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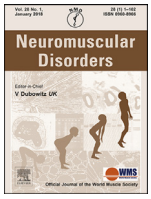

275th ENMC international workshop: Seronegative myasthenia gravis: An update paradigm for diagnosis and management, 9–11 February 2024, Hoofddorp, the Netherlands

Amelia Evoli[a], Jacqueline Palace[b], Gregorio Spagni[c], Marta Cheli[d], Annabel Ruiter[e], Jan Verschuuren[e], Lorenzo Maggi[d,*], the 275th ENMC study group[1]

[a] Neuroscience Department, Università Cattolica, Roma, Italy
[b] Department Clinical Neurology, Nuffiled Department of Clinical Neurology, John Radcliffe Hospital, Oxford OX3 9DU, UK
[c] Department of Neurosciences, Drugs and Child Health, University of Florence, Italy
[d] Neuroimmunology and Neuromuscular Diseases Unit, Fondazione IRCCS Instituto Neurologico Carlo Besta, Milano, Italy
[e] Department of Neurology, Leiden University Medical Center, Albinusdreef 2, Leiden 2333 ZA, the Netherlands

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ABSTRACT

The 275th ENMC workshop on the diagnosis and management of seronegative myasthenia gravis (SNMG) was held on February 9–11, 2024. The participants included experts in the field of adult and pediatric MG together with patient representatives. This workshop aimed to redefine SNMG in view of recent diagnostic and therapeutic updates and to identify patient unmet needs. The workshop has highlighted considerable challenges in the SNMG diagnostic work-up. To date, SNMG confirmation is often controversial, given the absence of specific diagnostic tests; no recommendations from international panels of experts are available in literature; myopathies, congenital myasthenic syndromes and functional disorders are the commonest misdiagnoses. Improving the disease diagnosis is crucial to avoid long delays in receiving appropriate treatment. To this purpose, a comprehensive diagnostic algorithm achieved consensus. Moreover, a remarkable variability in SNMG response to therapy and long-term prognosis has also been highlighted.

1. Introduction and background

Myasthenia gravis (MG) is an antibody (Ab)-mediated disorder of the neuromuscular junction (NMJ) characterized by fatigable muscle weakness. Early diagnosis is crucial as adequate therapy can restore muscle strength and significantly improve patients' quality of life. Patient sub-grouping, based on associated Abs, is a prerequisite for personalized treatment. Abs against the acetylcholine receptor (AChR, 80–85 %) and muscle-specific tyrosine kinase (MuSK, 0–60 %) are detected in around 90 % of patients [1]. Detection of either Ab by the standard radio-immunoprecipitation assay (RIPA) confirms MG in patients with congruous clinical signs. The pathogenic mechanisms of the Abs at the NMJ are well-defined [2].

Serum IgG Abs against the low-density lipoprotein receptor protein 4 (LRP4) are variably found in, 0–18 % of AChR/MuSK negative patients, representing around 1 % of the total MG population [3–5]; Abs to agrin are not routinely tested, and their frequency is unclear. The pathogenicities and specificies of agrin and LRP4 Abs are not yet established [4,6]. Lastly, in 10–15 % of MG patients no Ab can be detected (seronegative MG, SNMG). In these patients, MG confirmation can be challenging, and misdiagnosis is not rare.

Both the performance and availability of Ab assays are crucial to the MG serological diagnosis. While RIPA is still the gold-standard, AChR- and MuSK-Abs can also be tested by enzyme-linked immunosorbent assay (ELISA) but, especially for AChR-Abs, ELISA had lower sensitivity and specificity [7]. In-house cell-based assays (CBAs) proved specific and more sensitive than standard RIPA, detecting antibodies against AChR [8,9], and MuSK [9,10] in a proportion of RIPA-negative cases. Commercial CBAs based on fixed cells (F-CBA) have become available and show high sensitivity and specificity [11,12] and may be suitable for first-line testing.

In patients without detectable Abs, electrodiagnostic studies (repetitive nerve stimulation – RNS; single-fiber electromyography - SFEMG) and responsiveness to cholinesterase inhibitors (ChE-I) are generally used to confirm MG. However, both can yield "false positive" results in other disorders [13–15]. In addition,

* Corresponding author.
E-mail address: lorenzo.maggi@istituto-besta.it (L. Maggi).
[1] Listed at the end of this report.

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demonstration of post-synaptic neuromuscular transmission (NMT) defect and symptom improvement on ChE-I administration do not distinguish MG from most congenital myasthenic syndromes (CMS). Such misdiagnosis has been repeatedly reported particularly for CMS that manifest in adult life [16]. There is an unmet need to improve and evaluate diagnostic protocols for better identification and management of SNMG.

The 275th ENMC international workshop was held in February 9–11, 2024, in Hoofddorp, the Netherland with clinicians and researchers from Denmark, France, Germany, Italy, Norway, South Africa, Spain, Sweden, Switzerland, the Netherlands, UK and US, as well as patients and representatives (Italian MG Association, Dutch MG Association), and two invited participants from Argenx and UCB. A survey on Ab diagnostics in MG had been sent earlier to the parrticipants for discussion during the meeting.

The workshop was introduced by Patricia van Dongen, Programme Manager ENMC, who introduced the ENMC commitments and objectives. The organiser's main aims were: 1) to redefine SNMG in the view of the new Ab assays and provide a comprehensive diagnostic algorithm; 2) to gather information on the disease clinical pattern; 3) to learn about patients' unmet treatment needs; 4) to identify "open questions" that require collaborative studies.

2. SNMG definition, clinical aspects and current diagnostic guidelines

Amelia Evoli provided an overview on SNMG current definition and diagnostic challenges. From the current literature, SNMG generally refers to double-seronegative (dSN)-MG, i.e. MG with neither AChR nor MuSK Abs tested by RIPA or by ELISA [17,18]. In some reports, dSN-MG was confirmed by either commercial or in house CBAs [8,10,11,19]. In addition, MG could be termed "triple seronegative" (tSN) when AChR, MuSK and LRP4 Abs were all undetectable [4,5,9,20,21]. Collectively, there was considerable heterogeneity in the SNMG diagnostic work-up in these cohorts. MG diagnosis was confirmed by electrophysiologic studies and/or by clinical improvement on administration of ChE-Is, but the results were not detailed in all studies. It was not always clear whether SF-EMG had been performed as well as EMG. In some studies, the SNMG diagnosis had to be revised; mainly based on atypical clinical presentation and equivocal results of electrophysiological tests [18,22]. The most common SNMG mimickers were myopathies and functional disorders [4,18,22].

Renato Mantegazza discussed the demographic and clinical data of 105 tSN patients from a large cohort of 677 subjects with MG from an Italian reference center. When compared with the AChR-positive population, tSN patients did not differ as regard to sex ratio but had shorter disease duration and a shorter time to diagnosis ($p < 0.05$ in both variables). When the two groups were stratified according to age of onset, tSN-MG patients were more commonly affected by early-onset disease ($p < 0.040$). There was no difference in MGFA grade at presentation, while at maximum disease severity there was a higher rate of generalized MG among tSN cases ($p < 0.001$). Overall, the outcome was similar in the two groups with remarkable overlap in treatment results [23]. Some of these data, such as the higher prevalence of female patients with early-onset disease were confirmed by other studies [8,9,19,24], but other studies of dSN/tSNMG patients reported predominance of ocular or mild generalized MG [8,9]. Moreover, recent reports found high rates of unsatisfactory responses and refractory disease among dSN/tSNMG patients [17–19].

Jan Verschuuren reviewed the available diagnostic recommendations for SNMG. As above, the diagnosis of autoimmune MG s based on typical clinical signs and symptoms supported by either electrodiagnostic tests, or the presence of serum-Ab. Publicly available guidelines from different countries were screened for definition of SNMG and the preferred mode of serological or clinical testing to confirm the diagnosis. These included online available guidelines from Czech Republic, Germany, the Netherlands, Scotland, Unites States and published guidelines from the Japan [25], United Kingdom [26], United States [27,28].

