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False-positive acetylcholine receptor antibody results in patients without myasthenia gravis

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ABSTRACT

Acetylcholine receptor antibodies are very specific for myasthenia. During a large prospective cohort study of myasthenia, we encountered five patients, positive for acetylcholine receptor (AChR) antibodies by radioimmunoprecipitation assay (RIA), whose clinical course revealed diagnoses other than myasthenia. Two patients had transiently raised AChR antibodies associated with Guillain-Barré syndrome. Antibodies to clustered AChRs, in a live cell-based assay, were negative in all five patients, suggesting that results from the RIAs were false-positives. It is possible that the AChR antibodies detected by RIA in these cases were non-pathogenic, and directed to intracellular epitopes of the AChR.

1. Introduction

Myasthenia gravis (MG), in most patients, is caused by high affinity autoantibodies specific for the human nicotinic acetylcholine receptor (AChR), which result in impaired neuromuscular transmission and fatiguable muscle weakness. Typically, about 85% of patients with generalised symptoms of MG and 50% with pure ocular MG harbour specific AChR antibodies (Vincent et al., 2001). Up to two-thirds of patients who are seronegative for AChR antibodies in radioimmunoprecipitation assays have detectable antibodies to clustered AChRs in live cell-based assays (Leite et al., 2008; Jacob et al., 2012; Rodríguez Cruz et al., 2015), and these antibodies are highly specific for MG (Leite et al., 2008; Rodríguez Cruz et al., 2015).

During a prospective cohort study of MG patients, we encountered five patients whose clinical course suggested a diagnosis other than MG, despite the fact that they had antibodies to AChRs detected in a radioimmunoprecipitation assay (RIA). Subsequent results from live cell-based assays indicated likely false-positive results from the initial AChR RIAs.

2. Methods

Between March 2014 and May 2017 all patients with a possible, or definite, new diagnosis of MG, residing in the counties of Nottinghamshire, Derbyshire or Lincolnshire, UK, were seen at the specialist regional MG clinic at Queen's Medical Centre, Nottingham. Additional patients were recruited from specialist MG clinics in Birmingham and Oxford, UK.

The diagnosis of myasthenia was based on typical clinical features of fluctuating muscle weakness in the absence of other alternative diagnoses on follow-up, and presence of antibodies in either radioimmunoprecipitation (AChR or MuSK) or cell-based assays (for clustered AChR, MuSK or LRP4)(Leite et al., 2008; Rodríguez Cruz et al., 2015). Seronegative patients were included if symptoms and signs typical of MG responded to treatment with acetylcholinesterase inhibitors or immunosuppression, with or without findings of impairment of neuromuscular transmission on SFEMG (AAEM, 2001; Benatar, 2006). All patients were recruited at, or within several weeks of, diagnosis and examined initially and during follow-up by one or more of four of the authors (GS, PAA, SJ, PM). All patients provided signed, informed consent (ethical approval by NRES Committee West Midlands – South Birmingham (12/WM/0414). Serum samples were taken at recruitment and stored at −80 °C until analysis.

3. Results

A total of 149 patients were diagnosed with MG. Serum samples from 134/149 (90%) patients were obtained prior to commencement of

Abbreviations: AChR, acetylcholine receptor; CBA, cell-based assay; MG, myasthenia gravis; MuSK, muscle-specific kinase; RIA, radioimmunoprecipitation assay; SFEMG, single fibre electromyography

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Table 1
Prospective myasthenia patient cohort.

	Positive AChR antibodies (RIA)	Negative AChR antibodies (RIA)
Number	123/149 (82.6%)	26/149 (17.4%)
Males	74/123 (60.2%)	10/26 (39%)
Late-onset MG (> 50 years)	98/123 (79.7%)	12/26 (46%)
Positive AChR antibodies (RIA)		
Pure ocular symptoms[a]	61/123 (49.6%)	17/26 (65.4%)
Positive antibodies to clustered AChRs	121/123 (98.4%)	10/26 (39%)

[a] Patients' symptoms remained purely ocular for 3 months, or longer, from onset.

