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Bloodstream AQP4 Concentrations in Individuals Experiencing Intracerebral Hemorrhage Linked to Cerebral Amyloid Angiopathy

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Abstract

Cerebral amyloid angiopathy (CAA) is a prominent cause of lobar intracerebral hemorrhage (ICH) in elderly populations. Emerging research suggests aquaporin 4 (AQP4) may contribute to the pathology of amyloid-beta-related conditions, including CAA. This study assessed serum AQP4 levels in patients with CAA-associated lobar ICH. Using enzyme-linked immunosorbent assay (ELISA), AQP4 was measured in 60 patients with CAA-related ICH and 19 non-stroke control participants. The patient cohort was further subdivided according to the timing of functional outcome evaluation: mid-term (12 ± 18.6 months) and long-term (38.5 ± 32.9 months) post-ICH. Overall, AQP4 concentrations did not differ significantly between patients and controls; however, lower levels were detected in patients exhibiting specific hemorrhagic patterns, such as having ≥2 lobar ICHs or ≥5 lobar microbleeds on MRI. Notably, individuals who achieved favorable long-term functional recovery had higher circulating AQP4 than those with poor outcomes or controls. These results indicate that AQP4 may serve as a prognostic marker for long-term recovery and might have a protective effect following lobar ICH.

Keywords: Functional outcome, Aquaporin 4, Magnetic resonance imaging markers, Cerebral amyloid angiopathy, Intracerebral hemorrhage

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Introduction

Cerebral amyloid angiopathy (CAA) involves the deposition of amyloid proteins within the walls of cerebral vessels [1], with the most common form characterized by amyloid-beta (Aβ) accumulation in arterioles, capillaries, and leptomeningeal vessels, often coexisting with Alzheimer's disease (AD) [2, 3]. While AD is the leading cause of dementia worldwide, CAA represents the principal origin of lobar intracerebral hemorrhage (ICH) [4, 5]. Recurrent ICH is a major clinical concern in CAA, frequently resulting in high mortality and long-term disability [6]. Beyond overt hemorrhage, patients may experience transient focal neurological episodes or cognitive deficits unrelated to AD [7, 8]. Despite the significant clinical burden, effective therapies for CAA-related lobar ICH are lacking, and definitive diagnosis is generally confirmed postmortem [9, 10]. Clinically, diagnosis relies on the modified Boston criteria, which integrate patient history with specific MRI markers [11]. Common radiological indicators include lobar microbleeds, cortical superficial siderosis, enlarged perivascular spaces in the centrum semiovale, and white matter hyperintensities [12–15].

At a molecular level, Aβ peptides are produced via sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretases, yielding either Aβ40 or Aβ42. Aβ42 predominates in neuritic plaques within AD brain parenchyma, whereas Aβ40 primarily deposits along vessel walls, replacing smooth muscle cells and promoting vascular degeneration in CAA [16, 17]. The precise mechanisms leading to Aβ deposition remain uncertain, though an imbalance between production and clearance is the prevailing hypothesis. Clearance mechanisms include enzymatic degradation, microglial uptake, active BBB transport, and perivascular or lymphatic drainage [17–19]. Evidence suggests impaired perivascular clearance in CAA, resulting in Aβ accumulation within vascular basement membranes [20–22].

Aquaporins are transmembrane channels that facilitate water transport [23], with aquaporin 4 (AQP4) being the most abundant in the central nervous system, primarily expressed in astrocytes and ependymal cells. AQP4 is highly concentrated at perivascular astrocytic end-feet surrounding the glial limiting membrane [24, 25]. It plays a key role in solute exchange between cerebrospinal fluid (CSF) and interstitial fluid (IF) via perivascular pathways, including Aβ clearance [26, 27]. Altered AQP4 expression and localization have been observed in AD and CAA, but most studies rely on postmortem tissue, leaving the status of circulating AQP4 largely unexplored [28–30].

