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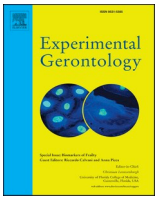

Associations of blood-based biomarkers of neurodegenerative diseases with mortality, cardio- and cerebrovascular events in persons with chronic coronary syndrome

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ABSTRACT

Background: In light of growing evidence highlighting interactions between cardiac and brain health, we investigated associations of biomarkers of neurodegenerative diseases with adverse outcomes (all-cause and cardiovascular mortality, major cardiovascular events, and stroke) in persons with chronic coronary syndrome (CCS).

Methods: We used data from a cohort of persons with CCS for whom major adverse events were recorded over a follow-up of 20 years. We measured biomarkers of neurodegenerative diseases in baseline blood samples, using the Single-Molecule Array Technology on a HD-1 Analyzer. These include biomarkers of neuronal (neurofilament light chain (NfL) ($n = 379$)) and glial neurodegeneration (glial fibrillary acidic protein (GFAP) ($n = 379$)), and Alzheimer's disease pathology (phosphorylated tau181 ($n = 379$), total tau ($n = 377$), and amyloid β ($A\beta_{40}$, $A\beta_{42}$, $A\beta_{42}/A\beta_{40}$) ($n = 377$)). We applied Cox-proportional hazards models to evaluate associations of these biomarkers with adverse outcomes, adjusting for covariates and exploring interactions with apolipoprotein E (*ApoE*) ε4 genotype.

Results: Participants with higher NfL levels had increased rates of all-cause and cardiovascular mortality (Hazard ratio per increase by one standard deviation (95 % confidence interval): all-cause mortality: 1.36 (1.10–1.68); cardiovascular mortality: 1.42 (1.05–1.93)). The $A\beta_{40}/A\beta_{42}$-ratio was linked to incident stroke (0.72 (0.52–1.00)). Associations of GFAP with all-cause mortality and incident stroke were depending on *ApoE* ε4 genotype. The other biomarkers were not significantly associated with the studied outcomes.

Conclusions: In persons with CSS, NfL and the $A\beta_{40}/A\beta_{42}$-ratio were related to mortality and incident stroke, respectively, whereas associations of GFAP with adverse outcomes varied by *ApoE* genotype. These biomarkers might play a role in linking aging, cardiovascular and neurodegenerative diseases.

1. Introduction

Cardiovascular and neurodegenerative diseases share common pathophysiological pathways (Casserly and Topol, 2004), and cardiovascular disease itself is a significant risk factor for neurodegeneration (Deckers et al., 2017). Possible explanations for this link include the relationship between neural activity and cerebral blood flow, known as "neurovascular unit" (Sweeney et al., 2018), and the role of the apolipoprotein E (*ApoE*) genotype, the most prominent genetic risk factor for Alzheimer's disease (AD), which, by impacting on lipid metabolism and

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risk of atherosclerosis, is crucial in linking cardiovascular disease and neurodegeneration (Tini et al., 2020). Epidemiological evidence even seems to suggest that the presence of cardiovascular pathologies and vascular risk factors might modify the association of the *ApoE* ε4 genotype with cognitive and neurodegenerative outcomes (Perna et al., 2016; Perna et al., 2023). Additionally, emerging data point to a role of amyloid-β (Aβ) in linking aging, cardiovascular disease and neurodegeneration (Stakos et al., 2020).

Accumulations of Aβ and phosphorylated tau (p-tau) in the brain are the hallmarks of AD, but vascular and cardiac depositions of Aβ also contribute to tissue inflammation and cardiac dysfunction (Stakos et al., 2020). Particularly $A\beta_{40}$ has been implicated in arterial disease due to its pro-inflammatory properties in both cerebral and peripheral vasculature (Stakos et al., 2020). Recent studies also identified a role of Neurofilament light chain (NfL) and Glial Fibrillary Acidic Protein (GFAP) in neurodegeneration (Li and Mielke, 2019; Stocker et al., 2022), with blood levels of p-tau and NfL interacting with cardiovascular health (Stocker et al., 2022). Additionally, blood-based biomarkers of neurodegenerative diseases have been linked to neurological outcomes and mortality. In population-based cohorts of older adults, elevated plasma $A\beta_{40}$ levels and $A\beta_{40}$ to $A\beta_{42}$ ratios, but not $A\beta_{42}$ levels alone, were associated with all-cause mortality (Gabelle et al., 2015). Increased levels of serum NfL were associated with all-cause mortality (Halloway et al., 2023; Rübsamen et al., 2021), cerebrovascular mortality (Rübsamen et al., 2021), and cardiovascular mortality (Halloway et al., 2023), while serum tau levels were associated with all-cause mortality (Rübsamen et al., 2021).

