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Neurologie, Neurochirurgie und Psychiatrie
Klinik für Neurologie und Experimenteller Neurologie
Direktor: Prof. Dr. med. Matthias Endres

Habilitationsschrift

Biomarker zur therapeutischen Stratifikation von Patient*innen mit Myasthenia gravis

zur Erlangung der Lehrbefähigung für
das Fach experimentelle Neurologie

vorgelegt dem Fakultätsrat der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

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Dekan: Professor Dr. med. Joachim Spranger

INHALTSVERZEICHNIS

Abkürzungen

AChR - Acetylcholinrezeptor

Ak- Antikörper

CLP - Calprotectin

CMS - kongenitales myasthenes Syndrom

COVID-19 - Coronavirus disease 2019

CT - Computertomografie

ELISA - Enzyme-linked Immunosorbent Assay

EMG - Elektromyographie

EOMG - Early-onset MG

HC - gesunde Kontrollen

gMG - generalisierte Myasthenia gravis

GWAS – genomweite Assoziationsstudie

iMZ - integriertes Myasthenie-Zentrum

LOMG - late onset MG

LRP4 - Low density lipoprotein receptor-related protein 4

MG - Myasthenia Gravis

MG-ADL - Myasthenia gravis Activities of daily living

MGFA - Myasthenia gravis Foundation of America

MMS - Minimaler Manifestationsstatus

MRT - Magnetresonanztomographie

MS - Multiple Sklerose

MuSK - Muskelspezifische Kinase

MyaReg - Deutsches Myasthenie-Register

NGS - next generation sequencing

NME - neuromuskuläre Endplatte

QMG - quantitative Myasthenia gravis Score

RA - rheumatoide Arthritis

sNfl - Serum Neurofilament light chain

SNMG - seronegative MG

TAMG - Thymom- assoziierte MG

WES - whole exome sequencing

1. Einleitung

1.1. Epidemiologie und Klassifikation der Myasthenia gravis

Die Myasthenia gravis (MG) ist die häufigste neuromuskuläre chronische Autoimmunerkrankung. Sie stellt mit einer Inzidenz von 8-10/ 1 Mio. Einwohnern und einer Prävalenz von ca. 150-250 Fälle pro 1 Mio. Einwohnern eine sogenannte seltene Erkrankung (*Orphan disease*) dar [1].

Das führende klinische Symptom ist die fluktuierende, belastungsabhängige, im Tagesverlauf zunehmende Muskelschwäche, die sich unter Ruhebedingungen bzw. Erholung bessert. In Abhängigkeit von der klinischen Manifestation unterteilt man die Erkrankung in eine okuläre, bulbäre oder generalisierte Verlaufsform. Der Verlauf und Schweregrad der Erkrankung ist individuell sehr unterschiedlich. Die Hälfte aller MG-Patient*innen präsentiert sich initial mit okulären Beschwerden (Doppelbilder, Ptosis). Aus dieser Patientengruppe präsentieren im Verlauf von 2-3 Jahren ca. 80% eine generalisierte Verlaufsform (gMG) [2]. Diese kann sämtliche Muskelgruppen des Körpers betreffen, zumeist findet sich jedoch eine Extremitätenschwäche (Extremitäten-betonte Verlaufsform) oder der oropharyngealen Muskulatur (bulbäre Verlaufsform). Nicht selten sind sowohl die Rumpf-Muskulatur als auch die Kopfhaltemuskulatur betroffen. Die Affektion der Atemmuskulatur kann zu einer partiellen und letztlich auch globalen respiratorischen Insuffizienz führen. Bei einer respiratorischen Globalinsuffizienz mit Beatmungspflichtigkeit spricht man von einer myasthenen Krise. Bekannte Trigger-Faktoren sind schwere bakterielle Infektionen, Operationen und Schwangerschaften und kommen bei bis zu 20% der Verläufe vor [3-5]. Eine myasthene Krise ist nach wie vor mit einer vergleichbar hohen Mortalität von ca. 5% verbunden [3, 4, 6].

Die MG wird heutzutage anhand des Antikörperstatus, der Thymuspathologie, des Alters (juvenile MG <18 Jahre, *early-onset* MG (EOMG, <50 Jahre, *late-onset* MG (LOMG, ≥50 Jahre) und der Art des Verlaufs (okulär versus generalisiert) klassifiziert [1]. Hiervon abzugrenzen sind die paraneoplastischen Myasthenien, welche Thymom-assoziiert (TAMG) sind und bei ca. 10-15% der MG-Patient*innen diagnostiziert werden.

Mit einer optimalen Behandlung sind die Prognosen, hinsichtlich der Letalität, gut. Jedoch leiden viele Patient*innen unter einer eingeschränkten Lebensqualität, welche nicht nur durch die medikamentösen Nebenwirkungen bedingt ist, sondern auch in den sozioökonomischen und psychosozialen Auswirkungen der Erkrankung begründet liegen [7,

8]. Ein wesentliches Problem bei der Behandlung der MG ist zudem, dass der Verlauf gerade in den ersten 3 Jahren schwer vorherzusehen ist und bisher keine prädiktiven Biomarker für einen hochaktiven Verlauf zur Verfügung stehen [9].

1.2. Pathophysiologie, Klinik und Diagnostik der Myasthenia gravis

Die MG gilt als Prototyp einer antikörpervermittelten Autoimmunerkrankung. Durch Bildung von Auto-Antikörpern (Ak) gegen postsynaptische Strukturen der neuromuskulären Endplatte (NME) kommt es zu einer belastungsabhängigen muskulären Schwäche.
Pathophysiologisch spielen zum einen genetische Faktoren eine wichtige Rolle. Es wurden zwar bislang keine groß angelegten Zwillingsstudien bei der MG durchgeführt, aber frühere genomweite Assoziationsstudien (GWAS) haben die auf dem Einzelnukleotidpolymorphismus basierende Heritabilität auf etwa 26% geschätzt [10]. Ungefähr 5% der Patient*innen haben eine familiäre Vorgeschichte von MG und in 28% der Fälle berichten Patient*innen, dass bei Verwandten andere Autoimmunerkrankungen vorliegen [11-13]. Frühere GWAS von MG haben genetische Loci am Haupthistokompatibilitätskomplex identifiziert [14] und spezifische Varianten gefunden, die mit der EOMG [14] und LOMG [15] assoziiert sind. Zudem wurde die MG mit mehreren Allelen des menschlichen Leukozytenantigens (HLA) in Verbindung gebracht, die eine entscheidende Rolle für die Immunfunktion ausüben [11, 15-17].
Bislang bleibt unklar, wodurch die MG ausgelöst wird, wobei die Thymuspathologie dabei eine zentrale Rolle spielt [18]. Gut verstanden sind jedoch die Ak-vermittelten Effektor-Mechanismen an der NME, die eine Erregungsstörung auslösen und klinisch zu einer belastungsabhängigen muskulären Schwäche führt.
Allen Ak gemeinsam ist, dass sie gegen postsynaptische Strukturen der NME gerichtet sind und damit Einfluss auf die Erregungsübertragung haben. Acetylcholinrezeptor (AChR)-Ak sind die häufigsten Auto-Ak und lassen sich bei ca. 80-85% der MG- Patient*innen finden [19] **(Abb. 1)**. In der Kohorte des Deutschen Myasthenie-Registers (MyaReg) (n=2485, Stand Januar 2024) liegt der Anteil allerdings nur bei ca. 75% (*unpublizierte Daten*).

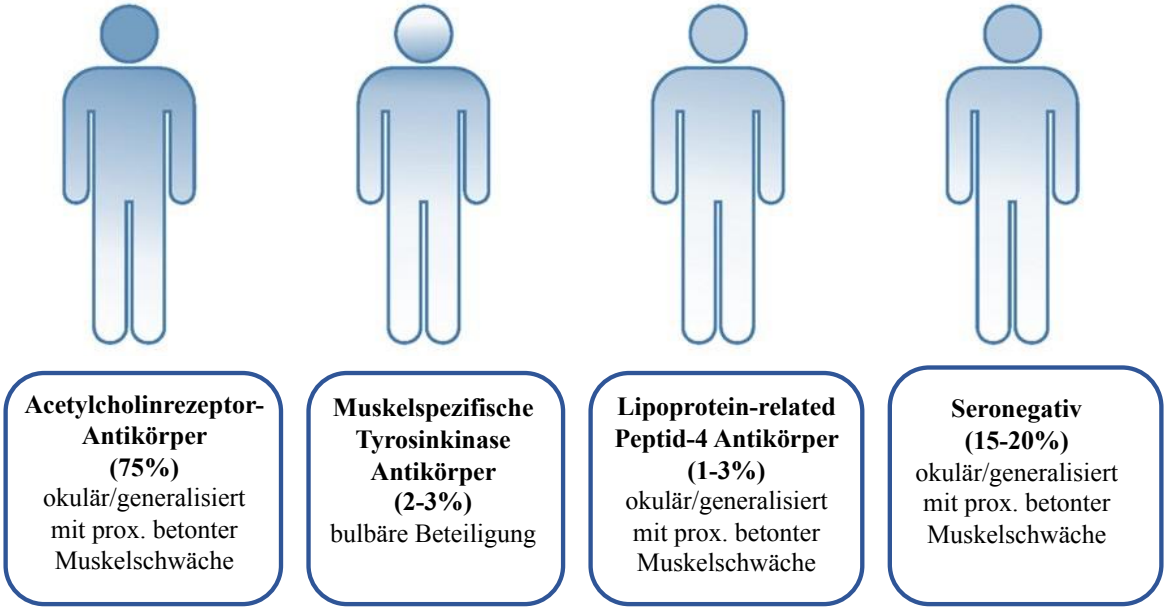

Abbildung 1: Antikörperverteilung und klinische Präsentation der Myasthenia gravis (eigene Abbildung).

Ein wichtiger Pathomechanismus der AChR-Ak, welche vorrangig vom IgG1- und IgG3-Typ sind, ist die Komplementaktivierung, welche durch die Bildung des Membranangriffkomplexes zur Zerstörung der postsynaptischen Membran führt [20] **(Abb 2)**. Ein weiterer wichtiger Pathomechanismus ist das Cross-Linking der AChR über Ak-Bindung. Dieser Mechanismus führt zur AChR-Internalisation und Zerstörung der Endplatte und kann auch durch eine vermehrte AChR-Synthese nicht kompensiert werden [21]. Selten spielt die direkte Blockade der AChR-Ak am Rezeptor ein Rolle [22]. Patienten mit Ak gegen die Muskelspezifische Tyrosinkinase (MuSK) weisen einen eigenen Pathomechanismus auf. Da die MuSK-Ak, im Gegensatz zu AChR-Ak, vom IgG4-Typ sind, können diese das Komplementsystem nicht aktivieren [23]. MuSK ist ein transmembranäres Protein, welches durch den LRP4-Agrin-Komplex aktiviert wird und für das Clustering des AChR an der NME eine wichtige Rolle spielt. MuSK-Ak werden bei ca. 1-10% der MG-Patient*innen gefunden. In Deutschland liegt der Anteil von MuSK-Ak positiven MG-Patient*innen bei ca. 3% (*unpublizierte Daten des MyaRegs*). Durch deren Bindung wird die Formation des AChR unterbunden, sodass deren Anzahl abnimmt [24]. Klinisch unterscheiden sich die MuSK-Ak positiven MG Patient*innen von AChR-Ak positiven Patient*innen: hier sind besonders Frauen mittleren Alters betroffen, welche häufiger als Patient*innen mit AChR-Ak eine bulbäre Beteiligung mit respiratorischen Krisen aufweisen [1]. Thymome, die bei ca. 10-15%

der AChR-Ak positiven MG-Patient*innen auftreten, werden bei MuSK-positiven Patient*innen in der Regel nicht beobachtet. Ebenso ist eine Thymektomie zur immunmodulatorischen Therapie bei MuSK-Ak positiven MG-Patient*innen nicht indiziert [25].

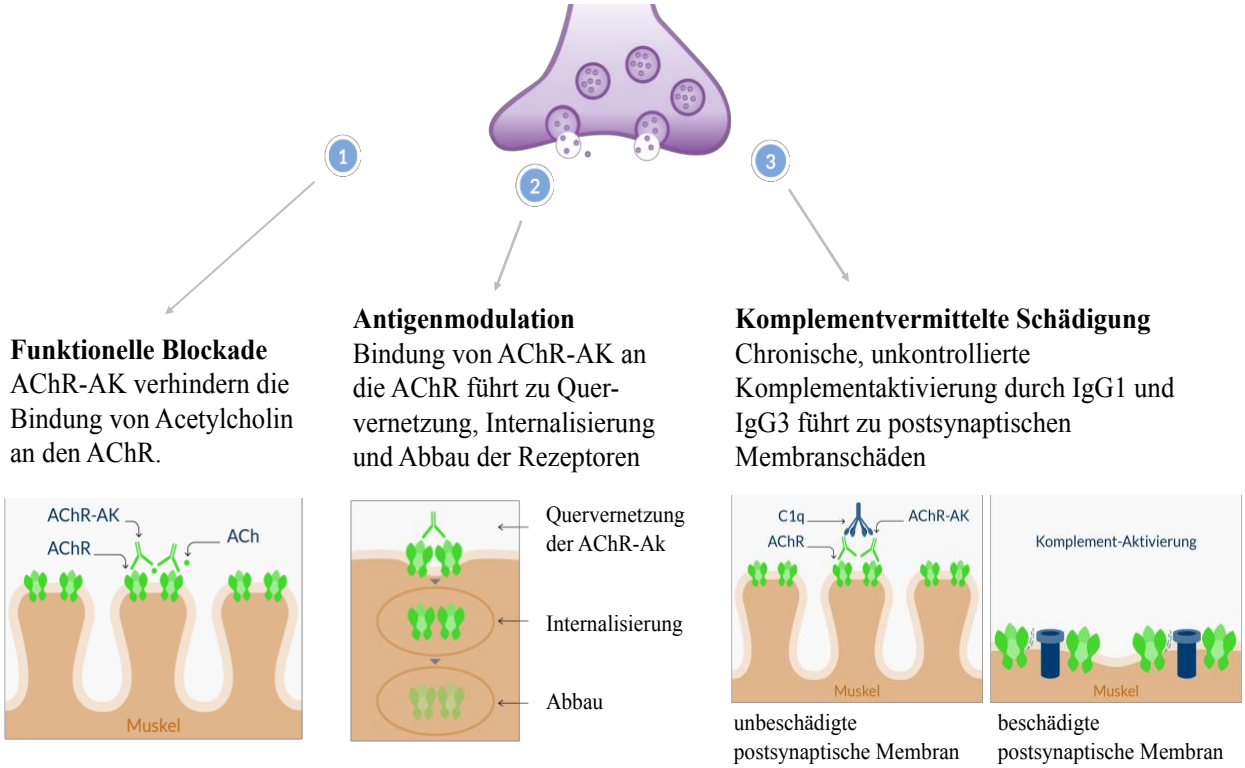

Abbildung 2: Pathophysiologische Mechanismen der Acetylcholinrezeptor-Antikörper (AChR-Ak) an der neuromuskulären Endplatte (eigene Abbildung).

