Diagnosis, Assessment
& Disease Monitoring

# Sex differences in CSF biomarkers for neurodegeneration and blood-brain barrier integrity

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## Abstract

**Introduction:** As cerebrospinal fluid (CSF) neurofilament light protein (NfL) and the CSF/serum albumin ratio ($Q_{Alb}$) are used in the clinical routine, the impact of demographic factors on these biomarkers is important to understand.

**Methods:** Participants were derived from two Swedish samples: the population-based H70 Study (n = 308, age 70) and a clinical routine cohort (CSF NfL, n = 8995, $Q_{Alb}$, n = 39252, age 0 to 95). In the population-based study, $Q_{Alb}$ and NfL were examined in relation to sex, cardiovascular risk factors, and cerebral white matter lesions (WMLs). In the clinical cohort, $Q_{Alb}$ and NfL sex differences were tested in relation to age.

**Results:** Men had higher $Q_{Alb}$ and NfL concentrations and had higher $Q_{Alb}$ and NfL concentrations from adolescence throughout life. NfL was not related to WML, but $Q_{Alb}$ correlated positively with WMLs.

**Discussion:** The CSF NfL sex difference could not be explained by vascular pathology. Future studies should consider using different reference limits for men and women.

**KEYWORDS**
albumin ratio, blood-brain barrier integrity, CSF biomarkers, neurofilament light, sex differences

# 1 | INTRODUCTION

Cerebrospinal fluid (CSF) neurofilament light protein (NfL) and CSF/serum albumin ratio ($Q_{Alb}$) are widely used biomarkers that aid diagnostics and disease monitoring of neurodegenerative and neuroinflammatory diseases in both research and clinical settings. CSF NfL is a biomarker signaling rate of ongoing neuronal decay in conditions such as multiple sclerosis (MS), Alzheimer's disease (AD), stroke, frontotemporal dementia (FTD), and Creutzfeldt-Jakob disease.[1–7] $Q_{Alb}$ is a biomarker of blood-brain barrier integrity and is altered in multiple neurodegenerative and inflammatory diseases.[8] In clinical chemistry, the possible influence of demographic factors such as age and sex on biomarker levels is examined, and if needed age- and sex-specific reference ranges are established, for example, plasma levels of hemoglobin, creatinine, and apolipoprotein A1 and B. It is known that CSF NfL increases with age, but it has been less examined if there are sex differences or other factors such as cardiovascular pathology that alter concentrations and reference ranges. A recent meta-analysis found higher NfL concentrations in men among healthy controls and some neurodegenerative conditions, and the same finding was observed in a small data set from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study.[9,10] However, the relation with possible vascular factors was not examined. Regarding $Q_{Alb}$, it was recently reported that men had higher levels of $Q_{Alb}$ than women from childhood and throughout life.[11] These differences might indicate a need to consider sex differences when constructing reference ranges. Currently, reference ranges for $Q_{Alb}$ and CSF NfL are commonly adjusted by age, but no adjustment due to sex is applied.[12,13]

Therefore, we aimed to determine whether there are sex differences in CSF NfL and $Q_{Alb}$ in non-demented population-based elderly, and in clinical patients across the lifespan. We hypothesized that CSF NfL and $Q_{Alb}$ levels would be higher in men. As a secondary aim, we sought to determine whether sex differences in these biomarkers could be explained by specific pathologies, such as cerebrovascular disease or demographic factors, to elucidate whether sex differences were due to sex-specific or non–sex-specific factors. In this aim, we hypothesized that CSF NfL concentration and $Q_{Alb}$ would be correlated, and that the sex differences could be explained by higher prevalence of cerebrovascular disease in men.

# 2 | METHODS

## 2.1 | Population-based study

This study included a population-based cohort comprising 70-year-olds recruited between 2014 and 2016, as part of the Gothenburg H70 Birth Cohort Studies, Sweden. Gothenburg residents were systematically selected from the local population based on their birthdates; 322 consented to a lumbar puncture (LP) as described previously.[14] Eight participants that lacked data on both CSF NfL and $Q_{Alb}$ were excluded. One participant was excluded due to an outlier level of CSF NfL, outside of 10 standard deviations (SD) from the mean. Furthermore, as the

## RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using traditional (eg, PubMed) sources and meeting abstracts and presentations. Cerebrospinal fluid (CSF) neurofilament light protein (NfL) concentrations are known to increase with age, but sex differences have been less examined. Higher CSF/serum albumin ratio ($Q_{Alb}$) and CSF NfL in males have recently been described. However, previous studies have not examined sex differences in CSF NfL in childhood or adolescence and have not examined the effect of vascular pathology.

