BRAIN COMMUNICATIONS

Serum neurofilament light in atrial fibrillation: clinical, neuroimaging and cognitive correlates

ⓘ Alexandros A Polymeris,[1] Michael Coslovksy,[2,3] Stefanie Aeschbacher,[3,4] Tim Sinnecker,[1,5] Pascal Benkert,[2] Richard Kobza,[6] Jürg Beer,[7,8] Nicolas Rodondi,[9,10] Urs Fischer,[11] Giorgio Moschovitis,[12] Andreas U Monsch,[13] Anne Springer,[3,4] Matthias Schwenkglenks,[14,15] Jens Wuerfel,[5] Gian Marco De Marchis,[1] Philippe A Lyrer,[1] Michael Kühne,[3,4] Stefan Osswald,[3,4] David Conen,[4,16] Jens Kuhle,[1,]* ⓘ Leo H Bonati[1,]* and for the Swiss-AF Investigators

*These authors contributed equally to this work.

Emerging evidence suggests that atrial fibrillation is associated with cognitive dysfunction independently of stroke, but the underlying mechanisms remain unclear. In this cross-sectional analysis from the Swiss-atrial fibrillation Study (NCT02105844), we investigated the association of serum neurofilament light protein, a neuronal injury biomarker, with (i) the CHA_2DS_2-VASc score (congestive heart failure, hypertension, age 65–74 or >75 years, diabetes mellitus, stroke or transient ischaemic attack, vascular disease, sex), clinical and neuroimaging parameters and (ii) cognitive measures in atrial fibrillation patients. We measured neurofilament light in serum using an ultrasensitive single-molecule array assay in a sample of 1379 atrial fibrillation patients (mean age, 72 years; female, 27%). Ischaemic infarcts, small vessel disease markers and normalized brain volume were assessed on brain MRI. Cognitive testing included the Montreal cognitive assessment, trail-making test, semantic verbal fluency and digit symbol substitution test, which were summarized using principal component analysis. Results were analysed using univariable and multivariable linear regression. Neurofilament light was associated with the CHA_2DS_2-VASc score, with an average 19.2% [95% confidence interval (17.2%, 21.3%)] higher neurofilament per unit CHA_2DS_2-VASc increase. This association persisted after adjustment for age and MRI characteristics. In multivariable analyses, clinical parameters associated with neurofilament light were higher age [32.5% (27.2%, 38%) neurofilament increase per 10 years], diabetes mellitus, heart failure and peripheral artery disease [26.8% (16.8%, 37.6%), 15.7% (8.1%, 23.9%) and 19.5% (6.8%, 33.7%) higher neurofilament, respectively]. Mean arterial pressure showed a curvilinear association with neurofilament, with evidence for both an inverse linear and a U-shaped association. MRI characteristics associated with neurofilament were white matter lesion volume and volume of large non-cortical or cortical infarcts [4.3% (1.8%, 6.8%) and 5.5% (2.5%, 8.7%) neurofilament increase per unit increase in log-volume of the respective lesion], as well as normalized brain volume [4.9% (1.7%, 8.1%) higher neurofilament per $100\,cm^3$ smaller brain volume]. Neurofilament light was inversely associated with all cognitive measures in univariable analyses. The effect sizes diminished after adjusting for clinical and MRI variables, but the association with the first principal component was still evident. Our results suggest that in atrial fibrillation patients, neuronal loss measured by serum neurofilament light is associated with age, diabetes mellitus, heart failure, blood pressure and vascular brain lesions, and inversely correlates with normalized brain volume and cognitive function.

1 Department of Neurology and Stroke Center, University Hospital Basel and University of Basel, Basel, Switzerland
2 Clinical Trial Unit, Department of Clinical Research, University Hospital Basel and University of Basel, Switzerland
3 Cardiovascular Research Institute Basel (CRIB), Basel, Switzerland
4 Cardiology Division, Department of Medicine, University Hospital Basel, Basel, Switzerland
5 Medical Image Analysis Center (MIAC) AG and Department of Biomedical Engineering, University of Basel, Basel, Switzerland
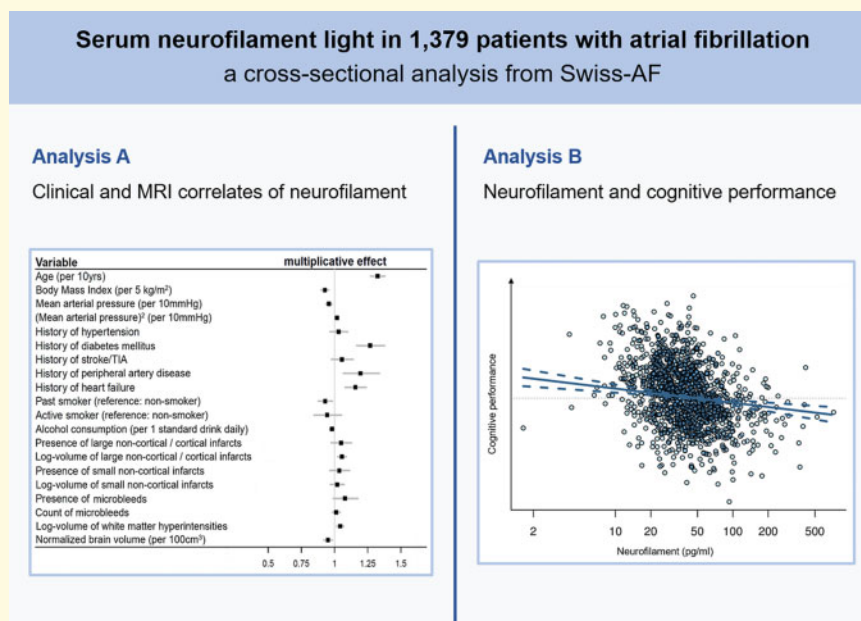6 Department of Cardiology, Cantonal Hospital Lucerne, Lucerne, Switzerland

7   Department of Medicine, Cantonal Hospital Baden, Baden, Switzerland
8   Molecular Cardiology, University Hospital Zurich, Zurich, Switzerland
9   Institute of Primary Health Care (BIHAM), University of Bern, Bern, Switzerland
10   Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland
11   Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland
12   Cardiology Division, Department of Medicine, EOC Ospedale Regionale di Lugano, Lugano, Switzerland
13   Memory Clinic, University Department of Geriatric Medicine Felix Platter and University of Basel, Basel, Switzerland
14   Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, Switzerland
15   Institute of Pharmaceutical Medicine (ECPM), University of Basel, Basel, Switzerland
16   Population Health Research Institute, McMaster University, Hamilton, Canada

Correspondence to: Leo H. Bonati, Department of Neurology and Stroke Center, University Hospital
Basel and University of Basel, Petersgraben 4, 4031 Basel, Switzerland
E-mail: Leo.Bonati@usb.ch

Graphical Abstract

Serum neurofilament light in 1,379 patients with atrial fibrillation
a cross-sectional analysis from Swiss-AF

Analysis A
Clinical and MRI correlates of neurofilament

Analysis B
Neurofilament and cognitive performance

Introduction

Atrial fibrillation (AF) and dementia are highly prevalent in the elderly. Atrial fibrillation is linked to dementia through ischaemic stroke, but evidence has emerged that even in the absence of clinically manifest stroke, the risk of cognitive impairment and dementia is increased in patients with AF (Chen et al., 2018; Madhavan et al., 2018; Kim et al., 2019). Several potential mechanisms have been postulated to explain this association,

including silent cerebral infarcts, cerebral small vessel disease (through shared risk factors such as diabetes and hypertension) and cerebral hypoperfusion (Madhavan et al., 2018; Diener et al., 2019), but tangible evidence is lacking. With the progressive ageing of the population, AF and dementia are a continuously growing public health concern, and a deeper understanding of the pathophysiological pathways underlying their association will be crucial in developing strategies to preserve cognitive function in the elderly (Kuhne et al., 2019).

