A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis

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Summary

Background **Neuromyelitis optica is an inflammatory demyelinating disease with generally poor prognosis that selectively targets optic nerves and spinal cord. It is commonly misdiagnosed as multiple sclerosis. Neither disease has a distinguishing biomarker, but optimum treatments differ. The relation of neuromyelitis optica to optic-spinal multiple sclerosis in Asia is uncertain. We assessed the capacity of a putative marker for neuromyelitis optica (NMO-IgG) to distinguish neuromyelitis optica and related disorders from multiple sclerosis.**

Methods **Indirect immunofluorescence with a composite substrate of mouse tissues identified a distinctive NMO-IgG staining pattern, which we characterised further by dual immunostaining. We tested masked serum samples from 102 North American patients with neuromyelitis optica or with syndromes that suggest high risk of the disorder, and 12 Japanese patients with optic-spinal multiple sclerosis. Control patients had multiple sclerosis, other myelopathies, optic neuropathies, and miscellaneous disorders. We also established clinical diagnoses for 14 patients incidentally shown to have NMO-IgG among 85 000 tested for suspected paraneoplastic autoimmunity.**

Findings **NMO-IgG outlines CNS microvessels, pia, subpia, and Virchow-Robin space. It partly colocalises with laminin. Sensitivity and specificity were 73% (95% CI 60–86) and 91% (79–100) for neuromyelitis optica and 58% (30–86) and 100% (66–100) for optic-spinal multiple sclerosis. NMO-IgG was detected in half of patients with high-risk syndromes. Of 14 seropositive cases identified incidentally, 12 had neuromyelitis optica or a high-risk syndrome for the disease.**

Interpretation **NMO-IgG is a specific marker autoantibody of neuromyelitis optica and binds at or near the blood-brain barrier. It distinguishes neuromyelitis optica from multiple sclerosis. Asian optic-spinal multiple sclerosis seems to be the same as neuromyelitis optica.**

Introduction

Neuromyelitis optica (Devic's syndrome) is a severe idiopathic inflammatory demyelinating disease that selectively affects optic nerves and spinal cord, typically spares the brain, and generally follows a relapsing course.[1–4] Within 5 years, 50% of patients lose functional vision in at least one eye or are unable to walk independently. In North America, the proportion of non-white individuals is higher among patients with neuromyelitis optica than among those with classic multiple sclerosis.[3,4] In Asia, an optic-spinal form of multiple sclerosis is a common inflammatory demyelinating disease. It accounts for 15–40% of multiple sclerosis cases in Japan.[5,6] Is the Asian optic-spinal form of multiple sclerosis the same entity as neuromyelitis optica seen in western populations?

When fully developed, neuromyelitis optica can be distinguished from multiple sclerosis by a combination of clinical, neuroimaging, and spinal-fluid findings.[3] Characteristics typical of neuromyelitis optica include episodic myelitis—commonly severe and frequently accompanied by paroxysmal tonic spasms—with longitudinally extensive spinal-cord lesions spanning three or more vertebral segments, absence of clinical evidence of brain involvement, and usually lack of multiple-sclerosis-type lesions on MRI of the brain.[7] During acute attacks, the spinal fluid contains inflammatory cells but there is no evidence of intrathecal IgG synthesis (panel). However, many patients showing initial symptoms of neuromyelitis optica are diagnosed with multiple sclerosis. Neither disorder has a specific diagnostic marker, but the prognosis and optimum treatments for the two diseases differ. Immunosuppressive drugs (eg, azathioprine and corticosteroids) are regarded as the best treatment for neuromyelitis optica,[8] whereas immunomodulatory treatments (eg, interferon beta and glatiramer acetate) are presently recommended for early treatment of multiple sclerosis.[9] When severe exacerbations of myelitis do not respond to corticosteroid therapy, plasmapheresis is more beneficial for patients with neuromyelitis optica than for those with multiple sclerosis.[10]

Early diagnosis and treatment are very important to reduce the morbidity of neuromyelitis optica. Early use of appropriate immunosuppressive therapy would be justified if this disorder could be reliably distinguished from multiple sclerosis at the initial onset of optic neuritis or transverse myelitis, before progression and fulfilment of all clinical diagnostic criteria. We describe a newly identified IgG autoantibody (NMO-IgG) that localises to the blood-brain barrier and seems to be a specific serological marker for neuromyelitis optica. We have assessed the diagnostic accuracy of this autoantibody for neuromyelitis optica and other high-risk syndromes that could lead to a diagnosis of the disorder (eg, longitudinally extensive transverse myelitis or recurrent optic neuritis).

