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RESEARCH ARTICLE

High cholesterol levels change the association of biomarkers of neurodegenerative diseases with dementia risk: Findings from a population-based cohort

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Abstract

Introduction: This study assessed whether in a population with comorbidity of neurodegenerative and cerebrovascular disease (mixed pathology) the association of glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and phosphorylated tau181 (p-tau181) with dementia risk varied depending on levels of total cholesterol and apolipoprotein E (*APOE*) ε4 genotype.

Methods: Plasma biomarkers were measured using Simoa technology in 768 participants of a nested case-control study embedded within an ongoing population-based cohort. Logistic and spline regression models, and receiver operating characteristic curves were calculated.

Results: The strength of the association between GFAP and NfL with risk of a clinical diagnosis of dementia changed depending on cholesterol levels and on *APOE* ε4 genotype. No significant association was seen with p-tau181.

Discussion: In individuals with mixed pathology blood GFAP and NfL are better predictors of dementia risk than p-tau181, and their associations with dementia risk are amplified by hypercholesterolemia, also depending on *APOE* ε4 genotype.

KEYWORDS
apolipoprotein E ε4 genotype, blood biomarkers, cholesterol, dementia, glial fibrillary acidic protein, neurofilament light chain, phosphorylated tau181

HIGHLIGHTS

1. Cholesterol levels changed the association of blood biomarkers with dementia risk.
2. Blood biomarkers seem to perform differently in community- and clinic-based cohorts.
3. Neurofilament light chain might be a biomarker candidate for dementia risk after stroke.

1 | BACKGROUND

Dementia is a progressive syndrome characterized by deterioration of cognitive, functional, and behavioral abilities. It has long been established that physical and mental health are fundamentally linked and that a healthy body is an essential precondition of a healthy brain and vice versa. One of the main mechanisms linking body and brain health is the maintenance of a good vascular health[1–4] and there is strong evidence indicating that several modifiable risk factors for dementia are also risk factors for vascular pathology[5] and that better cardiovascular health leads to better cognitive health.[1,6–8]

Raised plasma cholesterol, especially low-density lipoprotein cholesterol (LDL), is one of the major risk factors for ischemic heart disease and ischemic stroke and, according to worldwide estimates, it has a prevalence of approximately 39% among adults.[9] High cholesterol is also considered to be a potentially modifiable risk factor for dementia risk,[5] but the mechanisms linking high cholesterol to dementia are not clear and the findings relating to the association of cholesterol and dementia are mixed. In previous work, we found that the presence of high total cholesterol (TC), which is mostly composed of LDL, was associated with cognitive function only in the interplay with other risk factors, namely cardiovascular pathologies and apolipoprotein E (*APOE*) ε4 genotype.[10] Here we investigate the role of hypercholesterolemia for dementia risk in connection not only with vascular pathology and *APOE* ε4, but also with established blood biomarkers of neurodegenerative diseases. As biomarkers, we selected glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and phosphorylated tau181 (p-tau181), because all these biomarkers have been consistently associated with dementia or dementia risk, and GFAP and NfL also with cerebrovascular disease, such as stroke.[11–19] Furthermore, altered levels of GFAP could point to a possible dysregulation of brain cholesterol synthesized by astrocytes, whose interconnections with peripheral cholesterol are yet unexplored.[20]

Most dementia biomarker studies have been conducted in clinic-based research cohorts including very selected and well-characterized populations with evidence of amyloid beta (Aβ) plaques and tau tangles.[21–22] We believe that to understand the impact of blood biomarkers on dementia fully, we also need to focus on representative populations with mixed pathologies (comorbidity of neurodegenerative and cerebrovascular disease, including, inter alia, vascular encephalopathy, cerebral infarction, microangiopathy), and investigate how such markers unfold in synergism with other risk factors. Hence, here we investigate whether in a population-based cohort with high prevalence of mixed pathology, hypercholesterolemia changes the association of blood biomarkers of neurodegenerative diseases with the risk of receiving a clinical diagnosis of dementia across 17 years of follow-up and whether this association is further moderated depending on *APOE* ε4 genotype. A secondary aim of the study was to investigate whether the additional presence of stroke prior to or at the time of the measurement of the biomarkers changed the magnitude of the association between biomarkers of neurodegenerative diseases and dementia risk.

2 | METHODS

2.1 | Study population

Data are based on a nested case-control study within a community-based prospective cohort of White older adults followed for up to 17 years regarding clinical diagnosis of various age-related diseases and mortality (the ESTHER study). ESTHER participants (n = 9940) were recruited between 2000 and 2002 in Saarland, a southwestern German state. Eligibility criteria were age between 50 and 75 years, sufficient knowledge of the German language, residence in Saarland, and willingness to attend a general health examination performed by general practitioners (GPs).[23] No specific exclusion criteria were applied, as this would have impaired the generalizability of the ESTHER study.[24] Both at baseline and at follow-up participants provided health information and biological samples, including blood samples, which

were stored at −80°C. The ESTHER study was approved by the Ethics Committee of the Medical Faculty at Heidelberg University and the Physicians' Board of Saarland. All participants provided written informed consent.