Almost all guidelines define seronegative on absence of antibodies to AChR and MuSK Abs, not mentioning other antigens. Also the guidelines do not provide any advice regarding the assays that should be used to test for autoantibodies although a few mention cell-based assays. Guidelines do not provide advice on additional tests that are helpful to make a "definite" diagnosis of SNMG.

Online available guidelines on MG

- **Czech Republic:** https://www.myastheniagravis.cz./images/guidelines-euromyasthenia.pdf
- **Germany:** https://dgn.org/leitlinie/diagnostik-und-therapie-der-myasthenia-gravis-und-des-lambert-eaton-syndroms.
- **Netherlands:** MyastheniaGravis Autoimmuun- consensus richtlijn | Spierziekten Centrum Nederland.
- **Scotland:** MG-Info.pdf (scot.nhs.uk).
- **Unites States:** Seronegative MG Resource Center | MGFA (myasthenia.org).

3. Differential diagnosis

Jacqueline Palace reviewed congenital myasthenic syndromes (CMS), a heterogeneous group of genetic disorders causing nicotinic NMT dysfunction. CMS are much rarer than the acquired autoimmune form of myasthenia, except in younger children, with a varied clinical phenotype depending on the genotype and mutations. The majority are autosomally recessively inherited except for slow channel syndrome which has an autosomal dominant pattern of inheritance. Because CMS can present in older children and even in adults the term genetic myasthenic syndromes may be more appropriate.

CMS may be classified according to the location of the abnormal protein, ie presynaptic, synaptic and postsynaptic with glycosylation defects being included with the latter or separately. Mutations in the enzyme choline acetyltransferase (CHAT), the commonest presynaptic CMS, lead to a reduction in the recycling of choline to acetylcholine. Mutations in COLQ, the anchoring collagenic tail of acetylcholinesterase, is the commonest synaptic form of CMS and leads to reduced breakdown of ACh in the synapse and overstimulation. Postsynaptic defects are the most common and include those mutations causing AChR deficiency (predominantly the epsilon subunit), and AChR kinetic defects (slow channel and fast channel syndromes associated with abnormally prolonged and shortened opening times respectively). Mutations in Rapsyn that clusters the AChR also lead to an AChR deficiency syndrome, and glycosylation defects reduce the insertion of the AChR into the post-synaptic membrane. Additionally, pathogenic mutations of docking protein 7 (DOK7) and less commonly the other clustering complex proteins (MuSK, agrin and LRP4) allows dispersal of AChR in response to normal neurotransmitter release. This is worsened by drugs that further increase ACh at the NMJ such as pyridostigmine and even 3,4-DAP in some cases. Salbutamol and ephedrine are the drugs of choice for DOK7 (and MuSK, Agrin and LRP4) CMS and COLQ CMS, and fluoxetine (at high dose) or quinidine for slow channel syndrome.

In patients with a neuromuscular transmission defect without antibodies there are some features that may help differentiate between seronegative MG and CMS shown in Table 1. However, it is reasonable to do genetic testing on young onset patients within the first 3 years of life and those with a family history of CMS; it

Table 1
Clues in differentiating CMS from MG.

Congenital myasthenic syndromes	Acquired myasthenia
• Early onset (usually < 3 yrs), parental consanguinity • Life-long difficulties • More stable after early childhood • Rare isolate EOMG involvement • Ophthalmoplegia: static and since early life → lack diplopia • Symmetrical ptosis • Ankle dorsiflexion weakness common • No antibodies • Non NMJ involvement: arthrogryposis, myopathy, CNS • No response to immunotherapy • Negative response to AChE-Is in the slow channel syndrome, COLQ, DOK-7, MUSK, AGRIN, LRP4 mutations	• Later onset (neonates transient) • Acute or sub-acute onset • Acute exacerbations • Common isolated ocular form • Variable ophthalmoparesis → diplopia • Asymmetrical ptosis • Ankle dorsiflexion weakness uncommon • ~15 % antibody negative (more often in ocular MG and children) • Thymoma associated with AChR-MG • Response to immunotherapy • Response to AChE-Is, except in MuSK-MG

EOM: extrinsic ocular muscles; CNS: central nervous system; AChE-Is; acetylcholinesterase inhibitors; for the other abbreviations see text. *acute exacerbations more frequent in MG than in CMS, where they are usually limited to the first years of disease, rare in adult age.

should be considered in families where there is consanguinity or patients presenting in adolescence or adulthood with clinical signs suggestive of CMS.

Martijn Tannemaat discussed the challenges of diagnosing SNMG due to its similarity with various neuromuscular conditions and lack of serological evidence. Recently, a diagnostic flowchart for the diagnosis of MG has been published [29]. The typical clinical hallmarks of MG are fluctuating, fatigable skeletal muscle weakness, causing asymmetric ptosis, diplopia due to opthalmoparesis, bulbar weakness, limb weakness and respiratory weakness. When all or most of these typical clinical features are present, the diagnosis is straightforward, and the list of alternative diagnoses is short. However, differential diagnosis can be more difficult if only a subset of these features is present.

When considering the possibility of seronegative MG, it is crucial to distinguish it from other diseases like Graves Orbitopathy (GO), oculopharyngeal muscular dystrophy (OPMD), cranial nerve lesions, and chronic progressive external ophthalmoplegia (CPEO).

GO shares symptoms with MG such as diplopia. However, in GO, ocular symptoms are often accompanied by proptosis and may not exhibit fatigability or fluctuation in symptoms, which are typical in MG. OPMD is characterized by progressive swallowing difficulties and ptosis, resembling symptoms of MG. However, in OPMD, there is typically more symmetrical involvement, a more gradual progression and fewer fluctuations. CPEO presents with progressive weakness of the extraocular muscles leading to ptosis and ophthalmoparesis, similar to MG. Like OPMD, CPEO is more symmetrical, progresses slowly and does not fluctuate. Cranial nerve lesions can also mimic MG symptoms. However, cranial nerve lesions lead to neurological deficits related to the specific nerves affected. Red flags suggesting diagnoses other then SNMG are shown in Supplementary Table 1.

In summary, careful consideration of symptomatology, disease progression, associated features, and diagnostic tests such as electromyography and imaging studies is crucial for accurate diagnosis. Patients with pure ocular symptoms (ophthalmoparesis and ptosis) can present significant diagnostic challenges and time to diagnosis is generally longer in these cases. Misdiagnosis of MG is not uncommon and is often related to a failure to recognize atypical findings or technical errors in the performance of neurophysiological tests [18]. A recent review provides a useful diagnostic flowchart for these patients based on the presence of ptosis, symmetry, fluctuations and pain [30].

Sithara Ramdas discussed the characteristics of juvenile MG (JMG) defined as MG presenting below 18 years of age. JMG is divided based on age of onset to pre-pubertal and post pubertal. The pathophysiology of JMG is similar to adult-onset MG, presenting with fatiguable muscle weakness which can be generalised or ocular. Diagnosis is based on clinical phenotype, positive antibody and or abnormal neurophysiology. Between 10–40 % of JMG patients are seronegative, particularly the pre-pubertal children, but a proportion are positive for clustered-AChR Abs by CBA [9]. Current management of JMG is primarily based on adult guidelines and expert opinions. JMG management needs to be via a multidisciplinary team. AChE-Is (pyridostigmine) is usually first line treatment, but most JMG cases require addition of corticosteroids to achieve MG remission. Maintenance treatment usually include low dose corticosteroids, steroid sparing agents including rituximab and rarely regular intravenous immunoglobulin (IVIg) or plasma exchange (PLEX) [31]. Thymectomy should be offered in AChR positive peri- and post-pubertal generalized JMG and in refractory or steroid dependent ocular and pre-pubertal JMG [31], although possible extraocular muscle hypotrophy should be considered in the overall evaluation. There are significant issues related to the long-term impact of JMG and treatment-related side effects in the JMG patients compared to adults including impact on growth, childhood obesity, puberty, self-image, long-term immune function, late malignancy risk and psychological issues. These need to proactively monitored for and appropriate interventions put in place. There are several clinical trials of novel therapies in progress and planned which will hopefully bring more effective treatments to the JMG population in the future. All these considerations emphasize the need for an accurate diagnosis of MG in SN paediatric patients.