Methods

Patients

Our study included 124 clinically ascertained patients (figure 1) and 75 additional control patients (figure 2) with classic multiple sclerosis (19) and miscellaneous neurological disorders (56). The study was approved by the institutional review boards of the Mayo Clinic, Rochester, MN, USA, and Tohoku University School of Medicine, Sendai, Japan. Patients gave spoken consent for testing. All serum specimens were assayed and scored as positive or negative without knowledge of clinical diagnosis. Specimens from Japanese patients were subsequently decoded at a telephone conference between six of the investigators.

We obtained serum from three groups of patients. Group 1 included 102 consecutive North American patients, who were prospectively ascertained and were suspected to have either neuromyelitis optica or a syndrome with high risk of conversion to the disorder. At the most recent follow-up, 45 patients had met criteria for definite neuromyelitis optica. Of these, 37 (82%) had documented relapses (relapsing neuromyelitis optica), but of the remaining eight, only one had been followed up for 3 years after initial symptom onset (monophasic neuromyelitis optica). 22 patients initially suspected to have neuromyelitis optica were subsequently classified as having multiple sclerosis, because their myelitis was not longitudinally extensive, and they did not meet the criteria listed in the panel. 35 patients remained in the high-risk category (figure 1). Syndromes defined as high risk for neuromyelitis optica[11] or Japanese optic-spinal multiple sclerosis were: (1) one or more attacks of acute transverse myelitis with spinal-cord MRI showing continuous T2-weighted signal abnormality of at least three vertebral segments and no imaging evidence of brain parenchymal lesions that would satisfy criteria for a diagnosis of multiple sclerosis;[7] or (2) recurrent optic neuritis (simultaneous or sequential). We also included control serum samples from patients with miscellaneous disorders: classic multiple sclerosis (19), myasthenia gravis with thymoma (ten), paraneoplastic autoimmune vision loss with and without myelitis (16), Sjögren's syndrome (12), vasculitides with neurological complications (nine), and other disorders (nine; myelopathies due to autoimmunity to collapsin response-mediator protein 5,[12] vitamin B12 deficiency, sarcoidosis, lymphoma or glioma; idiopathic vision loss, normal-pressure hydrocephalus, and conversion reaction).

Group 2 included Japanese patients, retrospectively ascertained, who were diagnosed at Tohoku University Hospital multiple-sclerosis clinic. Of those with the optic-spinal form of multiple sclerosis, one had a single episode of longitudinally extensive myelitis and abnormal visual evoked responses, and was classified as having a high-risk syndrome (figure 1). The control patients with cerebral infarction were chosen to represent those with a CNS disorder lacking oligoclonal bands in the cerebrospinal fluid, whereas all those with classic multiple sclerosis did have oligoclonal bands. Patients in both control categories were typical for their respective diagnoses.

Group 3 included North American patients who were serologically ascertained by the incidental detection of a unique but unclassified CNS-restricted autoantibody. These patients had undergone paraneoplastic autoantibody assessment as a serological service at the Mayo Clinic Neuroimmunology Laboratory. They were among about 85 000 tested who had subacute multifocal neurological disorders that were initially suspected to be paraneoplastic. Three were seen at Mayo Clinic, and clinical information for the other 11 was obtained by communication with referring physicians. We ascertained these patients retrospectively, after we recognised, on the basis of the study of group 1, that NMO-IgG was their unclassified autoantibody.

Panel: **Criteria required for clinical diagnosis of neuromyelitis optica[3]**

Absolute (all required):
Optic neuritis
Acute myelitis
No demyelinating disease evident beyond optic nerve and spinal cord

Supportive (either one major criterion or two minor criteria required):
Major
Negative brain MRI at onset (or does not meet criteria for multiple sclerosis criteria[7])
Spinal-cord MRI signal abnormality extending three or more vertebral segments
Cerebrospinal fluid pleiocytosis >50×10^6 white blood cells per L, or >5×10^6 neutrophils per L
Minor
Bilateral optic neuritis
Severe optic neuritis with fixed visual acuity worse than 20/200 in at least one eye
Weakness in at least one limb; severe (Medical Research Council grade ⩽2), fixed, and attack-related

Procedures

We used the criteria of Poser and colleagues[13] to diagnose multiple sclerosis, but since these criteria do not specifically distinguish patients with neuromyelitis optica from those with multiple sclerosis, we diagnosed neuromyelitis optica only in those who additionally met the criteria in the panel. Three study neurologists (DMW, CFL, and BGW) assigned final diagnoses to North American patients by reviewing all case histories and laboratory data, without knowledge of the serological results. Another two neurologists (KF and IN) used the same criteria to diagnose the optic-spinal form of multiple sclerosis in the Japanese patients. We obtained demographic and clinical information, including race, sex, age at onset, neurological symptoms and signs, history of autoimmunity, and recorded laboratory and imaging data.

