.0)	93.5 (5.3)	88.1 (13.7)
MMSE	28.3 (1.5)	26.2 (4.3)	27.7 (1.6)	26.2 (5.0)
GIT	105.8 (11.7)	99.8 (16.9)[b]	103.8 (12.5)	100.6 (18.6)
TMT-A	30 (21 – 90)	37 (10 – 180)[e]	36 (10 – 92)[b]	40 (15 – 349)[c]
TMT-B	74.5 (38 – 216)	84 (36 – 540)[f]	82 (36 – 254)[b]	75.5 (44 – 300)[d]

[a] At baseline, one patient and one control did not consent to radiological examination. At follow-up, five patients did not consent to radiological examination.

[b] Missing data for one patient.

[c] Missing data for 3 patients.

[d] Missing data for 4 patients.

[e] Missing data for 5 patients.

[f] Missing data for 7 patients.

Results

NfL levels were higher in patients (median 6.31 pg/mL, range 1.22–107.7 pg/mL) than in controls (median 1.66 pg/mL, range 0.77 to 9.73 pg/mL) (P<0.001). NfL levels positively correlated with age in both patients and controls. However, this age-dependent increase was stronger in patients (7.6% increase per year, CI 4.6-10.7%) than in controls (3.7% increase per year, CI: 2.2%–5.3%)(P=0.012). In contrast to controls, patients showed a steeper increase in NfL levels from age 40 onwards, which coincided with the presence of lacunes (Figure 5.1).

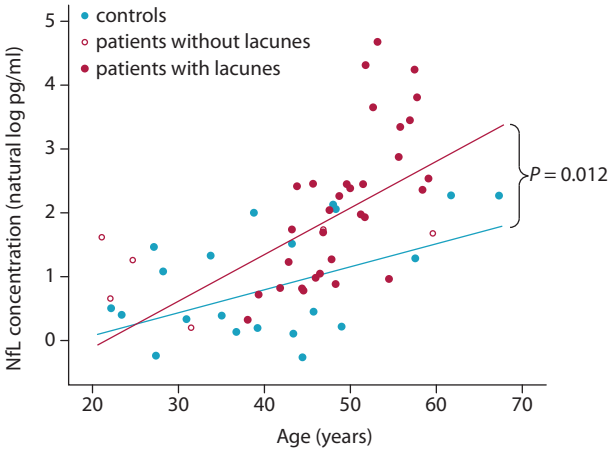

Figure 5.1: Serum NfL levels show an age-dependent increase in CADASIL patients and controls
CADASIL patients with lacunes (red dots) show a stronger age-dependent serum NfL increase than patients without lacunes (open circles) and controls (blue dots).

Correlation of serum NfL with disease severity at baseline

After correcting for age and sex, NfL levels correlated strongly with MRI lacune count (s=0.64, P=0.002), brain parenchymal fraction (r=-0.50, P=0.001) and microbleed count (s=0.48, P=0.044), but not with WMH_V levels (r=0.48, P=0.894) (Figure 5.2). Without correcting for age, NfL levels did correlate with WMH_V (r=0.48, P<0.001). When including all MRI markers in the model, NfL levels independently correlated with lacune count (β=0.20, P=0.002), brain parenchymal fraction (β=-0.14, P<0.001) and microbleed count (β=0.56, P=0.009). NfL levels did not correlate with time since last clinical stroke (data not shown).