2. Interpretation: Our findings show that CSF NfL and $Q_{Alb}$ are higher in men from adolescence throughout life and in CSF NFL irrespective of white matter pathology.

3. Future directions: The manuscript proposes that for clinical routine use of NfL and $Q_{Alb}$ demographic factors should be considered and that more studies examining possible influencing factors on CSF biomarkers are needed.

purpose of this study was to examine the possible underlying sex differences in CSF NfL and $Q_{Alb}$ concentrations, we chose to exclude all participants with a dementia diagnosis (n = 5). The final cohort for this study comprised 308 non-demented participants aged 70.

## 2.2 | Clinical routine patient cohort

A second cohort was used to study sex differences in CSF biomarker levels in relation to age and was compiled using archived data on all CSF NfL and $Q_{Alb}$ measurements for clinical purposes performed CSF sampling at the clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Sweden, from January 1, 2005 to June 1, 2012. All measurements of $Q_{Alb}$ and CSF NfL on patients ages 0 to 95 were included, regardless of clinical indication for the LP. For patients with repeated measurements, only the first measurement was included. The resulting data set consisted of 8995 subjects for the NfL analysis and 39,252 subjects for $Q_{Alb}$.

## 2.3 | Assessment of covariates

Several cardiovascular and related covariates in the H70 population-based study were assessed (diagnosis of hypertension, diabetes, previous stroke diagnosis, smoking in the past or present, presence of any heart disease, alcohol use as defined by the patients estimation of average total weekly consumption, apolipoprotein E $\varepsilon 4$ [$APOE$ $\varepsilon 4$)] carriership, clinical dementia rating [CDR] score, body mass index [BMI], current statin treatment, current renin-angiotensin-aldosterone

system [RAAS] treatment, current $\beta$-blocking and calcium blocking agent treatment, and total serum cholesterol) as described previously.[14–16] The neuropsychiatric and somatic examinations were conducted at the neuropsychiatric outpatient clinic at the Sahlgrenska University Hospital or in the participant's home by trained research nurses or medical doctors, as described previously.[15] Information on these covariates was not available for the clinical routine sample.

## 2.4 | CSF measurements

All CSF analyses were performed in clinical practice by board-certified laboratory technicians using procedures accredited by the Swedish Board for Accreditation and Conformity Assessment. CSF collection, processing, and storage procedures have been described previously.[17] CSF NfL was measured using a commercial enzyme-linked immunosorbent assay (ELISA; NF-light ELISA, Uman Diagnostics, Umeå, Sweden) as described by the manufacturer. CSF and serum albumin concentrations were measured by immunonephelometry on a Beckman Image Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA). $Q_{Alb}$ was calculated as CSF albumin (mg/L)/serum albumin (g/L). Longitudinal stability in the measurements over time was ascertained using an elaborate system of internal quality control samples and testing of incoming reagents. CSF amyloid beta 1-42 ($A\beta42$) was measured using a sandwich ELISA (INNOTEST $AB_{1-42}$), as described previously.[18]

## 2.5 | Magnetic resonance imaging (MRI) data acquisition and image processing

All H70 study participants (n = 308) were scanned on a 3T Philips Achieva system (Philips Medical Systems) at the Aleris Clinic in Gothenburg using a three-dimensional (3D) T1-weighted, a 3D fluid-attenuated inversion recovery (FLAIR), and a T2-weighted scan. The full magnetic resonance imaging (MRI) protocol and sequence detail are previously described.[15] The following MRI markers of white matter pathology were used as proxies for cerebrovascular pathology: total white matter lesion (WML) volume and WML volumes for sublobar regions, and the temporal, frontal, occipital, parietal, and limbic lobes.