We tested all serum samples by the indirect immunofluorescence assay described elsewhere.[12] To facilitate recognition of IgG that binds selectively to

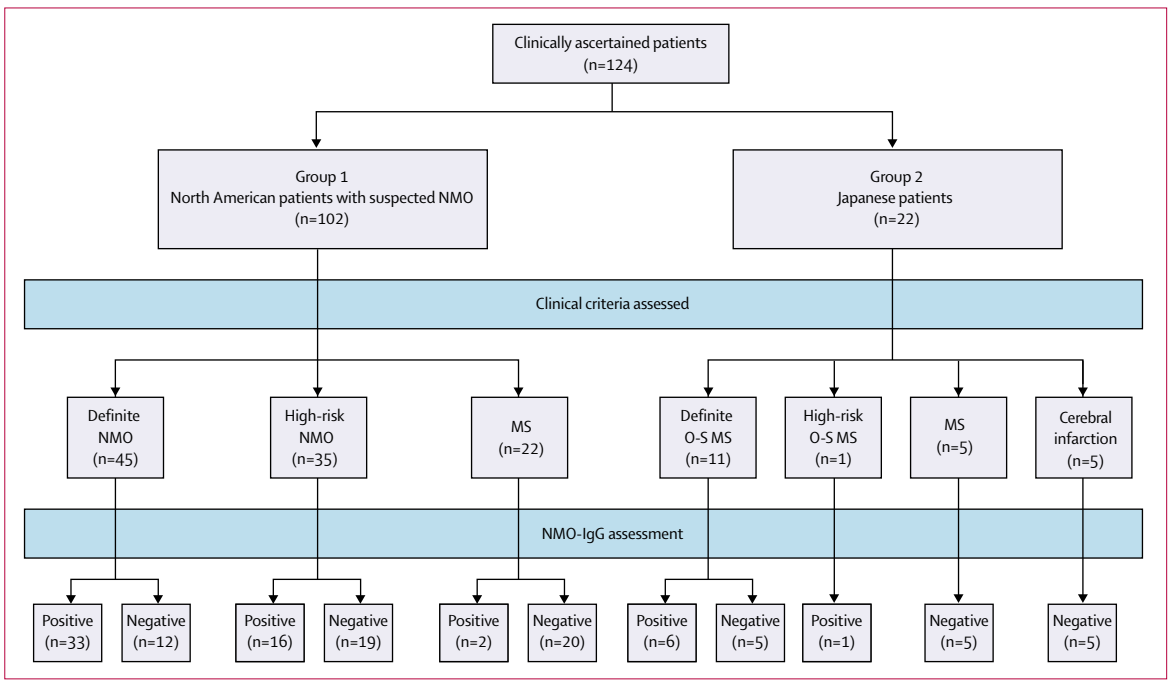

Figure 1: **Study design for recruitment of group 1 and 2 patients for NMO-IgG assessment**
Data for group 3 not shown. NMO=neuromyelitis optica. MS=multiple sclerosis. O-S MS=optic-spinal form of multiple sclerosis.

brain tissue, the procedure includes preabsorption of serum at 1 in 60 dilution with liver powder (to reduce interference from any coexisting non-organ-specific autoantibodies) and use of a composite substrate of adult mouse cerebellum, gut, and kidney. Bound IgG was detected with fluorescein-conjugated goat antibody to human IgG (Southern Biotechnology Associates, Birmingham, AL, USA). Positive serum samples were titrated in doubling dilutions, to ascertain the farthest dilution that was positive. Selected positive and negative serum samples were also tested on sections of mouse midbrain, spinal cord, and liver. To localise more precisely the regions to which the patients' IgG bound, we undertook dual immunostaining using rabbit IgG specific for factor VIII (Dako Corporation, Carpinteria, CA, USA), glial fibrillary acidic protein (Dako Corporation), or laminin (Sigma, St Louis, MO, USA), and rhodamine-conjugated antibody to rabbit IgG as detector reagent (Southern Biotechnology Associates). Serum samples from patients in groups 1 and 3 were tested as they became available, between 1998 and 2002. Those from group 2 patients were obtained between 2001 and 2002 and tested in 2003, interspersed with samples from North American control patients. Two independent assessors (VAL, SJP) classified every serum sample as positive or negative and were unaware of patients' diagnoses. No sample was classified as equivocal or indeterminate. Positive and negative scores were 100% concordant, and estimates of titration endpoints varied by no more than one doubling dilution.

