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A COMPARISON OF TISSUE-BASED AND RECOMBINANT PROTEIN-BASED ASSAYS FOR DETECTING PCA-TR/DNER-IGG

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Purkinje cell cytoplasmic antibody, type Tr (PCA-Tr) is typically encountered in patients with Hodgkin lymphoma-related paraneoplastic cerebellar ataxia.¹ Immunohistochemical techniques, including indirect immunofluorescence assays (IFAs), employing rodent brain as substrate, reveal a specific immunoglobulin G (IgG) binding pattern on cerebellar Purkinje cell cytoplasm and molecular layer dendritic spines.² PCA-Tr characterization as delta/notch-like epidermal growth factor-related receptor (DNER)-IgG has permitted antigen-specific assay development.³ With rare exception, the phenotype encountered is cerebellar ataxia among patients with Hodgkin lymphoma. Tr autoimmunity is occasionally reported in some patients with noncerebellar neurologic disorders, and in others with non-Hodgkin lymphoma.⁴

We report the performance of 2 DNER-IgG assays, with comparison to the Mayo Clinic Neuroimmunology Laboratory's IFA, among patients with known PCA-Tr autoimmunity, PCA-Tr-negative patients with lymphoma-related paraneoplastic disorders, and healthy controls.

Methods. *Standard protocol approvals, registrations, and patient consents.* The Mayo Clinic Institutional Review Board approved this study.

Patients. *PCA-Tr IgG-positive group.* In the course of clinical service evaluation for paraneoplastic neural autoantibodies in the Mayo Clinic Neuroimmunology Laboratory (1997–2016), PCA-Tr was identified in serum or CSF of 33 patients by in-house tissue-based IFA. Available archival specimens were serum only (16 patients), CSF only (5), or both specimen types (12). Clinical information, available for 23 patients, recorded the following neurologic diagnoses: cerebellar ataxia, 21; encephalopathy, 3; peripheral neuropathy, 2; tonic pupil, 2; dysgeusia, 1 and seizures, 1. All 15 patients with known cancer diagnoses had Hodgkin lymphoma.

PCA-Tr IgG-negative group. The Mayo Clinical Notes Search Tool was interrogated, using search terms

“lymphoma” and “paraneoplastic,” to find patients with likely paraneoplastic neurologic disorders related to lymphoma (2005–2015). Sixty-two patients were identified after reviewing 746 records. The remaining 684 patients were excluded (laboratory specimen unavailable, or neurologic disorder caused by lymphoma, treatment, or other). Neurologic presentations of the 62 patients were encephalopathy (16), ataxia (14), myelopathy (13), neuropathy (13), and multifocal neurologic disorder (6). Lymphoma types encountered were non-Hodgkin (52 patients) and Hodgkin (10 patients). Specimens were serum only, 33 patients; serum and CSF, 29 patients.

Controls. Serum controls included the following: for tissue-based IFA, 173 healthy (Olmsted County, Minnesota); for transfected cell-based assay (CBA), 100 healthy (University Clinic Schleswig-Holstein, Lübeck, Germany); for recombinant protein line blot assay, 98 healthy controls (Mayo Clinic Biobank). CSF controls were from patients with non-autoimmune neurologic disorders: tissue IFA, 77; line blot, 43.

Serum and CSF testing. Three assays were performed on all specimens: Mayo Clinic tissue-based IFA for PCA-Tr-IgG (including quantitation if positive), and kit assays (CBA and line blots for DNER-IgG [qualitative positive or negative results], Euroimmun, Lübeck, Germany).⁵ Optimum screening dilutions were determined by in-house verification (for all IFAs [serum 1:240; CSF 1:2] and CSF line blot [1:12.5]) or by manufacturer instructions (serum line blot [1:100] and all CBAs [serum, 1; 10; CSF, 1:2]). For the line blot, each patient specimen was incubated with a DNER-coated test strip. DNER purification was achieved by immobilized metal affinity chromatography.⁶ Bound IgG was detected by application of enzyme-conjugated anti-human IgG catalyzing a color reaction and densitometric data was acquired by EUROLINEScan software (Euroimmun).

