RESEARCH ARTICLE

Blood-brain barrier dysfunction and reduced cerebrospinal fluid levels of soluble amyloid precursor protein-β in patients with subcortical small-vessel disease

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Abstract

Introduction: Subcortical small-vessel disease (SSVD) is the most common vascular cognitive disorder. However, because no disease-specific cerebrospinal fluid (CSF) biomarkers are available for SSVD, our aim was to identify such markers.

Methods: We included 170 healthy controls and patients from the Gothenburg Mild Cognitive Impairment (MCI) study clinically diagnosed with SSVD dementia, Alzheimer's disease (AD), or mixed AD/SSVD. We quantified CSF levels of amyloid-β ($A\beta$)$_{x-38}$, $A\beta_{x-40}$, $A\beta_{x-42}$, as well as soluble amyloid precursor protein (sAPP)-α and sAPP-β.

Results: sAPP-β was lower in SSVD patients than in AD patients and controls. Receiver-operating characteristic (ROC) analyses showed that sAPP-β moderately separated SSVD from AD and controls. Moreover, the CSF/serum albumin ratio was elevated exclusively in SSVD and could moderately separate SSVD from the other groups in ROC analyses.

Discussion: SSVD has a biomarker profile that differs from that of AD and controls, and to some extent also from mixed AD/SSVD, suggesting that signs of blood-brain barrier (BBB) dysfunction and sAPP-β could be additional tools to diagnose SSVD.

Highlights:

- Patients with subcortical small-vessel disease (SSVD) exhibited reduced levels of sAPP-β and disturbances of the blood-brain barrier (BBB).
- This biochemical pattern is different from that of Alzheimer's disease (AD) and to some degree from that of mixed AD/SSVD.
- Our findings are speaking in favor of the concept that SSVD is a distinct vascular cognitive disorder (VCD) form.

1 | BACKGROUND

Vascular cognitive disorder (VCD) and Alzheimer's disease (AD) belong to the most common cognitive disorders in the elderly population. Several forms of VCD exist but in this article we use the singular denomination for all variants of VCD. VCD is similar to "vascular cognitive impairment" but refers more clearly to phenotypically characteristic subgroups and is broader than "vascular dementia," as milder forms of cognitive impairment also are included. The subcortical small-vessel type of disease (SSVD) has been estimated to be the most common form of VCD.[1,2] The disease affects the small vessels deep in the brain, including perforating arterioles, capillaries, and venules.[1,3] In these patients, magnetic resonance imaging (MRI) reveals increased occurrence of cerebral microbleeds (CMBs), infarcts, and lacunes, as well as white matter hyperintensities (WMHs) that correspond to lesions of the brain white matter. Moreover, SSVD patients exhibit reduced executive function, decreased processing speed, and only mild memory loss,[1] whereas patients with AD are characterized by disturbances in interpreting sensory information and pronounced loss of memory. However, the clinical phenotype may resemble that of AD. Especially, the continuously progressive disease course is characteristic of both SSVD and AD. There are so far no established disease-specific biochemical markers for SSVD, but the blood-brain barrier (BBB) has been suggested to be involved in the pathogenesis of SSVD.[4–7]

AD is characterized by the accumulation of extracellular plaques consisting of amyloid beta (Aβ), intracellular tangles composed of hyperphosphorylated tau (p-tau), and synapse degeneration. The clinical symptoms include predominantly memory and language impairments. The amyloid cascade hypothesis is the prevailing hypothesis of the origin of AD.[8] The presence of AD neuropathology can be identified by the use of cerebrospinal fluid (CSF) biomarkers. The typical CSF biomarker pattern includes decreased levels of Aβ_{1-42} (or A$\beta_{1-42/1-40}$ to increase specificity for plaque pathology), and increased levels of p-tau$_{181}$ and total tau (t-tau). Moreover, mixed pathology such as mixed AD/SSVD (also known as mixed dementia) is a common condition, where features of AD and SSVD coexist in the brain.[9] Biomarker studies investigating mixed AD/SSVD are few, but in one study, clinically defined mixed AD/SSVD was characterized by a typical AD CSF biomarker profile and an SSVD-like neuropsychological cognitive profile.[10]