immunosuppression: of the remaining 15 patients, almost all (13/15) had only received immunosuppression briefly, for less than 2 weeks, prior to blood sampling. In total, 123/149 (82.6%) patient samples were positive for AChR antibodies by RIA. Only 2/123 (1.6%) samples positive for AChR antibodies by RIA were subsequently negative for clustered AChR antibodies in a live cell-based assay (Table 1), both for the adult and fetal form of the AChR. Both of these patients had ocular symptoms of MG which improved on pyridostigmine, abnormal jitter with > 20% blocking on SFEMG of orbicularis oculi muscles, and low levels of AChR antibodies (RIA $< 10 \times 10^{-10}$ M) on repeated testing of pre-treatment diagnostic serum samples; one had thymic follicular hyperplasia at thymectomy). Eight patients with a new diagnosis of Lambert-Eaton myasthenic syndrome (LEMS), evaluated in the same clinic over the study period, were negative for AChR antibodies by both RIA and live cell-based assay (to clustered AChRs).

During the study time period, five additional patients were referred to us by other neurologists with a possible diagnosis of myasthenia, found to have positive AChR antibodies by RIA, but whose clinical course, and additional investigations, suggested an alternative diagnosis (Table 2). Two of the five patients had transiently raised AChR antibodies temporally associated with Guillain-Barré syndrome. Antibodies to clustered AChRs, in a live cell-based assay, were negative in all five patients, from original serum samples initially positive for AChR antibodies by RIA. Samples also tested negative for MuSK (by RIA and cell-based assay) and LRP4. Case histories of these five patients are detailed in supplementary data.

4. Discussion

During the course of a prospective study of patients with MG, we encountered five patients in whom the atypical clinical course and absence of antibodies to clustered AChRs suggested a diagnosis other than MG, despite the presence of AChR antibodies by RIA.

It is of particular interest that two of our cases with false-positive, or non-pathogenic, AChR antibodies had GBS with anti-ganglioside antibodies: presentation with asymmetrical ptosis in both patients led to investigations for myasthenia. Both our GBS patients had an infective prodrome, found to be *H. influenza* in one case. It is possible that, through molecular mimicry, a cross-reacting antibody response was generated not only to peripheral nerve myelin, but also to the AChR. Interestingly, AChR antibodies in our two GBS patients became undetectable after the acute illness, suggesting a monophasic immune trigger. Ptosis without ophthalmoplegia has been described rarely as a presenting feature in GBS (Teng and Sung, 2012). Although one of our patients subsequently described double vision, it is possible that he had developed mild extraocular muscle weakness, undetectable on bedside testing, given that he had GQ1b ganglioside antibodies, notably present in GBS patients with ophthalmoplegia (Odaka et al., 2001). There are a few case reports of patients having developed MG and GBS (Kraus et al., 2007; Carlander et al., 1991), but rarely simultaneously (Kizilay et al., 2008): although clustered AChR cell-based assays were not performed,

Table 2
Clinical features of patients with false-positive AChR antibodies.

Patient sex, age	Presenting symptoms	Initial AChR antibody titre ($\times 10^{-10}$M) (RIA)	Neurophysiology results	CT thorax imaging	Other antibodies	Follow-up AChR antibody titre ($\times 10^{-10}$M)(RIA)	Final diagnosis
1. M, 62	Dysphagia, bilateral non-fatiguable ptosis, proximal arm weakness	10	Normal SFEMG and RNS	Normal	None	12	OPMD
2. F, 19	Fatigue, fluctuating leg weakness and ptosis	6	Normal SFEMG and RNS	Not done	None	5	Functional neurological symptoms
3. M, 34	Fatigue, limb heaviness	9	Normal SFEMG and RNS	Normal	None	16	Spondyloarthropathy, Ankylosing spondylitis, OSA
4. M, 69	Asymmetrical ptosis, dysphagia, upper limb weakness	9	Absent upper limb SAPs. Normal RNS	Normal	GQ1b, GD1b	Negative ($< 5 \times 10^{-10}$ M)	GBS
5. F, 25	Asymmetrical ptosis, dysphagia, upper and lower limb weakness	13	Normal nerve conduction	Normal	GM1, GD1b	Negative ($< 5 \times 10^{-10}$ M)	GBS