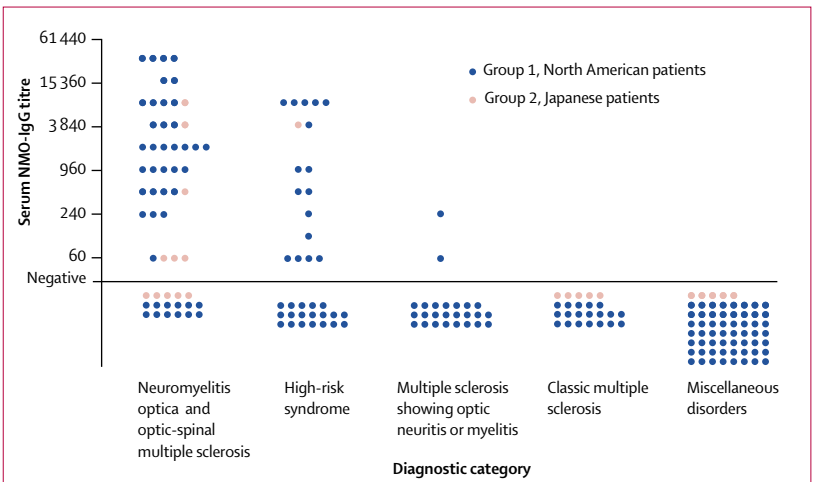

Figure 2: **Distribution of NMO-IgG titres in serum samples of patients**
Titres are expressed as the reciprocal of doubling serum dilutions. Serum samples below the horizontal line are negative. Lowest positive value is 1 in 60; highest is 1 in 30 720.

Statistical analysis

We compared the frequency of NMO-IgG detection in patients diagnosed with neuromyelitis optica or the optic-spinal form of multiple sclerosis (and in those defined as at high risk of those disorders) with the frequency in patients with classic multiple sclerosis and other neurological diagnoses. We assessed demographic and clinical features of seropositive and seronegative patients within every diagnostic category. χ^2 or Fisher exact tests and *t* test analyses (α=0·05) were used as appropriate. The sensitivity and specificity of autoantibody detection

