

EFNS Task Force Report: a questionnaire-based survey on the service provision and quality assurance for determination of diagnostic autoantibody tests in European neuroimmunology centres

H. J. Willison^a, W. Ang^b, N. E. Gilhus^c, F. Graus^d, R. Liblau^e, C. Vedeler^c and A. Vincent^f

^aUniversity Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow G51 4TF, UK; ^bUniversity Hospital Dijkzigt, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands; ^cDepartment of Neurology, Haukeland University Hospital, 5021 Bergen, Norway; ^dService of Neurology, Hospital Clinic de Barcelona, Villarroel 170, 08036 Barcelona, Spain; ^eHopital de la Salpetriere, 47 Boulevard de l'hopital, 75651 Paris Cedex 13, France; ^fDepartment of Clinical Neurology, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DU, UK

Keywords:

autoantibody,
neuroimmunology centres,
quality assurance, service
provision

Received 8 August 2000

Accepted 8 August 2000

Autoantibodies to a wide variety of neural components are frequently sought in the sera of patients with neurological diseases suspected to have an antibody-associated autoimmune basis. Variations in assay methodology and availability are likely to exist throughout European diagnostic immunology centres, and interlaboratory discrepancies in performance for some assays have been reported. The availability of quality assurance is largely unknown. In this questionnaire-based EFNS task force, all 18 national representatives of the Neuroimmunology Panel within the EFNS were invited to estimate the service provision within their country; 12 panel members responded. From these responses, it emerged that a range of assays are being performed throughout European centres, involving over 20 separate antigens, using a broad array of immunodetection techniques. With the exception of the estimation of anti-AChR antibodies for the diagnosis of myasthenia gravis, no systematic quality assurance schemes are available, this being conducted on an ad hoc basis, or not at all. Since quality is a central component of assay sensitivity and specificity, we conclude that there is an urgent need to introduce pan-European quality assurance schemes, based on provision of positive and negative test sera from a central source, and in which all neuroimmunology laboratories should participate.

Introduction

Over the last decade, there has been a steady increase in the use of antinerve antibody assays to aid both diagnosis and research into neurological diseases thought to have an antibody-mediated autoimmune basis (Giometto *et al.*, 1999; Vincent *et al.*, 1999). 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 in Table 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 has been thoroughly validated and both non-commercial and commercial quality assurance schemes

are available for laboratories to participate in. However, the procedures in place for identification of antibodies that mark paraneoplastic syndromes and for antiganglioside antibodies are less well developed. Efforts have been made to produce standard protocols, exchange samples and run workshops in both these latter areas, for example as manifested by the INCAT (Immune Neuropathy Cause and Treatment) group (Willison *et al.*, 1999). Such studies have principally involved researchers and laboratories with a specialized interest in these fields rather than clinical laboratories performing routine screening (Zielasek *et al.*, 1994).

The antineuronal 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. The clinical utility of these investigations is considerable, and the importance of accurate identification paramount to clinical decision-making. In addition, this spectrum of

Correspondence: Dr H. J. Willison, University Department of Neurology, Southern General Hospital, Glasgow G51 4TF, UK (fax: +44 141 2012993; e-mail: h.j.willison@udcf.gla.ac.uk).