4. Patients perspective in SNMG

Maria Bonaria (Maya) Uccheddu and Johan Voerman discussed the patient perspective on having SNMG rather than MG with detectable antibodies. They collected responses from 24 SNMG patients: 5 Dutch patients from the Netherlands MG group, 13 patients who are members of the Italian MG association (AIM), and 6 patients from different Countries in Europe. The main concerns were lack of access to treatments and to clinical trials. In addition, there was concern about the diagnostic delay (perceived to be longer for SNMG), and about the larger mental and economic impact compared to patients with seropositive MG. Delays between the clinical suspicion of MG and the final diagnosis, result in economic impact due to lack of access to national health system support or reimbursement.

Some patients found lack of experience of SNMG among health-care professionals (HCPs). One Dutch patient complained about

being left in the limbo of “probably MG.” Two Italian patients shared their journeys, underscoring the need for more reliable serological tests. One patient, initially found positive for AChR Abs, was retested years after the diagnosis and is now considered “seronegative,” with uncertainty about treatment options. The other patient endured a prolonged diagnostic delay, initially diagnosed with SNMG, until recently testing positive and gaining access to new treatments.

Another complaint was being doubted regarding their symptoms, mostly because SNMG is perceived as solely "ocular" by some HPCs. Patients attribute this lack of acknowledgment to the absence of detectable Abs and physicians’ uncertainty about the diagnosis

The perspectives of 24 SNMG patients highlight challenges such as limited treatment access, diagnostic delays, and insufficient HCP awareness. These insights underscore the need for improved diagnostic tools, medical education, and initiatives to address the unique needs of SNMG patients.

Sithara Ramdas gave an overview of patient-reported measures (PROM), standardized and validated questionnaires that collect information on health outcomes directly from patients. These can include disease-related symptoms, functional status and health-related quality of life. Whilst initially developed for research, PROMs are increasingly used not only in clinical decision making but also in service evaluation and comparing outcomes between health providers, service improvements and policy developments. PROMs can be generic or disease specific and both have their pros and cons. MG specific PROMs include Myasthenia Gravis Activities of Daily Living (MG-ADL), MG Quality of Life 15 revised and MG disability Scale, MG Impairment index, MG composite score. PROMs are particularly important in MG patients as MG symptoms fluctuate, so short objective assessments in clinic may not necessarily reflect patients’ experienced symptom burden, and current objective measures do not capture the impact of MG on patient’s day to day life like work, mood and social participation. In the past 5 years, PROMs have been used as a primary outcome measure in several of the novel therapy trials and are accepted by regulatory authorities. Newer simpler PROMs include single simple question (SSQ) and the patient acceptable symptom state (PASS) which can be delivered easily in clinics to evaluate a patient's satisfaction with their Myasthenia Gravis status. There are defined PASS thresholds for most of the MG outcome measures that can be used in research and clinics to guide disease control. It is also important to recognise that PROMs are subjective and reflect patients’ perspectives and experiences and should not be used in isolation in MG management. This is of crucial importance in patients with significant co-morbidity and when there is a clear non-organic component to the patient’s symptoms.

4. Antibody testing

Angela Vincent covered the Ab assays, particularly RIPA and CBA for AChR and MuSK Abs. The advantages of RIPA are the quantitative results, but to obtain accurate results it is essential to measure binding at different serum dilutions. This is time-consuming and unavailable in most clinical laboratories. As a result, antibody-determinations in serial samples are not necessarily suitable for guiding management. Disadvantages of RIPA are limited radioactivity facilities, and the low concentration of the antigens in solution that may reduce detection of some Abs – particularly early in the disease course [32]. CBAs, the main alternative nowadays, use transfected human embryonic kidney (HEK) cells to express the antigens. The L-CBAs measure only antibodies binding to extracellular epitopes and, by co-transfection with the intracellular clustering protein, rapsyn, were initially established to provide clusters of AChRs, suitable for divalent binding of low affinity antibodies to the clustered AChR, as at the NMJ [33,34]. L-CBAs can increase sensitivity [9]. There is now a commercial fixed version (F-CBA) available /being tested [35]. Tiny (mm) chips of fixed HEKs expressing, individually, adult AChR, fetal AChR, MuSK or no antigen, each mixed with excess fixed control cells, are placed into a small well on a glass or plastic slide. Binding of human IgG is detected with green, fluorescent anti-human IgG. The sensitivity is good or very good [35]. In addition, fetal AChR and MuSK should improve positivity in purely ocular MG [34,36] and guide management in mothers of offspring with joint contractures and other deformities [37]. These advances should help limit the number of MG sera who are seronegative but are unlikely to replace the need for their further study.

Valentina Damato described research use of Live- and Fixed-CBA (L-CBA, F-CBA) in clinical pracitce. The detection of clustered AChR and MuSK Abs in RIPA-negative (dSNMG) cohorts improved the rate of seropositivity particularly in juvenile, ocular and mild MG [8,10]. In a large cohort of well-defined RIPA-negative MG patients, L-CBA for clustered AChR, MuSK and LRP4 found clustered AChR and MuSK Abs in 28 % patientsand no LRP4 Abs. The remaining “triple” SNMG patients were mainly females with EOMG and mild disease and good clinical outcome [9].

Since 2021 a commercial, F-CBA has become available. F-CBA showed an excellent specificity and 4 % higher sensitivity compared to RIPA in SNMG [11] but, surprisingly, it was less sensitive than ELISA in other laboratory studies [38]. When compared to L-CBA, F-CBA had similar specificity but lower sensitivity [35]. Finally, in a large multicentre Chinese study, while the gain in sensitivity/specificity in the detection of MuSK antibodies by a commercial F-CBA was not significant different from RIPA or ELISA, F-CBA detected anti-AChR antibodies in 27.5 % of RIPA negative MG patients [12]. In conclusion, CBAs have improved the serological detection of Abs in MG. F-CBA represents a potential first-line option for antibody testing in MG potentially replacing RIPA or ELISA in clinical diagnostic laboratories worldwide. Live CBA remains the most sensitive assay for the detection of MG Abs and can be used as second-line test option for SNMG.

Gregorio Spagni presented the results of the pre-workshop survey that aimed to assess (1) the availability of Ab assays, (2) the perceived benefits and concerns related to different detection methods, and (3) the impact of Ab detection on the diagnosis and management of MG.

Nineteen/24 (79 %) experts from 15/16 (94 %) centers participated in the survey. Data regarding assay availability, use of external laboratories and turnaround time are summarized in Table 2. Overall, RIPA was reported as the most common first-step assay for both AChR and MuSK Abs (67 %), followed by AChR and MuSK ELISA (20 %). Regarding the second-step evaluation of RIPA/ELISA-seronegative samples, the use of AChR/MuSK CBA (live or fixed) was reported by 60 % of the participating Centers, and availability of LRP4 Abs testing (either on-site or through external laboratories) by 67 %. However, 37 % of survey responders reported that they do not include LRP4 Abs in the serological evaluation of MG, regardless of assay availability.

As for the perceived benefits and limitations of the different testing methods, most responders either agreed or strongly agreed that AChR and MuSK RIPAs are highly valuable for MG diagnosis, characterized by very good to excellent sensitivity and specificity. Strong consensus was also achieved for AChR RIPA as the best "first step" antibody assay (“agree” / “strongly agree” responses: 83.3 %). Regarding live CBA for clustered AChR and MuSK Abs, 87.5 % of responders considered the assay to have very good to excellent analytical characteristics and to be highly valuable for MG diagnosis, optimally as second-line assay for the evaluation of RIPA/ELISA-seronegative samples. As for the commercial fixed

Table 2
MG Ab assay availability, use of external laboratories and Ab results turnaround time.