In a cross-sectional analysis from the Swiss Atrial Fibrillation (Swiss-AF) cohort study, we previously showed that cortical and large non-cortical infarcts were common in AF patients and were independently associated with a lower score on the Montreal Cognitive Assessment (MoCA), lending support to the hypothesis that cognitive dysfunction in AF might—at least in part—be mediated through covert cerebral embolic infarcts (Conen et al., 2019).

Here, we used serum neurofilament light protein (sNfL) to further explore the mechanisms that underly neuronal damage and cognitive dysfunction in AF. Neurofilaments are neuron-exclusive cytoskeletal proteins that are released in the extracellular space, cerebrospinal fluid and eventually peripheral blood after neuroaxonal damage. sNfL has emerged as a biomarker for neuronal injury in inflammatory, degenerative, traumatic and vascular neurological disorders (Khalil et al., 2018), but has not yet been investigated as a marker of neurological disease in AF. In this analysis from the Swiss-AF cohort study, we investigated the association of sNfL with (i) clinical parameters and neuroimaging characteristics and (ii) measures of cognitive function.

Materials and methods

Study design, patient population and data collection

This was a cross-sectional analysis using baseline data from the ongoing prospective observational Swiss-AF cohort study (NCT02105844) that enrolled 2415 patients with AF between 2014 and 2017 across 14 centres in Switzerland. The detailed methodology of Swiss-AF has been described previously (Conen et al., 2017, 2019). In short, Swiss-AF included patients with documented AF aged 65 years or older, with an additional 15% of patients aged between 45 and 65 years. Patients with secondary forms of AF, those with a recent ischaemic stroke, transient ischaemic attack (TIA) or other acute illness (<4 weeks) and those unable to provide informed consent (e.g. patients with dementia, psychosis or delirium) were excluded. Baseline information on sociodemographic parameters and comorbidities was collected based on patients' history and/or medical chart review as applicable, using standardized case report forms. Upon inclusion, weight, height and the mean of three consecutive blood pressure measurements were obtained, and patients underwent blood sampling, brain MRI and cognitive testing.

Baseline blood samples were collected following standard operating procedures (Conen et al., 2017). After centrifugation, serum samples were aliquoted into cryotubes and stored at $-80°C$ in a centralized biobank. The concentrations of sNfL were measured in duplicate using a previously described ultrasensitive single-molecule array assay (Disanto et al., 2017). Inter-assay coefficients of variation were 10% for low (mean, 6.9 pg/mL), 12% for medium (mean, 19.6 pg/mL) and 5% for high (mean, 84.5 pg/mL) concentration quality control serum samples measured in duplicate in every run. The mean intra-assay coefficient of variation of duplicate determinations for concentration was 5%. Individuals performing sNfL measurements were blinded to clinical, MRI and cognitive patient data.

Baseline brain MRI was acquired on a 1.5 or 3.0 Tesla scanner using a standardized protocol including a 3D T1-weighted magnetization-prepared rapid gradient echo (MPRAGE), a 2D axial fluid-attenuated inversion recovery (FLAIR), a 2D axial diffusion-weighted imaging (DWI) and a 2D axial susceptibility-weighted imaging (SWI) or T2*-weighted sequence (Conen et al., 2017, 2019). All scans were analysed centrally in a core lab (Medical Image Analysis Center AG, Basel, Switzerland) by expert raters blinded to clinical and cognitive patients' data and measurements of sNfL. We evaluated the following vascular brain lesions, which we defined adapting the *Standards for reporting vascular changes on neuroimaging* classification of small vessel disease (Wardlaw et al., 2013), as in previous research (Conen et al., 2019): (i) small non-cortical infarcts (SNCIs), defined as hyperintense lesions on FLAIR, ≤ 20 mm in diameter on axial sections and not involving the cortex, consistent with ischaemic infarction in the territory of a perforating arteriole and located in the white matter, internal or external capsule, deep brain nuclei, thalamus or brainstem. (ii) Large non-cortical infarcts were non-cortical infarcts with a diameter of >20 mm. Cortical infarcts were defined as FLAIR hyperintense lesions involving the cortex irrespective of their size and whether they also involved subcortical areas. Large non-cortical and cortical infarcts (LNCCIs) were grouped together in the analyses. All infarcts were characterized as recent (hyperintense) or chronic according to their appearance on DWI. (iii) FLAIR hyperintensities not meeting the aforementioned criteria for infarcts were identified as white matter lesions (WMLs). (iv) Microbleeds (MBs) were identified and counted as nodular, strongly hypointense lesions on either SWI or T2*-weighted sequences. T2-weighted volumes of SNCIs, LNCCIs and WMLs were segmented and quantified semi-automatically in mm^3 using Amira (Mercury Computer Systems Inc., Chelmsford, MA, USA). Lesions with a central FLAIR hypointense core were segmented in total without differentiating between hyperintense and hypointense lesion areas. The normalized brain volume (nBV) was estimated in cm^3 on MPRAGE using SIENAX (Smith et al., 2002).

Cognitive testing was performed by trained study personnel in a standardized manner and included:

i. The MoCA, which assesses visuospatial and executive functions, confrontation naming, memory, attention, language and abstraction. Patients could obtain a

maximum of 30 points, with higher scores indicating better cognitive function. One point was added to the test score if the patient had ≤12 years of formal education (Nasreddine *et al.*, 2005).

ii. The Trail Making test (TMT), which assesses visual attention, processing speed and executive functioning. It consists of two parts (A and B), in which the patient was instructed to connect a set of 25 points, either circled numbers in ascending order (TMT-A) or circled numbers and letters in alternating numeric and alphabetic ascending order (TMT-B), as quickly as possible while maintaining accuracy. The number of correct connections and the time to test completion in seconds were measured, with a maximum allowed time of 180 and 300 s for TMT-A and TMT-B, respectively. The test metric was the number of correct answers per second, with higher scores indicating better cognitive function (Tombaugh, 2004).

iii. Semantic Verbal Fluency (SVF), which assesses semantic memory and language production. Patients were asked to name as many words as possible from the semantic category 'animals' within 60 s. The test metric was the number or correct responses, with higher scores indicating better cognitive function (Morris *et al.*, 1989).

iv. The Digit Symbol Substitution Test (DSST) of the Wechsler Adult Intelligence Scale, which assesses processing speed, visuomotor coordination and attention. Patients received a key grid of numbers and matching symbols and a test section with numbers and empty boxes. The test consisted of filling as many empty boxes as possible with the matching symbol from the key grid. The score was the number of correct number–symbol matches achieved within 120 s, with higher scores indicating better cognitive function (Petermann, 2011).