2.2 | Dementia assessment

During the 14- and 17-year follow-up, the GPs of 8353 ESTHER study participants (84%) could still be contacted and were asked to provide information relating to a diagnosis of dementia since enrollment and to send the corresponding medical records, if available.[25] GPs of participants who had dropped out of the study or had died were also contacted. From the available medical records it could be inferred that most dementia cases, independently of the dementia form reported by the GPs, also had cerebrovascular pathologies including encephalopathy, cerebral infarction, and microangiopathy. Both dementia diagnoses and cerebrovascular pathologies were mostly listed in the medical reports as an acquired diagnosis, but only limited details on the corresponding diagnostic procedures were provided. While the performance of a computed tomography or a magnetic resonance imaging was often reported, dementia diagnoses were rarely supported by biomarkers. All diagnostic procedures had to follow, as a minimum requirement, the International Classification of Diseases (ICD-10), because this is the official classification for the encoding of medical diagnoses in Germany.

In light of results of clinical–pathological studies performed among population-based cohorts showing that vascular pathologies are the most prevalent pathologies among demented patients,[26–27] and based on the observation that pure Alzheimer's disease (AD) pathology is very rare,[28] we hence assume that in the ESTHER cohort even in cases where AD dementia or vascular dementia is reported as primary diagnosis, mixed pathologies were likely to be present in the great majority of dementia cases. Hence, we will use the term dementia risk to indicate the risk of a clinical diagnosis of dementia reflecting a composite endpoint including a diagnosis of the clinical syndrome of AD (AD dementia), vascular dementia, and mixed dementia, with AD and vascular dementia indicating predominance of clinical symptoms closer to neurodegenerative or cerebrovascular pathologies, respectively. The present dataset included 507 controls (participants that remained without dementia diagnosis throughout follow-up) chosen at random and 261 dementia cases (dementia diagnosis occurring between baseline and the 17-year follow-up), as previously described.[25]

2.3 | Laboratory measurements

2.3.1 | Blood biomarkers for neurodegenerative diseases

GFAP, NfL, and p-tau181 were measured in a single batch in lithium-heparin plasma of baseline samples at the Center for Protein Diagnostics (PRODI) of Bochum University (Germany) using the single molecule array (Simoa) Neurology 4-Plex E Advantage Kit and Simoa pTau-181 Advantage V2 Kit (Quanterix) on a HD-X Analyzer as per manufacturer's instructions. Upon arrival at the laboratory the samples were thawed at room temperature and mixed thoroughly. After a centrifugation step at 10,000 × *g* for 5 minutes they were applied to a conical 96-well plate (Quanterix) and measured immediately, along with lot-specific calibrators and one low and one high concentrated lot-specific controls.[25] Measurements of Aβ (Aβ40 and Aβ42) were also performed, but the levels were below the limit of detection, possibly due to the use of lithium-heparin plasma[29] instead of ethylenediaminetetraacetic acid plasma as recommended by the manufacturer. Hence measurements of Aβ could not be used and were excluded from the analyses.

RESEARCH IN CONTEXT

1. **Systematic Review**: The literature was searched through pertinent databases, such as PubMed. Glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and phosphorylated tau181 (p-tau181) as blood biomarkers of neurodegenerative diseases have been widely studied, but how these biomarkers perform in community-based cohorts including individuals with comorbidity of neurodegenerative and cerebrovascular diseases (mixed dementia) and high cholesterol levels is largely unexplored.
2. **Interpretation**: The findings indicated that in this community-based cohort, GFAP and NfL were more promising than p-tau181 for predicting clinical diagnosis of dementia. The association with dementia risk changed depending on levels of total cholesterol. Apolipoprotein E ε4 genotype further modified the strength of the associations.
3. **Future Directions**: Future studies are required to evaluate whether the findings relating to cholesterol also apply to well-characterized, clinic-based cohorts with diagnoses of Alzheimer's disease and vascular dementia supported by biomarkers (imaging and biofluids).

2.3.2 | Total cholesterol

Cholesterol levels were determined in all ESTHER participants at baseline, with samples taken at the same time as the plasma used to measure the blood biomarkers. Cholesterol concentrations were measured in serum using a timed-endpoint method by which cholesterol esterase hydrolyzes esters to free cholesterol (Beckman Coulter SYNCHRON System[s]). The measurements were performed in the Laboratory of the University Clinic Heidelberg, Germany. Hypercholesterolemia was defined as TC ≥ 240 mg/dL.[30–31]

2.3.3 | *APOE* ε4 genotyping

ε alleles were determined based on allelic combinations of single nucleotide polymorphisms (SNP) rs7412 and rs429358 using TaqMan SNP genotyping assays. Genotypes were analyzed in an endpoint allelic discrimination read using a PRISM 7000 Sequence detection system (Applied Biosystems). In some cases with missing values ($n = 38$) the *APOE* genotype could be determined based on *APOE* SNP results from genome-wide association study data. Participants were divided into carriers of the ε4 allele (ε2/ε4, ε3/ε4, ε4/ε4) and non-carriers (ε2/ε2, ε2/ε3, ε3/ε3).