Results. In the PCA-Tr group with only a single specimen available (serum or CSF, 21 patients), all 3 assays were always positive (table). Where both specimens were available (12 patients), DNER-IgG was detected in CSF of all cases by IFA and CBA, and in 10 of 12 by line blot (83%), and in serum,

Table Comparison of tissue immunofluorescence assay (IFA) and recombinant line blot and cell-based assay (CBA) for Purkinje cell cytoplasmic antibody, type Tr (PCA-Tr)/delta/notch-like epidermal growth factor-related receptor (DNER)-immunoglobulin G (IgG) detection^a

Patient no.	Serum			CSF		
	IFA titer ^b	Line blot	CBA	IFA titer	Line blot	CBA
1	—	—	—	8	—	+
2	480	+	+	256	+	+
3	240	+	+	4	+	+
4	61,440	+	+	8	+	+
5	—	—	—	32	+	+
6	120	+	+	128	+	+
7	—	+	+	16	+	+
8	240	+	+	4	+	+
9	960	+	+	32	+	+
10	—	—	+	8	—	+
11	960	+	+	32	+	+
12	240	+	+	256	+	+
13	3,840	+	+	NA	NA	NA
14	240	+	+	NA	NA	NA
15	1,920	+	+	NA	NA	NA
16	7,680	+	+	NA	NA	NA
17	1,920	+	+	NA	NA	NA
18	1,920	+	+	NA	NA	NA
19	3,840	+	+	NA	NA	NA
20	3,840	+	+	NA	NA	NA
21	960	+	+	NA	NA	NA
22	1,920	+	+	NA	NA	NA
23	240	+	+	NA	NA	NA
24	960	+	+	NA	NA	NA
25	3,840	+	+	NA	NA	NA
26	30,720	+	+	NA	NA	NA
27	960	+	+	NA	NA	NA
28	1,920	+	+	NA	NA	NA
29	NA	NA	NA	32	+	+
30	NA	NA	NA	32	+	+
31	NA	NA	NA	16	+	+
32	NA	NA	NA	256	+	+
33	NA	NA	NA	256	+	+

Abbreviation: NA = not available.

^a All discrepant positive/negative results were repeated. Tissue IFA, serum from patient 1 was originally positive (1:240) but became negative in the course of this study.

^b Median PCA-Tr titer was 960 in serum (range 120–61,440) and 32 in CSF (range 4–256).

DNER-IgG was detected by IFA in 8 of 12 cases (66%), by line blot in 9 of 12 cases (75%), and by CBA in 10 of 12 cases (83%). The interassay sensitivity differences were not statistically significant ($p > 0.05$ for all comparisons, Fisher Exact test). In

the PCA-Tr-IgG-negative lymphoma group and healthy control groups, all specimens were negative by all 3 assays.

Discussion. PCA-Tr/DNER-IgG is one of the few (and rare) well-characterized lymphoma-associated paraneoplastic autoantibodies. Additional cases were not detected by DNER-specific assays among PCA-Tr-IgG-negative patients with lymphoma and potentially paraneoplastic neurologic disorders. Sensitivity of the recombinant protein-based assays may be an underestimate because our testing was limited to historical specimens (IgG reactivity might be lost with time and freeze-thaw cycles).

Some patients were PCA-Tr/DNER-IgG-negative in serum. Consistent with prior experience, additional evaluation of CSF improves diagnostic sensitivity for paraneoplastic disorders.⁷ Though interassay differences in sensitivity were not statistically significant, clinical importance was retained because PCA-Tr/DNER-IgG is rarely encountered, even in specialized laboratories. Recombinant DNER-IgG assay could serve as reflex confirmatory testing for suspected cases detected by tissue-based immunohistochemical assay (where that is the preferred antibody screening modality) or could be utilized for screening.

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