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

Jon P. Williams DO, PhD1,2,3, Meagan Street4, Jason K. Badger4, Lisa K. Peterson PhD, D(ABMLI)4, John E. Greenlee MD1,2, Noel G. Carlson PhD1,2,5, John W. Rose MD1,4, M. Mateo Paz Soldan MD, PhD1,2, and Stacey L. Clardy MD, PhD1,2
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.

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).

**Objective**

To identify patients with false-positive AQP4 results by ELISA, determine their clinical characteristics, and obtain a correct NMOSD diagnosis.

**Background**

- NMOSD 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.
- AQP4 has been demonstrated to have direct pathogenicity.
- Seropositivity to AQP4 is predictive of both a higher clinical relapse rate and a favorable response to therapeutic agents.
- AQP4 autoantibodies are detected by a variety of methods; the highest sensitivity is achieved with cell-based assays and flow cytometry.
- An estimated 5% of patients with this disorder have detectable antibodies to AQP4.

**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.
- 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.
- Systemic autoimmunity has been reported in seropositive individuals, 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.

**Conclusions**

- 39 yo otherwise healthy female with new headaches and diplopia
- Final diagnosis in cases with high positive AQP4 Ab negative by cell binding assay + FACS
- She had a complete clinical and radiographic response to prednisone followed by 28 days of IV ceftriaxone.

**Example case: low positive AQP4-IgG by ELISA**

**References**