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ORIGINAL ARTICLE

Profiling amyloid-β peptides as biomarkers for cerebral amyloid angiopathy

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Abstract

Brain amyloid-β (Aβ) deposits are key pathological hallmarks of both cerebral amyloid angiopathy (CAA) and Alzheimer's disease (AD). Microvascular deposits in CAA mainly consist of the $A\beta_{40}$ peptide, whereas $A\beta_{42}$ is the predominant variant in parenchymal plaques in AD. The relevance in pathogenesis and diagnostic accuracy of various other Aβ isoforms in CAA remain understudied. We aimed to investigate the biomarker potential of various Aβ isoforms in cerebrospinal fluid (CSF) to differentiate CAA from AD pathology. We included 25 patients with probable CAA, 50 subjects with a CSF profile indicative of AD pathology (AD-like), and 23 age- and sex-matched controls. CSF levels of $A\beta_{1\text{-}34}$, $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}39}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ were quantified by liquid chromatography mass spectrometry. Lower CSF levels of all six Aβ peptides were observed in CAA patients compared with controls ($p = 0.0005$–0.03). Except for $A\beta_{1\text{-}42}$ ($p = 1.0$), all peptides were decreased in CAA compared with AD-like subjects

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; ATN, amyloid/tau/neurodegeneration; AUC, area under the curve; Aβ, amyloid-β; CAA, cerebral amyloid angiopathy; CI, confidence interval; CSF, cerebrospinal fluid; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; LC, liquid chromatography; MoCA, Montreal Cognitive Assessment; MRI, magnetic resonance imaging; MS, mass spectrometry; QC, quality control; RUMC, Radboud University Medical Center.

($p = 0.007–0.03$). Besides $A\beta_{1-42}$, none of the Aβ peptides were decreased in AD-like subjects compared with controls. All Aβ peptides combined differentiated CAA from AD-like subjects better (area under the curve [AUC] 0.84) than individual peptide levels (AUC 0.51–0.75). Without $A\beta_{1-42}$ in the model (since decreased $A\beta_{1-42}$ served as AD-like selection criterion), the AUC was 0.78 for distinguishing CAA from AD-like subjects. CAA patients and AD-like subjects showed distinct disease-specific CSF Aβ profiles. Peptides shorter than $A\beta_{1-42}$ were decreased in CAA patients, but not AD-like subjects, which could suggest different pathological mechanisms between vascular and parenchymal Aβ accumulation. This study supports the potential use of this panel of CSF Aβ peptides to indicate presence of CAA pathology with high accuracy.

KEYWORDS
Alzheimer's disease, amyloid-β, biomarkers, cerebral amyloid angiopathy, cerebrospinal fluid, mass spectrometry

1 | INTRODUCTION

Amyloid-β (Aβ) aggregates within cortical arterioles, capillaries, and leptomeningeal blood vessel walls are the pathological hallmarks of cerebral amyloid angiopathy (CAA) (Charidimou et al., 2017). These vascular deposits increase the risk for blood vessels to eventually rupture, leading to subarachnoid or intracerebral hemorrhages (DeSimone et al., 2017). The prevalence of neuropathological CAA in the general elderly population is 23%, and as a result of the involvement of the Aβ protein, although this concerns different peptides, it is a common co-pathology in Alzheimer's disease (AD) (Jäkel et al., 2022). Establishing a proper diagnosis of CAA, however, proves to be difficult in clinical practice, since no sufficient definitive criteria to establish CAA during life are currently available.

In vivo clinical diagnosis of CAA is based on the Boston criteria which rely on imaging abnormalities. These include strictly lobar hemorrhagic lesions (i.e., intracerebral hemorrhages, cerebral microbleeds, and cortical superficial siderosis) and CAA-related white matter lesions as non-hemorrhagic magnetic resonance imaging (MRI) markers (Charidimou et al., 2022). Despite the high specificity and moderate sensitivity of these criteria (Charidimou & Boulouis, 2022), some notable limitations apply. Firstly, all MRI parameters included in the criteria reflect late-stage disease manifestations, since underlying disease pathology is already substantially advanced. Secondly, the criteria were not developed to grade the severity of CAA pathology. Thirdly, although diagnostically useful, it is unlikely that these MRI parameters may serve as biomarkers to monitor the efficacy of interventions aimed at reducing CAA burden. Fourthly, several markers are not disease-specific and may be present in non-CAA-related small vessel disease (e.g., hypertensive angiopathy (Das et al., 2023)) as well. Lastly, assessment of brain tissue is necessary to obtain a definitive CAA diagnosis, which is only infrequently performed, hence during life at best a probable diagnosis can be obtained. The unmet need for robust biomarkers to confidently diagnose CAA may be overcome by utilizing cerebrospinal fluid (CSF) as a more direct derivative of pathological processes occurring in the central nervous system. Moreover, abnormalities of CSF biomarkers in CAA may occur well before clinical symptom onset (van Etten et al., 2017).

