Neuromyelitis optica-IgG testing in an Indian cohort with neuromyelitis optica and related demyelinating disorders: Our experience

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Abstract

Background: Neuromyelitis optica (NMO) is an immune-mediated inflammatory demyelinating disorder of the central nervous system with a predilection for the optic nerves and the spinal cord. Immunopathological evidence suggests that the target antigen of the disease is aquaporin-4. An IgG antibody against this protein has been explored as a molecular marker for the disease and as a diagnostic tool due to its high sensitivity and specificity in various populations. **Objective:** To assess the value of NMO-IgG testing in Indian patients with clinical and magnetic resonance imaging features consistent with NMO and longitudinally extensive transverse myelitis (LETM). **Materials and Methods:** Forty-five patients with clinical and magnetic resonance imaging features consistent with NMO, LETM, and MS were tested for serum NMO-IgG. Of these patients, 22 patients satisfied revised (2006) Wingerchuk criteria for NMO (excluding NMO-IgG status) and 11 patients had LETM. Twelve patients satisfied the revised (2010) McDonald criteria for multiple sclerosis (MS). **Results:** Of the 21 patients, satisfying the criteria for NMO and for whom the test results were available, 17 were positive for NMO-IgG (80.9%), and of the 11 patients having LETM, 6 (54.5%) were positive for NMO-IgG. In one patient with NMO, the test result was not available. None of the 12 patients satisfying McDonald criteria for MS showed NMO-IgG seropositivity. **Conclusion:** Our study suggests that it is worthwhile to pursue NMO-IgG testing as a diagnostic tool for patients with clinical and Magnetic Resonance Imaging (MRI) features consistent with NMO and LETM in the Indian population.

Key Words

Anti-aquaporin-4 seropositivity, neuromyelitis optica, neuromyelitis optica-Immunoglobulin G seropositivity, transverse myelitis

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Introduction

Neuromyelitis optica (NMO) has several unique features which distinguish it from multiple sclerosis (MS), with respect to lesional topography, severity of exacerbations, MR imaging findings, cerebrospinal fluid (CSF) CSF abnormalities, immunopathology, therapy, and therapeutic response.[1]

The diagnosis is made by the revised Wingerchuk diagnostic criteria, which requires the presence of optic neuritis and myelitis, plus any two of the following: (a) brain MRI not satisfying the McDonald criteria; (b) MRI T2 lesions spanning three or more vertebral segments; and (c) positive serology for NMO-IgG (anti-Aquaporin-4 antibody).[2] NMO antibody is said to be 91% sensitive and 100% specific.[3]

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Although NMO is known to occur in India,[4] there is a paucity of data concerning NMO-IgG status. The objective of the study was to assess the value of NMO-IgG testing in Indian patients with clinical and magnetic resonance imaging features consistent with NMO and longitudinally extensive transverse myelitis (LETM).

Materials and Methods

During the study period from January 2010 to April 2012, 347 patients with demyelinating diseases were seen by us at the outpatient and inpatient department of a tertiary care hospital. These included 230 new cases and 117 follow-up cases. For this retrospective study, inclusion criteria were as follows:

- NMO: Patients with clinical and MRI features satisfying revised Wingerchuk criteria (2006).

- LETM: Patients having myelitis involving ≥ 3 spinal segments on MRI and brain MRI not satisfying revised McDonald criteria (2010).
- MS: Patients satisfying revised McDonald criteria (2010) for whom NMO-IgG test results were available from the records.

Forty-five patients (22 NMO, 11 LETM, and 12 MS) satisfied these criteria. Of the 22 patients with clinical and MRI features consistent with NMO, test results were available for 21 and unavailable for 1 patient. MS patients were included as internal controls. Patients came to the department mostly as referrals and therefore were seen by us at variable intervals from the onset of the disease and from the time of treatment for acute episodes.

NMO (anti-aquaporin-4) antibody testing was done by indirect immunofluorescence using the Euroimmun kit (Luebeck, Germany), a visual fluorescence-observation cell-based assay that incorporated fixed HEK293 cells transfected singly with either human AQP4-M1 or M23 isoform.[5] Testing was carried out at Metropolis Healthcare Limited, Mumbai. The study was approved by the institutional ethics committee.