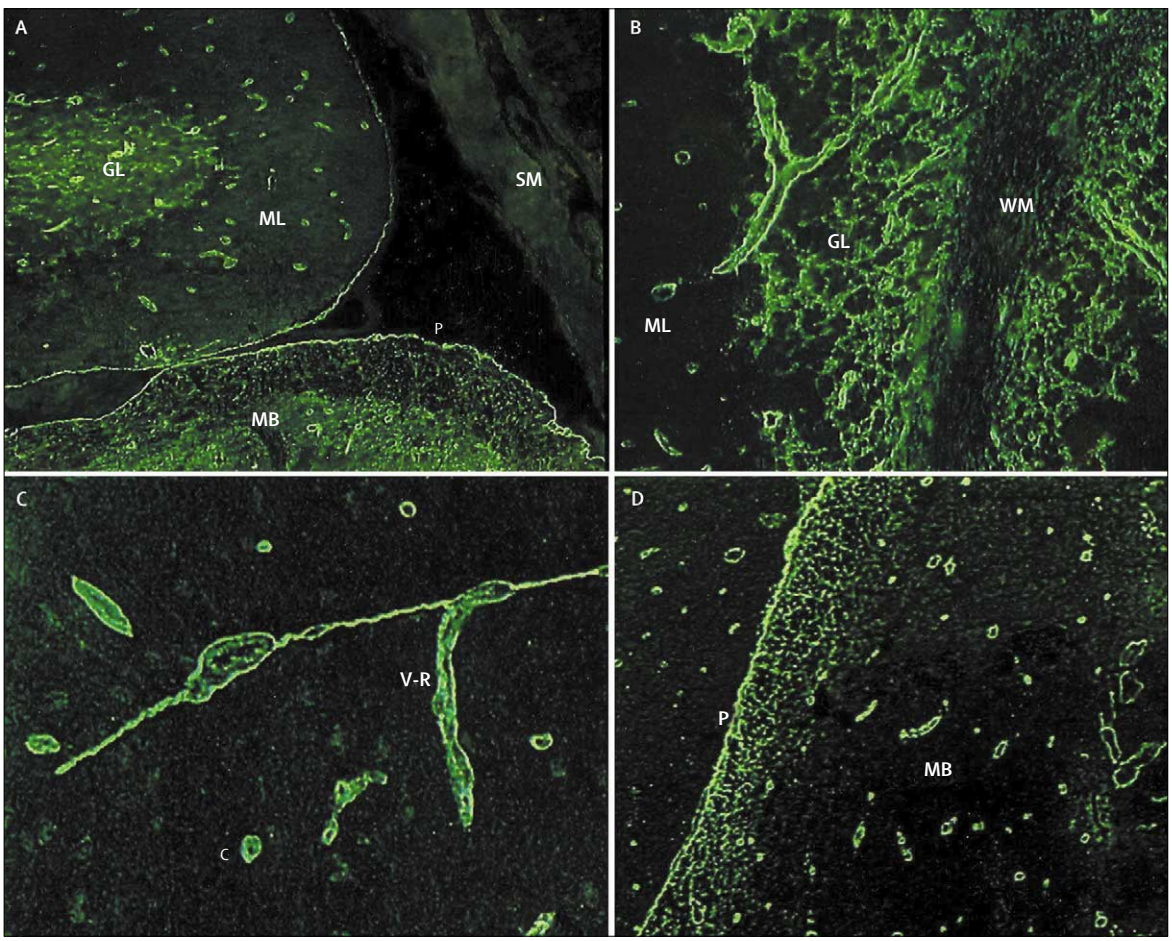

Figure 3: **Immunofluorescence pattern of bound NMO-IgG in mouse CNS**
A: Linear staining of juxtaposed pial membranes (P) of cerebellar cortex and midbrain (MB) and their microvessels. Adjacent gut smooth muscle, submucosa and vessels (SM) not stained (×200). B: Prominent microvessel staining in cerebellar molecular layer (ML), granular layer (GL), and white matter (WM) (×400). C: Linear staining in cerebellar cortex includes pia, pial lining of Virchow-Robin (V-R) spaces, and continues along microvessels, including capillaries (C). D: Staining of the subpia of midbrain (×400).

were calculated (with the clinical diagnosis as the gold standard), and used to determine likelihood ratios: for a positive autoantibody test result the likelihood ratio is the sensitivity divided by 1–specificity; that for a negative test result is 1–sensitivity divided by specificity. 95% CIs were calculated for all measures. We calculated the effect of disease duration (independent variable) on the likelihood of seropositive status for every disease group by logistic regression.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

Results

We identified a serum IgG that seems to be specific for patients with neuromyelitis optica or a high-risk syndrome. NMO-IgG yielded a characteristic immunohistochemical pattern of binding in mouse CNS tissues (figures 3 and 4). It was prominent in pia and subpia, and outlined the Virchow-Robin space and microvessels in white and grey matter of the cerebellum, midbrain, and spinal cord. It also bound to subependymal white matter and the subpial layer of midbrain in a mesh pattern. No staining was associated with subarachnoid vessels or choroid plexus. Capillary profiles were prominent in the central core of cerebellar white matter, and in the granular and molecular layers. They were not seen in gut mucosa or smooth muscle, kidney, or liver.

Dual labelling of endothelium (factor VIII) confirmed that NMO-IgG bound to the abluminal face of cerebral microvessels (figure 4). Dual labelling of astrocytic intermediate filaments (glial fibrillary acidic protein) revealed juxtaposition of the neuromyelitis-optica antigen in limited pericapillary regions (presumptive astrocytic foot processes, not shown). Extracellular matrix protein laminin showed colocalisation with the