The amyloid precursor protein (APP) is cleaved by β- and γ-secretases to produce various Aβ peptides (Haass et al., 2012). Vascular deposits in CAA mainly consist of the $A\beta_{40}$ peptide, whereas $A\beta_{42}$ dominates in parenchymal plaques (Greenberg et al., 2020), both of which are reflected in aberrant CSF levels. Decreased $A\beta_{40}$ and $A\beta_{42}$ CSF concentrations are observed in CAA relative to controls and AD patients (Verbeek et al., 2009). As compared to the commonly observed decrease in $A\beta_{42}$ CSF levels in AD (Motter et al., 1995), the $A\beta_{42/40}$ ratio differentiates AD patients from controls even better (Janelidze et al., 2016; Lewczuk et al., 2017). Neuropathological evidence proves that moderate-to-severe CAA may occur in 48% of patients with AD (Jäkel et al., 2022). This extensive overlap complicates the discrimination of both disease entities in a patient based on currently available biomarkers.

Other Aβ species of different amino acid lengths exist, which are less abundant than $A\beta_{40}$ and $A\beta_{42}$. However, their relevance in CAA pathogenesis remains understudied (Dunys et al., 2018). CAA likely occurs as a result of impaired Aβ clearance at the cerebral vasculature (McIntee et al., 2016). It is therefore conceivable that, like $A\beta_{40}$ and $A\beta_{42}$, multiple Aβ species accumulate in CAA differently from plaques in AD. $A\beta_{34}$, an intermediate form in Aβ degradation, has been located in microvessels especially surrounded by pericytes in early AD, which nevertheless vanished at later stages (Kirabali et al., 2019). $A\beta_{37}$, $A\beta_{38}$, and $A\beta_{39}$ were immunohistochemically detected in the cerebral vasculature of sporadic and familiar AD cases displaying abundant CAA (Moro et al., 2012; Reinert et al., 2014, 2016), as well as by mass spectrometry in cases with abundant CAA

(Brinkmalm et al., 2019; Gkanatsiou et al., 2019). Lower CSF $A\beta_{38}$ levels in CAA compared with AD and controls have been demonstrated in a rather small cohort (Banerjee et al., 2020). Levels of such truncated Aβ species have not yet been systematically studied in patients with CAA.

In this study, we aimed to investigate the biomarker potential of a large set of Aβ peptides ($A\beta_{1-34}$, $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-39}$, $A\beta_{1-40}$, and $A\beta_{1-42}$) for the differentiation of CAA patients from either AD-like subjects and controls. We used liquid chromatography and tandem mass spectrometry (LC–MS/MS) to simultaneously quantify the different Aβ peptide levels in CSF samples. Furthermore, we aimed to explore the relation of CSF levels of Aβ peptides to cerebrovascular imaging markers and cognitive decline in CAA. We hypothesized that CAA is reflected by a disease-specific profile of Aβ peptide levels in CSF as compared with AD-like subjects and controls.

2 | METHODS

2.1 | Cohorts

This study was approved by the local medical ethics committee Arnhem-Nijmegen (file numbers 2016-3011, 2017-3810 [BIONIC], and 2014-1401 [CAVIA]). The study was not pre-registered. We included CSF samples from 25 patients with probable CAA, 50 subjects with a CSF profile indicative of AD pathology (AD-like subjects), and 23 age- and sex-matched control subjects from the Radboud University Medical Center (RUMC, Nijmegen, the Netherlands; Table). CSF was collected via lumbar puncture after obtaining informed consent from all subjects or their legal representatives. See Supplementary Material for details on CSF sample collection.

Inclusion criteria for patients with CAA were a diagnosis of probable CAA and the availability of a CSF sample. Probable CAA diagnosis was established through MRI analysis based on the modified Boston criteria (Linn et al., 2010). Ten patients presented with an intracerebral hemorrhage. Twelve patients either had cognitive symptoms and/or transient focal neurological episodes. There was one patient who presented with a transient ischemic attack, and subsequent MRI was compatible with probable CAA. There was also one patient who had a seizure as a presenting symptom, with subsequent MRI compatible with probable CAA. Furthermore, there was one patient who presented with CAA-related inflammation. The lumbar puncture was performed more than 2 years later than the active phase of the inflammation. Cognitive function was assessed using the Montreal Cognitive Assessment (MoCA (Nasreddine et al., 2005)) in 21 of the CAA patients. None of the CAA patients had a concomitant clinical AD diagnosis.

AD-like subjects were selected from consecutive referrals to the RUMC for CSF diagnostics to assess the origin of their cognitive symptoms and were included based on having a positive CSF amyloid/tau/neurodegeneration (A+T+N+) biomarker profile that indicates presence of AD pathology (Jack Jr. et al., 2018; Vos et al., 2014), as defined by predefined local cut-off values for immunoassays of CSF $A\beta_{42} < 659$ pg/mL, phosphorylated $tau_{181} > 64$ pg/mL, and total tau >400 pg/mL (see Supplementary Material for details). No information on CAA imaging markers was available for AD-like subjects or controls.

The control subjects reported neither any cognitive complaints nor did they have a clinical CAA or AD diagnosis. All inclusion and exclusion criteria for control subjects are available in Supplementary Material.

For a subset of the CSF samples, $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ levels had been previously quantified by enzyme-linked immunosorbent assays (ELISAs) (De Kort et al., 2023). Details about these assays are described in Supplementary Material.

2.2 | LC–MS/MS analysis

LC–MS/MS analysis was performed as described previously (Leinenbach et al., 2014; Pannee et al., 2016) with some modifications; the main difference being an expansion to measure six Aβ peptides, $A\beta_{1-34}$, $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-39}$, $A\beta_{1-40}$, and $A\beta_{1-42}$.