This accumulating evidence points to the need of extending the focus to populations with cardiovascular diseases and of investigating the interactions of biomarkers of neurodegenerative diseases in such populations. Hence, we aimed to examine the associations of blood-based biomarkers of neurodegenerative diseases (i.e., GFAP, NfL, p-tau181, Aβ, and total tau) with all-cause mortality, and cardiovascular and cerebrovascular outcomes in persons with chronic coronary syndrome (CCS).

2. Methods

2.1. Study design and data collection

These analyses were based on the KAROLA cohort study ($n = 1206$), which has been set up in 1999/2000 with the aim of assessing determinants of long-term prognosis after in-patient rehabilitation among persons with CCS in two cooperating hospitals (Bad Nauheim and Isny, Germany) (Mons et al., 2014). Inclusion criteria were age between 30 and 70 years and the occurrence of an acute cardiovascular event or procedure within the past three months before admission to the rehabilitation clinic.

At baseline, participants filled in a self-administered health questionnaire and additional medical information was obtained from clinics' medical records. Smoking status was self-reported and corrected according to serum cotinine levels as previously described (Twardella et al., 2006). Body mass index (BMI), prevalence of diabetes mellitus, prevalence of hypertension, left ventricular ejection fraction, history of stroke, and the 1−/2−/3-vessel disease score were obtained from medical records. Kidney function was assessed by use of the estimated glomerular filtration rate (eGFR), which was calculated using the 2021 Chronic Kidney Disease Epidemiology Collaboration creatinine equation based on serum creatinine levels (Inker et al., 2021). High density lipoprotein (HDL), low density lipoprotein (LDL), use of statins and antiplatelets were measured at the end of the rehabilitation. *ApoE* genotypes were determined using allele-specific restriction enzyme analysis with *Afl* III and *Hae* II as described elsewhere (Vossen et al., 2008). Participants were categorised into carriers of the ε4 allele (ε2/ε4, ε3/ε4, ε4/ε4) and non-carriers (ε2/ε2, ε2/ε3, ε3/ε3).

Participants were followed-up for 20 years. We retrieved information about vital status and potential dates of death of participants from registration offices. We then ascertained causes of fatal events by analysing death certificates obtained from health authorities. Subsequently, we classified the cause of death according to the respective current versions of the International Statistical Classification of Diseases and Related Health Problems (ICD-9 or ICD-10). Cardiovascular causes of death were defined according to ICD-9 items 390-459, ICD-10 items I00-I99 and R57.0; cerebrovascular causes of death according to ICD-9 items 430-438 and ICD-10 items I60-I69. Data on non-fatal events were gathered from the participants' general practitioners. Non-fatal cardiovascular events and stroke were defined as myocardial infarction and stroke (including both ischemic and haemorrhagic stroke), respectively. Both major cardiovascular events and stroke were defined as combined endpoints including occurrence of both fatal and non-fatal events. It should be noted that the term major cardiovascular events refers to both cardiovascular events and stroke. Cardiovascular mortality included deaths with cardiovascular disease or cerebrovascular disease as underlying cause. All-cause mortality included deaths of any cause.

The KAROLA study was approved by the Ethics Committees of the Universities of Ulm (186/98) and Heidelberg (S-351/2001) and the state medical boards of Baden-Württemberg and Hessen (Germany). We obtained written informed consent from all participants in accordance with the Declaration of Helsinki at the time of enrolment.

2.2. Selection of study participants for this analysis

For the present study, we used a subset of KAROLA participants who also participated in a voluntary cognitive assessment at the ten-year follow-up measurement and from whom stored frozen baseline blood samples were available ($n = 390$). This subset of participants with cognitive data was selected due to financial constraints, which limited the feasibility of biomarker measurements for the entire cohort. By focusing on participants with cognitive data, we aimed to maximise the value of the measurements, as this approach allowed us to investigate associations of these biomarkers not only with adverse outcomes, but also with cognitive outcomes, which is part of a separate study.

Among this subset, 379 participants had valid frozen baseline serum samples, and 256 had valid frozen baseline EDTA plasma samples. This differentiation by sample type is crucial, as some of the biomarkers we aimed to measure required serum (NfL, GFAP, p-tau181), while others required plasma (Aβ, t-tau). As our budget allowed us to analyse up to 385 plasma samples, we included an additional 129 samples to enhance the study subset, randomly chosen from all participants of the KAROLA study (including those with no cognitive assessment) aged 50 years and above with available frozen EDTA plasma samples. Among the participants with EDTA plasma, we encountered a lack of outcome data for two participants. Additionally, biomarkers could not be measured for six participants due to issues related to blood quality ($n = 5$) and transport ($n = 1$). Consequently, our analysis was based on two subsets: 379 participants in the serum marker subset, and 377 participants in the plasma marker subset. Notably, 241 participants were included in both subsets (see Additional Fig. 1 for a flow chart of the selection of the study participants).