The number and volume of WMLs were automatically segmented with LST and FreeSurfer. LST is an open source segmentation toolbox implemented in the SPM software (https://www.fil.ion.ucl.ac.uk/spm/). LST utilizes a lesion-prediction algorithm (LPA) based on FLAIR image intensity distribution (hyperintensities). MRI data management and processing was done with the HiveDB system.[19]

## 2.6 | Statistical analysis

Differences between men and women in demographic variables, biomarker concentrations, MRI measures, and possible covariates were analyzed using $t$ tests (age, BMI, serum cholesterol, and all MRI measures) and Mann-Whitney $U$ tests (NFL, $Q_{Alb}$) for continuous variables and chi-square statistics for categorical variables. To identify variables associated with NfL and $Q_{Alb}$ levels in the population-based cohort, we performed Spearman correlation analyses between NfL and $Q_{Alb}$ with age, hypertension, diabetes, stroke, smoking, alcohol use, APOE $\varepsilon4$ carriership, BMI, statin treatment, current $\beta$-blocking and calcium-blocking agent treatment, RAAS treatment, total cholesterol, CDR scores, and CSF $A\beta42$. The variables that were significantly associated with NfL and $Q_{Alb}$ in these analyses were then included as predictors in forward linear regression models using NfL and $Q_{Alb}$ as dependent variables and sex as a covariate. In addition, because higher NfL concentrations were associated with increasing age, we included age as an additional covariate in the NfL model. Due to significantly skewed distributions, biomarker levels were logarithmically transformed where appropriate. In all analyses, a two-tailed, $P < 0.05$ was considered significant. All statistical analyses were performed in SPSS version 25 (IBM, New York).

## 2.7 | Ethics—standard protocol approvals, registrations, and patient consent

This study was approved by the Ethics Committee for Medical Research at the University of Gothenburg. All participants in the population-based study gave informed consent before taking part in the study. Written informed consent was obtainend from all participants in the H70 study. In the second clinical routine patient cohort, the biomarker data was compiled using archived data for clinical purposes performed CSF sampling at the clinical Neurochemistry Laboratory, Sahlgrenska University Hospital. The regional ethical committee at the University of Gothenburg has approved both studies.

## 3 | RESULTS

### 3.1 | Demographics and covariates

The population-based study sample consisted of 146 women and 162 men, without dementia (Table 1). Compared to women, men were more often diagnosed with diabetes (12% vs 5%, $P = 0.028$) and had higher alcohol consumption in grams per week (chi-square = 23.18, $P = 0.003$). Men also received RAAS-blocking treatment (36% vs 22%, $P = 0.017$) and statin treatment (28% vs 16%, $P = 0.015$) more often than women, and their total serum cholesterol levels were lower (mean 5.21 vs 5.98, $P < 0.001$).

There were no significant differences between men and women in age (mean 70.9 vs 70.9, $P = 0.98$), BMI (mean 26.3 vs 25.5, $P = 0.09$), APOE $\varepsilon4$ carriership (chi-square = 5.4, $P = 0.07$), smoking (chi-square = 6.3, $P = 0.18$), previous stroke diagnosis (chi-square = 1.8, $P = 0.17$), or presence of hypertension (chi-square = 0.67, $P = 0.41$). Men and women were also as frequently treated with diuretics (chi-square = 23, $P = 0.63$), $\beta$-blocking (chi-square = 3.0, $P = 0.23$), and calcium-blocking agents (chi-square = 0.99, $P = 0.61$) Table 1 gives median (IQR).

**TABLE 1** Characteristics and cerebrospinal fluid levels according to biological sex in cognitively normal 70-year-old participants of the Gothenburg H70 Birth Cohort studies

| Characteristics | Median (IQR)/N(%) |
|---|---|
| Female (n = 146) | |
| Age | 70.8 (70.7, 71.2) |
| Presence of *APOE* ε4 allele | 44 (30.6%) |
| Body mass index | 24.5 (22.3, 27.4) |
| Diabetes | 7 (4.8%)[*] |
| Hypertension | 68 (46.6%) |
| Serum total cholesterol | 6.0 (5.2, 6.6)[**] |
| Statin treatment | 23 (15.7%)[*] |
| CSF NfL (pg/mL) | 696.0 (525.0, 857.0)[*] |
| CSF $Q_{Alb}$ (pg/mL) | 5.7 (4.5, 6.7)[**] |
| Male (n = 162) | |
| Age | 70.9 (70.7, 71.1) |
| Presence of *APOE* ε4 allele | 66 (40.7%) |
| Body mass index | 25.9 (23.6, 28.3) |
| Diabetes | 19 (11.8%)[*] |
| Hypertension | 83 (51.2%) |
| Serum total cholesterol | 5.2 (4.4, 5.8)[**] |
| Statin treatment | 45 (27.7%)[*] |
| CSF NfL (pg/mL) | 742.5 (576.0, 976.0)[*] |
| CSF $Q_{Alb}$ (pg/mL) | 6.7 (5.3, 8.3)[**] |

NfL, neurofilament light protein, serum total cholesterol in mmol/L; QAlb, CSF/serum albumin ratio.