AChR = acetylcholine receptor; GBS = Guillain-Barré syndrome; OPMD = oculopharyngeal muscular dystrophy; OSA = obstructive sleep apnoea; RIA = radioimmunoprecipitation assay; RNS = repetitive nerve stimulation; SAP = sensory action potential; SFEMG = single-fibre electromyography.

it is reasonable to assume that the AChR antibodies found by RIA in these reported cases were pathogenic given that there was neurophysiological evidence of abnormal neuromuscular transmission, unlike in one of our GBS patients who had normal RNS. Nevertheless, it has been reported that IgG from GBS patients, negative for AChR antibodies by RIA, is capable of blocking post-synaptic nicotinic AChRs (Krampfl et al., 2003) and binds to the post-synaptic membrane (Wessig et al., 2001) although the exact antigenic IgG binding sites remain unknown. Ultimately, the tempo of recovery and sustained improvement in the absence of ongoing treatment in our two patients with GBS would be atypical for myasthenia, presenting in crisis.

False positive AChR antibodies by RIA are extremely rare (< 1%), whether this be due to technical assay effects (Apiwattanakul et al., 2010) or the finding of AChR antibodies in conditions other than MG (Lindstrom et al., 1976; Somnier, 1993). Understandably, most cases reported are in conditions where presentation may mimic MG, such as LEMS (Tim et al., 1998; Lennon, 1997; Oh, 2016) or motor neuron disease (Abbott et al., 1986; Okuyama et al., 1997). Where MG is said to have occurred concurrently with other neuromuscular disorders, the pathogenicity of the AChR antibodies has been determined by the clinical course of the patient (including response to specific MG treatments), thought to be compatible with MG, and neurophysiology findings of post-synaptic impaired neuromuscular transmission (Tai et al., 2017; Oh, 2016).

Some patients with MG harbour antibodies that are mainly specific for the fetal form of the AChR (Riemersma et al., 1996). These antibodies may occur in asymptomatic female patients with a history of pregnancy loss due to arthrogryposis, where AChR antibodies that are positive by RIA bind more avidly to fetal, than adult, AChRs in cell-based assays (Vincent et al., 2012). Although we did not test for fetal-specific AChR antibodies by cell-based assay in our five false-positive patients, it is known that serum from patients which bind to fetal AChRs also bind to clustered AChRs in live cell-based assays (Leite et al., 2008; Jacob et al., 2012). Thus, it would be unlikely for any of our patients to have antibodies entirely specific for the fetal form of the AChR and therefore be negative for antibodies to adult clustered AChRs.

We predict that the AChR antibodies found in our cases were non-pathogenic for several reasons: firstly, the initial presentation and subsequent clinical course, in the absence of requiring specific treatments for MG, was most compatible with another diagnosis in each case; secondly, there was no neurophysiological evidence of a disorder of neuromuscular transmission in four of five cases examined; and thirdly, the absence of antibodies to clustered AChRs in a live cell-based assay, found in > 98% of our contemporaneously enrolled MG patients, may suggest that the AChR antibodies detected by RIA are non-pathogenic, and directed to intracellular epitopes of the AChR in these five patients. We know from work performed in our laboratories that many patients positive for voltage-gated potassium channel (VGKC) complex antibodies by RIA, but negative for LGI-1 and CASPR2 antibodies in a cell-based assay, have non-pathogenic antibodies that bind to intracellular epitopes of the VGKC (Lang et al., 2017).

We have shown that, almost without exception, a patient with typical clinical features of MG, and positive AChR antibodies by RIA, will also have detectable antibodies to clustered AChRs. Where the clinical course may be atypical, the absence of antibodies to clustered AChRs suggests that the presentation is not due to MG. Future studies should determine whether or not there is binding to intracellular epitopes of the AChR by these probable non-pathogenic antibodies.

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Conflicts of interest

The University of Oxford and AV hold a patent for MuSK antibodies, licenced to Athena Diagnostics; AV receives a proportion of royalties.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2019.04.001.

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