CHAPTER 6

Use of antibody testing in nervous system disorders

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Objective

To evaluate service provision and quality assurance schemes for clinically useful autoantibody tests in neurology.

Background

Over the past 20 years there has been a steady increase in the use of anti-nerve antibody assays to aid diagnosis or research into neurological diseases thought to have an antibody-associated or antibody-mediated autoimmune basis [1–9]. The range of antigens tested and their associated diseases includes nerve and neuromuscular junction disorders, and paraneoplastic disorders affecting the central nervous system, as listed and referenced in table 6.1. With respect to the use of the anti-acetylcholine receptor (anti-AChR) antibody assay to aid in the diagnosis in myasthenia gravis, the radioimmunoassay in standard use [29] has been thoroughly validated for many years. Both non-commercial and commercial quality assurance schemes for laboratories to participate in are available. However, the procedures in place for quality assurance in the identification of antibodies that mark paraneoplastic syndromes and for anti-ganglioside antibodies are less well developed. Efforts have been made to produce standard protocols, exchange samples, and run workshops in both these latter areas, as manifested by the INCAT (Immune Neuropathy Cause and Treatment) group [39] and the Paraneoplastic Neurological Syndrome Euronetwork [5]. Such studies have principally involved researchers and laboratories with a specialized interest in these fields rather than clinical laboratories performing routine screening.

The anti-neuronal antibodies associated with paraneoplastic syndromes, anti-Hu anti-Yo, and anti-Ri (ANNA-1, PCA-1, ANNA-2 respectively), were initially demonstrated by immunohistochemistry of brain sections and more recently by blotting of recombinant proteins, as listed in table 6.1. The clinical utility of these investigations is considerable, and the importance of accurate identification paramount to clinical decision making. In addition, this spectrum of autoantibodies is the subject of important research developments. This has been recently discussed in a detailed workshop report [40].

The determination of anti-ganglioside and glycolipid antibodies has increasingly entered clinical practice over recent years [8]. Anti-glycolipid antibodies are associated with acute and chronic peripheral neuropathies and may be useful in diagnosis of clinical subtypes of neuropathy. They are widely measured by enzyme-linked immunosorbent assay (ELISA), dot blot, and thin layer chromatography overlay [39, 41, 42].

Both anti-neuronal and anti-glycolipid antibody assays are being conducted in laboratories throughout Europe. Until recently, this has been without any externally or independently monitored quality assurance, although

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Table 6.1 Antigens tested and their associated diseases

Antibody specificity	Associated neurological disorders	Detection method	References
Anti-Hu (ANNA-1)	Subacute sensory neuronopathy, limbic encephalitis, brain stem encephalitis, paraneoplastic encephalomyelitis, chronic pseudoobstruction	IMH/IMF, confirmed by WB on recombinant protein or neuronal extracts	10, 11
Anti-Yo (PCA-1)	Paraneoplastic cerebellar degeneration	IMH/IMF, confirmed by WB as above	12, 13
Anti-Ri (ANNA-2)	Myoclonus/opsoclonus	IMH/IMF, confirmed by WB as above	14
Anti-Tr	Paraneoplastic cerebellar degeneration	IMH/IMF (requires fixed tissue),	15
Anti-amphiphysin	Stiff person syndrome, encephalomyelitis, subacute sensory neuronopathy	IMH/IMF (requires fixed tissue), confirmed by WB as above	16, 17
Anti-CV2/CRMP5	Cerebellar degeneration, encephalomyelitis, limbic encephalitis	IMH/IMF (requires fixed tissue), confirmed by WB as above	18
Anti-VGKC	Acquired neuromyotonia, limbic encephalitis (usually not paraneoplastic)	RIA	19
Anti-VGCC	Lambert-Eaton myasthenic syndrome, paraneoplastic cerebellar degeneration	RIA	20, 21
Anti-Aquaporin 4	Neuromyelitis optica	IMH/IMF. IMF on unpermeabilized cells transfected with the antigen is most sensitive	22, 23
Anti-NMDAR	NMDAR-antibody encephalopathy	IMF on unpermeabilized cells transfected with the antigen is most sensitive	24
Anti-ganglionic AChR	Autonomic neuropathy	RIA	25
Anti-(TA) Ma2	Limbic encephalitis	IMH/IMF, confirmed by WB as above	26, 27
Anti-AChR, MuSK	Myasthenia gravis	RIA	28, 29
Anti-GM1, GD1b (IgM) Anti-GM2 (IgM)	Multifocal motor neuropathy, chronic motor neuropathy	ELISA, TLC	9, 30, 31
Anti-GM1a, GM1b GD1a, GalNAcGD1a, (IgG)	Acute motor axonal neuropathy	ELISA, TLC	8, 32
Anti-GQ1b, GT1a	Miller Fisher syndrome	ELISA, TLC	8, 33
Anti-GD3, GD1b	Acute ataxic neuropathies	ELISA, TLC	34
Anti-GD1b and other disialylated gangliosides (IgM)	Paraproteinemic neuropathies, CANOMAD	ELISA, TLC	35, 36
Anti-MAG/SGPG (IgM)	IgM paraproteinaemic neuropathy	WB of CNS myelin, ELISA	37
Anti-GAD	Stiff person syndrome/cerebellar ataxia	IMH/IMF (requires fixed tissue), confirmed by WB, RIA	17, 38