Metabolites derived from the amyloid precursor protein (APP) including different Aβ peptides (e.g., Aβ_{x-38}, Aβ_{x-40}, and Aβ_{x-42}, where "x" indicates any lengths of the peptides), as well as the soluble APP fragments cleaved by α- and β-secretases (sAPP-α and sAPP-β, respectively) have been used to improve the understanding of the amyloid cascade hypothesis in AD, but it is not yet known whether they are altered in SSVD. Although most of the studied APP fragments, excluding Aβ_{1-42}, are unaltered in AD,[11] the results of several studies suggest that these biomarkers (including Aβ_{1-42}) may be associated with the amount of WMHs.[12–16] Consequently, these biomarkers may be altered in SSVD that is characterized by the presence of WMHs. To our knowledge, no studies have yet investigated CSF levels of the whole spectrum of APP metabolites in patients with pure SSVD. Therefore, we investigated a panel of CSF APP metabolites, and in addition, CSF/serum albumin ratio in participants of the Gothenburg Mild Cognitive Impairment (MCI) study.[17] We included healthy controls and patients diagnosed with SSVD, AD, and mixed AD/SSVD mild dementia using clinical and biomarker tools.

2 | METHODS

2.1 | Study participants

In this cross-sectional study, we evaluated CSF amyloid biomarkers in 45 healthy controls and 125 patients (SSVD, n = 30; AD, n = 60; and mixed AD/SSVD, n = 35). Patients with other forms of dementia (cortical vascular dementia, primary progressive aphasia, Lewy body dementia, frontotemporal dementia, or unspecified dementia) were excluded. The participants were recruited from the Gothenburg MCI study, a mono-center study of patients seeking help for cognitive complaints at the memory clinic at Sahlgrenska University Hospital.[17] The inclusion and exclusion criteria were designed to exclude somatic and psychiatric conditions associated with increased risk of cognitive impairment. Thus the inclusion criteria comprised age >40 and <79 years, Mini Mental State Examination (MMSE) score >19, and self- or informant-reported cognitive decline with a duration ≥6 months. The

exclusion criteria included severe somatic disease (e.g., subdural hemorrhage, brain tumor, untreated hypothyroid state, encephalitis, and unstable heart disease), psychiatric disorder (e.g., major affective disorder or schizophrenia), substance abuse, and confusion. The healthy controls were primarily recruited through senior citizen organizations, for example, information meetings on cognitive disorders, and some controls were relatives of the patients. Present, or history of, cognitive decline was an exclusion criterion in the controls; otherwise the exclusion criteria as well as the study procedures were similar as those applied for the patients.

2.2 | Diagnostic procedures

The patients were classified using the global deterioration scale (GDS), in which a GDS score of 4 equals mild dementia.[18] The classification into GDS group 4 was based on medical history, checklists, and instruments for cognitive symptoms[17]: (1) Stepwise Comparative Status Analysis (STEP), variables 13 to 20 (memory disturbance, disorientation, reduced abstract thinking, visuospatial disturbance, poverty of language, sensory aphasia, visual agnosia and apraxia); (2) I-FLEX, a short form of the Executive Interview (EXIT) (number-letter task, word fluency, anomalous sentence repetition, interference task, Luria hand sequences, counting task); (3) MMSE; and (4) clinical dementia rating (CDR). The CDR assessment was based on information from both the subject and an informant. Guidelines for GDS 4 were: STEP >1, I-FLEX >3, CDR >1.0, MMSE ≤25. Finally, a consensus decision among

the physicians at the clinic was made to determine the appropriate GDS score. Only patients with mild dementia were included in the present study.

The physicians who determined the specific dementia diagnoses according to the research protocol had access to clinical symptomatology and MRI data, but were blinded to neuropsychological test results and CSF biomarker data. AD was diagnosed using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[19] More specifically: an AD diagnosis required predominant parietotemporal lobe symptoms and no or mild WMHs using magnetic resonance imaging (MRI).[17] Although diagnoses in the umbrella study were set without taking into account CSF biomarkers, they were revised in the present substudy using these markers. Hence, it was required that AD patients had CSF biomarker levels consistent with an AD profile in agreement with the International Working Group-2 (IWG-2) criteria.[20] Furthermore, clinical SSVD patients with CSF biomarker evidence of AD pathology were considered to fulfill a mixed AD/SSVD diagnosis, and patients with clinical mixed AD/SSVD without CSF biomarker evidence were reclassified to SSVD. Patients with a clinical AD diagnosis, but without CSF biomarker evidence of pathology, were excluded as well as controls with biomarker evidence of AD pathology, when diagnoses were revised using biomarkers. The applied cut-offs for abnormality for the CSF AD biomarkers were t-tau >350 ng/L, p-tau$_{181}$ >59 ng/L, and Aβ_{1-42} <530 ng/L.[21]

A clinical SSVD diagnosis was set according to the Erkinjuntti criteria.[22] More specifically: for SSVD, the patient had to have MRI-detected cerebral WMHs, (mild, moderate, or severe according to Fazekas classification)[23] and predominant frontal lobe symptoms. If WMHs were only mild, then SSVD was set only if parietotemporal lobe syndromes were not marked. For an SSVD diagnosis to be made, the CSF AD biomarkers had to be negative (same criteria as above). Mixed AD/SSVD was diagnosed if AD patients also exhibited MRI findings of cerebral WMHs (moderate or severe according to Fazekas classification)[23] with no predominant frontal lobe syndrome, if AD patients exhibited mild degree of WMHs in combination with a marked frontal lobe syndrome, or if SSVD patients showed CSF AD biomarker evidence of AD pathology.

