Decreased cerebrospinal fluid amyloid β 38, 40, 42, and 43 levels in sporadic and hereditary cerebral amyloid angiopathy

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Decreased Cerebrospinal Fluid Amyloid β 38, 40, 42, and 43 Levels in Sporadic and Hereditary Cerebral Amyloid Angiopathy

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Objective: Vascular amyloid β (Aβ) accumulation is the hallmark of cerebral amyloid angiopathy (CAA). The composition of cerebrospinal fluid (CSF) of CAA patients may serve as a diagnostic biomarker of CAA. We studied the diagnostic potential of the peptides Aβ38, Aβ40, Aβ42, and Aβ43 in patients with sporadic CAA (sCAA), hereditary Dutch-type CAA (D-CAA), and Alzheimer disease (AD).

Methods: Aβ peptides were quantified by immunoassays in a discovery group (26 patients with sCAA and 40 controls), a validation group (40 patients with sCAA, 40 patients with AD, and 37 controls), and a group of 22 patients with D-CAA and 54 controls. To determine the diagnostic accuracy, the area under the curve (AUC) was calculated using a receiver operating characteristic curve with 95% confidence interval (CI).

Results: We found decreased levels of all Aβ peptides in sCAA patients and D-CAA patients compared to controls. The difference was most prominent for Aβ42 (AUC of sCAA vs controls for discovery: 0.90, 95% CI = 0.82–0.99; for validation: 0.94, 95% CI = 0.89–0.99) and Aβ43 (AUC of sCAA vs controls for discovery: 0.95, 95% CI = 0.88–1.00; for validation: 0.91, 95% CI = 0.83–1.0). All Aβ peptides except Aβ43 were also decreased in sCAA compared to AD (CSF Aβ38: AUC = 0.82, 95% CI = 0.71–0.93; CSF Aβ40: AUC = 0.88, 95% CI = 0.80–0.96; CSF Aβ42: AUC = 0.79, 95% CI = 0.66–0.92).

Interpretation: A combined biomarker panel of CSF Aβ38, Aβ40, Aβ42, and Aβ43 has potential to differentiate sCAA from AD and controls, and D-CAA from controls.

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Cerebral amyloid angiopathy (CAA) is pathologically defined by the accumulation of amyloid β (Aβ) in the arterioles, leptomeningeal vessels, and capillaries of the cerebral cortex.[1] The clinical spectrum is characterized by intracerebral hemorrhage (ICH), cognitive impairment or dementia, and transient focal neurological episodes.[2] Transient focal neurological episodes may mimic transient ischemic attacks, migraine auras, and focal seizures, but can be recognized by recurrent, stereotyped, transient episodes of spreading paresthesias, numbness, or weakness, typically lasting seconds to minutes, usually resolving over a similar period.[1,3,4] Histopathologically, moderate-to-severe CAA can be found in approximately one quarter of the elderly population upon examination of (postmortem) brain tissue,[5] although only a minority of them will develop symptoms. Dutch-type CAA (D-CAA) is a hereditary form of CAA, caused by a point mutation at codon 693 of the amyloid precursor protein (*APP*) gene on chromosome 21, with a young onset but with clinical symptoms similar to sporadic CAA (sCAA).[6]

The diagnosis of sCAA during life relies on the modified Boston criteria, which include the presence of lobar hemorrhage(s), strictly lobar microbleeds, and superficial siderosis on magnetic resonance imaging (MRI).[7] An update of these criteria, which additionally incorporates convexity subarachnoid hemorrhage and nonhemorrhagic markers, was recently published.[8] Although these criteria are quite sensitive, they have some limitations. The imaging markers in these criteria are likely to reflect end-stage disease, and they are indirect reflections of CAA, because they represent the consequences of CAA instead of its etiology, that is, vascular amyloid deposition. In addition, they lack specificity, because the incorporated imaging markers can also be (partially) caused by arteriosclerotic small vessel disease (SVD). Moreover, they cannot provide a definitive diagnosis without a brain biopsy, which is rarely performed. Lastly, the updated criteria have a sensitivity of only 55% in patients without ICH.

Cerebrospinal fluid (CSF) biomarkers may provide an opportunity to identify CAA at an earlier stage and can be used to monitor disease progression. It has previously been demonstrated that CSF Aβ40 and Aβ42 levels are decreased in patients with sCAA compared to both controls and patients with Alzheimer disease (AD).[9–11] Furthermore, CSF Aβ40 and Aβ42 levels were decreased in patients with presymptomatic D-CAA before CAA-related imaging abnormalities were found, and they were further decreased in patients with symptomatic D-CAA,[12] which indicates that these markers may serve as biomarkers of early stages of CAA.

In addition, reduced levels of CSF Aβ38 have been demonstrated in a single report on patients with sCAA versus controls.[13] CSF levels of another common Aβ peptide, Aβ43, have not been studied in CSF from patients with sCAA, but reduced levels have been reported in patients with AD when compared to controls.[14,15]

The aim of our study was to examine whether our previous findings of decreased CSF Aβ40 and Aβ42 can be extended to a larger panel of Aβ peptides in the CSF of patients with sCAA and D-CAA as compared to patients with AD and controls. For this, we studied separate discovery and validation cohorts of patients with sCAA and controls, and additionally validated our findings in patients with (pre)symptomatic D-CAA and controls. Furthermore, we assessed CSF Aβ levels in patients with a CSF biomarker profile indicative of AD.

Subjects and Methods

Participants

Patients with sCAA and Controls: Discovery. We included 26 patients with sCAA and 40 controls from Radboud University Medical Center (RUMC), Nijmegen, the Netherlands (see Fig S1). The inclusion criteria for the patients with sCAA included a diagnosis of probable (n = 19) or possible (n = 3) CAA according to the modified Boston criteria.[7] Additionally, we included patients (n = 4) with mixed lobar and deep hemorrhages/microbleeds. Availability of lumbar CSF was a prerequisite for inclusion. The CSF was collected in the context of either routine clinical workup (n = 4) or 2 cross-sectional studies investigating new CSF biomarkers for CAA (Cerebral Amyloid Angiopathy: Vascular Imaging and Fluid Markers of Amyloid deposition [CAVIA], n = 11 or Biomarkers for Cognitive Impairment Due to Cerebral Amyloid Angiopathy [BIONIC], www.radboudumc.nl/BCS, n = 11). All but 2 CSF samples were taken at least 3 months after symptomatic ICH.

Some of the patients with sCAA (n = 12) underwent a Montreal Cognitive Assessment (MoCA).

Some of the controls (n = 30) underwent a lumbar puncture as part of diagnostic workup to exclude central nervous system involvement of a systemic disease, a (central) neurological cause for their symptoms, or a neurological infection or inflammation. Exclusion criteria were neurodegenerative disease, known cognitive impairment, sepsis, a recent stroke (<6 months), and a malignancy in the central nervous system. The other controls (n = 10) were patients who underwent thoracoabdominal aortic aneurism repair, for which they had an external lumbar drain, from which CSF was sampled before the operation. They did not have known cognitive impairment or recent (<3 months) stroke or traumatic brain injury. The controls were sex-matched to the patients with sCAA (Table 1).