The detailed patients' flowchart for the analyses of this study is shown in Supplementary Fig. 1. We included all Swiss-AF patients with quantifiable sNfL measurement and complete MRI data and excluded those with recent subclinical ischaemic infarcts on DWI (which would expectedly raise the concentrations of sNfL disproportionately (Gattringer *et al.*, 2017; De Marchis *et al.*, 2018; Tiedt *et al.*, 2018)). The Ethics Committee of Northwest and Central Switzerland approved Swiss-AF including this study (PB_2016-00793). Written informed consent was obtained from all study participants according to the Declaration of Helsinki. This study was conducted in accordance with the STROBE Statement for cross-sectional studies (von Elm *et al.*, 2007).

Statistical analyses

Analysis A: association of the CHA_2DS_2-VASc score, clinical and MRI characteristics with sNfL

To investigate the association of patients' characteristics with sNfL, we fitted uni- and multivariable linear regression models with various sets of clinical and MRI variables as independent variables and the log-transformed sNfL concentration as dependent variable. Continuous independent variables were centred on their mean (or, in case of skewed data, median) values. We report the back-transformed model-based estimates, which represent multiplicative effects on the geometric mean of sNfL and are denoted by β_{mult} (so that a one-unit increase in the independent variable is associated with an average β_{mult}-fold change in sNfL), along with 95% confidence intervals (CI) and two-sided *P*-values. We interpret *P*-values as a continuous measure, with smaller values indicating stronger evidence for an association, but without specifying a threshold value. To compare between models, we used the Akaike's information criterion (AIC), which estimates the relative quality of different models fitted to a given dataset, while penalizing models for larger number of independent variables. Lower AIC values indicate a better fit. Additionally, we provide the coefficient of determination (R^2) of each model as a measure of the proportion of the observed sNfL variance explained by the model. Since R^2 tends to increase with the number of independent variables, we also provide the adjusted R^2 (R^2_{adj}), which penalizes R^2 for larger numbers of variables. We fitted the following predefined models with log-sNfL as the dependent variable:

i. The CHA_2DS_2-VASc score (congestive heart failure, hypertension, age 65–74 or ≥75 years, diabetes mellitus, stroke or TIA, vascular disease, sex) models: CHA_2DS_2-VASc is a validated clinical score predicting stroke risk in AF patients (Lip *et al.*, 2010; Friberg *et al.*, 2012). We opted to first investigate the association of sNfL with this risk score as a whole, independent of its individual components. Given the known association of sNfL with age (Khalil *et al.*, 2018), we fitted univariable models for the association of sNfL with age, with the CHA_2DS_2-VASc score and with the CHA_2DS_2-VASc score after exclusion of its age component. Additionally, we fitted bivariable models for the age-adjusted association of sNfL with the CHA_2DS_2-VASc score and with the CHA_2DS_2-VASc score after exclusion of its age component. We selected the best fitting CHA_2DS_2-VASc score model based on AIC values, and proceeded to further adjust it for MRI markers of small vessel disease (SNCIs, MBs and WMLs) (Wardlaw *et al.*, 2013) as well as for all MRI variables, as detailed below, in two additional multivariable models.

ii. The clinical model: We fitted a multivariable model for the association of sNfL with the following predefined clinical variables: age, sex, history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, peripheral artery disease (PAD), heart failure, obstructive sleep apnoea, AF type (paroxysmal, persistent and permanent), body mass index (BMI, calculated as weight in kg/height in m^2), smoking status (active, past, non-smoker), alcohol consumption (number of standard drinks daily) and mean arterial pressure [MAP,

calculated as $1/3 \times$ systolic blood pressure $+ 2/3 \times$ diastolic blood pressure (DeMers and Wachs, 2019)]. We opted to use the MAP instead of including both systolic and diastolic blood pressure in the models due to collinearity between those variables. There was no evidence of collinearity upon visual inspection of scatter plots for any of the other continuous clinical variables, but there was evidence for a curvilinear association between sNfL and MAP, which we modelled by introducing an additional quadratic term (MAP^2). We reduced the clinical model to a smaller set of variables via stepwise backward elimination based on AIC values. We imputed the few missing values in the clinical variables with simple single imputation, using the mode (i.e., the most common category) for categorical variables and the mean (or, in case of skewed data, the median) for continuous variables (Table 1; one missing value in smoking status and alcohol consumption, imputed with 'past' and 0.6 standard drinks daily, respectively; six missing values in systolic and diastolic blood pressure, imputed with 134.7 and 78.4 mmHg, respectively).

iii. The MRI model: We fitted a multivariable model for the age-adjusted association of sNfL with the following predefined MRI variables: nBV, SNCIs' presence and log-transformed volume, LNCCIs' presence and log-volume, MBs' presence and count (truncated at 20 to reduce the influence of outliers) and WMLs' log-volume.

iv. The combined clinical and MRI model: We fitted a final combined model for the association of sNfL with the chosen clinical and all MRI variables from the models ii and iii.

Analysis B: association of sNfL with measures of cognitive function

To investigate the association of sNfL with cognitive function, we fitted linear regression models with the score of each of the cognitive tests (MoCA, TMT-A, TMT-B, SVF and DSST) as the dependent variable and the log-transformed sNfL concentration as independent variable. We report the model-based estimates, which represent additive effects on the mean of the test score and are denoted by β, along with the 95% CI and two-sided P-values. A one-unit increase in log-sNfL is associated with an average change in the test score of β units (or a 10% increase in sNfL is associated with a change of $0.095 \times \beta$ units in the test score). For each cognitive test, we fitted the following predefined models with test score as the dependent variable:

i. univariable model (including only log-sNfL as independent variable);

ii. age-adjusted model (including log-sNfL and age as independent variables);

iii. clinical multivariable model, including log-sNfL, age, sex, education level (basic, middle and advanced), history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, PAD, heart failure, obstructive sleep apnoea, BMI, smoking status (active, past, non-smoker) and alcohol consumption (number of standard drinks daily) as independent variables. We imputed the few missing values in the clinical variables with simple single imputation, as described above.

iv. MRI multivariable model, including log-sNfL, age, nBV, SNCIs' presence and log-volume, LNCCIs' presence and log-volume, burden of MBs (categorized as 0, 1, 2 and ≥ 3) and WMLs' log-volume as independent variables;

v. combined clinical and MRI multivariable model, including all aforementioned variables.