IMH/IMF, immunohistochemistry/immunofluorescence; WB, Western blot; RIA, radioimmunoassay; TLC, thin layer chromatography overlay; ELISA, enzyme-linked immunosorbent assay; MAG, myelin associated glycoprotein; SGPG, sulphated glucuronyl paragloboside; CANOMAD, chronic ataxic neuropathy, ophthalmoplegia, M protein, cold agglutinins, anti-disialosyl antibodies; VGCC, voltage-gated calcium channels; VGKC, voltage-gated potassium channel; Ach-R, acetylcholine receptor; MuSK, muscle specific kinase; GAD, glutamic acid decarboxylase; NMDAR, N-methyl-D-aspartate receptor.

such schemes are now becoming available. To investigate the scale of this issue and to identify the perceived needs of neuroimmunology laboratories in assay availability and quality, we conducted a questionnaire-based survey of European neuroimmunology centres and here report and discuss the findings.

Methods

Under the auspices of the EFNS Scientific Panel on Neuroimmunology, an anti-nerve antibody task force was established to conduct the review. Eighteen national representatives were invited to participate in a questionnaire-based survey. The questionnaire requested information on: (a) the availability of tests both within the individual's institution and nationally; (b) an approximation of the number of tests conducted annually; (c) the methodology used; (d) the availability of quality assurance schemes; (e) the availability of positive and negative control sera; (f) the interest in setting up and participating in a pan-European quality assurance scheme.

Results

The questionnaire was distributed in 1999 to 18 national members of the EFNS Scientific Panel on Neuroimmunology, of which 12 responded. The range of assays being conducted is summarized in table 6.1, as are the associated neurological disorders and key references. In addition, novel assays routinely available in 2010, e.g. anti-aquaporin 4 antibody screening to aid in the diagnosis of neuromyelitis optica [22, 23] and NMDAR antibodies for the diagnosis of a newly described encephalopathy [24] are also included in the updated table.

Antibody assays for anti-AChR antibodies are widely available, being conducted in at least one centre in most of the countries that responded (10 out of 12). Quality assurance schemes were used either nationally or internationally and the exclusive method used was the standard radioimmunoassay, using iodinated bungarotoxin bound to acetylcholine receptors extracted either from muscle or from muscle-like cell lines. Commercial kits are available for AChR and MuSK antibodies from RSR Ltd, Cardiff, UK. A recent unpublished survey of centres in Europe conducted by Euromyasthenia indicated that most groups used these tests and all groups had concordant results.

Antibodies to glutamic acid decarboxylase (GAD), found in autoimmune stiff person syndrome [17], were conducted in five of 12 neuroimmunology laboratories in responding countries and estimated using a variety of methods including immunohistology, ELISA, radioimmunoassay, and Western blot. At present it is difficult to compare values between different laboratories despite the use of international units in some cases. Because these assays are designed principally for use in investigation of diabetes, and because titres are much higher in stiff person syndrome and some cases of cerebellar ataxia than in diabetes, it will be important to ensure that laboratories performing this test for neurological disorders use techniques designed to measure high titres.

Antibody assays to voltage-gated calcium channels (VGCC) and potassium channels (VGKC) were rarely conducted, being available in three and one surveyed centres respectively. A commercial kit for the VGCC test is now available (RSR Ltd, Cardiff, UK) and results from different laboratories should be comparable.