Patient*innen mit Ak gegen das Low-Density-Lipoprotein-Rezeptor-verwandte Protein 4 (LRP4), welche bei ca. 3% der Patient*innen nachgewiesen werden können (*unpublizierte Daten des MyaRegs)*, weisen klinisch ähnlich wie die AChR-Ak positiven Patient*innen eine okuläre und generalisierte Beteiligung auf. Ebenso wie bei der MuSK-Ak positiven MG liegt auch bei der LRP4-positiven MG eine Thymom-Assoziation nicht vor [1]. Der klinische Schweregrad und das therapeutische Ansprechen bleibt Bestandteil aktueller Forschung. Der Anteil sogenannter seronegativer Patient*innen (SNMG) (keine AChR, MuSK oder LRP4-Ak) liegt bei ca. 15-20% (*unpublizierte Daten des MyaRegs*). Insbesondere bei diesen Patient*innen sind weitere diagnostische Maßnahmen, wie die elektrophysiologische Untersuchung, pharmakologische Testung und eine erweitere Ak-Diagnostik mittels zellbasierter Assays zum Nachweis von sogenannten Cluster-AChR-Ak indiziert [26-28]. In spezialisierten Zentren kann mittels Interkostalmuskelbiopsie der Nachweis von

Immunglobulin- und Komplementablagerungen als pathognomisches Merkmal autoimmuner myasthener Syndrome erfolgen. Im Falle eines seronegativen Ak-Status kann somit die Diagnose bestätigt und SNMG-Patient*innen mit hochaktiven Verläufen eine Therapie mit komplementinhibierenden Therapien ermöglicht werden [29]. Bei positiver Familienanamnese oder fehlendem Nachweis von Komplementablagerungen in der Interkostalmuskelbiopsie sollte zudem ein kongenitales myasthenes Syndrom (CMS) mittels next generation sequencing (NGS), besser jedoch mittels whole genome sequencing (WES) ausgeschlossen werden (**Abb. 3**).

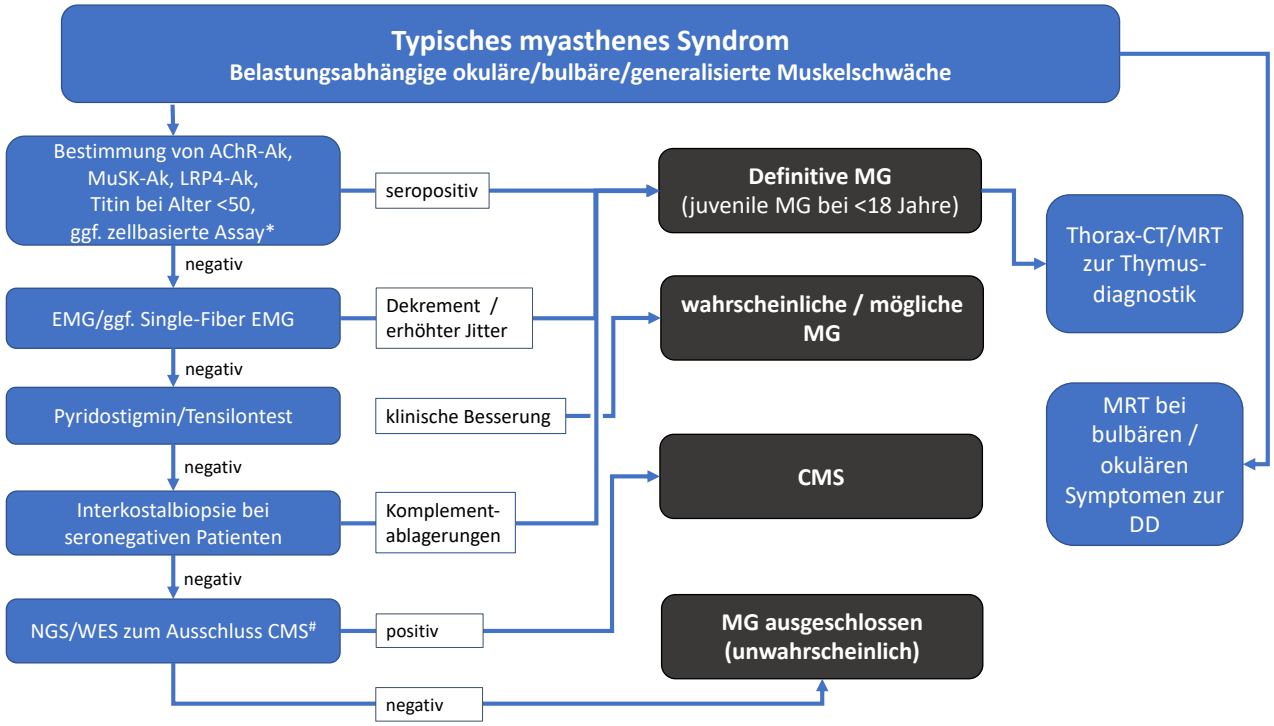

Abbildung 3: Diagnostischer Algorithmus der Myasthenia gravis (eigene Abbildung).

Abkürzungen: CMS=kongenitales myasthenes Syndrom, CT=Computertomographie, DD=Differentialdiagnose, EMG=Elektromyographie, MRT=Magnetresonanztomographie, NGS=next generation sequencing, WES=whole genome sequencing

1.3. Therapie

Aufgrund der aktuell dynamischen Entwicklung neuer Medikamente ist die Leitlinie zur Therapie von myasthenen Syndromen grundlegend angepasst worden [30]. Die neue Leitlinie ermöglicht nun über die klinische Definition der Krankheitsaktivität den Einsatz der modernen Immunmodulatoren auf Basis eines Stufenansatzes. Die Detektion der

Erkrankungsaktivität erfolgt rein klinisch und orientiert sich an der Myasthenia gravis Foundation of America (MGFA)-Klassifikation. Diese unterscheidet die (aktuelle) Erkrankungsschwere und unterteilt in eine mild/moderate versus eine hochaktive MG. Letztere umfasst auch den Begriff der „therapierefraktären" gMG. Hier ist ein entsprechender Score erarbeitet worden, der diese Definition im klinischen Alltag erleichtern soll [31]. Das Therapieziel ist nach der neuen Myasthenie-Leitlinie die „bestmögliche Krankheitskontrolle unter Wiederherstellung der Lebensqualität der Patient*innen" [30]. Darüber hinaus orientiert sich die Therapie neben der Krankheitsaktivität zunehmend am Ak-Status.

Neben der symptomatischen Therapie kommen regelhaft immunsuppressive Therapieverfahren zum Einsatz, die neben Steroiden klassische Langzeit-Immunsuppressiva umfassen (Azathioprin, Mycophenolat Mofetil, Methotrexat, Ciclosporin). Die Thymektomie ist Bestandteil der multimodalen Therapie, vor allem bei der AChR-Ak positiven, generalisierten *EOMG* und sollte frühzeitig im Krankheitsverlauf durchgeführt werden [32].

In den letzten Jahren sind neue therapeutische Ansätze entwickelt worden, von denen vor allem die AChR-Ak positiven MG-Patient*innen mit hochaktiven Krankheitsverläufen profitieren. Während der C5-Komplementinhibitor Eculizumab der sogenannten therapierefraktären AChR-Ak positiven gMG vorbehalten war, wurden kürzlich zwei neue Wirkstoffe, der neonatale FcRezeptor (FcRn)-Inhibitor Efgartigimod [33] sowie der weiterentwickelte C5-Komplementinhibitor Ravulizumab [34] als *add-on* Therapie der AChR-Ak positiven gMG zugelassen. Zudem wurden für beide Substanzgruppen kürzlich subkutane Formulierungen zugelassen, um eine höhere Patientenautonomie zu ermöglichen [35, 36]. Darüber hinaus mehren sich die Hinweise, dass bereits frühzeitig nach Diagnosestellung eine immunmodulierende Therapie erfolgen sollte, um das Erreichen eines minimalen klinischen Manifestationsstatus (MMS) zu ermöglichen. Dies legen die Ergebnisse der RINOMAX-Studie nahe, die den Einsatz von Rituximab bei früh diagnostizieren AChR-Ak positiven MG-Patient*innen untersuchte [37]. Aber auch ältere Studien über den frühzeitigen Einsatz von Steroiden zeigen ähnliche Ergebnisse [38]. Bei hochaktiven Verläufen der MG mit Ak gegen MuSK sollte frühzeitig der Einsatz von Rituximab erwogen werden [39]. Weitere wesentliche Fortschritte sind von spezifischen Immunmodulatoren zu erwarten, die auf die B- und T-Zellimmunologie zielen [40, 41].

Aufgrund der Seltenheit der Erkrankung wird die Behandlung bzw. deren Koordination über spezialisierte Zentren, wie die integrierten Myasthenie-Zentren (iMZ) empfohlen. Aktuell gibt es in Deutschland 19 iMZ, die durch die Deutsche Myasthenie Gesellschaft und das Bundesinstitut für Qualität und Patientensicherheit zertifiziert wurden. Seit Februar 2019 besteht mit dem MyaReg erstmalig ein zentrales Register, welches longitudinal Daten zu soziodemografischen Faktoren, Diagnose, Therapie, Nebenwirkungen und Patient- reported Outcome Parametern erhebt, um genauere Einblicke in die Versorgungsrealität von Myasthenie-Patient*innen zu erhalten. Bislang konnten knapp 2500 Patient*innen mit myasthenen Syndromen eingeschlossen werden. Darüber hinaus wurde eine zentrale Biobank etabliert, um die multizentrische Entwicklung prognostischer Biomarker zu ermöglichen.
Neben der Verbesserung der Versorgungsqualität dienen die Daten des MyaRegs darüber hinaus zur Beantwortung wichtiger epidemiologischer Fragestellungen. So untersuchten wir zu Beginn der COVID-19 Pandemie das Outcome einer COVID-19 Infektion bei MG-Patient*innen mit bestehender Immunsuppression [42]. Aktuell laufen eine Vielzahl weiterer, multizentrischer Projekte, wie z.B. die klinische Phänotypisierung von LRP4-positiven MG-Patient*innen, Identifikation von geschlechterspezifischen Unterschieden bei der MG oder die Erhebung der Erkrankungslast bei der SNMG.

1.4. Zielstellung der Habilitationsschrift

Mit den neuen Immunmodulatoren ergeben sich Möglichkeiten für eine frühe intensivierte Immuntherapie. Im Gegensatz zu den klassischen Langzeit-Immunsuppressiva führen diese Therapien zur raschen Verbesserung der Krankheitsverläufe und das, soweit bisher beurteilbar, mit geringeren Nebenwirkungen. Um diese Medikamente zunehmend gezielter und damit effektiver einsetzen zu können, müssen prädiktive Biomarker für die Krankheitsaktivität, das Therapieansprechen und die Krankheitsprognose entwickelt werden. Darüber hinaus bieten die Daten des MyaRegs die Möglichkeit, multizentrische epidemiologische und Biomarker-Studien durchzuführen, um somit zu einem besseren Verständnis der Pathogenese, Krankheitsverläufe und des Therapieansprechens beizutragen. Im Rahmen meiner Forschungstätigkeit und der damit hier vorgelegten Habilitationsschrift stehen daher folgende Fragestellungen im Fokus:

1. Identifizierung von klinischen Risikofaktoren für einen schweren und hochaktiven Verlauf der MG.

2. Identifikation von blutbasierten Biomarkern zur Detektion der Erkrankungsaktivität, therapeutischen Stratifizierung und Prädiktion des Erkrankungsverlaufs von Patient*innen mit MG.

2. Eigene Arbeiten

2.1. Unabhängige Risikofaktoren für myasthene Krise und Exazerbation in einer retrospektiven Myasthenia gravis-Kohortenstudie

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Die myasthene Exazerbation und Krise kommt bei bis zu 20% der Verläufe vor und ist trotz einer verbesserten intensivmedizinischen Therapie immer noch mit einer vergleichbar hohen Mortalität von ca. 5% verbunden [3, 4, 6].