The classification used in the study is in line with the results of the Vascular Impairment of Cognition Classification Consensus Study (VICCCS) in which SSVD dementia, denominated subcortical ischemic vascular dementia, is one of the entities.[24] No patients exhibited poststroke dementia or multi-infarct dementia.

2.3 | Neuropsychological testing

In addition to the tests used for GDS classification, a neuropsychological test battery was administered.[17,25] We used the delayed recall from the Rey Auditory Verbal Learning Test (RAVLT) to assess episodic memory and the Trail Making Test A (TMT-A) and B (TMT-B) to evaluate visual scanning and complex attention.

2.4 | Cerebrospinal fluid sampling and biomarker assessments

CSF samples were drawn at the lumbar vertebrae L3/L4 or L4/L5 interspace with the patients in a fasted state between 08:00 and 10:00 a.m. The first portion of the CSF sample was discarded to avoid blood contamination. In all, 20 mL of CSF was collected in polypropylene tubes and gently mixed by inverting the tube. The CSF was centrifuged at room temperature at 2000 × g for 10 minutes, and then stored at −80°C pending analyses.[17]

All biomarkers were analyzed at the Clinical Neurochemistry Laboratory in Mölndal, Sweden, by board-certified laboratory technicians who were blinded to the clinical diagnoses and other clinical information. CSF concentrations of $A\beta_{1-42}$, the axonal damage marker t-tau, and p-tau$_{181}$ were measured using sandwich enzyme-linked immunosorbent assays (ELISAs; INNOTEST β-AMYLOID (1-42), INNOTEST hTAU Ag, and INNOTEST PHOSPHO-TAU (181P), respectively; Fujirebio, Gent, Belgium). CSF $A\beta_{x-38}$, $A\beta_{x-40}$, and $A\beta_{x-42}$ concentrations were measured using the MSD V-PLEX $A\beta$ Peptide Panel 1 (4G8) kit (Meso Scale Diagnostics, Rockville, MD, USA). CSF sAPP-α and sAPP-β concentrations were measured using the MSD sAPP-α/sAPP-β duplex assay (Meso Scale Diagnostics). Two internal control CSF samples (aliquots of pooled CSF) were analyzed in each run as internal quality controls to control for between-assay variability, which was <10% for all markers.[17] Serum and CSF albumin were measured using immunonephelometry on a Beckman Immage immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA). The ratio between CSF albumin (mg/L) and serum albumin (g/L) was used as a measure of the BBB function. Apolipoprotein E gene (APOE) genotyping was performed by minisequencing.[26]

2.5 | Statistical analysis

SPSS version 25 (IBM Corp., Armonk, NY, USA) was used for all statistical procedures. The descriptive statistical results are given as the mean and SD, if not otherwise stated. Between-group differences were assessed using the Kruskal-Wallis test for multiple variables, followed by the Mann-Whitney U test for pairwise comparisons of continuous variables and using chi-square tests for categorical variables. The relationships between sensitivity and specificity between study groups were described using receiver-operation characteristic (ROC) analysis. In these analyses, we calculated area under the ROC curve (AUROC) and 95%CIs. Correlations were sought using the Spearman rank order correlation test. Statistical significance was obtained if the two-tailed P-value was < .05.

2.6 | Ethical considerations

The study was approved by the regional ethical committee in Gothenburg (# 091-99, T479-11 and 2020-06733). Oral and written informed consent was obtained from all study participants. The study was performed in compliance with the Declaration of Helsinki.