2.4 | Stroke and sociodemographic data

Medical diagnoses of stroke were made either at or prior to baseline and were self-reported. Detailed information on diagnostic procedures was not available. Age, sex, and educational level (less/equal to, or higher than 9 years of school education) were collected through the self-administered questionnaire administered at baseline.

2.5 | Statistical analysis

Main baseline characteristics of the included ESTHER cases and controls were described for the whole population and according to levels of TC. Multivariable logistic regression models adjusted for age, sex, educational level, TC, and *APOE* ε4 genotype were estimated for the outcome dementia. Biomarker values were divided into quartiles (Q), and odds ratios (OR) with 95% confidence intervals (CI) were calculated for the highest quartile (Q_4) compared to the other three quartiles ($Q_{1\text{-}3}$), serving as the reference group. All regression models were run for the overall sample, by levels of TC (high: ≥ 240 mg/dL; low < 240 mg/dL)[30–31] and by *APOE* ε4 genotype (carriers/non-carriers). Additional regression models adjusted for age and sex were performed stratified by stroke at baseline. The ORs in the main analyses were also calculated per one standard deviation (SD) increase of each biomarker. To assess whether outliers affected associations, the main results were repeated after excluding the outliers according to the interquartile range (IQR) method (Q_3 +1.5 x IQR). Interaction terms between biomarkers and cholesterol, *APOE* ε4, and stroke were also calculated and added to the regression models.

Dose-response analyses using restricted cubic spline functions (RCS) with knots at the 5th, 35th, 65th, and 95th percentiles were conducted. A receiver operating characteristic (ROC) curve was calculated to explore the discriminative power of the blood biomarkers in addition to age, sex, educational level, and *APOE* ε4 (main model). RCS functions and ROC curves were derived separately in subgroups with high and low TC; the values of GFAP, NfL, and p-tau181 were inserted as continuous variables. For all three biomarkers, the distributions were moderately right skewed. However, because the data were reasonably close to a normal distribution and for better interpretability, we used the original values. All analyses were performed with the statistical software SAS, version 9.4.

3 | RESULTS

3.1 | General characteristics of the study population

In this nested case-control study, most cases and controls were older than 60 years and a large majority had a low educational level (Table 1). In total, $n = 94$ (36%) cases and $n = 194$ (38%) controls had high TC and both among cases and controls this percentage was higher among *APOE* ε4 carriers than among non-carriers (cases: 42% vs. 32%, controls: 41% vs. 38%). For all three biomarkers, levels were higher among cases. Mean follow-up time from recruitment in the study until clinical diagnosis of dementia was 9.9 years.

3.2 | Markers of neurodegenerative diseases and total cholesterol

In the whole study population the values separating Q3 from Q4 were 123.50 pg/ml for GFAP, 21.50 pg/ml for NfL, and 2.07 pg/ml for p-tau181. Results of regression analyses showed that the odds of a dementia diagnosis were higher among participants with both high baseline levels of blood biomarkers and high TC levels than among participants with low TC (Table 2). This pattern was particularly evident for GFAP and NfL, for which ORs among participants with high TC were 5.10 (CI 2.45–10.60) and 2.96 (CI 1.43–6.14), respectively. For comparison, the ORs among participants with low TC were 2.44 (CI 1.47–4.07) and 1.15 (CI 0.69–1.92), respectively. After excluding the outliers (GFAP: ≥ 214.7pg/ml, NfL ≥ 36.5pg/ml, p-tau181 ≥ 3.5pg/ml) the values for Q4 in this restricted population were ≥ 118.0 pg/ml for GFAP, ≥ 20.5 pg/ml for NfL, and ≥ 1.9pg/ml for p-tau181. The odds for dementia were 2.63 (95% CI 1.74–3.99) for GFAP, 1.49 (95% CI 0.98–2.27) for NfL, and 1.32 (95% CI 0.87–2.00) for p-tau181. The pattern of the odds relating to high and low TC remained stable (GFAP: 3.93, 95% CI 1.92–8.05; NfL: 2.89, 95% CI 1.36–6.16; p-tau181: 1.55, 95% CI 0.77–3.14 and GFAP = 2.16, 95% CI 1.29–3.61; NfL = 1.09, 95% CI 0.65–1.82; p-tau181: 1.21, 95% CI 0.71–2.04, respectively). In a regression model not including the biomarkers and adjusted for age, sex, educational level, and *APOE* ε4, hypercholesteremia was not independently associated with increased odds of dementia (OR = 0.95; 95% CI 0.66–1.37).