TABLE 1. Sporadic CAA Patients and Controls in the Discovery Experiments: Characteristics and Results of Magnetic Resonance Imaging and CSF Analysis

Characteristic	sCAA, n = 26	Probable CAA, n = 19	Controls, n = 40	sCAA vs Controls, *p*	Probable CAA vs Controls, *p*
Age, years	72 ± 7	72 ± 8	63 ± 8	$p < 0.001$[a]	$p < 0.001$[a]
Sex, M/F, n	18/8	7/12	28/12	$p = 0.95$[b]	$p = 0.60$[b]
MoCA	24 [20–28][c]	24 [20–28][d]	NA	—	—
Hypertension	48%[e]	44%[e]	43%[f]	$p = 0.72$[b]	$p = 0.94$[b]
CSF Aβ38, pg/ml	1,411 ± 520	1,400 ± 396	1,679 ± 526	$p = 0.015$[g]	$p = 0.013$[g]
CSF Aβ40, ng/ml	7.55 [4.91–9.10]	7.58 [6.07–9.04]	9.08 [6.14–12.5]	$p = 0.015$[g]	$p = 0.024$[g]
CSF Aβ42, pg/ml	356 [292–3,447]	353 [299–406]	822 [572–1,225]	$p < 0.001$[g]	$p < 0.001$[g]
CSF Aβ43, pg/ml	11.5 [9.27–14.6]	11.5 [10.6–12.8]	40.2 [20.9–53.6]	$p < 0.001$[g]	$p < 0.001$[g]

Note: Values are median [interquartile range] except for age (mean ± SD), Aβ38 (mean ± SD), sex (n), and hypertension prevalence (%). The sCAA patients consist of 19 patients with probable CAA, 4 patients with mixed microbleeds/intracerebral hemorrhage, and 3 patients with possible CAA. The probable CAA patients are a subset of sCAA patients.

Abbreviations: Aβ = amyloid beta; CAA = cerebral amyloid angiopathy; CSF = cerebrospinal fluid; F = female; M = male; MoCA: Montreal Cognitive Assessment; NA = not available; sCAA = sporadic CAA; SD = standard deviation.

[a]Student t test.

[b]Chi-squared test.

[c]n = 12.

[d]n = 11.

[e]Hypertension status unknown for 1 patient.

[f]Hypertension status unknown for 10 controls.

[g]Linear regression with age and sex as covariates.

Patients with sCAA and Controls: Validation. For validation purposes, we included a total of 40 patients with sCAA, 12 new patients from RUMC, 19 patients from Massachusetts General Hospital (MGH; Harvard Medical School, Boston, MA), and 9 patients from Leiden University Medical Center (LUMC; Leiden, the Netherlands). Of the 40 patients, 1 patient had definite CAA, 5 patients had probable CAA with supporting pathology, and 34 patients had probable CAA (modified Boston criteria).[7] The CSF of the patients with sCAA from RUMC was collected in the context of a clinical workup (n = 1) or the BIONIC study (n = 11; see Fig S1). The CSF of the patients with sCAA from LUMC was collected in the context of a natural history study (Following Sporadic CAA Study [FOCAS][1]), and the CSF from the patients with sCAA from MGH was also taken in the context of a research study. CSF samples were also taken at least 3 months after symptomatic ICH. Due to a limited amount of available CSF, Aβ38 was not measured in n = 7 and Aβ43 was not measured in n = 13 patients from MGH. Some of the patients with sCAA (n = 21) underwent an MoCA.

We included 37 controls from RUMC that met the same criteria as defined above for the discovery group. The controls were age- and sex-matched to the patients with sCAA (Table 2). Details about the final diagnosis of the controls are specified in the Methods section of the Supplementary Material.

Patients with AD. We included 40 patients with a CSF profile indicative of AD. These were patients who referred to RUMC for CSF diagnostics to investigate the etiology of their cognitive symptoms (see Fig S1, Table 2). Patients were selected based on CSF biomarker evidence of amyloid deposition (A), tau accumulation (T), and neurodegeneration (N).[16] We used the following predefined local cutoff values: (CSF Aβ42 (A+): <659pg/ml; phosphorylated tau (T+): >64pg/ml; total tau (N+): >400pg/ml).

Patients with D-CAA. We included 10 patients with presymptomatic D-CAA from LUMC and n = 26 age-matched controls from RUMC, and 12 patients with symptomatic D-CAA from LUMC and n = 28 age-matched controls from RUMC (see Fig S1, Table 3). Patients with D-CAA were included if they had an available CSF sample. Presymptomatic D-CAA was defined as

TABLE 2. Sporadic CAA Patients, AD Patients, and Controls in the Validation Experiments: Characteristics and Results of Magnetic Resonance Imaging

Characteristic	sCAA, n = 40	AD Patients, n = 40	Controls, n = 37	Probable CAA vs Controls, *p*	Probable CAA vs AD, *p*	AD vs Controls, *p*
Age, yr	68 ± 9	70 ± 8	70 ± 8	0.41[a]	0.65[a]	0.78[a]
Sex, M/F, n	19/21	17/23	15/22	0.54[b]	0.27[b]	0.86[b]
MoCA	25 [22–26][c]	NA	NA	—	—	—
Hypertension, %	50%	NA	35%[d]	0.20[b]	—	—
SVD burden score	4 [3–6][c]	NA	NA	—	—	—
CSF Aβ38, pg/ml	2,349 [1,611–3,153][e]	3,355 [2,893–4,084]	3,947 [2,567–5,206]	<0.0001[f]	<0.0001[f]	0.11[f]
CSF Aβ40, ng/ml	4.86 [2.97–6.97]	9.86 [7.73–12.34]	11.1 [6.67–15.05]	<0.0001[f]	<0.0001[f]	0.28[f]
CSF Aβ42, pg/ml	240 [120–323]	429 [312–486]	755 [544–1,068]	<0.0001[f]	0.012[f]	<0.0001[f]
CSF Aβ43, pg/ml	12.0 [7.49–16.5][g]	16.8 [12.3–20.7]	42.6 [30.0–64.5]	<0.0001[f]	0.86[f]	<0.0001[f]

Note: Values are median [interquartile range] except for age (mean ± standard deviation), sex (n), and hypertension prevalence (%).
Abbreviations: AD = Alzheimer disease; Aβ = amyloid beta; CAA = cerebral amyloid angiopathy; CSF = cerebrospinal fluid; F = female; M = male; MoCA: Montreal Cognitive Assessment; NA = not available; sCAA = sporadic CAA; SVD = small vessel disease.
[a]Student *t* test.
[b]Chi-squared test.
[c]n = 21.
[d]Hypertension status unknown for 3 controls.
[e]n = 33.
[f]Linear regression with age and sex as covariates.
[g]n = 27.

carriership of the c.2077G > C mutation in the Aβ precursor protein gene (resulting in p.Glu693Gln) and absence of symptomatic (hemorrhagic) strokes. Symptomatic D-CAA was defined by the presence of one or more symptomatic ICH(s) in combination with either confirmed carriership of the c.2077G > C mutation or one or more first-degree relatives with D-CAA. The D-CAA CSF samples were collected in the context of a D-CAA natural history study of LUMC (the AURORA study).[17]

The prevalence of hypertension was assessed in all patient groups and controls, except in the patients with AD (see Tables 1–3).

CSF Analysis. All participants in this study underwent a lumbar puncture according to state-of-the-art local protocols. At all participating hospitals, the CSF was collected in polypropylene tubes, centrifuged, aliquoted, and stored in polypropylene tubes at −80°C.

For all CSF analyses, patient and control samples were randomly analyzed to avoid bias. CSF Aβ40, Aβ42, tau phosphorylated at threonine 181, and total tau levels were quantified using the Lumipulse chemiluminescent immunoassay (Fujirebio, Ghent, Belgium). The samples were analyzed in different batches; however, we adhered to strict guidelines under the ISO15189 guidance to control that interassay variation was kept within predefined limits of variation for each assay.

CSF Aβ38 was quantified using enzyme-linked immunosorbent assays (ELISAs; Euroimmun, Lübeck, Germany for the discovery groups; due to a production stop that occurred between the execution of the discovery and validation stages of the study, we used an ELISA produced by IBL [Fujioka, Japan] for all other samples). CSF Aβ43 was quantified using an ELISA (IBL). The Aβ38 and Aβ43 ELISAs were done within a limited time window for both study cohorts separately (ie, discovery and validation stage, respectively).