3 | RESULTS

3.1 | Clinical characteristics

The clinical characteristics of the patients and controls are given in Table 1 and Figure S1. The proportion of men was higher in the SSVD group than in the AD group. Mean age was higher in all the patient groups compared with the controls (Figure S1A). In addition, patients with mixed AD/SSVD had higher mean age than SSVD and AD patients. Education level was lower (Figure S1B), and the scores of the neuropsychological tests (MMSE, RAVLT delayed recall, TMT-A, and TMT-B; Figure S1C-SF) were more impaired in the patient groups compared with the control group. Furthermore, SSVD patients had higher MMSE scores than patients with mixed AD/SSVD and higher RAVLT delayed-recall scores than AD and mixed AD/SSVD patients. Per design, the core CSF AD biomarker levels ($A\beta_{1-42}$, t-tau, and p-tau$_{181}$) were abnormal in the AD or AD/SSVD groups compared with the SSVD and control groups (Figure S1G-SI). Nevertheless, SSVD patients had higher CSF t-tau level than the controls. Finally, the AD and SSVD groups had higher prevalence of APOE ε4 allele than the control group, and AD patients also had higher prevalence of APOE ε4 allele than SSVD patients (Table 1).

3.2 | Fluid biomarkers

CSF levels of $A\beta_{x-38}$, $A\beta_{x-40}$, $A\beta_{x-42}$, sAPP-α, and sAPP-β are presented in Figure 1 and Table 2. SSVD patients had lower CSF sAPP-β levels compared with the controls, whereas there was no difference between these two groups in terms of other CSF biomarkers (Figure 1E). In addition, CSF sAPP-β levels were significantly lower in SSVD patients compared with AD patients. The average levels of CSF sAPP-β were lower in SSVD than in mixed AD/SSVD, but this difference was not statistically significant. As expected, patients with AD and mixed AD/SSVD had reduced CSF $A\beta_{x-42}$ levels (Figure 1C), and also lower $A\beta_{x-42}/A\beta_{x-38}$ and $A\beta_{x-42}/A\beta_{x-40}$ ratios than both SSVD patients and healthy controls (Figure 1F-G). Moreover, the CSF/serum albumin ratio was elevated in the SSVD group (Figure 1H).

3.3 | Biomarker correlations

CSF/serum albumin ratio was not correlated with CSF levels of sAPP-β or sAPP-α in the study population (n = 170; r = −0.11 and r = −0.03, respectively) or in any of the study groups (data not shown). CSF sAPP-β level correlated positively with CSF sAPP-α level in the total study population (r = 0.66, P < .001) as well as in all study groups (SSVD: r = 0.75, P < .001; AD: r = 0.75, P < .001; mixed AD/SSVD: r = 0.57, P < .001; and control: r = 0.48, P < .001).

TABLE 1   Baseline characteristics in patients and controls

Variable	SSVD (n = 30)	AD (n = 60)	Mixed AD/SSVD (n = 35)	Control (n = 45)	P-value between groups
Men/women (n, %)	18/12 (60/40)[a]	16/44 (27/73)	14/21 (40/60)	19/26 (42/58)	.02
Age (years)	67.4 (6.4)[b,c]	66.8 (7.2)[b,c]	71.4 (6.2)[d]	62.3 (6.2)	<.001
Education (years)	10.7 (3.1)[b]	10.6 (3.2)[d]	11.0 (3.5)[e]	12.6 (2.4)	<.01
MMSE score	25.5 (1.9)[d,f]	24.3 (2.9)[d]	24.2 (2.6)[d]	29.5 (0.6)	<.001
RAVLT delayed recall	5.62 (3.90)[a,b,c]	2.66 (1.62)[d]	2.35 (1.63)[d]	8.88 (3.13)	<.001
TMT-A (seconds)	63.7 (23.4)[d]	78.1 (49.6)[d]	78.8 (56.9)[d]	32.8 (10.1)	<.001
TMT-B (seconds)	173 (60)[d]	191 (108)[d]	223 (101)[d]	75 (17)	<.001
CSF $A\beta_{1-42}$ (ng/L)	679 (187)[g,h]	333 (83)[d]	331 (110)[d]	679 (260)	<.001
CSF t-tau (ng/L)	372 (188)[e,g,h]	710 (294)[d]	733 (332)[d]	260 (103)	<.001
CSF p-tau$_{181}$ (ng/L)	51 (19)[g,h]	95 (34)[d]	104 (50)[d]	46 (15)	<.001
Number of APOE ε4 alleles, 0/1/2 (n, %)	15/12/3 (50/40/10)[i]	12/34/12 (21/59/21)[b]	9/17/9 (26/49/26)[b]	23/18/2 (53/42/5)	<.01

Values are given as means (SD). Between-group differences for continuous variables were examined using the Kruskal-Wallis test for multiple comparisons followed by the Mann-Whitney U test for pairwise comparisons. For categorical variables, chi-square tests were used to assess differences between groups. APOE genotyping was not performed in four patients. SSVD, subcortical small vessel disease; AD, Alzheimer's disease; MMSE, Mini Mental State Examination; RAVLT, Rey Auditory Verbal Learning Test; TMT, Trail Making Test; Aβ, amyloid-beta; t-tau, total tau; p-tau, phosphorylated tau; sAPP, soluble amyloid precursor protein; APOE, apolipoprotein E.