2.3. Assessment of blood biomarkers of neurodegenerative diseases

Blood-based markers of neurodegenerative diseases were measured either in serum or EDTA plasma samples through single-molecule arrays (Simoa) Technology (Quanterix, MA, USA) on a HD-1 Analyzer as per manufacturer's instructions (Thijssen et al., 2020; Asken et al., 2020; Rissin et al., 2010). Levels of p-tau181 and of GFAP and NfL were measured in baseline serum with the Simoa pTau-181 Advantage V2.1 kit and the Simoa Human Neurology 2-Plex E assay, respectively. T-tau, $A\beta_{40}$, $A\beta_{42}$ were measured in baseline EDTA plasma with the Simoa Human Neurology 3-Plex A assay. Upon arrival at the laboratory, the samples were thawed at room temperature and mixed thoroughly.

Afterwards they were put through a centrifugation step at 10,000 ×*g* for 5 min and applied to a conical 96-well plate (Quanterix, MA, USA). At the end of this procedure, the samples were measured immediately with lot specific calibrators and one low and one high concentrated lot specific control.

2.4. Data analysis

Data for GFAP, NfL, p-tau181, and t-tau were highly skewed and therefore log-transformed. We z-standardised all biomarkers for interpretability. We used right-censored Cox proportional-hazard models to explore associations of biomarkers of neurodegenerative diseases with incidence of major cardiovascular events and stroke. With exception of the random sample in the plasma marker subset, measurement of markers of neurodegenerative diseases were conditional upon participation in the ten-year follow-up cognitive assessment and hence to be alive at that point, therefore their survival times were left-truncated. We adjusted all models for age, sex, study centre, eGFR, *ApoE* ε4 genotype, smoking status, BMI and comorbidities (diabetes mellitus, hypertension, left ventricular ejection fraction, HDL, LDL, use of statins, use of antiplatelets, history of stroke, and the 1-/2-/3-vessel disease score). In addition, we checked for interactions between biomarkers of neurodegenerative diseases and *ApoE* ε4 genotype by adding a biomarker x *ApoE* ε4 genotype interaction term to the model, and further stratified for *ApoE* ε4 genotype when this interaction was significant.

To ensure that log-transforming data for GFAP, NfL, p-tau181, and t-tau did not lead to any bias, we performed a sensitivity analysis utilising the same models as described above except for including the non-log-transformed data for GFAP, NfL, p-tau181, and t-tau. Moreover, given the influence of age on both cardiovascular and neurodegenerative processes, we performed a sensitivity analysis stratifying participants by age (<65 and ≥65 years) to assess whether the associations of biomarkers with the studied outcomes differed by age group.

All analyses were performed in R version 4.2.1 (R Core Team, 2020). *P*-values <0.05 were considered statistically significant. Missing values ranged from 0.5 % for eGFR to 12.7 % for left-ventricular ejection fraction in the serum marker subset, and from 0.5 % for eGFR to 9.2 % for left-ventricular ejection fraction in the plasma marker subset, and were imputed based on nonparametric missing value imputation applying random forest using the R package missForest (version 1.4) (Stekhoven and Bühlmann, 2011).

3. Results

3.1. Characteristics of the study population

The baseline characteristics of the study subsets are presented in Table 1. Characteristics stratified by *ApoE* ε4 status can be found in Additional Table 1. Overall, more than half of participants of the KAROLA study were older than 60 years when they entered the study and a large majority were men. Less than one third of the participants were *ApoE* ε4 carriers. The serum and plasma marker subsets differed only with respect to eGFR levels and the study centres (Table 1). Table 2 presents an overview of all-cause and cause-specific mortality and incidence of cardiovascular events and stroke within 20 years.

3.2. Associations between biomarkers of neurodegenerative diseases and all-cause mortality

Participants with higher levels of NfL had increased rates of all-cause mortality (Hazard Ratio (HR) per increase by one SD (95 % confidence interval (CI)): 1.36 (1.10–1.68); Table 3). We found that the association between GFAP and all-cause mortality changed depending on *ApoE* ε4 genotype (GFAP x *ApoE* ε4 genotype interaction: $p = 0.035$). Stratification for *ApoE* ε4 genotype revealed that *ApoE* ε4 non-carriers with higher levels of GFAP had higher rates of all-cause mortality (HR per one

Table 1
Characteristics of the analysis subsets.

