

**Title:** Aquaporin-4 IgG antibody related disorders in patients with juvenile systemic lupus erythematosus

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**Abstract:**

**Objective:** The aim of this study was to: (i) screen a large group of unselected patients with juvenile systemic lupus erythematosus for anti-aquaporin 4 antibodies (AQP4-Ab); (ii) identify clinical and laboratory predictors of presence of AQP4-Ab positivity in jSLE.

**Methods:** Sera from ninety patients with jSLE were tested for the presence of AQP4-Ab using a cell-based assay. Demographics, clinical and immunological features, treatment received were summarized. Fisher's exact test was used to identify clinical predictors of positivity for AQP4-Ab.

**Results:** Five of 90 (5.5%) patients tested positive for AQP4-Ab, all of which had neurological involvement, mainly transverse myelitis and optic neuritis. AQP4-Ab positive patients were more likely to have neurological symptoms ( $p=0.002$ ), less likely to experience dermatological manifestations ( $p=0.045$ ), and less likely to have detectable anti-dsDNA antibodies ( $p=0.022$ ). These patients were also more likely to have received anti-epileptic ( $p=0.023$ ) and anti-coagulant ( $p=0.007$ ) drugs.

**Conclusions:** The findings of this study indicate that some patients with jSLE develop antibodies against AQP4 and may be at risk of developing a neurological clinical phenotype. We suggest that all jSLE patients should be systematically screened for presence of AQP4-Ab and this may help identify a high risk for neurological involvement in jSLE.

**Introduction**

Aquaporin-4 (AQP4) is a water channel found on the foot processes of astrocyte cells in the brain and is also expressed in the renal tract, the gastrointestinal system, skeletal muscles, lung and blood system [1,2].

In the central nervous system, AQP4 are involved in water movement, cell migration and neuroexcitation [3]. Antibodies are predominantly of the IgG1 subtype and bind three-dimensional conformational epitopes on the extracellular loops of AQP4. This produces astrocyte damage by complement-dependent cytotoxicity which leads to blood–brain barrier disruption and cause leukocyte infiltration and cytokine release resulting in damage to oligodendrocyte, myelin and neurons [3].

Antibodies against AQP4 (AQP4-Ab) were first identified in 2005 in patients with neuroinflammation best now classified as having neuromyelitis optica spectrum disorders (NMOSD) [1]. These NMOSD are a group of inflammatory disorders of the central nervous system that often present with a relapsing remitting course and are characterized by optic neuritis and longitudinally extensive transverse myelitis (LETM). They are typically associated with serum antibodies that selectively bind to AQP4 or myelin oligodendrocyte glycoprotein 2 (MOG) [1].

The discovery of pathogenic AQP4-Ab resulted in expansion of the clinical phenotypes associated with NMSOD to include patients with more atypical neuroinflammation even in the absence of optic neuritis and transverse myelitis but who test positive for these antibodies. Notably, in recent years, growing evidence suggests that AQP4-IgG may also causes damage to peripheral

AQP4-expressing organs beyond the CNS such as skeletal muscle, vestibulocochlear nerves, gastrointestinal tract, blood system, kidney, lung and placenta [2]. So the spectrum of disease associated with these autoantibodies may expand over the years.

Notably, the presence of AQP4-Ab has already been described in the context of systemic autoimmune diseases such as adult onset systemic lupus erythematosus (SLE) [4,5,6] but no studies have systematically assessed the presence of AQP4-Ab in children and adolescents with juvenile onset SLE (jSLE). While jSLE shares clinical and serological features with adult-onset SLE, jSLE typically exhibits a much more severe protracted disease course. Notably the incidence of neuropsychiatric manifestations in jSLE is much more common than in adult patients [7]. Whether this increased frequency of neurological manifestations in paediatric patients relates to the presence of neurotoxic antibodies such as AQP4-Ab remains unclear.

The aim of this study was therefore to: (i) screen a large group of unselected patients with jSLE for AQP4-Ab; (ii) identify clinical and laboratory predictors of presence of AQP4-Ab positivity in jSLE.