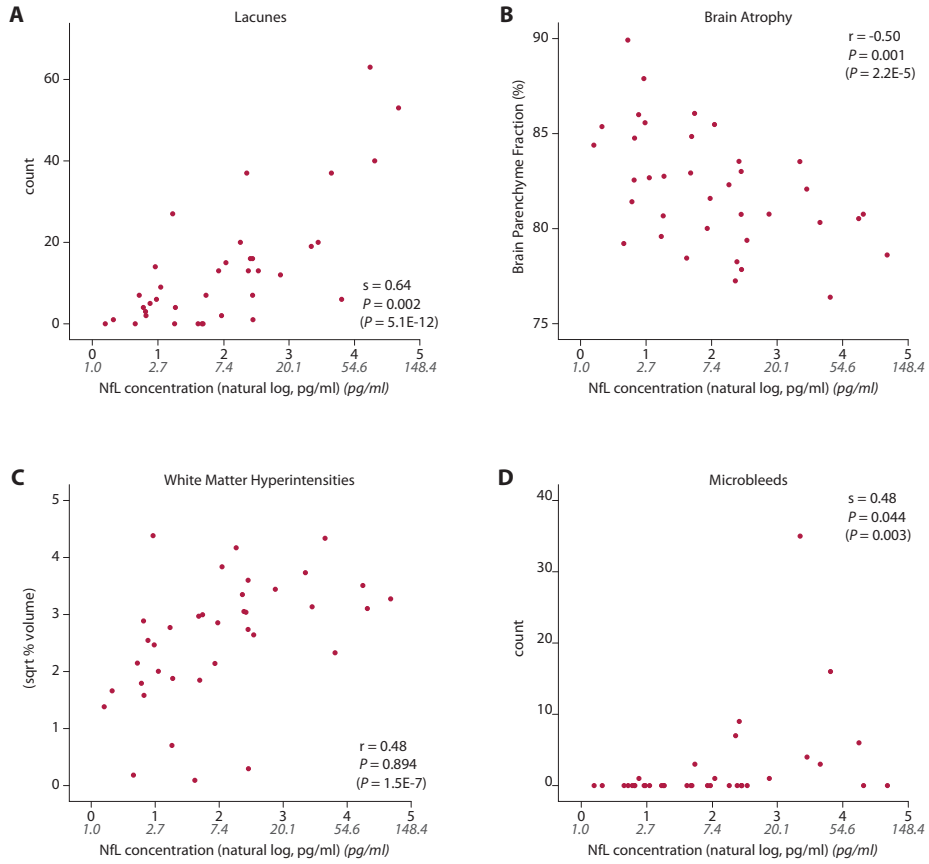

Figure 5.2: Cross-sectional associations between serum NfL levels and MRI markers

After correction for age and sex, NfL levels significantly correlate with lacune count (A), brain atrophy (B), and microbleeds (D), but not with white matter hyperintensity volume (C). *P*-values in parentheses represent *P*-value before correction for age and sex. NfL levels were natural log transformed for analyses, non-transformed NfL levels are shown in gray. NfL, Neurofilament light-chain.

Next, the relation of NfL with cognitive outcome measures was assessed. After correcting for sex and age, NfL levels correlated with disability scores of mRS (r=0.70, P=0.002), and cognitive function determined by CAMCOG (s=-0.45, P =0.010), MMSE (r=-0.61, P=0.003), GIT score (r=-0.61, P<0.001), and TMT-A (r=0.70, P<0.001), but not with TMT- B (s=0.39, P=0.115) (Figure 5.3). Serum NfL correlated more strongly with cognitive function (CAMCOG, MMSE and GIT) than any of the MRI markers correlated with cognitive function. NfL was associated less strongly with disability (mRS) and executive function (TMT-A and TMT-B) than lacune count (Table 5.2).