[*]Significant difference at the <0.05 level.

[**]Significant difference at the <0.001 level.

In the population-based cohort, men had higher occipital WML volumes than women (mean 0.15 vs 0.06, $P < 0.001$).

## 3.2 | CSF biomarkers in the population based sample

There were significant correlations between CSF NfL and $Q_{Alb}$ in the whole sample ($r_s = 0.27$, $P < 0.001$), as well as for both men ($r_s = 0.22$, $P = 0.004$) and women ($r_s = 0.25$, $P = 0.002$) separately. Men had higher CSF NfL concentrations (median 742.5 vs 696.0, $P = 0.011$) and higher $Q_{Alb}$ (median 6.7 vs 5.7; $P < 0.001$) (Table 1, Figure 1) than women.

## 3.3 | Association between CSF $Q_{Alb}$ and NfL and MRI evidence of cerebral vascular pathology in the population-based cohort

Among all participants, after adjusting for TIV, $Q_{Alb}$ correlated significantly with total WML volume ($r_s = 0.13$, $P = 0.032$), temporal WML volume ($r_s = 0.14$, $P = 0.016$), frontal WML volume ($r_s = 0.12$, $P = 0.038$), and parietal WML ($r_s = 0.13$, $P = 0.034$). There were no sig-

nificant correlations between $Q_{Alb}$ and any markers of white matter pathology in men. Among women, $Q_{Alb}$ correlated with frontal WML volume ($r = 0.18$, $P = 0.033$). However, there were no significant interactions between $Q_{Alb}$, sex, and frontal WML.

No significant correlations were found between CSF NfL and markers of white matter pathology in the whole group, or in men or women alone.

## 3.4 | Association between NfL and $Q_{Alb}$ with clinical variables in the population-based cohort

We found a significant correlation between $Q_{Alb}$ and RAAS treatment ($r_s = 0.143$, $P = 0.011$), statin treatment ($r_s = 0.136$, $P = 0.015$), cholesterol ($r_s = -0.147$, $P = 0.009$), diabetes ($r_s = 0.148$, $P = 0.008$), and BMI ($r_s = 0.180$, $P = 0.001$). In addition, NfL was significantly associated with BMI ($r_s = -0.126$, $P = 0.025$).

When the previous variables were entered in separate linear regression models to predict $Q_{Alb}$ and NfL concentrations while adjusting for sex ($Q_{Alb}$, NfL) and age (NfL), we found that BMI was associated significantly with higher $Q_{Alb}$ concentrations ($R^2 = 0.086$, B = 0.006, t = 3.316, $P = 0.001$) and lower NfL levels ($R^2 = 0.041$, BMI: B = −0.007, t = −2.421, $P = 0.016$).

## 3.5 | Sex differences in biomarkers and BMI in the population-based study

BMI was available for all participants in the population-based study (Table 1), but not for subjects in the clinical routine cohort. BMI correlated positively with $Q_{Alb}$ ($r_s = 0.16$, $P = 0.10$) and negatively with CSF NfL ($r_s = -0.17$, $P = 0.008$) in the whole population-based study. In men, BMI correlated with both $Q_{Alb}$ ($r_s = 0.20$, $P = 0.010$) and CSF NfL ($r_s = -0.19$, $P = 0.018$), but in women BMI did not significantly correlate with either $Q_{Alb}$ or CSF NfL.

## 3.6 | Sex differences in CSF biomarkers in the clinical study sample

We then examined sex differences in CSF biomarkers concentrations in 8982 (CSF NfL) and 39252 ($Q_{Alb}$) patients, ages 0 to 95 years, collected for clinical diagnostic purposes. Subject counts for all age categories in the clinical routine cohort are detailed in Table 2. Among all patients, men had higher CSF NfL concentrations (median 1460 vs 1180, $P < 0.001$) and higher $Q_{Alb}$ (median 6.7 vs 5.2, $P < 0.001$) than women. However, correlations between CSF NfL and $Q_{Alb}$ were similar when examining the whole cohort ($r_s = 0.44$, $P < 0.001$), men only ($r_s = 0.41$, $P < 0.001$), and women only ($r_s = 0.43$, $P < 0.001$).

