Clinical and serological characteristics of patients with double seronegative Myasthenia Gravis

V. Damato, MD[1,2], A. Evoli, MD[2], T. Smith, MSc[1], M.R. Woodhall, PhD[1], P.E. Alboini, MD[2], R. Iorio, MD, PhD[2] ,S.R. Irani, MD, DPhil[1], and A. Vincent, FRCPath[1]

[1] Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom

[2] Institute of Neurology, Catholic University, Rome, Italy

Introduction

Patients without AChR and MuSK antibodies by radioimmunoprecipitation assay (RIPA) are referred to as having seronegative myasthenia gravis (SNMG). Cell-based assays (CBAs) for detection of clustered AChR, MuSK,LRP4 and agrin antibodies in SNMG patients have been established, but their frequencies in well studied clinical cohorts are not clear. Aim of this study is to report the clinical and immunological features of a well, characterized Italian SNMG cohort of patients.

Methods

Study subjects

The diagnosis of MG was based on typical clinical features, evidence of neuromuscular junction impairment on electroneuromyography, response to cholinesterase inhibitors. The MGFA postintervention status (MGFA-PIS) classification was used to assess the clinical state after institution of the recorded treatment.

Immunofluorescence CBA

The sera (dilution 1:20) were retrospectively tested by CBAs on HEK 293T cells trasfected with:

•cDNAs expressing human AChR α, β, δ, and ε/γ subunits and rapsyn-enhanced green fluorescent protein (EGFP) in a ratio 2:1:1:1:1. for the clustered AChR assay;

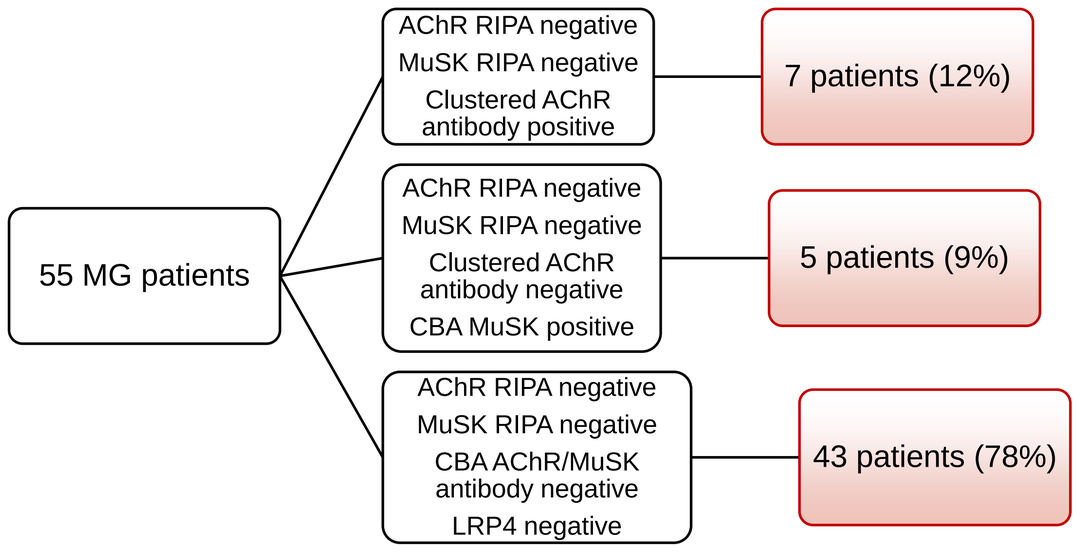
•full- length MuSK–EGFP DNA For MuSK transfections;

•cDNAs expressing human LRP4 and a chaperone protein to enhance cell surface expressio for the LRP4 assay,

•cDNA expressing agrin and EGFP in a ratio 3:0.1

Results

Fifity-five MG patients were enrolled in the study. Thirty-seven patients were females, the median age of onset was 37 years old (range: 6-86) and the median follow-up 11.5 years (range: 0.5-31years). Twenty-nine/55 (53%) patients were untreated at sampling time, with 15/55 (29%) patients at disease onset. Generalised myasthenia gravis was present in the majoirity of patients (51/55, 93%)



- **Patients with clustered AChR-antibodies** (five men/two women), aged 18-64 years at onset, had an early onset, ocular symptoms at onset and a mild disease course in 67% of cases. Serum of one generalised patient bound both to AChR adult and foetal form, while one patient was positive just for the AChR foetal form. Repetitive nerve stimulation was positive in all patients. Two patients had thymic hyperplasia. Immunosuppressants were required in four patients. The outcome was good, but none achieved complete stable remission or pharmacological remission.
- **Patients with CBA MuSK antibodies** were all females except for one case. Bulbar symptoms and a less severe course than RIPA-positive MuSK were present in all the patients. They all responded well to prednisone, with remission in 20% of cases.
- No patient was positive for LRP4 and agrin antibodies

Conclusion

Patients with antibodies to clustered AChR and MuSK account for a significant proportion of SNMG patients and resemble patients with antibodies detected by RIPA, but appear to have a milder course. The significant proportion of SNMG patients might reflects that the diagnostic and clinical use of CBAs will depend on applying these test to sera at onset and before immunotherapies.