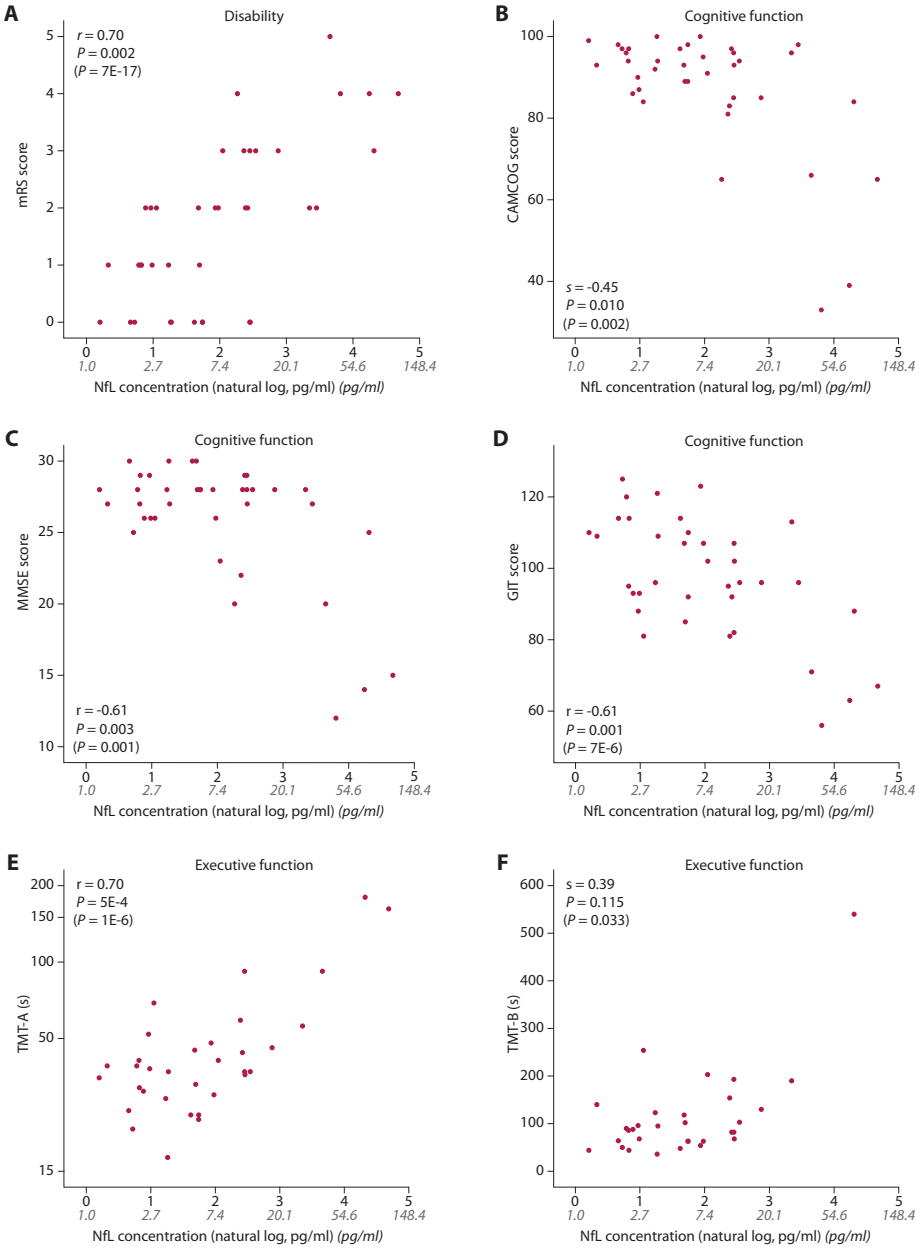

Figure 5.3: Cross-sectional association between serum NfL levels and disability, cognitive function, and executive function

NfL levels correlate with disability score mRS (A), cognitive function scores CAMCOG (B), MMSE (C), GIT (D), and executive function score TMT-A (E), but not with TMT-B (F). *P*-values in parentheses represent *P*-value before correction for age and sex. Serum NfL levels were natural log transformed for analyses. Non-transformed NfL levels are shown in gray. NfL, Neurofilament light-chain; mRS, modified Rankin Scale; CAMCOG, Cambridge Cognitive Examination; MMSE, Mini–mental state examination; GIT, Groningen Intelligence Test; TMT-A, Trail Making Test part A; TMT-B, Trail Making Test part B.