Briefly, uniformly labeled isotope-labeled standards ^{15}N-$A\beta_{1-38}$, ^{15}N-$A\beta_{1-40}$, and ^{13}C-$A\beta_{1-42}$, (rPeptide, Bogart, GA, USA), as well as $A\beta_{1-34}$, $A\beta_{1-37}$, and $A\beta_{1-39}$ labeled with $^{13}C^{15}N$ at Arg-5 in the Aβ sequence (CASLO, Kongens Lyngby, Denmark), were added to 180 µL CSF, followed by addition of 200 µL 5 M guanidine hydrochloride, vortexing for 20 min, and addition of 200 µL 4.7% phosphoric acid before solid-phase extraction on an Oasis MCX µElution plate (Waters Corporation, Milford, MA, USA). Samples were then eluted in 75% acetonitrile/2.5% NH_4OH, dried in a vacuum centrifuge, and stored at −80°C pending analysis. Prior to analysis, samples were reconstituted in 25 µL 20% acetonitrile/1.0% NH_4OH and shaken for 20 min. The injected volume was 20 µL.

Analysis was performed on a Dionex 3000 system coupled to a Q Exactive (both Thermo Fisher Scientific). Reverse-phase separation was performed under alkaline conditions with a monolithic ProSwift RP-4H column (length 250 mm, diameter 1.0 mm; Thermo Fisher Scientific) at a flow rate of 300 µL/min using a 5 min linear gradient from 5 to 20% B (mobile phase A was 5% acetonitrile/0.075% NH_4OH, and B was 95% acetonitrile/0.025% NH_4OH). The mass spectrometer was operated in parallel reaction monitoring mode collecting $[M+4H]^{4+}$ peptide ions using an isolation window of 2.5 m/z units, a maximum injection time of 250 ms, and an automatic gain control target of 2×10^5 charges. A normalized collision energy setting of 19 was employed and fragment ion spectra were acquired with a resolution setting of 17 500.

Calibration curves were obtained for all six Aβ peptides. Two sets of quality controls (QCs) were used to monitor the performance of the parallel reaction monitoring assay, a low and a high concentration QC sample, which consisted of unlabeled peptide standards in artificial CSF (4 mg/mL bovine serum albumin [Sigma Aldrich, Saint Louis, MO, USA] in artificial CSF perfusion fluid [Harvard Apparatus, Holliston, MA]).

2.3 | MRI acquisition and analysis

All CAA patients underwent an MRI scan of the brain according to previously published protocols (De Kort et al., 2023). MRIs from the CAA patients were rated independently by AMK and HBS (see Acknowledgements). In case of disagreement between AMK and HBS, FHMBS (senior vascular neurologist) was consulted before final consensus was reached. See Supplementary Material for details on MRI sequences and the various rated imaging markers.

2.4 | Data processing and statistical analyses

Acquired spectra were processed using the built-in Xcalibur QuanBrowser (version 4.1.31.9, Thermo Fisher Scientific) by summing between 9 and 17 b-ion (b_{23}-b_{41}) fragment ion peak areas for each peptide. Ratios were then obtained by dividing the sum of peak areas of the endogenous peptide by the sum of peak areas of the corresponding isotope-labeled standard. Finally, the concentration for each sample was calculated based on the amount of isotope-labeled standard added and a 6-point calibration curve.

Data were analyzed using GraphPad Prism version 9.0.0 (GraphPad Software, Inc., San Diego, CA, USA). Power analyses were performed a posteriori through G*Power version 3.1.9.4 (Faul et al., 2007) for our primary outcome; the difference in individual Aβ peptide concentrations among the three groups. Details on the performed power analyses can be found in Table S1.

Parametric data are displayed as mean values ± standard deviation, and non-parametric data as median values and interquartile range. Data normality was analyzed using Shapiro–Wilk tests. No test for outliers was conducted. Sex distribution differences were analyzed via Chi-square test. Statistical group differences were analyzed with either an analysis of variance with Bonferroni's post hoc test, or Kruskal–Wallis with Dunn's post hoc test, as appropriate. Correlations between variables were analyzed with either Pearson's or Spearman's correlation, as appropriate. The diagnostic accuracy of individual peptides and all six peptides combined was assessed using receiver operator characteristics to discriminate CAA patients from controls and AD-like subjects. Area under the curve (AUC) was calculated for pairwise group comparisons. The threshold for statistical significance was set at $p \leq 0.05$.

3 | RESULTS

Post hoc power calculations showed a power of 0.71 for $A\beta_{1-34}$, 0.95 for $A\beta_{1-37}$, 0.84 for $A\beta_{1-38}$, 0.90 for $A\beta_{1-39}$, 0.95 for $A\beta_{1-40}$, and 1.0 for $A\beta_{1-42}$, respectively. Used input parameters for power calculations can be found in Table S1.

Coefficient of variation (CV) was determined for each peptide by using QC samples. Two low-concentration and two high-concentration QCs per plate were used, over two plates in total. The overall mean CV was 3.6% and was below 16% for all evaluated QC pairs.

There were no differences in age between the three diagnostic groups ($p = 0.08$; Table 1). Sex was equally distributed across the groups ($p = 0.89$; Table 1).

3.1 | Aβ peptide concentrations

CSF levels of all six Aβ peptides were decreased in CAA patients compared with controls (Figure 1; Table S2). Furthermore, all peptide levels, except for $A\beta_{1-42}$, were decreased in CAA compared with AD-like subjects. Finally, apart from decreased $A\beta_{1-42}$ levels, none of the other five peptides were decreased in subjects compared with controls.