On-site Ab assay available on-site	*n. of centers, (%)*
AChR RIPA	8 (53 %)
MuSK RIPA	6 (40 %)
AChR ELISA	4 (27 %)
MuSK ELISA	3 (20 %)
AChR/MuSK[a] commercial fixed CBA	3 (20 %)
AChR/MuSK live "in-house" CBA	2 (13 %)
LRP4 "in-house" live CBA	2 (13 %)
LRP4 fixed CBA	1 (7 %)
No MG Ab assay available on-site	4 (27 %)
Use of external laboratories for MG Ab testing by each Center	***n. of centers, (%)***
No use of external laboratories	5 (36 %)
Use of one laboratory	4 (29 %)
Use of two laboratories	6 (40 %)
Ab assay requested to external laboratories	***n. of centers requesting each assay, (%)[a]***
AChR RIPA	4 (40 %)
MuSK RIPA	4 (40 %)
LRP4 live "in-house" CBA	3 (30 %)
AChR/MuSK live "in-house" CBA	2 (20 %)
LRP4 fixed CBA	2 (20 %)
AChR ELISA, MuSK ELISA, LRP4 commercial IIFT, AChR/MuSK commercial fixed CBA	each requested to external labs by 1 (10 %) center
Antibody assay result turnaround time	***n. of replies per categor[b], (%)***
"First step" on-site assay	
< 2 weeks	8 (73 %)
2–4 weeks	2 (18 %)
4 weeks – 3 months	1 (9 %)
"Second step" on-site assay	
< 2 weeks	2 (22 %)
2–4 weeks	7 (78 %)
Assay performed by external laboratory	
2–4 weeks	9 (70 %)
4 weeks-3 months	2 (15 %)
>3 months	2 (15 %)

AChR/MuSK CBA, there was a high level of agreement on its optimal use as a second step assay, and its analytical characteristics and diagnostic value were also rated positively, although with a lower level of agreement compared to the live CBA (with 67 % and 69 % of "agree" or "strongly agree" responses, respectively). No need for radioactivity was seen as a major advantage of both CBAs. On the other hand, most responders disagreed that AChR and MuSK ELISAs are the best first-step tests (67 % and 54 %, respectively), with concerns mostly regarding their analytical characteristics in terms of optimal sensitivity and specificity. Overall, there was a very strong agreement about Ab detection having a significant impact on the confidence in MG diagnosis and treatment choices. All survey responders agreed that Ab detection by CBA in seronegative MG patients (previously tested by RIPA or ELISA) has a significant impact on treatment decisions; accordingly, a wider adoption of CBAs was considered to potentially improve the management of SNMG.

Future efforts should focus on making cAChR/MuSK CBA more widely available, reducing turnaround time and on clarifying the role of LRP4 Ab testing in the serological diagnosis of MG, as well as the optimal detection method for these Abs.

Amelia Evoli further discussed the specificity and sensitivity of serologic assays. Provided the patient has typical history and signs, a clear-cut positivity of RIPA, ELISA, or CBA for AChR or MuSK Abs can confirm MG diagnosis. When compared to RIPA, for AChR Ab detection, ELISA was found to be as sensitive but less specific [39], while both commercial and in-house CBAs appear to be as specific and more sensitive [11,35]. For MuSK Abs, ELISA and RIPA perform comparably [40], while live CBA appears to be more sensitive than fixed CBA [35].

RIPA positivity for AChR Abs can occur in non-MG thymoma patients and very rarely in other neurological diseases (ONDs) that can mimic MG, for instance amyotrophic lateral sclerosis (ALS) and mitochondrial myopathies. The Ab results have high relevance in clinical practice but broad screening for Abswithout appropriate clinical features should be discouraged, as it increases the risk of false positive results [41].

The current prevalence of LRP4 Abs in AChR and MuSK-negative patients is very low. Only LRP4-Abs have been reported in patients without electrodiagnostic abnormalities [42] and also in ALS, in ONDs and even in healthy controls [4]; there should be caution regarding diagnosis in these cases. Abs to Agrin have been reported in some patients but the associated clinical aspects are not yet defined. A conclusive demonstration of pathogenicity of LRP4 and Agrin Abs by passive transfer studies is still lacking. Finally, Abs against intracellular antigens such as titin, ryanodine receptor, and cortactin are not diagnostic of MG and are unlikely to be pathogenic.

5. Electrodiagnostic in SNMG

Anna Rostedt Punga covered RNS in the diagnostic workup of generalized MG, including SNMG.

RNS is the first-line non-invasive electrodiagnostic tool for confirming NMT failure in MG. Several considerations, such as stimulation frequency, medication timing, and patient preparation, influence the reliability of RNS results.

Key practical aspects of RNS include employing low-frequency stimulation (3 Hz), ensuring at least 12-hour intervals since the last dose of acetylcholine esterase inhibitors, allowing adequate rest before the exam, and maintaining optimal skin temperature. In cases of uncertainty, redoing the test may be necessary. RNS measures the difference in amplitude between the 1st and 4th compound muscle action potential (CMAP), with an abnormal decrement typically defined as 6–10 %. However, cutoff values may vary across different laboratories. The standard protocol involves stimulation at rest, immediately after 20 s of muscle activation to discover facilitation, then after 1 and 3 min to see

the reappearance of a decrement. Commonly examined muscles include the deltoid, trapezius, anconeus, nasalis, abductor digiti quinti, and frontalis.

RNS sensitivity varies across MG subtypes and patient populations; generalized MG sensitivity ranges from 53 % to 95 % [43]. Sensitivity tends to be higher in clinically affected muscles [44] and in patients with AChR Abs [45]. However, even in SNMG, abnormal decrements are observed in approximately two-thirds of cases, particularly in muscles such as the deltoid [46]. Recommendations advocate testing at least three muscles to enhance diagnostic sensitivity. Lowering cutoff values for abnormal decrement while maintaining specificity has improved diagnostic accuracy [47–49].

Despite its diagnostic utility in both seropositive MG and seronegative SNMG, RNS has limited prognostic value. While RNS decrement may correlate with the worst recorded clinical status, it does not reliably predict long-term outcomes or correlate with AChR antibody titers [48]. Moreover, a normal RNS does not exclude the possibility of acute-onset MG [49], highlighting the need for SF-EMG in these cases.

Donald Sanders discussed SFEMG in generalized MG. SFEMG electrodes (SFEs) record action potentials from single muscle fibers (SFAPs), which permits measuring fiber density (FD), a sensitive measure of reinnervation, and jitter, a sensitive measure of abnormal NMT.

When measuring jitter, avoid pitfalls due to inconstant firing rates during voluntary activation and subliminal stimulation during activation with axonal stimulation. When measuring jitter with concentric needle electrodes (CNEs), exclude spikes with notches, shoulders or rising phases that are not parallel - these are produced by summated SFAPs. Reference jitter values for CNEs should be used [50] - they are about 5 μsec lower than those for SFEs . CNE and SFE recordings are both highly sensitive in detecting increased jitter, as in MG. Jitter is also increased in reinnervation and some myopathies. With SFEs, these conditions can be identified by increased FD. FD cannot be measured with CNEs; thus, if jitter is increased, conventional electrodiagnostic procedures must be performed to detect neurogenic or myogenic abnormalities. Jitter is abnormal after botulinum toxin injections, even in muscles remote from the injection site, and can persist for 6 months or more. In laboratories with SFEMG capability, measurement of jitter may be the initial NMT test as it is more sensitive than RNS. Jitter may also be measured as the initial NMT test in patients with mild symptoms in whom RNS is likely to be normal, or if discomfort prevents completion of RNS [51].

Jitter is increased in almost all patients with generalized MG, even in muscles with normal strength. No one muscle is more abnormal or more likely to be abnormal in every MG patient–muscles should be tested based on clinical findings. If jitter is normal in the first muscles tested, examine a weak muscle.

In some patients with MuSK Abs, weakness and abnormal jitter are distributed in patterns different from other MG patients. For example, in MuSK-MG patients with weakness predominantly in neck or shoulder muscles, it may be necessary to examine those muscles to demonstrate abnormal jitter. If jitter is normal in a weak muscle, the weakness is not due to MG.