The present study aimed to investigate whether plasma AQP4 could serve as a biomarker for CAA pathology and assist in disease diagnosis and prognosis. We analyzed associations between circulating AQP4 levels and key neuroimaging markers of CAA in a multicenter cohort of patients with lobar ICH and explored its relationship with functional outcomes.

Methods

Study population

This study enrolled 60 patients presenting with symptomatic ICH and a clinical diagnosis of CAA, along with 19 age- and sex-matched healthy controls. CAA–ICH patients met modified Boston criteria for possible, probable, or probable CAA with supporting pathology [11]. Controls were stroke-free individuals from the ISSYS cohort (Investigating Silent Strokes in Hypertensives) who had brain MRI at follow-up and no hemorrhagic findings [31].

Patients were recruited from neurology and stroke units across 10 Spanish centers, were over 55 years of age, and had experienced at least one lobar ICH. Exclusion criteria included deep ICH, microbleeds in basal ganglia, internal/external capsule, thalamus, or brainstem, and anticoagulant therapy. Collected data included patient identifiers, inclusion date, demographics, vascular risk factors (hypertension, diabetes, dyslipidemia), and MRI findings.

Blood samples were collected during the chronic phase (mean 13.6 ± 17.8 months post-ICH) to reduce confounding from acute inflammation. Cognitive impairment was evaluated at baseline via clinical and neurological assessment. Functional outcome was measured using the modified Rankin Scale (mRS).

For detailed analysis, patients were stratified by timing of outcome assessment. Subcohort 1 (n = 35) had mid-term evaluation at 12 ± 18.6 months post-ICH, concurrent with blood collection. Subcohort 2 (n = 25) had blood drawn 15.8 ± 17 months after the last ICH, with long-term outcomes assessed 34.4 ± 24.8 months later. Outcomes were dichotomized as good (mRS ≤ 3) or poor (mRS > 3). Appendix A, Figure A1 illustrates the study timeline.

Table 1. Demographic, clinical, and radiological characteristics of the total cohort

Variable	Control (n = 19)	CAA–ICH (n = 60)	p-Value
Age, years, median (IQR)	74 (73.5–74)	76.5 (71.5–70)	0.130
Sex, female, n (%)	10 (52.6%)	30 (50%)	1
Hypertension	19 (100%)	29 (48.3%)	**0.000**
Diabetes	6 (31.3%)	7 (11.7%)	0.063
Dyslipidemia	16 (78.9%)	17 (28.3%)	**0.000**
APOE genotype, ε2 carriers	1 (5.3%)	8 (13.3%)	0.679
APOE genotype, ε4 carriers	7 (36.8%)	14 (23.3%)	0.251
Lobar ICH	0 (0.0%)	60 (100%)	**0.000**
WMH, **n** (%)	9 (47.4%)	57 (95.0%)	**0.000**
CMB	0 (0.0%)	40 (66.7%)	**0.000**
Serum AQP4, ng/mL, median (IQR)	2.12 (1.63–2.67)	2.15 (1.44–4.12)	0.626

CAA, cerebral amyloid angiopathy; IQR, interquartile range; APOE, apolipoprotein E; WMH, white matter hyperintensity; ICH, intracerebral hemorrhage; CMB, cerebral microbleed; AQP4, aquaporin 4. p-Values below 0.05 are shown in bold.

The research received formal approval from the Clinical Investigation Ethics Committee of Vall d'Hebron University Hospital, Barcelona (PR(AG)326/2014), as well as from the ethics boards of all collaborating centers, adhering strictly to the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from every participant before enrollment.

MRI acquisition and imaging analysis

All participants underwent brain MRI, performed on average 1.5 ± 16.4 months after their most recent ICH event, using a 1.5-T whole-body scanner. Imaging protocols included axial T1-weighted spin-echo, T2-weighted turbo spin-echo, T2-weighted FLAIR, and T2*-weighted echo-planar gradient-echo sequences. A single experienced neuroradiologist at Vall d'Hebron Hospital, blinded to patients' clinical history and laboratory results, reviewed all scans to ensure uniformity in assessment.