## **Methods**

We screened for the presence of AQP4-Ab a total of 90 patients with jSLE fulfilling the 1997 American College of Rheumatology (ACR) criteria [8] seen in the Paediatric and Adolescent Rheumatology and Paediatric Neurology

departments at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London Hospitals between 2009 and 2017. Ethical approval for the study was obtained from the Regional Ethical Committee (Study reference 16IR33).

AQP4-IgG antibodies were measured in serum using a validated commercially available cell-based assay (Euroimmun, United Kingdom) as per manufacturer's instructions [9]. In addition, testing for MOG antibodies was also completed for those patients with positive AQP4-Ab.

The demographic, clinical, and laboratory characteristics recorded were as follows: sex, age, ethnicity (established via the information provided by patients/parents to register with the hospital), neurological symptoms, previous organ involvement, manifestations of jSLE, erythrocyte sedimentation rate (ESR, in the first hour; normal <10 mm/h) and serum C-reactive protein (CRP; normal range < 10 mg/L), presence of anti-dsDNA antibodies (normal range 0–9.9 IU/ml), anti-nuclear antibodies (ANA), antibodies to extractable nuclear antigens (ENA) and its subtypes, antiphospholipid antibodies (ACL), lupus anticoagulant (LA), and complement C3 and C4 levels. Therapies used were also recorded.

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA). Fisher's exact test was used for comparison between groups. Categorical data were summarised as percentages. Any continuous variables were summarised as median and range. 2-sided p-values less than 0.05 were considered significant.

## Results

We studied a total of 90 patients (11/90, 12% male) with jSLE with a median age of 18.7 (range 11 to 27) years old. Nineteen/90 (21%) were White British, 11/90 (12%) White Other, 3/90 (3%) Black British, 7/90 (8%) Black African, 11/90 (12%) Black Caribbean, 24/90 (27%) Asian, 15/90 (17%) of mixed background or of other or unknown ethnicity. The median age of disease onset was 8 (range 0.3 –15) years old.

Five/90 patients tested positive for AQP4-Ab. All patients but one who tested positive for AQP4-IgG at diagnosis remained positive on repeat measurements during follow up: median 3 measurements (range 1-8) over a median of 18.5 months (range 9-20 months).

Table 1 summarises the clinical characteristics of the 5 patients who tested positive for AQP4-Ab at the time of initial presentation and latest follow up, brain magnetic resonance (MRI) imaging findings and treatment received. These 5 patients had a median age of 15 (range 11-17.5) years old; a median disease duration of 3 (range 0.2 - 8.5) years, and a median length of follow up of 3 (range 1- 9) years.

Predominant neurological presentation was observed in 3/5 (60%) at onset with the other two developing neurological symptoms over time. Four/5 (80%) patients fulfilled the diagnostic criteria for NMOSD (table 2, supplementary material) [10]. MOG antibodies tested negative in 5/5 patients. Two/4 patients with spinal lesions had recurrence of transverse myelitis and optic neuritis in more than one occasion (Figure 1). One child presented with acute psychosis, in context of normal neuroimaging. Cerebrospinal fluid analysis was performed

in two patients. CSF was acellular, with normal protein and no detection of CSF oligoclonal bands.

Treatment received included corticosteroids in all patients; cyclophosphamide (2/5); rituximab (3/5); plasma exchange (2/5); azathioprine (3/5); and mycophenolate mofetil (MMF) (2/5).

### ***Predictors of presence of AQP4-Ab in jSLE***

Patients with positive AQP4-Ab were more likely to have neurological involvement compared to those who tested negative, ( $p=0.002$ ), and they were less likely to have skin involvement ( $p=0.045$ ) (Table 3). There appeared to be no other significant differences between groups of patients when the remaining clinical features were considered.

Patients with positive AQP4-Ab were less likely to have positive anti-dsDNA antibodies than those with negative AQP4-Ab ( $p=0.022$ ) (Table 3), with no other significant differences between the two groups of patients when the remaining laboratory characteristics were considered.

All treatments received prior to the sample collection were included in the analysis. Patients with AQP4-Ab antibodies were more likely to receive anti-epileptics ( $p=0.023$ ), and anti-coagulants ( $p=0.007$ ), and they were less likely to have received hydroxychloroquine ( $p<0.001$ ) (Table 3).