Martijn Tannemaat discussed the role of repetitive ocular vestibular evoked myogenic potential (ROVEMP), ice pack test, or orthoptic measurements in MG diagnosis. SNMG poses a diagnostic challenge due to its similarity with various neuromuscular conditions and lack of serological evidence. A non-invasive, quick and easy to perform test in patients presenting with ptosis is the ice pack test [43]. A recent study showed that the diagnostic value of the ice pack test is almost comparable to that of SF-EMG: the sensitivity of the ice cube test in suspected ocular MG was 86 % and specificity 79 % [52].

Recently, ROVEMP has been proposed as a novel method to test NMT failure of the extrinsic ocular muscles (EOM), the most commonly affected muscles in MG [53]. ROVEMP does indeed show a decrement in some patients, but the test can be quite challenging to perform, and test-retest reliability appears to be suboptimal [54], and arecent follow-up study found only 30 % specificity for ocular MG [55].

Orthoptic measurements such as the Hess chart can also be used to quantify EOM weakness, and typical MG-associated fatigability can be quantified by asking patients to maintain their gaze for one minute and to observe whether the eyes “drift”. A recent study found that the sensitivity was 81 % and specificity was 100 % of the presence of drift in MG [ref?], compared to both healthy subjects and disease controls. Drift during persistent gaze on a Hess chart is specific for MG and could be used for diagnostic purposes, as the Hess chart examination is widely available, inexpensive and fast [56].

6. Other diagnostic tools/investigations

Ulrike Schara-Schmidt discussed the clinical characteristics and response to therapy of SNMG in children and adolescents. She first described two patients who illustrated the challenges in defining SNMG, the variablility of clinical symptoms and effects of pyridostigmine, and other therapies including intravenous immunoglobulins (IVIg) and plasmapheresis [31].

She described the Essen cohort of SN (RIPAs and ELISAs negative) JMG patients from the last 20 years. Diagnostic work-up in patients with suspected JMG followed a standard operating procedure, including, clinical examinations and MGFA score, serum antibody testing (AChR, MuSK, LRP-4) and RNS tests [16,31]. Twenty SN-JMG patients aged 3–16 years were reported. The MGFA scores at maximum severity were I (5 %), II (50 %), III (30 %), IV(5), V (2 %). 5

Response to pyridostigmine therapy was observed in 18/20 (90 %); this was not always found at onset or with severe symptoms, but sometimes only during long-term treatment. Additional therapy was adopted on the basis of clinical symptoms and RNS tests. All patients received piridostigmine, prednisolone was admistered to 19 (95 %), azathioprine or mycophenolate mofetil to 9 (45 %) patients. Thymectomy was performed in 8 cases (40 %), thymic hyperplasia was found in 4; all thymectomized patients showed clinical benefit.

Overall from our experience: 1) the algorithm for diagnostic work-up cannot address all situations in clinic [16,31,57]; 2) re-testing can disclose a positive antibody titer in patients previously diagnosed with SNMG (up to one year after presentation, or even longer?) [9,35,57]; 3) although in SN-JMG ocular and mild generalized disease are frequent, clinical course should be monitored carefully [31]; 4) In SN-JMG, CMSs are an important differential diagnosis [16,31].

There are still some open issues in SN-JMG: 1) there are few data from literature on clinical features and response to treatments, including pyridostigmine; 2) establishing the diagnosis of SNMG also in children and adolescents is necessary for an appropriate therapy and counselling; 3) characterization of patients with seronegative MG in more detail is necessary (which are the similarities and differences between SN and seropositive JMG?); 4) The diagnostic role of CBA and muscle biopsy in RIPA/ELISA SN-JMG is not yet established.

Jeannine Heckmann covered the utility of brain MRI/CT in patients with ocular signs. Fatigable weakness of EOMs is often the earliest manifestation of MG. In a proportion of patients, symptoms and signs remain confined to the EOMs and half of these patients have no detectable AChR or MuSK? Abs. Although childhood-onset ocular MG is often a transient benign disease,

some cases, particularly Asian and African juveniles with MG, may develop severe treatment-resistant ophthalmoplegia [58]. Treatment-resistant ophthalmoplegia may also occur in patients with generalized MG in which other affected muscles respond to treatment in contrast to the EOMs. To better understand the refractoriness of EOMs in MG, several groups performed magnetic resonance imaging (MRI) and compared EOM volume and fat content of MG cases (with and without AChR Abs) with age-matched controls and with other conditions with restricted eye movements such as CPEO [56,59,60]. Although different generations of MRI machines were used (7 and 3 Tesla), the results were similar. Chronic EOM dysfunction in MG resulted in EOM volume loss and fatty replacement compared to age-matched controls, similar to CPEO. Two reports found no difference in the EOM imaging findings between adults with AChR Abs and SN-MG, but both EOM atrophy and fat replacement appeared more severe in older patients compared to middle-aged adults. In isolated MG patients with treatment-resistant EOM paralysis who underwent ocular re-alignment surgery, the histopathology of EOMs excised during surgery also showed muscle atrophy and fibrofatty replacement as well as ultrastructural mitochondrial changes [61]. However, not all MG patients with chronic EOM dysfunction showed EOM atrophy on imaging, suggesting a potential for functional recovery [56] which may also be detected in the clinic (JMH personal observation). Observational data suggested that a shorter latency between onset of ocular MG symptoms (diplopia) and prednisone treatment initiation, as well as higher prednisone doses, can result in earlier resolution of ocular dysfunction [62].

Daniel Natera-de Benito further discussed when genetic analysis for CMS should be performed. CMS patients may be misdiagnosed among the broader SNMG cohort; however, their proper identification is crucial, as CMS are treatable conditions with treatment approaches markedly different from MG [63].

In individuals under 18 years of age, the incidence of CMS is higher than that of MG. Therefore, an early onset of symptoms should prompt consideration for genetic testing. Other clues suggesting a genetic etiology include a generalized phenotype without severe bulbar involvement, a family history of myasthenic symptoms or consanguinity, and unresponsiveness to immunomodulatory and immunosuppressive therapies. Conversely, a genetic origin is less likely in individuals with acute onset, exclusively ocular involvement, or adult-onset myasthenia gravis. However, certain subtypes of CMS, such as those related to mutations in *DOK7, COLQ,* glutamine-fructose-6-phosphate transaminase 1(*GFPT1)* or guanosine diphosphate mannose (GDP-mannose) pyrophosphorylase B (*GMPPB)* genes, often present with a late-onset proximal weakness phenotype, leading to potential confusion with MG more frequently than other CMS subtypes [64].

7. SNMG demographic and clinical data (What do we know from current data?)

Erik Niks covered the clinical pattern in ocular vs generalized MG at the maximum disease severity. The description of clinical patterns in SNMG largely depends on cohort studies and the methodology by which the absence of Abs was determined. This has evolved in the last two decades due to the growing availability of RIPA followed by CBA. A literature review was performed from the first comparison between the clinical patterns of generalized SNMG and MuSK-MG (2003–2024). Eight out of 24 studies contained information on symptoms during maximum disease severity. Using AChR and MuSK RIPA only, milder disease forms and less frequent bulbar symptoms and crises were found in SNMG using MGFA classification in Italian, Turkish and US populations respectively [65–67]. A milder longitudinal trajectory of SNMG compared to AChR-MG was found using the Osserman scale in a follow-up study of 20 years [68]. By contrast, there were more asymptomatic patients in AChR-MG versus SNMG after 5 years in one small study [69]. Using both RIA for AChR and MuSK and CBA for AChR, MuSK, and LRP4 Abs, milder weakness at maximum disease severity was reported in triple SNMG in South-Africa and Italy respectively [9,70]. Only one study on ocular MG showed a decreased risk of generalization in SNMG patients compared to AChR- and MuSK-MG [71]. During the meeting, data were also presented from a retrospective Dutch natural history study in which 97 patients with a diagnosis of generalized MG without AChR Abs by RIPA were included [72]. MuSK Abs were found using RIPA in 35. From the remaining 62, 47 were reexamined and the clinical diagnosis confirmed in 41. Using CBAs, 10 had Abs to clustered AChR and another 10 had Abs to MuSK. The clinical pattern in the 21 SNMG patients in the first 5 years after symptom onset was that of a mild limb-girdle weakness with diplopia and ptosis, but significantly less bulbar and axial symptoms.