[a]$P < .01$ versus AD.
[b]$P < .01$ versus control.
[c]$P < .01$ versus mixed AD/SSVD.
[d]$P < .001$ versus control.
[e]$P < .05$ versus control.
[f]$P < .05$ versus mixed AD/SSVD.
[g]$P < .001$ versus AD.
[h]$P < .001$ versus mixed AD/SSVD.
[i]$P < .05$ versus AD.

## 3.4 | Diagnostic accuracy of CSF sAPP-β and CSF/serum album ratio

Next, we performed ROC curve analyses to further evaluate CSF sAPP-β, which was different in SSVD compared with healthy controls and AD patients. We found that CSF sAPP-β moderately differentiated SSVD from controls (AUROC 0.65, 95% CI: 0.53 to 0.78) and SSVD from AD (AUROC 0.69, 95% CI: 0.58 to 0.81), whereas it did not distinguish SSVD from mixed AD/SSVD (AUROC 0.59, 95% CI: 0.45 to 0.73).

We observed that CSF/serum albumin ratio, which was different in SSVD compared with all other study groups, had moderate ability to separate SSVD from controls (AUROC 0.70, 95% CI: 0.58 to 0.83), SSVD from AD (AUROC 0.75, 95% CI: 0.64 to 0.85), and SSVD from mixed AD/SSVD (AUROC 0.66, 95% CI: 0.53 to 0.79).

Finally, we included both CSF sAPP-β and CSF/serum albumin ratio in the analyses. This panel (CSF sAPP-β and CSF/serum albumin ratio) had a somewhat improved ability to distinguish SSVD from controls (AUROC 0.73, 95% CI: 0.61 to 0.85), SSVD from AD (AUROC 0.78, 95% CI: 0.68 to 0.88), and SSVD from mixed AD/SSVD (AUROC 0.66, 95% CI: 0.53 to 0.79).