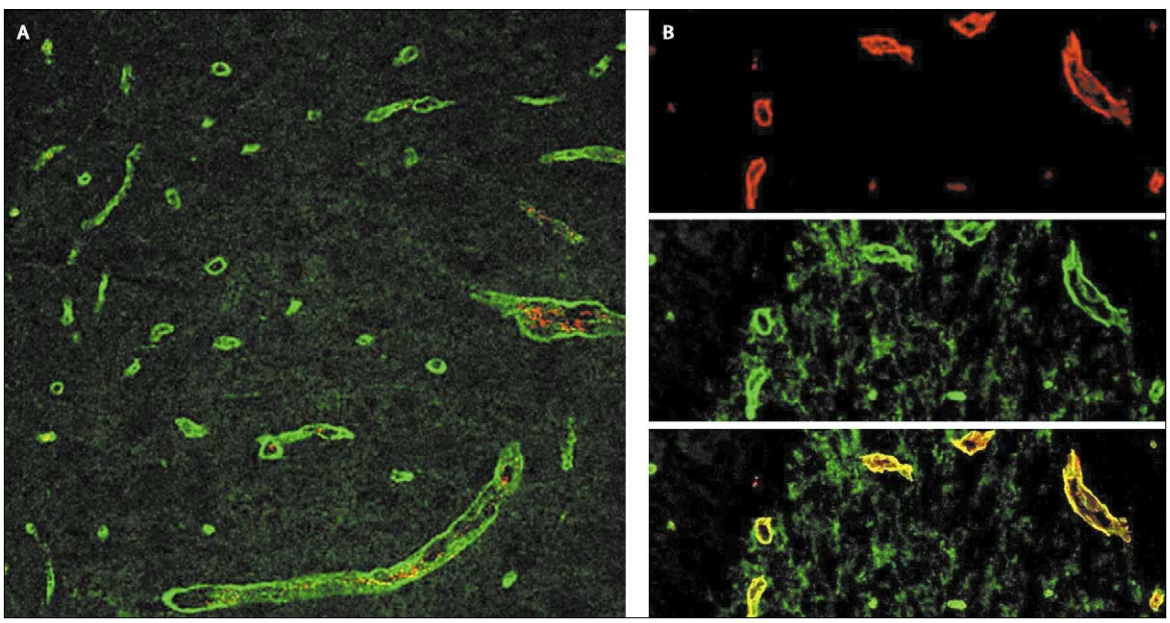

Figure 4: **Dual-immunofluorescence staining of mouse spinal cord and cerebellar cortex**
A: Endothelium of spinal-cord microvessels binding rabbit-IgG-specific for factor VIII (red). Binding of NMO-IgG (green) is external to endothelium (×400).
B: Microvessel profiles in cerebellar granular layer and white matter stained with rabbit anti-laminin IgG (top, red), NMO-IgG (middle, green), and both antibodies together (bottom, yellow) (×400).

neuromyelitis-optica antigen in pial (not shown) and perivascular locations (figure 4).

Clinical characteristics of the main comparison categories of group 1 patients are shown in the table. 33 (73%) of 45 patients diagnosed with neuromyelitis optica, and 16 (46%) of 35 classified as high risk were seropositive for NMO-IgG (figures 1 and 2). In each of these categories we recorded no significant differences between seropositive and seronegative patients with respect to sex, ethnicity, clinical status (occurrence of bilateral optic neuritis, presence of severe myelitis-associated weakness, number of attacks, or presence of coexisting autoimmune disease), brain MRI findings, or spinal-fluid abnormalities. Of 22 patients who had a final diagnosis of multiple sclerosis but presented with myelitis and optic neuritis, two (9%) were seropositive ($p<0·0001$, comparison of neuromyelitis optica with multiple sclerosis). Thus, a positive result had sensitivity of 73% (95% CI 60–86) and specificity of 91% (79–100) when used to discriminate patients with clinically defined neuromyelitis optica from those who have optic neuritis or myelitis but do not meet the strict criteria for neuromyelitis optica (likelihood ratio for a positive result 8·07 [95% CI 2·13–30·6]; likelihood ratio for a negative result 0·29 [0·18–0·48]). There were no positive results among the 19 patients diagnosed with classic multiple sclerosis or the 56 control patients with miscellaneous autoimmune and paraneoplastic neurological disorders (figure 2). The sensitivity of the assay was 61·3% (50·6–71·9) and specificity 90·9% (78·9–100) when neuromyelitis optica and high-risk symptoms were considered together. A logistic regression analysis showed no effect of disease duration on the likelihood of seropositive status ($p=0·2697$ for neuromyelitis optica; $p=0·7553$ for high-risk neuromyelitis optica syndromes; $p=0·5321$ for multiple sclerosis).