	Total analysis subset (*N* = 517)	Serum marker subset (N = 379)	Plasma marker subset (*N* = 377)	p-Value*
Age group, n (%)				0.302
30–49 years	54 (10.4)	52 (13.7)	38 (10.1)	
50–59 years	175 (33.8)	128 (33.8)	132 (35.0)	
60 years and above	288 (55.7)	199 (52.5)	207 (54.9)	
Sex, n (%)				
Men	442 (85.5)	321 (84.7)	332 (88.1)	0.214
Women	75 (14.5)	58 (15.3)	45 (11.9)	
Study Centre, n (%)				
Isny	139 (26.9)	240 (63.3)	342 (90.7)	<0.001
Bad Nauheim	378 (73.1)	139 (36.7)	35 (9.3)	
eGFR [mL/min/1.73 m^2], mean (SD)	85.2 (16.3)	87.0 (16.0)	82.6 (15.7)	<0.001
ApoE ε4 status				
ApoE ε4 carrier	147 (28.4)	113 (29.8)	116 (30.8)	0.960
ApoE ε4 non-carrier	364 (70.4)	263 (69.4)	258 (68.4)	
BMI [kg/m^2], mean (SD)	26.6 (3.0)	26.7 (3.1)	26.6 (3.1)	0.589
Smoking, n (%)				0.639
Never	181 (35.0)	123 (32.5)	133 (35.3)	
Long-term quitter	204 (39.5)	154 (40.6)	153 (40.6)	
Recent quitter[a]	63 (12.2)	50 (13.2)	50 (13.3)	
Smoker	69 (13.3)	52 (13.7)	41 (10.9)	
Left ventricular ejection fraction, n (%)				0.124
No or mild dysfunction	364 (70.4)	281 (74.1)	255 (67.6)	
Moderate or severe dysfunction	97 (18.8)	63 (16.6)	74 (19.6)	
Number of affected vessels, n (%)				0.926
1	113 (21.9)	83 (21.9)	84 (22.3)	
2	150 (29.0)	117 (30.9)	108 (28.6)	
3 or 4	228 (44.1)	162 (42.7)	167 (44.3)	
History of myocardial infarction, n (%)	274 (53.0)	195 (51.5)	197 (52.3)	0.882
Prevalent type 2 diabetes, n (%)	70 (13.5)	48 (12.7)	51 (13.5)	0.881
Prevalent hypertension, n (%)	340 (65.8)	245 (64.6)	246 (65.3)	0.927
History of stroke, n (%)	4 (0.8)	3 (0.8)	2 (0.5)	0.157
High density lipoprotein, mean (SD)	38.6 (10.5)	39.2 (10.3)	38.1 (10.3)	0.130
Low density lipoprotein, mean (SD)	100.7 (29.2)	101.0 (29.3)	100.4 (29.1)	0.791
Statin use, n (%)	414 (80.1)	299 (78.9)	315 (83.6)	0.122
Antiplatelet use, n (%)	55 (10.6)	43 (11.3)	40 (10.6)	0.836
Biomarkers of neurodegenerative diseases[b]				
GFAP, median [IQR]		99.0 [71.1, 135.3]		
NfL, mean (SD)		24.3 (30.9)		
p-tau181, median [IQR]		1.1 [0.8, 1.6]		
$A\beta_{40}$, mean (SD)			225.7 (57.5)	
$A\beta_{42}$, mean (SD)			30.5 (8.8)	
$A\beta_{40}/A\beta_{42}$, mean (SD)			0.1 (0.0)	
t-tau, median [IQR]			1.6 [1.3, 2.2]	

Missing data: *ApoE* ε4 status: subset with either serum or plasma samples n = 6 (1.2 %), serum marker subset n = 3 (0.8 %), plasma marker subset n = 3 (0.8 %); left ventricular ejection fraction: subset with either serum or plasma samples *n* = 56 (10.8 %), serum marker subset *n* = 35 (9.2 %), plasma marker subset *n* = 48 (12.7 %); number of affected vessels: subset with either serum or plasma samples *n* = 26 (5.0 %), serum marker subset *n* = 17 (4.5 %), plasma marker subset *n* = 18 (4.8 %); diabetes: subset with either serum or plasma samples n = 4 (0.8 %), serum marker subset n = 4 (1.1 %), plasma marker subset n = 3 (0.8 %); hypertension: subset with either serum or plasma samples *n* = 6 (1.2 %), serum marker subset *n* = 5 (1.3 %), plasma marker subset n = 6 (1.6 %); history of stroke: subset with either serum or plasma samples *n* = 38 (7.4 %), serum marker subset *n* = 16 (4.2 %), plasma marker subset *n* = 28 (7.4 %); HDL: subset with either serum or plasma samples *n* = 7 (1.4 %), serum marker subset n = 7 (1.8

%), plasma marker subset n = 3 (0.8 %); LDL: subset with either serum or plasma samples n = 14 (2.7 %), serum marker subset n = 13 (3.4 %), plasma marker subset n = 7 (1.9 %).