Intracerebral hemorrhages were identified as hypointense lesions exceeding 5 mm on T2*-weighted images, with both count and anatomical location recorded. Smaller hypointense foci under 5 mm were classified as cerebral microbleeds (CMBs) and evaluated using the Brain Observer Microbleed Scale [32]. White matter hyperintensities (WMHs) appeared as hyperintense areas on T2-FLAIR or T2*-weighted sequences and were graded in deep and periventricular regions according to the Fazekas scale [33], where 0 indicates absent or tiny isolated foci (≤3 mm), 1 represents foci under 5 mm (excluding periventricular caps), 2 corresponds to foci larger than 5 mm (excluding caps), 3 reflects early coalescence, and 4 denotes large confluent lesions (>20 mm or multiple fused lesions). WMHs with scores of 3 or 4 were considered severe. Evaluation was performed in the hemisphere spared from hemorrhage, except when both hemispheres were affected.

Perivascular spaces (Virchow–Robin spaces), visualized as CSF-like signal abnormalities along penetrating arteries, were quantified in the basal ganglia and centrum semiovale (CSO). EPVS were categorized as moderate (≤20) or severe (≥21) per axial T2 images [13,34]. Cortical superficial siderosis (cSS) was defined by subpial deposition of hemosiderin; lesions were classified as focal if confined to ≤3 sulci and disseminated if affecting >4 sulci [11]. Siderosis contiguous with ICH sites was excluded.

To assess the cumulative burden of small vessel disease (SVD), a composite score was assigned based on the presence and severity of four key MRI features associated with CAA: lobar CMBs, WMHs, EPVS, and cSS [35]. Total SVD scores ranged from 0 to 6, with values ≥4 indicating a high overall disease burden.

Table 2. Radiological characteristics of the CAA–ICH cohort

CAA–ICH (n = 60)	
Boston Criteria	
Possible	12 (20.0%)
Probable	45 (75.0%)
Probable with supporting pathology	3 (5.0%)
WMH	57 (95.0%)
Periventricular	51 (85.0%)
Moderate (1–2 Fazekas)	10 (16.7%)
Severe (3–4 Fazekas)	41 (68.3%)
Deep subcortical WMH	52 (86.7%)
Moderate (1–2 Fazekas)	21 (35.0%)
Severe (3–4 Fazekas)	31 (51.6%)
CMB	40 (66.7%)
Lobar CMB	40 (66.7%)
1–5	14 (23.3%)
6–10	9 (15.0%)
10–20	3 (5.0%)
>20	14 (23.3%)
Deep CMB	0 (0.0%)
Cerebellar CMB	4 (6.7%)
EPVS	53 (88.3%)
EPVS basal ganglia	52 (86.7%)
Moderate (1–20)	43 (71.7%)
Severe (21 to >40)	9 (15.0%)
EPVS CSO	41 (68.3%)
Moderate (1–20)	19 (31.7%)
Severe (21 to >40)	22 (36.7%)
cSS	30 (50.0%)
Focal	9 (15.0%)
Disseminated	21 (35.0%)
Atrophy	23 (38.3%)
Small vessel disease burden	
Low (0–3)	22 (36.7%)
High (4–6)	38 (63.3%)

Results are presented as number and percentage (n [%]). Abbreviations: WMH= white matter hyperintensity; CMB= cerebral microbleed; EPVS= enlarged perivascular space; CSO= centrum semiovale; cSS= cortical superficial siderosis.