Ziel dieser multizentrischen Arbeit an acht iMZ in Deutschland war es deshalb, sowohl potenzielle Prädiktoren für eine myasthene Exazerbation und Krise zu detektieren als auch Faktoren zu identifizieren, die mit einem schlechten Verlauf assoziiert waren. Im Zeitraum von 2000-2021 wurden in dieser retrospektiven Beobachtungsstudie 815 MG-Patient*innen mit einer durchschnittlichen Nachbeobachtungszeit von 62,6 Monaten eingeschlossen. Mittels multivariaten Regressionsanalysen erfolgte die Bestimmung von Risikofaktoren für eine myasthenen Krise und Krankheitsexazerbation.

Der Schweregrad der Erkrankung zum Zeitpunkt der Diagnose gemessen am quantitativen Myasthenia gravis (QMG)-Score und der MGFA-Klassifikation, das Vorhandensein von Thymomen sowie ein MuSK-positiver Ak-Status waren unabhängige Prädiktoren für eine myasthene Krise oder Exazerbation. Patient*innen mit einem MMS 12 Monate nach der Diagnose hatten ein geringeres Risiko für eine myasthenen Krise oder Exazerbation.

Prädiktoren für einen schlechten Verlauf waren neben einem höheren Patientenalter und klinischen Schweregrad, die Anzahl der Komorbiditäten, eine niedrige Vitalwert-Kapazität bei Aufnahme, die Notwendigkeit einer Intubation und eine myasthenen Krise, die durch eine Infektion ausgelöst wurde. Bezüglich der Therapie fanden wir keinen Unterschied im Outcome im Vergleich der Behandlungen mit intravenöses Immunglobulinen vs. Plasmaaustausch oder einer Kombinationstherapie.

Die Ergebnisse dieser bislang größten, retrospektiven Beobachtungsstudie zeigen, dass eine myasthene Krise und Exazerbation eine erhebliche Krankheitslast für MG-Patient*innen darstellt. Neben dem Ak-Status und dem Vorhandensein eines Thymoms stellt insbesondere die klinische Erkrankungsschwere zum Zeitpunkt der Diagnose einen robusten Risikofaktor für die Entwicklung einer myasthenen Krise dar, sodass diese Patientengruppe intensiver monitoriert werden sollte. Darüber hinaus unterstreicht diese Arbeit, dass die Prävention und rasche Behandlung von Infektionen eine wichtige Rolle für das Outcome einer myasthenen Krise spielt.

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RESEARCH

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Independent risk factors for myasthenic crisis and disease exacerbation in a retrospective cohort of myasthenia gravis patients

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Abstract

Background: Myasthenic crisis (MC) and disease exacerbation in myasthenia gravis (MG) are associated with significant lethality and continue to impose a high disease burden on affected patients. Therefore, we sought to determine potential predictors for MC and exacerbation as well as to identify factors affecting outcome.

Methods: We examined a retrospective, observational cohort study of patients diagnosed with MG between 2000 and 2021 with a mean follow-up of 62.6 months after diagnosis from eight tertiary hospitals in Germany. A multivariate Cox regression model with follow-up duration as the time variable was used to determine independent risk factors for MC and disease exacerbation.

Results: 815 patients diagnosed with MG according to national guidelines were included. Disease severity at diagnosis (quantitative MG score or Myasthenia Gravis Foundation of America class), the presence of thymoma and anti-muscle specific tyrosine kinase-antibodies were independent predictors of MC or disease exacerbation. Patients with minimal manifestation status 12 months after diagnosis had a lower risk of MC and disease exacerbation than those without. The timespan between diagnosis and the start of immunosuppressive therapy did not affect risk. Patients with a worse outcome of MC were older, had higher MGFA class before MC and at admission, and had lower vital capacity before and at admission. The number of comorbidities, requirement for intubation, prolonged mechanical ventilation, and MC triggered by infection were associated with worse outcome. No differences between outcomes were observed comparing treatments with IVIG (intravenous immunoglobulin) vs. plasma exchange vs. IVIG together with plasma exchange.

Conclusions: MC and disease exacerbations inflict a substantial burden of disease on MG patients. Disease severity at diagnosis and antibody status predicted the occurrence of MC and disease exacerbation. Intensified monitoring

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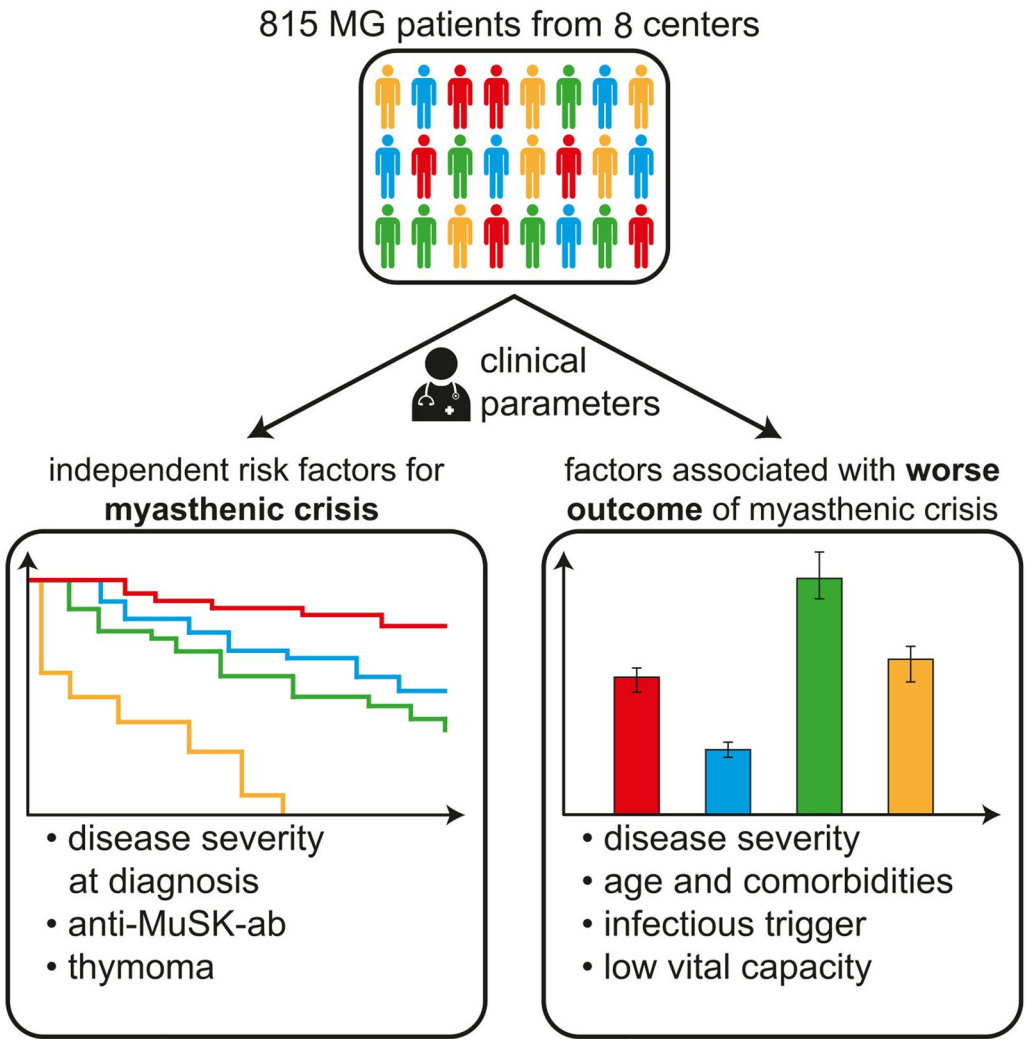
with emphasis on the prevention of infectious complications could be of value to prevent uncontrolled disease in MG patients.

Keywords: Myasthenic crisis, Myasthenia gravis, Disease exacerbation, Risk factors, Predictors

Graphical Abstract



Background

Myasthenia gravis (MG) is an acquired autoimmune disorder of the neuromuscular junction characterized by dysfunction of the post-synaptic membrane [1]. Owing to improved treatment strategies and diagnostic tools, therapeutic outcomes have improved for the majority of MG patients [2]. However, a clinically distinct subgroup of patients, often referred to as refractory, remains symptomatic despite therapy [2, 3]. Exacerbation of disease and myasthenic crisis (MC) are frequent in these patients and substantially contribute to disease burden [4]. Despite diagnostic and therapeutic advances for the management of MG, patients experiencing MC continue to face a substantial mortality rate of approximately 5–12% [5, 6]. The requirement for hospitalisation, the associated burden of disease and the cost of available rescue therapies, underline the importance of the prevention and management of MC [7, 8].

Hindered by the rarity of MG, our understanding of the underlying pathophysiological mechanisms related to insufficient disease control remains fragmented. A range of potential triggers for the manifestation of MC

or disease exacerbations have been observed including infections, surgery, adverse effects of medication, comorbidity, pregnancy or tapering of immunosuppressive medication [9, 10]. Prognostic factors identifying patients at risk for MC or disease exacerbations remain incompletely understood and have only been characterized for MG patients presenting with a thymoma [11, 12]. However, factors predicting the occurrence of MC especially in patients without thymoma remain largely elusive. Finally, factors defining the outcome of MC are incompletely identified, but urgently needed to guide the clinical management of these patients. Our analysis aims at understanding factors predicting clinical deteriorations. We therefore analysed a cohort of 815 MG patients to identify potential risk factors for MC and disease exacerbations.

Methods

Study design and participants

Our cohort study is a retrospective analysis of 815 patients from eight university hospitals in Germany (Charité—Universitätsmedizin Berlin and University Hospitals Cologne, Duesseldorf, Essen, Freiburg, Magdeburg, Muenster and Regensburg). Patients requiring intensive care were treated on specialized neurological intensive care units (NICU). Patients were identified by searching the on-site database for the corresponding ICD-10 code (ICD-10-GM-2019 G70.-). Overall, 1645 patients were screened, of whom 815 were included in the analysis (Fig. 1). Diagnosis of MG was established by characteristic clinical presentation in accordance with national guidelines [13], independent of disease duration or severity. All centres are certified as integrated myasthenia centre (iMC) by the German Myasthenia Gravis Society applying standardised clinical pathways for patient management. Diagnosis was supported by antibody findings and repetitive nerve stimulation. Antibody testing was performed by enzyme-linked- or radio-immunoassay (Euroline). Suspected cases without established diagnosis, with a change to their diagnosis ($n=609$) or with insufficient case documentation were excluded (<6 months of longitudinal documentation) ($n=127$) (Fig. 1). The final cohort consisted of patients diagnosed between January 2000 and July 2021. Patients with an established diagnosis and sufficient longitudinal documentation of >6 months were included during this time period. Socio-demographics (age, sex, disease duration), antibody (ab) status (acetylcholine-receptor (AChR), muscle specific receptor tyrosine kinase (MuSK), lipoprotein-related protein 4 (LRP4), seronegative), MG specific medication (cholinesterase-inhibitors, glucocorticoids, and long-term immunosuppressant's), history of thymoma-status, and comorbidities were collected from patient' charts. The follow-up strategy was standardized across centres. According to iMC standards, patients with a stable course were seen every 6 months and instable patients more frequently. MG-specific scoring was performed by the treating neurologist at the time of presentation.

Definitions

For this cohort analysis, we differentiated between MC and disease exacerbation as distinct clinical events.

A MC was defined as a rapid clinical decline requiring non-invasive ventilation, intubation or parenteral nutrition [14]. Dysphagia severe enough to require a nasogastric tube was also included as criterion for MC.

A disease exacerbation was defined as fulfilment all of the following criteria as adapted from national guidelines [15]:

- Objective: QMG (quantitative myasthenia gravis) score [16] of ≥8 points and a minimum increase of ≥5 points from the previous visit. Ocular findings must not account for more than 5 points on the QMG score.
- Subjective: progressive clinical deterioration due to weakness of bulbo-pharyngeal or limb muscles or reduced respiratory function impacting activities of daily living.
- Period of time: progress of symptoms no longer than 30 days.

A clinical event matching both the definition of MC and disease exacerbation was classified as MC. The outcome of MC was defined according to the MGFA (Myasthenia Gravis Foundation of America) post-intervention status (MGFA–PIS) [17, 18]: Specifically, improved signifies that QMG score at hospital discharge was reduced by ≥3 points compared to pre-admission, worse signifies that QMG score hospital discharge was increased by ≥3 points compared to before the admission and unchanged signifies that neither the criteria for improved nor worse were met. Patients with worse outcome were discharged for further rehabilitation. The threshold was defined to be that a score of 3 points in a single item of the QMG score reflects severe impairment [18]. The cutoff between early-(EOMG) and late-onset (LOMG) MG was set at 50 years as previously defined [19]. Minimal manifestation status (MMS) was defined in accordance with the MGFA–PIS as no symptoms of functional limitation from MG but weakness on examination only detectable by examination [17, 20, 21]. For MMS, immunosuppressive therapy and symptomatic therapy, e.g., cholinesterase inhibitors, were permitted (analogous to MMS-3 as proposed by the MGFA–PIS) [17, 20, 21].