To summarize performance over all cognitive tests, we used principal component analysis. The first principal component (PC1) explained 61.3% of the observed variance and the loading of each test on PC1 (representing the covariance between each test and PC1) was positive (MoCA: +0.40, TMT-A: +0.45, TMT-B: +0.50, SVF: +0.39, DSST: +0.49), thereby allowing for the use of PC1 as a single, summary measure of cognitive function, with higher values indicating better cognitive performance. We additionally fitted all the above-described models i–v with PC1 as the dependent variable.

As a sensitivity analysis, we repeated all models described under analyses A and B in the subset of patients without history of stroke or TIA.

All analyses were performed with R version 3.5.2 (2018-12-20).

Data availability

The Swiss-AF consent forms, as approved by the ethics committee, do not allow for the data to be made publicly available. Researchers may contact the authors for the potential submission of research proposals for future analyses or independent verification of our results.

Results

A total of 1379 patients [mean (SD) age, 72.3 (8.6) years, 27.1% female] with quantifiable sNfL measurement and complete MRI data were available for analysis A (Supplementary Fig. 1). The median (IQR) sNfL concentration was 38.2 (26.6–56.4) pg/ml. The detailed demographic, clinical and MRI characteristics of all patients are summarized in Table 1.

Association of the CHA$_2$DS$_2$-VASc score with sNfL

The CHA$_2$DS$_2$-VASc score was associated with sNfL in univariable analysis, with an average 19.2% increase in sNfL concentration per point increase in the CHA$_2$DS$_2$-VASc score [$\beta_{mult} = 1.192$, 95% CI (1.172, 1.213), $P < 0.001$; Fig. 1]. Age was also strongly associated with sNfL in univariable analysis [$\beta_{mult} = 1.489$ per 10 years, 95% CI (1.440, 1.539), $P < 0.001$]. The association

Table 1 Patient demographic, clinical and MRI characteristics

| Demographic and clinical data	All patients (N = 1379)	Missing values rate (%)	Patients without stroke/TIA (N = 1125)
Age, years, mean (SD)	72.3 (8.6)	0	71.7 (8.8)
Sex, female, N (%)	374 (27.1)	0	297 (26.4)
AF type, *N* (%)		0	
Paroxysmal	636 (46.1)		510 (45.3)
Persistent	408 (29.6)		349 (31.0)
Permanent	335 (24.3)		266 (23.6)
History of			
Hypertension, *N* (%)	930 (67.4)	0	741 (65.9)
Diabetes mellitus, *N* (%)	190 (13.8)	0	146 (13.0)
Stroke or transient ischaemic attack, *N* (%)	254 (18.4)	0	0 (0)
Coronary heart disease, *N* (%)	364 (26.4)	0	292 (26.0)
Peripheral artery disease, *N* (%)	87 (6.3)	0	64 (5.7)
Heart failure, *N* (%)	297 (21.5)	0	238 (21.2)
Obstructive sleep apnoea, *N* (%)	171 (12.4)	0	128 (11.4)
CHA_2DS_2-VASc score, median (IQR)	3 (2–4)	0	3 (2–4)
Smoking status, *N* (%)		0.1	
Non-smoker	603 (43.8)		488 (43.4)
Past smoker	671 (48.7)		553 (49.2)
Active smoker	104 (7.5)		84 (7.5)
Alcohol consumption, standard drinks/day, median (IQR)	0.6 (0.1–1.3)	0.1	0.6 (0.1–1.3)
Education level, *N* (%)		0.1	
Basic	157 (11.4)		128 (11.4)
Middle	679 (49.3)		555 (49.3)
Advanced	541 (39.3)		442 (39.3)
Body mass index (kg/m^2), mean (SD)	27.5 (4.6)	0	27.6 (4.7)
Systolic blood pressure (mmHg) mean (SD)	134.7 (18.7)	0.4	134.7 (18.6)
Diastolic blood pressure (mmHg) mean (SD)	78.4 (11.9)	0.4	78.7 (11.9)
Mean arterial pressure (mmHg) mean (SD)	97.2 (12.6)	0.4	97.3 (12.7)
Oral anticoagulation, *N* (%)	1240 (89.9)	0	1,004 (89.2)
MRI data			
Small non-cortical infarcts, *N* (%)	293 (21.2)	0	200 (17.8)
Volume (if present) (mm^3), median (IQR)	60 (30–150)		56 (30–123)
Large non-cortical and cortical infarcts, *N* (%)	288 (20.9)	0	153 (13.6)
Volume (if present) (mm^3), median (IQR)	1374 (252–7454)		585 (162–4002)
White matter lesions, *N* (%)	1368 (99.2)	0	1116 (99.2)
Volume (if present) (mm^3) median (IQR)	3662 (1350–9197)		3335 (1224–8252)
Microbleeds, *N* (%)	291 (21.1)	0	220 (19.6)
Count (if present), median (IQR)	1 (1–2)		1 (1–2)
Normalized brain volume (cm^3), median (IQR)	1411 (1354–1478)	0	1417 (1358–1487)

SD, standard deviation; IQR, interquartile range.

between the CHA_2DS_2-VASc score and sNfL persisted after excluding the age component of the score, after adjusting for age and after both excluding the age component and adjusting for age. The model with the best fit was the one including the unmodified CHA_2DS_2-VASc score and adjusting for age, which was used in the rest of the analyses. The CHA_2DS_2-VASc score remained associated with sNfL after adjusting for MRI markers of small vessel disease and for all MRI variables combined (Supplementary Table 1).

Association of clinical and MRI characteristics with sNfL

The detailed results of the clinical, MRI and combined models are summarized in Table 2. The clinical model fitted the data better and explained a larger proportion of the observed sNfL variance compared to the MRI model. The combined clinical and MRI model fitted the data best. Figure 2 shows the effect size estimates for the association of all clinical and MRI variables with sNfL from the combined model: Parameters positively associated with sNfL were age (on average 32.5% higher sNfL per 10 years), history of diabetes mellitus (26.8% higher sNfL), PAD (19.5% higher sNfL) and heart failure (15.7% higher sNfL), as well as volume of LNCCIs and WMLs (5.5% and 4.3% higher sNfL per unit increase in log-volume of the respective lesion). Mean arterial pressure showed a curvilinear association with sNfL, with an inverse linear and U-shaped component (Fig. 3A). Parameters inversely associated with sNfL were BMI (7.3% lower sNfL per 5 kg/m^2 higher BMI), past smoker
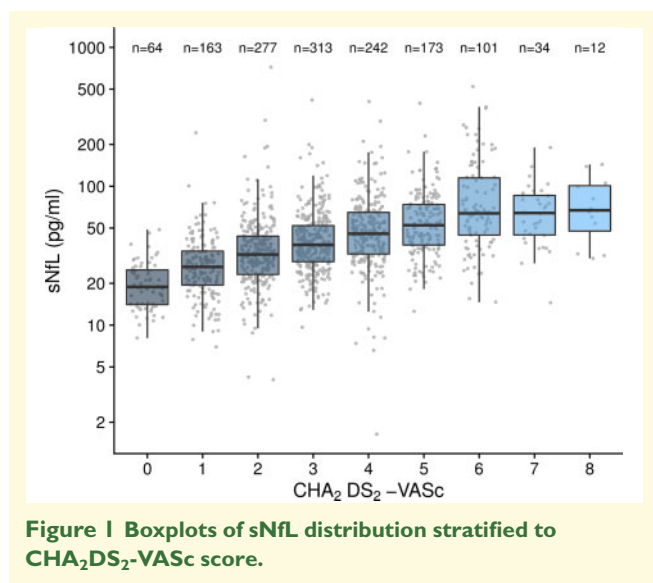
Figure 1 Boxplots of sNfL distribution stratified to CHA₂DS₂-VASc score.