Serum AQP4 assessment

Blood samples were collected in EDTA-containing tubes, and serum was separated immediately by centrifugation at 1500 g for 15 minutes before being stored at –80 °C. Only samples obtained during follow-up visits—ensuring at least 1.5 months had passed since the ICH event—were analyzed. Total serum AQP4 concentrations were measured using the AQP4 Human ELISA Kit (Cusabio Biotech, Wuhan, China) according to the manufacturer's protocol. Optical density readings were recorded at 450 nm using a Synergy™ Mx microplate reader (BioTek Instruments, Vermont, USA). All assays were run in duplicate, and any sample with a coefficient of variation above 20% was excluded from subsequent analyses.

Statistical approach

All analyses were carried out using SPSS version 20.0 (IBM, Armonk, NY, USA), while figures were prepared in GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA). Demographics, clinical characteristics, and MRI findings were summarized descriptively. The Kolmogorov–Smirnov test was applied to assess whether continuous variables followed a normal distribution. Group comparisons for non-normally distributed data were performed with the Mann–Whitney U-test or Kruskal–Wallis test, while Spearman's rho assessed correlations between continuous variables. Categorical

variables were compared using the chi-squared test. Binary logistic regression models were applied to evaluate predictors of ≥2 ICHs and cognitive impairment, including variables that were significant in univariate analysis, with a stepwise forward selection method to determine the final model. Data are reported as mean ± SEM or median with interquartile range. A p-value <0.05 was considered statistically significant.

Results

Baseline characteristics

Table 1 details the demographic, clinical, and imaging profiles of the 60 patients with CAA-related ICH and 19 control participants. Both groups were comparable in terms of age, sex, and APOE genotype. Controls were selected to exclude any history of ICH or detectable cerebral microbleeds on MRI. While a subset of controls displayed periventricular and deep WMHs, the frequency and severity of these lesions were markedly higher in the CAA–ICH group. Serum AQP4 was detectable in all participants, but concentrations did not differ significantly between patients and controls.

MRI assessments of the CAA–ICH cohort are summarized in **Table 2**. All patients satisfied the Boston criteria for probable or possible CAA. Lobar CMBs were observed in 66.7% of patients, whereas no deep-region CMBs were detected. Severe SVD burden was noted in 63.3% of patients. High-grade WMHs were present in 68.3% of periventricular regions and 51.6% of deep white matter. Enlarged EPVS in the CSO (36.7%) and disseminated cSS (35%) were also common.

Relationship of AQP4 levels with clinical and imaging findings

Serum AQP4 levels were examined in relation to clinical and radiological features. Univariate analysis indicated lower AQP4 concentrations were associated with the presence of Apoε4 and cognitive impairment **(Table 3)**. After adjusting for confounding factors in binary logistic regression, only periventricular WMHs remained independently linked to cognitive dysfunction **(Table A1 and Table 5)**. Patients with a history of prior hemorrhagic stroke exhibited significantly reduced AQP4 levels, whereas previous ischemic events did not appear to influence serum AQP4 concentrations **(Table 3)**.

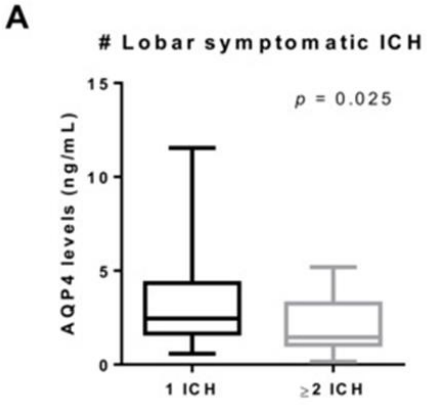
Table 3. Univariate analysis of AQP4 levels according to CAA–ICH demographic and clinical characteristics

