#420

Characterization and Alternative Diagnoses in Patients with False-Positive Aquaporin-4 Autoantibody Detection by Enzyme Linked Immunosorbent Assay (ELISA)

Jon P. Williams DO, PhD[1,2,3], Meagan Street[4], Jaron K. Badger[4], Lisa K. Peterson PhD, D(ABMLI)[4], John E. Greenlee MD[1,2], Noel G. Carlson PhD[1,2,5], John W. Rose MD[1,5], M. Mateo Paz Soldan MD, PhD[1,2], and Stacey L. Clardy MD, PhD[1,2]

1. University of Utah, Department of Neurology, Salt Lake City, UT. 2. George E. Wahlen Department of Veterans Affairs Medical Center, Salt Lake City, UT. 3. US Air Force Institute of Technology.
4. ARUP Laboratories, Immunology, Salt Lake City, UT. 5. University of Utah, Department of Neurobiology and Anatomy, Salt Lake City, UT.

Objective

To determine the rate and characteristics of patients not meeting diagnostic criteria for neuromyelitis optica spectrum disorders (NMOSD) who tested positive for autoantibodies to aquaporin 4 (AQP4).

Background

- NMSOD includes a family of inflammatory central nervous system syndromes, variable in both clinical presentation and paraclinical markers, including the presence of autoantibodies, primarily to AQP4[1,2].
- AQP4 has been demonstrated to have direct pathogenicity[2].
- Seropositivity to AQP4 is predictive of both a higher clinical relapse rate and a favorable response to therapeutics[3,4].
- AQP4 autoantibodies are detected by a variety of methods; the highest sensitivity is achieved with cell-based assays and flow cytometry[5].
- An estimated 88% of patients with this disorder have detectable antibodies to AQP4. However, a subset of patients with reported positive tests do not meet clinical criteria for NMOSD[6].

Design and Methods

- Approved U of Utah/VA IRB # IRB_00108537
- We queried the medical record at the University of Utah for patients with a diagnosis of NMOSD by ICD code.
- We pulled all orders for and patients positive for AQP4 by ELISA by test code at the regional reference laboratory, ARUP.
- The data were cross-referenced and we included all subjects with a positive result from Aug 2010 through September 2017.

Results

- Identified 750 tests ordered, of which 75 were positive, corresponding to 48 unique patients within the University of Utah system.
- Of these 48 unique patients, 20 met clinical criteria for NMOSD

Figure 1. 20/48 Meet clinical criteria for NMOSD: Characterization of detected AQP4-IgG by ELISA

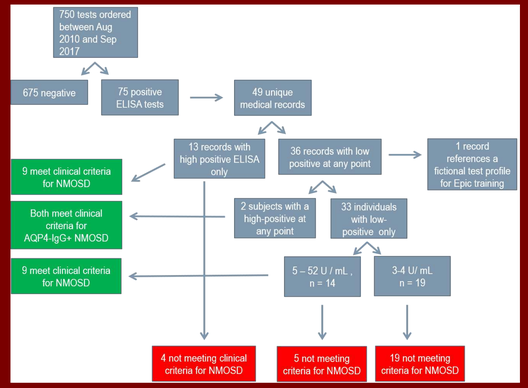

Table 1. Final diagnosis with detectable AQP4 IgG by ELISA (<4 U/mL)

Diagnosis	n (total =18)
MS	10
Isolated ON	2
Isolated TM	2
Cyclic vomiting syndrome	1
Spinal cord infarct	1
Autoimmune thyroiditis	1
Migraine	1
Presumed ON	1

Table 2. Final diagnosis in low-positive ELISA (5–52 U/mL)

Diagnosis	n (total = 14)
NMOSD	9
Multiple sclerosis	2
Disseminated Lyme	1
Limited TM	1
Migraine/ fibromyalgia	1

Table 3. Additional testing for low-positive ELISA (5–52 U/mL). CBA, cell-based assay; FACS, fluorescent activated cell sorting.

Diagnosis	Total	Retested	CBA		FACS	
			+	-	+	-
NMOSD	9	5	2, 1		1, 1	
Multiple sclerosis	2	1	0, 0		0, 1	
Disseminated Lyme	1	1	0, 1		0, 1	
Limited TM	1	1	0, 0		0, 1	
Migraine/ fibro	1	1	0, 0		0, 1	

Table 4. Distribution of low-positives (5-52 U/mL)

Diagnosis	Total	Average (U/mL)	Range (U/mL)
NMOSD	9	16.04	8 – 36
Other	5	15.25	8.1-23.9

Table 5. Final diagnosis in cases with high-positive ELISA (> 160 U/mL).

Diagnosis	n (total =15)
NMOSD	14
Sarcoidosis	1

Example case: low positive AQP4-IgG by ELISA

39 yo otherwise healthy female with new headaches and diplopia

Examination: Significant for right sixth nerve palsy and sensory disturbance over her right face

Laboratory findings
CSF: (x2) WBC 248, 244 (Lymph 87-97%);
Protein 132, 112; Glucose 45, 56;
cytology with reactive lymphocytes but no malignant cells x2; Meningoencephalitis PCR negative
3 unique oligoclonal bands
IgG synthesis increased at 12.8

Serum: ACE/ ionized Calcium normal,
Anti-SSA/SSB normal, RF normal, ANA negative
TSH normal, ant-TPO/TG both normal
AQP-4 Ab low positive by ELISA 23.9
Lyme IgG Band(s) present: 93, 58, 30, 28, 23, 18 kDa
Lyme IgM Band(s) present: 41, 23 kDa

AQP-4 Ab negative by cell binding assay + FACS
She had a complete clinical and radiographic response to prednisone followed by 28 days of IV ceftriaxone.

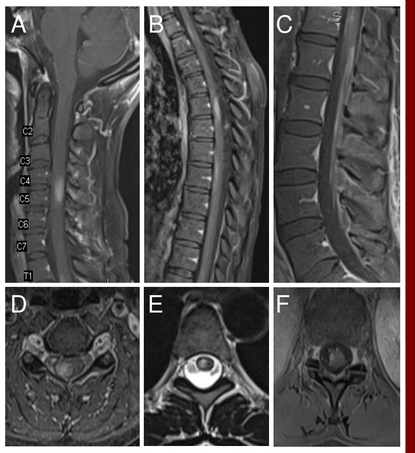

Figure 2. T1-weighted post-gadolinium MRI of spinal cord demonstrating multifocal enhancing lesions at presentation. A, D cervical spine. B,E thoracic spine, and C,F cauda equina.

Conclusions

- We describe detection of AQP4 antibodies by ELISA in patients not meeting diagnostic criteria for NMOSD.
- More sensitive assays are available, the best of which is limited to 71% sensitivity[5].
- Systemic autoimmunity has been reported in seropositive individuals[7], compelling consideration of either alternative solitary processes or overlap with early or atypical NMOSD.
- Iterative testing via different methodologies should be considered in such cases, given the significant implications of incorrect diagnoses and immunosuppressive treatment[5].

References

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