Table 5.2: Independent cross-sectional predictors for disability, cognition and executive function

		First predictor			*Second predictor*		
	Clinical scores	Covariate	Std. β	*P*-value	Covariate	Std. β	*P*-value
Cognition	CAMCOG (sqrt)	NfL	-0.593	<0.001*			
Cognition	MMSE	NfL	-0.609	<0.001*			
Cognition	GIT	NfL	-0.611	<0.001*			
Executive function	TMT-A (log)	Lacunes	0.466	0.008*	NfL	0.382	0.027*
Executive function	TMT-B	Lacunes	0.401	0.002*	NfL	0.362	0.013*
Disability	mRS	Lacunes	0.455	0.006*	NfL	0.381	0.019*

For clinical scored, the covariate with the best correlation is shown in the 'first predictor' column. A second predictor is only shown if it made a significant contribution. Results of forward regression are shown, but were validated with backward regression (not shown). NfL = serum concentration Neurofilament light-chain; mRS = modified Rankin Scale; CAMCOG = Cambridge Cognitive Examination; MMSE = Mini–mental state examination; GIT = Groningen Intelligence Test; TMT-A = Trail Making Test part A; TMT-B = Trail Making Test part B.

Prediction of seven year disease progression

To determine whether serum NfL levels predict disease progression, we assessed whether baseline serum NfL levels correlated with changes over 7 years in MRI lesion load, disability and cognition. Baseline NfL levels correlated with increased disability (mRS; β=0.34, *P*<0.001), decline in cognitive function (CAMCOG; β=-4.94, *P*=0.032), and decline in executive function (TMT-B time; β=24.55, *P*=0.035) (Table 5.3A). After correction for baseline values of the clinical measures, the correlation between NfL and disability (mRS; β=0.28, *P*=0.044) and executive dysfunction (TMT-B; β=29.03, *P*=0.025) remained significant, suggesting that NfL levels measured at any stage of the disease are associated with future disability.

NfL was not associated with an increase in lesion load of any of the MRI measures at follow-up. However, in patients with disability at baseline (mRS>0), baseline NfL levels were associated with an increase in lacune count (β=0.371, *P*<0.001), as well as with increased disability (mRS; β=0.33, *P*<0.001), decline in cognitive function (CAMCOG; β=-5.71, *P*=0.018; MMSE; -2.37, *P*=0.037) and decline in executive function (TMT-B time; β=33.5, *P*=0.030) (Table 5.3B). In patients who had no disability at baseline (mRS=0), serum NfL did not associate with disease progression.

Prediction of 17-year survival

After correction for age, serum NfL levels significantly predicted 17-year survival (HR 2.3 per 1 unit natural log pg/ml NfL increase, CI:1.3–4.2, *P*=0.006; corresponding to HR 1.8 per 2-fold increase in absolute NfL levels, CI: 1.2–2.7, *P*=0.006). In patients with the highest

Table 5.3: Correlation between baseline serum NfL levels and 7-year changes in neuroimaging markers and clinical scores in all patients (A) and in patients with dis- ability at baseline (mRS>0) (B)