The diagnosis of SNMG thus requires multiple levels of confirmation combining clinical criteria, EMG, and repeated testing for antibodies using both RIPA and CBA. Generalized SNMG may be milder throughout the course of the disease with predominant limb-girdle and extra-ocular weakness. Respiratory crises are less common, but if present, do not discriminate between AChR positive MG and SNMG, and both warrant proactive management [24].

Sarah Hoffmann covered gender- and age-specific aspects of MG. Epidemiological differences in female and male MG patients are widely accepted. There is an overall increasing incidence of MG with age but women often show a bimodal distribution in incidence rates with a first peak in their 3rd decade of life [73]. Hence, early-onset MG (EOMG) is more frequent in women whereas late-onset MG (LOMG) is more frequent in men. This makes gender-related differences in pathogenesis likely. In fact, women show higher rates of thymus hyperplasia compared to men [74].

Data from a large Spanish cohort study suggested that patients with late-onset MG (age at onset $\geq$50 and $<$65 years) and verylate onset MG (age at onset $\geq$65 years) more frequently have purely ocular MG. However, the very-late onset group also more often presented with life-threatening events at disease onset, although they were more likely to achieve pharmacological remission over the course of their disease and were less frequently therapy-refractory than patients with earlier presentation. A recent review showed a gender bias in the autoantibody distribution with a female predominance in MuSK- and LRP4-positive MG as well as seronegative MG. In turn, various studies indicate that male MG patients are significantly more likely to have AChR-positive MG.

Overall, literature on gender-specific differences in the prognosis of MG is scarce. The few studies addressing gender-related differences in disease severity report a lower quality of life, a higher impairment in activities of daily living and a higher risk for myasthenic exacerbations in female compared to male MG patients [75–77]. Various studies consistently reported a delayed diagnosis in female compared to male MG patients. There is accumulating data suggesting that time to diagnosis and, thus, initiation of therapy influence clinical outcome in MG patients. In the light of a growing focus on laboratory diagnostics, this might be in part explainable by the higher rate of seronegativity in women compared to men. The resulting delay in diagnosis might also partially explain the higher disease severity in female MG patients.

In conclusion, gender and age at onset are inter-related in MG but are two different factors. More insights into the pathogenesis

and clinical patterns in EOMG vs. LOMG as well as female vs. male MG patients will hopefully allow for earlier diagnosis and more personalized treatment options in MG in the future.

Annabel Ruiter discussed fatigue in SNMG versus seropositive MG. There are two types of fatigue in MG: peripheral fatigue as a direct result of muscle fatigability, and central fatigue as an experienced lack of energy/feeling of tiredness, interfering with mental or physical activities. Prevalence of central fatigue in MG is 44–82 % compared to 18–40 % in control groups [78]. The prevalence of fatigue increases with disease severity. It is hypothesized that muscle damage/ disease leads to CNS induced fatigue to downregulate physical activities to (further) protect the muscles of damage. The pathophysiology is multifactorial: disease severity, gender, depression, sleep, restriction of physical activities and antibody status have previously been associated with fatigue. Only two studies investigated the association between fatigue and Ab status in MG [79,80]. One study found associations between fatigue and AChR and Musk antibodies but not with seronegative antibody status through univariate statistics. However, there associations disappeared in multivariate analysis [79]. Unpublished data of the Dutch-Belgian MG patient registry among 420 patients showed higher patient-reported fatigue rates among patients with a SNMG status compared to seropositive patients. This association also disappeared in multivariate analyses. One hypothesis is that SN patients reported higher rates of fatigue because they feel neglected.

Anthony Behin covered ocular SNMG, which is highly variable in terms of clinical severity, involvement of extraocular muscles, fluctuations, and response to treatments. Among diagnostic tests in ocular MG, SF-EMG showed the highest sensitivity, followed by sleep test and Tensilon test. On the other hand, AChR Ab assay, RNS and ice pack test yelded higher specificity [80].

In a Japanese cohort of 73 ocular MG patients tested with live CBA, 26 (36 %) were negative for AChR, MuSK and LRP4 antibodies. Interestingly, among the 44 (60 %) patients with antibodies to AChR, 7 (16 %) and 2 (5 %) were positive only for fetal or adult AChR Abs, respectively [36].

In the differential diagnosis of ocular SNMG mitochondrial disorders and CMS should be considered.

Treatment of ocular MG in France includes cholinesterase inhibitors as first line therapy, followed by corticosteroids in case of inefficacy and azathioprine if needed. In case of lack of efficacy after these treatments, the diagnosis should be rediscussed in a multidisciplinary setting.

Lorenzo Maggi discussed the prognosis and the response to treatment in SNMG. Traditionally, the prognosis of SNMG has been considered similar to that of AChR-MG and better than MuSK-MG [23,66]. However, recent studies do not confirm these data [19,24,48]. Response to conventional treatments, evaluated by the Myasthenia Gravis Impairment Index (MGII) and Myasthenia Gravis Foundation of America Post-Intervention Status (MGFA-PIS), was significantly better in AChR-MG than in SNMG though the initial disease severity was similar in the two groups [19]. In the study by Tomschik et al. [48], SNMG patients had lower remission rates than MuSK-MG, the highest rate of symptomatic patients compared to all other subgroups and were more likely to be treatment-resistant. Conversely, MG crisis treatment efficacy and outcome do not differ between SNMG and AChR-MG [24].

Then, Lorenzo Maggi presented unpublished data from a retrospective study investigating MGFA-PIS in a large cohort including 617 MG Italian patients. Apart from thymoma-associated MG, SNMG and MuSK-MG subgroup had the lowest mean cumulative complete stable remission (CSR) rate (4.0 %) compared to generalised non-thymoma AChR-MG (%) and ocular MG (%). No significant difference in terms of satisfactory (CSR, pharmacological remission, minimal manifestations) versus unsatisfactory (worsened and unchanged) MGFA-PIS were observed across the different MG subtypes in the 10-year follow-up.

Moreover, a multicenter Spanish study investigating the outcome of refractory MG showed that only 10 % of drug-refractory SNMG patients achieved a satisfactory MGFA-PIS compared to 42.6 % of AChR-MG and 100 % MuSK-MG patients [81]. A further study found that refractory MG was significantly associated with SNMG compared to AChR-MG and MuSK-MG [17]. These data differ from an earlier report by Suh et al. showing lower rates of refractory disease in SNMG patients [82]. Finally, unpublished data from the Italian cohort [see above] found that antibody status did not significantly correlate with refractory status. Overall, SNMG outcomes deserve further study.

Marta Cheli presented retrospective data on clinical follow-up of SNMG patients aiming at describing the fluctuating severity of symptoms through the use of the Neurological Institute Foundation of Milan (INCB) scale and fatigability score [83]. In this cohort including 74 SNMG patients followed at the Neurological Institute Carlo Besta in Milan, the median age of onset was 44 years (range: 10–83 years) and the median follow-up 9.5 years (1–20.8 years). The rate of patients with normal INCB score increased from 21 % to 45 % during the first three years and then slightly decreased, reaching 36 % in the fifth year of follow-up, with fatigability following a similar trend. Among 30 (40.5 %) patients presenting with isolated ocular myasthenia, 23 (76.6 %) developed generalized symptoms: 15 (55.5 %) in the first year of follow-up, 3 (11 %) between the second and the fifth year and finally 9 (33.3 %) after 5 years of clinical observation. Seventeen patients (56.6 %) of this subgroup developed also bulbar weakness, in most cases (15/17 – 88.4 %) after 5 years of follow-up. No predictor of generalization in patients with ocular-onset SNMG were detected. Of the 44 patients with generalized onset, 27 (61 %) developed bulbar symptoms and 9 (20 %) patients also respiratory involvement. Notably, none of those patients needed mechanical ventilation. Risk of bulbar involvement in patients with generalized onset was not predicted by disease severity expressed as INCB score, but it was associated with longer disease duration ($p = 0.034$). Ocular onset ($p = 0.012$) and bulbar involvement during follow-up ($p = 0.022$) were associated with better and worst clinical outcomes (expressed as MGFA PIS) at the last observation, respectively.