3.3 | *APOE* ε4 genotype

The additional stratification of the models by *APOE* ε4 genotype revealed that the strongest association between GFAP and dementia risk was among non-carriers and between NfL and dementia risk among carriers. These results were supported by interaction effects between GFAP x TC and NfL x TC, which were higher among *APOE* ε4− and *APOE* ε4+, respectively (Table S1 in supporting information). In a model adjusted for age and sex the interaction terms *APOE* ε4 x $TC_{\geq 240\ mg/dL/<240\ mg/dL}$ and *APOE* ε4 x $TC_{per\ 1SD}$ for odds of dementia were 1.66 (95% CI 0.78–3.51) and 1.39 (95% CI 0.96–2.02),

TABLE 1 Main baseline characteristics of the study population

Dementia cases				Controls		
ESTHER study (*n* = 768)	**Overall *n* = 261**	**TC ≥ 240 mg/dL *n* = 94**	**TC < 240 mg/dL *n* = 167**	**Overall *n* = 507**	**TC ≥ 240 mg/dL *n* = 194***	**TC < 240 mg/dL *n* = 312***
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Age groups						
50–59	20 (7.7)	8 (8.5)	12 (7.2)	202 (39.8)	79 (40.7)	123 (39.4)
60–70	152 (58.2)	54 (57.5)	98 (58.7)	258 (50.9)	101 (52.1)	156 (50.0)
71–75	89 (34.1)	32 (34.0)	57 (34.1)	47 (9.3)	14 (7.2)	33 (10.6)
Sex						
Women	151 (57.9)	64 (68.1)	87 (52.1)	278 (54.8)	120 (61.9)	158 (50.6)
Men	110 (42.1)	30 (31.9)	80 (47.9)	229 (45.2)	74 (38.1)	154 (49.4)
Educational level						
Low [a]	214 (82.0)	75 (79.8)	139 (83.2)	394 (77.7)	156 (80.4)	238 (76.3)
High [b]	37 (14.2)	15 (16.0)	22 (13.2)	102 (20.1)	34 (17.5)	67 (21.5)
Missing values	10 (3.8)	4 (4.3)	6 (3.6)	11 (2.2)	4 (2.1)	7 (2.2)
APOE ε4						
Carriers [c]	103 (39.5)	43 (45.7)	60 (35.9)	131 (25.8)	54 (27.8)	77 (24.7)
Non-carriers [d]	143 (54.8)	46 (48.9)	97 (58.1)	364 (71.8)	137 (70.6)	226 (72.4)
Missing values	15 (5.8)	5 (5.3)	10 (6.0)	12 (2.4)	3 (1.6)	9 (2.9)
Stroke						
Yes	18 (6.9)	4 (4.3)	14 (8.4)	9 (1.8)	2 (1.0)	7 (2.2)
No	229 (87.7)	88 (9.4)	141 (84.4)	481 (94.9)	186 (95.9)	294 (94.2)
Missing values	14 (5.4)	2 (2.1)	12 (7.2)	17 (3.4)	6 (3.1)	11 (3.5)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
GFAP	133.2 (78.2)	146.0 (99.1)	126.0 (62.6)	87.0 (46.7)	83.4 (40.8)	89.3 (50.0)
NfL	22.8 (13.4)	24.1 (14.8)	22.1 (12.6)	15.8 (8.4)	15.8 (9.9)	15.8 (7.4)
P-tau181	2.1 (1.5)	2.3 (1.5)	2.1 (1.5)	1.7 (1.2)	1.7 (1.5)	1.7 (1.0)

Abbreviations: *APOE*, apolipoprotein E; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-tau181, tau phosphorylated at threonine 181; SD, standard deviation; TC, total cholesterol.

*The numbers sum up to 506 and not to 507 because one control measurement of cholesterol was not available.

[a] ≤ 9 years of school education.

[b] > 9 years of school education.

[c] Participants carrying *APOE ε4* genotype (ε2/ε4, ε3/ε4, ε4/ε4).

[d] Participants not carrying *APOE ε4* genotype (ε2/ε2, ε3/ε2, ε3/ε3).

respectively. Interaction terms between markers of neurodegeneration and *APOE ε4* did not indicate any increase in the odds for dementia and the estimates did not reach a significance level < 0.05 for any biomarker (results not shown).

3.4 | Diagnosis of stroke at baseline

Participants with lifetime history of stroke and high biomarker levels, especially NfL, had substantially increased odds of dementia (Table S2 in supporting information), a finding which was also reflected in increased odds for the interaction term between biomarkers and stroke in the whole cohort, but the small sample size of the study population and the very large CIs limit the interpretation of these results. Hence, for comparison, we also calculated the odds for dementia among participants with lifetime history of myocardial infarction (n = 44) and we observed that they were much lower than those seen for stroke (GFAP: 1.46 [95% CI 0.66–3.20], NfL: 1.09 [95% CI 0.61–1.93], p-tau181: 1.04 [95% CI 0.70–1.53]).

