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Autoantibody diversity in paraneoplastic syndromes and related disorders: The need for a more guided screening approach

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ABSTRACT

Background: Significant progress has been made in understanding the role and diversity of autoantibodies in the pathogenesis, diagnosis and management of paraneoplastic syndrome (PNS) and related autoimmune neurologic diseases. We evaluated the positivity rates for diverse autoantibody panels to rationalize testing strategies and utilization.
Methods: The result patterns for different autoantibody panels for PNS offered at 2 reference laboratories in the U.S. were retrospectively reviewed for the same period. The positivity rates were evaluated and compared for specific autoantibodies within panels offered at both laboratories.
Results: For the Hu, Ri, Yo, and amphiphysin antibodies offered by both laboratories, no significant difference in positivity rates was observed. The positivity rates for non-classic PNS markers were 0% [AGNA and PCCA-Tr], and 0.06% [ANNA-3 and PCAC-2] while the prevalence of antibodies associated with neuromuscular autoimmunity varied from 1.40% to 4.44% [Striated muscle, AChR binding, ganglionic AChR, VKCC, P/Q- and N-type VGCC].
Conclusions: The data suggest that test utilization could be substantially improved based on ordering practice geared towards clinical manifestations and prevalence of autoantibodies. Concerted efforts towards streamlining diagnostic algorithms based on risk, clinical manifestations, characterization of autoantibodies and their associations as well as therapeutic strategies are needed.

1. Introduction

Paraneoplastic neurological syndromes (PNS) are indirect remote effects of cancer on the nervous system, often associated with the presence of specific serum antibodies. In recent decades a number of such antibodies have been characterized and added to commercially available PNS diagnostic panels and/or platforms. Many of these antibodies are extremely rare, raising questions about the cost-effectiveness of large PNS testing panels. This study presents a retrospective analysis of PNS- and/or PNS-related autoantibody results (acquired neuromuscular junction diseases) in the context of test utilization. This information may be useful in informing more selective ordering of these tests as well as the development of evidence-based guidelines for use in clinical practice.

Association between specific autoantibodies in PNS and autoimmune neurologic diseases may be classified based on their clinical and analytical characterization and/or the cellular location of the recognized antigen [1,2]. Antibodies that target intracellular antigens have been associated with specific cancers in PNS. Amongst these, antibodies to Hu (anti-neuronal nuclear antibody type 1; ANNA-1), Ri (anti-neuronal nuclear antibody type 2; ANNA-2), Yo (Purkinje cell cytoplasmic antibody type 1; PCA-1), CRMP5 (collapsin response mediator protein 5; also referred to as CV2), Ma_2/Ta and amphiphysin are thought to be classical and considered diagnostic [1,3]. Of these, Hu and Yo antibodies are the most commonly found with a prevalence of 38.8% and 13.4% respectively as reported in the PNS Euronetwork Database [3]. Based on this database, Ri (5.1%), Ma_2/Ta (4.5%), CV2 (6.5%) and amphiphysin (3.4%) antibodies appear to be less common [3]. Furthermore, antibodies such as the Yo and Ma_2/Ta are predominantly found in female and male subjects respectively [1,3,4]. Except for Ma_2/Ta, these antibodies tend to occur in older adults. These indications provide strong clinical rationale that may guide their selection in disease evaluation [1,3–4]. Immunohistochemical analysis may reveal the presence of more than one of these autoantibodies. This is particularly true for Hu, CV2, Ri, amphiphysin, Yo, VGCC or Zic4 autoantibodies [5–9].

Recently, it has become clear that certain neurologic disorders may be associated with antibodies that bind to surface antigens on neuronal cells referred to as neuronal surface antibodies (NSAbs) [2,4,10]. These include antibodies to voltage-gated potassium channel (VGKC) complex [including contactin associated protein 2 (CASPR2) and leucine-rich

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glioma inactivated 1 protein (LGI1)], *N*-methyl-D-aspartate receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), γ-amino butyric acid B receptor (GABA(B)R), glycine receptor (GlyR), and metabotropic glutamate receptor (mGluR) [2,4]. These antibodies are not age-dependent; may or may not be associated with cancers, are thought to be pathogenic and usually treatment-responsive. For patients with autoimmune limbic encephalitis who are positive for NSAb or onconeuronal antibodies, their clinical manifestations may be indistinguishable [2,4]. Although these diseases are beginning to be widely recognized, there are likely other treatment-responsive types with no defined NSAb(s). For example, in a systematic study of pediatric patients approximately 44% (21/48) with eventual diagnosis of probable autoimmune encephalopathy were positive for known antibodies (NMDAR, VGKC and GAD65) [10].

In addition to antibodies directed towards intracellular and neuronal surface proteins, a diversity of other autoantibodies for acquired neuromuscular junction diseases with significant associations with certain cancers are well recognized [8,11,12]. In addition to anti-VGKC for acquired neuromyotonia (Isaac's syndrome), other major autoantibodies include voltage gated calcium channel (VGCC) associated with Lambert-Eaton myasthenic syndrome, striated muscle (STM) and acetylcholine receptor (AChR) binding and blocking (myasthenia gravis, MG), ganglionic nicotinic acetylcholine receptor (gAChR) in patients with autoimmune autonomic ganglionopathy [11–13]. Recently, antibodies directed against dipeptidyl peptidase-like protein 6 (DPPX), a regulatory subunit of the Kv4.2 potassium channels on the surface of neurons were reported in a novel and distinct variant of progressive encephalomyelitis with rigidity and myoclonus (PERM) [14].