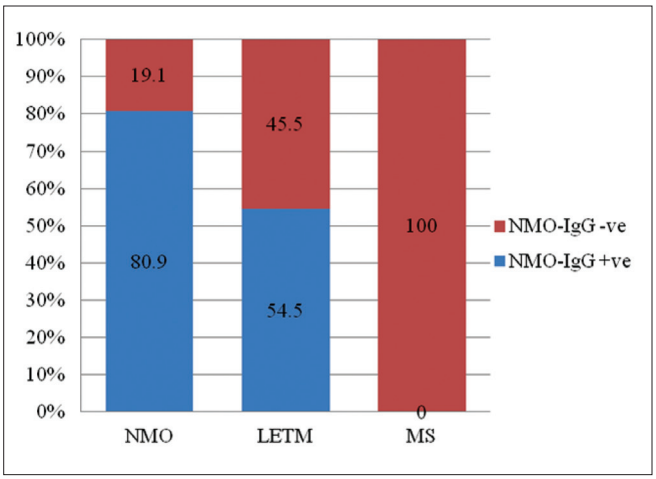
Results

Seventeen of 21 (80.9%) patients with clinical and MRI features consistent with NMO and 6 of 11 (54.5%) patients with LETM were positive for NMO-IgG [Graph 1]. None of the MS patients was positive for NMO-IgG. One female NMO patient was lost to follow-up and NMO-IgG result was not available. She had presented at the age of 24 with unilateral optic neuritis and had one subsequent relapse of optic neuritis and one of myelitis.

Clinical details of NMO and LETM patients are summarized in Tables 1 and 2, respectively.

Discussion

Historically, NMO was characterized by near-simultaneous development of bilateral optic neuritis and acute transverse myelitis with no other CNS involvement.[6] It was postulated to have a monophasic course resulting in significant neurodeficit.[7] Furthermore, whether NMO is a subtype of MS or a separate entity remained controversial till recently.[7] There



Graph 1: NMO-IgG percentage seropositivity in the three study groups (NMO = Neuromyelitis Optica, LETM = Longitudinally Extensive Transverse Myelitis, MS = Multiple Sclerosis)

Table 1: Clinical details of neuromyelitis optica group

Clinical parameters	NMO-IgG+ve NMO (*n*=17)	NMO-IgG−ve NMO (*n*=4)
Age at onset (mean±SD)	30.47±16.78	32.5±20.43
Male/female ratio	1/16	0/4
Initial presentation		
Myelitis	9	1
Optic neuritis	5	2
Both	2	1
Brainstem involvement	1*	0
Number of attacks: Mean (range)	6.09 (1-16)	2.75 (2-4)
Number of attacks of myelitis: Mean (range)	3.53 (1-12)	1.75 (1-3)
Number of attacks of optic neuritis: Mean (range)	2.18 (1-5)	0.75 (1-2)
Timing of NMO-IgG testing		
At time of attack	7	1
Later†	8	3

NMO=Neuromyelitis optica, IgG=Immunoglobulin G, *This patient had an atypical initial presentation of isolated brainstem involvement but subsequently had attacks of myelitis and optic neuritis, †The rest of the patients had the test done at variable intervals from a relapse, depending on when they were referred to us, One patient had the test immediately after steroid treatment, at which time it was negative. It was repeated 14 months later, at which time it was positive, In the NMO-IgG negative group, the interval between NMO-IgG testing and the last steroid treatment ranged from 1 to 6 months

Table 2: Clinical details of longitudinally extensive transverse myelitis group

Clinical parameters	NMO-IgG+ve LETM (*n*=6)	NMO-IgG-ve LETM (*n*=5)
Age at onset (mean±SD)	35.60±9.04	38.83±15.38
Male/female ratio	0/6	4/1
Initial presentation		
Cervicodorsal	2	3
Cervical	3	1
Dorsal	1	1
Number of attacks: Mean (range)	3.16 (1-6)	2 (1-3)
Duration of follow-up in years (mean)	4.08	1
Timing of VEP*		
At time of attack	5†	4
Later	1	1
Timing of NMO-IgG testing‡		
At time of attack	5	3
Later	1§	2‖
History of fever	1¶	None

NMO=Neuromyelitis optica, LETM=Longitudinally extensive transverse myelitis, IgG=immunoglobulin G, VEP=visual evoked potential, *All VEPs were normal, †Repeated in two patients, also at the time of an attack, ‡Of the NMO-IgG negative LETM patients, three had NMO-IgG testing within 6 months of the last steroid treatment; in two patients, it was done before any steroid treatment. §6 months after an attack. ‖Within 2 months of an attack, ¶History of fever was present in one patient in two of six attacks; there was evidence of long segment involvement (>3 vertebral segments) on all MRIs

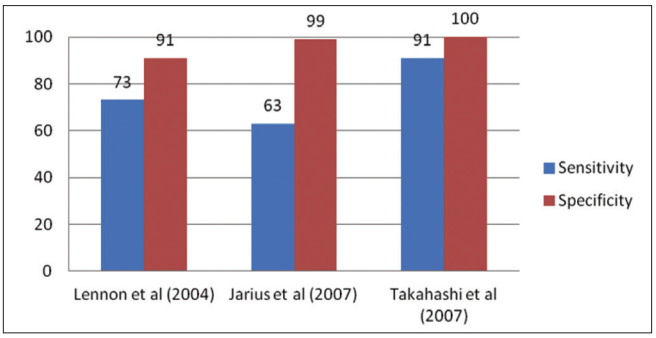
were many reports about the higher incidence of NMO in Asia, especially in Japan, than in Western populations.[6] A similarly high rate of incidence of NMO was reported in India, 7.1% of total number of MS patients, comparable to 7.6% in a series in Japan.[8] Subsequently, NMO was reported to have a relapsing course[7] and has been recognized as a separate entity from MS with distinct clinical, radiological, and pathological features.[9]