Overall, this study sheds light on the relevance of longitudinal quantitative assessment and of evaluation of prognostic factors in a large cohort of SNMG.

8. Treatment of SNMG

John Vissing discussed the use of intravenous immunoglobulin (IVIG) and plasma exchange (PLX) in MG. He reported the data of an unpublished study in Denmark where SNMG patients were found to be only 15 of 350 (4 %) patients with MG. In this cohort, 20 patients had originally tested negative, with later seroconversion in 5. The remaining 15 patients were all triple SN. In this study, SNMG patients generally needed less aggressive treatment to achieve good control of their disease, but otherwise did not differ demographically, in terms of clinical outcome or onset-to-diagnosis time interval compared to seropositive patients.

There is no solid evidence for the use of IVIG and PLEX in SNMG. Case studies generally showed similar efficacy as in seropositive MG. The evidence for effect of IVIG in the treatment of MG crisis and exacerbations is well documented, but recent evidence shows that IVIG is inappropriate as maintenance treatment for MG [84], although it is still used frequently [85]. Evidence for PLEX is comparable to IVIG, but side-effects may be more serious, and onset of action may be faster using PLEX vs. IVIG [86].

Nils Erik Gilhus covered conventional immunosuppressive therapy and thymectomy. SNMG represents an autoimmune disease and should be treated with immunosuppressive treatment. Treatment aims should be ambitious, and most patients achieve a status of minimal or moderate symptoms or even symptom freedom. Conventional immunosuppressive treatment is believed to increase the risk for infections and for more severe infections by a rate of around 50 %. Treatment studies show that SNMG deterioration during the first year after diagnosis is common [69]. Thus, early active immunosuppressive therapy is important.

The combination of prednisolone and azathioprine represents a favored first-line SNMG treatment [1,87]. To induce a remission, high corticosteroid daily doses (40–60 mg) are necessary. The dose should be gradually reduced after a few weeks to the lowest possible daily dose. The beneficial effect of azathioprine appears slowly over months. Rituximab represents an alternative first-line treatment in SNMG. Mycophenolate mofetil is sometimes used instead of azathioprine, or as a secondary alternative if that drug fails. Tacrolimus and methotrexate are also used in SNMG.

Thymectomy within 4 months after diagnosis is recommended for early onset MG with acetylcholine receptor antibodies, but not with MuSK antibodies [1]. There are no data supporting thymectomy in SNMG. However, SNMG patients who may have acetylcholine receptor antibodies that were not detected by the available tests should be treated in the same way as those with proven antibodies.

An advantage of conventional immunosuppressive therapy is that it is relatively cheap. Cost-benefit aspects are relevant for treatment decisions [88]. Patient preference is another key factor. The patient's view on the balance between optimal muscle strength, experienced side-effects, and risk of future side-effects is important. Immunosuppressive SNMG therapy should be in accordance with recent local, national, and international guidelines. It should at the same time be personalized, taking into consideration aspects such as comorbidities, potential pregnancies, and age. Globally access to immunosuppressive therapies varies. SNMG is a fluctuating disease, and the doses and type of immunosuppressive therapy should therefore be changed over time.

Jan Verschuuren discussed new treatments. Recently, two new classes of drugs have become available for the treatment of MG. These include antibodies against the neonatal Fc-receptor (FcRn) and complement inhibitors (CI). For both classes at least three different drugs are being developed. The FcRn inhibitors include efgartigimod [89], rozanolixizumab [90], nipocalimab [91] and batoclimab [92,93]. For several drugs phase 2 or 3 clinical studies have been completed, showing that FcRn inhibition leads to a fast decline of all serum IgG. Within about 4 weeks serum IgG is decreased to about 70 % of normal values and accompanied by a clinically significant improvement. CIs are eculizumab [94], ravulizumab [95], and zilucoplan [96]. They also show produce clinical improvement within 4 weeks after start of the treatment. Thus, both new classes of drugs have a rather fast mode of onset and a good safety profile, but the high price of these drugs are likely to restrict their wide use. SNMG patients ave been included in few trials, limiting SNMG as an indication on the label of these new drugs. Also, the role of complement-mediated damage in SNMG is not yet clear, restricting the choice of CIs.

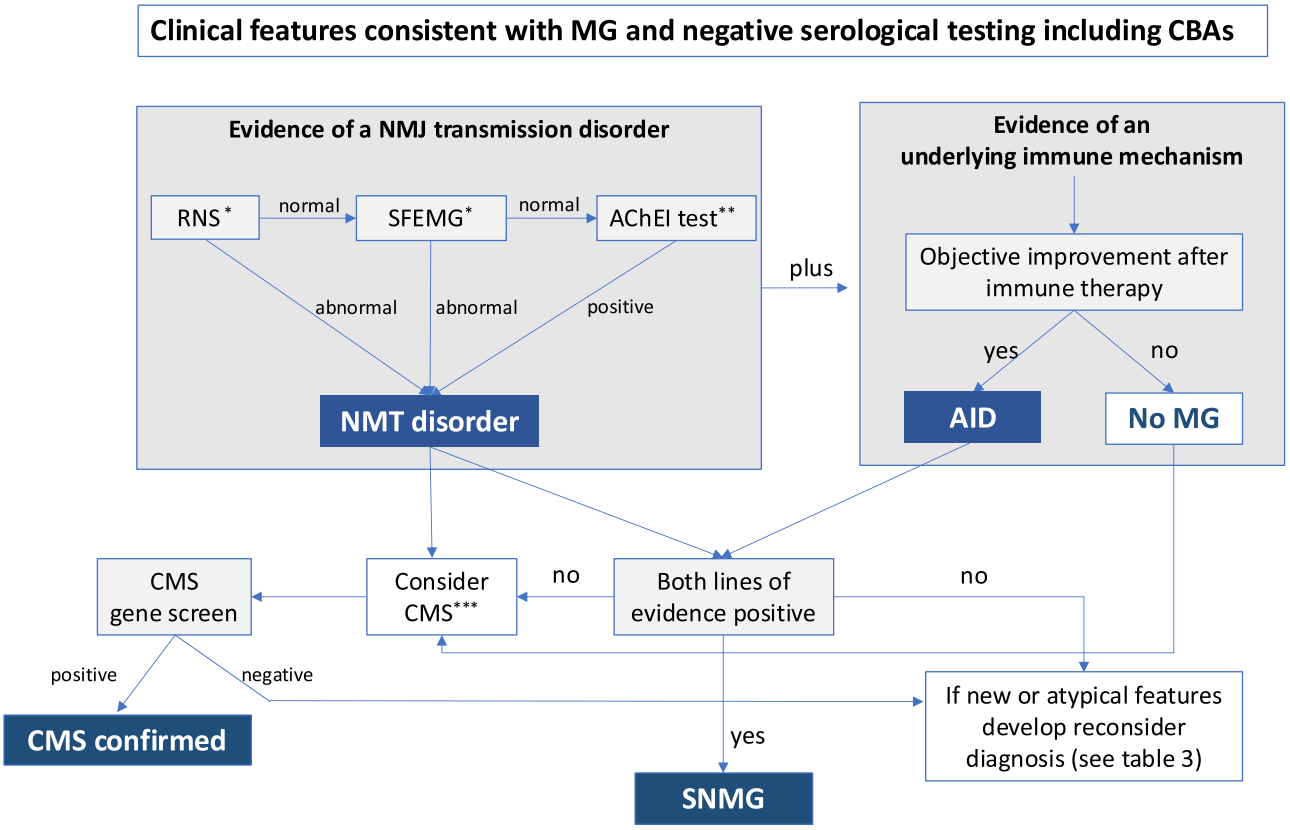

Fig. 1. Algorithm for the diagnosis of SNMG. *Normal results of jitter studies in a clinically weak muscle exclude MG as the cause of weakness in that muscle. In a clinically strong muscle RNS may be normal. In ocular MG with only extraocular muscle involvement neurophysiology can be normal. **Objective visualization of muscle fatiguability or observed response by the neurologist to a standardized cholinesterase inhibitor test supports a NMJ disturbance. ***For clues for the diagnosis of CMS see text and Table 2. AID=autoimmune disease; AChEI= acetylcholine esterase inhibitors; CBAs: cell-based assays; CMS: congenital myasthenic syndromes; RNS: repetitive nerve stimulation; SFEMG: single-fiber electromyography; SNMG: seronegative myasthenia gravis.

