#420

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

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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

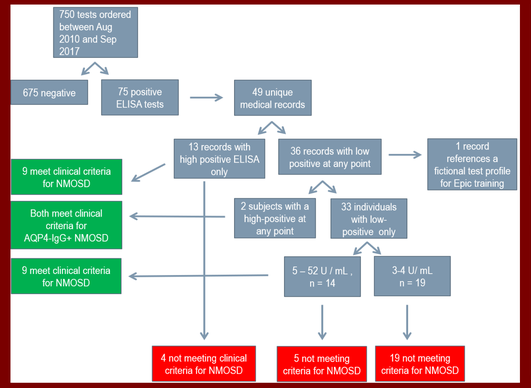

Table 1. Final diagnosis with detectable AQP4 IgG by ELISA (<4 U/mL)

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.

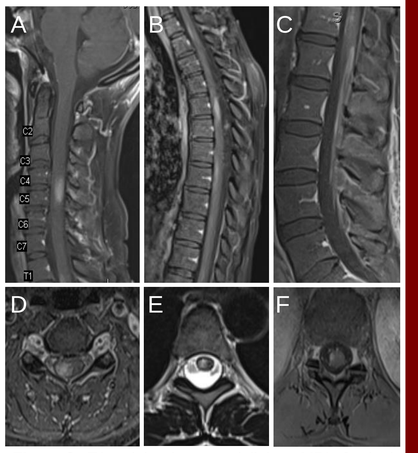

Figure 2. T1-weighted post-gadolinium MRI of spinal cord demonstrating multifocal enhancing lesions at presentation. A, D cervical spine. B,E thoracic spine, and C,F cauda equina.

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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