TABLE 3. Characteristics and Results of Cerebrospinal Fluid Analysis in Patients with D-CAA and Controls

Characteristic	Presymp D-CAA, n = 10	Young Controls, n = 26	Symp D-CAA, n = 12	Old Controls, n = 28	Presymp vs Young, *p*	Symp vs Old, *p*	Presymp vs Symp, *p*
Age, yr	40 ± 8	43 ± 8	59 ± 8	58 ± 8	0.37[a]	0.88[a]	<0.0001[a]
Sex, M/F, n	7/3	15/11	7/5	11/17	0.50[b]	0.27[b]	0.18[b]
Hypertension, %	20%	8%[c]	33%	19%[d]	0.31[b]	0.34[b]	0.48[b]
Aβ38, pg/ml	1,240 [1,043–1,701]	3,647 [3,196–4,833]	747 [667–1,087]	3,387 [2,594–4,318]	<0.0001[e]	<0.0001[e]	0.14[e]
Aβ40, ng/ml	2.34 [1.88–3.34]	9.71 [7.12–11.2]	1.55 [1.17–2.07]	10.2 [6.52–12.2]	<0.0001[e]	<0.0001[e]	0.047[e]
Aβ42, pg/ml	108 [86.0–148]	903 [643–1,087]	70.5 [53.3–89.8]	782 [597–1,300]	<0.0001[e]	<0.0001[e]	0.026[e]
Aβ43, pg/ml	9.77 [7.49–12.64]	63.4 [48.9–63.4]	6.53 [4.95–8.07]	47.3 [35.4–68.5]	<0.0001[e]	<0.0001[e]	0.14[e]

Note: Values are median [interquartile range] except for age (mean ± standard deviation), sex (n), and hypertension prevalence (%).
Abbreviations: Aβ = amyloid beta; D-CAA = Dutch-type cerebral amyloid angiopathy; F = female; M = male; Presymp = presymptomatic; Symp = symptomatic.
[a]Student *t* test.
[b]Fisher exact test.
[c]Hypertension status unknown for 1 control.
[d]Hypertension status unknown for 2 controls.
[e]Linear regression with age and sex as covariates.

For all ELISAs, 5 quality control samples were included on each plate to correct for any inconsistencies between plates. These controls consisted of pooled CSF samples that were stored in aliquots at −80°C. For each analysis, a fresh aliquot was used.

All CSF analysis was done at RUMC.

MRI Analysis

MRI Acquisition. Patients with sCAA from the BIONIC study underwent a 3.0T MRI scan (Siemens Magnetom Prisma; Siemens Healthineers, Erlangen, Germany) using a 32-channel head coil. Participants were examined using a comprehensive protocol, and for the current study, the 3-dimensional (3D) multiecho gradient echo T2*-weighted sequence (voxel size = 0.8 × 0.8 × 0.8mm), 3D T2-weighted sequence (voxel size = 0.8 × 0.8 × 0.8mm), and 3D fluid-attenuated inversion recovery (FLAIR) sequence (voxel size = 0.8 × 0.8 × 0.8mm) were analyzed. Magnitude and phase data from the multiecho gradient sequence were processed to susceptibility-weighted imaging (SWI) using the CLEAR-SWI (contrast-weighted, Laplace-unwrapped, bipolar multiecho, ASPIRE-combined, homogeneous, improved resolution SWI) method.[18] Patients with sCAA and D-CAA from the FOCAS and AURORA studies were scanned on a 3.0T MRI scanner (Philips Healthcare, Best, the Netherlands) with a 32-channel head coil. This protocol included SWI, T2, and FLAIR sequences. Further details are described in Koemans et al.[17]

MRIs from the BIONIC study were rated independently by A.M.D.K. and H.E.P.v.B.-S. and MRIs from the AURORA and FOCAS studies by E.A.K. In the case of disagreement between A.M.D.K. and H.E.P.v.B.-S., F.H.B.M.S. (senior vascular neurologist) was consulted before final consensus was reached.

The following markers were assessed following the STRIVE (Standards for Reporting Vascular Changes on Neuroimaging) criteria: cerebral microbleeds (CMBs),[19] cortical superficial siderosis (CSS),[7] enlarged perivascular spaces (EPVS) in the centrum semiovale (CSO; using a dichotomized classification: high [≥21 EPVS] or low [≤20 EPVS]), and white matter hyperintensities (WMH) according to the Fazekas Scale.[20,21] In addition, we assessed the total burden score of SVD in CAA,[22] henceforth referred to as SVD burden score. This is an ordinal composite score, ranging from 0 to 6 points, which represents the SVD burden in CAA and incorporates lobar CMBs, CSS, CSO perivascular spaces (PVS), and WMH. In the case of 2 to 4 lobar CMBs, 1 point is awarded; in the case of 5 of more CMBs, 2 points are awarded. Focal CSS is awarded 1 point, and disseminated CSS 2 points. Periventricular WMH Fazekas score of 3 and/or deep WMH Fazekas score of ≥2[23] was awarded 1 point. Lastly, CSO PVS ≥ 21 was also awarded with 1 point.[22]