When stratifying by age, men had higher $Q_{Alb}$ in all age groups ($P < 0.05$) except in 5- to 10-year-olds ($P = 0.163$) (Figures 2A and 3A).

Men also had significantly ($P < 0.05$) higher CSF NfL concentrations in most age groups, except in the groups between 0 and 25 and 90 and 95 years of age (Figures 2B and 3B).
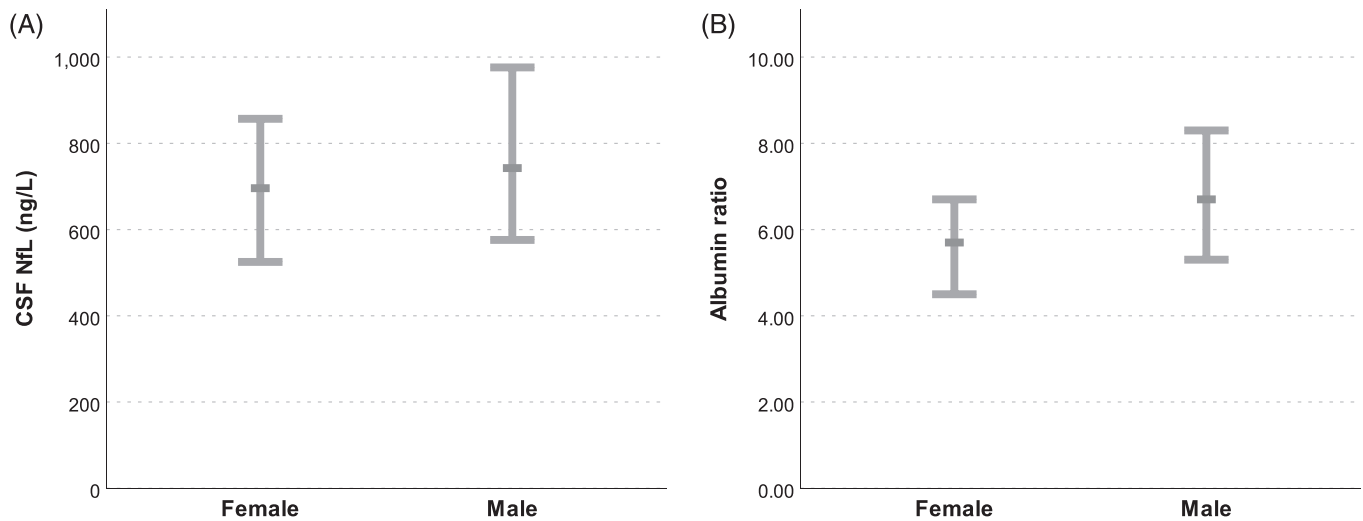
**FIGURE 1** (A) Cognitively normal 70-year-old men exhibit higher cerebrospinal fluid neurofilament light protein concentrations than women in the population-based Gothenburg H70 Birth Cohort studies. Median concentrations and interquartile ranges are displayed in the chart.
(B) Cognitively normal 70-year-old men exhibit higher $Q_{Alb}$ than women in the population-based Gothenburg H70 Birth Cohort studies. Medians and interquartile ranges are displayed in the chart

