ACKNOWLEDGMENT

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CSF COMPLEMENTS SERUM FOR EVALUATING PARANEOPLASTIC ANTIBODIES AND NMO-IgG

The detection of neural-reactive immunoglobulin G (IgG) autoantibodies aids the diagnosis of organ-specific autoimmune neurologic disorders. Many paraneoplastic autoantibodies reliably predict a particular cancer type and are accompanied by varied neurologic presentations of subacute onset.¹ The detection of neuromyelitis optica (NMO)-IgG predicts a relapsing inflammatory demyelinating disorder predominated by optic neuritis and transverse myelitis.² When an autoimmune neurologic disorder is suspected, serologic testing of serum is frequently undertaken before more invasive CSF evaluation. However, CSF evaluation can complement testing of serum when suspicion for an autoimmune etiology persists despite a negative serum result. Here we report, for a 25-year period of testing by standardized indirect immunofluorescence protocols, the frequency of neural autoantibody detection in serum and CSF.

Methods. The immunofluorescence protocols we used were validated in this laboratory for detection of paraneoplastic antibodies (anti-neuronal nuclear antibody [ANNA]-1; ANNA-2; ANNA-3; Purkinje cell cytoplasmic antibody [PCA]-1; PCA-2; PCA-Tr; collapsin response-mediator protein [CRMP]-5-IgG; amphiphysin antibody; antigial/ neuronal nuclear antibody [AGNA]-1; NMDA receptor antibody) and NMO-IgG. We searched the Mayo Clinic Neuroimmunology Laboratory database (January 1986 to March 2010) for all patient samples submitted for service evaluation of paraneoplastic or NMO-IgG. We included both Mayo Clinic and non-Mayo patients for whom both serum and CSF were submitted, and reviewed available oncologic data for patients with antibodies identified by CSF testing.

Results. Testing was performed on a clinical service basis for a median of 12 years (range 2–25 years). The antibody detection rate in all specimens ranged from 0.01% for PCA-Tr to 7% for NMO-IgG (table).

In patients for whom paired serum and CSF samples were tested, the antibody detection rate ranged from 0.08% (PCA-Tr) to 9% (NMDA receptor antibody). One or more neural autoantibodies were detected in 462 patients (497 antibodies detected). In 405 of those 462 patients, both serum and CSF yielded a positive result (88%). In 57 patients, serum or CSF alone was positive (12%). Among those patients, serum alone yielded a positive result in 31 (54%) and CSF alone in 26 (46%). For classic paraneoplastic antibodies, CSF alone yielded a positive result in 20 patients, twice as commonly as serum alone (10 patients). For NMO-IgG, serum alone yielded a positive result in 21 patients 3.5 times more commonly than CSF alone (6 patients).

Discussion. From our review of a 25-year experience with immunofluorescence testing on a service basis in the Mayo Clinic Neuroimmunology Laboratory, we found that the rate of clinically pertinent autoantibody detection was highest when both serum and CSF were tested. It is plausible that this finding may reflect a greater likelihood of physicians deciding to test both serum and CSF in patients with the highest index of clinical suspicion.

When both serum and CSF were tested, CSF was more commonly informative than serum for paraneoplastic antibody detection. This raises concern that clinically important neural antibodies may be missed when only serum is tested. This finding was most prominent for NMDA receptor-specific IgG. Consistent with this finding, Kumar et al.³ recently reported 3 patients, each of whom had NMDA receptor IgG detected in CSF but not in serum. Where there is a high suspicion for

Table Results of immunofluorescence testing for paraneoplastic autoantibodies and NMO-IgG, 1986–2010

IgG detected	Years of service testing	Total specimens tested, n	Total specimens positive, n (%)	Paired CSF and serum specimens tested				
				Patients tested, n	Patients positive total, n (%)	Patients positive in both serum and CSF, n	Patients positive in serum only, n	Patients positive in CSF only, n
ANNA-1 (anti-Hu)	19	214,000	1,735 (0.8)	7,000	147 (2)	141 ^a	3	3
ANNA-2 (anti-Ri)	15	187,000	123 (0.07)	6,200	12 (0.2)	9 ^b	1	2
ANNA-3	9	134,000	30 (0.02)	4,400	1 (0.02)	1	0	0
PCA-1 (anti-Yo)	24	227,000	943 (0.4)	7,500	86 (1)	80	1	5
PCA-2	9	134,000	140 (0.1)	4,400	15 (0.3)	13 ^c	2 ^{d,e}	0
PCA-Tr	8	122,968	16 (0.01)	3,937	3 (0.08)	3	0	0
CRMP-5 IgG (anti-CV2)	13	171,000	798 (0.5)	5,600	62 (1)	58 ^f	2 ^{a,g}	2
Amphiphysin antibody	12	162,000	237 (0.15)	5,300	21 (0.4)	16 ^h	1	4
AGNA-1 (SOX-1 IgG)	12	162,000	131 (0.08)	5,300	12 (0.2)	9	2 ^{d,g}	1
NMDA receptor antibody	2	412	21 (5)	109	10 (9)	7	0	3
NMO-IgG (aquaporin-4 IgG)	5	51,298	3,587 (7)	1,604	128 (8)	101	21	6