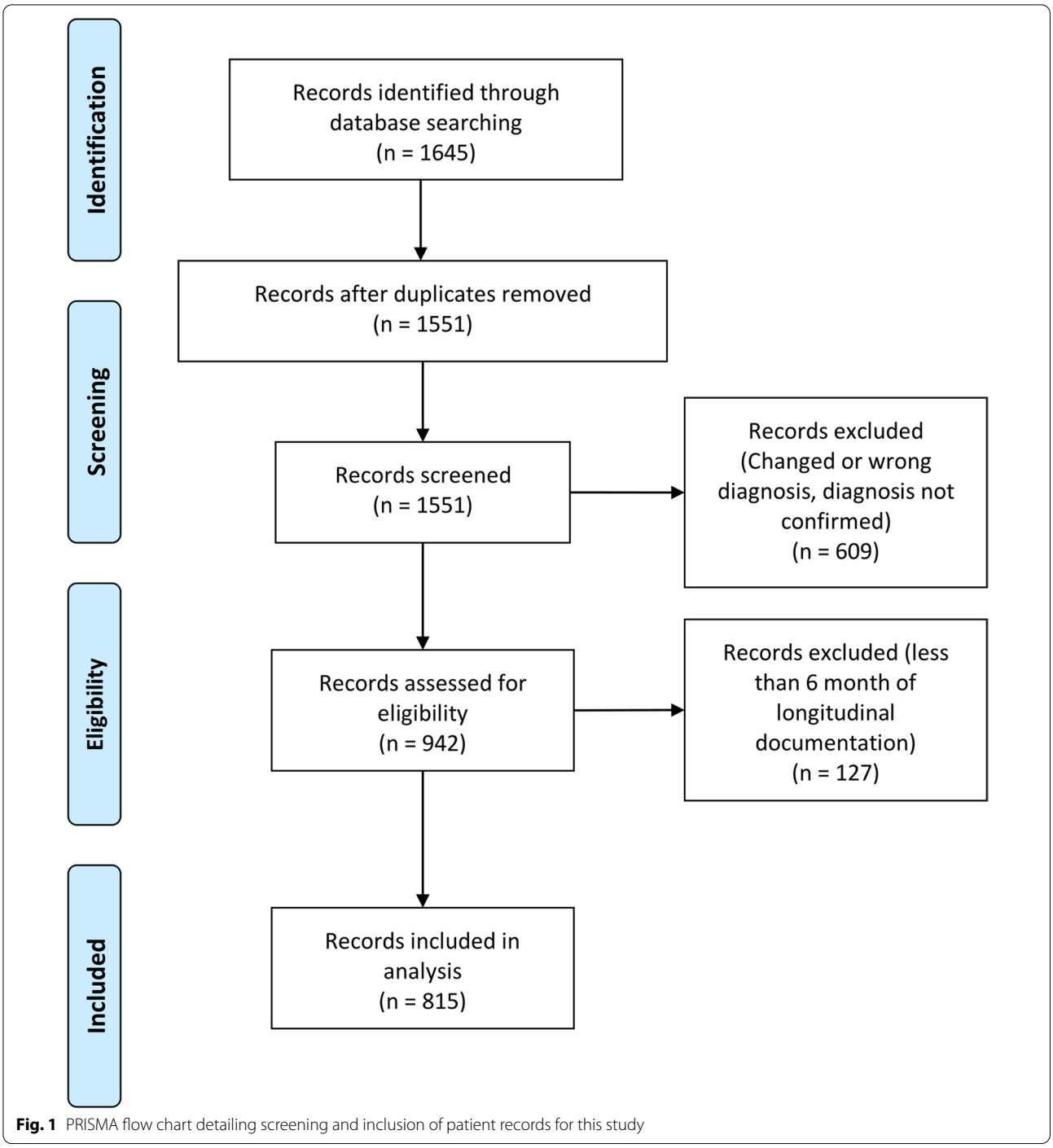


Fig. 1 PRISMA flow chart detailing screening and inclusion of patient records for this study

Standard protocol approvals, registrations, and patient consents

The study was approved by the local ethics committee and institutional review boards (no. AZ 2020-010-f-S, no. AZ 07/2017, 19-8973-BO, AZ 21-1265, AZ 21-1331). Data were anonymized and collected retrospectively according to the standardized requirements of the German register for myasthenia.

Statistical analysis

Statistical Analysis was performed using GraphPad Prism 9.3 (GraphPad Software, Inc., San Diego, CA) and

R (R Core Team, 2020). Data were presented as median (IQR = interquartile range), mean (standard deviation = SD), or *n* (%). For univariate logistic regression, goodness of fit was assessed by Cox-Snell's generalized R squared or Tjur's Pseudo R squared as appropriate. Significance was assessed by the likelihood ratio test. The odds ratio (OR) was assessed using a multivariate Cox regression model with follow-up as the time variable. Experiencing at least one MC or disease exacerbation compared to no event was used as the status variable. For analysis of time between diagnosis and MC or disease exacerbation the Kaplan–Meier method was used. Statistical significance between survival curves was determined by a pairwise log rank test. Analysis of variance (ANOVA) testing was performed for the analysis of groups for continuous variables and Fisher's exact test for categorial variables. To account for multiple comparisons, statistical significance was corrected by the false discovery rate (FDR). Anonymized data will be shared by request from any qualified investigator. For regression analysis of MGFA class II to IV, MGFA classes A and B were combined to allow for statistical analysis. Therefore, analysis is limited to MGFA classes without distinguishing the distribution of muscle weakness.

Results

Baseline characteristics and clinical features

Clinical and demographic data are presented in Table 1. Mean age at disease onset was 52.7 years (SD 20.0) and at diagnosis 53.5 years (SD 19.8). Early disease onset before the age of 50 years occurred in 300 patients (36.8%), while 510 cases (62.6%) were LOMG. The follow-up time was 62.6 months (SD 73.3) after diagnosis.

MGFA class at diagnosis was available for 782 patients (96.3%). 236 (28.9%) patients presented with ocular weakness (Class I); 309 (37.9%) with mild symptoms (Class II); 169 (20.8%) with moderate symptoms (Class III); 43 patients (5.3%) with severe muscle weakness (Class IV) and for 25 patients a history of intubation (3.0%) (Class V) was documented. Disease severity at diagnosis was classified by assessment of QMG score and was available for 687 patients (84.4%) [22]. Median QMG score at diagnosis was 4 points (IQR 2.0–8.0).

With respect to ab status, 714 (87.6%) patients were seropositive, whereas 86 (10.5%) were seronegative. The ab-status included anti-AChR-ab ($n = 641$), anti-MuSK-ab ($n = 71$), and anti-LRP4-ab ($n = 2$). 436 patients (53.5%) received corticosteroids following diagnosis with a mean dosage of 15 mg (SD 10). The average time between diagnosis and the start of the first immunosuppressive therapy (IST) was 1.3 years (SD 3.7). 451 patients (54.6%) received their first IST less than 1 year after diagnosis and were considered as early IST, while 111 patients

Table 1 Clinical and demographic baseline characteristics of patients

Characteristic	*n*	%
Total	**815**	**100**
Sex		
Male/Female	361/454	44.4/55.6
Age, y		
Mean age at first manifestation, years	52.7 ± 20.0	
Mean age at diagnosis, years	53.5 ± 19.8	
Early-onset MG (< 50 years)	300	36.9
Decremental response		
Positive	368	45.2
Negative	446	54.8
Increment		
Positive	6	0.7
Generalized MG at diagnosis		
Ocular MG	215	26.3
Generalized MG	589	72.7
MGFA class at diagnosis		
I (ocular)	236	28.9
II	309	37.9
III	169	20.8
IV	43	5.3
V	25	3.0
Missing	30	3.7
QMG-score at diagnosis, median ± IQR	4.0 (2.0–8.0)	
Antibody status		
Seronegative	86	10.5
Seropositive	714	87.6
Anti-AchR-ab	641	89.9
Anti-MuSK-ab	71	9.9
Anti-LRP4-ab	2	0.3
Anti-Titin-ab	156	21.8
Missing	15	1.8
Thymectomy	294	36.1
MRI or CT		
Thymoma-suspect	98	12.0
Histology		
Thymoma	158	19.4
First IST		
Azathioprine	475	58.2
MMF	46	5.6
Methotrexate	41	5.0
Cyclosporine	0	0
Mean corticosteroid dosage following diagnosis, mg	15 ± 10	
Concomitant diseases		
Cardiovascular	379	46.6
Arterial hypertension	289	35.4
Heart failure (any cause)	59	7.3
Aortic stenosis	64	7.8
Cardiac arrythmia	45	5.5

Table 1 (continued)

Characteristic	*n*	%
Other	111	13.6
Pulmonary	133	16.3
Chronic obstructive pulmonary disease	74	9.1
Asthma	28	3.5
Smoking	89	10.9
Other	23	2.8
Metabolic	185	22.7
Diabetes mellitus (type 1 or 2)	166	20.4
Hypercholesterolemia	155	19.0
Other	21	2.5
Gastrointestinal	167	20.5
Celiac disease	18	2.2
Gastroesophageal reflux disease	91	11.1
Liver failure (any cause)	15	18.4
Inflammatory bowel disease	8	0.9
Other	12	14.7
Malignancy other than thymoma	91	11.2
Lung cancer	22	2.7
Prostate cancer	33	4.0
Breast cancer	12	1.4
Other	15	1.8
Autoimmune disease	152	18.7
Hashimoto's disease	44	5.3
Rheumatoid arthritis	32	3.9
Psoriasis	34	4.2
Multiple sclerosis	3	0.3
Other	22	2.7
Months of follow-up	62.6 ± 73.3	

Baseline characteristics of included patients with myasthenic syndromes. *ab* antibody, *anti-AChR-ab* anti-acetylcholine-receptor-ab, *anti-MuSK-ab* anti-muscle-specific tyrosine kinase-ab, *anti-LRP4-ab* anti-low-density lipoprotein receptor-related protein 4-ab, *MC* myasthenic crisis, *MG* myasthenia gravis, *MMF* mycophenolate-mofetil, *IST* immunosuppressive therapy, *IQR* interquartile range, *SD* standard deviation. Unless otherwise reported, values are mean ± SD (range), median ± IQR (range) or *n* (%); QMG-score = quantitative myasthenia gravis-score

(13.5%) received IST after 1 year or more and were considered late IST. The remaining patients did not receive IST during the observation period.

Predictive factors for MC and disease exacerbation

Overall, 217 patients (26.3%) experienced a MC during their disease course while 225 patients (27.6%) experienced a disease exacerbation. To assess potential risk factors for the occurrence of MC or disease exacerbation, we employed a model of univariate logistic regression (Additional file 1: Table S1). We assessed the risk for experiencing at least one MC or disease exacerbation compared to patients experiencing no event. Aiming to identify independent risk factors, we entered risk factors reaching statistical significance ($p < 0.05$) in univariate analysis in a model of multivariate Cox regression. In addition, we included clinical parameters (sex and age) as they were related to clinical outcomes in previous studies [5]. To avoid overfitting, factors facing high collinearity were excluded (age at manifestation, thymectomy, imaging suggestive of thymoma). Accordingly, multivariate analysis revealed that QMG score at diagnosis [OR 1.23 95% confidence interval (95%CI) 1.14–1.66, $p < 0.0001$], MGFA class at diagnosis (OR 1.83 95% CI 1.65–1.97, $p < 0.001$), anti-MuSK-ab (OR 2.18 95% CI 1.76–2.59, $p < 0.05$) and the presence of a thymoma (OR 3.71 95% CI 3.01–4.41, $p < 0.0001$) predicted the occurrence of MC as independent risk factors (Table 2). Multivariate analysis of risk factors for disease exacerbation identified generalized disease (OR 1.83 95% CI 1.23–2.39, $p < 0.05$), QMG score at diagnosis (OR 1.12 95% CI 1.09 to 1.44, $p < 0.001$), anti-MuSK-ab (OR 1.07 95% CI 1.01–1.28,

Table 2 Risk factors for MC and exacerbation—Multivariate analysis

	Odds ratio	95%CI	*p*-value
MC			
Age at diagnosis	1.01	0.87–1.25	0.32
Sex	0.96	0.81–1.43	0.86
QMG score at diagnosis	1.23	1.14–1.66	**< 0.0001**
MGFA status at diagnosis	1.83	1.65–1.97	**< 0.0001**
Anti-MuSK-ab	2.18	1.76–2.59	**0.02**
Thymoma	3.71	3.01–4.41	**< 0.0001**
Cardiovascular disease	1.29	0.72–1.66	0.35
Heart failure (any cause)	1.11	0.71–1.78	0.48
Pulmonary disease	1.36	0.88–1.44	0.25
Chronic obstructive pulmonary disease	1.41	0.91–1.48	0.11
Exacerbation			
Sex	0.82	0.66–1.17	0.24
Age at diagnosis	1.03	0.76–1.51	0.45
Generalized disease at diagnosis	1.83	1.23–2.39	**0.03**
QMG score at diagnosis	1.12	1.09–1.44	**< 0.0001**
MGFA status at diagnosis	1.03	0.75–1.48	0.11
Anti-MuSK-ab	1.07	1.01–1.28	**0.003**
Thymoma	1.64	1.29–2.07	**0.02**
Pulmonary disease	1.22	0.71–1.47	0.32
Chronic obstructive pulmonary disease	1.32	0.92–1.41	0.12

Risk factors for MC and exacerbation in multivariate Cox regression analysis. *anti-Musk-ab* anti-muscle-specific tyrosine kinase-ab, *MGFA* Myasthenia Gravis Foundation of America, *SD* standard deviation, *QMG* quantitative myasthenia gravis score. Variables with a *p*-value < 0.05 in the univariate analysis and clinically relevant variables (sex, age) were included in the multivariate analysis. For highly collinear factors (thymoma, thymectomy and imaging suspect for thymoma as well as age at onset, age at diagnosis and early onset) we included only one variable to avoid overfitting. Risk is presented as odds ratio. A *p*-value below 0.05 was considered statistically significant. Statistically significant results are bold. 95% CI = 95% confidence interval

$p < 0.01$) and the presence of a thymoma (OR 1.56 95% CI 1.29–2.07, $p < 0.05$) as independent risk factors. Next, we applied the Kaplan–Meier method to our data set. Here, we observed an inverse relation between MGFA class and the occurrence of MC (Fig. 2A, B). In addition, we observed that anti-MuSK-ab status correlates with the risk for experiencing MC (Fig. 2C) or disease exacerbation (Fig. 2D) (Table 2).