3.2 | Association of Aβ peptides with CAA and AD pathology

Receiver operator characteristics analyses of single peptides showed that $A\beta_{1-42}$ performed best (AUC 0.85; 95% confidence interval [CI]: 0.74–0.97) in discriminating patients with CAA from controls, followed by $A\beta_{1-37}$ and $A\beta_{1-40}$ (both AUC 0.73; both 95% CI: 0.57–0.89; Figure 2a). AUC values for all peptides to discriminate CAA from AD-like subjects were very similar (range AUC 0.69–0.75; range 95% CI: 0.55–0.86; Figure 2b), except $A\beta_{1-42}$ (AUC 0.51; 95% CI: 0.37–0.65), which did not differentiate between these groups.

Since the CSF $A\beta_{42/40}$ ratio is suggested to mirror cerebral Aβ deposition better than CSF $A\beta_{42}$ alone (Lewczuk et al., 2017), we compared the association with either CAA or AD pathology of this ratio to that of all single Aβ peptides. The $A\beta_{42/40}$ ratio did not yield a better differentiation of CAA patients from controls (AUC 0.75; 95% CI: 0.59–0.91; data not shown). For the differentiation of CAA and AD-like subjects, the $A\beta_{42/40}$ ratio performed similar (AUC 0.74; 95% CI: 0.62–0.86; data not shown) to $A\beta_{1-37}$ and $A\beta_{1-39}$.

A logistic regression model including all six Aβ peptides yielded higher AUC values than based on a single Aβ peptide for CAA versus controls (single peptides: range AUC 0.69 to 0.85; combination of peptides: AUC 0.91 [95% CI: 0.83–1.0]), and for CAA versus AD-like subjects (single peptides: range AUC 0.51–0.75; combination of peptides: AUC 0.84 [95% CI: 0.74–0.94]). Since age was not perfectly matched between groups, we added age as covariate into the combined regression model. This resulted in even better group differentiations: AUC 0.95 (95% CI: 0.89–1.0) for CAA versus controls, and AUC 0.85 (95% CI: 0.75–0.95) for CAA versus AD-like subjects. When excluding $A\beta_{1-42}$ from the regression model for CAA vs. AD-like subjects (given its use as a priori inclusion criterion for AD-like subjects), the AUC was 0.78 without age as covariate (95% CI: 0.67–0.89), and 0.80 with age as covariate (95% CI: 0.70–0.91). The remaining five peptides still better discriminated CAA patients from AD-like subjects compared with $A\beta_{1-42}$ alone (AUC 0.51; 95% CI: 0.37–0.65).

TABLE 1 Cohort demographics.

	Controls	CAA	AD-like	*p* value
Demographics				
Number of patients	23	25	50	-
Age (years)	72.7 ± 6.9	73.2 ± 6.6	69.8 ± 7.3	0.08[a] ($F = 2.6$, DFn = 2, DFd = 95)
Sex, M/F	11/12	13/12	23/27	0.89[b] ($\chi^2 = 0.24$, DF = 2)
Aβ and tau levels[d]				
$A\beta_{38}$ (pg/mL)	3899 ± 1361 ($n = 20$)	3002 ± 762 ($n = 12$)	3587 ± 835 ($n = 40$)	**0.007**[a,e] ($F = 5.3$, DFn = 2, DFd = 85)
$A\beta_{40}$ (ng/mL)	10.9 [6.46–14.7]	7.53 [6.13–8.39]	9.75 [7.41–11.7]	**0.002**[c,e,g] ($H = 12.5$)
$A\beta_{42}$ (pg/mL)	641 [529–1080]	346 [288–410]	417 [298–479]	**<0.0001**[c,e,f] ($H = 34.6$)
t-tau (pg/mL)	256 [186–367]	391 [272–537]	861 [727–958]	**<0.0001**[c,f,g] ($H = 61.2$)
p-tau_{181} (pg/mL)	32.8 [25.9–51.3]	47.2 [34.6–61.8]	132 [114–148]	**<0.0001**[c,f,g] ($H = 64.3$)

Note: Age and $A\beta_{38}$ levels (quantified by immunoassays) are presented as means ± standard deviations. $A\beta_{40}$, $A\beta_{42}$, t-tau, and p-tau_{181} levels are presented as medians and interquartile range. Statistical values are reported for analysis of variance (*F* value, DFn, and DFd), Kruskal–Wallis tests (*H* value), and Chi-square test (χ^2 and DF). Bold *p* values indicate statistical significance.

Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; DFd, degrees of freedom denominator; DFn, degrees of freedom numerator; F, female; M, male; p-tau_{181}, phosphorylated tau; t-tau, total tau.

[a]Analysis of variance with Bonferroni's post hoc test.

[b]Chi-square test.

[c]Kruskal–Wallis test with Dunn's post hoc test.

[d]Quantified with immunoassay.

[e]Statistically significant for controls versus CAA.

[f]Statistically significant for controls versus AD-like.

[g]Statistically significant for CAA versus AD-like.