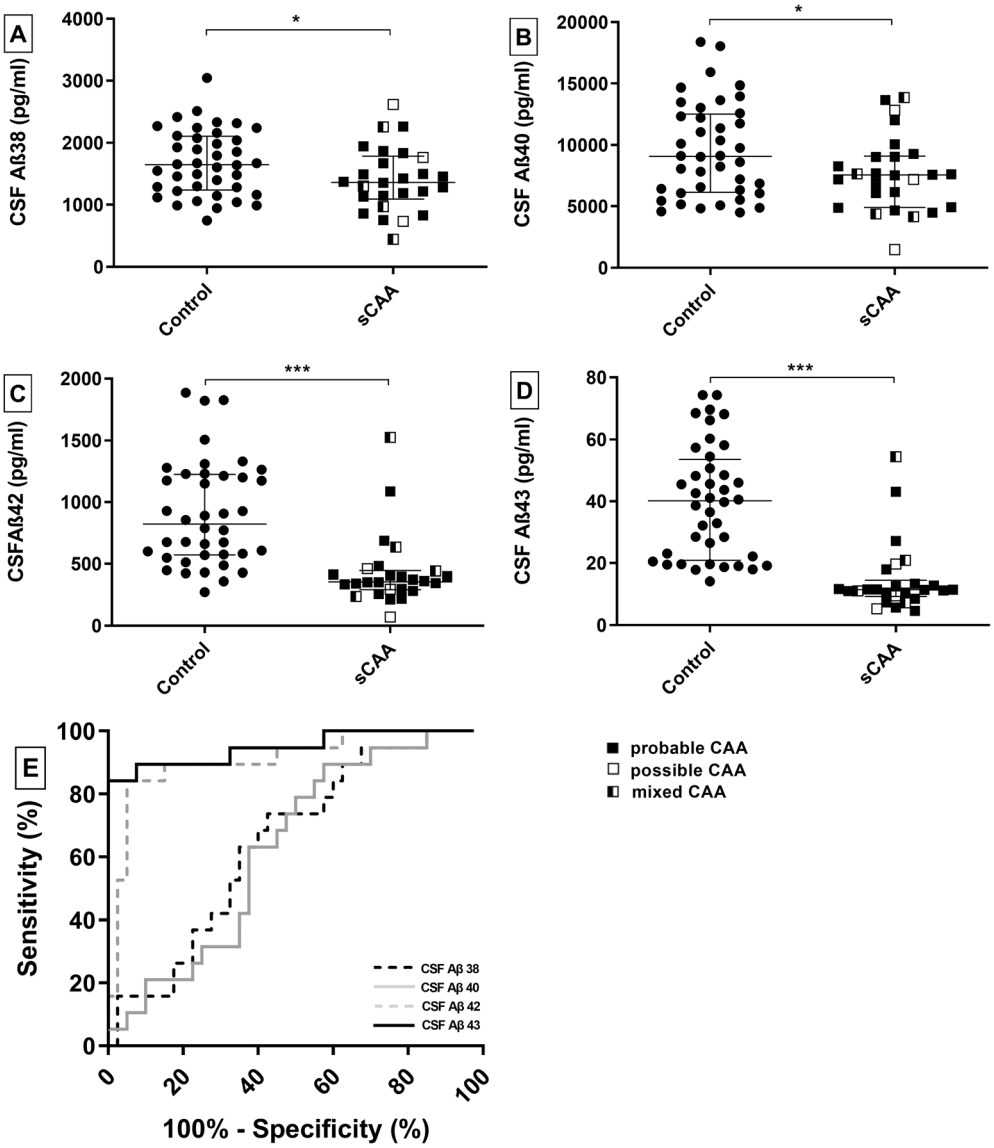

FIGURE 1: Cerebrospinal fluid (CSF) amyloid β (Aβ) 38, Aβ40, Aβ42, and Aβ43 levels in sporadic cerebral amyloid angiopathy (sCAA) patients and controls for discovery. (A) Scatterplots in all panels depict median and interquartile range; *p*-values are adjusted for age and sex. CSF Aβ38 levels were decreased in sCAA patients ($p = 0.015$). (B) CSF Aβ40 levels were decreased in sCAA patients ($p = 0.015$). (C) CSF Aβ42 levels were decreased in sCAA patients ($p < 0.0001$). (D) CSF Aβ43 levels were decreased in sCAA patients ($p < 0.0001$). (E) Receiver operating characteristic curve analysis showed moderately high to high accuracy levels for discrimination of probable CAA from controls in the discovery group (CSF Aβ38: area under the curve [AUC] = 0.65, 95% confidence interval [CI] = 0.51–0.79; CSF Aβ40: AUC = 0.63, 95% CI = 0.49–0.75; CSF Aβ42: AUC = 0.90, 95% CI = 0.82–0.99; CSF Aβ43: AUC = 0.95, 95% CI = 0.88–1.00). *$p < 0.05$, ***$p < 0.001$.

Data Analysis

The Shapiro–Wilk test was used to analyze the normality of the data. If parameters were normally distributed, they were depicted as mean ± standard deviation, and group differences were analyzed with a Student *t* test or analysis of variance. Otherwise, they were stated as median with interquartile range (IQR), and differences were analyzed with a Mann–Whitney *U* test or a Kruskal–Wallis test. Depending on group size, sex frequency was analyzed by a chi-squared test or Fisher exact test.

To measure the magnitude of difference between patients and controls, we compared the mean biomarker levels of the different groups, expressed as a fold change (FC; patients/controls). When comparing group differences of CSF Aβ levels, we adjusted for age and sex by performing multiple regression analysis with patient group, age, and sex as independent variables. In the patients with sCAA and controls used for discovery, we performed a sensitivity analysis comparing probable CAA patients with controls. To determine the diagnostic accuracy of CSF Aβ38, Aβ40, Aβ42, and Aβ43 for the distinction between groups, we determined the area under the curve (AUC) using a receiver operating characteristic curve (ROC) with 95% confidence interval (CI). The Youden index was determined (sensitivity + specificity − 1.0) to find the optimal cutoff value, and the sensitivity and specificity for that cutoff were calculated.

