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Research article

Circulating Aquaporin-4 as A biomarker of early neurological improvement in stroke patients: A pilot study

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ABSTRACT

Patients' outcome prediction after ischemic stroke is still challenging. Aquaporin-4 (AQP4) is a water channel that is up-regulated in the brain after the ischemic event, but its presence in bloodstream of stroke patients has not been previously studied. The aim of this pilot study was to investigate circulating AQP4 levels after stroke and its correlation with infarct growth and neurological outcome.

AQP4 level was determined by ELISA in serum from 42 t-PA-treated ischemic stroke patients at admission (before t-PA) and 13 healthy subjects. To assess infarct growth, serial brain diffusion-weighted magnetic resonance images were performed at hospital admission and 1–3 days after. Neurological improvement was defined as a ≥4-point decrease in NIHSS score compared to baseline score.

Despite stroke patients and healthy controls had similar baseline circulating AQP4 levels, among strokes AQP4 level negatively correlated with NIHSS score at admission ($R = -0.34$, $p = 0.029$) and with infarct growth after 1–3 days of stroke onset ($R = -0.36$; $p = 0.018$). Furthermore, baseline AQP4 level was higher in those stroke patients showing a neurological improvement 48 h after stroke onset ($p = 0.030$) and at hospital discharge ($p = 0.037$). Baseline AQP4 levels also resulted to be an independent predictor of good neurological outcome at both studied time points (OR_{adj}: 14.33[1.82–112.92], $p = 0.012$ at 48 h; OR_{adj}: 4.86[0.98–24.12], $p = 0.053$ at discharge) in logistic regression analysis, adjusted by age, sex, baseline NIHSS and significant variables in the univariate analysis.

Overall, we have explored circulating AQP4 levels, and our data suggest that AQP4 could be used as a biomarker of neurological recovery in the acute-subacute phase of ischemic stroke.

1. Introduction

Ischemic stroke is among the first causes of death and long-term disability worldwide [1]. The prediction of patients' outcome after this fatal disease is essential to ensure optimal stroke patients management. Nowadays, the prediction of patients' outcome is exclusively based on clinical scores that include variables such as stroke severity and age. However, the identification of blood biomarkers that add value to this prediction might substantially improve the actual decision-making processes and optimize patients' care and healthcare resources to ultimately have an impact on outcome after ischemic stroke [2].

Aquaporin 4 (AQP4) is the most abundant water channel in the brain, and plays a crucial role in maintaining cerebral water balance [3]. To date, various studies have reported increased brain expression of AQP4 after ischemic stroke, both in humans [4,5] and animal models [6–8]. However, the presence of this protein in circulation after stroke

Abbreviation: AQP4, Aquaporin-4; AUC, areas under the ROC curve; BBB, brain-blood barrier; Cc, cubic centimeters; DWI, diffusion-weighted image; ELISA, enzyme-linked immunosorbent assay; IDI, integrated discrimination improvement; MRI, magnetic resonance imaging; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; PWI, perfusion-weighted image; ROC, Receiver operating characteristics; TOAST, etiology stroke subtype classification; t-PA, tissue plasminogen activator

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has never been studied before. In fact, AQP4 has only been detected in blood in the context of bacterial meningitis [9].

Overall, our aim was to conduct a pilot study to investigate circulating AQP4 levels after stroke as well as its correlation with progression of infarct size and neurological outcome in ischemic stroke patients.

2. Materials and methods

2.1. Study population

Patients with an acute stroke admitted to the Emergency Department of Vall d'Hebron University Hospital (Barcelona, Spain) were prospectively studied. A total of 133 patients with a cerebral artery occlusion documented by a Transcranial Doppler who received tissue plasminogen activator (t-PA) in a standard 0.9-mg/kg dose (10% bolus, 90% continuous infusion during 1 h) within 4.5 h of symptoms onset were recruited. In 57 patients, serial magnetic resonance imaging (MRI) exams were performed, including diffusion- (DWI) and perfusion-weighted image (PWI) sequences within the first 4.5 h after symptoms onset. For the present study, 42 patients with MRI data and blood sample at admission were selected. Moreover, in these patients, a second MRI was performed 1–3 days after the ischemic event to evaluate lesion increase. Patients with a known or suspected inflammatory or malignant disease were excluded from the study. Mechanical thrombectomy was not performed in any patient.