4 | DISCUSSION

In this study, CSF APP metabolites and CSF/serum albumin ratio were examined in patients with SSVD, mixed AD/SSVD, or AD at an outpatient memory clinic. We found that sAPP-β was decreased in the SSVD group compared with both the control and AD groups. BBB function, as measured by the CSF/serum albumin ratio, was increased in the SSVD in comparison with all other study groups, whereas the groups with AD pathology did not differ from healthy controls. Overall, ROC curve analyses using sAPP-β, CSF/serum albumin ratio, and their combinations showed a moderate ability to separate SSVD from the other groups. These findings support the notion of SSVD being a separate dementia form and is line with Consensus Statement of Subcortical Small Vessel Disease[27] and the classification of the Vascular Impairment of Cognition Classification Consensus Study (VICCCS) in which SSVD dementia, denominated subcortical ischemic vascular dementia, is one of the VCD forms.[24] VICCCS used the Delphi method among clinicians and researchers in the field to determine the nosological structure of major VCI. In addition to SSVD, the following were identified as relevant major VCI subtypes: poststroke dementia (PSD), multi-infarct dementia (MID), and mixed dementias. Because
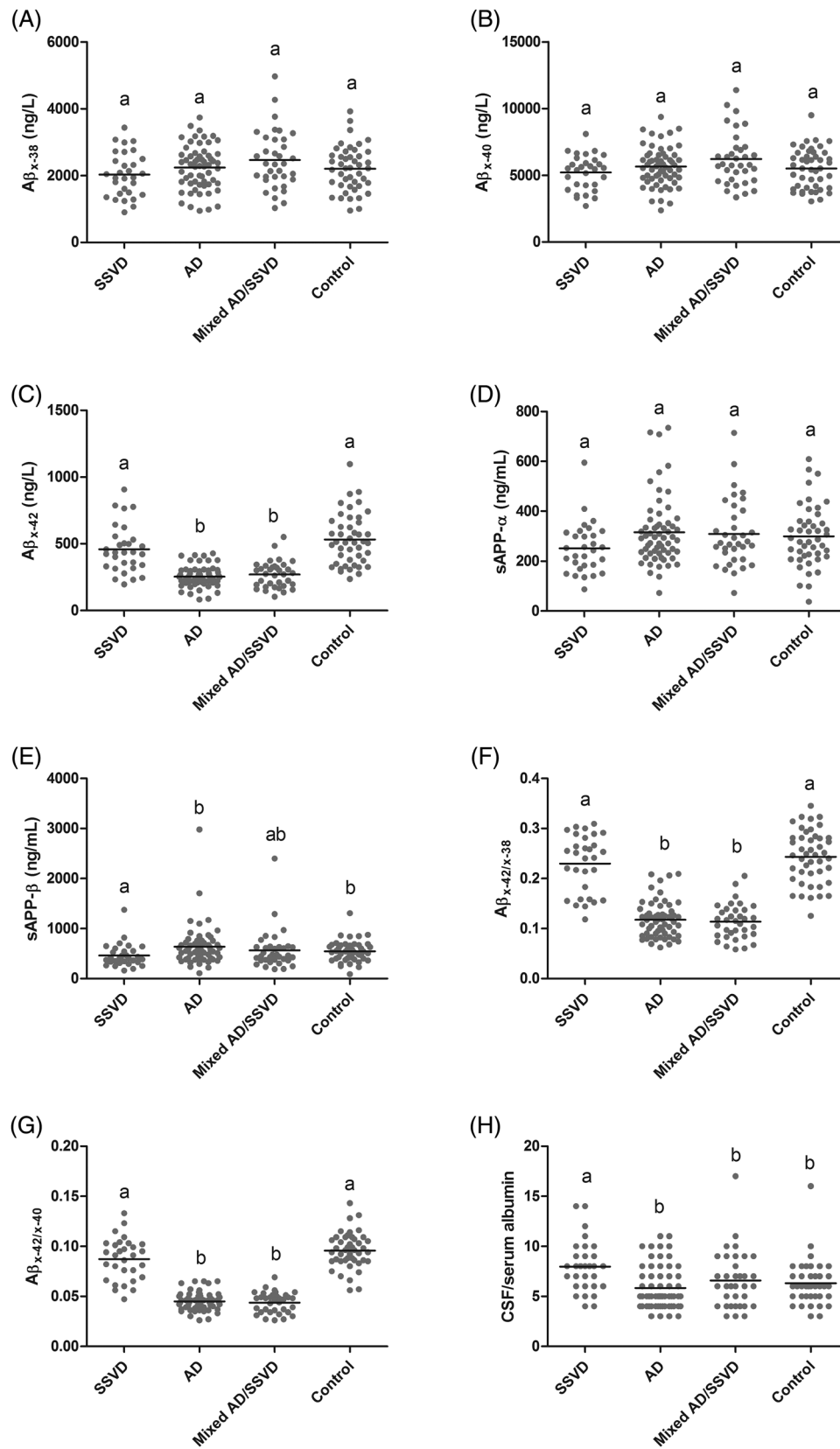
FIGURE 1 Cerebrospinal fluid (CSF) levels of $A\beta_{x-38}$, $A\beta_{x-40}$, $A\beta_{x-42}$, sAPP-α, and sAPP-β, as well as $A\beta_{x-42/x-38}$, $A\beta_{x-42/x-40}$, and CSF/serum albumin ratios in patients and controls. Values are given as means (SD). Different letters above each group correspond to significant differences of $P < .05$. For details regarding significance values, see Table 2. SSVD, subcortical small vessel disease; AD, Alzheimer's disease; $A\beta$, amyloidbeta; sAPP, soluble amyloid precursor protein.

TABLE 2 Cerebrospinal fluid (CSF) levels of $A\beta_{x-38}$, $A\beta_{x-40}$, $A\beta_{x-42}$, sAPP-α, and sAPP-β, as well as $A\beta_{x-42/x-38}$, $A\beta_{x-42/x-40}$, and CSF/serum albumin ratios in patients and controls

Variable	SSVD (n = 30)	AD (n = 60)	Mixed AD/SSVD (n = 35)	Control (n = 45)	P-value between groups
$A\beta_{x-38}$ (ng/L)	2035 (664)	2242 (658)	2467 (874)	2208 (672)	.21
$A\beta_{x-40}$ (ng/L)	5210 (1304)	5639 (1454)	6200 (1967)	5506 (1480)	.29
$A\beta_{x-42}$ (ng/L)	459 (180)[a,b]	254 (77)[c]	269 (99)[c]	532 (192)	<.001
sAPP-α (ng/mL)	252 (102)	316 (136)	309 (134)	300 (123)	.13
sAPP-β (ng/mL)	462 (235)[d,e]	636 (405)	566 (394)	546 (211)	.02
Ratios					
$A\beta_{x-42/x-38}$	0.229 (0.058)[a,b]	0.118 (0.036)[c]	0.114 (0.035)[c]	0.244 (0.053)	<.001
$A\beta_{x-42/x-40}$	0.087 (0.021)[a,b]	0.045 (0.009)[c]	0.044 (0.010)[c]	0.096 (0.018)	<.001
CSF/serum albumin	7.9 (2.6)[a,f,g]	5.8 (2.2)	6.6 (2.8)	6.3 (2.3)	.001