### **Discussion**

In this retrospective case series, we have shown that there is a subgroup of patients with jSLE that develop AQP4-Ab and these patients are more likely to develop neurological involvement. The main clinical phenotype associated with

AQP4-IgG seropositivity was transverse myelitis and optic neuritis. We would recommend that clinicians have a low threshold for screening for presence of these antibodies in patients with jSLE and have a low threshold for undertaking neuroimaging investigations to screen for neurological involvement.

A previous study in adults with SLE that systematically assessed for the presence of anti AQP4-IgG antibodies in a large cohort of patients, indicated that only SLE patients with NMOSD features tested positive for these antibodies [4]. These results therefore were supportive of the specificity of a positive test for AQP4-Ab rather than this being an epiphenomenon of autoimmunity, and were highlighting a unique neurological clinical phenotype in SLE. We now also demonstrate that younger patients with jSLE may also develop these antibodies and are also at risk of neurological involvement.

We note the lack of classical NMOSD clinical features in one patient positive for AQP4-Ab. This patient however had already received significant immunosuppressive treatment for other jSLE features at the time of AQP4-Ab testing, which may have influenced his clinical presentation. Although false positivity cannot be ruled out, it is possible that NMOSD symptomatology may differ in patients with established autoimmunity. Alternatively, antibody positivity may precede the onset of severe neurological phenotype similar to reports in case of patients with myasthenia gravis [11].

The therapeutic approach with respects to immunosuppression did not differ between groups and this is unsurprising as rituximab, cyclophosphamide and



corticosteroids are effective therapies for NMOSD as well as SLE. The patients with AQP4-Ab positive were less likely to be treated with HCQ. Although HCQ is a safe drug and generally recommended for the treatment of all patients with SLE [12], the decreased use in the patients with AQP4-Ab positivity could reflect the reluctance of the clinicians to add a medication with a risk of retinal toxicity to the patients with optic neuritis.

We acknowledge the retrospective nature of our cohort and likely bias by case severity as patients were included from two tertiary centres. Larger prospective longitudinal studies are now needed to confirm our observations and to establish whether the AQP4-Ab are persistent or transient and the effect of immunotherapy on these antibodies. In our study we used a qualitative assay for detection of anti AQP4-Ab; future studies using quantitative assays and temporal correlation with the clinical status would be of interest. Nevertheless, our results highlight that the association between jSLE and AQP4 autoimmunity and support the screening for these antibodies in all patients with jSLE to establish the risk of neurological involvement.

#### **Conflict of interest statement**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Table 1. Clinical and laboratory characteristics, imaging, treatment and follow up for the patients with jSLE and anti-AQP4 antibody positivity.**