Additionally, a control group of 13 healthy subjects, age-matched with stroke patients and free from inflammatory or infectious disease was studied to set reference AQP4 levels in serum.

2.2. Clinical protocol

The study was approved by the Ethics Committee of Vall d'Hebron Hospital (PR(HG)89/2003) and conducted in accordance with the Declaration of Helsinki. All patients or relatives gave informed consent. A detailed history of vascular risk factors was obtained from each patient. Clinical examination was performed on admission, at 12, 24 and 48 h of symptoms onset and at discharge. Stroke severity and neurological outcome were assessed using the National Institutes of Health Stroke Scale (NIHSS). Neurological improvement was defined as a $\geq$4-point decrease and neurological deterioration as $\geq$4-point increase in NIHSS score at any time compared to the baseline score or death. Functional outcome 3 months after stroke was assessed using the modified Rankin Scale (mRS) in all 42 patients. Transcranial Doppler assessments were performed by an experienced neurologist using a Multi-Dop® X4 (DWL Elektroniche Systeme GmbH, Sipplingen, Germany) device as previously described [10].

2.3. MRI protocol

All MRI studies were performed as previously described [10] with a 1.5 T whole-body imaging system with 24-mT/m gradient strength, 300-ms rise time and an echo-planar-capable receiver equipped with a gradient overdrive (Magnetom Vision Plus, Siemens Medical Systems, Germany).

DWI was obtained with a single-shot spin-echo echo-planar pulse sequence with diffusion gradient b-values of 0, 500, and 1000s/mm2 along all 3 orthogonal axes over 15 axial sections, with 5-mm slice thickness (interslice gap of 1.5 mm), a field of view of 230 mm, and 96 × 128 matrix. The acquisition time for the DWI equaled 56 s. PWI was acquired by using a bolus of gadolinium-based contrast material (Magnevist; Schering AG, Berlin, Germany) for selected 13- to 15-section positions measured 40 times sequentially. The perfusion-weighted sequence generated a time-to-peak map for each section position that was immediately available for interpretation at the console with all the other images. PWI was obtained using 5-mm-thick sections, an interslice gap of 1.5 mm, a field of view of 240 mm, and 128 × 128 matrix. The extent of ischemic penumbra was estimated as the difference between baseline PWI and DWI volumes, and the increase in DWI lesion was assessed as the difference between final DWI and initial DWI, divided per initial DWI and expressed as a percentage (%).

2.4. Aquaporin 4 immunoassays

Peripheral blood samples were drawn in all 42 patients at hospital admission before any treatment was given. In a subset of 12 patients, additional serial extractions were performed at 1 and 2 h after t-PA infusion, and at 12 and 24 h after stroke onset to obtain a temporal profile. Blood samples from 13 healthy controls were also obtained. All samples were handled in the same way: blood was collected and serum was immediately separated by centrifugation at 1500 g for 15 min at 4 °C and stored at −80 ∘C. Total AQP4 levels were determined by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (Cusabio Biotech., CO LTD.; sensitivity: 0.039 ng/ml) and blinded to clinical

data. Optical density was measured at 450 nm in a Synergy™ Mx microplate reader (BioTek Instruments Inc., USA). Each serum sample was assayed in duplicate and the mean value of both determinations was used; replicates with a coefficient of variation > 30% were discarded for statistical analyses.

2.5. Statistical analyses

SPSS statistical package 20.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analyses, and graphs were generated using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA). Normality was assessed by Kolmogorov–Smirnov test. For normal distributed continuous variables *t*-test (mean ± SD) was used, whereas for variables with non-normal distribution, Mann–Whitney test was used (median and interquartile range). AQP4 repeated measurements for the temporal profile were analysed with Friedman test. Chi-squared test was used to assess intergroup differences for categorical variables, expressed as frequencies. Correlations between continuous variables were calculated using Spearman's test. Receiver operating characteristics (ROC) curves were used to obtain the cut-off points of AQP4 for discriminating IS outcome with optimal accuracy (both sensitivity and specificity).