Antibody assays for Hu (ANNA-1) and Yo (APCA-1) were widely available and frequently conducted in many centres in most countries (nine of 12), using a combination of immunhistochemistry and Western blot analysis. Anti-Ri (ANNA-2), -Tr, and -amphiphysin antibodies were sought less frequently. The less frequent paraneoplastic antibodies, anti-Ma2/anti-Ta, anti-CV2/CRMP5, can also be detected by immunohistochemistry, but in many cases fixed rather than fresh frozen tissue is required, and not all laboratories do this routinely. There is a need to distribute positive sera to help in the recognition of these antibodies. There is increasing use of comprehensive commercial immunoblots such as those marketed by Ravo-Diagnostika (Freiburg, Germany) and Euroimmun (Lubeck, Germany) that detect antibodies to a broad panel of recombinant antigens including Hu, Yo, Ri, Ma1, Ma2/Ta, CV2/CRMP5, and amphiphysin.

Anti-myelin-associated glycoprotein (MAG) antibodies were determined in laboratories in at least one centre in seven of 12 countries, using a commercial kit that has good standardization (Buhlmann Laboratories, Basel, Switzerland), or using Western blot of myelin [43]. Measurement of anti-ganglioside antibodies was also widely available in many centres and included a wide range of gangliosides and glycolipids (e.g. GM1, GM2, GA1,

GD1a, GD1b, GQ1b, and sulphatides), but the details of the ELISAs used differ considerably between laboratories [39, 41, 42, 44, 45].

In response to questions on quality assurance, most centres reported that they conducted in-house quality assurance, although information on their precise nature was not sought. However, the only assay in which national or international quality assurance was widely used was the anti-AChR antibody assay. With respect to quality assurance schemes for other antigens, all laboratories indicated that they would join a quality assurance scheme for at least some, if not all, the investigations they were conducting.

Among the newer antibodies, AQP4 antibody testing is now conducted in several centres in Europe, but a formal survey has not been conducted. NMDAR antibodies are performed in at least two centres. Both these antibody tests have recently been established by Euroimmun (Lubeck, Germany) and there is also an ELISA for AQP4 antibodies (RSR Ltd, UK).

Discussion and Good Practice Points

It is evident from this survey that a wide variety of antibody assays used in the diagnosis of neuroimmunological diseases are being conducted in many centres throughout Europe. This survey was restricted to major antigens and their respective antibodies, but did not consider the very wide array of emerging tests that have yet to be fully validated for clinical utility. This represents a healthy perception of the value of such investigations among clinical neurologists, but also highlights the need for a high degree of inter-laboratory uniformity and standards of practice.

A number of co-operative inter-laboratory studies have previously been conducted through distribution of coded positive and negative samples to participating laboratories. These have demonstrated marked variations in the ability to detect accurately positive or negative samples for both anti-ganglioside antibodies and antibodies marking paraneoplastic syndromes, particularly for borderline samples. This particular issue was not addressed in this survey. However, information was sought on methodology and in this context it is evident that methodologies being used vary quite widely among different laboratories.

Since the survey was originally conducted there has been steady progress in understanding the nature and role of antibodies to nervous system components in neurological diseases. The clinical utility of testing remains to be determined.

The most striking finding of this survey was the lack of any organized quality assurance schemes for the great majority of these autoantibodies, the exception being for anti-AChR antibodies, and more recently some anti-neuronal and anti-glycolipid antibodies. The survey indicated a very strong demand for such quality assurance schemes to be instituted. The mechanism by which such schemes should be organized is a matter for debate. Our Good Practice Points are thus summarized as follows.

1 The determination of anti-neuronal antibodies should be conducted using protocols agreed during the course of multi-centre comparative studies, such as the INCAT study for anti-glycolipid antibodies.

2 Laboratories conducting immunassays for anti-AChR antibodies should join existing quality assurance schemes.

3 Where no official scheme is available (i.e. for the majority of assays covered in this survey) laboratories should develop arrangements for exchanging coded positive and negative samples at least biannually, to ensure sensitivity and specificity are being maintained.

4 A quality assurance scheme for the most commonly measured anti-glycolipid antibodies (GM1 and GQ1b) and paraneoplastic antibodies (Hu and Yo) should be established as a matter of priority (this has now been done).

5 The EFNS should consider how open-access quality control schemes in Europe are best established, both for laboratory and other measures, and should actively promote such schemes.

Conflicts of interest

A. Vincent and Clinical Neurology, Oxford, receive royalties and payments for antibody tests.

The other authors have reported no conflicts of interest.

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