status (7% lower sNfL compared to non-smoker), alcohol consumption (1.9% lower sNfL per 1 standard drink daily) and nBV (4.9% lower sNfL per 100 cm³ larger nBV; Fig. 3B).

Association of sNfL with measures of cognitive function

Of the 1379 patients with sNfL and MRI data, cognitive testing was incomplete in 16, leaving 1363 patients available for analysis B. The median (IQR) MoCA score was 26 (24–28) points, TMT-A and TMT-B scores were 0.53 (0.39–0.68) and 0.21 (0.14–0.28) correct connections per second, respectively, SVF score was 19 (15–23) correct responses and DSST score was 45 (36–54) correct matches. The detailed results of all models for the association of sNfL with all cognitive measures are summarized in Table 3. In univariable analyses, log-sNfL was strongly associated with all cognitive tests and the PC1, with higher sNfL concentrations indicating worse cognitive performance. The effect sizes generally diminished for all cognitive measures after adjusting for age, clinical variables and MRI characteristics. In the combined models adjusting for all aforementioned parameters, the association of log-sNfL with TMT-A, TMT-B and PC1 persisted. Figure 4 shows the model-based estimates for the association of sNfL with PC1. The scatter plot of the association of sNfL with PC1 is shown in Fig. 5.

Sensitivity analyses in patients without history of stroke or transient ischaemic attack

After excluding those with history of stroke or TIA, 1125 patients were available for sensitivity analysis A. Their median (IQR) sNfL concentration was 36.7 (25.5–

53.2) pg/ml and their detailed demographic, clinical and MRI characteristics are summarized in Table 1. Of those, 12 patients had incomplete cognitive testing, leaving 1113 patients available for the sensitivity analysis B. Both sensitivity analyses in patients without history of stroke or TIA yielded consistent results with the main analysis (Supplementary Tables 2–4).

Discussion

This cross-sectional study on the clinical, neuroimaging and cognitive correlates of sNfL in a large sample of AF patients showed the following key findings: (i) Higher CHA₂DS₂-VASc scores indicated increasing neuronal injury, independent of age and vascular brain lesions visible on MRI. (ii) Besides age, clinical factors associated with increased neuronal loss were diabetes mellitus, PAD, heart failure and lower MAP. (iii) MRI characteristics associated with higher sNfL were higher volume of WMLs and LNCCIs, as well as lower nBV. (iv) sNfL was associated with worse cognitive performance, an association which was largely but not exclusively explained by age, comorbidities and vascular brain lesions.

The CHA₂DS₂-VASc score, a validated clinical score predicting ischaemic stroke risk in AF patients (Lip *et al.*, 2010; Friberg *et al.*, 2012), was associated with sNfL. This was independent of age, history of stroke and ischaemic infarcts visible on MRI, as well as MRI markers of small vessel disease, and might therefore reflect ongoing ischaemic brain injury in AF that evades detection on conventional MRI (Brundel *et al.*, 2012). Higher CHA₂DS₂-VASc scores have also been previously associated with an increasing risk for dementia in stroke-free AF patients (Kim *et al.*, 2019).

Our study in AF patients confirms the strong independent association of sNfL with age, which has been demonstrated across a wide variety of patient populations and healthy controls, probably reflecting neurodegenerative processes associated with normal ageing (Khalil *et al.*, 2018, 2020). Furthermore, we found that diabetes mellitus was associated with a sNfL increase by a similar magnitude as 10 years of age. In line with this, poor glycemic control was independently associated with sNfL in a previous study on sNfL among diabetics (Korley *et al.*, 2019). The association of diabetes mellitus with sNfL was independent of small vessel disease markers and potentially embolic infarcts on MRI. It remains, therefore, unknown whether this association reflects increasing ischaemic neuronal injury in the presence of diabetes mellitus due to micro-infarcts that go undetected on conventional MRI (Brundel *et al.*, 2012), some other non-ischaemic, diabetes-induced mechanism of neuronal damage in the central nervous system (Malone, 2016) or the potential contribution of diabetic neuropathy in the peripheral nervous system (Mariotto *et al.*, 2018). Peripheral artery disease was another independent

Table 2 Association of patients' clinical and MRI characteristics with sNfL

Variables (N = 1379)	Clinical model[a] AIC = 2079.05 $R^2 = 0.36$, $R^2_{adj} = 0.36$		MRI model AIC = 2148.29 $R^2 = 0.33$, $R^2_{adj} = 0.32$		Combined model AIC = 2040.56 $R^2 = 0.39$, $R^2_{adj} = 0.38$	
	β_{mult}[b] (95% CI)	P-value	β_{mult}[b] (95% CI)	P-value	β_{mult}[b] (95% CI)	P-value
Age (per 10 years)	1.411 (1.365, 1.460)	<0.001	1.367 (1.312, 1.424)	<0.001	1.325 (1.272, 1.380)	<0.001
BMI (per 5 kg/m²)	0.925 (0.897, 0.955)	<0.001			0.927 (0.899, 0.957)	<0.001
MAP (per 10 mmHg)	0.961 (0.939, 0.983)	<0.001			0.958 (0.937, 0.980)	<0.001
MAP² (per 10 mmHg)	1.019 (1.008, 1.031)	0.001			1.019 (1.007, 1.030)	0.002
History of hypertension	1.068 (1.003, 1.138)	0.042			1.030 (0.967, 1.098)	0.351
History of diabetes mellitus	1.283 (1.181, 1.394)	<.001			1.268 (1.168, 1.376)	<0.001
History of stroke or TIA	1.137 (1.059, 1.220)	<0.001			1.056 (0.978, 1.141)	0.166
History of peripheral artery disease	1.231 (1.098, 1.380)	<0.001			1.195 (1.068, 1.337)	0.002
History of heart failure	1.181 (1.102, 1.266)	<0.001			1.157 (1.081, 1.239)	<0.001
Past smoker (ref: non-smoker)	0.925 (0.873, 0.980)	0.008			0.930 (0.878, 0.984)	0.012
Active smoker (ref: non-smoker)	0.950 (0.850, 1.060)	0.358			0.944 (0.847, 1.053)	0.301
Alcohol consumption (per 1 standard drink daily)	0.984 (0.965, 1.002)	0.086			0.981 (0.963, 1.000)	0.045
Presence of LNCCIs			1.100 (1.025, 1.180)	0.008	1.049 (0.975, 1.128)	0.199
Log-volume of LNCCIs			1.066 (1.035, 1.099)	<0.001	1.055 (1.025, 1.087)	<0.001
Presence of SNCIs			1.055 (0.980, 1.136)	0.156	1.036 (0.965, 1.113)	0.330
Log-volume of SNCIs			1.030 (0.978, 1.085)	0.262	1.020 (0.970, 1.072)	0.442
Presence of MBs			1.104 (1.011, 1.207)	0.028	1.079 (0.991, 1.176)	0.079
Count of MBs			1.017 (0.987, 1.047)	0.266	1.013 (0.985, 1.042)	0.372
Log-volume of WMLs			1.045 (1.019, 1.071)	<0.001	1.043 (1.018, 1.068)	<0.001
nBV (per 100 cm³)			0.945 (0.914, 0.978)	0.001	0.951 (0.919, 0.983)	0.003