Forward stepwise multivariate logistic regression analyses for functional outcome were performed with all clinical variables associated with each end-point. Using the selected cut-off points, baseline AQP4 levels were added to the clinical model at the last step to assess its independent association and to build new predictive models.

The R software (v3.4.4; R Development Core Team 2012, Vienna, Austria) was used to compare the areas under the ROC curve (AUC) from the predictive models, using likelihood ratio method (lmtest package). The integrated discrimination improvement (IDI) index determined the added value of AQP4 to the clinical models for the studied end-points (Hmsic R package).

In all cases, a p-value < 0.05 was considered statistically significant at a 95% confidence level.

3. Results

The demographic characteristics and risk factor profile of the 42 stroke patients and the 13 healthy control subjects included in the study are described in Table 1. In brief, there were no significant differences between strokes and controls, except for a higher prevalence of atrial fibrillation in ischemic strokes.

Planimetric measurements revealed an admission median DWI volume of 17.6 (10.7–46.8) cubic centimeters (cc), an initial mean PWI volume of 212.37 ± 80.46 cc and a mean calculated volume of ischemic penumbra of 182.12 ± 71.78 cc (Table 1). The follow-up DWI

Table 1
Clinical characteristics of acute ischemic stroke patients included in the study. Data are expressed as n(%), mean ± SD or median (interquartile range). cc: cubic centimeters; DWI: diffusion-weighted imaging; NIHSS: National Instituted of Health Stroke Scale; PWI: perfusion-weighted imaging; TOAST: etiology stroke subtype classification.

	Stroke patients (n = 42)	Control subjects (n = 13)	p-value
Age, years	74 (66-78)	76 (70-83)	0.258
Sex (male)	20 (47.5%)	5 (38.5%)	0.562
Smoker	10 (23.8%)	2 (15.4%)	0.508
Arterial hypertension	25 (59.5%)	6 (46.2%)	0.396
Glycemia (mg/dl)	124.85 ± 30.80	–	–
Diabetes mellitus	3 (7.1%)	2 (15.4%)	0.392
Dyslipidemia	13 (31.0%)	5 (38.5%)	0.617
Atrial fibrillation	17 (40.5%)	1 (7.7%)	**0.016**
Ischemic cardiopathy	8 (19.0%)	2 (15.4%)	0.761
Previous stroke	3 (7.1%)	0 (0%)	0.196
TOAST classification			
Atherothrombotic	21 (50.0%)	–	–
Cardioembolic	10 (23.8%)	–	–
Undetermined	10 (23.8%)	–	–
Missing	1 (2.38%)	–	–
Admission NIHSS score	17 (14-18)	–	–
Admission DWI (cc)	17.6 (10.7-46.8)	–	–
Admission PWI (cc)	212.37 ± 80.46	–	–
Penumbra (cc)	182.12 ± 71.78	–	–
Admission aquaporin 4 levels (ng/ml)	1.70 (1.32-2.85)	1.63 (1.05-2.27)	0.332

study performed 1–3 days after symptoms onset showed a median DWI volume of 54.5 (23.46–103.00) cc, which corresponds to a median percentage of DWI lesion increase of 112.5% (42.00%–263.00%). Only 6 patients out of 42 showed a decrease in the lesion volume at the follow-up MRI study.

AQP4 was detected in serum from both control subjects and stroke patients. At hospital admission, median value of circulating AQP4 levels was similar between stroke patients and control subjects (p = 0.332) (Fig. 1A). Temporal profile analysis revealed that stroke patients presented higher levels of serum AQP4 at 1 h and 2 h after the thrombolytic treatment when compared to admission (p = 0.006 and p = 0.041 respectively) as well as when compared with control subjects (p = 0.012 and p = 0.087 respectively). However, this observed increase was not further maintained at 12 h and 24 h after symptoms onset (Fig. 1B).

Searching for factors that could influence AQP4 in circulation, we found that baseline NIHSS score and AQP4 levels at admission were negatively correlated (R = -0.336, p = 0.029). However, no correlation was found between AQP4 levels at admission and the other risk factors studied. Moreover, no differences were found in AQP4 baseline levels between patients that achieved artery recanalization within 1 h and 24 h and patients who did not (data not shown). In reference to MRI parameters, we found that AQP4 baseline levels inversely correlate with the percentage of DWI increase (R = −0.364 p = 0.018).