3.5 | Dose-response association and ROC curves

Restricted cubic spline curves indicated a steadily increasing dose-response association between higher GFAP levels and dementia among

TABLE 2 Associations of GFAP, NfL, and p-tau181 with dementia risk by total cholesterol and *APOE* ε4 status (ESTHER cohort study 2000–2017)

	Overall	TC ≥ 240 mg/dL	TC < 240 mg/dL
General population **ESTHER study (*n* = 768)**	**Odds ratio*** **(95% CI; *n* = cases)**	**Odds ratio*** **(95% CI; *n* = cases)**	**Odds ratio*** **(95% CI; *n* = cases)**
Glial fibrillary acidic protein			
$GFAP_{Q4\ (vs.\ Q1\text{-}3)}$	3.08 (2.04–4.66) (*n* = 261)	5.10 (2.45–10.60) (*n* = 94)	2.44 (1.47–4.07) (*n* = 167)
$GFAP_{per\ 1\ SD\ increase}$	1.66 (1.3-2-2.08)	2.54 (1.62–3.98)	1.41 (1.08–1.83)
APOE ε4+ [a]			
$GFAP_{Q4\ (vs.\ Q1\text{-}3)}$	3.13 (1.63–6.04) (*n* = 103)	2.40 (0.82–7.01) (*n* = 43)	3.90 (1.67-9.13)(*n* = 60)
$GFAP_{per\ 1\ SD\ increase}$	1.85 (1.30–2.62)	2.15 (1.21–3.83)	1.72 (1.10-2.68)
APOE ε4− [b]			
$GFAP_{Q4\ (vs.\ Q1\text{-}3)}$	3.41 (1.98–5.88) (*n* = 143)	10.02 (3.57–28.15) (*n* = 46)	2.05 (1.06–3.97) (*n* = 97)
$GFAP_{per\ 1\ SD\ increase}$	1.64 (1.20–2.23)	3.15 (1.56–6.35)	1.35 (0.97–1.89)
Neurofilament light			
$NfL_{Q4\ (vs.\ Q1\text{-}3)}$	1.57 (1.04–2.38) (*n* = 261)	2.96 (1.43–6.14) (*n* = 94)	1.15 (0.69–1.92) (*n* = 167)
$NfL_{per\ 1\ SD\ increase}$	1.45 (1.15–1.82)	1.56 (1.09–2.24)	1.40 (1.03–1.89)
APOE ε4+ [a]			
$NfL_{Q4\ (vs.\ Q1\text{-}3)}$	1.30 (0.62–2.70) (*n* = 103)	4.21 (1.15–15.40) (*n* = 43)	0.67 (0.26–1.74) (*n* = 60)
$NfL_{per\ 1\ SD\ increase}$	1.24 (0.81–1.90)	2.04 (0.89–4.68)	1.04 (0.65–1.67)
APOE ε4− [b]			
$NfL_{Q4\ (vs.\ Q1\text{-}3)}$	1.82 (1.10–3.01) (*n* = 143)	2.42 (0.98–5.97) (*n* = 46)	1.55 (0.84–2.85) (*n* = 97)
$NfL_{per\ 1\ SD\ increase}$	1.59 (1.21–2.09)	1.44 (0.96–2.17)	1.71 (1.17–2.52)
p-tau181			
$p\text{-tau181}_{Q4\ (vs.\ Q1\text{-}3)}$	1.29 (0.86–1.93) (*n* = 261)	1.51 (0.77–2.98) (*n* = 94)	1.19 (0.72–1.97) (*n* = 167)
$p\text{-tau181}_{per\ 1\ SD\ increase}$	1.18 (0.99–1.40)	1.23 (0.97–1.55)	1.13 (0.87–1.48)
APOE ε4+ [a]			
$p\text{-tau181}_{Q4\ (vs.\ Q1\text{-}3)}$	0.99 (0.52=-1.91) (*n* = 103)	1.10 (0.38=-3.18) (*n* = 43)	0.97 (0.41–2.26) (*n* = 60)
$p\text{-tau181}_{per\ 1\ SD\ increase}$	1.18 (0.86–1.61)	1.67 (0.88–3.18)	1.05 (0.74–1.47)
APOE ε4− [b]			
$p\text{-tau181}_{Q4\ (vs.\ Q1\text{-}3)}$	1.67 (1.00–2.79) (*n* = 143)	2.02 (0.83–4.94) (*n* = 46)	1.55 (0.82–2.93) (*n* = 97)
$p\text{-tau181}_{per\ 1\ SD\ increase}$	1.23 (0.99–1.52)	1.18 (0.89–1.57)	1.38 (0.95-1.99)

Abbreviations: *APOE*, apolipoprotein E; CI, confidence interval; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-tau181, tau phosphorylated at threonine 181; SD, standard deviation; TC, total cholesterol.

*All logistic regression models were adjusted for age, sex, educational level, TC (exception: stratified analyses by TC), and *APOE* ε4 genotype (exception: stratified analyses by *APOE* ε4).

[a]Participants carrying *APOE* ε4 genotype (ε2/ε4, ε3/ε4, ε4/ε4).