Variable	YES	NO	p-Value
Age	r = 0.147		0.264
Sex, female	2.19 (1.46–4.20) n = 30	2.08 (1.41–4.04) n = 30	0.684
Hypertension	1.85 (1.45–3.29) n = 29	2.89 (1.77–4.27) n = 28	0.102
Diabetes	2.60 (2.11–3.58) n = 7	2.14 (1.44–4.27) n = 48	0.435
Dyslipidemia	1.81 (1.41–2.89) n = 17	2.11 (1.44–4.41) n = 36	0.331
APOE genotype, ε2 carriers	1.75 (1.18–3.00) n = 8	2.30 (1.45–4.27) n = 52	0.317
APOE genotype, ε4 carriers	1.46 (1.03–2.60) n = 14	2.41 (1.65–4.20) n = 46	**0.028**
Cognitive impairment	1.69 (1.27–2.76) n = 30	3.09 (1.81–4.38) n = 30	**0.030**
Previous stroke	1.28 (0.99–1.67) n = 12	2.68 (1.69–4.35) n = 48	**0.002**
Previous ischemic stroke	1.53 (1.26–3.27) n = 4	2.68 (1.69–4.35) n = 48	0.261
Previous hemorrhagic stroke	1.12 (0.79–1.61) n = 8	2.68 (1.69–4.35) n = 48	**0.001**

Data are presented as median serum AQP4 concentrations in nanograms per milliliter, with interquartile ranges. Spearman's rho (r) was used to evaluate correlations within the CAA–ICH cohort (n = 60), and p-values below 0.05 are highlighted in bold.

Neuroimaging analysis revealed an inverse relationship between the number of lobar ICH events and circulating AQP4 levels **(Table 4)**. Patients who had experienced two or more lobar ICHs exhibited significantly lower serum AQP4 compared to those with a single lobar ICH (Figure 1A). Logistic regression, adjusted for variables identified in the univariate analysis **(Table 5)**, confirmed that lower AQP4 levels, the presence of the Apoε2 allele, high SVD burden, and cerebral atrophy were independent predictors of having ≥2 lobar ICHs **(Table 5)**. A trend toward reduced AQP4 levels in patients with lobar CMBs was also observed (p = 0.052). When patients were stratified by CMB count, those with ≥5 lobar microbleeds had significantly lower AQP4 concentrations **(Figure 1B)**.

Additionally, patients with deep-region WMHs displayed decreased serum AQP4 levels **(Table 4)**. Notably, no significant association was found between AQP4 levels and the overall MRI-based SVD score validated for CAA with symptomatic ICH [35].



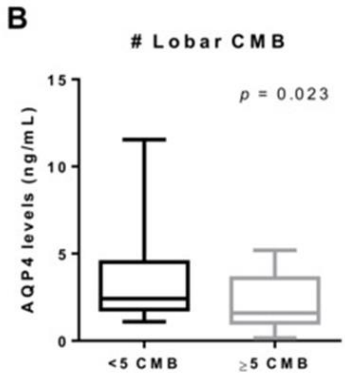


Figure 1. Relationship between serum AQP4 concentrations and hemorrhagic manifestations in the CAA–ICH cohort. (A) Boxplots showing AQP4 levels stratified by the number of symptomatic ICH events (1 ICH, n = 43; ≥2 ICHs, n = 17). (B) Boxplots illustrating AQP4 levels according to lobar CMB count (<5 CMBs, n = 34; ≥5 CMBs, n = 26). Abbreviations: ICH= intracerebral hemorrhage; CMB= cerebral microbleed

Table 4. Univariate analysis of AQP4 levels according to CAA–ICH radiological characteristics