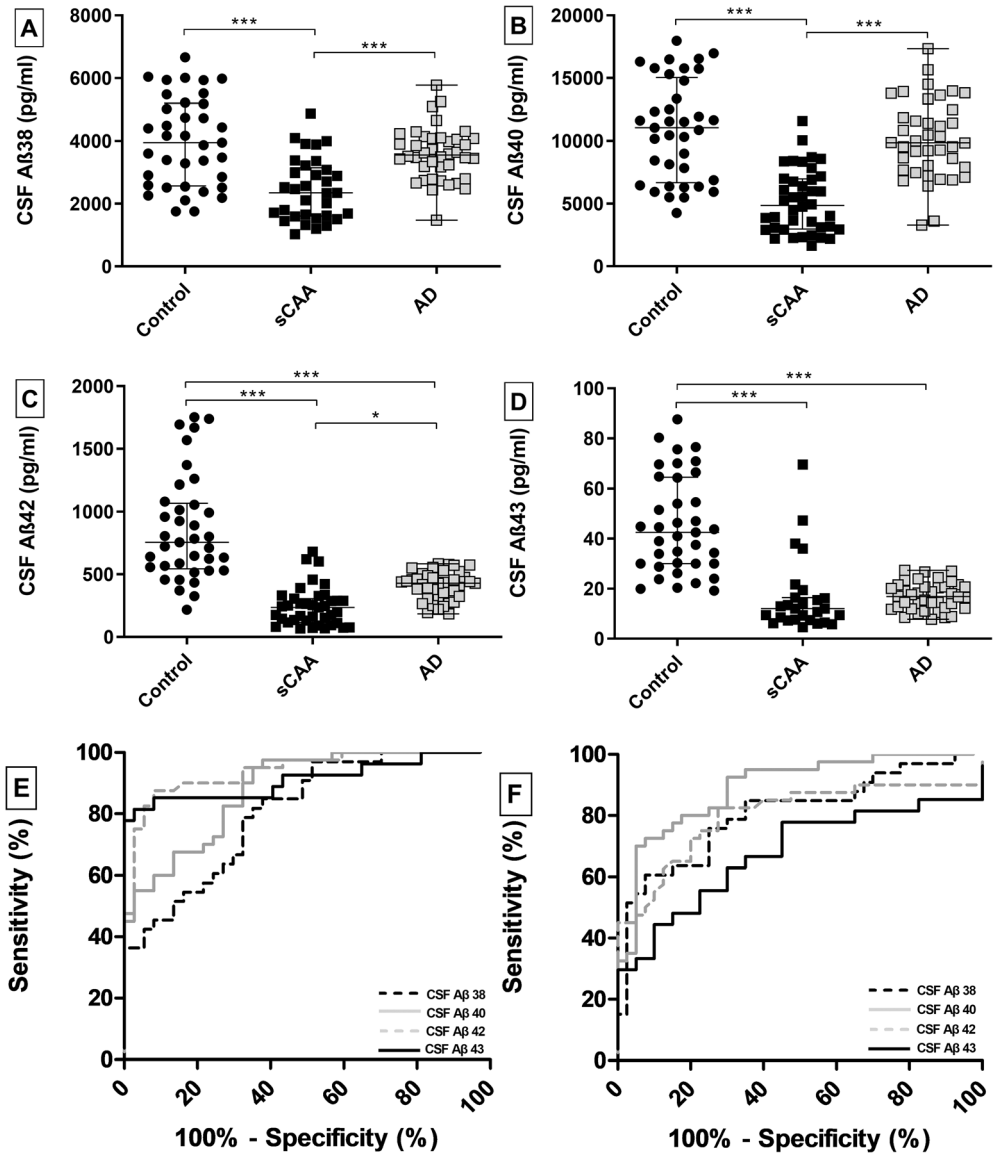

FIGURE 2: Cerebrospinal fluid (CSF) amyloid β (Aβ) 38, Aβ40, Aβ42, and Aβ43 levels in sporadic cerebral amyloid angiopathy (sCAA) patients, Alzheimer disease (AD) patients, and controls for validation. Scatterplots in all panels depict median and interquartile range; *p*-values are adjusted for age and sex. (A) CSF Aβ38 levels were decreased in sCAA patients compared to controls ($p < 0.0001$) and compared to AD patients ($p < 0.0001$). (B) CSF Aβ40 levels were decreased in sCAA patients compared to controls ($p < 0.001$) and compared to AD patients ($p < 0.0001$). (C) CSF Aβ42 levels were decreased in sCAA patients compared to controls ($p < 0.0001$) and compared to AD patients ($p = 0.012$), and decreased in AD patients compared to controls ($p < 0.0001$). (D) CSF Aβ43 levels were significantly decreased in sCAA patients compared to controls ($p < 0.001$) and in AD patients compared to controls ($p < 0.001$), but similar between AD and sCAA patients ($p = 0.86$). (E) Receiver operating characteristic curve (ROC) analysis showed moderately high to high accuracy levels for discrimination of probable sCAA from controls in the validation group (CSF Aβ38: area under the curve [AUC] = 0.81, 95% confidence interval [CI] = 0.71–0.91; CSF Aβ40: AUC = 0.61, 95% CI = 0.46–0.75; CSF Aβ42: AUC = 0.94, 95% CI = 0.89–0.99; CSF Aβ43: AUC = 0.91, 95% CI = 0.83–1.0). (F) ROC analysis showed moderately high to high accuracy levels for discrimination of probable sCAA from AD patients (CSF Aβ38: AUC = 0.82, 95% CI = 0.71–0.93; CSF Aβ40: AUC = 0.88, 95% CI = 0.80–0.96; CSF Aβ42: AUC = 0.79, 95% CI = 0.66–0.92; CSF Aβ43: AUC = 0.68, 95% CI = 0.53–0.82). *$p < 0.05$, ***$p < 0.001$.

We also evaluated whether ratios or combinations of the various Aβ peptides yielded a higher AUC than a single Aβ peptide.

Spearman rank correlation (r_{SP}) was used to evaluate correlations of the CSF Aβ peptides with each other, age, and MoCA scores. Using partial correlation, the correlation between the CSF Aβ peptides and SVD burden score was adjusted for age.

Ethical Statement

Lumbar punctures were performed after informed consent from the patients themselves. CAA patients underwent a lumbar puncture in the context of cross-sectional studies on biomarkers for CAA, which were approved by the local medical ethics committees (CAVIA, 2014–1401; BIONIC, 2017–3810; AURORA,

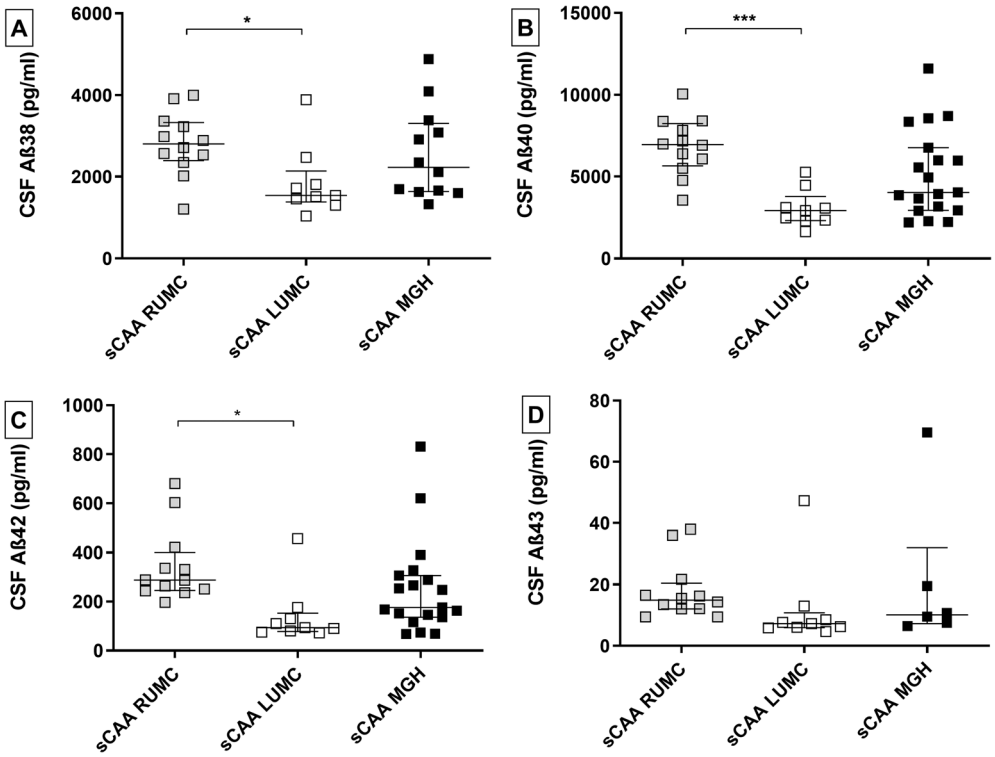

FIGURE 3: Effect of center on cerebrospinal fluid (CSF) amyloid β (Aβ) 38, Aβ40, Aβ42, and Aβ43 levels. CSF Aβ38, Aβ40, Aβ42, and Aβ43 levels are shown in sporadic cerebral amyloid angiopathy (sCAA) patients from the validation cohort and CAA patients from Radboud University Medical Center, Nijmegen, the Netherlands (RUMC), Leiden University Medical Center, Leiden, the Netherlands (LUMC), and Massachusetts General Hospital, Boston, Massachusetts (MGH). All panels scatterplots depict median and interquartile range. *p*-values are adjusted for age and sex. The mean age of the sCAA patients from RUMC (n = 12) was 71 ± 7 years, and 33% were male; the mean age of the sCAA patients from LUMC (n = 9) was 74 ± 9 years, and 30% were male; and the mean age of the sCAA patients from MGH (n = 19) was 63 ± 8 years, and 63% were male. (A) CSF Aβ38 levels in patients with sCAA from LUMC were decreased compared to the patients with sCAA from RUMC ($p = 0.046$). (B) CSF Aβ40 levels were decreased in patients with sCAA from LUMC compared to patients with sCAA from RUMC ($p < 0.0001$). (C) CSF Aβ42 levels were decreased in patients with sCAA from LUMC compared to patients with sCAA from RUMC ($p = 0.013$). (D) CSF Aβ43 levels were similar in patients with sCAA from LUMC, RUMC, and MGH. *$p < 0.05$, ***$p < 0.001$.

NL62670.058.17; FOCAS, NL63256.058.17; patients from MGH, 2006P000664). For AD patients and controls, CSF was used that remained after a clinical diagnostic workup (2016–3011).

Results

CSF Aβ Levels in Patients with sCAA and Controls: Discovery

Patients with sCAA (age = 72 ± 7 years) were older than the controls (age = 63 ± 8 years, $p < 0.0001$). The sex distribution was similar (see Table 1).

CSF Aβ38 (FC = 0.84), Aβ40 (FC = 0.80), Aβ42 (FC = 0.48), and Aβ43 (FC = 0.37) levels, adjusted for age and sex, were all significantly lower in patients with sCAA compared with controls (see Table 1, Fig 1A–D; results of unadjusted comparisons are presented in Table S1). The differences were most pronounced for Aβ42 and Aβ43. The comparison between the subset of patients with probable CAA and controls yielded similar results (see Table 1). ROC analysis (probable CAA vs controls) yielded AUC values between 0.65 (Aβ38) and 0.95 (Aβ43; for details, see Fig 1E and Table S3). Any ratio or any combination of Aβ biomarkers did not yield a higher AUC than calculated for any of the individual peptides (data not shown).

CSF Aβ Levels in Patients with sCAA and Controls: Validation

The age and sex distribution of the patients with sCAA was similar (see Table 2).

CSF Aβ38 (FC = 0.61), Aβ40 (FC = 0.48), Aβ42 (FC = 0.30), and Aβ43 (FC = 0.36) levels, adjusted for age and sex, were all significantly decreased in patients with sCAA compared to controls (see Table 2, Fig 2A–D; results of unadjusted comparisons are presented in Table S2). The differences were most pronounced for Aβ42 and Aβ43. ROC analysis (probable CAA vs controls) yielded AUC values between 0.81 (Aβ38) and 0.94 (Aβ42; for details, see Fig 2E and Table S3). Any ratio or any combination of Aβ biomarkers did not yield a higher AUC than calculated for any individual peptides (data not shown).