[b]Participants not carrying *APOE* ε4 genotype (ε2/ε2, ε3/ε2, ε3/ε3).

participants with TC ≥ 240 mg/dL (Figure 1). For the other markers, dementia risk seemed to increase more strongly in participants with high TC levels, but leveled off with higher biomarker levels or even showed a tendency to decrease at higher NfL levels. ROC curve analyses for dementia showed that GFAP was the marker with the strongest discriminative ability in this cohort. However, the change in the area under the ROC curve (AUC) was small and the CIs were largely overlapping. Specifically, the AUC increased from 0.811 (95% CI 0.758—0.864) for the main model to 0.841 (95% CI 0.791–0.891) after the inclusion of GFAP in the group of participants with high TC and from 0.768 (95% CI 0.724–0.813) to 0.780 (95% CI 0.737–0.824) in the group with low TC (Figure 2). The inclusion of NfL and p-tau181 to the main model increased the AUC only marginally.

4 | DISCUSSION

In this study, we examined associations of biomarkers of neurodegenerative diseases with dementia risk in a population with a high prevalence of mixed pathology and found that, especially for GFAP, the strength of the association changed depending on levels of TC and, partially, also on *APOE* ε4 genotype. Furthermore, the association of

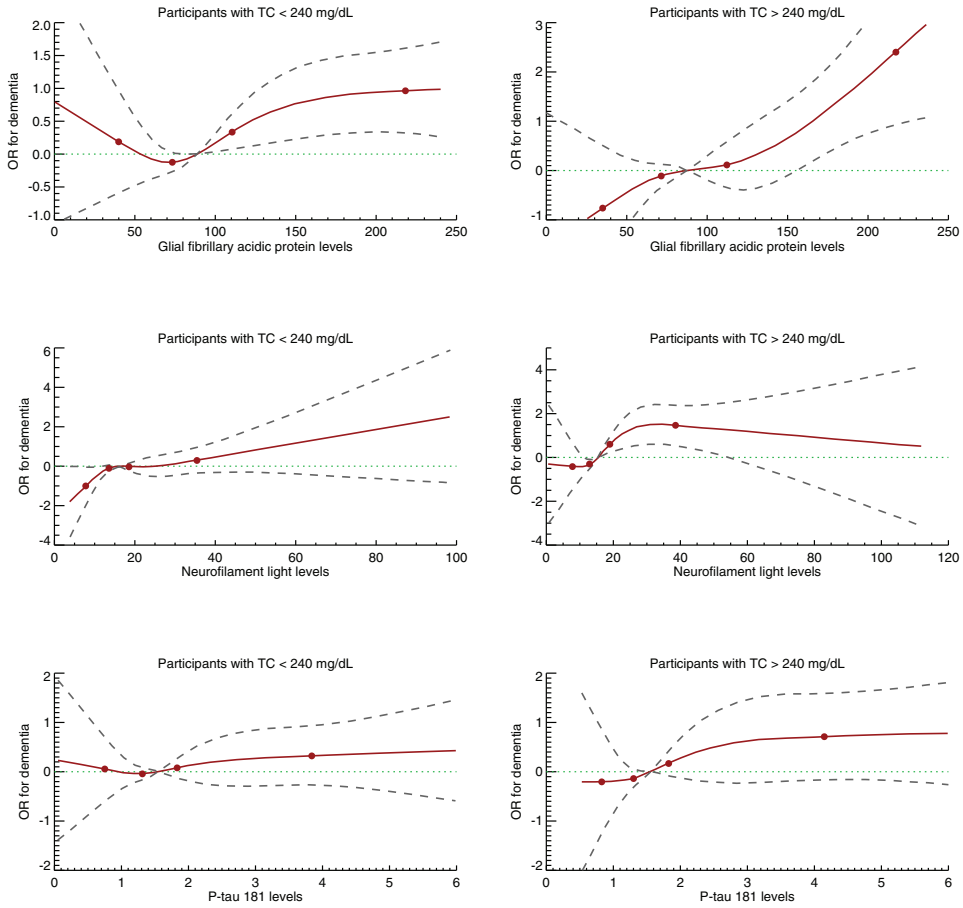

FIGURE 1 Dose-response associations of dementia by levels of total cholesterol; results of restricted cubic spline regression models. Top row: Association of dementia with GFAP (glial fibrillary acidic protein) among participants with low (< 240 mg/dL) and high (≥ 240 mg/dL) total cholesterol (TC) levels. Middle row: Association of dementia with NfL (neurofilament light chain) among participants with low (< 240 mg/dL) and high (≥ 240 mg/dL) TC levels. Bottom row: Association of dementia with p-tau 181 (tau phosphorylated at threonine 181) among participants with low (< 240 mg/dL) and high (≥ 240 mg/dL) TC levels. Results of spline regression models adjusted for age, sex, educational level, and apolipoprotein E ε4 polymorphism (ε2/ε4, ε3/ε4, ε4/ε4 vs. ε2/ε2, ε3/ε2, ε3/ε3). GFAP, NfL, and p-tau 181 were inserted in the models as continuous variables. Solid line: point estimates; dashed curved lines: 95% confidence interval limits; dashed horizontal line: reference line (odds ratio = 1); dots: knots (5th, 35th, 65th, and 95th)

all three biomarkers, especially NfL, with dementia risk was stronger among people with stroke. Altogether, the findings indicate that GFAP and, to a lesser extent, NfL are more promising than p-tau181 for predicting dementia risk in the older White general population, and they suggest that high TC as risk factor for dementia might become evident only in synergism with other markers or pathologies. This observation would explain the inconsistent findings reported in the literature relating to associations of hypercholesterolemia and dementia.[32–33]