Overall, there has been significant progress in our understanding of the types and role of antibodies in the pathogenesis, diagnosis and management of PNS and autoimmune neurologic diseases. Nevertheless, their lack of specificities (overlap with clinical syndromes and/or tumors) and accessibility in routine laboratories pose a challenge in appropriate test utilization. To address these, expert guidelines have been proposed to direct testing practice based on specific populations [1,3–4]. Despite these efforts, there exist far-reaching autoantibody panels with obscure clinical relevance considering the rarity of some antibody types as well as disorders. Höftberger and colleagues recently defined a screening approach suitable for detecting antibodies based the cellular location of the binding antibodies, that is, intracellular or neuronal surface antigens [15]. Graus et al. in 2004 proposed two levels of evidence to define a neurological syndrome as paraneoplastic: “definite” and “possible” [1]. Based on these recommendations, each level can be reached by combining a set of criteria based on the presence or absence of cancer and the definitions of “classical” syndrome and “well characterized” onconeural antibody [1]. For a diagnosis of “definite PNS” four criteria must be met one of which includes the use of well characterized antibodies (Hu, Yo, CV2, Ri, Ma2, or amphiphysin) and no known cancer in presence a neurological syndrome (classical or not). In the case of “possible PNS”, three criteria were proposed. Of these, the presence of a neurological syndrome (classical or not) with partially characterized onconeural antibodies and no history cancer is of diagnostic relevance. The two others include a classical syndrome, no onconeural antibodies, no history cancer but a high risk to have an underlying tumor and a non-classical syndrome, no onconeural antibodies and cancer present within two years of diagnosis. In addition to PNS, it is well recognized that conditions such as myasthenia gravis (MG) and the Lambert-Eaton myasthenic syndrome (LEMS) have a strong autoimmune involvement with variable cancer associations [11,12]. Antibodies in both MG and LEMS patients are directed against essential membrane receptors or

Table 1
Autoantibody markers and methods for their detection in the clinical laboratory.

Category	Specific antibodies	Detection methods[a]					
		IHC/IFA[a]	WB	LIA	RIA	ELISA	CBA
Intracellular antigens	ANNA-1 (Hu)	x	x	x	–	x	–
	ANNA-2 (Ri)	x	x	x	–	x	–
	ANNA-3	x	–	–	–	–	–
	CRMP-5 (CV2)	x	x	x	–	x	–
	Ma/Ta	x	x	x	–	–	–
	AGNA-1 (Sox-1)	x	x	x	–	x	–
	PCCA-1 (Yo)	x	x	x	–	x	–
	PCCA-2	x	–	–	–	–	–
	PCCA-Tr (DNER)	x	x	x	–	x	–
	GAD65	x	x	x	x	x	–
	Amphiphysin	x	x	x	–	x	–
	Recoverin	x	x	x	–	x	–
Neuronal surface antigens	NMDAR	x	–	–	–	x	x
	LGI1	x	–	–	–	–	x
	CASPR2	x	–	–	–	–	x
	AMPAR	x	–	–	–	–	x
	$GABA_BR$	x	–	–	–	–	x
	mGluR	x	–	–	–	–	x
	GyR	x	–	–	–	–	x
Neuromuscular junction or channel antigen	STR	x	–	–	–	x	–
	AQP4	x	x	x	–	x	x
	PQ-VGCC	–	–	–	x	–	–
	N-VGCC	–	–	–	x	–	–
	AChRBIN	–	–	–	x	–	–
	gAChR	–	–	–	x	–	–
	VGKC	–	–	–	x	–	–
	DPPX	x	–	–	x	–	x

IHC/IFA, indirect immunohistochemistry/indirect fluorescence antibody assay; WB, Western blot; LIA, line immunoassay; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; CBA, cell-based assay; anti-neuronal nuclear antibody type 1: ANNA-1 (Hu); anti-neuronal nuclear antibody type 2: ANNA-2 (Ri); anti-neuronal nuclear antibody type 3: ANNA-3; collapsin response mediator protein 5: CRMP5, also referred to as CV2; anti-glial/neuronal nuclear: AGNA-1, also referred to as Sox1; Purkinje cell cytoplasmic antibody type 1: PCA-1(Yo); Purkinje cell cytoplasmic antibody type 2: PCA-2; Purkinje cell cytoplasmic antibody Tr: PCA-Tr also referred to as DNER (delta/notch-like epidermal growth factor-related receptor); GAD: glutamic acid decarboxylase; *N*-methyl-D-aspartate receptor: NMDAR; leucine-rich glioma inactivated 1: LGI1; contacting associated protein 2: CASPR2; alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor: AMPAR; gamma-amino butyric acid B receptor: $GABA_BR$; metabotropic glutamate receptor: mGluR; glycine receptor: GlyR; striational muscle: STM; aquaporin-4: AQP4; PQ- and N-type voltage-gated calcium channel: PQ-VGCC and N-VGCC; voltage-gated potassium channel: VGKC; acetylcholine receptor binding: AChRBIN; ganglionic nicotinic acetylcholine receptor: gAChR; dipeptidyl-peptidase-like protein-6: DPPX antibodies.

[a] IMH/IFA results always require confirmatory testing if available by another method.