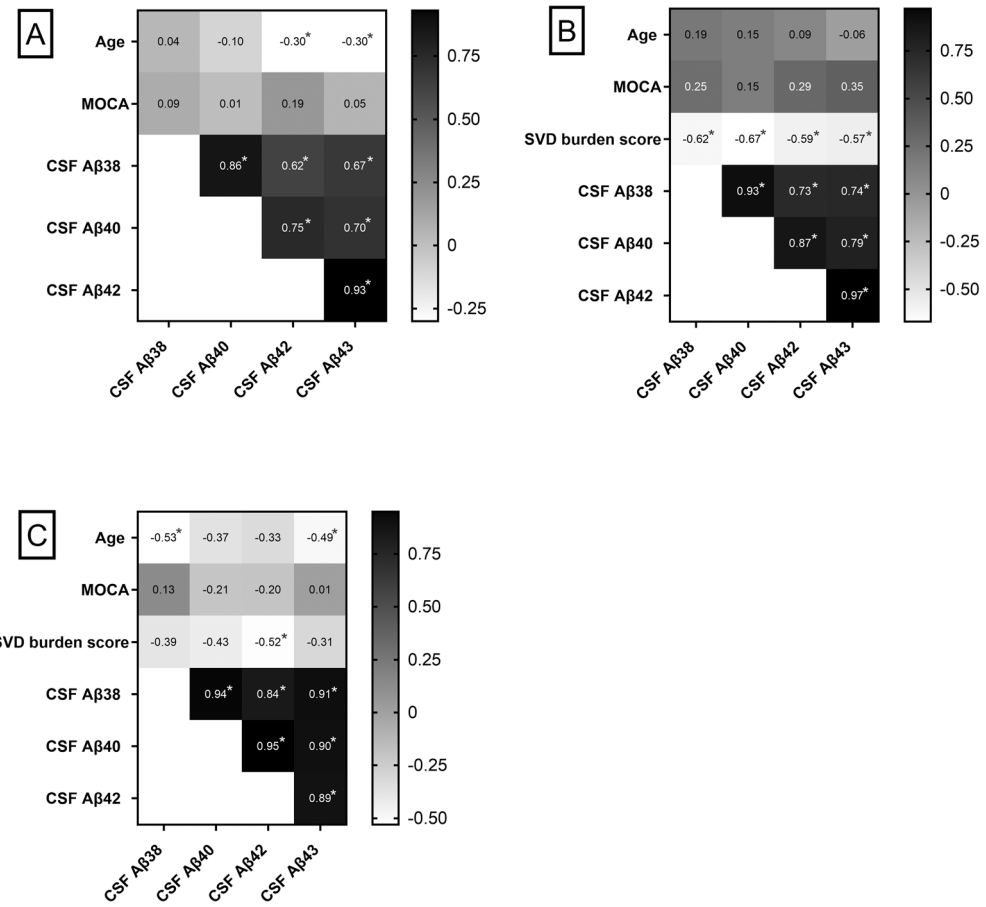

FIGURE 4: (A) Correlation of cerebrospinal fluid (CSF) amyloid β (Aβ) 38, Aβ40, Aβ42, and Aβ43 with age, and Montreal Cognitive Assessment (MoCA) score, and among the Aβ peptides (patients with sporadic cerebral amyloid angiopathy [sCAA] and controls for discovery [n = 67]). MoCa score (n = 12) was only available for a subset of sCAA patients. Spearman correlation coefficients are shown. (B) Correlation of CSF Aβ38, Aβ40, Aβ42, and Aβ43 with age, MoCa score, and small vessel disease (SVD) burden score, and among the Aβ peptides (patients with sCAA and controls for validation [n = 78]). Spearman correlation coefficients are shown. MoCA and SVD burden scores were only available for a subset of sCAA patients (both n = 21). (C) Correlation of CSF Aβ38, Aβ40, Aβ42, and Aβ43 with age, MoCA score, and SVD burden score, and among the Aβ peptides in patients with Dutch-type cerebral amyloid angiopathy (D-CAA) and controls. Spearman correlation coefficients are shown. MoCA (n = 21) and SVD burden scores (n = 19) were only available for a subset of D-CAA patients. The correlation of SVD burden score with the Aβ peptides was adjusted for age. The correlation between CSF Aβ40 and SVD burden score showed a trend ($p = 0.075$). *Significant correlation ($p < 0.05$).

Comparison of Aβ Levels of Patients with sCAA at Different Centers

CSF Aβ38, CSF Aβ40, and CSF Aβ42, adjusted for age and sex, were significantly decreased in the patients with sCAA from LUMC compared to the patients with sCAA from RUMC (see Fig 3). CSF Aβ43, however, was similar in patients with sCAA from LUMC and RUMC. There were no significant differences in any CSF Aβ peptide between the patients with sCAA from MGH and patients with sCAA from RUMC or LUMC (see Fig 3).

Correlations of CSF Aβ Isoforms with Each Other, Age, MoCa, and MRI Parameters in sCAA and Controls

In the patients with sCAA and controls for discovery, both CSF Aβ42 ($r_{SP} = -0.30$, $p = 0.02$) and CSF Aβ43 ($r_{SP} = -0.30$, $p = 0.02$) correlated with age, whereas CSF Aβ38 and CSF Aβ40 did not (see Fig 4A,B). In the patients with sCAA and controls for validation, none of the CSF Aβ peptides correlated with age. Furthermore, all CSF Aβ peptides correlated with each other, with the strongest correlations between CSF Aβ38 and Aβ40 (discovery: $r_{SP} = 0.86$, $p < 0.001$; validation: $r_{SP} = 0.93$, $p < 0.001$) and CSF Aβ42 and Aβ43 (discovery: $r_{SP} = 0.93$, $p < 0.001$; validation: $r_{SP} = 0.97$, $p < 0.001$; see Fig 4A,B). There were no significant correlations between MoCA scores and CSF Aβ levels in patients with sCAA of both groups. In the validation cohort, there were significant age-adjusted correlations between all Aβ peptides and SVD burden ($r_{SP} = -0.57$ to -0.67, $p = 0.001$–0.009; see Fig 4A, B). Lastly, we found no significant correlations between the time (in days) between most recent ICH and lumbar