Variable	YES	NO	p-Value
Interval between the last ICH and the date of blood collection	r = –0.052		0.703
Number of lobar ICHs	r = –0.307		**0.017**
WMH	2.04 (1.43–3.71) n = 57	4.46 (3.15–5.7) n = 3	0.163
Periventricular	2.02 (1.42–3.69) n = 51	3.29 (1.84–5.02) n = 9	0.092
Deep subcortical WMH	1.88 (1.42–3.67) n = 50	3.41 (2.04–5.19) n = 10	**0.045**
Lobar CMB	1.83 (1.41–3.79) n = 40	2.84 (1.93–4.25) n = 20	0.052
EPVS	2.26 (1.46–4.04) n = 53	1.81 (1.27–3.78) n = 7	0.718
EPVS basal ganglia	2.30 (1.47–4.12) n = 52	1.63 (1.27–3.78) n = 8	0.521
EPVS CSO	2.45 (1.50–4.36) n = 41	1.84 (1.10–3.00) n = 19	0.144
cSS	2.11 (1.16–4.38) n = 30	2.15 (1.65–2.90) n = 30	0.988
Chronic Infarct	1.53 (1.27–2.02) n = 13	2.52 (1.55–4.19) n = 44	0.146
Atrophy	2.26 (1.43–4.00) n = 23	2.04 (1.50–4.04) n = 37	0.715
High SVD burden (score 4–6)	2.35 (1.27–4.34) n = 23	2.03 (1.81–2.90) n = 37	0.570

Values are presented as median serum concentrations in nanograms per milliliter (interquartile range). Spearman's rho (r) was used for correlation analysis. CAA–ICH cohort: n = 60. Abbreviations: ICH= intracerebral hemorrhage; WMH= white matter hyperintensity; CMB= cerebral microbleed; EPVS= enlarged perivascular space; CSO= centrum semiovale; cSS= cortical superficial siderosis; SVD= small vessel disease burden. Statistically significant p-values (<0.05) are highlighted in bold.

Table 5. Binary logistic regression for cognitive impairment and ≥2 ICHs

	Regression Cognitive Impairment	Regression ≥ 2 ICHs
Variable	OR (95% CI) p-value	OR (95% CI) p-value
AQP4	-	0.520 (0.286–0.976) **p = 0.042**
APOE genotype, ε2 carriers	-	22.536 (1.989–255.296) **p = 0.034**
WMH periventricular	10.545 (1.227–90.662) p = 0.032	-

Atrophy	-	6.167 (1.080–35.213) **p = 0.004**
High SVD burden (score 4–6)	-	11.280 (1.109–114.739) **p = 0.025**

Binary logistic regression was conducted including variables that showed significant associations with cognitive impairment and/or ≥2 ICHs in the univariate analysis. Results are reported as odds ratios (ORs) with 95 percent confidence intervals (CIs) and corresponding p-values. Abbreviation: SVD= small vessel disease. P-values less than 0.05 are highlighted in bold.

AQP4 and functional outcomes

We examined the relationship between circulating AQP4 levels and functional outcomes in two subcohorts defined by the timing of outcome assessment (Appendix A, Figure A1). In subcohort 1, poor outcomes were observed in seven out of 35 patients (20%), while in subcohort 2, eight of 25 patients (32%) had poor outcomes. Serum AQP4 concentrations did not differ significantly between subcohort 1 (median 1.87 [1.29–3.53] ng/mL) and subcohort 2 (median 2.38 [1.65–4.38] ng/mL; p = 0.165). Interestingly, the association between AQP4 levels and functional outcome varied depending on when the outcome was assessed. In subcohort 1, where blood sampling and functional evaluation occurred concurrently, AQP4 levels were similar between patients with good versus poor outcomes **(Figure 2A)**. Conversely, in subcohort 2, when functional outcomes were evaluated over the long term, patients with favorable outcomes exhibited significantly higher AQP4 levels compared to those with poor outcomes and to control subjects (Figure 2B). In this subcohort, univariate analysis identified AQP4 as the only variable significantly associated with poor functional outcome (Appendix A, Table A2).