In group 2, none of the patients with a diagnosis of classic multiple sclerosis or cerebral infarction was positive (figures 1 and 2). Of the seven we identified as seropositive, six met the diagnostic criteria for neuromyelitis optica, and one had a high-risk syndrome (longitudinally extensive transverse myelitis and abnormal visual evoked responses). If we assumed that this high-risk patient actually has neuromyelitis optica, the sensitivity of the assay was 58% (95% CI 30–86) and the specificity 100% (66–100) for the Asian optic-spinal form of multiple sclerosis. As with the North American patients who had neuromyelitis optica, there were no significant differences between seropositive and seronegative Japanese patients who had the optic-spinal form of multiple sclerosis, with respect to clinical or standard laboratory indices. The sensitivity ($p=0·4783$; Fisher's exact test) and specificity ($p=1·0$) of NMO-IgG seropositivity also did not differ between North American patients with neuromyelitis optica and Japanese patients with the optic-spinal form of multiple sclerosis.

Results for the 14 NMO-IgG-positive patients in group 3 are excluded from figures 1 and 2 and the table. 12 of the 14 met clinical criteria for the diagnosis of neuromyelitis optica (three) or a high-risk syndrome (seven for longitudinally extensive myelitis and two for recurrent optic neuritis). Of those who did not meet the criteria, one had an unspecified myelopathy of recent onset, and the other had an inflammatory, steroid-

	Neuromyelitis optica (n=45)	Multiple sclerosis (n=22)	p	High-risk syndromes	
				Recurrent optic neuritis (n=8)	Recurrent transverse myelitis (n=27)
Demography					
Male/female	7 (16%)/38 (84%)	6 (27%)/16 (73%)	0·3271	1 (13%)/7 (87%)	9 (33%)/18 (67%)
Median age at onset, years (IQR)	41 (30–50)	32 (28–38)	0·0092	43 (25–51)	42 (37–53)
White (% of those recorded)	32 (76%)	18 (95%)	0·1481	7 (100%)	17 (68%)
Clinical features					
Bilateral optic neuritis	30 (67%)	9 (41%)	0·0447	7 (88%)	n/a
Bilateral simultaneous optic neuritis	16 (36%)	4 (18%)	0·1444	1 (13%)	n/a
Severe attack-related weakness	32 (71%)	3 (14%)	<0·0001	n/a	15 (56%)
Imaging and CSF					
Initial MRI brain, normal (% of those tested)	31 (77%)	9 (47%)	0·0309	7 (88%)	17 (71%)
Initial MRI brain, does not meet multiple sclerosis criteria[7] (% of those tested)	38 (84%)	14 (64%)	0·0953	7 (88%)	23 (85%)
MRI spinal-cord lesion >3 segments (% of those tested)	42 (98%)	3 (15%)	<0·0001	0	26 (100%)
CSF OB or raised IgG index (% of those tested)	4 (17%)	8 (67%)	0·0074	0	2 (13%)
NMO-IgG detected	33 (73%)	2 (9%)	<0·0001	2 (25%)	14 (52%)

CSF=cerebrospinal fluid. p values compare patients with neuromyelitis optica with those with index symptoms of optic neuritis and myelitis (subsequently diagnosed with multiple sclerosis). n/a=not applicable.

Table: **Clinical characteristics and seropositivity rates in main comparison categories of group 1 (North American) patients (n= 102)**

responsive disorder of the CNS. 12 (86%) were women. The median age of group 3 patients was 56 years (range 31–91). Nine were white, four African, and one hispanic.

Discussion

We describe an IgG autoantibody (NMO-IgG) that has high specificity for neuromyelitis optica. No biomarker has previously been described to aid diagnosis of this disorder. Our assay detected NMO-IgG in almost three-quarters of patients with this diagnosis, in nearly half of those at high risk of developing neuromyelitis optica, and in about a tenth of those showing optic neuritis or myelitis as the initial manifestation of multiple sclerosis. As yet we have not detected NMO-IgG in any patient with classic multiple sclerosis. The specificity of this antibody is strongly supported by our incidental serological identification, in fully masked conditions, of 14 NMO-IgG-positive patients (group 3), of whom 12 were subsequently shown to have a disorder related to neuromyelitis optica.

We used stringent criteria to assign a final diagnosis of neuromyelitis optica in this study. High-risk patients were assigned a final diagnosis of multiple sclerosis if, during follow-up, brainstem symptoms had developed (eg, vertigo) or minor brain MRI abnormalities met radiological criteria. This approach might explain our finding of NMO-IgG in two of 46 patients with a diagnosis of multiple sclerosis. We recognise that the clinical spectrum of neuromyelitis optica and multiple sclerosis overlap and that the clinical criteria proposed are imperfect arbiters of the diagnosis.