Next, we aimed to investigate the use of AQP4 as a biomarker of early neurological outcome after stroke. We found that patients showing neurological improvement at 48 h after stroke symptoms onset had higher baseline levels of AQP4 compared to those not improving [2.67 ng/ml (1.50–3.40) vs. 1.45 ng/ml (1.16–1.89), p = 0.030] (Fig. 2A). In the same line, patients improving their neurological state at discharge had also higher levels of AQP4 at admission in comparison with those not improving at that later time-point [2.06 ng/ml (1.48–2.92) vs. 1.38 ng/ml (1.01–1.70), p = 0.037] (Fig. 2B). In contrast, we did not found any association between AQP4 levels at baseline and long-term outcome, measured by mRS at 3rd month. However, we did find that neurological improvement both at 48 h and at discharge positively correlated with good outcome at third month (R = 0.37 p = 0.018; R = 0.48 p = 0.001 respectively).

The univariate analysis revealed that patients showing neurological improvement at 48 h had a lower prevalence of atrial fibrillation than those patients not improving, whereas no demographic and clinical differences were found between the subset of patients that did and did not ameliorate their neurological state by the time of hospital discharge (Table 2).

Through a ROC curve analysis, we determined a cut-off point for AQP4 (2.52 ng/ml) associated to patients' neurological improvement 48 h after stroke with a 54.5% sensitivity and 89.9% specificity (Fig. 3A). A clinical model to predict early neurological improvement at 48 h was then created by multivariate logistic regression analysis, with adjustment by age, sex, basal NIHSS and atrial fibrillation, which confirmed that only absence of atrial fibrillation trend to be a predictor for improvement at the studied time-point (p = 0.066). The addition of AQP4 to this predictive model showed that AQP4 strongly remained an independent predictor of neurological improvement at 48 h after stroke onset (p = 0.012) (Table 3). Moreover, the addition of AQP4 improved significantly the predictive capacity of the clinical model (p = 0.0025), and notably enhanced the discriminatory ability of the model by 8.93% (p = 0.0054) (Table 3).

Next, we also determined a cut-off point for AQP4 (1.72 ng/ml) that was associated with neurological improvement by the time of hospital discharge with a 58.6% sensitivity and 76.9% specificity (Fig. 3B). The multivariate logistic regression analysis, adjusted by sex, age and NIHSS score at baseline, confirmed that AQP4 also tended to remain as an independent predictor of neurological improvement by the time of hospital discharge, (p = 0.053). At this later time-point, the addition of AQP4 also improved the predictive capacity of the clinical model (p = 0.04) as well as it increased substantially the discriminatory ability of the clinical model by 11.39% (p = 0.0089) (Table 3).