3.3 | Correlations between Aβ peptides and with age for all groups

Overall, the correlation between all Aβ peptides was high when all groups were combined (Figure S1). The strongest correlations were observed for $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}39}$, and $A\beta_{1\text{-}40}$ ($r_s = 0.71$–0.97, all $p < 0.0001$). The correlation of $A\beta_{1\text{-}42}$ with any of the other peptides was relatively weak ($r_s = 0.26$–0.67, $p = 0.0001$–0.008). Also, for $A\beta_{1\text{-}34}$, lower correlations with other peptides were observed than for correlations between the first mentioned peptides ($r_s = 0.26$–0.75, $p = 0.0001$–0.009).

A weak correlation with age was observed for $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ ($r_s = 0.21$–0.24, $p = 0.02$–0.04; Figure S1).

Aβ concentrations previously quantified by ELISA were available for a subset of samples for $A\beta_{38}$ ($n = 90$), and all samples for $A\beta_{40}$ ($n = 98$) and $A\beta_{42}$ ($n = 98$; Table 1). All three peptide concentrations displayed a strong correlation between levels quantified by ELISA versus LC–MS/MS ($r_s = 0.85$–0.91, all $p < 0.0001$; Figure S2).

3.4 | Correlations of Aβ peptides with MoCA and imaging parameters in CAA patients

The $A\beta_{1\text{-}42}$ concentration displayed a moderate correlation with MoCA scores in CAA patients (Figure 3; $r_s = 0.51$; $p = 0.02$). A trend was observed for the correlation with the $A\beta_{42/40}$ ratio ($r_s = 0.42$; $p = 0.06$). No other Aβ peptide correlated with the MoCA score.

A moderate correlation between $A\beta_{1\text{-}37}$ concentration with the presence of an intracerebral hemorrhage was observed ($r_s = -0.42$; $p = 0.04$; Figure 3). None of the other individual Aβ peptides correlated with any MRI parameter. The $A\beta_{42/40}$ ratio, however, correlated with lobar cerebral microbleeds ($r_s = -0.50$; $p = 0.01$) and cortical superficial siderosis ($r_s = 0.42$; $p = 0.04$). A trend toward significance was observed for the correlations between $A\beta_{1\text{-}38}$ and presence of an intracerebral hemorrhage ($r_s = -0.36$; $p = 0.08$) and between $A\beta_{1\text{-}42}$ and lobar cerebral microbleeds ($r_s = -0.38$; $p = 0.06$). The scores of the MRI parameters can be observed in Table S3.

4 | DISCUSSION

In the current study, we investigated the biomarker potential of CSF $A\beta_{1\text{-}34}$, $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}39}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ in patients with CAA, AD-like subjects, and in controls. Most importantly, we found lower levels of all Aβ peptides in CAA patients compared with controls, and similarly of all peptides, except for $A\beta_{1\text{-}42}$, when compared with AD-like subjects. The combination of all Aβ peptides differentiated CAA better from controls or AD-like subjects than individual peptide levels, even after omitting $A\beta_{1\text{-}42}$ from the model and only the remaining five peptides were utilized. Among the six Aβ peptides, results obtained for $A\beta_{1\text{-}42}$ were deviant since it yielded the lowest AUC values for the comparisons between CAA and either controls or AD-like subjects, and it correlated relatively weakly with the other peptides, whereas the remaining peptides yielded alike results.