Values are given as means (SD). Between-group differences were examined using the Kruskal-Wallis test for multiple comparisons followed by the Mann-Whitney U test for pairwise comparisons. SSVD, subcortical small vessel disease; AD, Alzheimer's disease; Aβ, amyloid-beta; sAPP, soluble amyloid precursor protein.
[a] $P < .001$ versus AD.
[b] $P < .001$ versus mixed AD/SSVD.
[c] $P < .001$ versus control.
[d] $P < .05$ versus control.
[e] $P < .01$ versus AD.
[f] $P < .01$ versus control.
[g] $P < .05$ versus mixed AD/SSVD.

there was an absence of stroke episodes or no close time relationship between stroke episodes and development of cognitive impairment in the present study, no patients exhibited PSD or MID.

Our study is the first one measuring CSF sAPP-β in SSVD. Previously, sAPP-β has been investigated in only a few patient cohorts. In the LADIS (Leukoaraiosis and Disability in the Elderly) study, sAPP-β was negatively correlated with WMH volume.[15,16] In another study, CSF sAPP-β levels were lower in poststroke patients than in patients with subjective cognitive impairment (SCI)/MCI patients, and the chronic white matter lesions correlated with reduced levels of sAPP-β in both patient groups.[28] Furthermore, CSF sAPP-β was reduced in patients with frontotemporal lobar degeneration compared to AD and healthy controls,[29,30] and sAPP-β was correlated positively with cortical thickness.[30] Of interest, it was recently shown that β-secretase is expressed less in postmortem hippocampus of CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) patients compared to sporadic and familial AD cases, which could explain low levels of sAPP-β in diseases affecting the brain vasculature.[31] Although the earlier studies indicate that low levels of sAPP-β are involved in the pathogenesis of vascular diseases, more knowledge regarding the neurobiological functions of sAPP-β is needed to explain the results in our study.

APP is expressed abundantly in the brain and has synaptic adhesion and neurotrophic properties, but limited knowledge is available on neuropathological functions of soluble APP species.[32] Both sAPP-α and sAPP-β have been shown to decrease cell adhesion and increase axon elongation when given to neuronal cultures.[33] However, although sAPP-α has been shown to protect neurons against Aβ oligomer–induced dendritic spine loss and increased tau phosphorylation, sAPP-

β does not have such protective functions.[34] On the other hand, sAPP-β serves as a death receptor 6 ligand and regulates neuronal cell death and axonal pruning.[35] Considering the role of sAPP-β in axonal elongation, the low levels of sAPP-β in SSVD patients could suggest that these patients lack a so far unknown protective repair mechanism mediated by sAPP-β.

The present study adds to the involvement of BBB dysfunction in VCD[36–38] and SSVD.[39] However, to our knowledge, there is only one previous CSF study on pure SSVD, which also includes pure AD and mixed AD/SSVD.[7] In that study, albumin ratio was increased in cerebral small vessel disease with cognitive impairment without a CSF AD signature, whereas in patients with small vessel disease and a CSF AD signature, albumin ratio was altered to a smaller degree.[7] Although the diagnostic entrance was partially different from that in our study, the results are in agreement with our findings, suggesting that increased albumin ratio is more typical for SSVD compared with mixed dementia.

To date, the relationship between sAPP-β and BBB dysfunction has not been clarified, but APP metabolites have been shown to regulate BBB function (reviewed in Ristori et al.[40]). The presence of BBB dysfunction in SSVD suggests that the pathology may be closely related to changes in the tight junctions of the vessel wall. A possible link between BBB and APP processing could be matrix metalloproteinases (MMP), which in previous studies have been found to be increased in SSVD[41] and VCD.[42] MMPs are thought to contribute to the BBB dysfunction by disrupting the integrity of the BBB,[43] and MMPs have been shown to be associated with BBB dysfunction in SSVD.[42] In the last years, MMPs have also been suggested to alter the metabolism and processing of APP,[44] which could underlie the altered CSF levels of sAPP-β in SSVD.

In our study, we could not find any statistical correlation between BBB function and either of the two sAPP proteins, indicating that they are not directly connected.