Any patient who shows recurrent optic neuritis or myelitis or an isolated episode of myelitis associated with a longitudinally extensive spinal cord lesion poses a difficult diagnostic and therapeutic problem. Our finding that half of this high-risk group have NMO-IgG suggests that a range of inflammatory disorders involving the spinal cord and optic nerve might share a common autoimmune pathogenesis. Whether the inflammatory optic-spinal disorder that currently accounts for 30% of multiple sclerosis cases in Asia is the same entity as neuromyelitis optica in western populations has long been questioned.[5,6] Our finding that the frequency of NMO-IgG is similar in Japanese patients with the optic-spinal form of multiple sclerosis and in North American patients with neuromyelitis optica is consistent with these disorders being the same entity.

Our dual-immunofluorescence study showed that the putative target autoantigen of neuromyelitis optica is associated with both the glia limitans of the subarachnoid and Virchow-Robin spaces and the extracellular matrix of parenchymal-penetrating microvessels in the CNS. This distribution is consistent with an antigen localised at the blood-brain barrier.[14] The vasculocentric pattern of immunoglobulin and complement deposition seen in spinal-cord tissues of patients who had died of neuromyelitis optica[15] was similar to the pattern we saw for patients' serum IgG binding to mouse spinal-cord tissue. The autopsy study showed co-localisation of IgM (more than that of IgG) with complement C9 neoantigen on the outer rim of vessels that were abnormally thickened and hyalinised in patients who were otherwise healthy and young.

These findings implicate a role for specific autoantibody and local activation of complement in the pathogenesis of neuromyelitis optica. Chemotactic byproducts of complement activation plausibly explain the frequent presence of polymorphonuclear leucocytes in spinal fluid obtained during acute attacks. The characteristic absence of high IgG concentrations or oligoclonal immunoglobulin banding in spinal fluid of patients with neuromyelitis optica,[2,3,16] and the frequent therapeutic

benefit of early plasma exchange during acute exacerbations of disease,[10] argue against intrathecal synthesis of immunoglobulin in this disorder but support a peripheral lymphoid source for the CNS tissue-bound immunoglobulin. We have not yet determined whether an NMO-IgM autoantibody might be detectable in some of the 27% of patients with definite neuromyelitis optica in whom we did not find NMO-IgG. Other explanations for this seronegativity rate might include lack of sensitivity inherent in immunofluorescence assays; potential species differences between neuromyelitis optica antigens of rodent tissue (the substrate we used in this study) and primate tissues; or the existence of more than one neuromyelitis optica antigen, shown elsewhere for autoimmune myasthenia gravis.[17] Our study does not explain why neuromyelitis optica mainly affects optic nerves and spinal cord. In addition to potentially increasing the assay's sensitivity, future use of primate tissues as substrate might show a species-restricted neuroanatomical distribution of the antigen.

Moreover, detection of the antibody enables early diagnosis of neuromyelitis optica before all clinical criteria are fulfilled, thus justifying early initiation of appropriate immunosuppressive therapy. NMO-IgG also holds promise as a quantifiable biomarker to monitor disease progression and response to treatment, and as an investigative tool for classifying disorders that are related to neuromyelitis optica and presumably share a common autoimmune pathogenesis. Proof of autoimmune pathogenesis will need induction of the characteristic vasculocentric lesions in the spinal cord and optic nerve of animals by passive transfer of NMO-IgG, or by active immunisation with the appropriate autoantigen, once it is defined.[18]

Contributors

D M Wingerchuk analysed the significance of results and participated in design of the data management base, assignment of final clinical diagnoses for North American patients, and writing of the report. T J Kryzer developed, optimised, and supervised the assay used to detect NMO-IgG, and prepared figures 2–4. S J Pittock participated in the study design, masked scoring of serum samples, database management, and writing of the report, and prepared figure 1. C F Lucchinetti provided valuable pathological insights, and participated in the study design, recruitment of North American patients, and assignment of final clinical diagnoses. K Fujihara and I Nakashima recruited Japanese patients and assigned their final clinical diagnoses. V A Lennon recognised and defined the unique tissue binding pattern of NMO-IgG, provided control serum samples from North American patients (other than those diagnosed with multiple sclerosis), and participated in masked scoring of serum samples, figure selection, and writing of the report. B G Weinshenker participated in the design of the study and the data management base, recruitment of North American patients, collection of missing clinical information, assignment of final clinical diagnoses, and writing of the report.

Conflict of interest statement

VAL and TJK are named inventors on a patent application filed by Mayo Foundation for Medical Education and Research that relates to the neuromyelitis optica antigen and its application for the detection of NMO autoantibody.

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