In the near future several other drugs might become available. Several immunomodulating drugs are tested in clinical trials, for example anti-IL-6 drugs, like satralizumab [97]. For other immunosuppressive drugs only case series are available. Another new class of drugs are the Selective Glucocorticoid Receptor Agonists and Modulators (SEGRAMs) [98]. These are not yet in clinical trials for autoimmune MG but might be useful as an alternative for classical corticosteroids. Vamorolone is an oral compound for daily use and was recently approved by EMA for use in Duchenne Muscular Dystrophy in boys older than 4 years. The efficacy appeared comparable to daily use of corticosteroids, while there was less effect on bone health and growth reduction [99].

At last, also new symptomatic drugs for MG are under development. NMD670, an orally administered muscle chloride channel inhibitor, is being developed for symptomatic treatment in MG [Dutch Trial Register (onderzoekmetmensen.nl)].

Altogether, a large group of new drugs will be available in the next years for treatment of autoimmune MG. For both MG patients with or without known serum antibodies, the new drugs most likely will become available first for patients with more severe or refractory disease. Healthcare costs will also play an important role in many countries. In addition, especially for the patients with SNMG the most likely pathogenic mechanism and previous participation of SNMG patients in a particular clinical trial might form additional factors.

9. Diagnostic algorithm in SNMG

As discussed above, diagnosis of SNMG may be challenging and no specific diagnostic recommendations from international panel of experts are available in literature. Hence, a diagnostic algorithm for SNMG was discussed among the participants in a dedicated session moderated by Elena Cortes-Vicente and Bettina Schreiner and finalized as Fig. 1.

The starting point of this algorithm is the presence of clinical features strongly suggestive of MG associated with negative serological testing including CBAs. Literature data show that CBAs are very sensitive and can be good alternatives to RIPA, but the latter remains the most standardized serological test to date. According to the algorithm, two lines of evidence are required to make a definite diagnosis of SNMG, combining the evidence of a neuromuscular transmission defect with prove of an immune mechanism of the disorder. In agreement with literature reports, an unclear response to immunotherapy and borderline results of electrophysiology are associated with high risk of misdiagnosis.

10. Conclusions and workshop deliverables

Overall, this workshop has shown some heterogeneity in the SNMG diagnostic work-up. New Ab assays improve the serological diagnosis, but the fixed CBAs are not standardized and live-CBA has limited availability. A multicenter study comparing sensitivity and specificity of the different Ab assays is in preparation. In addition, an international lab network allowing exchanges among centers can improve the access to optimal serological diagnosis that currently represents a patients' major unmet need. SNMG confirmation is difficult, and myopathies, CMS and functional disorders are the commonest misdiagnoses. Improving the neurologist's awareness seems crucial to avoid long diagnostic delays and inappropriate treatment.

This workshop has clearly shown a remarkable variability in SNMG response to therapy and long-term prognosis. While this may be due, at least in part, to differences in diagnostic criteria, the absence of detectable Abs should advice caution in prescribing treatment. Studies on the disease natural history and precise prospective data on treatment response are very important in further delineation of the disorder and in directing treatment choices.

List of participants

Erik Niks (NET) department of Neurology, Leiden University Medical Center, Leiden, the Netherlands.

Sithara Ramdas (UK), Department of Paediatric Neurology, John Radcliffe Hospital, Oxford, UK; MDUK neuromuscular centre, Department of Paediatrics, University of Oxford, UK.

Daniel Natera-de Benito (SPA), Neuromuscular Unit, Pediatric Neurology Department, Hospital Sant Joan de Déu, Barcelona, Spain.

Ulrike Schara-Schmidt (GER), Department of Pediatric Neurology, Developmental Neurology and Social Pediatrics, University of Essen, Essen, Germany.

Elena Cortes-Vicente (SPA), Unitat Patologia Neuromuscular Servei Neurologia, Hospital Santa Creu i Sant Pau, Barcelona, Spain.

Anthony Behin (FRA), Institut de Myologie GH Pitié-Salpêtrière, Paris, France

Sarah Hoffmann (GER), Center for Stroke Research Berlin, Charité -Universitätsmedizin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Berlin, Germany.

Nils Erik Gilhus (NOR), Department of Clinical Medicine, University of Bergen, Bergen, Norway; Department of Neurology, Haukeland University Hospital, Bergen, Norway.

John Vissing (DEN), Copenhagen Neuromuscular Center, Department of Neurology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.

Jeannine Heckmann (South Africa), Division of Neurology, University of Cape Town, Cape Town, South Africa.

Donald Sanders (USA), Department of Neurology, Duke University Medical Center, Durham, USA.

Anna Rostedt Punga (SWE), Clinical Neurophysiology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

Martijn Tannemaat (NET), Department of Neurology, Leiden University Medical Center, Leiden, the Netherlands.

Renato Mantegazza (ITA), Neuroimmunology and Neuromuscular Diseases Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

Bettina Schreiner (SWI), Department of Neurology, University Hospital Zürich, Zürich, Switzerland.

Angela Vincent (UK), Nuffield Department of Clinical Neurosciences, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom.

Valentina Damato (ITA), Department of Neurosciences, Drugs and Child Health, University of Florence, Italy.

Patients'representatives

Maria Bonaria (Maya) Uccheddu. AIM| Associazione Italiana Miastenia ODV, Italy

EuMGA (European Myasthenia Gravis Association)

Johan Voerman (Dutch MG association, Netherlands)

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

AE received honoraria for serving as SPIN Award jury member from Grifols, as speaker from UCB and Argenx, for participating in meetings and advisory boards from UCB and Alexion. She declares no competing interest regarding this manuscript.

JP has received support for scientific meetings and honorariums for advisory work from Merck Serono, Chugai, Alexion, Roche, Amgen, UCB, Amplo. Grants from Alexion, Argenx, Amplo biotechnology. Shares in AstraZenica. She acknowledges partial funding to the trust by Highly specialised services NHS England for the CMS service. She is on the UK NHSE IVIG Committee and until April 2024 was on the ABN advisory group for neuromuscular diseases.

JJGMV has been involved MG research sponsored by the Princes Beatrix Fonds, Health Holland and consultancies for Argen-X, Alexion, and NMD Pharma. Reimbursements were received by the LUMC. He is coinventor on patent applications based on MuSK-related research. The LUMC receives royalties for MuSK antibody assays. He is a member of the European Reference Network for Rare Neuromuscular Diseases [ERN EURO-NMD].

LM has received honoraria for speaking, advisory boards and compensation for congress participations from Sanofi Genzyme, Roche, Biogen, Amicus Therapeutics, Alexion, Argenx, UCB, Janssen, Lupin, outside the submitted work.

US received honoraria for aboard attendance and invited talks from Alexion.

DS has no relevant Conflicts of Interest to declare.

NEG has received honoraria for advisory work and as a speaker from Aera, Alexion, Amgen, Argenx, Denka, Dianthus, Grifols, Huma, Immunovant, Johnson & Johnson (Janssen), Merck, Roche, Takeda, UCB.

ECV has received public speaking honoraria and compensation for advisory boards and/or consultations fees from UCB, argenx, Alexion, Janssen and Lundbeck. She declares no competing interest regarding this manuscript.

JH has received honoraria for serving as speaker for Roche and for participating in meetings and advisory boards for Merck and Argenx. She declares no competing interest regarding this manuscript.

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CRediT authorship contribution statement

Amelia Evoli: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. **Jacqueline Palace:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Gregorio Spagni:** Data curation, Investigation, Writing – review & editing. **Marta Cheli:** Data curation, Investigation, Writing – review & editing. **Annabel Ruiter:** Data curation, Investigation, Writing – review & editing. **Jan Verschuuren:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Lorenzo Maggi:** Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing.

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Supplementary materials

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