The results obtained in this cohort, which yielded GFAP as the strongest predictor for dementia diagnosis, shall be interpreted in relation to the nature of the ESTHER cohort, which mainly included dementia cases with both cerebrovascular and neurodegenerative injury. In the presence of brain injury astrocytes undergo structural, molecular, and functional changes and become "reactive."[34] Individuals with diffuse cerebrovascular injury might be more subject to widespread astrogliosis than patients with more localized neurodegenerative brain lesions, such as individuals with AD pathology alone, and this might accelerate cognitive decline because, among other things, reactive astrocytes might also lose their ability to regulate adult neurogenesis and to control circuits involved in learning and memory.[35–36] Additionally, the strong predictive value of GFAP in this cohort supports a vascular link between brain and body health, possibly driven by neurovascular coupling,[2] a multidimensional process involving several agents and signals, with astrocytes playing an instrumental role.[37–38]

In this cohort the association between biomarkers, especially GFAP and NfL, and risk of a dementia diagnosis seems to depend also on cholesterol levels. These findings could be observed both in the analyses with categorical and continuous values of biomarkers, and they remained stable after excluding outliers, which suggests that the observations relating to cholesterol patterns are particularly robust. Because high GFAP levels might also point to a possible dysregulation in brain cholesterol produced by astrocytes, these results might suggest a mechanism involving a cross-talk between central and peripheral cholesterol metabolism, possibly driven by the permeabilization of the

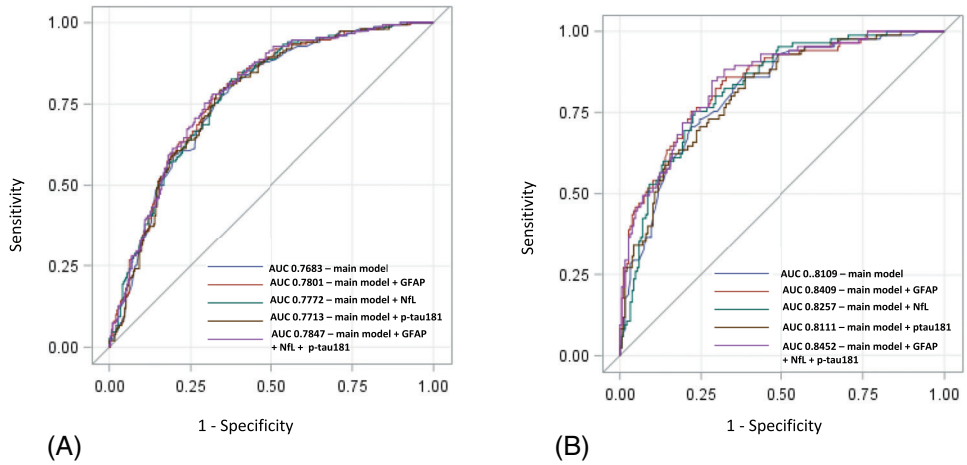

FIGURE 2 Results of receiver operating characteristic (ROC) curve analyses for dementia by levels of total cholesterol. A, ROC curve analyses for dementia with values of the corresponding areas under the curve (AUC) among participants with total cholesterol levels < 240 mg/dL. The main model includes age, sex, educational level, and apolipoprotein E (*APOE*) ε4 polymorphism (ε2/ε4, ε3/ε4, ε4/ε4 vs. ε2/ε2, ε3/ε2, ε3/ε3). Glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and tau phosphorylated at threonine 181 (p-tau 181) were inserted in the models as continuous variables. B, ROC curve analyses for dementia with values of the corresponding AUC among participants with total cholesterol levels ≥ 240 mg/dL. The main model includes age, sex, educational level, and *APOE* ε4 polymorphism (ε2/ε4, ε3/ε4, ε4/ε4 vs. ε2/ε2, ε3/ε2, ε3/ε3). GFAP, NfL, and p-tau 181 were inserted in the models as continuous variables

blood–brain barrier.[20,34] Another possible explaining factor could be the possible over-production of astrocyte-derived cholesterol driven by abnormal activation and proliferation of astrocytes, which in turn leads to increased AD-like pathology, as shown in lab mice,[39] a hypothesis that could not be tested here because of the lack of valid Aβ measurements.