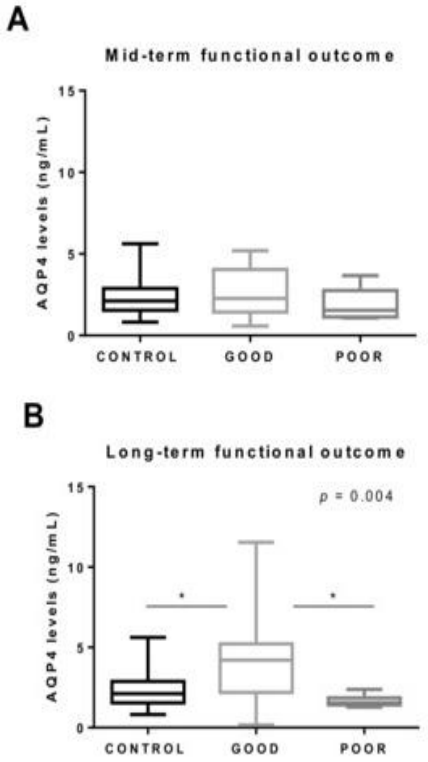


Figure 2. Relationship between AQP4 concentrations and functional neurological outcomes in the CAA–ICH cohort. (A) Boxplots illustrating AQP4 levels in patients with good (n = 27) versus poor (n = 7) mid-term functional outcomes (subcohort 1), compared with controls (n = 19). (B) Boxplots showing AQP4 levels in patients with good (n = 17) versus poor (n = 8) long-term outcomes (subcohort 2), compared with controls (n = 19). Outcomes were dichotomized as good or poor, with good outcomes defined as a modified Rankin Scale score of 0–3. Abbreviations: ICH, intracerebral hemorrhage; CMB, cerebral microbleed. *p < 0.05

Discussion

This study provides the first evidence of detectable circulating AQP4 in patients with CAA-related ICH. Although serum AQP4 concentrations did not differ significantly between patients and non-stroke controls, we observed a relationship between circulating AQP4 and cerebral hemorrhagic burden. Moreover, our findings suggest that AQP4 may serve as a predictor of long-term functional outcomes following lobar ICH in CAA patients. Interest in AQP4 has grown considerably due to its critical role in maintaining brain water homeostasis under both physiological and pathological conditions [24,29]. Previous studies have implicated AQP4 in cerebral edema, ischemic stroke, traumatic brain injury, tumors, and neuromyelitis optica, among other conditions [24,28,35]. Additionally, AQP4 has been shown to facilitate the clearance of interstitial solutes, including amyloid-beta (Aβ), via the glymphatic system, indicating a potential contribution to AD and CAA pathophysiology [26,36].

Most research on AQP4 in AD and CAA has relied on postmortem analyses of brain tissue, which have reported altered protein distribution. Wilcock *et al.* observed reduced AQP4 expression in AD patients with moderate-to-severe CAA [37], whereas subsequent studies reported elevated AQP4 immunoreactivity in AD and CAA brains relative to controls [38–40]. Evidence suggests that AQP4 expression may vary according to disease stage, with higher detection in CAA cases exhibiting severe AD pathology [41]. Furthermore, AQP4 immunoreactivity differs between gray and white matter and is influenced by age and CAA severity [42]. Collectively, these findings indicate that brain AQP4 levels are modulated by multiple factors, including patient age, disease severity, and neuroanatomical location. However, to date, no studies have examined AQP4 modulation in the circulation of AD or CAA patients.

In our study, AQP4 was measurable in the serum of CAA–ICH patients, although levels were comparable to non-stroke controls. Consistent with prior reports [43,44], periventricular WMHs were independently associated

with cognitive impairment in our cohort. Univariate analysis also revealed lower circulating AQP4 in patients with cognitive deficits, aligning with preclinical evidence: AQP4 knockout mice exhibit behavioral impairments [45–47], and transgenic AD and CAA models confirm that AQP4 deficiency exacerbates cognitive deficits, increases Aβ deposition, and promotes synaptic damage [48]. Conversely, in 5xFAD mice—an accelerated AD model with minimal vascular injury—AQP4 deletion did not affect Aβ accumulation or memory performance [49]. Overall, experimental data suggest that AQP4 may contribute to Aβ clearance via perivascular drainage, potentially offering neuroprotection in CAA.