Table 1 Antigens tested and their associated diseases

Antibody specificity	Associated neurological disorders	Detection method	References
Anti-Hu (ANNA-1)	Subacute sensory neuroneopathy, limbic encephalitis, Brain stem encephalitis, paraneoplastic encephalomyelitis, Chronic pseudoobstruction	IMH/IMF, confirmed by WB on recombinant protein or neuronal extracts	Dalmau <i>et al.</i> (1992) Lucchinetti <i>et al.</i> (1998) Giometto <i>et al.</i> (1999)
Anti-Yo (PCA-1)	Paraneoplastic cerebellar degeneration	IMH/IMF, confirmed by WB, as above	Peterson <i>et al.</i> , 1992 Furieux <i>et al.</i> , 1990
Anti-Ri (ANNA-2)	Myoclonus/opsoclonus	IMH/IMF, confirmed by WB as above	Luque <i>et al.</i> (1991)
Anti-Tr	Paraneoplastic cerebellar degeneration	IMH/IMF (requires fixed tissue)	Graus <i>et al.</i> (1997)
Anti-amphiphysin	Stiff man (person) syndrome, encephalomyelitis, subacute sensory neuroneopathy	IMH/IMF (requires fixed tissue), confirmed by WB as above	Saiz <i>et al.</i> , 1999 Folli <i>et al.</i> , 1993
Anti-CV2	Cerebellar degeneration, encephalomyelitis, limbic encephalitis	IMH/IMF (requires fixed tissue), confirmed by WB, as above	Honnorat <i>et al.</i> , 1996;
Anti-VGKC	Acquired neuromyotonia	R/A	Hart <i>et al.</i> (1994)
Anti-VGCC	Lambert-Eaton myasthenic syndrome, paraneoplastic cerebellar degeneration	R/A	Motomur <i>et al.</i> (1995) Mason <i>et al.</i> (1997) Vincent (1999)
Anti-(TA) Ma2	Limbic encephalitis	IMH/IMF, confirmed by WB, as above	Voltz <i>et al.</i> (1999)
Anti-AChR	Myasthenia gravis	R/A	Vincent and Newsom Davis (1985)
Anti-GQ1b(IgG/IgM)	Miller Fisher Syndrome, Guillain-Barré syndrome with ophthalmoplegia	ELISA, TLC	Willison <i>et al.</i> , 1999
Anti-GM1, GD1b (IgM)	Multifocal motor neuropathy,	ELISA, TLC	Willison <i>et al.</i> , 1999
Anti-GM1 (IgG/IgM)	Motor forms of Guillain-Barré syndrome		
Anti-GD1a (IgG)	Acute motor axonal neuropathy	ELISA, TLC	Ho <i>et al.</i> (1999)
Anti-GD1b and other disialylated gangliosides (IgM)	Paraproteinemic neuropathies	ELISA, TLC	Willison <i>et al.</i> (1996)
Anti-MAG/SGPG (IgM)	IgM paraproteinaemic neuropathy	WB of CNS myelin, ELISA	Weiss <i>et al.</i> (1999)
Anti-GAD	Stiff person syndrome/cerebellar ataxia	IMH/IMF (requires fixed tissue), confirmed by WB, R/A	Solimena <i>et al.</i> , 1990 Saiz <i>et al.</i> (1997)

IMH/IMF: immunohistochemistry/immunofluorescence; WB: Western blot; requires fixed tissue: requires paraformaldehyde fixed tissue; R/A: 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, antidiisialosyl antibodies.

autoantibodies is the subject of important research developments. This has been recently discussed in a detailed workshop report (Vincent *et al.*, 1998).

The determination of antiganglioside and glycolipid antibodies has increasingly entered the clinical domain over recent years. Antiglycolipid 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, dot blot, and thin-layer chromatography overlay.

Both antineuronal and antiglycolipid antibody assays are being conducted in laboratories throughout Europe

without any externally or independently monitored quality assurance being widely available, to our knowledge. In order 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 antinerve antibody Task Force was established to conduct the review. Eighteen national re-

representatives were invited to participate in a questionnaire-based survey. The questionnaire requested information on the following.

- 1 The availability of tests both within the individual's institution and nationally.
- 2 An approximation of the number of tests conducted annually.
- 3 The methodology used.
- 4 The availability of quality assurance schemes.
- 5 The availability of positive and negative control sera.
- 6 The interest in setting up and participating in a pan-European quality assurance scheme.

Results

The questionnaire was distributed 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 1, as are the associated neurological disorders. 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 muscle-like cell lines.

Antibodies to glutamic acid decarboxylase (GAD), found in autoimmune stiff man syndrome, were conducted in five out of 12 neuroimmunology laboratories in responding countries and were 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 man 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.