In the present study, we also measured $A\beta$ isoforms $A\beta_{x-38}$, $A\beta_{x-40}$, $A\beta_{x-42}$ and their ratios in CSF. $A\beta_{38}$ and $A\beta_{42}$ are produced at relatively low levels compared with $A\beta_{40}$, which is the most abundant $A\beta$ isoform.[45] $A\beta_{38}$ is the most soluble of the three peptides and is seldom seen in senile plaques in sporadic AD postmortem brains, but can be detected in the brain vasculature.[46] Levels of $A\beta_{40}$ and $A\beta_{42}$ are high in postmortem AD brain tissue.[47] $A\beta_{40}$ is moderately aggregation-prone and the main component of vascular amyloid.[48] However, the most aggregation-oriented $A\beta$ peptide is $A\beta_{42}$, which is accumulated in amyloid plaques linked to AD.[49] A previous study has shown that CSF $A\beta_{42}$ was lower in MCI patients later developing SSVD, than in controls, but CSF $A\beta_{42}$ levels were even lower in MCI patients later developing AD or mixed AD/SSVD.[50] In our study, we used $A\beta_{42}$ for diagnostic purposes. $A\beta_{42}$ could therefore not be used for outcome evaluation, and as a consequence, the low levels of $A\beta_{x-42}$ in both AD and mixed AD/SSVD are expected.

The absence of differences between the patients and controls with regard to $A\beta_{x-38}$ and $A\beta_{x-40}$ suggests that these proteins are not involved in disease processes resulting in AD or SSVD. In a study of patients with AD and vascular dementia without stroke,[48] which does not directly correspond to our specific SSVD group, increased levels of $A\beta_{40}$ were found in comparison with nondemented controls. Furthermore, one study showed lower CSF levels of $A\beta_{38}$ and $A\beta_{40}$ in subcortical vascular dementia patients compared to levels in controls and AD patients,[12] which are at variance with our results. Different aims, diagnostic procedures, and analysis methods between the studies may explain the divergent results.

In our study, the neuropsychological outcome measures TMT-A and TMT-B did not differ between the various disease entities, which may question the notion that executive dysfunction and mental speed are key features of SSVD dementia.[51] However, executive dysfunction is a complex phenomenon with several components. One possibility could be that our SSVD and mixed AD/SSVD groups exhibited other difficulties in planning, initiating, and implementing than those represented by the TMT tests. Another explanation for the lack of differences between groups with regard to TMT could be that the degree of the disease was sufficiently pronounced as to affect the whole range of cognitive domains.

4.1 | Strengths and limitations

A strength of our study is that the patients were systematically diagnosed using a combination of basic characteristics and biomarkers in patients seeking help at a single memory clinic. Limitations include the lack of neuropathological verification of the clinical entities and that the biomarkers used for diagnosis of SSVD dementia and AD dementia belonged to different modalities and varied with regard to precision of scaling (semiquantitative assessment of WMHs vs quantitative measurement of biochemical markers). Another limitation is the lack of

longitudinal data, which makes it difficult to examine cause-and-effect relationships.

4.2 | Future research

The establishment of a systematic diagnosis protocol, including the use of AD biomarkers, will in the future allow for continuing biomarker analyses on this cohort. This is of potential importance because biomarker studies on pure SSVD cases are still limited. Moreover, the Gothenburg MCI cohort being a longitudinal study could allow for future investigation of BBB dysfunction and sAPP-β in controls and in patients over time, both before and after the conversion to dementia stages. Moreover, the cohort could also be of importance for detailed molecular analyses of disease processing in the future, by using state-of-the-art molecular techniques, including proteomics and lipidomics.

5 | CONCLUSION

In conclusion, at an outpatient memory clinic, patients with SSVD exhibited reduced levels of sAPP-β and disturbances of the BBB. This biochemical pattern is different from that of AD patients and to some degree also from that of mixed AD/SSVD. Our findings are speaking in favor of the conception that SSVD is a distinct VCD form.

CONFLICTS OF INTEREST

Petronella Kettunen, Maria Bjerke, Carl Eckerström, and Johan Svensson: no conflicts of interest. Michael Jonsson: Consulting fees for assignment as paid advisory board member for Biogen 2020-2021. Henrik Zetterberg has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Eisai, Denali, Roche, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx, and Red Abbey Labs; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, and Biogen; 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). Kaj Blennow has served as a consultant, on advisory boards, or on data-monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, 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, all unrelated to the work presented in this article. Anders Wallin: Lecture fees from Lundbeck.

AUTHOR CONTRIBUTIONS

Petronella Kettunen, Maria Bjerke, Johan Svensson, and Anders Wallin: study design, data analysis, data interpretation, drafting of manuscript, and manuscript revision. Henrik Zetterberg and Kaj Blennow: sample analysis. Carl Eckerström, Michael Jonsson, Henrik Zetterberg, and Kaj Blennow: manuscript revision. All authors have accepted the last version of the manuscript.

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