Finally, we investigated whether therapeutic management of MG influences the occurrence of MC and exacerbation during the disease course. To assess the time between diagnosis and treatment as a potential risk factor, we separated the patient cohort by the time between diagnosis and the start of the first standard IST. Standard IST comprised of azathioprine, MMF, methotrexate and cyclosporine. Here, the risk for MC and disease exacerbation was not different for patients with early vs. late IST, respectively (MC: OR 0.38 95% CI 0.22–0.87, $p = 0.79$, exacerbation: OR 0.86 95% CI 0.65–0.99, $p = 0.38$). In addition to the time to treatment, we investigated the effect of treatment response on the occurrence of MC or exacerbation. We analysed the risk for MC and exacerbation for patients achieving MMS at 12 months after diagnosis and those who did not. To exclude bias due to patients presenting with MC or exacerbation as first manifestation, patients with a clinical event up to 6 months after diagnosis were excluded from the analysis of treatment response. Indeed, the risk was reduced for achieving MMS for MC (OR 0.32 95% CI 0.17–0.61, $p = 0.002$) and for exacerbation (OR 0.50 95% CI 0.34–0.70, $p < 0.001$). Next, using the Kaplan–Meier method we observed treatment non-responders as at risk to experience MC and exacerbations early in their disease as compared to treatment responders (Fig. 2E, F). To further dissect the importance of therapeutic management, we analyzed both cortisone treatment, as binary variable, and dosage, as continuous variable, as predictors for MC or exacerbation. Here, the risk for MC (OR 1.12 95% CI 1.05–1.33, $p = 0.16$) and exacerbation (OR 1.09 95% CI 1.01–1.45, $p = 0.42$) were similar for patients receiving cortisone following diagnosis compared with those who did not. In the group of cortisone-treated patients, assessment of cortisone dose did not reveal an association with the risk for MC (OR 1.27 95% CI 1.16–1.65, $p = 0.23$) or exacerbation (OR 1.52 95% CI 1.34–1.72, $p = 0.18$).

Factors determining the outcome of MC

Given the substantial mortality and lasting functional impairment associated with MC [5, 23], we further investigated potential factors affecting the outcome of MC. As detailed above, patients experiencing MC were grouped into three cohorts (improved, unchanged and worse). Clinical, demographic, diagnostic and therapeutic data were assessed for each cohort (Additional file 2: Table S2). Overall, 235 MC were recorded. In-hospital mortality was recorded for 6 patients (0.25%). To prevent bias (i.e., shorter ventilation time despite worse outcome), patients had died to MC were not included. Comparison of groups was performed on the remaining 229 MC. Recorded trigger factors are presented in Additional file 3: Table S3. Outcomes after MC were defined as improved for 143 MC (62.4%), unchanged for 33 MC (14.4%) and worse for 53 MC (23.2%) (Table 3).

Patients experiencing a worse outcome of following MC were older at the time of MC as compared to improved patients, while sex displayed no association with the outcome. MGFA class at admission as well as the last most recent measurement of MGFA class before prior to admission were lower in patients improving who improved. Interestingly, MC triggered by infections were was associated with a worse outcome. Consistent with previous reports, patients with a high number of comorbidities at admission had a worse outcome. Of note, vital capacity (VC) at admission, as well as the last recorded VC before MC, were was lower in patients worseningwho worsened. In addition, patients who were intubated, who had a longer time of mechanical ventilation or total hospital stay, and who developed pneumonia or sepsis had a poor outcome.

Finally, we analysed the impact of the available rescue therapies on the outcome of MC. Here, we compared the effect of IVIG (43 patients) vs. plasma exchange [PLEX (plasmapheresis) or IA (immunoadsorption)] (90 patients) vs. IVIG combined with plasma exchange (47 patients) vs. no rescue therapy (49 patients) (Table 3). Out of 49 patients with no rescue therapy, 31 were unable to receive therapy due to comorbidities (e.g., sepsis, renal failure), while 18 patient charts contained insufficient data on rescue treatments. Assessing the outcome of different rescue therapies revealed no differences between IVIG, plasma exchange, and the combination of both. However, patients receiving no rescue therapy had

(See figure on next page.)

Fig. 2 Survival analysis of MC and disease exacerbation. Survival curves displaying the time (in months) between diagnosis and the first MC (myasthenic crisis) or exacerbation. (**A**) Survival graph displaying the time to MC according to MGFA class. (**B**) Survival graph displaying the time to exacerbation according to MGFA class. (**C**) Survival graph displaying the time to MC according to anti-Musk-ab status. (**D**)Survival graph displaying the time to exacerbation according to anti-Musk-ab status. (**E**) Survival graph displaying the time to MC according to minimal manifestation status (MMS) at 12 months after diagnosis. (**F**) Survival graph displaying the time to exacerbation according to MMS at 12 months after diagnosis. Significance between survival curves was assessed by logrank testing. ****$p < 0.0001$ ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

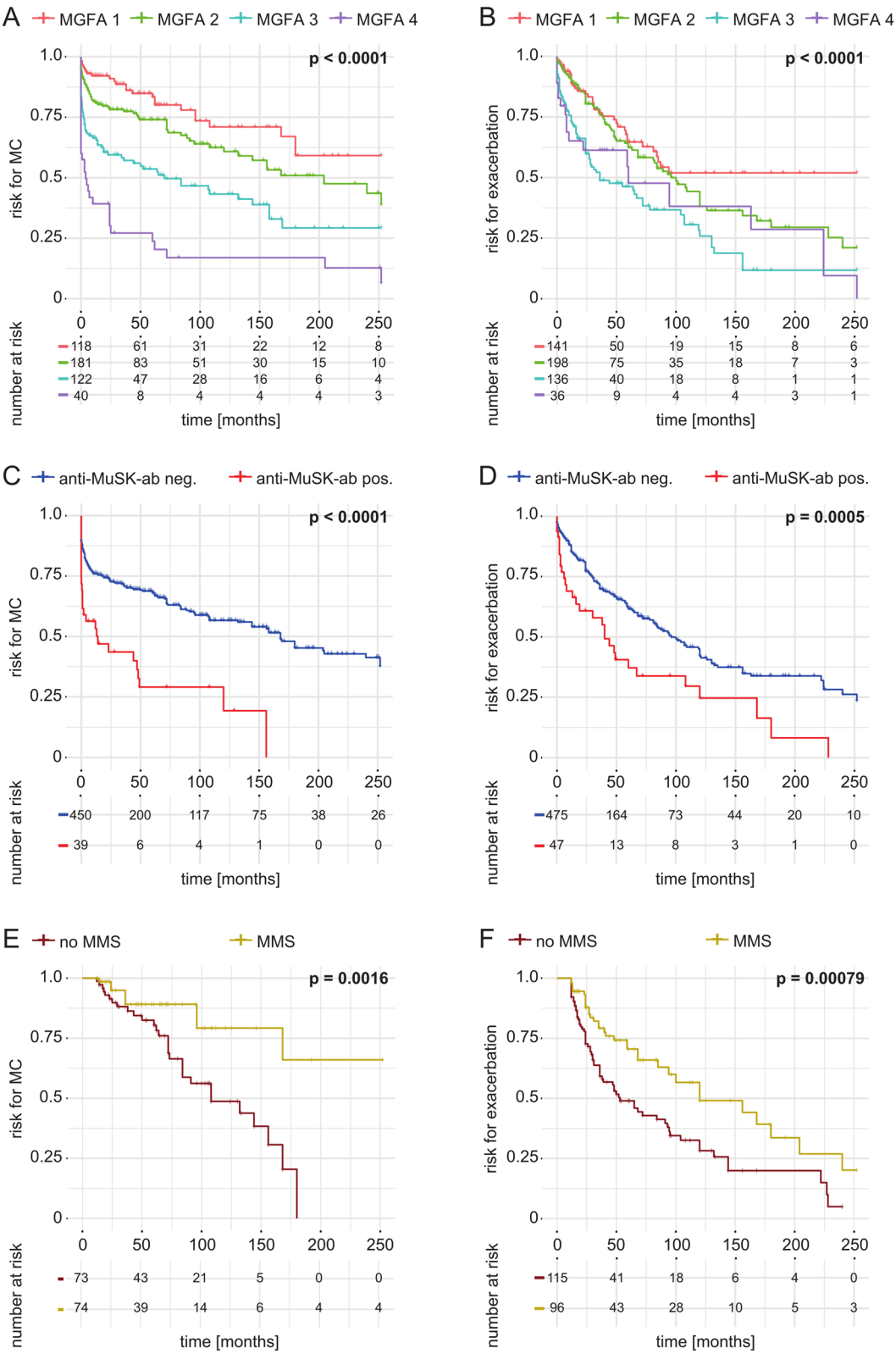


Fig. 2 (See legend on previous page.)

Table 3 Factors affecting outcome of MC

	Improved (*p* value compared to worse)	Unchanged (*p* value compared to worse)	Worse
Female (% of patients)	51.5% (0.89$^{\#}$)	40.5% (0.62$^{\#}$)	48.1%
Age at MC [Year, mean (SD)]	57.9 (21.8) (**0.01**$^{+}$)	60.7 (17.8) (0.14^{+})	67.5 (15.0)
Time between diagnosis and MC [Months, mean (SD)]	31.1 (50.7) (0.77^{+})	27.5 (61.0) (0.77^{+})	36.4 (52.5)
MGFA before MC [MGFA, median (IQR)]	2 (1) (**0.006**$^{+}$)	2 (1) (0.38 +)	3 (2)
MGFA at admission [MGFA, median (IQR)]	3 (1) (**< 0.001**$^{+}$)	3 (1) (**0.008**$^{+}$)	4 (2)
Treated with IST at start of MD (% of patients)	63.1% (0.13$^{\#}$)	60.0% (0.49$^{\#}$)	50.9%
MC triggered by infection (% of MC triggered by infections)	33.5% (**0.005**$^{\#}$)	42.4% (0.26$^{\#}$)	56.6%
VC before MC [VC in ml, mean (SD)]	2192 (822) (0.14^{+})	1938 (739) (**< 0.001**$^{+}$)	1533 (581)
VC at admission [VC in ml, mean (SD)]	1292 (800) (**0.04**$^{+}$)	1263 (598) (**0.006**$^{+}$)	871 (348)
Comorbidities [Number of comorbidities at admission, median (IQR)]	2 (2) (**< 0.001**$^{+}$)	2 (2) (**< 0.001**$^{+}$)	4 (2)
Time of hospitalisation [days, mean (SD)]	20.2 (16.1) (**< 0.001**$^{+}$)	24.7 (25.8) (0.11^{+})	34.1 (32.4)
Intubated (% of patients)	26.9% (**< 0.001**$^{\#}$)	26.7% (**0.002**$^{\#}$)	64.2%
Time of invasive ventilation (days, mean)	7.8 (13.8) (**0.002**$^{+}$)	4.1 (8.9) (**0.001**$^{+}$)	22.6 (39.2)
Pneumonia (% of patients)	26.1% (**< 0.001**$^{\#}$)	20.0% (**< 0.001**$^{\#}$)	57.1%
Sepsis (% of patients)	6.6% (**0.002**$^{\#}$)	3.3% (**0.013**$^{\#}$)	25.0%
Treated with PLEX or IA (% of patients)	57.8% (0.11$^{\#}$)	45.5% (0.99$^{\#}$)	34.2%
Treated with IVIG and PLEX or IA (% of patients)	24.3% (0.24$^{\#}$)	12.2% (0.76$^{\#}$)	15.3%
Treated with no IVIG, PLEX or IA (% of patients)	18.0% (**< 0.001**$^{\#}$)	15.2% (**< 0.001**$^{\#}$)	60.3%

Factors affecting the outcome of MC. *SD* standard deviation, *IQR* interquartile range, *MC* myasthenic crisis, *MGFA* Myasthenia Gravis Foundation of America, *IST* immunosuppressive therapy, *IVIG* intravenous immunoglobulin, *IA* immunoadsorption, *PLEX* plasmapheresis, *VC* vital capacity. Patients who died during the MC were excluded from the analysis as to prevent bias of data due to early death. Significance for groups was assessed by ANOVA (denoted by $^{+}$) or Fisher's exact test (denoted by $^{\#}$). To account for multiple comparisons, statistical significance was corrected by the false discovery rate (FDR). A *p*-value below 0.05 was considered statistically significant. Statistically significant results are bold. Unless otherwise specified, values are mean ± SD (range), median ± IQR (range) or *n* (%)

worse outcome compared to patients that received rescue therapy.

The six patients not surviving MC were on average 70 (SD 11.6) years. All 6 patients were intubated at admission, and the treating physician recorded an infection as the trigger for MC (pneumonia in all 6 cases). The average time of ventilation was 36.3 (SD32.5) days. Four patients died due to sepsis. One patient was treated with PLEX, one patient received both PLEX and IVIGs, while 4 patients did not receive rescue therapies.

Discussion

Despite therapeutic advances, 10–20% of MG patients experience MC during their disease course [3, 6, 24]. To ameliorate the burden of disease incurred by uncontrolled disease, identification of patients at risk for these events as well as factors and strategies promoting MC remission are of high importance for clinical practice. To guide identification and—by extension—management of patients at risk, we analysed a large cohort of MG patients, which reflected previously reported demographic and clinical characteristics [6]. In essence, our data implicate disease severity at diagnosis as a readily accessible and reliable predictor for MC. Treatment strategies should be tailored to the severity of initial symptoms, potentially reducing the likelihood for MC or exacerbation. In addition, our data underlines that the prevention and resolution of infections are pivotal factors defining MC outcome.