Studies to isolate a molecular marker for NMO identified an NMO-specific human IgG[1] that binds selectively to aquaporin-4, a water channel.[10] Distribution of aquaporin-4-rich sites in the CNS is highly compatible with that of NMO lesions.[11] The identification of the anti-aquaporin-4 antibody led to revision of the diagnostic criteria for NMO to include NMO-IgG (anti-aquaporin-4) seropositivity status.[2] This increased the sensitivity and specificity of the criteria.[2]

The advent of the NMO antibody refined the diagnosis of NMO and NMO spectrum disorders to include various limited syndromes with NMO-IgG seropositivity.[9] NMO-IgG testing aids the diagnosis of limited syndromes such as severe optic neuritis or isolated LETM, or when the MRI shows a long lesion less than three vertebral segments in length. This has significant therapeutic implications.[12] Relying solely on MRI appearances for diagnosis can be misleading for two reasons: The first is that MRI should preferably be performed in the acute myelitis stage, as long lesions may resolve entirely or atrophy may ensue in later stages.[13] The second is that coalescence of multiple plaques can occur in MS, which may be mistaken for a long cord lesion.[12]

NMO antibody seropositivity needs to be tested within diverse populations to assess its usefulness as a diagnostic tool. Jarius *et al*.[14] found a seropositivity of 61.11% (22 of 36) among NMO patients as defined by the 1999 Wingerchuk criteria; 35 of these 36 patients had long cord lesions. A Brazilian study[15] found NMO-IgG seropositivity in 18 of 28 (64.3%) of NMO patients as defined by 1999 Wingerchuk criteria. In a study by Takahashi *et al*.,[3]20 of 22 (90.9%) NMO patients, defined by the 2006 Wingerchuk criteria, were positive for NMO-IgG. Our study found that 80.9% of patients with NMO and 54.5% patients with LETM were positive for NMO-IgG. Our result contrasts with the findings in a study by Pandit[16] where only 1 out of 8 patients of NMO (12.5%) was positive. Our study suggests that it is worthwhile to consider the diagnostic usefulness of NMO-IgG testing even in Indian patients, given that NMO-IgG testing has been shown to be highly sensitive and specific in several studies[1,3,17] [Graph 2].

Takahashi *et al*.[3] found a positive correlation between antibody titers and lesion length at the nadir of exacerbation. They also found a decrease in antibody titer during relapse-free periods under immunosuppressive therapy, as well as after high-dose intravenous methylprednisolone. This has not been systematically examined in this study. However, one patient was seronegative when tested post-treatment with methylprednisolone and plasmapheresis, but tested positive 14 months later. Therefore, there is evidence of fluctuation in antibody titer depending on the state of the disease. Hence, the timing of sample collection for NMO testing would be crucial if it is to be accepted as a diagnostic marker.



Graph 2: Comparison of sensitivity and specificity of NMO-IgG test in various studies (NMO = Neuromyelitis Optica, LETM = Longitudinally Extensive Transverse Myelitis, MS = Multiple Sclerosis)

Another factor in the use of NMO-IgG status as a diagnostic tool is the sensitivity and specificity of the assay method used. A multicenter study conducted by Waters *et al*.[5] compared commercially available transfected cell-based assays (CBA), a commercially available ELISA-based assay, a fluorescence immunoprecipitation assay, a tissue-based immunofluorescence assay, an in-house quantitative flow cytometry assay, and a combination of commercially available cell-based and ELISA assays. The most sensitive assays were those detecting IgG binding to cells expressing recombinant AQP4 with quantitative flow cytometry (77%; 46 of 60) or visual observation (CBA, 73%; 44 of 60).[5] The present study employed the commercially available CBA.

This retrospective study has the limitation that most patients came to us in the form of referrals. Therefore, NMO-IgG status at the time of the first relapse could not be determined. In addition, our study included only NMO patients with clinical and MRI features satisfying revised Wingerchuk criteria (2006). This brings in a selection bias which influences NMO-IgG seropositivity in our study group. Previous studies have indicated the influence of selection bias in reported NMO-IgG seropositivity.[18] The higher proportion of patients with relapsing forms of the disease[19] and the female preponderance[19] in our data set may also have contributed to the high seropositivity. Further investigation with a larger sample size should help to clarify the Indian scenario with regard to NMO-IgG seropostivity.

Conclusion

Our study suggests that it is worthwhile to pursue NMO-IgG testing as a diagnostic tool for patients presenting with clinical and MRI features consistent with NMO and LETM in the Indian population.

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