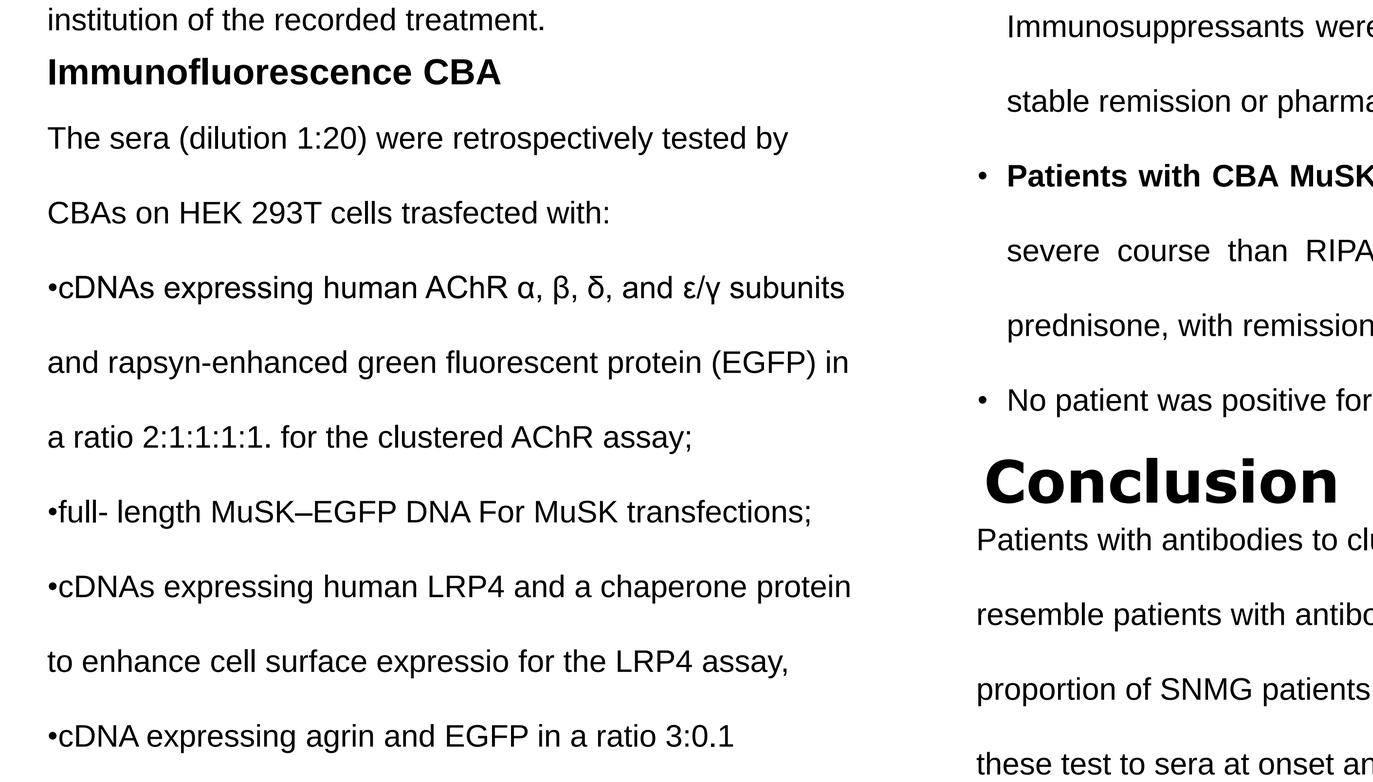
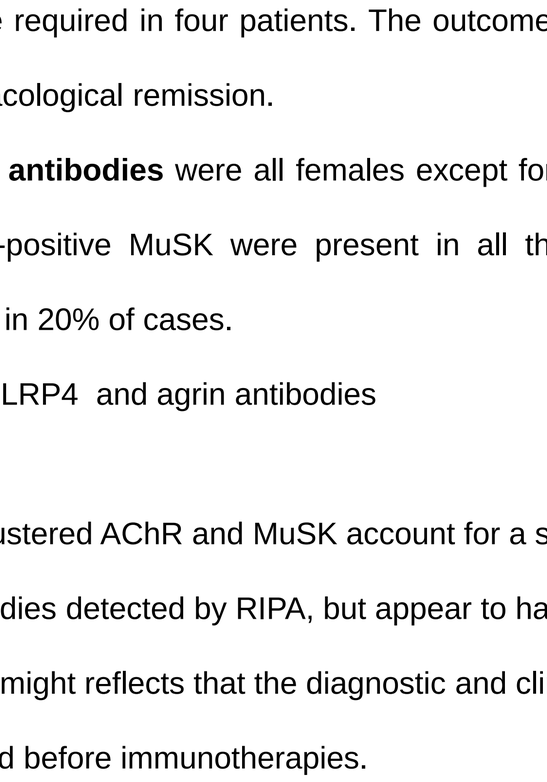


Figure 1

AChRs clustered by rapsyn-EGFP on HEK cells surface (green) and detected by anti human IgG (red) (A.) . Detection of MuSK- antibodies binding to MuSK-EGFP transfected HEK cells in a RIPA neg. patient (C). Negative binding of serum from a SNMG patient (B-D)



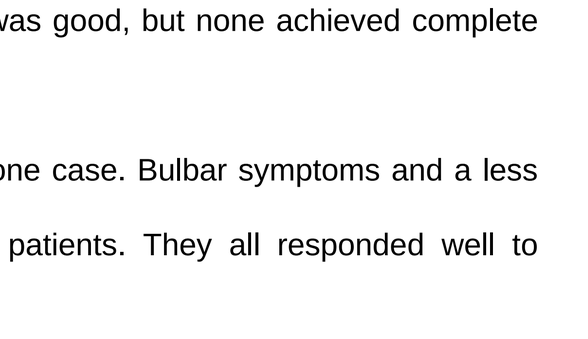


Figure 2

Clinical characteristics (A) and outcome (B) of the different populations included. Distribution of the patients as a function of the score they obtained in the CBA (C).