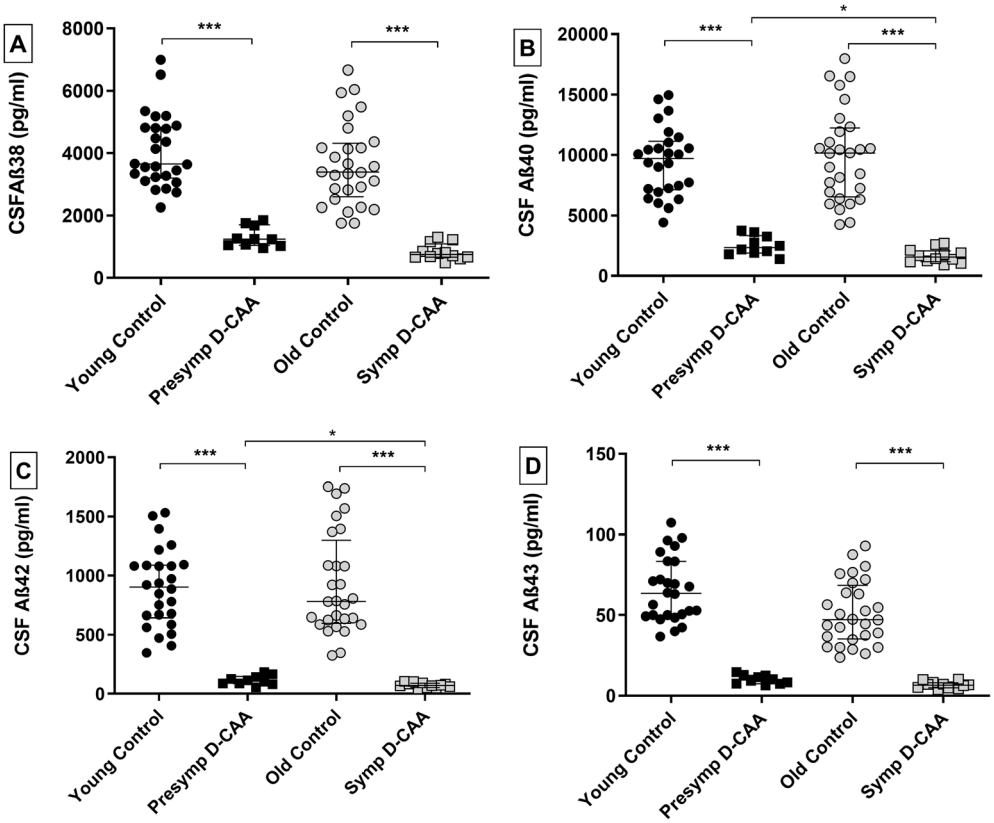

FIGURE 5: Cerebrospinal fluid (CSF) amyloid β (Aβ) 38, Aβ40, Aβ42, and Aβ43 levels in Dutch-type cerebral amyloid angiopathy (D-CAA) patients and controls. Scatterplots in all panels depict median and interquartile range; *p*-values are adjusted for age and sex. (A) CSF Aβ38 levels were significantly decreased in presymptomatic (Presymp) and symptomatic (Symp) D-CAA patients versus their respective controls (both $p < 0.0001$). (B) CSF Aβ40 levels were significantly decreased in presymptomatic and presymptomatic D-CAA patients versus their respective controls (both $p < 0.001$) and in symptomatic versus presymptomatic patients ($p = 0.047$). (C) CSF Aβ42 levels were significantly decreased in presymptomatic and symptomatic D-CAA patients versus their respective controls (both $p < 0.001$), in symptomatic D-CAA patients versus controls ($p < 0.001$) and in symptomatic versus presymptomatic patients ($p = 0.026$). (D) CSF Aβ43 levels were significantly decreased in presymptomatic and presymptomatic D-CAA patients versus their respective controls (both $p < 0.0001$). Abbreviations: D-CAA = Dutch-type cerebral amyloid angiopathy, CSF = cerebrospinal fluid. Presymp D-CAA = presymptomatic D-CAA patients, Symp D-CAA = symptomatic D-CAA patients. *$p < 0.05$, ***$p < 0.001$.

puncture and CSF Aβ isoforms (data not shown). The median time between lumbar puncture and most recent ICH in the patients with sCAA in the discovery cohort was 476 days (IQR = 189–1,320) and in the validation cohort 425 days (IQR = 249–1,228).

CSF Aβ Levels in Patients with AD Compared to Patients with sCAA and Controls: Validation Group

The age of the patients with AD was similar to the age of controls and of patients with sCAA, and sex distribution was similar as well (see Table 2).

CSF Aβ38 and CSF Aβ40 levels, adjusted for age and sex, were significantly higher in patients with AD compared to patients with sCAA, but similar to controls (see Table 2, Fig 2A,B; results of unadjusted comparisons are presented in Table S2). Furthermore, CSF Aβ42 levels were significantly higher in patients with AD compared to patients with sCAA, and were significantly decreased in both sCAA and AD compared to controls (see Table 2, Fig 2C). Lastly, CSF Aβ43 levels were similar in patients with AD and patients with sCAA, but were decreased compared to controls (see Table 2, Fig 2D).

ROC analysis (AD vs probable CAA) yielded AUC values between 0.68 (Aβ43) and 0.88 (Aβ40; for details, see Fig 2F and Table S3). The ratio of CSF Aβ42/Aβ43 yielded an AUC of 0.92 (95% CI = 0.85–0.99). The combination of CSF Aβ40 and Aβ43 in combination with either Aβ38 or Aβ42 both yielded the highest AUC (0.96, 95% CI = 0.92–1.0).

CSF Aβ Levels in D-CAA

Age and sex were statistically similar in patients with presymptomatic D-CAA (8 of these patients did not have microbleeds or CSS) versus the young controls, and in the patients with symptomatic D-CAA patients versus the old

controls (see Table 3). CSF Aβ38, Aβ40, Aβ42, and 43 levels, adjusted for age and sex, were all significantly decreased in both presymptomatic and symptomatic patients versus controls. Furthermore, all CSF Aβ peptides were decreased in patients with symptomatic D-CAA compared to patients with asymptomatic D-CAA in unadjusted analysis, whereas analyses adjusted for age and sex showed a decrease of CSF Aβ40 and Aβ42. (see Table 3 Fig 5, Table S4).

All ROC analyses, for both presymptomatic and symptomatic D-CAA patients compared to age-matched controls, and for all Aβ peptides, yielded an AUC of 1 (95% CI = 1–1).

CSF Aβ38 ($r_{SP} = -0.53$, $p = 0.01$) and Aβ43 correlated with age ($r_{SP} = -0.49$, $p < 0.001$).

Furthermore, all CSF Aβ peptides correlated with each other, with the strongest correlations between CSF Aβ38 and Aβ40 ($r_{SP} = 0.94$, $p < 0.001$; see Fig 4C) and CSF Aβ40 and Aβ42 ($r_{SP} = 0.95$, $p < 0.001$). There were no correlations between CSF Aβ isoforms and MoCA scores (see Fig 4C). Lastly, there was a significant age-adjusted correlation of SVD score with Aβ42 ($r_{SP} = -0.52$, $p = 0.026$; see Fig 4C). Lastly, we found no significant correlations between the time (in days) between most recent ICH and lumbar puncture (data not shown) CSF Aβ isoforms in the patients with symptomatic D-CAA. The median time between most recent ICH and lumbar puncture was 392 days (IQR = 264–969).

Discussion

The main findings of our study are as follows. (1) In patients with sCAA, levels of CSF Aβ38, Aβ40, Aβ42, and Aβ43 are significantly decreased compared to controls, and the decreases in CSF levels of Aβ42 and Aβ43 (AUC value of up to 0.94) appeared stronger than in the other CSF Aβ species, as established in 2 independent sample sets. (2) All four Aβ peptide levels are also decreased in presymptomatic and symptomatic D-CAA patients, discriminating patients from controls with 100% specificity and 100% sensitivity. (3) Levels of CSF Aβ38, Aβ40, and Aβ42, but not Aβ43, are lower in sCAA as compared to AD. (4) The combination of Aβ40, Aβ43, and either Aβ38 or Aβ42 yielded an AUC of 0.96 to discriminate sCAA from AD patients.

With our results, we confirm previous findings of decreased CSF Aβ40 and Aβ42 levels in patients with sCAA compared to patients with AD and controls.[9,24] In addition, we demonstrate reduced CSF levels of Aβ38 and Aβ43 in patients with sCAA compared to controls. In our study, Aβ42 and Aβ43 yield a better AUC compared to Aβ38 and Aβ40, and also compared to earlier reported AUCs of Aβ40 (0.74) and Aβ42 (0.68).[9] Thus, Aβ42 and Aβ43 seem superior in their discrimination of sCAA and controls. These markers may be used in clinical practice when the diagnosis of CAA is uncertain, for instance, in patients with possible CAA, with an unusual age at onset, or with a contraindication for MRI. In addition, because the Boston criteria v2.0 only have 55% sensitivity in patients without ICH, the CSF Aβ panel may add more diagnostic certainty in this patient group. Furthermore, this panel of 4 Aβ peptides could aid in the discrimination of sCAA from AD when there is uncertainty about the predominant pathology in patients presenting with cognitive symptoms; a decrease in all 4 peptides would be more indicative of CAA, whereas a decrease only in CSF Aβ42 and Aβ43, but not in Aβ38 and Aβ40, would be more indicative of AD. Another possible application of our results could be the detection of CAA in healthy subjects or patients with (mild) AD, although this should be validated first in a study specifically designed for this aim. If CSF Aβ peptides are capable of detecting CAA in patients with AD, this would be highly relevant in light of the emergence of anti-Aβ immunotherapy, especially with regard to the (controversial) approvals in 2022/2023 of aducanumab and lecanumab by the US Food and Drug Administration.[25] These therapies are accompanied by a risk of side effects, namely, the so-called "anti-amyloid therapy-related imaging abnormalities"—brain edema and hemorrhages, which occurred more frequently in AD patients with concomitant CAA.[26] Thus, CSF biomarkers that detect CAA in patients with AD may potentially help with patient selection and exclusion of patients with (a high load of) CAA from these and other anti-Aβ immunotherapy trails.