Previous observational studies regarding possible risk factors are mostly available for the subgroup of MG patients that received thymectomy [11, 12]. Investigating patients with and without thymoma, our study corroborates the presence of thymoma as a risk factor. Corroborating previous studies [25, 26], we also identified anti-MuSK-ab positivity as an independent risk factor for disease deterioration. Anti-MuSK-ab positive has also been associated with poor outcome of MC [27]. Interestingly, disease severity as assessed by clinical scoring was a robust predictor for patients at risk for MC or exacerbation, underlining the importance of standardized clinical evaluation of MG patients. Patients presenting with severe disease should receive intensified disease monitoring to recognize and, if possible, prevent the occurrence of MC.

Analysing the impact of disease management, we observed that treatment response influenced the risk for MC. Here, patients achieving MMS were at an reduced risk for MC and exacerbation than those who did not. MMS was proposed by the International Consensus

Guidance for Management of MG as treatment target [17, 20]. We analyzed this parameter to understand if achieving the proposed treatment target is associated with a reduced risk for MC [17]. Treatment strategies were previously suggested to affect the course of disease in MG. As such, a recent meta-analysis suggested that cortisone treatment reduces the risk for secondary generalization for MG patients with ocular manifestation [28]. Thymectomy is also evidenced to improve clinical readouts over a 3-year time span as demonstrated in a recent, randomized, controlled trial [19, 20]. Taken together, successful treatment approaches appear to influence long-term outcomes.

Knowledge of factors affecting the outcome of MC are of high clinical importance to promote remission and functional independence [6], with most studies reporting factors associated with prolonged ventilation as a surrogate marker for clinical outcome of MC [6, 23, 29]. Following analysis of patients experiencing MC according to the MGFA post-intervention-status [17], we observed an association between prolonged ventilation time and a worse outcome, suggesting that ventilation time correlates with functional status at discharge. However, our cohort also revealed that VC might be a valuable biomarker for risk stratification of MC as VC predicted the outcome if assessed at admission. Interestingly, a previous retrospective cohort analysing 5 patients with MC found no link between VC and the need for mechanical ventilation [30]. Corroborating VC as a predictive biomarker in other neuromuscular diseases such as Guillain-Barré syndrome [31], our study contrasts the findings from the previous cohort with the difference potentially attributed to the substantial variance in cohort size implicating that monitoring and improvement of ventilation might allow clinicians to avert severe courses of MC. Intriguingly, an infectious trigger of MC was both frequent and associated with an unfavourable outcome compared to other triggers. Hence, prevention and early management of infection in MG patients, notably in MG patients with impaired ventilatory capacities, constitutes a cornerstone in the management of MC. We suggest that treatment of comorbidities making patients vulnerable to infection and resolute adhesion to vaccination protocols should be employed to reduce the risk of infection for MG patients.

The retrospective design of this study might be vulnerable to confounding factors as data were collected during routine clinical practice rather than a formal study setting making data sensitive to variation both in quantity and quality between individual patients and time points. Nonetheless, data quality was improved by collection according to standardised requirements of the German Myasthenia register. A focus on tertiary centers might introduce a bias towards severe cases. However, given the rarity of the disorder, most MG patients are treated in specialized centres [32]. Thus, our cohort is likely to be representative of the general MG population. Regarding the analysis of predictors, a potential limitation is that patients initially presenting with MC or exacerbation could not be included. These patients potentially constitute a distinct clinical subtype as they are expected to have fewer co-morbidities and are likely to be treated more aggressively [6]. Furthermore, definitions for MG exacerbation are heterogenous and diverging interpretations have been previously proposed, e.g., de Meel et al. included an increase in immunosuppressive therapy in their operational definition for exacerbation [33]. A caveat to the analysis of rescue therapies is that the subgroup of patients receiving no treatment for MC is biased to severe cases as these patients were often unable to be treated due to comorbidities (e.g., sepsis or renal failure).

Conclusions

Our study highlights that disease severity at diagnosis is a valuable clinical marker to identify patients at risk for MC or disease exacerbation. Intensified monitoring with emphasis on the prevention of infectious complications is pivotal for management of patients at risk.

Abbreviations

ANOVA: Analysis of variance; ab: Antibody; anti-AChR-ab: Anti-acetylcholine-receptor-ab; anti-LRP4-ab: Anti-low-density lipoprotein receptor-related protein 4-ab; anti-MuSK-ab: Anti-muscle-specific tyrosine kinase-ab; anti-VGCC-ab: Anti-voltage-gated calcium channel-ab; EOMG: Early-onset MG; FDR: False discovery rate; IA: Immunoadsorption; iMC: Integrated Myasthenia Centre; IQR: Interquartile range; IST: Immunosuppressive therapy; IVIG: Intravenous immunoglobulin; LOMG: Late-onset MG; MC: Myasthenic crisis; MG: Myasthenia gravis; MGFA: Myasthenia Gravis Foundation of America; MGFA–PIS: Myasthenia Gravis Foundation of America post-intervention status; MMF: Mycophenolate-mofetil; NICU: Neurological Intensive Care Unit; OR: Odds ratio; PLEX: Plasmapheresis; SD: Standard deviation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12974-022-02448-4.

Additional file 1. Suppl. Table 1. Risk factors for MC and exacerbation – Univariate analysis.

Additional file 2. Suppl. Table 2. Clinical and demographic characteristics of included MC.

Additional file 3. Suppl. Table 3. Trigger factors.

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Author contributions

CN, FS and TR designed the study and methods. Formal analysis was done by CN and FS. Clinical data was provided by FS, CE, MP, CBS, NH, PM, EA, MÖ, DF, SS, SV, AG, HS, MS, BB, AT and TH. Resources were provided by SGM, AM, HW and TR. CN, FS and TR wrote the original draft. CE, MP, CBS, NH, PM, EA, EÖ, DF, SS, SV, AG, HS, MS, BB, AT, TH, SGM, AM and HW reviewed and edited

the manuscript. Figures were created by CN, CBS and NH. Subversion was provided by SGM, AM, HW and TR. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the local ethics committee and institutional review boards (no. AZ 2020-010-f-S, no. AZ 07/2017, 19-8973-BO, AZ 21-1265, AZ 21-1331). Data were anonymized and collected retrospectively according to the standardized requirements of the German register for myasthenia.

Consent for publication
Not applicable.

Competing interests
Dr. Nelke reports no disclosures. Dr. Stascheit received speaking Honoria from Biogen and Alexion. Cand. med. Eckert reports no disclosures. Dr. Pawlitzki received speaker honoraria and travel/accommodation/meeting expenses from Novartis. Dr. Mergenthaler is on the Advisory Board of HealthNextGen Inc. and has equity interest in the company. His research is funded by the Bundesministerium für Bildung und Forschung (BMBF), the European Union, the Else Kröner-Fresenius Stiftung, the Volkswagen Stiftung, and the Einstein Foundation Berlin. Dr Schroeter reports no disclosures. Dr. Arat reports no disclosures. Dr. Özturk reports no disclosures. Prof. Föll reports no disclosures. Prof. Schreiber reports no disclosures. Prof. Vielhaber reports no disclosures. Dr. Gassa reports no disclosures. Dr. Stetefeld reports not disclosures. Prof. Schroeter reports no disclosures. Dr. Berger received travel grants and/or training expenses from Bayer Vital GmbH, IpsenPharma GmbH, Norvartis, Biogen GmbH and Genzyme, as well as lecture fees from Ipsen Pharma GmbH, Alexion Pharma GmbH, Merck, Sanofi Genzyme and Roche. Dr. Totzeck reports no disclosures. Dr. Hagenacker received speaker and advisory board honoraria from Alexion, Biogen and Roche. Prof. Meuth receives honoraria for lecturing, and travel expenses for attending meetings from Almirall, Amicus Therapeutics Germany, Bayer Health Care, Biogen, Celgene, Diamed, Genzyme, MedDay Pharmaceuticals, Merck Serono, Novartis, Novo Nordisk, ONO Pharma, Roche, Sanofi-Aventis, Chugai Pharma, QuintilesIMS and Teva. His research is funded by the German Ministry for Education and Research (BMBF), Bundesinstitut für Risikobewertung (BfR), Deutsche Forschungsgemeinschaft (DFG), Deutsche Multiple Sklerose Gesellschaft (DMSG), Else Kröner Fresenius Foundation, Gemeinsamer Bundesausschuss (G-BA), German Academic Exchange Service, Hertie Foundation, Interdisciplinary Center for Clinical Studies (IZKF) Muenster, German Foundation Neurology and Alexion, Almirall, Amicus Therapeutics Germany, Biogen, Diamed, Fresenius Medical Care, Genzyme, HERZ Burgdorf, Merck Serono, Novartis, ONO Pharma, Roche, and Teva. Prof. Wiendl receives honoraria for acting as a member of Scientific Advisory Boards, Biogen, Evgen, Genzyme, MedDay Pharmaceuticals, Merck Serono, Novartis, Roche Pharma AG, and Sanofi-Aventis as well as speaker honoraria and travel support from Alexion, Biogen, Cognomed, F. Hoffmann-La Roche Ltd., Gemeinnützige Hertie-Stiftung, Merck Serono, Novartis, Roche Pharma AG, Genzyme, TEVA, and WebMD Global. Prof. Wiendl is acting as a paid consultant for Abbvie, Actelion, Biogen, IGES, Johnson & Johnson, Novartis, Roche, Sanofi-Aventis, and the Swiss Multiple Sclerosis Society. His research is funded by the German Ministry for Education and Research (BMBF), Deutsche Forschungsgemeinschaft (DFG), Else Kröner Fresenius Foundation, Fresenius Foundation, the European Union, Hertie Foundation, NRW Ministry of Education and Research, Interdisciplinary Center for Clinical Studies (IZKF) Muenster and RE Children's Foundation, Biogen, GlaxoSmithKline GmbH, Roche Pharma AG, Sanofi-Genzyme. Prof. Meisel received speaker honoraria from Alexion, argnx, GRIFOLS and Hormosan. He received honoraria from Alexion, argnx, UCB, Janssen and Vitaccess for consulting services and financial research support from Octapharma and Alexion. Andreas Meisel is chairman of the medical advisory board of the German Myasthenia Gravis Society. Dr. Ruck reports grants from German Ministry of Education, Science, Research and Technology, grants and personal fees from Sanofi-Genzyme and Alexion; personal fees from Biogen, Roche and Teva; personal fees and nonfinancial support from Merck Serono, outside the submitted work.

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Publisher's Note

2.2. Risiko und Verlauf von COVID-19 bei immunsupprimierten Patient*innen mit Myasthenia gravis

Stascheit F, Grittner U, Hoffmann S, Mergenthaler P, Schroeter M, Ruck T, Pawlitzki M, Blaes F, Kaiser J, Schara U, Della-Marina A, Thieme A, Hagenacker T, Jacobi C, Berger B, Urban PP, Knop KC, Schalke B, Lee DH, Kalischewski P, Wiendl H, Meisel A. Risk and course of COVID-19 in immunosuppressed patients with myasthenia gravis. J Neurol. 2023 Jan;270(1):1-12. **https://doi.org/10.1007/s00415-022-11389-0**

Zu Beginn der COVID-19 Pandemie waren die mit COVID-19 verbundenen Risiken für Patient*innen mit MG unbekannt. Erste fallbasierte Analysen legten nahe, dass MG-Patient*innen potenziell anfälliger für einen schweren COVID-19-Verlauf sind, aufgrund einer vorbestehenden bulbären und/oder respiratorischen, muskulären Schwäche sowie einer erhöhten Infektanfälligkeit durch eine vorbestehende immunsuppressive Therapie. Zudem war bekannt, dass Infektionen eine myasthene Krise triggern können [6, 43, 44].
Wir untersuchten in dieser Arbeit daher, ob eine bestehende immunsuppressive Therapie das Risiko einer SARS-CoV-2-Infektion und den Schweregrad der COVID-19 Infektion beeinflusst. Hierfür wurden die Daten des MyaReg (DRKS00024099) von Mai 2020 bis Juni 2021 analysiert, wobei der Großteil der untersuchten Patient*innen zum Zeitpunkt der Datenerfassung noch nicht gegen COVID-19 geimpft war. Dabei wurde mittels Propensity Score Matching der Zusammenhang einer symptomatischen SARS-CoV-2-Infektion und schwerem COVID-19-Verlauf (Krankenhausaufenthalt, Tod) bei MG-Patient*innen mit bestehender immunsuppressiver Therapie versus MG-Patient*innen ohne immunsuppressiver Therapie untersucht.
Von 1379 Patient*innen lag bei 95 Patient*innen (7%) eine COVID-19 Infektion vor, von denen 76 (80%) zum Zeitpunkt der Infektion eine immunsuppressive Therapie erhielten. 32 Patient*innen (34%) wurden aufgrund von COVID-19 ins Krankenhaus eingeliefert; insgesamt verstarben 11 Patient*innen (12%). Eine vorbestehende immunsuppressive Therapie stellte somit einen Risikofaktor für einen Krankenhausaufenthalt oder Tod in der Gruppe der von COVID-19 betroffenen MG-Patient*innen dar. Eine bestehende immunsuppressive Therapie war jedoch nicht mit einem erhöhten Risiko für eine SARS-CoV-2-Infektion verbunden. Die Ergebnisse dieser Arbeit unterstützten die konsequente Umsetzung wirksamer Strategien zur Prävention einer COVID-19 Infektion, um das Risiko für einen schweren Verlauf in dieser Hochrisikogruppe zu minimieren.