Patient ID, gender and ethnicity	Age at onset (years)	Clinical features	Ocular features	Antibody profile	Spine MRI	Brain MRI	CSF studies	Treatment	Disease course and imaging during follow up	Follow-up (years)
<b>Patient 1</b> Male, Afro-Caribbean	12	<ul style="list-style-type: none"> <li>Arthritis</li> <li>Pancytopenia with haematological picture of secondary macrophage activation syndrome</li> <li>Lung vasculitis on biopsy, retinal vasculitis,</li> <li>Psychotic episode with hallucinations</li> <li>No focal neurological deficit</li> </ul>	Retinal vasculitis	<ul style="list-style-type: none"> <li>ANA positive</li> <li>Anti-dsDNA positive</li> <li>Anti-C1q antibodies positive</li> <li>Anti-ENA negative</li> <li>AQP4-Ab positive</li> <li>NMDAR-Ab negative</li> <li>MOG-Abs negative</li> </ul>	Normal	<p>Long standing infarcts of right caudate nucleus and dorsal right lentiform nucleus</p> <p>Generalised progressive atrophy</p>	Not done	<ul style="list-style-type: none"> <li>Induction: IVMP, Cyclophosphamide, Rituximab; Etoposide</li> <li>Maintenance therapy: Mycophenolate mofetil; hydroxychloroquine</li> </ul>	Disease controlled on medication, no evidence of clinical or radiological disease activity.	3
<b>Patient 2</b> Female, Afro-Caribbean	11	<ul style="list-style-type: none"> <li>Malar rash</li> <li>Raynaud phenomenon</li> <li>Myositis</li> <li>Lung involvement</li> <li>Haematological disease (leukopenia, neutropenia)</li> <li>Family history of SLE (father, paternal aunt)</li> </ul>	No	<ul style="list-style-type: none"> <li>ANA positive</li> <li>Anti ds-DNA negative</li> <li>Anti-RNP positive</li> <li>Anti-Ro positive</li> <li>AQP4 positive</li> <li>NMDAR-Ab negative</li> <li>MOG-Abs negative</li> </ul>	LETM	Non-specific lesion subcortical frontal and parietal white matter	Not done	<ul style="list-style-type: none"> <li>Induction: Corticosteroids; Rituximab</li> <li>Maintenance: Azathioprine; Methotrexate</li> </ul>	Persistent headaches but no further relapse with stable neuroimaging	2
<b>Patient 3</b> Female, Caucasian	9	<ul style="list-style-type: none"> <li>Non-specific gastrointestinal symptoms</li> <li>Tubulo-interstitial nephritis on renal biopsy</li> <li>Right sided monoparesis</li> </ul>	Unilateral optic atrophy diagnosed at 2yrs	<ul style="list-style-type: none"> <li>ANA positive</li> <li>Anti-dsDNA weakly positive</li> <li>Anti-RNP positive</li> <li>Anti-Ro positive</li> <li>AQP4-Ab positive</li> <li>MOG-Ab negative</li> </ul>	LETM C3-T1 central lesion	Normal intracranial appearances	Acellular normal protein matched OCB	<ul style="list-style-type: none"> <li>Induction: Corticosteroids, plasma exchange, cyclophosphamide</li> </ul>	Five relapses with LETM  Movement disorder	9

		<ul style="list-style-type: none"> <li>Unilateral optic atrophy (secondary to previous optic neuritis)</li> </ul>						<ul style="list-style-type: none"> <li>Maintenance: Azathioprine; Mycophenolate mofetil</li> </ul>		
<b>Patient 4</b> Female, Asian	10	<ul style="list-style-type: none"> <li>Optic neuritis (ON)</li> </ul>	Four episodes of ON	<ul style="list-style-type: none"> <li>ANA weakly positive</li> <li>Anti-dsDNA positive</li> <li>AQP4-Ab positive</li> <li>MOG-Ab negative</li> </ul>	LETM C1-C5 central lesion	Abnormal signal right temporal cortex and amigdala and left thalamus and cervicomedullar junction.	acellular normal protein OCB negative.	<ul style="list-style-type: none"> <li>Induction: Corticosteroids</li> <li>Maintenance: Methotrexate, Azathioprine</li> </ul>	Multiple relapses of optic neuritis and transverse myelitis.	8
<b>Patient 5</b> Female, Asian	12	<p>Acute onset</p> <ul style="list-style-type: none"> <li>Vasculitic rash</li> <li>Haematological disease with anaemia, lymphopenia, thrombocytopenia</li> <li>Paresthesias with arms and legs weakness</li> <li>Respiratory failure</li> </ul>	No	<ul style="list-style-type: none"> <li>ANA positive</li> <li>ENA screen positive, typing negative</li> <li>AQP4-Ab positive</li> <li>MOG-Ab negative</li> <li>NMDAR Abs negative</li> </ul>	LETM C1-T1 central lesion	Single non-specific white matter lesion in the right frontal lobe	Not done	<ul style="list-style-type: none"> <li>Induction: Corticosteroids, plasma exchange, IVIG</li> <li>Maintenance: Rituximab</li> </ul>	Clinical improvement independently mobile. No clinical relapses	1

ANA=antinuclear antibodies, dsDNA=double stranded DNA, ENA=extractable nuclear antigens, AQP4=aquaporin 4, IVMP=intravenous methylprednisolone, NMDA=N-methyl D-aspartate receptor, MOG=myelin oligodendrocyte glycoprotein, RNP=ribonucleoprotein, VGKC=voltage gated potassium channel, SLE=systemic lupus erythematosus, C=cervical vertebrae, T=thoracic vertebrae, EEG=electroencephalogram, MRI=magnetic resonance imaging, CSF=cerebrospinal fluid, LETM= Longitudinal extensive transverse myelitis, OCB=oligoclonal bands, IVIG=intravenous immunoglobulin