In patients with D-CAA, we also found decreased levels of Aβ38, Aβ40, Aβ42, and Aβ43 peptides, with no overlap between both presymptomatic and symptomatic patients and their respective controls. This confirms earlier findings of decreased Aβ40 and Aβ42 levels in D-CAA patients[12] and extends these findings to decreased levels of Aβ38 and Aβ43. Our results also show that CAA pathogenesis and the consequent alterations in all 4 CSF Aβ isoforms occur before symptomatic ICH, and even before the occurrence of neuroimaging features of CAA such as microbleeds or superficial siderosis.

We observe for D-CAA the strongest decreases in Aβ42 and Aβ43, similar to the pattern in patients with sCAA. This finding supports the hypothesis that in the process of vascular Aβ accumulation, Aβ42 deposits early and may serve as a "seed" in cerebral vessels, followed by growth of these deposits by Aβ40 deposition.[27–31] There is also some evidence that Aβ43 can act as a seed in the parenchyma,[32] although it has not been established

whether this occurs in vessels as well. Our findings indicate that CSF Aβ42 and Aβ43 in patients with D-CAA (and sCAA) may be used for monitoring of the efficacy of disease-modifying drugs in clinical trials.

It is known that a proportion of patients with AD have concurrent CAA pathology; based on MRI, a prevalence of 14% has been described for probable CAA, 22% for strictly lobar microbleeds, and 5% for CSS, and based on neuropathology, the prevalence is even higher at almost 50%.[5] Despite the finding that AD and CAA are not two entirely separate entities and often co-occur, we show that levels of CSF Aβ38, Aβ40, and Aβ42, but not Aβ43, are lower in sCAA as compared to AD. The use of CSF Aβ40 and Aβ42 to discriminate sCAA from AD has been described earlier.[9] However, we show that the combination of Aβ40, Aβ43, and either Aβ38 or Aβ42 yielded an AUC of 0.96 to discriminate sCAA from AD. We unfortunately lack information on imaging markers associated with CAA in the patients with AD, but interestingly, although a proportion of our AD patients may have CAA, nonetheless the aforementioned distinct CSF Aβ pattern (normal levels of Aβ38 and Aβ40, decreased levels of Aβ42 and Aβ43) was found at the group level in AD patients.

Our observations of aberrant CSF Aβ levels may also provide insights into the underlying pathophysiology of CAA. Alterations in the dynamic equilibrium of Aβ production, clearance, and accumulation may lead to changes in CSF Aβ concentrations.[9] Decreased Aβ levels in the CSF are thought to be the result of a net reduction of Aβ peptides eluted with interstitial fluid toward the CSF. This may be partly explained by trapping of the Aβ peptides in the cerebral vasculature or parenchyma. Longer Aβ peptides such as Aβ42 and Aβ43 are less soluble, more prone to aggregate, and mostly found in amyloid plaques in the brain parenchyma,[33–35] whereas shorter peptides such as Aβ38 and Aβ40 are more soluble, can diffuse along perivascular drainage pathways, and accumulate predominantly in CAA.[36–38] This corresponds to our finding that Aβ38 and Aβ40 are decreased only in patients with sCAA and not in AD patients. Furthermore, we show that Aβ42 and Aβ43 are decreased in AD patients, which may be a result of the predominant accumulation of these peptides in amyloid plaques.

We found inconsistent results regarding the correlation between SVD burden score and CSF Aβ levels in the patients with sCAA and D-CAA. The SVD burden score is an ordinal score, based on the presence of 4 SVD imaging markers, and has been associated with the severity of postmortem CAA-associated vasculopathic changes.[22] In patients with sCAA, we found strong correlations with all Aβ peptides, which indicates that CSF Aβ may be a reflection of CAA-related pathologic changes. In the D-CAA group, we found only a weak correlation between SVD score and CSF Aβ42, and a trend for Aβ40. This may be explained by the relatively small sample size of this group. Another explanation may be that the CSF Aβ levels in symptomatic D-CAA reach a plateau level earlier than the SVD burden score.

It is notable that the absolute values of Aβ38, Aβ40, Aβ42, and Aβ43 differed between the discovery and validation cohorts. Furthermore, levels of the Aβ peptides were lower in patients with sCAA from LUMC compared to patients with sCAA from RUMC. It is well known that preanalytical factors may affect the results of CSF Aβ analysis.[39] Although we harmonized CSF protocols in terms of, for example, centrifugation, storage temperature, and tube use across the centers, it cannot be ruled out that unknown preanalytical factors may have affected our results. Furthermore, it cannot be excluded that the observed differences were partly caused by a difference in patient characteristics in the various cohorts. Nevertheless, a center effect on Aβ measurements cannot be ruled out, which complicates the interpretation of the results in the validation cohort. Future studies that combine cohorts from different centers should also take this possible center effect into account.

Strengths of our study are that we could validate our findings in 2 independent, extensively characterized, cohorts of patients with sCAA from various centers, and in the biggest group of patients with D-CAA to date.

A limitation of this study is that we did not have controls from centers other than RUMC, which complicates interpretation of results, because the origin of the CSF samples may affect the concentration of CSF Aβ peptides. In addition, although neuroimaging was not the primary type of investigation of our study, we did not have standardized MRI available for the patients with AD and controls. Another limitation is that we did not have information on the final clinical diagnosis in our patients with AD, although the ATN classification has been accepted as a proxy for AD pathology.[16] Furthermore, we lacked information on the presence of strictly lobar microbleeds or CSS in our controls, although the reported prevalence of these markers in the general population is quite low and likely did not affect our results (7% for lobar microbleeds and 0.5% for CSS[5]). Another limitation is that we had to use a different Aβ38 assay for the validation experiment, because the Aβ38 assay that we used in the discovery experiment went out of production during our study.

Conclusions

A biomarker panel consisting of CSF Aβ38, Aβ40, Aβ42, and Aβ43 has great potential to distinguish sCAA from controls and from AD patients; Aβ38 and Aβ40 are only decreased in CAA, but not in AD. On the other hand, all

4 peptides are decreased in CAA compared to controls. Furthermore, Aβ38 and Aβ40 are most specific for sCAA in comparison with AD, whereas Aβ42 and Aβ43 are superior for the distinction between sCAA and controls.

Author Contributions

H.B.K., F.H.B.M.S., C.J.M.K., and M.M.V. contributed to the conception and design of the study. A.M.D.K., H.B.K., T.M.M., L.J. E.v.d.B., I.K., H.E.P.v.B.-S., M. D., E.S., W.F.A., I.R., S.V., E.A.K., K.K., A.D.W., S.M. G., G.B., G.M.T., M.J.H.W., F.H.B.M.S., C.J.M.K., and M.M.V. contributed to data acquisition and data analysis. A.M.D.K., H.B.K., and M.M.V. contributed to drafting the text or preparing the figures.

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Potential Conflicts of Interest

Nothing to report.

Data Availability Statement

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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