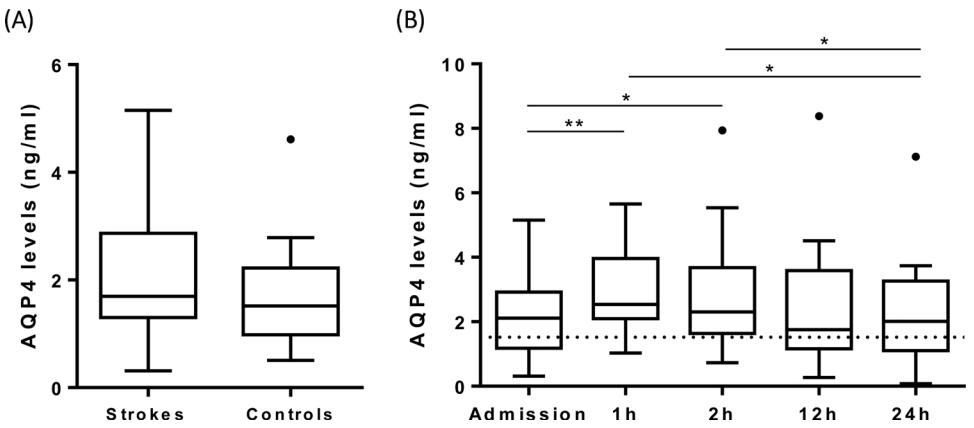

Fig. 1. AQP4 temporal profile. Boxplots represent serum AQP4 levels, median (interquartile range). **(A)** Baseline AQP4 levels in ischemic stroke patients (n = 42; 1.70 (1.32–2.85) ng/ml) and control subjects (n = 13, 1.52 (0.98–2.17) ng/ml). **(B)** Temporal profile of AQP4 measured at admission (2.11 (1.20–2.92) ng/ml), 1 h (2.53 (2.12–3.90) ng/ml) and 2 h (2.30 (1.77–3.37) ng/ml) after t-PA administration, and at 12 h (1.75 (1.21–3.29) ng/ml) and 24 h (2.02 (1.13–3.22) ng/ml) after symptoms onset (n = 12). Dashed line indicates median value for AQP4 in the control group. (*) Indicates p < 0.05 and (**) p < 0.01.

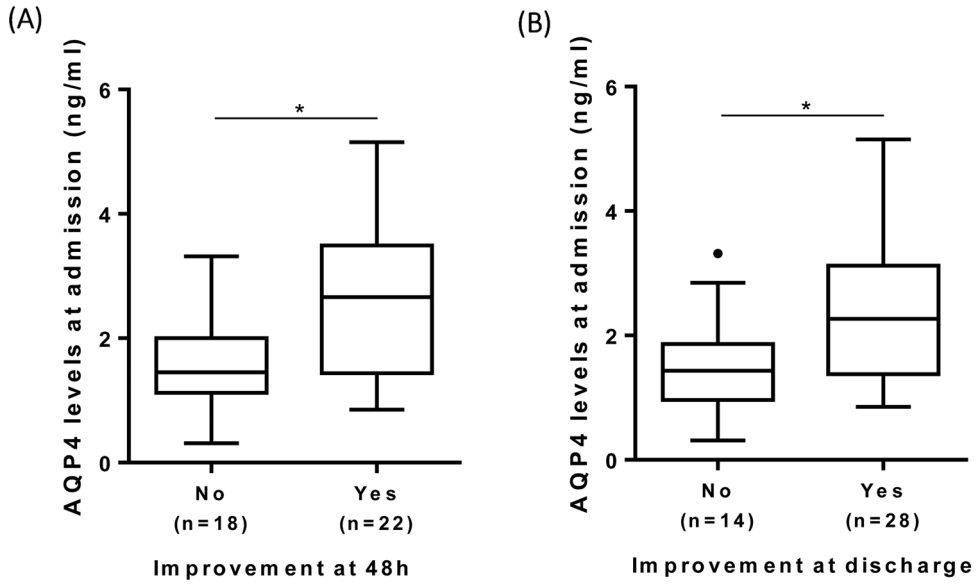

Fig. 2. Association between AQP4 levels at admission and functional outcome **(A)** 48 h after symptoms onset (p = 0.030) and **(B)** at hospital discharge (p = 0.037). (*) Indicates p < 0.05. Boxplots represent serum AQP4 levels, median (interquartile range).

4. Discussion

To the best of our knowledge, this is the first time that AQP4 protein levels have been measured in circulation of ischemic stroke patients. Our study provides new evidence that AQP4 rapidly increases in bloodstream of ischemic stroke patients after the acute phase of the disease. Moreover, our study reveals that circulating AQP4 levels measured at patients' admission to the hospital is an independent predictor of early neurological improvement in stroke patients treated with t-PA.

AQP4 is the most abundant water channel in the brain [3] and its implication in ischemic stroke pathophysiology has been previously studied. In fact, following cerebral ischemia there is a pronounced up-regulation of AQP4 mRNA and protein levels in the brain, both in rodents [6] and ischemic stroke patients [4,5]. Moreover, it has been reported that AQP4 expression distinctively increases on the astrocytic end-feet within the ischemic core [4,5,7,8,11]. Since these end-feet surround blood vessels in the brain, it has been suggested that AQP4 could play a role in brain-blood barrier (BBB) integrity [12,13]. However, recent research pointed out that the deletion of AQP4 did not alter BBB integrity [14,15], so uncertainty regarding the exact functions of AQP4 in brain following ischemic stroke still exist. Moreover, AQP4 also plays a crucial role in maintaining cerebral water balance after an ischemic event [3,16], being implicated in edema formation, an aggravating factor among ischemic stroke patients related to poor neurological and functional outcome [17–19]. However, beyond these previous findings in brain, the measurement of AQP4 in circulation after ischemic stroke has not been studied before in stroke models nor in stroke patients.