AIC, Akaike's information criterion; BMI, body mass index; MAP, mean arterial pressure; TIA, transient ischaemic attack; LNCCIs, large non-cortical or cortical infarcts; SNCIs, small non-cortical infarcts; MBs, micro-bleeds; WMLs, white-matter lesions; nBV, normalized brain volume.
[a]Sex, atrial fibrillation type, history of coronary heart disease and obstructive sleep apnoea were eliminated from the final, reduced clinical model.
[b]The back-transformed model-based estimates β_{mult} represent multiplicative effects on sNfL (e.g. $\beta_{mult} = 1.325$ for age denotes an average 1.325-fold increase in sNfL concentration, that is an average 32.5% sNfL increase, per 10 years older age).

determinant of sNfL, even after adjustment for vascular MRI brain lesions, which might again reflect increasing ischaemic brain injury that evades detection on conventional MRI (Brundel *et al.*, 2012) in the presence of manifest atherosclerotic disease and is in line with cumulating evidence for the association of PAD with cognitive dysfunction independently of manifest cerebrovascular disease (Rafnsson *et al.*, 2009).

Interestingly, we found a curvilinear association of MAP with sNfL, with evidence for both an inverse linear and a U-shaped relationship, indicating increasing neuronal loss with lower MAP. This novel finding is in contrast to a previous smaller study in diabetics, which found a positive linear association of systolic blood pressure and no association of diastolic blood pressure with sNfL (Korley *et al.*, 2019). As MAP is a measure of the organ perfusion pressure (DeMers and Wachs, 2019), our finding suggests that neuronal damage in AF may be partly attributable to cerebral hypoperfusion. This was independent of history of heart failure, which was another independent determinant of sNfL, suggesting that hemodynamic changes in AF might adversely affect brain health above and beyond clinically manifest heart failure. Taken together, these findings refine and further support the hypoperfusion hypothesis for the association of AF with cognitive dysfunction (Madhavan *et al.*, 2018;

Diener *et al.*, 2019), which has been proposed based on the known associations of cerebral hypoperfusion with dementia (Austin *et al.*, 2011; Wolters *et al.*, 2017; Iadecola *et al.*, 2019), AF with cerebral hypoperfusion (Lavy *et al.*, 1980; Gardarsdottir *et al.*, 2018), and heart failure with cerebral hypoperfusion (Roy *et al.*, 2017) and cognitive dysfunction (Vogels *et al.*, 2007). Of note, recent studies in healthy individuals provide evidence for a U-shaped association of blood pressure with cognitive dysfunction (Lv *et al.*, 2017) and for an inverse association of diastolic blood pressure with white matter disease (Fuhrmann *et al.*, 2019) and cognitive decline (Levine *et al.*, 2019). Putting our findings in this context, the curvilinear association of MAP with sNfL that we observed might not be specific to AF, but rather reflect a universal effect of blood pressure on brain health.

Of all vascular MRI brain lesions, the volume of WMLs and chronic LNCCIs were the strongest independent determinants of sNfL, also after excluding patients with stroke history. This indicates that both small vessel disease and infarcts of potentially embolic origin, even clinically silent ones, contribute to neuronal injury in AF. Our finding is in line with previous research on the association of sNfL with the burden of small vessel disease (Gattringer *et al.*, 2017; Duering *et al.*, 2018; Uphaus *et al.*, 2019) and the size of acute ischaemic infarcts
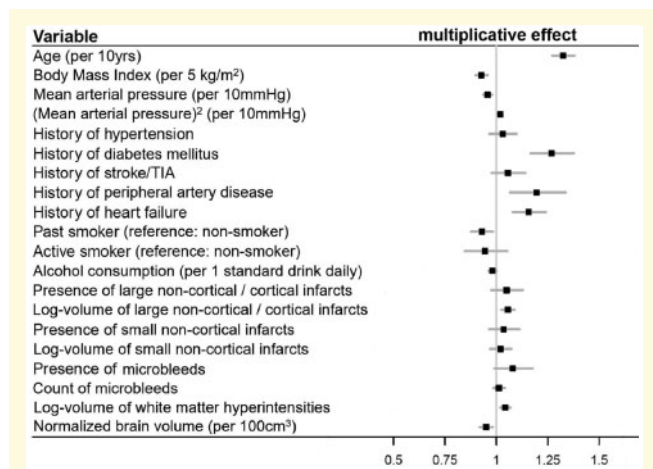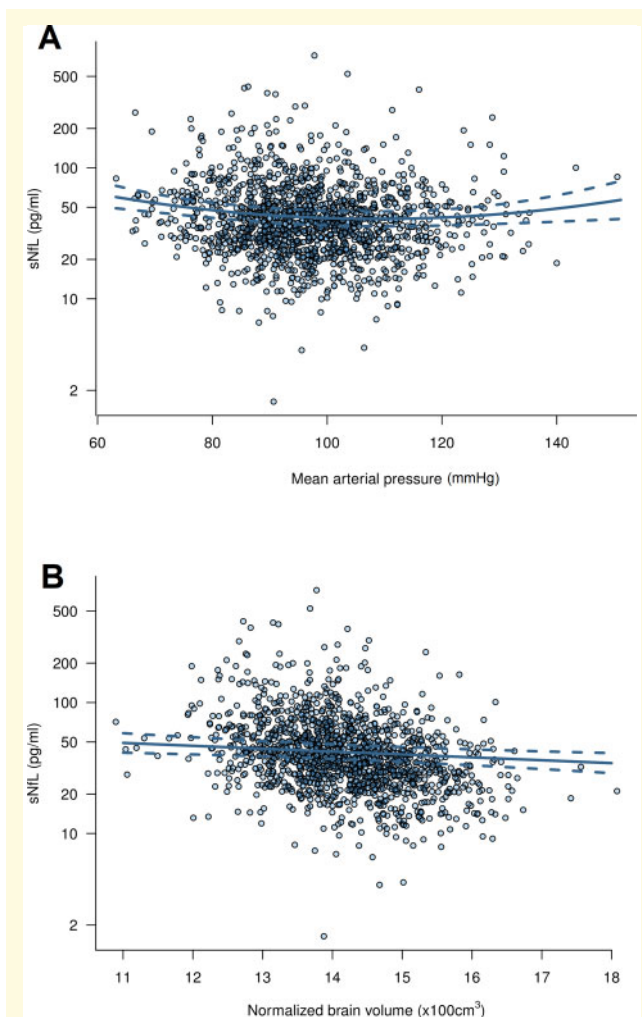
Figure 2 Multiplicative effect sizes of the association of clinical and MRI variables with sNfL from the combined model.