[a] Cessation after acute cardiovascular event or procedure leading to admission to the rehabilitation clinic.

[b] For normally distributed biomarkers of neurodegenerative diseases mean (SD) are presented, otherwise median [interquartile range (IQR)].

[*] *P*-values comparing the serum and plasma marker subsets where categorical variables were compared using a chi-square test, and continuous variables utilising *t*-tests.

Table 2
Numbers of events according to time since baseline blood sampling in the serum marker subset and the plasma marker subset.

	Serum marker subset (N = 379)		Plasma marker subset (N = 377)	
	<10 years	10–20 years	<10 years	10–20 years
All-cause mortality	0	107	28	99
Cardiovascular mortality	0	50	16	44
Major cardiovascular events	45	87	56	77
Stroke	27	25	24	19

Note: In the serum marker subset, all participants were included based on their participation in a cognitive assessment at ten-year-follow-up, which is why no fatal events occurred over the first ten years in this particular subset. In the plasma marker subset, we additionally included a random sample of 129 participants independent of their participation in a cognitive assessment, in which fatal events occurred over the whole follow-up period.

Table 3
Associations of biomarkers of neurodegenerative diseases with all-cause and cardiovascular mortality in the serum (n = 379) and plasma (n = 377) marker subset.

	All-cause mortality			Cardiovascular mortality		
	Events	Adjusted HR per one SD (95 % CI)	p	Events	Adjusted HR per one SD (95 % CI)	p
Serum marker						
GFAP	107	1.21 (0.96–1.53)	0.098	50	1.11 (0.80–1.55)	0.531
NfL	107	1.36 (1.10–1.68)	0.005	50	1.42 (1.05–1.93)	0.022
p-tau181	107	0.93 (0.76–1.15)	0.507	50	0.99 (0.73–1.33)	0.937
Plasma marker						
$A\beta_{40}$	127	1.17 (0.98–1.42)	0.090	60	1.27 (0.97–1.67)	0.087
$A\beta_{42}$	127	1.09 (0.89–1.33)	0.408	60	1.06 (0.78–1.44)	0.691
$A\beta_{40}$/$A\beta_{42}$	127	0.89 (0.74–1.08)	0.231	60	0.82 (0.60–1.12)	0.202
t-tau	127	1.03 (0.86–1.24)	0.746	60	1.09 (0.82–1.45)	0.565

Note. Models were adjusted for age, sex, study centre, estimated glomerular filtration rate, *ApoE* ε4 genotype, smoking status, body mass index and comorbidities (diabetes mellitus, hypertension, left ventricular ejection fraction, high density lipoprotein, low density lipoprotein, statin use, antiplatelet use, history of stroke, and the 1-/2-/3-vessel disease score). HR = Hazard ratio; SD = standard deviation; CI = confidence interval.

SD (95 % CI): 1.37 (1.05–1.79)), whereas *ApoE* ε4 carriers did not (HR per one SD (95 % CI): 0.59 (0.32–1.06); Table 5). The other biomarkers of neurodegenerative diseases were not associated with rates of all-cause mortality (Table 3).

3.3. Associations between biomarkers of neurodegenerative diseases and cardiovascular mortality

Higher levels of NfL were associated with increased risk for cardiovascular mortality (HR per one SD (95 % CI): 1.42 (1.05–1.93); Table 3). The other biomarkers were not associated with rates of cardiovascular mortality, and there were no significant interactions between biomarkers of neurodegenerative diseases and *ApoE* ε4 genotype.

3.4. Associations between biomarkers of neurodegenerative diseases and incident major cardiovascular events

Biomarkers of neurodegenerative diseases were not associated with incident major cardiovascular events (Table 4).

3.5. Associations between biomarkers of neurodegenerative diseases and incident stroke

A higher $A\beta_{40}/A\beta_{42}$ ratio was associated with reduced risks of incident stroke (HR per one SD (95 % CI): 0.72 (0.52–1.00)). We found an interaction effect of GFAP with *ApoE* ε4 genotype (GFAP x *ApoE* ε4 genotype interaction: p = 0.027). Higher levels of GFAP were associated with higher rates of incident cerebrovascular events in *ApoE* ε4 carriers (HR per one SD (95 % CI): 2.72 (1.22–6.03)), but not in *ApoE* ε4 non-carriers (HR per one SD (95 % CI): 0.95 (0.64–1.43); Table 5).