**Drug doses:** Corticosteroids= methylprednisolone given intravenous at 30 mg/kg/day for 3 consecutive days followed by oral prednisolone 1-2 mg/kg/day for 3-6 months; Azathioprine at 2 mg/kg/day; Rituximab intravenous given at two doses at 750 mg/m<sup>2</sup> 2 weeks apart; Mycophenolate mophetil at 600 mg/m<sup>2</sup>/day; intravenous Cyclophosphamide 4-6 doses at 500-750 mg/m<sup>2</sup> given monthly; Subcutaneous Methotrexate given at 15 mg/m<sup>2</sup> once a week; Hydroxychloroquine at 6mg/kg/day: plasma exchange 2 volume exchanges/day for 5-7 consecutive days.

**Table 3. Predictors of presence of anti-Aq4 antibodies in patients with juvenile systemic lupus erythematosus jSLE. Groups were compared using Fisher's exact test; p < 0.05 was considered significant.**

Features		Anti-AQP4 positive	Anti-AQP4 negative	p-value	
Sex	Male	1/11 (9.1%)	10/11 (90.9%)	0.487	
	Female	4/79 (5.1%)	75/79 (94.9%)		
Clinical features	Skin involvement	Absent	3/17(17.6%)	0.045	
		Present	2/73 (2.7%)		71/73 (97.3%)
	Malar rash	Absent	4/67 (6.0%)	63/67 (94.0%)	1.000
		Present	1/23 (4.3%)	22/23 (95.7%)	
	Discoid rash	Absent	5/87 (5.7%)	82/87 (94.3%)	1.000
		Present	0/3 (0%)	3/3 (100%)	
	Photosensitivity	Absent	5/78 (6.4%)	73/78 (93.6%)	1.000
		Present	0/12 (0%)	12/12 (100%)	
	Mucocutaneous involvement	Absent	3/63 (4.8%)	60/63 (95.2%)	0.634
		Present	2/27 (7.4%)	25/27 (92.6%)	
	Oral ulcers	Absent	4/64 (6.3%)	60/64 (93.8%)	1.000
		Present	1/26 (3.8%)	25/26 (96.2%)	
	Musculoskeletal involvement	Absent	2/18 (11.1%)	16/18 (88.9%)	0.260
		Present	3/72 (4.2%)	69/72 (95.8%)	
	Arthritis	Absent	3/56 (5.4%)	53/56 (94.6%)	1.000
		Present	2/34 (5.9%)	32/34 (94.1%)	
	Serositis	Absent	4/76 (5.3%)	72/76 (94.7%)	0.580
		Present	1/14 (7.1%)	13/14 (92.9%)	
	Renal involvement	Absent	4/62 (6.5%)	58/62 (93.5%)	1.000
		Present	1/28 (3.6%)	27/28 (96.4%)	
	Neurological involvement	Absent	0/62 (0%)	62/62 (100%)	0.002
		Present	5/28 (17.9%)	23/28 (82.1%)	
Haematological involvement	Absent	3/54 (5.6%)	51/54 (94.4%)	1.000	
	Present	2/36 (5.6%)	34/36 (94.4%)		
Lymphadenopathy	Absent	4/81 (4.9%)	77/81 (95.1%)	0.417	
	Present	1/9 (11.1%)	8/9 (88.9%)		