Despite these observations, we did not find a correlation between circulating AQP4 and the total MRI-based SVD burden, which is associated with pathological CAA [35,50], suggesting that serum AQP4 may not serve as a biomarker for CAA pathology. Nevertheless, given AQP4's role in solute clearance, future studies should explore its levels in cerebrospinal fluid to better understand its pathophysiological relevance [26,27].

Importantly, we observed a notable relationship between serum AQP4 concentrations and CAA-related hemorrhagic lesions, including symptomatic ICH and cerebral microbleeds (CMBs), within our study cohort. Specifically, patients experiencing ≥2 lobar ICHs and/or ≥5 lobar CMBs exhibited lower AQP4 levels compared to those without such hemorrhagic events, suggesting a potential neuroprotective role for this protein. Additionally, we identified an inverse correlation between the number of lobar ICHs and circulating AQP4, consistent with previous reports indicating that AQP4 deficiency exacerbates neurological deficits and neuronal death following ICH [51,52]. Supporting this, AQP4 gene variants have been recently reported as independent predictors of outcomes after ICH in diverse populations [53,54]. Moreover, our analysis revealed that lower serum AQP4 levels, together with the presence of the Apoε2 allele, cerebral atrophy, and elevated small vessel disease (SVD) burden, independently predicted the occurrence of ≥2 ICHs. The Apoε2 allele is well-established as a risk factor for CAA-related ICH, predisposing patients to recurrent hemorrhages [55–57]. Given AQP4's localization at astrocytic end-feet, it has been proposed that this protein may help preserve blood–brain barrier (BBB) integrity [58]. While many studies suggest a protective function for AQP4, the impact of AQP4 deletion on BBB integrity after ICH remains debated [59–62]. Further investigations are needed to clarify AQP4's role in the post-ICH brain, and our findings should be validated in cohorts with ICH of non-CAA etiology.

Our findings also indicate that serum AQP4 levels may serve as a predictor of long-term functional outcomes in CAA–ICH patients. Individuals with favorable long-term outcomes had higher circulating AQP4 than both patients with poor outcomes and healthy controls. To date, only one study has examined circulating AQP4 in neurological disorders, reporting elevated levels in ischemic stroke patients during the post-acute phase [63]. That study also identified AQP4 as an independent predictor of favorable neurological outcomes, aligning with our observations [63]. Collectively, the association of higher circulating AQP4 with good long-term outcomes and its reduction in patients with greater hemorrhagic burden supports the notion that AQP4 may have a protective role following ICH.

This study has several limitations. The relatively small sample size may have limited the detection of some associations, particularly after correction for multiple testing. Other constraints include the cross-sectional study design and variability in the timing of recruitment and functional outcome assessment after hemorrhage. To address this, the cohort was divided into two subgroups, further reducing sample sizes. Future studies should involve larger patient populations with repeated functional assessments at multiple time points to determine whether circulating AQP4 could serve as a reliable predictor of long-term outcomes after ICH. It would also be valuable to investigate the temporal dynamics of AQP4 and examine its relationship with the progression of neuroimaging markers using follow-up blood samples and serial MRI evaluations.

Conclusions

This study provides the first evidence that AQP4 is detectable in the serum of patients with CAA-related lobar ICH. Our findings revealed a relationship between serum AQP4 levels and particular hemorrhagic neuroimaging characteristics. Specifically, AQP4 levels were lower in patients with more than two symptomatic lobar ICHs and in those with over five lobar CMBs identified by MRI. These results indicate that AQP4 may exert a protective effect in CAA patients following ICH, potentially helping to maintain BBB integrity and representing a possible therapeutic target. Additionally, since higher circulating AQP4 levels were observed in patients with favorable long-term outcomes, this serum biomarker may serve as a promising candidate for further research aimed at enhancing prognostic accuracy in individuals with lobar ICH.

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