The detection of antibodies against AQP4 in blood is routinely used and has been already clinically approved to diagnose neuromyelitis optica, an autoimmune inflammatory disease of the central nervous system that mainly affects optic nerves and the spinal cord [20,21]. However, concerning the expression of AQP4 in bloodstream, only one single study has explored the presence of this protein in circulation in bacterial meningitis patients, reporting increased levels of AQP4 in

Table 2
Univariate analysis. Clinical characteristics associated with improvement at 48 h after symptoms onset and at hospital discharge. Statistical significant differences between groups are highlighted with bold p-value.

	Improvement at 48 h			**Improvement at hospital discharge**		
Factors	**No** (n = 22)	**Yes** (n = 18)	**p-value**	**No** (n = 13)	**Yes** (n = 29)	**p-value**
Age, years	78 (71-79)	74 (66-77)	0.276	78 (73-80)	74 (65-77)	0.111
Sex (male)	7 (38.9%)	12 (54.5%)	0.324	4 (30.8%)	16 (55.2%)	0.143
Smokers	4 (22.2%)	5 (22.7%)	0.970	3 (27.1%)	7 (24.1%)	0.940
Arterial hypertension	11 (61.1%)	12 (59.1%)	0.897	9 (69.2%)	16 (55.2%)	0.391
Glycemia (mg/dl)	127.06 ± 31.17	124.18 ± 31.06	0.780	129.00 ± 35.88	123.07 ± 28.90	0.854
Diabetes mellitus	2 (11.1%)	1 (4.5%)	0.432	1 (7.7%)	2 (6.9%)	0.927
Dyslipidemia	5 (27.8%)	6 (27.3%)	0.972	4 (30.8%)	9 831.0%)	0.986
Atrial fibrillation	11 (61.1%)	6 (27.3%)	**0.031**	6 (46.2%)	11 (37.9%)	0.616
Ischemic cardiopathy	2 (11.1%)	6 (27.3%)	0.193	2 (15.4%)	6 (20.7%)	0.681
Previous stroke	1 (5.6%)	2 (9.1%)	0.669	1 (7.7%)	2 (6.9%)	0.927
Admission NIHSS score	18 (14-20)	13 (17-18)	0.538	18 (14-20)	17 (13-18)	0.158
Admission DWI (cc)	12.85 (5.4-40.7)	23.55 (10.7-52.0)	0.242	16.3 (5.4-40.7)	22.0 (10.7-52.0)	0.471
Admission PWI (cc)	185.34 ± 54.36	234.39 ± 95.43	0.060	195.53 ± 47.11	219.92 ± 91.31	0.261
Penumbra (cc)	159.91 ± 53.90	201.49 ± 82.16	0.073	168.85 ± 53.86	188.07 ± 78.63	0.429
Admission AQP4 levels (ng/ml)	1.45 (1.16-1.89)	2.67 (1.50-3.40)	**0.030**	1.38 (1.01-1.70)	2.06 (1.48-2.92)	**0.037**
AQP4 > 2.52 ng/ml	2 (9.1%)	12 (66.6%)	**0.004**	–	–	–
AQP4 > 1.72 ng/ml	–	–	–	3 (23.1%)	17 (58.6%)	**0.033**