**TABLE 2** Clinical routine cohort description

| | NfL | | | $Q_{Alb}$ | | |
|---|---|---|---|---|---|---|
| Age group | Total | Male (%) | Female (%) | Total | Male (%) | Female (%) |
| 0-4 | 228 | 130 (57%) | 98 (43%) | 1083 | 564 (52%) | 519 (48%) |
| 5-9 | 111 | 67 (60%) | 44 (40%) | 661 | 365 (55%) | 296 (45%) |
| 10-14 | 116 | 56 (48%) | 60 (52%) | 756 | 330 (44%) | 426 (56%) |
| 15-19 | 126 | 54 (43%) | 72 (57%) | 1063 | 386 (36%) | 677 (64%) |
| 20-24 | 140 | 43 (31%) | 97 (69%) | 1299 | 484 (37%) | 815 (63%) |
| 25-29 | 183 | 59 (32%) | 124 (68%) | 1818 | 623 (34%) | 1195 (66%) |
| 30-34 | 192 | 64 (33%) | 128 (67%) | 2226 | 802 (36%) | 1424 (64%) |
| 35-39 | 297 | 119 (40%) | 178 (60%) | 2524 | 966 (38%) | 1558 (62%) |
| 40-44 | 358 | 137 (38%) | 221 (62%) | 2503 | 986 (39%) | 1517 (61%) |
| 45-49 | 410 | 182 (44%) | 228 (56%) | 2428 | 1062 (44%) | 1366 (56%) |
| 50-54 | 481 | 232 (48%) | 249 (52%) | 2870 | 1261 (44%) | 1609 (56%) |
| 55-59 | 692 | 340 (49%) | 352 (51%) | 3281 | 1526 (47%) | 1755 (53%) |
| 60-64 | 985 | 504 (51%) | 481 (49%) | 3333 | 1633 (49%) | 1700 (51%) |
| 65-69 | 1220 | 603 (49%) | 617 (51%) | 3461 | 1676 (48%) | 1785 (52%) |
| 70-74 | 1321 | 691 (52%) | 630 (48%) | 3861 | 1863 (48%) | 1998 (52%) |
| 75-79 | 1152 | 579 (50%) | 573 (50%) | 3572 | 1626 (46%) | 1946 (54%) |
| 80-84 | 721 | 323 (45%) | 398 (55%) | 1902 | 838 (44%) | 1064 (56%) |
| 85-89 | 244 | 98 (40%) | 146 (60%) | 564 | 229 (41%) | 335 (59%) |
| 90-94 | 18 | 7 (39%) | 11 (61%) | 47 | 20 (43%) | 27 (57%) |
| Total | 8995 | 4288 (48%) | 4707 (52%) | 39252 | 17240 (44%) | 22012 (56%) |

Subjects counts in each age group and with data available on cerebrospinal fluid neurofilament light protein level and $Q_{Alb}$ ratio.
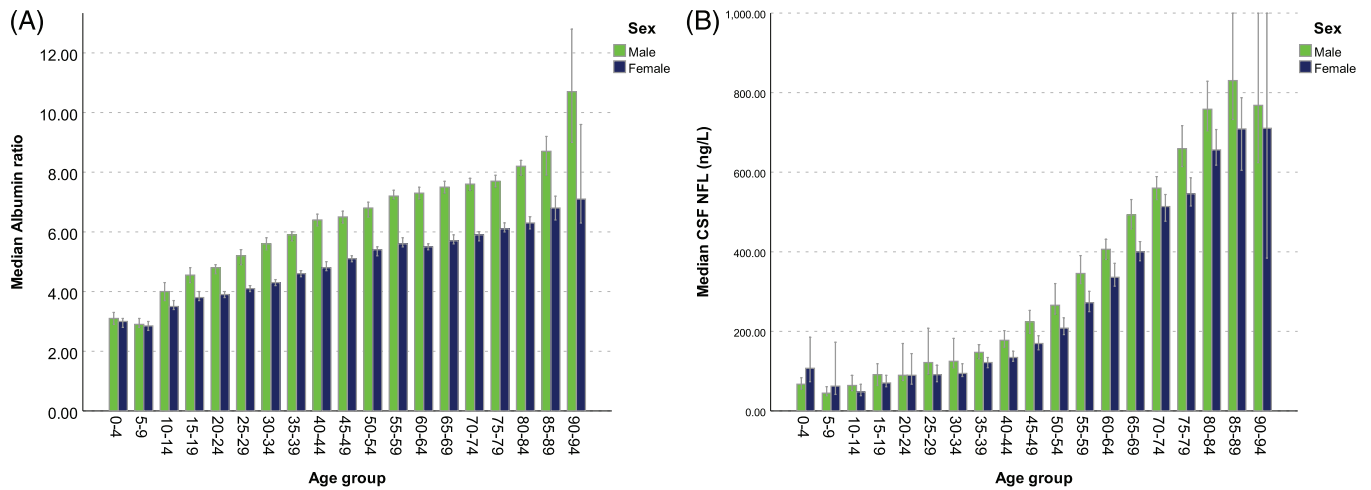NfL, Neurofilament light protein, $Q_{Alb}$, CSF/serum albumin ratio.