Antibodies 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, 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 out of the 12 neuroimmunology laboratories), using a combination of immunohistochemistry and Western blot analysis. Anti-Ri (ANNA-2), anti-Tr and anti-amphiphysin antibodies were sought

less frequently. The less frequent paraneoplastic antibodies, anti-Ma, anti-Ta, anti-CV2, can also be detected by immunohistochemistry, but in many cases fixed rather than fresh frozen tissue is required. There is a need to distribute positive sera to help in the recognition of these antibodies.

Antimyelin-associated glycoprotein (MAG) antibodies were determined in laboratories in at least one centre in seven of the 12 countries, using a commercial kit which has good standardization, or using Western blot of myelin. Measurement of antiganglioside antibodies was also widely available in many centres and included a wide range of gangliosides and glycolipids (e.g. GM1, GM2, GA1, GD1b, GQ1b and sulphatides), but the details of the ELISAs used differ considerably between different laboratories.

In response to questions on quality assurance, most of the 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 available 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, of the investigations they were conducting.

Discussion and recommendations

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 represents a healthy perception of the clinical utility of such investigations amongst clinical neurologists but also highlights the need for a high degree of interlaboratory uniformity and standards of practice.

A number of co-operative interlaboratory 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 antiganglioside 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 the methodologies being used vary quite widely amongst different laboratories.

The most striking finding of this survey was the lack of any organized quality assurance scheme for the great majority of these autoantibodies, the exception being for anti-AChR antibodies. The survey indicated a very strong demand for such quality assurance schemes to be insti-

tuted. The mechanism by which such schemes should be organized is a matter for debate. Our recommendations are thus summarized as follows:

- The determination of antineuronal antibodies should be conducted using protocols agreed during the course of multicentre comparative studies, such as the INCAT study for antiglycolipid antibodies.
- Laboratories conducting immunoassays for anti-AChR antibodies should join existing quality assurance schemes.
- 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.
- A quality assurance scheme for the most commonly measured antiglycolipid antibodies (GM1 and GQ1b) and paraneoplastic antibodies (Hu and Yo) should be established as a matter of priority.
- The EFNS should consider how optimal quality control schemes in Europe are best established, both for laboratory and other measures, and should actively promote such schemes.