Regarding *APOE* ε4, dementia risk was higher in non-carriers with high GFAP and hypercholesterolemia than in carriers. These results are counterintuitive, but the statistically significant interaction term between GFAP and cholesterol in the group of non-carriers supports such observations. In a recent study, although GFAP was not associated with *APOE* ε4, significantly higher GFAP levels were observed only among *APOE* ε4 non-carriers with AD pathology and not among *APOE* ε4 carriers,[12] and in a large population of older adults with mixed dementia pathologies hypercholesterolemia was linked to a lower dementia risk among *APOE* ε4 carriers.[33] Furthermore, in the population-based Rotterdam cohort hypercholesterolemia was associated with lower Aβ levels in carriers but not in non-carriers.[40] These results are in line with our findings obtained with GFAP, which point to lower odds for dementia among carriers than among non-carriers. The interaction term between TC and *APOE* ε4, although not statistically significant, possibly due to insufficient statistical power, also yielded increased odds for dementia, further pointing to a possible synergism between markers of neurodegeneration, especially GFAP and NfL, cholesterol, and *APOE* ε4 status in relation to dementia. However, it cannot be ruled out that the results are driven by the small number of participants in the *APOE* ε4 subgroups, hence they shall be interpreted with caution.

NfL is a blood marker of axonal damage and has been found elevated both in dementia, including AD, and in cerebrovascular disease,[19] and high NfL levels have been associated with an increased risk of all-cause dementia, AD, and AD progression.[11,41–42] Furthermore, it has been shown that an elevated vascular risk factor burden might synergistically interact with AD pathophysiology contributing to longitudinal increases in plasma NfL and cognitive decline.[43] Our results expand these previous observations by pointing to NfL as a marker for dementia with mixed pathology, especially among *APOE* ε4 carriers with hypercholesterolemia. Furthermore, we also show that NfL might be a candidate marker for risk of dementia among individuals with stroke and this supports previous observations pointing to NfL as a predictive marker for long-term outcome after ischemic stroke.[17]

Blood p-tau181 has been shown to be a marker associated with progressive AD-related neurodegeneration and capable of distinguishing AD from vascular dementia and other neurodegenerative disorders in previous research.[11,15] These results have been found in well-characterized cohorts with evidence of AD pathology in the brain. In our community-based cohort including both participants with AD and vascular dementia without neuropathological or biomarker evidence, p-tau181 was weakly associated with dementia and the results were not statistically significant. This might point to a strong contribution of cerebrovascular diseases to the clinical diagnoses of dementia in the ESTHER cohort and to the discrepancy between biological versus clinically defined AD.[44] These findings would also support p-tau181 as a biomarker specifically increased in AD and not in other dementias. The presence of hypercholesterolemia had a marginal impact on the strength of the association, even if patterns comparable to those observed with the other biomarkers could be noticed, but the associations were not statistically significant. However, the low performance of p-tau 181 in this cohort might also be explained with the platform used, because the performance of plasma phosphorylated tau may be platform dependent.[45]

The findings of the present study also support the results of our previous study showing that hypercholesterolemia and cardiovascular disease changed the association of *APOE ε4* with cognitive function in two independent cohorts[10] and also seem to suggest that the use of a population-based cohort with high circulating peripheral cholesterol, as opposed to a well-characterized, but potentially selective cohort, might reveal different insights on the interaction between vascular and neurological diseases. However, to fully understand the relationship between markers of neurodegenerative disease and cholesterol it is important that future studies focus on the different components of TC, especially LDL cholesterol, which was not available for the whole cohort. Furthermore, in this study it was not possible to assess possible effects of cholesterol levels on biochemical reactions and the measurements of TC and blood biomarkers were performed at the same time point, which prevented a temporal exploration of the observed association and limited its interpretation. It would also be important to disentangle effects of *APOE ε4*, cholesterol, and vascular pathology, and investigate possible therapeutic opportunities, as suggested by a recent study that established a functional link among *APOE ε4*, cholesterol, myelination, and memory.[46]

The findings of this cohort should also be replicated in populations of different racial and ethnical compositions,[47] because the results of this study can only be generalized to the older White population. Other important limitations were the lack of brain biomarkers for AD pathology and of Aβ measurements in blood, and the dementia diagnoses made in community settings, which did not allow differentiation between AD and vascular dementia with certainty. Despite such limitations relating to the diagnoses made in a community setting it is to be noted that, if the ultimate goal of blood biomarkers of neurodegenerative diseases is to replace brain biomarkers, and if such biomarkers shall also be used in clinical settings, the use of a population-based cohort with mixed dementia pathologies and with diagnoses reflecting real-word clinical practice is of outmost importance to assess the predictive performance of the biomarkers with greater external validity. This study showed that the informative and diagnostic value of biomarkers developed in research cohorts with highly selected participants might be different in community-based cohorts and that the interplay with cerebrovascular injuries, and vascular and genetic risk factors shall also be considered when such biomarkers are used in community settings.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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