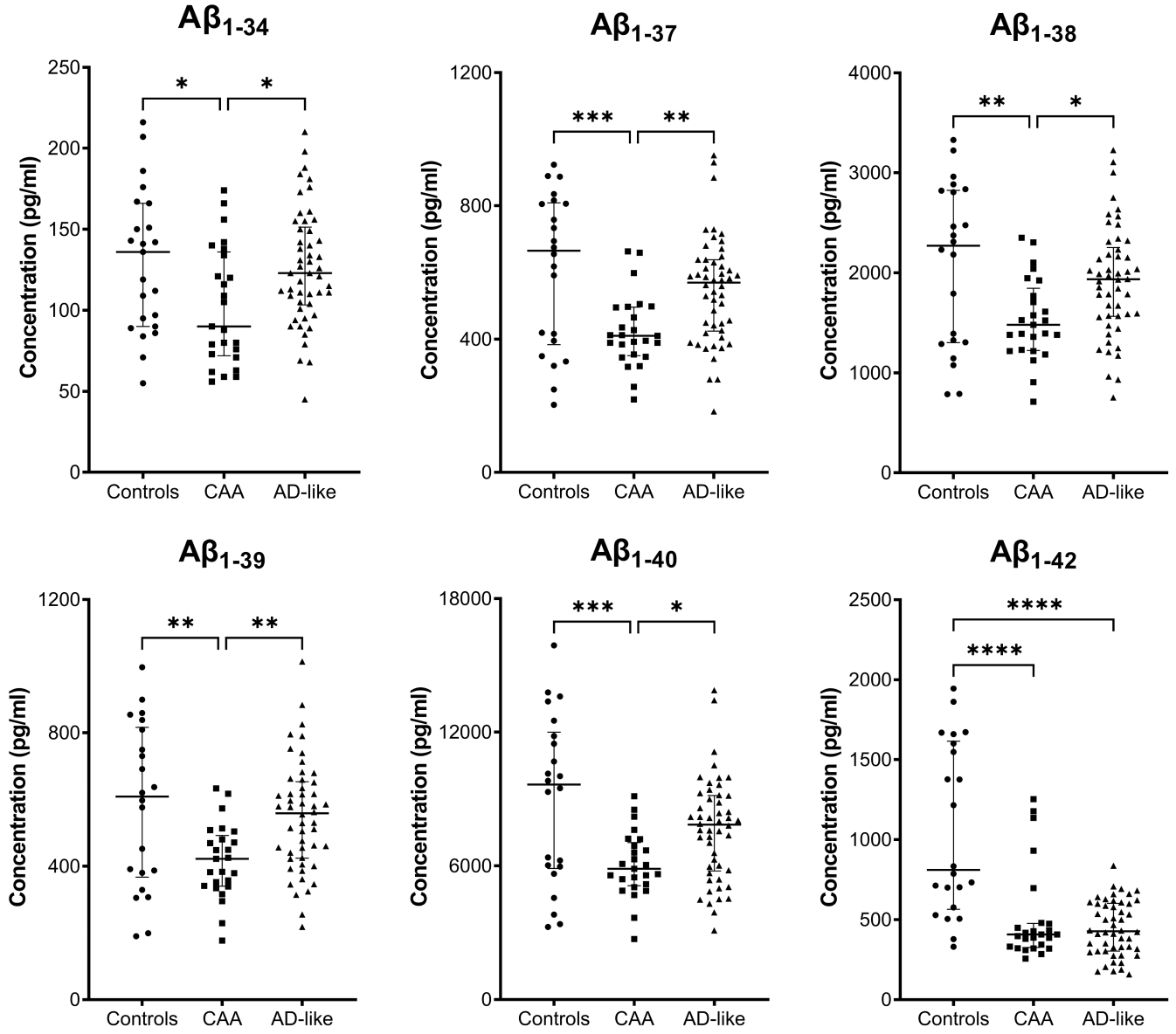

FIGURE 1 Cerebrospinal fluid levels (pg/mL) of amyloid-β (Aβ) peptides in controls ($n = 23$ for $A\beta_{1\text{-}34}$, $n = 22$ for other peptides), cerebral amyloid angiopathy (CAA) patients ($n = 25$), and Alzheimer's disease-like (AD-like) subjects ($n = 50$). Statistical comparison was performed with analysis of variance with Bonferroni's post hoc test (for $A\beta_{1\text{-}34}$, $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}39}$, and $A\beta_{1\text{-}40}$), or Kruskal–Wallis with Dunn's post hoc test (for $A\beta_{1\text{-}42}$), as appropriate. p values: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, and $^{****}p \leq 0.0001$. Median values and interquartile range are indicated.

Correspondings with previous studies (Banerjee et al., 2020; De Kort et al., 2023; Verbeek et al., 2009), we observed lower CSF concentrations of $A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}40}$ in CAA compared with AD-like subjects and controls, and lower $A\beta_{1\text{-}42}$ compared with controls only. In addition, the concentrations of shorter Aβ peptides were consistently decreased in patients with CAA as well, as opposed to AD-like subjects and controls. $A\beta_{34}$, $A\beta_{37}$, and $A\beta_{39}$ have previously been detected in the cerebral vasculature of transgenic AD mice and human AD brain tissue, but not in parenchymal plaques (Kirabali et al., 2019; Reinert et al., 2016). It is widely accepted that $A\beta_{42}$ is the most abundant peptide in human AD brains without CAA, as opposed to $A\beta_{40}$ in AD brains with CAA pathology (Gkanatsiou et al., 2019). In line with this fact, deposition of Aβ peptides shorter than 42 amino acids specifically in the cerebral vasculature could explain the lower peptide concentrations in the CSF of patients with CAA, as previously suggested using a mass spectrometry imaging approach (Kakuda et al., 2017). Immunohistochemical studies using C-terminal-specific antibodies may provide more support for this suggestion. Nevertheless, as a result of the unavailability of imaging information for AD-like subjects, we cannot rule out the presence of CAA in this group, emphasizing the need for a cautious interpretation of the current results. However, if a certain proportion of our AD-like group would have CAA, it is expected that the current differences in CSF Aβ levels between CAA patients and AD-like subjects would be smaller, in contrast to when CAA would have been excluded in the AD-like group.

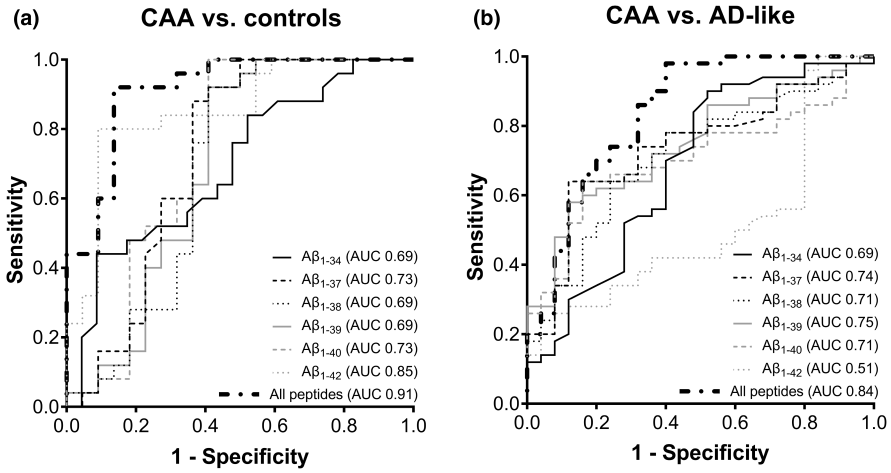

FIGURE 2 Receiver operator characteristics curves for discrimination of (a) cerebral amyloid angiopathy (CAA) patients from controls and (b) CAA patients from Alzheimer's disease-like (AD-like) subjects. Area under the curve (AUC) was calculated for all individual amyloid-β (Aβ) peptides and the combination of all six peptides.