There were no significant associations between other biomarkers of neurodegenerative diseases and incident stroke (Table 4).

3.6. Sensitivity analysis including non-log-transformed data for GFAP, NfL, p-tau181, and t-tau

Overall, this sensitivity analysis showed similar patterns to the results retrieved from the models including log-transformed biomarkers of neurodegenerative diseases and supports the robustness of these associations (data not shown).

Table 4
Associations of biomarkers of neurodegenerative diseases with major cardiovascular events and stroke in the serum (n = 379) and plasma (n = 377) marker subset.

	Major cardiovascular events			Stroke		
	Events	Adjusted HR per one SD (95 % CI)	p	Events	Adjusted HR per one SD (95 % CI)	p
Serum marker						
GFAP	132	1.08 (0.89–1.31)	0.431	52	1.29 (0.93–1.78)	0.127
NfL	132	1.11 (0.91–1.36)	0.301	52	1.18 (0.85–1.65)	0.322
p-tau181	132	0.94 (0.75–1.18)	0.598	52	0.97 (0.70–1.34)	0.840
Plasma marker						
$A\beta_{40}$	134	1.09 (0.90–1.32)	0.391	43	1.14 (0.81–1.62)	0.448
$A\beta_{42}$	134	0.95 (0.78–1.17)	0.631	43	0.80 (0.55–1.16)	0.240
$A\beta_{40}$/$A\beta_{42}$	134	0.90 (0.75–1.08)	0.241	43	0.72 (0.52–1.00)	0.049
t-tau	134	1.07 (0.89–1.29)	0.468	43	1.01 (0.72–1.43)	0.940

Note. Models were adjusted for age, sex, study centre, estimated glomerular filtration rate, *ApoE* ε4 genotype, smoking status, body mass index and comorbidities (diabetes mellitus, hypertension, left ventricular ejection fraction, high density lipoprotein, low density lipoprotein, statin use, antiplatelet use, history of stroke, and the 1-/2-/3-vessel disease score). HR = Hazard ratio; SD = standard deviation; CI = confidence interval.

Table 5
Associations of GFAP with all-cause mortality and stroke, stratified for *ApoE* ε4 status.

	ApoE ε4 carrier ($n = 113$)			*ApoE* ε4 non-carrier ($n = 266$)		
	Events	Adjusted HR per one SD (95 % CI)	p	Events	Adjusted HR per one SD (95 % CI)	p
All-cause mortality						
GFAP	23	0.59 (0.32–1.06)	0.077	84	1.37 (1.05–1.79)	0.022
Stroke						
GFAP	16	2.72 (1.22–6.03)	0.014	36	0.95 (0.64–1.43)	0.819

Note. Models were adjusted for age, sex, study centre, estimated glomerular filtration rate, *ApoE* ε4 genotype, smoking status, body mass index and comorbidities (diabetes mellitus, hypertension, left ventricular ejection fraction, high density lipoprotein, low density lipoprotein, statin use, antiplatelet use, history of stroke, and the 1-/2-/3-vessel disease score). HR = Hazard ratio; SD = standard deviation; CI = confidence interval.

3.7. Sensitivity analysis stratified by age (cut-off 65 years)

In persons aged <65 years ($n = 289$ in the serum marker subset; $n = 286$ in the plasma marker subset), higher levels of NfL were associated with increased risks of all-cause mortality (HR per one SD (95 % CI): 1.52 (1.17–1.97)) and cardiovascular mortality (HR per one SD (95 % CI): 1.56 (1.08–2.28)). A higher $A\beta_{40}/A\beta_{42}$ ratio was associated with reduced risks of major cardiovascular events (HR per one SD (95 % CI): 0.77 (0.61–0.97)), while elevated GFAP levels were linked to higher risks of incident stroke (HR per one SD (95 % CI): 1.49 (1.04–2.13); Additional Table 2).

In persons aged ≥65 years ($n = 90$ in serum marker subset; $n = 91$ in plasma marker subset), elevated levels of $A\beta_{40}$ and $A\beta_{42}$ were associated with all-cause mortality (HR per one SD (95 % CI): $A\beta_{40}$: 1.56 (1.11–2.19); $A\beta_{42}$: 1.51 (1.05–2.17)), NfL and $A\beta_{40}$ with cardiovascular mortality (HR per one SD (95 % CI): NfL: 2.22 (1.01–4.88); $A\beta_{40}$: 1.83 (1.04–3.23)), higher levels of NfL with incident major cardiovascular events (HR per one SD (95 % CI): 1.72 (1.01–2.94)), and higher levels of $A\beta_{40}$ and a higher $A\beta_{40}/A\beta_{42}$ ratio with incident stroke (HR per one SD (95 % CI): $A\beta_{40}$: 1.89 (1.10–3.25); $A\beta_{40}/A\beta_{42}$ ratio: 0.34 (0.18–0.64); Additional Table 2).