<b>Laboratory results</b>	Anti double stranded DNA antibodies	Negative	5/43 (11.6%)	38/43 (88.4%)	<b>0.022</b>
		Positive	0/47 (0%)	47/47 (100%)	
	Anti-nuclear antibodies	Negative	1/12 (8.3%)	11/12 (91.7%)	0.520
		Positive	4/78 (5.1%)	74/78 (94.9%)	
	Lupus anticoagulant	Negative	4/82 (4.9%)	78/82 (95.1%)	1.000
		Positive	0/4 (0%)	4/4 (100%)	
	Anti-cardiolipin antibodies	Negative	5/86 (5.8%)	81/86 (94.2%)	1.000
		Positive	0/3 (0%)	3/3 (100%)	
	Complement C3 levels	Normal or high	5/73 (6.8%)	68/73 (93.2%)	0.579
		Low	0/17 (0%)	17/17 (100%)	
	Antibodies to extractable nuclear antigens (ENA)	Negative	2/35 (5.7%)	33/35 (94.3%)	1.000
		Positive	3/55 (5.5%)	52/55 (94.5%)	
	ENA, anti-La/SSB	Negative	3/40 (7.5%)	37/40 (92.5%)	0.554
		Positive	0/15 (0%)	15/15 (100%)	
	ENA, anti-RNP	Negative	1/20 (5%)	19/20 (95%)	1.000
		Positive	2/34 (5.9%)	32/34 (94.1%)	
	ENA, anti-Ro/SSA	Negative	2/21 (9.5%)	19/21 (90.5%)	0.551
		Positive	1/34 (2.9%)	33/34 (97.1%)	
	ENA, anti-Sm	Negative	3/41 (7.3%)	38/41 (92.7%)	0.562
		Positive	0/14 (0%)	14/14 (100%)	
Other ENA sub-type	Negative	2/52 (3.8%)	50/52 (96.2%)	0.158	
	Positive	1/3 (33.3%)	2/3 (66.7%)		
<b>Treatment received</b>	Rituximab	Not Prescribed	2/5 (40%)	55/85 (64.7%)	0.352
		Prescribed	3/5 (60%)	30/85 (35.3%)	
	Cyclophosphamide	Not Prescribed	3/5 (60%)	70/85 (82.4%)	0.237
		Prescribed	2/5 (40%)	15/85 (17.6%)	
	Hydroxychloroquine	Not Prescribed	3/5 (60%)	2/85 (2.4%)	<b>0.001</b>
		Prescribed	2/5 (40%)	83/85 (97.6%)	
	Methotrexate	Not Prescribed	3/5 (60%)	72/85 (84.7%)	0.192
		Prescribed	2/5 (40%)	13/85 (15.3%)	
	Corticosteroids	Not Prescribed	0/5 (0%)	42/85 (49.4%)	0.058
		Prescribed	5/5 (100%)	43/85 (50.6%)	
	Azathioprine	Not Prescribed	2/5 (40%)	56/85 (65.9%)	0.343
		Prescribed	3/5 (60%)	29/85 (34.1%)	
	Mycophenolate Mofetil	Not Prescribed	3/5 (60%)	54/85 (63.5%)	1.000
		Prescribed	2/5 (40%)	31/85 (36.5%)	



Other immunosuppressive agent	Not Prescribed	4/5 (80%)	81/85 (95.3%)	0.254
	Prescribed	1/5 (20%)	4/85 (4.7%)	
Anti-coagulant drug	Not Prescribed	3/5 (60%)	84/85 (98.8%)	<b>0.007</b>
	Prescribed	2/5 (40%)	1/85 (1.2%)	
Anti-hypertensive drug	Not Prescribed	3/5 (60%)	64/85 (75.3%)	0.599
	Prescribed	2/5 (40%)	21/85 (24.7%)	
Aspirin	Not Prescribed	5/5 (100%)	75/85 (88.2%)	1.000
	Prescribed	0/5 (0%)	10/85 (11.8%)	
Plasma exchange	Not Undertaken	4/5 (80%)	84/85 (98.8%)	0.109
	Undertaken	1/5 (20%)	1/85 (1.2%)	
Anti-epileptics	Not Prescribed	3/5 (60%)	82/85 (96.5%)	<b>0.023</b>
	Prescribed	2/5 (40%)	3/85 (3.5%)	
Anti-psychotics or anti-depressants	Not Prescribed	5/5 (100%)	80/85 (94.1%)	1.000
	Prescribed	0/5 (0%)	5/85 (5.9%)	

ANA=antinuclear antibodies, dsDNA=double stranded deoxyribonucleic acid,

ENA=extractable nuclear antigens, AQP4=aquaporin 4, RNP

=ribonucleoprotein, anti-Sm=anti-Smith antibodies, anti-Ro/SSA=anti Sjogren

syndrome A antibodies, anti-La/SSB=anti Sjogren syndrome B antibodies