**FIGURE 2** (A) Median $Q_{Alb}$ and age in the clinical routine cohort. Men exhibit 25% to 30% higher $Q_{Alb}$ in all age group from ages 10 and up. Error bars represent 95% confidence intervals. (B) Median cerebrospinal fluid neurofilament light concentrations and age in the clinical routine cohort. Men exhibit 15% to 30% higher neurofilament light concentrations in most age groups from ages 10 and up. Error bars represent 95% confidence intervals
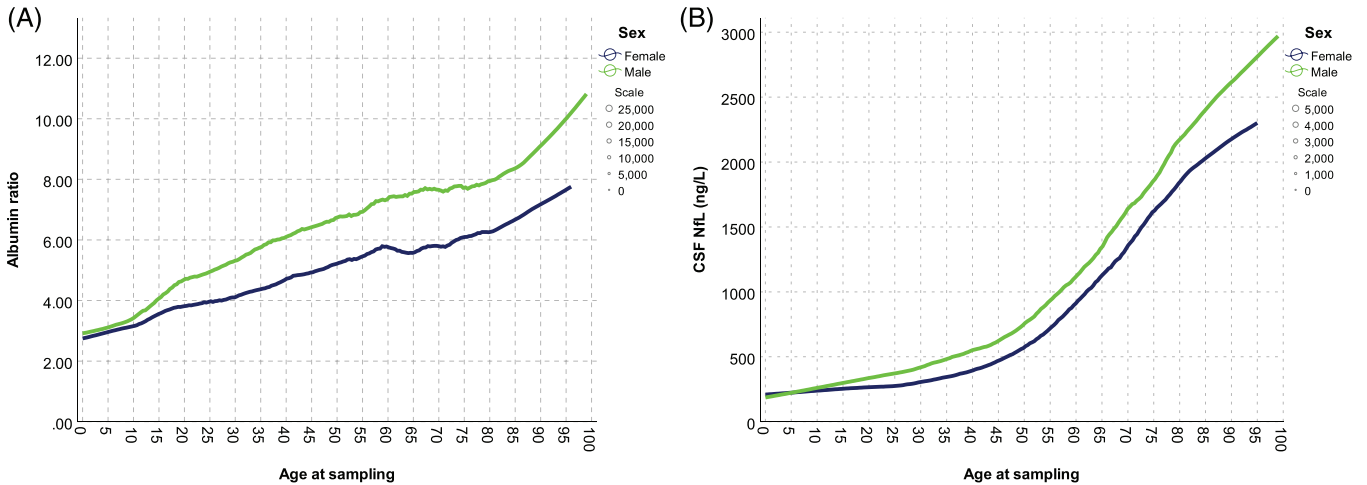


**FIGURE 3** (A) $Q_{Alb}$ and age in the clinical routine cohort. Local regression trend lines show $Q_{Alb}$ starting to diverge in the early teens and remain constantly separated at ages after about 15 to 20. (B) Cerebrospinal fluid neurofilament light and age in the clinical routine cohort. Both sexes exhibit accelerating increases in neurofilament light concentrations with age, and men have higher concentrations than women from teen years and throughout life.

## 4 | DISCUSSION

In this study we aimed to identify and examine sex differences in CSF NfL, a non-specific biomarker for neurodegeneration, and $Q_{Alb}$, a biomarker for BBB integrity, among non-demented 70-year-olds derived from a population-based sample and a clinical routine cohort, spanning ages 0 to 95. In both samples, men had higher $Q_{Alb}$ and CSF NfL levels than women. We initially hypothesized that higher $Q_{Alb}$ and CSF NfL levels in men could be due to the higher prevalence of vascular disease in men. However, after adjustment for cerebrovascular pathology and vascular risk factors, the sex difference in $Q_{Alb}$ and CSF NfL remained. Furthermore, when exploring CSF NfL and $Q_{Alb}$ in relation to age in the large sample of clinical routine measurements, we found that males had higher levels starting in adolescence and

remaining throughout the lifespan. These results suggest that the higher levels of CSF NfL and $Q_{Alb}$ are not due to cardio- or cerebrovascular disease, as vascular pathology increases with age and is rare in younger age groups. The phenomenon of higher $Q_{Alb}$ in males across all ages has recently been described.[11] However, previous studies have not examined sex differences in CSF NfL in childhood or adolescence.