	Model 1, corrected for sex		Model 2, corrected for sex and baseline values of tested covariate			
	NfL		Baseline		NfL	
	β	*P*	β	*P*	β	*P*
(A) All patients						
MRI markers						
Δ Lacune count (sqrt)	0.325	0.178	0.128	0.001*	-0.149	0.341
Δ BPF	-0.059	0.879	-0.180	0.249	-0.323	0.504
Δ WMH_V (sqrt)	0.074	0.593	0.487	<0.001*	-0.165	0.291
Δ MB count (log)	0.350	0.643	5.871	0.071	-0.874	0.476
Clinical scores						
Δ mRS score	0.338	<0.001*	0.072	0.551	0.281	0.044*
Δ CAMCOG score (sqrt)	-4.944	0.032*	0.782	0.040*	-4.297	0.061
Δ MMSE score	-2.109	0.051	-0.132	0.595	-2.100	0.053
Δ GIT score	-1.714	0.462	0.157	0.538	-1.073	0.727
Δ TMT-A	20.028	0.087	132.81	0.276	9.152	0.306
Δ TMT-B	24.551	0.035*	-0.175	0.421	29.025	0.025*
(B) Patients with disability at baseline (mRS>0)						
MRI markers						
Δ Lacune count (sqrt)	0.371	0.007*	0.112	0.003*	-0.187	0.187
Δ BPF	-0.005	0.989	0.087	0.458	0.106	0.681
Δ WMH_V (sqrt)	0.102	0.576	0.774	<0.001	-0.273	0.115
Δ MB count (log)	0.586	0.479	6.169	0.140	-1.272	0.471
Clinical scores						
Δ mRS score	0.330	<0.001*	-0.035	0.864	0.356	0.076
Δ CAMCOG score (sqrt)	-5.705	0.018*	0.528	0.265	-5.879	0.013*
Δ MMSE score	-2.374	0.037*	-0.412	0.459	-2.311	0.046*
Δ GIT score	-2.280	0.397	-0.038	0.847	-2.347	0.422
Δ TMT-A	24.545	0.154	296.25	0.224	4.957	0.747
Δ TMT-B	33.488	0.030*	-0.548	0.054	53.286	0.001*

Delta indicates the difference between baseline and 7-year follow-up for the respective MRI marker or clinical score; positive value indicate an increase in the score, while negative values indicate a decrease in the score. Data of all patients who participated at seven year follow-up is shown (A), as well as the subset of patients with disability at baseline (B). In model 1 the prognostic value of baseline NfL for the differences in MRI markers and clinical scores are shown, after correcting for sex. In model 2, the prognostic value of baseline NfL and the baseline score of the tested variable are shown, after correcting for sex. NfL levels were natural log transformed. Behind MRI markers and clinical scores the type of transformation of the variable is indicated, if applicable. NfL = serum concentration Neurofilament light-chain; mRS = modified Rankin Scale; CAMCOG = Cambridge Cognitive Examination; MMSE = Mini–mental state examination; GIT = Groningen Intelligence Test; TMT-A = Trail Making Test A; TMT-B = Trail Making Test B.