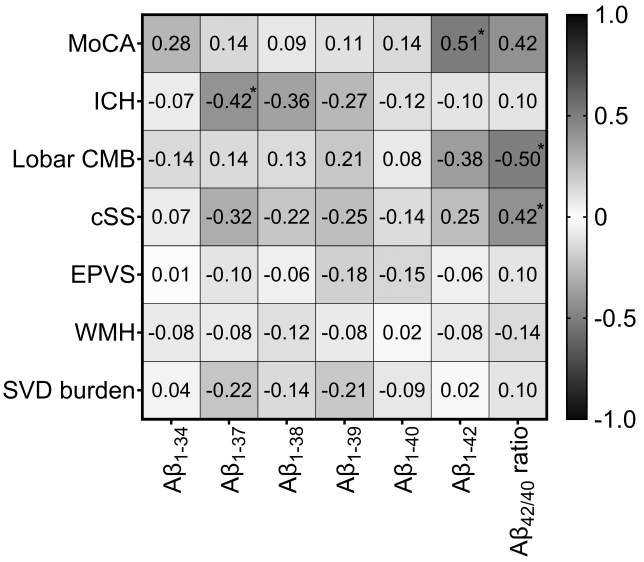

FIGURE 3 Correlations of individual amyloid-β (Aβ) peptide levels and $A\beta_{42/40}$ ratio with Montreal Cognitive Assessment (MoCA) score and cerebrovascular imaging markers in patients with cerebral amyloid angiopathy (CAA). Spearman rank correlation coefficients are displayed. MoCA was available in a subset ($n = 21$) of CAA patients. Asterisk indicates a significant p value (≤0.05). CMB, cerebral microbleeds; cSS, cortical superficial siderosis; EPVS, enlarged perivascular spaces; ICH, intracerebral hemorrhage; SVD, small vessel disease; WMH, white matter hyperintensities.

Decreased CSF $A\beta_{42}$ levels in CAA patients compared to AD were previously reported (Banerjee et al., 2020; De Kort et al., 2023), but we did not observe a similar decrease in AD-like subjects, for which there could be several explanations. This may be as a result of the selection of AD-like subjects having decreased $A\beta_{1\text{-}42}$ levels (and also increased total tau and phosphorylated tau_{181} levels). Moreover, our relatively small sample size may have contributed to this observation, although the obtained power for $A\beta_{1\text{-}42}$ analyses was very high. In addition, each technique will have its own analytical limitations (e.g., matrix effect could interfere with ELISA efficiency and ion suppression could impact quantification in LC–MS/MS). Of note, the reported correlations between levels quantified by ELISA versus LC–MS/MS for $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ were high.

Ample evidence suggests that failed Aβ clearance across the blood–brain barrier causes the shorter C-terminally truncated Aβ peptides to deposit within cerebral vessel walls (Cabrera et al., 2018; Qi & Ma, 2017; Weller et al., 1998). On the other hand, $A\beta_{42}$ is more rigid (Dong et al., 2016) and hydrophobic (Jarrett et al., 1993), and likely to oligomerize and aggregate in the form of parenchymal plaques. Our observations of high correlations and similar AUC values between all shorter Aβ peptides support a possible distinct biological function or fate of $A\beta_{42}$. Besides this proposed difference in perivascular drainage between short (40 amino acids or shorter) and long (42 amino acids or longer) Aβ peptides, sequential APP cleavage routes might also contribute to this observed difference. Various APP fragments are formed by secretases (Dunys et al., 2018), and reduced production of shorter fragments could also explain the observed decreased CSF levels in patients with CAA.

Diagnostic accuracies of CSF $A\beta_{40}$ and $A\beta_{42}$ concentrations in CAA were recently evaluated as part of a quantitative meta-analysis (Margraf et al., 2022) and in a large cohort study (Grangeon et al., 2022). In our study, $A\beta_{1\text{-}40}$ (AUC 0.72) and $A\beta_{1\text{-}42}$ (AUC 0.85) discriminated CAA from controls similarly well to these earlier reports (AUC 0.69–0.76 for $A\beta_{40}$; AUC 0.79–0.89 for $A\beta_{42}$). Overall, the $A\beta_{42/40}$ ratio yields more variable accuracies (AUC 0.56–0.90) (Grangeon et al., 2022; Margraf et al., 2022). For differentiation of CAA from AD-like subjects, our AUCs (0.71 for $A\beta_{1\text{-}40}$; 0.51 for $A\beta_{1\text{-}42}$) were comparable to these earlier reports as well (AUC 0.72–0.73 for $A\beta_{40}$; AUC 0.54–0.62 for $A\beta_{42}$). Notably, our combined panel of six Aβ peptides performed better in differentiating CAA patients from either controls (AUC 0.91) or AD-like subjects (AUC 0.84) than these core CSF biomarkers individually, irrespective of age (AUC 0.95 for CAA vs. controls; AUC 0.85 for CAA vs. AD-like subjects). Hence, CSF analysis of a panel of Aβ peptides may have the future potential to support clinicians in determining the most prominently present type of pathology (i.e., AD or CAA) with high accuracy in a patient, despite the substantial and often observed neuropathological overlap. When our findings have been independently validated in other cohorts including

clinically diagnosed AD patients, they might be implemented in clinical practice for diagnostic purposes. An important application may be to aid the detection of CAA in AD patients which may help to select patients for inclusion in anti-Aβ immunotherapy trials, which are known to be hampered by CAA-related side effects in the form of amyloid-related imaging abnormalities (Sveikata et al., 2022). Nevertheless, the aforementioned limitations of the Boston criteria (i.e., reflection of late-stage CAA, and not severity) should be considered, raising uncertainty about whether the panel of Aβ peptides is sufficiently sensitive and specific to detect early-stage CAA.