Abbreviations: IgG = immunoglobulin G; NMO = neuromyelitis optica.

Coexisting antibodies, n of patients for each:

^a CRMP-5 IgG, 16; PCA-2, 1; amphiphysin antibody, 1.

^b Amphiphysin antibody, 1; CRMP-5 IgG, 1.

^c CRMP-5 IgG, 8; ANNA-1, 1.

^d CRMP-5 IgG, 1.

^e AGNA-1.

^f ANNA-1, 16; PCA-2, 8; amphiphysin antibody, 3; ANNA-2, 1.

^g PCA-2, 1.

^h CRMP-5 IgG, 3; ANNA-1, 1; ANNA-2, 1.

cancer, additional testing for paraneoplastic antibodies on CSF may be helpful.

For detection of NMO-IgG, the positivity rate was greater for serum alone than for CSF alone. However, testing of CSF is helpful when the serum is negative. Klawiter et al.⁴ reported 3 patients who had an NMO spectrum disorder but were seronegative for NMO-IgG (those patients are included in this report also). Detection of NMO-IgG in CSF allowed unambiguous distinction of NMO from MS. CSF testing offers the additional advantage of generally lacking the non-organ-specific IgG autoantibodies (e.g., antinuclear, antimitochondrial, and smooth muscle) that are common in serum of patients with NMO and also with classic paraneoplastic autoimmune disorders.

Testing of serum or CSF for paraneoplastic autoantibodies or NMO-IgG should be reserved for clinically suspicious cases. Risk factors for a paraneoplastic disorder which may prompt testing include a subacute onset of neurologic symptoms and signs, a personal or family history of autoimmune or cancer, and smoking history. Seronegativity for paraneoplastic antibodies does not exclude a diagnosis of cancer, and testing for occult malignancy should proceed in highly suspicious cases.⁵ The low positivity rates for most

paraneoplastic antibodies in this study are consistent with their rarity, and reflects the ordering of this testing in patients with diverse neurologic presentations, where no cause has been established.¹ The more frequent detection of NMO-IgG and NMDA receptor antibody is consistent with the more narrow clinical contexts for ordering these 2 tests.^{2,6}

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Editor's Note to Authors and Readers: Levels of Evidence coming to *Neurology*®

Effective January 15, 2009, authors submitting Articles or Clinical/Scientific Notes to *Neurology*® that report on clinical therapeutic studies must state the study type, the primary research question(s), and the classification of level of evidence assigned to each question based on the classification scheme requirements shown below (left). While the authors will initially assign a level of evidence, the final level will be adjudicated by an independent team prior to publication. Ultimately, these levels can be translated into classes of recommendations for clinical care, as shown below (right). For more information, please access the articles and the editorial on the use of classification of levels of evidence published in *Neurology*.^{1–3}

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Classification scheme requirements for therapeutic questions

Class I. A randomized, controlled clinical trial of the intervention of interest with masked or objective outcome assessment, in a representative population. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class II. A randomized, controlled clinical trial of the intervention of interest in a representative population with masked or objective outcome assessment that lacks one criterion a-e in Class I or a prospective matched cohort study with masked or objective outcome assessment in a representative population that meets b-e in Class I. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class III. All other controlled trials (including well-defined natural history controls or patients serving as their own controls) in a representative population, where outcome is independently assessed, or independently derived by objective outcome measurements.

Class IV. Studies not meeting Class I, II, or III criteria including consensus or expert opinion.

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A = Established as effective, ineffective, or harmful (or established as useful/predictive or not useful/predictive) for the given condition in the specified population. (Level A rating requires at least two consistent Class I studies.)

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C = Possibly effective, ineffective, or harmful (or possibly useful/predictive or not useful/predictive) for the given condition in the specified population. (Level C rating requires at least one Class II study or two consistent Class III studies.)

U = Data inadequate or conflicting; given current knowledge, treatment (test, predictor) is unproven.