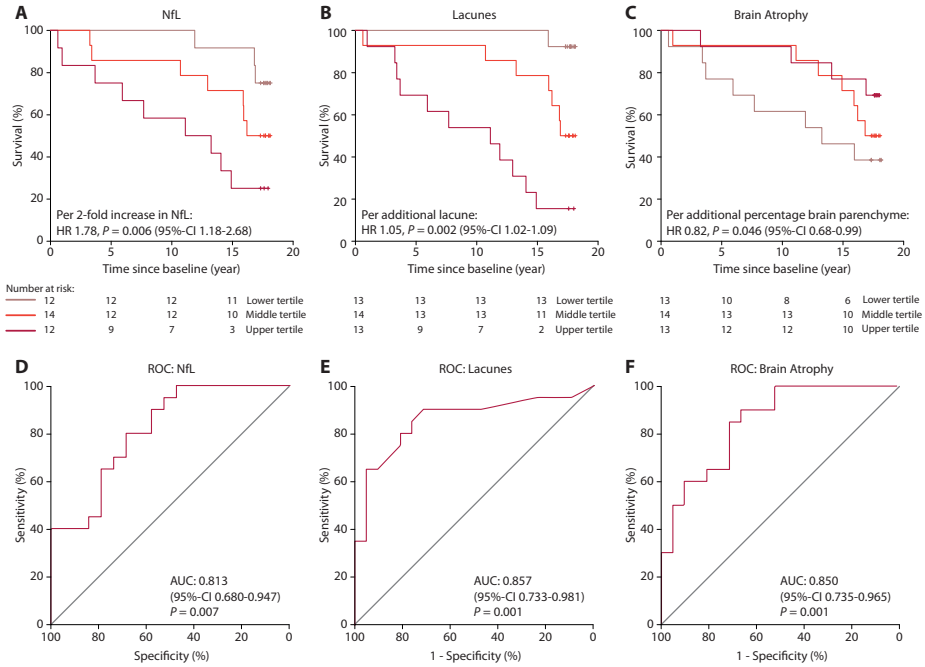

Figure 5.4: Association between serum NfL levels and 17-year survival

Kaplan–Meier plots show longitudinal survival divided into tertiles for serum NfL levels (upper tertile >11.2 pg/mL, middle tertile 3.53–11.22 pg/mL, lower tertile <3.53 pg/mL) (A), lacune count (upper tertile >13, middle tertile 4–13, lower tertile ≤3) (B), and brain atrophy (upper tertile > 83.4%, middle tertile 80.7–83.4%, lower tertile <80.7%) (C). Receiver Operating Characteristic curves illustrate the prognostic ability for serum NfL levels (D), lacune count (E), and brain atrophy (F) at baseline to discriminate between live and deceased patients after 17-year follow-up. *P*-values indicate testing with null-hypothesis AUC 0.50 as indicated by the diagonal line. NfL = Neurofilament light-chain; AUC = area under the curve; 95%-CI = 95% confidence interval.

NfL levels at baseline (upper tertile; >11.2 pg/mL), the survival after 17 years was 25%, while survival was 50% and 75% in patients with NfL levels in the middle and lower tertile (<3.55 pg/ml) (log rank *P*=0.012), respectively (Figure 5.4). To determine whether NfL was better in predicting 17-year survival than MRI markers or age, receiver operating characteristic (ROC) analyses were performed. The area under the curve (AUC) was similar for all parameters: 0.813 for serum NfL (CI: 0.680–0.947), 0.857 for lacune count (CI: 0.733–0.981), 0.850 for brain atrophy (CI: 0.735–0.965) and 0.843 for age (CI: 0.726–0.960). In both ROC analyses and logistic regression, the combination of NfL with any MRI marker or with age was not significantly better in predicating 17 year survival than NfL alone.

Discussion

Here, we validate a recent study showing that serum NfL levels correlate with disease severity in CADASIL. Moreover, we show that serum NfL levels at baseline also predict disease progression as well as 17-year survival in a cohort of well-characterized CADASIL patients.

We found that serum NfL levels correlated with all neuroimaging markers. After correction for age, serum NfL levels at baseline correlated independently with lacune count, brain atrophy and to a lesser extent with microbleed count, but not with WMH_V. These findings are in line with the recent study by Duering *et al.*, showing serum NfL correlates with all neuroimaging markers in CADASIL.[20]

Although WMH are widely present in patients with SVD, such as CADASIL, and seem to correlate to some extent with NfL in serum or CSF,[20,22,38] other neuroimaging markers, namely brain atrophy and lacunes, correlate more strongly with disease severity.[11–17] In line with this, mean diffusivity from diffusion tensor imaging (DTI) was shown to be most strongly associated with serum NfL levels in CADASIL.[20] Together with our finding that NfL independently correlates with lacunes and brain atrophy, this indicates that serum NfL reflects structural axonal damage, irrespective of the cause, and probably integrates the effect of lacunar infarcts and brain atrophy in a single measure, in a similar way as mean diffusivity does. This may implicate that serum NfL may be a suitable biomarker for manifest CADASIL, but not for the pre-manifest stages of CADASIL *i.e.* when patients only have WMH. However, this needs to be further clarified in larger cohorts of pre-manifest patients.

In agreement with the study by Duering *et al.*, serum NfL levels correlated with disease severity as reflected by disability scores, global cognitive function and executive function. Global cognitive function correlated more strongly with serum NfL levels than with lacune count or BPF, which have been considered to be the best predictors of cognitive function and cognitive decline in CADASIL to date.[11–17] Taken together, the correlation of serum NfL levels with MRI lesion load, cognition and disability, suggests that serum NfL may serve as a feasible fluid biomarker to facilitate assessment of CADASIL disease severity in a single measure at a given time-point.