Certain limitations apply to the current study. Classification of AD-like subjects was based on the ATN classification system, with no information on their definitive clinical diagnosis. However, it has been acknowledged that this biological classification system identifies AD pathology accurately (Jack Jr. et al., 2018). Moreover, it is necessary to validate our findings in an independent cohort (including patients with hypertensive arteriopathy who exhibit lobar and/or deep microbleeds) prior to implementation in clinical practice. Furthermore, presence of CAA pathology and information on cognitive functioning in AD-like subjects and controls was unknown, since no MRI scan or standardized cognitive assessments were available for these subjects. Since 22% of patients with AD display lobar microbleeds (as a sign of concomitant CAA) (Jäkel et al., 2022), based on our observations on CSF Aβ peptides in CAA patients, co-pathology of CAA in our AD-like subjects may have led to decreased Aβ levels in a proportion of subjects. Thus, our observed AUC values in the comparison between AD-like subjects and CAA patients may turn out to be even higher when we would have been able to compare AD subjects without CAA to CAA patients. Future studies should include AD patients with and without imaging indications of CAA pathology to study the effect of CAA pathology in AD patients on Aβ levels, which will likely improve the discrimination of CAA patients from pure AD patients. Moreover, our relatively small sample size should be increased, but we would like to emphasize that with the current sample sizes, sufficient statistical power (84–100%) was achieved for most peptides (except for $A\beta_{1\text{-}34}$). Lastly, the *APOE* ε4 allele is a known major risk factor for the development of both vascular and parenchymal Aβ deposits (Greenberg et al., 2020), but *APOE* genotype status was not available to include as variable. A major strength includes the use of a robust and validated LC–MS/MS quantification method (Leinenbach et al., 2014; Pannee et al., 2016), with high sensitivity and selectivity for the targeted peptides. Additionally, excellent correlations among $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ concentrations quantified by our LC–MS/MS approach compared to concentrations quantified by ELISAs were observed, corroborating the high performance of our applied technique, which allows simultaneous quantification of multiple Aβ species in a small sample volume.

In conclusion, CSF levels of $A\beta_{1\text{-}34}$, $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}39}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ are clearly decreased in CSF from patients with CAA compared with controls, and all peptides, except for $A\beta_{1\text{-}42}$, are decreased in CAA compared with AD-like subjects as well. This represents a distinct disease-specific Aβ profile for CAA patients compared to both AD-like subjects and controls. The complete panel of Aβ species differentiated CAA from controls and AD-like subjects with high accuracy. Future studies may include the investigation of this Aβ peptide panel in patients with clinical AD with and without evidence of CAA, to evaluate the effect of CAA on CSF Aβ levels in these patients, and to select patients for immunotherapy trials. Moreover, via immunohistochemical studies, the possible association of different Aβ peptides with CAA should be studied in more detail to assess the correlation with observations in CSF. Finally, other Aβ species (e.g., N-terminal truncated peptides and post-translationally modified peptides) would be of interest to study in CAA populations to obtain more detailed mechanistic insight into Aβ metabolism in CAA pathogenesis.

AUTHOR CONTRIBUTIONS

Emma van den Berg: Writing – original draft; writing – review and editing; visualization; formal analysis; data curation. **Iris Kersten:** Methodology; data curation; writing – review and editing. **Gunnar Brinkmalm:** Writing – original draft; methodology; validation; writing – review and editing; data curation; resources. **Kjell Johansson:** Methodology; writing – review and editing; validation; data curation. **Anna M. de Kort:** Resources; writing – review and editing. **Catharina J. M. Klijn:** Conceptualization; funding acquisition; writing – review and editing; resources. **Floris H. B. M. Schreuder:** Conceptualization; funding acquisition; writing – review and editing; resources. **Johan Gobom:** Writing – original draft; methodology; writing – review and editing; validation. **Erik Stoops:** Writing – review and editing; resources. **Erik Portelius:** Conceptualization; funding acquisition; writing – review and editing. **Eleni Gkanatsiou:** Methodology; writing – review and editing; validation. **Henrik Zetterberg:** Writing – review and editing. **Kaj Blennow:** Writing – review and editing. **Hinke B. Kuiperij:** Conceptualization; writing – review and editing; funding acquisition; supervision. **Marcel M. Verbeek:** Writing – review and editing; conceptualization; funding acquisition; supervision; project administration.

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CONFLICT OF INTEREST STATEMENT

HZ has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). ES is employee of ADx NeuroSciences. KB has served as a consultant on advisory boards or on data monitoring committees for BioArctic, Biogen, Julius Clinical, Lilly, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. The other authors declare that they have no competing interests.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jnc.16074.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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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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