4. Discussion

In these subsets of persons with CCS, we found that elevated baseline serum NfL levels were associated with increased rates of all-cause and cardiovascular mortality. The $A\beta_{40}/A\beta_{42}$ ratio was associated with incident stroke. We also observed trends suggestive of a link between baseline plasma $A\beta_{40}$ levels and cardiovascular mortality, and between serum GFAP levels and incident stroke in *ApoE* ε4 carriers. Our results imply that, in persons with CCS, NfL levels might be used to identify patients at higher risk of adverse outcomes. Furthermore, serum GFAP levels and the $A\beta_{40}/A\beta_{42}$ ratio might serve as a potential biomarker for identifying patients at increased risk of stroke.

NfL is released upon neuroaxonal degeneration and injury, irrespective of cause, and therefore considered a general marker of neurodegeneration (Khalil et al., 2018). Our findings are in line with this definition and with previous studies showing associations of NfL with all-cause mortality in other populations (Halloway et al., 2023; Rübsamen et al., 2021; Kaeser et al., 2021; Amrein et al., 2023), and broadens the application of NfL as a marker of all-cause mortality for persons with CCS. Additionally, we found an association between high levels of NfL with higher rates of cardiovascular mortality. This aligns with a population-based study showing that levels of NfL interacted with cardiovascular health, defined by use of a score for ten-year risk for fatal CVD (Stocker et al., 2022), supporting a potential role of NfL as a marker for cardiovascular mortality.

However, we did not find associations between NfL and incidence of major cardiovascular events or stroke, which contradicts results from other studies. These studies reported NfL to be predictive of incident stroke within eleven years in the general population (Heshmatollah et al., 2022), and within seven years in persons with diabetes mellitus (Korley et al., 2019). Additionally, NfL has been linked to higher rates of cerebrovascular five-year mortality in the general population (Rübsamen et al., 2021). We cannot confirm these associations in our subset of persons with CCS.

This study indicated that the $A\beta_{40}/A\beta_{42}$ ratio was associated with incident stroke. This is in line with current developments in the field of neurodegenerative diseases, acknowledging the role of cerebrovascular disease burden in AD by recognising this relationship in the most recent revised criteria for diagnosing and staging AD (Jack Jr. et al., 2024). Additionally, the $A\beta_{40}/A\beta_{42}$ ratio is a well-known predictor of cerebral amyloid angiopathy, a form of cerebral small vessel disease, which is a risk factor for future stroke and neurodegeneration (Janelidze et al., 2016; Vergallo et al., 2019).

Our data pointed to associations of GFAP with all-cause mortality and stroke in *ApoE* ε4 carriers. GFAP is a marker of inflammation and astrocytic activation and our results might indicate a potential interplay between genetic risk and glial neurodegeneration, supporting previous studies demonstrating associations between GFAP and *ApoE* ε4 genotype in different populations (Stocker et al., 2023; Gonzales et al., 2023), suggesting that GFAP might also be a marker of the heritable component of a disease (Stocker et al., 2023). While recent studies have indicated that GFAP might be detectable in early states of neurodegeneration and a potential marker for disease progression (Lin et al., 2023; Meier et al., 2023; Shen et al., 2023), the clinical utility of GFAP for vascular diseases needs to be further elaborated. Other studies found that the interplay of biomarkers of neurodegenerative diseases, including GFAP, with *ApoE* ε4 genotype might also depend on other risk factors (Perna et al., 2023; van Arendonk et al., 2023). These emerging observations contribute to explaining our results indicating that *ApoE* ε4 non-carriers with higher levels of GFAP had higher rates of all-cause mortality. In fact, the observed association might depend upon the distribution of other risk factors interacting with GFAP and *ApoE* ε4. However, given the small sample of this subset, which also prevented finer stratified analyses, such results shall be interpreted with caution. Taken together, our findings relating to GFAP highlight the possibility that cardiovascular and neurodegenerative diseases have overlapping aetiologies, and underscore the need for future studies to further explore the interplay between GFAP and *ApoE* ε4 genotype.