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ORIGINAL COMMUNICATION

Risk and course of COVID-19 in immunosuppressed patients with myasthenia gravis

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Abstract

Background Patients with myasthenia gravis (MG) are potentially prone for a severe COVID-19 course, but there are limited real-world data available on the risk associated with COVID-19 for patients with MG. Here, we investigate whether current immunosuppressive therapy (IST) influences the risk of SARS-CoV-2 infection and COVID-19 severity.

Methods Data from the German myasthenia gravis registry were analyzed from May 2020 until June 2021 and included patient demographics, MG disease duration, comorbidities, current IST use, COVID-19 characteristics, and outcomes. Propensity score matching was employed to match MG patients with IST to those without, and multivariable binary logistic regression models were used to determine associations between IST with (1) symptomatic SARS-CoV-2 infection and (2) severe COVID-19 course, as measured by hospitalization or death.

Results Of 1379 patients with MG, 95 (7%) patients (mean age 58 (standard deviation [SD] 18) presented with COVID-19, of which 76 (80%) received IST at time of infection. 32 patients (34%) were hospitalized due to COVID-19; a total of 11 patients (12%) died. IST was a risk factor for hospitalization or death in the group of COVID-19-affected MG patients (odds ratio [OR] 3.04, 95% confidence interval [CI] = 1.02–9.06, $p = 0.046$), but current IST was not associated with a higher risk for SARS-CoV-2 infection itself.

Discussion In this national MG cohort study, current IST use was a risk factor for a severe disease course of COVID-19 but not for SARS-CoV-2 infection itself. These data support the consequent implementation of effective strategies to prevent COVID-19 in this high-risk group.

Trial registration information German clinical trial registry (https://www.drks.de), DRKS00024099, first patient enrolled: February 4th, 2019.

Keywords Myasthenia gravis · COVID-19 · Immunosuppressive therapies · Outcome · German myasthenia gravis registry

Introduction

Coronavirus disease 2019 (COVID-19) is a global pandemic and raised concerns about the risk of severe infections in patients with myasthenia gravis (MG) due to several factors, such as preexistent bulbar and respiratory muscle fatigability, exacerbation of symptoms due to infections [16, 28], and an immunocompromised state due to the immunosuppressive therapies (IST) that can be found in up to 80% of patients with MG [33]. There is increasing evidence that patients receiving corticosteroids [17, 49] or rituximab for treatment of different rheumatological [5, 43] and neurological conditions [44, 47] have a higher risk of severe disease courses of COVID-19 than patients without such medications. In addition, many comorbidities associated with a high mortality in COVID-19 [11], such as cardiovascular diseases and type II diabetes, are common among patients with MG [14].

On the other hand, it is well recognized that the severity and outcome of COVID-19 might be associated with the excessive production of pro-inflammatory cytokines [51]

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and IST is considered as treatment of choice in patients severely affected by COVID-19 [34]. Thus, the associated risk of COVID-19 severity related to prior IST remains uncertain.

To date, there are limited real-world data on the risk associated with COVID-19 for patients with MG [2, 6, 8, 25, 36, 46]. Management of patients with MG during COVID-19 pandemic has been guided by expert consensus [24]. Apart from a series of case reports [2, 6, 8], the largest studies to date, the international CARE-MG registry [36], and the Czech-MG study [25] reported a mortality of COVID-19 in patients with MG of 24% and 11%, respectively. These data suggest a higher mortality in MG than in the general population with COVID-19 infection, where it is approximately 2% [12, 15, 53]. However, these numbers may be affected by reporting bias, because severe courses are usually hospitalized and therefore easier to capture. This likely has a greater impact when rare diseases are analyzed in small patient populations. Therefore, it is important to understand whether IST is an additional factor favoring the risk of SARS-CoV-2 infection as well as disease severity and poor outcome of COVID-19 in patients with MG. Taking into account known risk factors for severe COVID-19 courses, such as higher age, sex, and comorbidities [15, 53], we here investigated whether patients with MG receiving IST are at higher risk for symptomatic SARS-CoV-2 infection and prone to a worse outcome in case of COVID-19 compared to patients with MG without IST.

Methods

Study design and data collection

This multicenter national cohort study analyzed data from the German Myasthenia gravis Registry (MyaReg), which was established in February 2019 by the national patient support organization for patients with MG (German Myasthenia gravis Society; DMG). MyaReg is assessing longitudinal clinical data on diagnostics, therapy, adverse events, socioeconomic status, and patient-reported outcome parameters of patients with myasthenic syndromes including MG and Lambert–Eaton myasthenic syndrome (LEMS), which were diagnosed based on the current German guidelines [35].

Patients were followed up with entry into the registry in specialized MG clinics on an ongoing basis until June 2021. Socio-demographics (age, sex), MG disease duration, current MG-specific medication, which includes cholinesterase inhibitors, first-line IST (corticosteroids, azathioprine, mycophenolate mofetil, methotrexate, cyclosporine), escalation therapy (rituximab, eculizumab), history of thymectomy, and comorbidities were collected in an electronic database (asthesis®). SARS-CoV-2 infection-related data encompassed COVID-19 severity (hospitalization, stay on intensive care unit, invasive/non-invasive ventilation, myasthenic exacerbation/crisis, exacerbation therapies, change of preexistent IST, antiviral therapy), and outcome (death, rehabilitation, nursing home, discharge to home). Exclusion criteria were missing information on COVID-19 diagnosis (by polymerase chain reaction or antibody testing), MG disease duration, comorbidities, and current MG-specific medication.

Outcome

Endpoints were symptomatic SARS-CoV-2 infection (COVID-19 of any severity) and severity of COVID-19. The severity of COVID-19 course was classified as mild (defined as outpatient treatment), moderate (defined as hospitalization without ICU treatment), and severe (defined as ICU treatment and deceased). We analyzed whether a current IST use was associated with (1) a symptomatic SARS-CoV-2 infection, and (2) with a worse course of COVID-19 defined by hospitalization or death.

Statistical analysis

As descriptive statistics, means and standard deviation (SD) or median and interquartile ranges (IQR) for continuous variables, and absolute and relative frequencies for nominal data were reported. Standardized mean differences (SMD) were calculated as standardized effect sizes. To evaluate the association of IST with the risk of SARS-CoV-2 infection, propensity score matching was employed to match MG patients with IST (exposure) to those without IST. Variables that were used for matching were age, sex, specific diagnosis of myasthenic syndrome, arterial hypertension, thymectomy, heart failure, obesity, chronic obstructive pulmonary disease (COPD), diabetes, and Myasthenia Gravis Foundation of America (MGFA) score. A caliper of 0.2 (of the standard deviation of the logit) was applied [4]. The matching ratio for matching patients with and without IST ranged from 1 to 9, resulting in 203 patients without IST matched to 949 patients with IST. Within the matched subgroup of patients with MG, a multivariable binary logistic regression and a generalized estimation equation (GEE) regression model with adjustment for age, sex, presence of comorbidities, and MGFA clinical classification score was used to analyze the association of IST to risk of infection. Additionally, to evaluate the association of current IST use and COVID-19 severity within the group of COVID-19 patients, SMD were calculated, and multivariable binary logistic regression models adjusted for age, sex and the presence of comorbidities were performed. Patients receiving eculizumab were excluded for risk analysis due to mode of action and the small number

of subjects treated with eculizumab in our population. Odds ratios (OR) and 95% confidence interval (CI) are reported. Statistical analyses were performed using SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) and R (R Core Team 2020) [50], package MatchIt [18], and the R package geepack [19].

Data availability

All analyzed data are presented in the manuscript and are available on reasonable request from qualified investigators.

Results

Demographics and clinical characteristics

We included 1379 patients with myasthenic syndromes (mean age 59 [SD 18]). Because of missing details on COVID-19 diagnosis, comorbidities, and current MG-specific therapy status, four patients with COVID-19 had to be excluded. Overall, 95 patients with MG and COVID-19 could be included for further analysis, of which the majority was infected with SARS-CoV-2 until March 2021, and only 2% ($n=2$) were partly vaccinated against SARS-CoV-2 at the time of infection. MG patients with COVID-19 were comparable to those without COVID-19 regarding age (58 vs. 59 years, SMD: 0.04), sex (41% males vs. 44% males, SMD: 0.05) and disease duration (median 65 vs. 55 months, SMD: 0.13). Atrial fibrillation (11% vs. 5%, SMD: 0.20) and COPD (11% vs. 4%, SMD: 0.24) were more prevalent in MG patients with SARS-CoV-2 infection compared to those without infection. The vast majority of MG patients with and without COVID-19 received IST (80% vs 84%, SMD: 0.19), of which first-line IST (azathioprine, mycophenolate mofetil, methotrexate, cyclosporine) was the most common (52% vs. 61%), followed by corticosteroid monotherapy (19% vs. 16%) and escalation therapy with rituximab (7.4 vs. 6.7) or eculizumab (2.1% vs. 1.4%). A history of thymectomy was similar in both patient groups (43% vs 40%).

Risk of SARS-CoV-2 infection is not associated with immunosuppressive treatment

To investigate whether a current use of corticosteroid monotherapy, first line, and escalation (only rituximab) IST was associated with SARS-CoV-2 infection, we compared MG patients with and without IST including corticosteroids considering relevant covariates (Table 1). In the group of all MG patients before matching, SARS-CoV-2 infections occurred in 76 of 1167 patients treated with IST (6.5%) compared to 19 of 221 treated without IST (8.6%; Table 1). In the matched subgroup, SARS-CoV-2 infections occurred in 5.1% of patients treated with IST ($n=949$ in 203 clusters) compared to 7.9% of those treated without IST ($n=203$). A multivariable binary logistic regression model in the non-matched MG group as well as a GEE model in the matched MG group revealed no substantial association of current IST to the risk of symptomatic SARS-CoV-2 infection (all OR below 1, all 95% CIs included 1; Table 2).

Immunosuppressive treatment is associated with COVID-19 severity

To investigate a potential impact of current IST on the course of COVID-19, we included only the subset of all COVID-19-affected MG patients ($n=95$) in the further analysis. Thirty-two (34%) of those MG patients were hospitalized. Twelve (13%) patients were admitted to the ICU, and six of them survived (Table 3). Eight patients required non-invasive ventilation or respiratory support, and ten received invasive ventilation. Myasthenic exacerbation was observed in nine patients, of which five were treated with intravenous immunoglobulins (IVIG), three with plasmapheresis, and one with high-dose corticosteroids (data not shown). Eleven (12%) patients died (Table 3).

Patients hospitalized or deceased compared with non-hospitalized patients were older (68 vs. 53 years, SMD: 0.94), more frequently male (56% vs. 33% males, SMD: 0.47), more severely affected by MG according to MGFA classification score (SMD: 0.83), more frequently receiving MG related IST (84.4% vs. 77.8%, SMD: 0.48) and had a higher prevalence of comorbidities (88% vs. 73%, SMD: 0.37). Several known risk factors for poor outcome after COVID-19 were more frequent in the group of hospitalized or deceased patients compared with the group of non-hospitalized patients: arterial hypertension (50% vs. 33%, SMD: 0.34), COPD (25% vs. 3%, SMD: 0.66), and cancer (19% vs 6%, SMD: 0.38). A history of thymectomy (36% vs 47%; SMD: 0.23) was less frequently present in patients who were hospitalized or deceased. Disease duration (median 65 vs. 64 months, SMD: 0.11) was similar in both groups. Patients with MG who died due to COVID-19 where more likely to be older with 76 (SD 11) vs. 53 (SD 18) years and more likely to be male (55% vs. 33%) compared to non-hospitalized MG patients with COVID-19. All deceased patients had at least one concomitant disease, with arterial hypertension ($n=6$) and type two diabetes type II ($n=4$) being the most common. The majority of these patients had MG-specific IST at time of infection ($n=9$), of whom 2 received rituximab (Table 3).

After pooling rituximab-treated patients ($n=7$) with first-line IST due to the low case number of this subgroup, multivariable binary logistic regression adjusted for age, sex, and comorbidities showed that SARS-CoV-2-infected patients with MG and current IST had a higher risk for

Table 1 Characteristics of patients with myasthenia gravis and with or without immunosuppressive therapy before and after propensity score matching