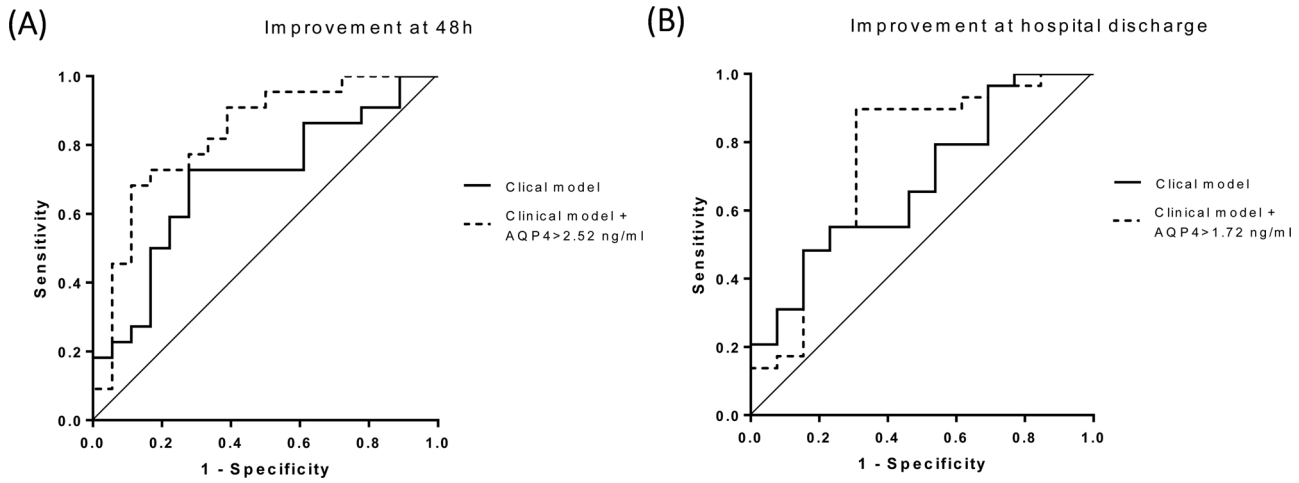

Fig. 3. ROC curve of the multivariate regression models developed to predict improvement at 48 h and at hospital discharge in our study population. Clinical model was adjusted by age, sex, basal NIHSS in both cases as well as by atrial fibrillation in the case of improvement at 48 h. **(A)** ROC curves to predict improvement at 48 h. The AUC of the clinical model was 69.7% and when including the AQP4 > 2.52 ng/ml to this clinical model the AUC increased to 82.8%. **(B)** ROC curves to predict improvement at discharge. The AUC of the clinical model was 68.2% and when including the AQP4 > 1.72 ng/ml to this clinical model the AUC increased to 75.3%.

patients when compared to controls [9].

In the present study we have explored for the first time the presence of AQP4 protein in the bloodstream of ischemic stroke patients. Despite AQP4 circulating levels did not differ between ischemic stroke patients and controls by the time of hospital admission, we have found that ischemic strokes presented a peak of AQP4 levels in blood within the very early phase of the disease. This increase suggests that the stroke-related changes previously described at the brain level might be somehow reflected in circulation within the first hours after ischemic stroke. However, the exact source of AQP4 in circulation has not been elucidated yet. One possible explanation would be that AQP4 may be released from cell membrane to circulation trough a mechanism that has not been described yet. In this line, the increase on AQP4 circulating levels observed 1 and 2 h after the hospital admission could be explained by an increase on brain-blood-barrier leakiness, leading to an augmented release of AQP4 to the circulation. On the other hand, another possibility is that what is being detected in circulation is the presence of brain-derived vesicles containing AQP4. In fact, the presence of vesicles containing aquaporins in bloodstream has been described before [22], as well as the presence of brain-derived vesicles, indicating that these vesicles can cross the brain-blood-barrier both in healthy and pathological conditions [23,24].

In addition to this, the present study also demonstrates that baseline AQP4 levels might potentially serve as a predictor of early neurological improvement during the acute and sub-acute phase of stroke. In this regard, various pre-clinical studies have previously explored the implication of brain AQP4 in stroke outcome by using AQP4 knockout mice. Although the majority of these studies seem to point out a protective role of AQP4 [25–27], controversies still exist regarding the role of AQP4 in stroke prognosis [28]. Here we have found that ischemic stroke patients showing early neurological improvement, either within the acute phase or during the recovery phase had higher circulating AQP4 levels at baseline, which suggest a plausible protective role of this protein against stroke, as reported by the vast majority of preclinical studies. It is important to know that this effect is not related with recanalization induced by t-PA, rather other mechanisms should be involved. In this sense, AQP4 levels at baseline inversely correlate with the percentage of DWI increase and with the NIHSS score at admission, which also support that increased AQP4 levels in circulation by the time patients arrive to the hospital seem to be beneficial for stroke prognosis. Taking all this into account, the early measurement of circulating AQP4 in ischemic stroke patients may facilitate the decision-making process

Table 3
Predictive models comparative for improvement at 48 h and at hospital discharge. For logistic regression models, adjusted odds ratio (OR_{adj}) (95% CI) and p-values are shown. AQP4 was added to the clinical logistic regression model using the cut-off point of 2.52 ng/ml for neurological improvement at 48 h and 1.72 ng/ml for neurological improvement at discharge. AUC: area under the curve; area is given for each model with 95% CI. IDI: integrated discrimination improvement index; index is given for events, nonevents and for the sum of both (with 95% CI).