Moreover, baseline serum NfL predicted seven-year deterioration in disability (mRS), global cognitive function (CAMCOG), and executive function (TMT-B), but did not correlate with seven-year progression in MRI lesion load. Previous studies have shown that brain atrophy and lacunes predict disease progression in CADASIL,[11–17] and both of these strongly

correlate with serum NfL levels. Here, the lack of correlation between NfL and progressive MRI lesions at follow-up is likely explained by the loss to follow up of more severely affected patients. Therefore, patients who were pre-manifest or mildly affected at baseline, were over-represented at seven year follow-up. Indeed, when only including in the analysis those patients who had disability at baseline, serum NfL did correlate with an increase in MRI lesion load (lacune count).

A general weakness of this study is that it was not originally designed for the purpose of a longitudinal study of serum biomarkers, and in this regard the cohort is relatively small.

In several studies, serum NfL has been shown to predict survival in neurodegenerative disease, but not yet in SVD.[25,39,40] Here, we show that NfL also predicts survival in CADASIL, a pure model of SVD. However, as the follow-up period was relatively long and our study lacks longitudinal assessment of serum NfL levels, we were not able to determine whether NfL levels can be used as a disease monitoring biomarker at shorter time intervals or as a potential marker for therapeutic response in future clinical trials. We do find that NfL correlates strongly with lacunes and BPF, which have both been shown to significantly change in a three year timeframe.[14,17] This suggests that serum NfL levels may also show significant changes at shorter intervals in manifest patients with progressive disease.

Like Duering *et al.*, we used the ultra-sensitive single-molecule array (Simoa) for NfL measurements into the pg/mL range at high precision and sensitivity, which outperforms the conventional ELISA and chemiluminescence-based methods.[20,36] Serum NfL levels were lower for both patients and controls in our study. A single-measure serum biomarker such as NfL clearly has many advantages over more complex measures such as MRI markers or neuropsychological testing, which are currently used to assess CADASIL disease severity and predict disease progression.

In conclusion, we validate the recent finding that serum NfL levels correlate cross-sectionally with relevant measures of disease severity in an independent CADASIL sample. Furthermore, we show that serum NfL is predictive of seven year deterioration in cognition and disability, and is associated with 17-year survival. These findings suggest that serum NfL may be useful marker to monitor and predict disease course in this variable disorder, as well as potentially providing a feasible marker for therapeutic intervention in future clinical trials for CADASIL.

Appendix

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Contribution

GG, JR and SLO conceived and designed the study. ML and JG performed the MRI studies. IV performed the serum NfL quantification. GG, JR, SB and SLO analysed the data. GG, JR and SLO drafted the manuscript. All authors reviewed the manuscript.

Disclosures

We report no relevant conflict of interest. GG and JW reports grants from The Netherlands Brain Foundation during the conduct of the study. SLO reports grants from The Netherlands Brain Foundation, during the conduct of the study; grants from VIDI ZonMW, outside the submitted work. In addition, SLO has a patent Means and methods for modulating NOTCH3 protein expression and/or the coding region of NOTCH3 licensed to LUMC. IV reports a grant from Health~Holland via Alzheimer Nederland to support a research collaboration with Crossbeta Biosciences. Non-financial support in the form of research consumables was received from Crossbeta Biosciences for this same project, outside the submitted work. CT reports personal fees from advisory board of Fujirebio and Roche, non-financial support from research consumables from ADxNeurosciences, other from performed contract research or received grants from Janssen prevention center, Boehringer, EIP farma, Roche and Probiodrug, PeopleBio, Charles River, outside the submitted work. AAR reports grants from Netherlands Brain Foundation, during the conduct of the study; grants from EU, grants from ZonMw (Dutch Government), grants from Duchenne Parent Project, grants from Prinses Beatrix Spierfonds, outside the submitted work. In addition, AAR has a patent issued. JG and SB have nothing to disclose.

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