Previous studies have shown correlations between $Q_{Alb}$ and CSF NfL concentrations, and that blood-brain barrier leakage is common in dementia, especially in vascular dementia.[20] Studies have also shown associations between white matter pathology and both CSF NfL and $Q_{Alb}$.[21–23] In this study we found correlations between CSF NfL and $Q_{Alb}$ in cognitively normal 70-year-olds without evidence of dementia, and only a weak correlation between $Q_{Alb}$, but not NfL, and

vascular pathology and risk factors. These results, together with the elevated CSF NfL and $Q_{Alb}$ levels in young age groups, suggest that other factors are contributing to the observed sex differences. Data from a recent study did not indicate any sex difference changes at puberty or menopause, and propose that their findings point away from hormonal factors as explanatory factors.[11] Our data, which was also derived from clinical routine samples, partly contradicts this notion by showing that $Q_{Alb}$ and CSF NfL concentrations in males and females start to diverge in the early teens, roughly coinciding with puberty, and that the separation remains relatively constant at ages over 20 years. Notably, the ratio of CSF $Q_{Alb}$ and NfL between the sexes did not differ at or after the age of natural menopause. This suggests that changes in estrogen levels are not the cause of the sex difference. It should be noted that the data in the clinical routine sample is taken from subjects who had an LP on clinical indication and cannot be generalized to healthy populations.

Both NfL and $Q_{Alb}$ increased with age in our clinical routine cohort, corroborating previous studies.[6,24] CSF NfL has been shown to reflect rate of neurodegeneration in several pathological processes such as stroke, MS, FTD, and Creutzfeldt-Jakob disease but also in normal aging measured as loss of brain parenchymal fraction (BPF).[1–6] However, faster age-related decline in BPF in men has, to our knowledge, not been observed, contradicting it as the driver of the gap between sexes in CSF NfL. Taken together, this may indicate that higher CSF NfL concentrations in men reflect a higher axonal turnover.

BMI has previously been observationally reported to be associated with higher $Q_{Alb}$.[25] Of interest, both obesity and AD are affected by sex steroid hormones and differ between sexes.[26,27] In this study, BMI was associated with both $Q_{Alb}$ and CSF NfL in regression modeling. BMI also correlated weakly with both CSF NfL and $Q_{Alb}$, but not in all groups. Unfortunately, BMI data were not available in the clinical routine cohort prohibiting analysis across age categories. In any case, adjusting for BMI did not eradicate sex as a predictor of $Q_{Alb}$.

The main strengths of this study included the use of the well-characterized and population-based H70 cohort for detailed analysis of confounders, in combination with access to the high number of subjects in the clinical routine cohort. The clinical routine sample also provided means of studying the effect of age on biomarker levels. This study also had limitations. The lack of detailed clinical data on the clinical routine cohort might lead to bias, as we had no information on the reason for each individual LP. It is, however, unlikely that this would bias differences in relation to sex, as there are no major indications for an LP that differ greatly between sexes. An exception is MS, which is roughly twice as common in women. Disease onset in MS usually occurs in the third to fourth decade of life, probably explaining the high proportion of women in the clinical routine cohort with CSF NfL measurements around those age categories.[28] Another limitation is that although we have previously shown that participants in LP were similar regarding a number of factors in the H70 study, we cannot exclude the possibility that participants with and without LP differed in health status, even though it is unlikely that this selection bias should affect sex differences.[14] Although the H70 CSF sample was relatively large for a population-based cohort, it was small in overall size. In addition, men were slightly over-represented compared to women, but this should also not affect sex differences in CSF levels. Finally, it is plausible that the studied pathologies do not capture all possible biological determinants of CSF NfL and $Q_{Alb}$. Thus there may still be unknown factors that could explain the sex differences. However, this study highlights the need for further studies in large cohorts of healthy individuals to evaluate the need for sex-specific reference ranges.

In conclusion, this study corroborated previous findings of higher $Q_{Alb}$ in men in most age groups, and could add the finding that this difference emerges in ages 10 to 20, coinciding with puberty. This study could also reveal a similar age gap between men and women in CSF NfL concentrations, that also develops in adolescence or early adult years and remains constant throughout life. Vascular pathology as a main driver of these sex differences was contradicted by our results. The results of this study suggest that hormonal factors influence blood-brain barrier integrity, and patterns of neuronal life cycles. Further studies into the influence of hormonal factors on blood-brain barrier integrity and CSF NfL are needed. Our results suggest that reference limits for both $Q_{Alb}$ and CSF NfL concentration might need to include a consideration of sex.

## STATISTICAL ANALYSIS

Tobias Skillbäck and Joana Pereira.

## CONFLICT OF INTEREST

HZ has served at scientific advisory boards for Roche Diagnostics, Wave, CogRx, and Samumed, and has given lectures in symposia sponsored by Biogen and Alzecure and is a co-founder of Brain Biomarker

## Data availability statement

Anonymized data will be shared by request from any qualified investigator.

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## SUPPORTING INFORMATION

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