	Improvement at 48 h		**Improvement at hospital discharge**	
	Only clinical	**Clinical + AQP4**	**Only clinical**	**Clinical + AQP4**
Logistic regression (OR_{adj})				
Age	0.99 (0.92-1.08) p = 0.881	1.02 (0.93-1.12) p = 0.664	0.97 (0.89-1.05) p = 0.446	0.97 (0.89-1.05) p = 0.461
Sex	1.20 (0.27-5.27) p = 0.811	1.81 (0.32-10.17) p = 0.500	1.94 (0.42-8.88) p = 0.394	2.37 (0.45-12.56) p = 0.312
Admission NIHSS score	0.98 (0.85-1.14) p = 0.811	1.05 (0.88-1.26) p = 0.600	0.90 (0.75-1.07) p = 0.226	0.94 (0.78-1.13)
Atrial fibrillation	0.26 (0.06-1.09) p = 0.066	0.20 (0.04-1.07) p = 0.059	–	–
AQP4 > 2.52 ng/ml	–	14.33 (1.92-112.92) **p = 0.012**	–	–
AQP4 > 1.72 ng/ml	–	–	–	4.86 (0.98-24.12) p = 0.053
ROC curves				
Area under curve (AUC)	0.70 (0.53-0.86)	0.83 (0.70-0.96)	0.68 (0.51-0.86)	0.75 (0.58-0.93)
Likelihood ratio	**p = 0.0025**		**p = 0.04**	
IDI statistics				
IDI	Ref	8.93% (2.63-15.23) **p = 0.0054**	Ref	11.39% (2.86-19.92) **p = 0.0089**
IDI events	–	4.02%	–	3.52%
IDI nonevents	–	4.91%	–	7.86%

of clinicians, such as in admitting patients to specialized stroke units or recovery programs, evaluating complementary treatments, or recruiting patients into clinical trials. Moreover, early neurological improvement in ischemic stroke patients treated with thrombolytic therapies has been also suggested to be a straightforward surrogate indicator of good outcome [29,30]. Therefore, the usage of AQP4 as a biomarker for early stroke prognosis might help in optimal patients' management in order to improve their functional outcome and quality of life after ischemic stroke. In addition to this, given the role of AQP4 in stroke prognosis it would be interesting to explore whether the pharmacological modulation of this protein as a complementary therapeutic tool could be a possible approach to improve stroke patients' outcome.

Our study also stands with various limitations that should be improved in future studies. First, we are aware that our sample size, although deeply phenotyped, is small, especially for the temporal profile. In this line, future studies should use a larger number of stroke patients as well as healthy subjects. Second, our data exploring early neurological outcome has been derived from a cohort of patients that have been treated with t-PA, so it would also be interesting to explore these findings in non t-PA-treated patients. Third, since AQP4 has been previously related to brain edema [3,16], it would also be interesting to evaluate the association between AQP4 levels in circulation and the extent of brain edema in ischemic or hemorrhagic stroke patients.

In conclusion, circulating AQP4 could be a suitable biomarker for predicting early neurological outcome in the acute-subacute phase of ischemic stroke after t-PA, improving the prediction that is currently done only with clinical information, and subsequently easing the decision-making process regarding the management and treatment of the affected patients.

Author contribution statement

L.R., A.B. and J.M. conceived and designed the experiments. A.P. and F.M. performed the ELISAs with human blood samples. L.R. and A.S. performed the statistical analysis. A.B. and A.G-T. helped in recruiting and selecting the cohort of patients and analysed the clinical data. A.R carried out the magnetic resonance images. A.S. A.B. and J.M. supervised the experiments and L.R. drafted the manuscript. All authors have critically reviewed the article content and approved it in its final version.

Declaration of Competing Interest

Authors declare no conflict of interests.

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