Our explorative sensitivity analysis stratifying for age (cut-off 65 years) suggested that in the older subgroup, plasma $A\beta_{40}$ and $A\beta_{42}$ were associated with all-cause mortality, serum NfL and plasma $A\beta_4$ with cardiovascular mortality, serum NfL with major cardiovascular events, and plasma $A\beta_{40}$ and the $A\beta_{40}/A\beta_{42}$ ratio with incident stroke. The association between NfL and all-cause mortality seemed to be driven by persons younger than 65 years. An association between NfL and all-cause mortality among younger adults has also been found in another large study conducted among the US general population (Ciardullo et al., 2023). In our cohort of persons with CCS this finding might point to subtle neuronal degeneration already in younger age, which might lead to cognitive decline and a worse general health status leading to earlier mortality. Unfortunately, the lack of brain imaging and repeated cognitive testing over the observational time does not allow to further investigate these findings. However, the results of this sensitivity analysis need to be interpreted with caution due to the restricted sample size, particularly in the subgroup older than 65 years.

In this study, p-tau181 and t-tau were not associated with any outcomes. By contrast, in population-based cohorts, p-tau181 was found to interact with ten-year risk of fatal CVD (Stocker et al., 2022), while t-tau was associated with all-cause and non-stroke related mortality

(Rübsamen et al., 2021). These discrepancies highlight the need for further research to resolve these inconsistencies.

While there is mounting evidence of potential interplays between cardiac and neurodegenerative disease particularly emphasising a key role of Aβ in arterial diseases and neurodegeneration (Stakos et al., 2020), future studies would benefit from multimodal approaches to further distinguish between vascular and neurodegenerative diseases. These multimodal approaches should include vascular neuroimaging data or additional genetic data to facilitate identification of disease-specific markers that might be applied in clinical settings. Additionally, these might shed more light into the mechanisms underlying the associations between the biomarkers of neurodegenerative diseases and cardiovascular events.

In interpreting the results of this study, several limitations need to be considered. The relatively small sample size reduced our statistical power, especially for subgroup analyses. We had only a few cases with fatal stroke ($n = 5$ in each subset) and could therefore not investigate associations between biomarkers of neurodegenerative diseases with cerebrovascular mortality. Furthermore, we included only a subset of the KAROLA study population and, with exception of the random sample included in the plasma marker subset, this subset was chosen based on participation in a cognitive assessment at the ten-year follow-up, hence participants in this study had to be alive for at least ten years after baseline assessment and were more likely to be (cognitively) healthy at that time point. While we adequately dealt with this in the statistical analysis by taking the left-truncation into account, this nevertheless introduces a potential for selection bias. Compared to the overall study population of the KAROLA study, the two study subsets included less smokers, and less participants with a history of myocardial infarction and stroke (data not shown). Nevertheless, the subsets of participants analysed in this study are to some extent representative of a general population of persons with CCS, including a substantial proportion (44 %) of participants with 3- or 4-vessel disease, and results shown in this study might rather be potential underestimations of the true associations. Further, this study did not collect data for incident neurodegenerative disease. Therefore, we were unable to distinguish between the occurrence of outcomes related to neurodegeneration, cardio- or cerebrovascular diseases. Lastly, given the observational nature of this study design, our analyses do not allow a causal interpretation.

Despite such limitations, this study is a unique prospective study assessing risk factors of prognosis in a well-characterised cohort of persons with CCS with major strengths including the long-term follow-up period for vital status, cardiovascular events and stroke.

5. Conclusions

In conclusion, our findings suggest that NfL and the $A\beta_{40}/A\beta_{42}$-ratio might be promising biomarkers for predicting all-cause and cardiovascular mortality and incident stroke, respectively, in persons with CCS. Additionally, relations of GFAP with all-cause mortality and incident stroke were dependent on *ApoE* genotype. Taken together, these results might suggest a possible role of blood-based biomarkers of neurodegenerative diseases in linking aging, cardiovascular and brain health. Further studies are needed to elucidate the pathophysiology of this interplay, taking into account multimodal approaches including neuroimaging data, and to further explore the utility of these biomarkers for disease monitoring and, eventually, treatment response.

Declaration competing interest

RP has received honoraria for advisory boards and speaker engagements from Roche, EISAI, Eli Lilly, Biogen, Janssen-Cilag, Astra Zeneca, Schwabe, Grifols, Novo Nordisk and Tabuk. All other authors declare no conflicts of interest.

CRediT authorship contribution statement

Valerie Lohner: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Laura Perna:** Writing – review & editing, Methodology, Conceptualization. **Ben Schöttker:** Writing – review & editing, Methodology, Conceptualization. **Robert Perneczky:** Writing – review & editing, Conceptualization. **Hermann Brenner:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Ute Mons:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2025.112684.

Data availability

Data will be made available on request.

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