Figure 3 Scatter plot of the association of (A) mean arterial pressure and (B) normalized brain volume with sNfL. The solid line represents the predicted values from the combined clinical and MRI model and the dashed lines represent the 95% pointwise confidence intervals.

(Gattringer *et al.*, 2017; Tiedt *et al.*, 2018), and might suggest a greater severity of ongoing neurodegenerative processes secondary to ischaemia (Tiedt *et al.*, 2018) or persistent, active microischaemic phenomena in the brain of AF patients with a higher burden of established, embolic or microangiopathic, ischaemic MRI lesions. These findings demonstrate the potential of sNfL as a blood biomarker to select AF patients who would benefit from further MRI investigations to uncover potential subclinical vascular brain disease, considering that mass screening with MRI is not feasible and that sNfL in our cohort appeared to be sensitive to potential mechanisms of brain injury independent of structural changes visualized on MRI. Of note, the presence of MBs was only marginally associated with sNfL after adjustment for other brain lesions, an association that was further weakened in the combined model. Thus, MBs might represent a proxy marker of vascular brain disease and contribute little if any to neuronal injury *per se*, in line with the previous observations (Akoudad *et al.*, 2016; Conen *et al.*, 2019).

Another major finding was the inverse association of nBV with sNfL, which was independent of age, history of stroke and vascular MRI brain lesions. This association, which has been described in neurological diseases including multiple sclerosis and dementias (Khalil *et al.*, 2018), might reflect an underlying ongoing neurodegenerative process in AF. Indeed, AF has been previously associated with reduced brain volume independent of ischaemic infarcts, with putative explanations being cerebral microinfarcts or hypoperfusion leading to brain atrophy (Stefansdottir *et al.*, 2013; Piers *et al.*, 2016).

Despite the in-depth neuroimaging patients' characterization, the clinical model still explained a larger proportion of the sNfL variance than the MRI model. We can only speculate on the reasons for this: It is possible that microischaemic, hemodynamic, degenerative or other, yet unknown processes lead to neuronal damage in AF while remaining undetected on conventional MRI.

Finally, sNfL was inversely associated with cognitive performance in patients with AF. This is in line with previous research on the association of sNfL with cognitive measures in patients with small vessel (Duering *et al.*, 2018) and neurodegenerative diseases (Byrne *et al.*, 2017; Mattsson *et al.*, 2017; Lin *et al.*, 2018; van der Ende *et al.*, 2019), and suggests that sNfL is a non-disease-specific marker of neuronal damage resulting in cognitive dysfunction. The association of sNfL with cognitive measures grew markedly weaker after adjusting for age and was further attenuated in the multivariable clinical and MRI models, indicating that a multitude of factors including ageing, comorbidities and vascular brain lesions contribute to or mediate the association of neuronal damage with cognitive

Table 3 Association of log-sNfL with measures of cognitive function

| Cognitive measures (N = 1363)	Univariable β[d] (95% CI)	P-value	Age-adjusted β[d] (95% CI)	P-value	Multivariable clinical model[a] β[d] (95% CI)	P-value	Multivariable MRI model[b] β[d] (95% CI)	P-value	Multivariable combined model[c] β[d] (95% CI)	P-value
MoCA	−0.93 (−1.17, −0.69)	<0.001	−0.37 (−0.65, −0.10)	0.008	−0.23 (−0.51, 0.05)	0.114	−0.22 (−0.51, 0.06)	0.123	−0.15 (−0.44, 0.14)	0.307
TMT-A	−0.11 (−0.13, −0.10)	<0.001	−0.04 (−0.06, −0.02)	<0.001	−0.04 (−0.06, −0.02)	<0.001	−0.03 (−0.04, −0.01)	0.009	−0.03 (−0.04, −0.01)	0.012
TMT-B	−0.06 (−0.07, −0.05)	<0.001	−0.02 (−0.03, −0.01)	<0.001	−0.02 (−0.03, −0.01)	<0.001	−0.01 (−0.02, −0.00)	0.015	−0.01 (−0.02, −0.00)	0.023
SVF	−1.56 (−2.00, −1.12)	<0.001	−0.46 (−0.97, 0.05)	0.076	−0.29 (−0.81, 0.24)	0.283	−0.14 (−0.67, 0.38)	0.587	−0.09 (−0.62, 0.45)	0.752
DSST	−6.95 (−8.07, −5.83)	<0.001	−2.40 (−3.64, −1.16)	<0.001	−1.75 (−2.96, −0.54)	0.005	−0.98 (−2.23, 0.27)	0.124	−0.84 (−2.05, 0.37)	0.175
PCI	−0.98 (−1.11, −0.84)	<0.001	−0.34 (−0.49, −0.20)	<0.001	−0.27 (−0.41, −0.12)	<0.001	−0.18 (−0.33, −0.03)	0.016	−0.16 (−0.31, −0.01)	0.032

MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test; SVF, Semantic Verbal Fluency; DSST, Digit Symbol Substitution Test; PC1, first principal component.
[a]Adjusted for age, sex, education level, history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, peripheral artery disease, heart failure, obstructive sleep apnoea, BMI, smoking status and alcohol consumption.
[b]Adjusted for age, normalized brain volume, presence and volume of small non-cortical infarcts, presence and volume of large non-cortical or cortical infarcts, burden of MBs and volume of white matter lesions.
[c]Adjusted for age, sex, education level, history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, peripheral artery disease, heart failure, obstructive sleep apnoea, BMI, smoking status, alcohol consumption, normalized brain volume, presence and volume of small non-cortical infarcts, presence and volume of large non-cortical or cortical infarcts, burden of MBs and volume of white matter lesions.
[d]The model-based estimates β represent additive effects on test score (e.g. $\beta = -0.93$ for the association of log-sNfL with MoCA denotes an average decrease of 0.93 points in the MoCA score per unit higher log-sNfL, or an average decrease of approx. 0.09 points in the MoCA score per 10% higher sNfL concentration).
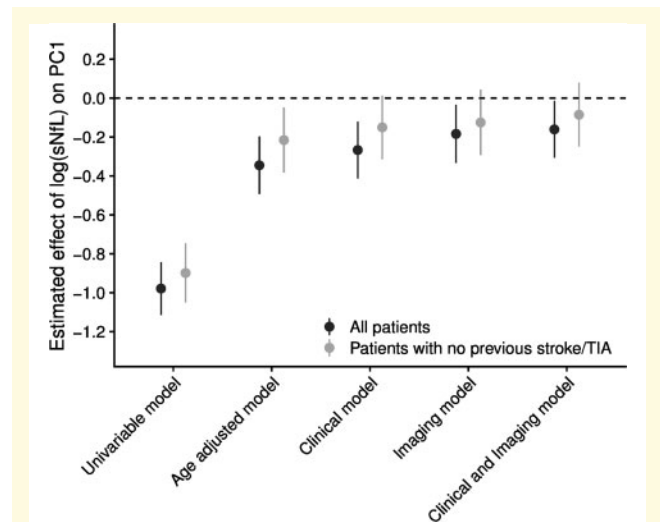
Figure 4 Model-based estimates for the association of log-sNfL with PC1.
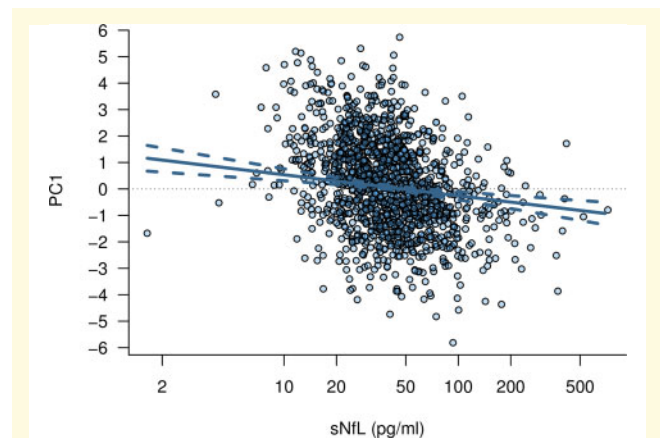
Figure 5 Scatter plot of the age-adjusted association of sNfL with PC1. The solid line represents the model-based predicted values and the dashed lines represent the pointwise 95% confidence intervals.

dysfunction in AF. Importantly, the association of sNfL with PC1, the summary cognitive measure, persisted after adjustment for all clinical and neuroimaging parameters, suggesting that additional unknown factors might contribute to this association. Among the separate cognitive measures, the strongest associations of sNfL were with TMT-A, TMT-B and—to a lesser extent—DSST, all measures of executive function, processing speed and attention. Preferential changes in these cognitive domains are known to reflect a vascular profile of cognitive dysfunction (O'Brien *et al.*, 2003) and have been previously associated with AF (Nishtala *et al.*, 2018).

The strengths of our study include (i) its large sample size of AF patients with detailed clinical, neuroimaging and

cognitive characterization, allowing for adjustment for several confounding factors and thus reducing the risk of spurious findings, (ii) the standardized manner of data acquisition, high rate of data completeness and blinded MRI assessment and sNfL measurement, reducing the risk of bias and (iii) its multicentre design, indicating a certain generalizability of our results, at least within the Caucasian population of central Europe. However, the following limitations must be acknowledged: (i) The study's cross-sectional design, which allows only for the assessment of association but not causality thereof. (ii) As Swiss-AF included exclusively patients with AF, we did not have a comparison group of patients with other heart diseases or healthy controls. It is, therefore, unknown whether our results are specific to AF. (iii) A large number of patients with Swiss-AF did not undergo brain MRI due to contraindications or claustrophobia and were thus ineligible for this study. It is, therefore, unknown whether our results are generalizable to patients with AF unsuited for brain MRI. (iv) We were not able to adjust our analyses for diseases of the peripheral nervous system, which were not systematically collected in Swiss-AF but might contribute to sNfL (Khalil *et al.*, 2018). (v) Neuroimaging was performed on 1.5 or 3.0 Tesla scanners, which might miss a relevant proportion of microinfarcts compared to higher resolution MRI (van Veluw *et al.*, 2013).

Conclusion

In conclusion, our study demonstrates the potential of sNfL as a tool to explore the mechanisms that underly cognitive dysfunction in AF. It seems likely that neuronal damage in AF results from a complex interplay between subclinical brain ischaemia, altered hemodynamics and neurodegeneration. Serum neurofilament light holds promise not only as an instrument to investigate the intricate mechanisms underlying the heart–brain interactions, but also as a surrogate outcome parameter for brain health and cognitive function in cardiovascular research. In future Swiss-AF analyses, we plan to investigate the prognostic significance of sNfL and other blood-based biomarkers of cardiovascular disease longitudinally with regard to the development of vascular brain lesions, brain atrophy and cognitive dysfunction over time.

Supplementary material

Supplementary material is available at *Brain Communications* online.

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Competing interests

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Appendix I

List of all Swiss-AF investigators according to participating center:

University Hospital Basel and University of Basel: Stefan Osswald, Michael Kühne, Stefanie Aeschbacher, Chloe Auberson, Steffen Blum, Leo Bonati, Selinda Ceylan, David Conen, Simone Doerpfeld, Ceylan Eken, Marc Girod, Peter Hämmerle, Philipp Krisai, Michael Kühne, Christine Meyer-Zürn, Pascal Meyre, Andreas U. Monsch, Christian Müller, Stefan Osswald, Philipp Reddiess, Anne Springer, Fabienne Steiner, Christian Sticherling, Thomas Szucs, Gian Voellmin, Leon Zwimpfer. University Hospital Bern: Nicolas Rodondi, Drahomir Aujesky, Urs Fischer, Juerg Fuhrer, Laurent Roten, Simon Jung, Heinrich Mattle; Luise Adam, Carole Elodie Aubert, Martin Feller, Claudio Schneider, Axel Loewe, Elisavet Moutzouri, Tanja Flückiger, Cindy Groen, Damiana Rakovic, Rylana Wenger, Lukas Ehrsam, Alexandra Nuoffer, Nathalie Schwab. Triemli Hospital Zurich: Andreas Müller, Christopher Beynon, Roger Dillier, Michèle Deubelbeiss, Franz Eberli, Christine Franzini, Isabel Juchli, Claudia Liedtke, Jacqueline Nadler, Thayze Obst, Noreen Tynan, Xiaoye Schneider, Katrin Studerus, Dominik Weishaupt. Cantonal Hospital Baden: Jürg-Hans Beer, Simone Fontana, Silke Kuest, Karin Scheuch, Denise Hischier, Nicole Bonetti, Alexandra Grau, Jonas Villinger, Eva Laube, Philipp Baumgartner, Mark Filipovic, Marcel Frick, Giulia Montrasio, Stefanie Leuenberger, Franziska Rutz. Cardiocentro Lugano: Tiziano Moccetti, Angelo Auricchio, Adriana Anesini, Cristina Camporini, Giulio Conte, Maria Luce Caputo, Francois Regoli. Cantonal Hospital St.Gallen: Peter Ammann, Roman Brenner,

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