References

- Dalmau J, Graus F, Rosenblum MK, Posner JB (1992). Anti-Hu-associated paraneoplastic encephalomyelitis/sensory neuropathy. A clinical study of 71 patients. *Medicine* **71**:59–72.
- Folli F, Solimena M, Cofield R *et al.* (1993). Autoantibodies to a 128-kd synaptic protein in three women with the stiff-man syndrome and breast cancer. *N Engl J Med* **328**:546–551.
- Furueux HM, Rosenblum MK, Dalmau J *et al.* (1990). Selective expression of Purkinje-cell antigens in tumor tissue from patients with paraneoplastic cerebellar degeneration. *N Engl J Med* **322**:1844–1851.
- Giometto B, Taraloto B, Graus F (1999). Autoimmunity in paraneoplastic neurological syndromes. *Brain Pathol* **9**:261–273.
- Graus F, Dalmau J, Valldeoriola F *et al.* (1997). Immunological characterization of a neuronal antibody (anti-Tr) associated with paraneoplastic cerebellar degeneration and Hodgkin's disease. *J Neuroimmunol* **74**:55–61.
- Hart IK, Vincent A, Leys K *et al.* (1994). Serum autoantibodies bind to voltage-gated potassium channels in acquired neuromyotonia. *Ann Neurol* **36**:325.
- Ho TW, Willison HJ, Nachamkin I *et al.* (1999). Anti-GD1a antibody distinguishes axonal from demyelinating forms of Guillain-Barré syndromes. *Ann Neurol* **45**:168–173.
- Honorat J, Antoine JC, Derrington E, Aguera M, Belin MF (1996). Antibodies to a subpopulation of glial cells and a 66 kDa developmental protein in patients with paraneoplastic neurological syndromes. *J Neurol Neurosurg Psychiatry* **61**:270–278.
- Lucchinetti CF, Kimmel DW, Lennon VA (1998). Paraneoplastic and oncologic profile of patients seropositive for type I antineuronal nuclear autoantibodies. *Neurology* **50**:652–657.
- Luque A, Furueux HM, Ferziger R *et al.* (1991). Anti-Ri: an antibody associated with paraneoplastic opsoclonus and breast cancer. *Ann Neurol* **29**:241–251.
- Mason WP, Graus F, Lang B *et al.* (1997). Small-cell lung cancer, paraneoplastic cerebellar degeneration and the Lambert–Eaton myasthenic syndrome. *Brain* **120**:1279–1300.
- Motomura M, Johnston I, Lang B, Vincent A, Newsom-Davis J (1995). An improved diagnostic assay for Lambert–Eaton myasthenic syndrome. *J Neurol Neurosurg Psychiatry* **58**:85–87.
- Peterson K, Rosenblum MK, Kotanides H, Posner JB (1992). Paraneoplastic cerebellar degeneration. I. A clinical analysis of 55 anti-Yo antibody-positive patients. *Neurology* **42**:1931–1937.
- Saiz A, Arpa J, Sagasta A *et al.* (1997). Autoantibodies to glutamic acid decarboxylase in three patients with cerebellar ataxia, late-onset insulin-dependent diabetes mellitus, and polyendocrine autoimmunity. *Neurology* **49**:1025–1030.
- Saiz A, Dalmau J, Butler MH *et al.* (1999). Anti-amphiphysin I antibodies in patients with paraneoplastic neurologic disorders associated with small cell lung carcinoma. *J Neurol Neurosurg Psychiatry* **66**:214–217.
- Solimena M, Folli F, Aparisi R, Pozza G, De Camilli P (1990). Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. *N Engl J Med* **322**:1555–1560.
- Vincent A (1999). Antibodies to ion channels in paraneoplastic disorders. *Brain Pathol* **9**:285–291.
- Vincent A, Newsom Davis J (1985). Acetylcholine receptor antibody as a diagnostic test for myasthenia gravis: results in 153 validated cases and 1967 diagnostic assays. *J Neurol Neurosurg Psychiatry* **48**:1246–1252.
- Vincent A, Honnorat J, Antoine JC, Giometto B, Dalmau J, Lang B (1998). Autoimmunity in paraneoplastic neurological disorders. *J Neuroimmunol* **84**:105–109.
- Vincent A, Lily O, Palace J (1999). Pathogenic autoantibodies to neuronal proteins in neurological disorders. *J Neuroimmunol* **100**:169–180.
- Voltz R, Gultekin SH, Rosenfeld MR *et al.* (1999). A serological marker for paraneoplastic limbic and brain-stem encephalitis in patients with testicular cancer. *N Engl J Med* **340**:1788–1795.
- Weiss MD, Dalakas MC, Lauter CJ, Willison HJ, Quarles RH (1999). Variability in the binding of anti-MAG and anti-SGPG antibodies to target antigens in demyelinating neuropathy and IgM paraproteinaemia. *J Neuroimmunol* **95**:175–184.
- Willison HJ, O'Hanlon GM, Paterson GJ *et al.* (1996). A somatically mutated human anti-ganglioside antibody that induces experimental neuropathy in mice is encoded by the variable region heavy chain gene, V1–18. *J Clin Invest* **97**:1155–1164.
- Willison HJ, Veitch J, Swan AV *et al.* (1999). Inter-laboratory validation of an ELISA for the determination of serum anti-ganglioside antibodies. *Eur J Neurol* **6**:71–79.
- Zielasek J, Ritter G, Magi S *et al.* (1994). A comparative trial of anti-glycoconjugate antibody assays: IgM antibodies to GM1. *J Neurol* **241**:475–480.