	Patients with IST	Patients without IST	SMD	Matched patients with IST[a]	Matched patients without IST	SMD
n	1164	215		949 patients in 203 clusters	203	
Sex male, *n* (%)	531 (45.5%)	71 (32.1%)	0.28	66.6 (32.8%)	68 (33.5%)	0.02
Age in years, mean (SD)	59 (18)	56 (19)	0.19	57 (10)	57 (18)	0.02
diagnosis						
MG	1145 (98.4%)	208 (96.7%)	0.23	196.3 (96.7%)	197 (97.0%)	
LEMS	19 (1.6%)	7 (3.3%)		5.9 (2.9%)	5 (2.5%)	
Disease duration in months, median (IQR) (34 missing's)	59 (23–123)	42 (10–133)	0.05	79 (55–132)	42 (11–134)	0.02
Thymectomy, n (%) (4 missing's)	473 (40.7%)	79 (35.7%)	0.10	82.0 (40.4%)	78 (38.4%)	0.05
MGFA (13 missing's)			0.53			0.05
0	61 (5.0%)	12 (5.5%)		11.8 (5.8%)	11 (5.4%)	
I	210 (18.2%)	73 (33.3%)		62.7 (30.9%)	67 (33.0%)	
II A	298 (25.8%)	57 (26.0%)		58.3 (28.7%)	55 (27.1%)	
II B	310 (26.8%)	60 (27.4%)		58.3 (28.7%)	57 (28.1%)	
III A	92 (8.0%)	7 (3.2%)		3.2 (1.6%)	4 (2.0%)	
III B	121 (10.5%)	7 (3.2%)		7.1 (3.5%)	7 (3.4%)	
IV A/IV B /V	64 (5.5%)	3 (1.4%)		1.6 (0.8%)	2 (1.0%)	
Comorbidities present, *n* (%) (2 missing's)	945 (81.0%)	181(81.9%)	0.02	162.0 (79.8%)	166 (81.8%)	0.06
Autoimmune disease, *n* (%)	273 (23.4%)	50 (22.6%)	0.02	52.8 (26.0%)	45 (22.2%)	0.11
Diabetes mellitus type II, *n* (%)	154 (13.2%)	28 (12.7%)	0.02	24.6 (12.1%)	26 (12.8%)	0.03
Arterial hypertension, *n* (%)	413 (35.4%)	73 (33.0%)	0.05	65.4 (32.2%)	71 (35.0%)	0.07
Heart failure, *n* (%)	23 (2.0%)	1 (0.5%)	0.14	1.2 (0.6%)	1 (0.5%)	0.02
Atrial fibrillation, *n* (%)	67 (5.7%)	10 (4.5%)	0.06	9.3 (4.6%)	10 (4.9%)	0.02
Obesity, *n* (%)	24 (2.1%)	5 (2.3%)	0.01	3.9 (1.9%)	4 (2.0%)	0.01
COPD, *n* (%)	62 (5.3%)	3 (1.4%)	0.22	4.1 (2.0%)	3 (1.5%)	0.05
Asthma, *n* (%)	119 (10.2%)	28 (12.7%)	0.08	23.3 (11.5%)	27 (13.3%)	0.07
Cancer, *n* (%)	123 (10.5%)	22 (10.0%)	0.02	19.7 (9.7%)	22 (10.8%)	0.05
Stroke, *n* (%)	34 (2.9%)	7 (3.2%)	0.01	5.9 (2.9%)	7 (3.4%)	0.04
Dementia, *n* (%)	6 (0.5%)	1 (0.5%)	< 0.01	0.8 (0.4%)	1 (0.5%)	0.02
SARS-CoV-2 infection	76 (6.5%)	19 (8.6%)	0.08	10.4 (5.1%)	16 (7.9%)	0.13

Data are presented as mean (SD), *n* (%) or median (IQR) showing differences in numbers and characteristics of MG patients with or without immunosuppressive therapy (IST) before and after propensity score matching and were compared by standardized mean differences (SMD). Variables used for matching were age, diagnosis, thymectomy, presence of autoimmune disease, diabetes, arterial hypertension, heart failure, atrial fibrillation, obesity, chronic obstructive lung disease (COPD), asthma, cancer, stroke, and dementia. For COPD and atrial fibrillation exact matching was used. For age, a caliper of 0.5 was chosen. Disease duration is the time from diagnosis until examination date

Abbreviations: *COPD* chronic obstructive pulmonary disease, *LEMS* Lambert–Eaton-Myasthenic Syndrome, *IST* immunosuppressive therapy, *IQR* interquartile range, *MG* myasthenia gravis, *MGFA* Myasthenia gravis foundation of America classification, *n* number of included patients, *SD* standard deviation, *SMD* standardized mean differences

[a]Based on average measures on cluster level

[b]Based on log-transformed values

hospitalization or death in comparison to patients without IST or only corticosteroid use (OR 3.04, 95% CI 1.02–9.06, $p = 0.046$; Table 4, Model 2). Age was an independent additional risk factor for COVID-19 severity in both models (OR 1.8, 95% CI 1.22–2.64, respectively, OR 1.76, 95% CI 1.21–2.56, $p = 0.003$).

Discussion

To our knowledge, this study is to date the largest to evaluate the risk of COVID-19 in MG patients in regard to current IST use with an appropriate control group of uninfected MG patients. We identified current IST use not to be a substantial

Table 2 Multivariable binary logistic regression model in total and generalized estimation equation binary logistic regression model in matched myasthenia gravis patients for SARS-CoV-2 infection with regard to immunosuppression status

	Non-matched MG patients ($n=1352$[a]) Nagelkerke $R^2=0.02$		Matched MG patients ($n=203$ groups, 936 individuals[a]) Marginal $R^2=0.01$	
	OR (95% CI) for COVID-19 infection	*p*	OR (95% CI) for COVID-19 infection	*p*
No corticosteroids and no immune suppression (reference)	1		1	
Only corticosteroids	0.82 (0.41–1.66)	0.589	0.74 (0.35–1.60)	0.445
First line IST (AZT, MMF, MTX, CSA)	0.56 (0.31–1.01)	0.052	0.60 (0.32–1.12)	0.107
Escalation therapy with rituximab	0.59 (0.23–1.56)	0.289	0.85 (0.29–2.51)	0.769
Age in decades	1.00 (0.99–1.02)	0.692	–	
Sex male, ref: female	1.10 (0.68–2.09)	0.541	–	
Comorbidities present, ref: no comorbidities present	1.19 (0.68–2.09)	0.541	–	
MGFA, ref: 0			–	
I	0.39 (0.16–0.97)	0.043	–	
II A	0.53 (0.22–1.25)	0.146	–	
II B	0.50 (0.21–1.18)	0.112	–	
III A	0.80 (0.28–2.28)	0.672	–	
III B	0.70 (0.25–1.94)	0.491	–	
IV A/IV B/V	1.10 (0.36–3.33)	0.868	–	

Odds ratios (OR) and 95% confidence interval (CI) for SARS-CoV-2-infected myasthenia gravis (MG) patients from multivariable binary logistic regression (left side) adjusted for age, sex, MGFA score, and thymectomy status ($n=1352$, Nagelkerke R Square: 0.02), and from generalized estimation equation (GEE) binary logistic regression model (right side) after propensity score matching ($n=203$ matched groups, 936 individuals) without further adjustment

Abbreviations: *AZT* azathioprine, *CI* confidence interval, *CSA* cyclosporine A, *GEE* generalized estimation equation, *IST* immunosuppressive therapy, *MG* myasthenia gravis, *MGFA* Myasthenia gravis foundation of America classification, *MMF* mycophenolate mofetil, *MTX* methotrexate, *n* number of included patients, *OR* odds ratio

[a]Patients treated with eculizumab ($n=20$) or missing covariate data ($n=16$) were excluded from matched and non-matched analysis

risk factor for SARS-CoV-2 infection. However, IST, including first line and escalation therapy, was a risk factor for poor COVID-19 prognosis with a higher likelihood of hospitalization and death. Corticosteroid therapy alone was not a relevant risk factor for COVID-19 severity, which is in contrast to other studies examining the impact of IST in MG for outcome after COVID-19 [25, 36, 46]. However, our data indicate that known risk factors for severe COVID-19, such as patient age [11], have a greater impact on the severity of COVID-19 in MG patients than does IST.

Although data on COVID-19 and MG are still scarce, experts agreed early in the pandemic that caution was needed in patients with MG [16, 28, 38]. First, there is the risk of exacerbation of myasthenic symptoms from SARS-CoV-2 infection, compounded by the potentially higher risk of severe courses of COVID-19 due to the immunocompromised state from IST. Approximately 80% of patients with MG receive IST [33], which is in line with our data. Second, discontinuation of IST in patients with MG would likely lead to worsening of myasthenic symptoms. Our data suggest that IST is not per se associated with a higher risk of SARS-CoV-2 infection. This might be due to MG patients with IST being more concerned about COVID-19 than MG patients without IST leading to better compliance with hygiene recommendations in Germany during the study period (e.g., lockdowns, social distancing rules) [26, 31].

Nevertheless, expert consensus agreed that especially MG patients receiving rituximab were more prone to worse COVID-19 outcome [24], as there was evidence of higher risk for hospitalization and mortality in patients with rheumatoid arthritis (RA) [13] or multiple sclerosis (MS) [47] receiving anti-CD20 therapy, which has now been confirmed by large meta-analysis studies [40, 42]. Rituximab treatment is often required long term in MG and may then be associated with a higher risk of severe infections, as recently shown in a Swedish cohort study of MS patients compared with other IST [32]. In our population, seven COVID-19-affected patients received rituximab therapy prior to their infection, of which two patients died. While the association between rituximab therapy and severe outcome is not pronounced in our study (OR 2.35; 95% CI 0.29–19.08), the Czech-MG-COVID-19 study revealed a poor outcome after COVID-19 with three deaths in four MG patients treated with rituximab (OR 35.14; 95% CI 3.2–383.9) [25].

Table 3 Characteristics of myasthenia gravis patients with COVID-19

	Non-hospitalized patients	Hospitalized and/or deceased patients	SMD	Hospitalized patients (survivors, no intensive care)	Patients with intensive care (survivors)	Deceased patients
n	63	32		15	6	11
Sex male, *n* (%)	21 (33.3%)	18 (56.3%)	0.47	8 (53.3%)	4 (66.7%)	6 (54.5%)
Age in years, mean (SD)	53 (18)	68 (15)	0.94	63 (13)	66 (21)	76 (11)
Disease duration in months, median (IQR) (3 missing's)	64 (26–123)	65 (29–163)	0.11[a]	51 (27–232)	31 (7–111)	109 (47–160)
Thymectomy, *n* (%) (4 missing's)	28 of 60 (46.7%)	11 of 31 (35.5%)	0.23	8 (53.3%)	2 (33.3%)	1 (10.0%)
MGFA			0.83			
0	4 (6.3%)	4 (12.5%)		2 (13.3%)	1 (16.7%)	1 (9.1%)
I	12 (19.0%)	3 (9.4%)		1 (6.7%)	–	2 (18.2%)
II A	15 (23.8%)	8 (25.0%)		6 (40.0%)	–	2 (18.2%)
II B	17 (27.0%)	5 (15.6%)		3 (20.0%)	2 (33.3%)	–
III A	8 (12.7%)	1 (3.1%)		–	–	1 (9.1%)
III B	5 (7.9%)	4 (12.5%)		1 (6.7%)	3 (50.0%)	–
IV A/IV B/V	2 (3.2%)	7 (21.9%)		2 (13.3%)	–	5 (45.5%)
Comorbidities present, *n* (%)	46 (73.0%)	28 (87.5%)	0.37	12 (80.0%)	5 (83.3%)	11 (100.0%)
Autoimmune disease, *n* (%)	14 (22.2%)	5 (15.6%)	0.17	2 (13.3%)	1 (16.7%)	2 (18.2%)
Diabetes type II, *n* (%)	5 (7.9%)	5 (15.6%)	0.24	–	1 (16.7%)	4 (36.4%)
Arterial hypertension, *n* (%)	21 (33.3%)	16 (50.0%)	0.34	6 (40.0%)	4 (66.7%)	6 (54.5%)
Heart failure, *n* (%)	–	3 (9.4%)	0.46	2 (13.3%)	–	1 (9.1%)
Atrial fibrillation, *n* (%)	6 (9.5%)	4 (12.5%)	0.10	–	–	4 (36.4%)
Obesity, *n* (%)	1 (1.6%)	3 (9.4%)	0.35	1 (6.7%)	1 (16.7%)	1 (9.1%)
COPD, *n* (%)	2 (3.2%)	8 (25.0%)	0.66	3 (20.0%)	2 (33.3%)	3 (27.3%)
Asthma, *n* (%)	9 (14.3%)	2 (6.3%)	0.27	1 (6.7%)	–	1 (9.1%)
Cancer, *n* (%)	4 (6.3%)	6 (18.8%)	0.38	4 (26.7%)	–	2 (18.2%)
Stroke, *n* (%)	1 (1.6%)	2 (6.3%)	0.24	1 (6.7%)	1 (16.7%)	–
Dementia, *n* (%)	–	2 (6.3%)	0.37	–	1 (16.7%)	1 (9.1%)
Current MG-specific IST			0.48			
No immune suppression, *n* (%)	14 (22.2%)	5 (15.6%)		2 (13.3%)	1 (16.7%)	2 (18.2%)
Only steroids, *n* (%)	15 (23.8%)	3 (9.4%)		–	1 (16.7%)	2 (18.2%)
Standard IST (AZT, MMF, MTX, CSA), *n* (%)	29 (46.0%)	20 (62.5%)		11 (73.3%)	4 (66.7%)	5 (45.5%)
Escalation therapy						
Rituximab, *n* (%)	4 (6.3%)	3 (9.4%)		1 (6.7%)	–	2 (18.2%)
Eculizumab, *n* (%)	1 (1.6%)	1 (3.1%)		1 (6.7%)	–	–

Data are presented as mean (SD), n (%) or median (IQR) showing standardized mean differences (SDM) of hospitalized versus non-hospitalized MG patients with COVID-19 and clinical characteristics of hospitalized and deceased patients

Abbreviations: *AZT* azathioprine, *COPD* chronic obstructive pulmonary disease, *CSA* cyclosporine A, *IST* immunosuppressive therapy, *IQR* interquartile range, *MG* myasthenia gravis, *MGFA* Myasthenia Gravis Foundation of America classification, *MMF* mycophenolate mofetil, *MTX* methotrexate, *n* number of included patients, *SD* standard deviation, *SDM* standardized mean differences

[a] based on log-transformed data

Table 4 Multivariable binary logistic regression models for risk of hospitalization or death in patients with COVID-19 with regard to immunosuppressive status

	Model 1 $N=93$, Nagelkerke $R^2=0.30$			Model 2 $N=93$, Nagelkerke $R^2=0.29$	
	OR (95% CI) for hospitalization or death	*p*		OR (95% CI) for hospitalization or death	*p*
No IST or only corticosteroids (reference)	1		No IST or only corticosteroids (reference)	1	
First line IST (AZT, MMF, MTX, CSA)	2.86 (0.94–8.73)	0.064	First line IST (AZT, MMF, MTX, CSA) or escalation therapy (rituximab)	3.04 (1.02–9.06)	0.046
Escalation therapy (rituximab)	5.03 (0.72–35.01)	0.103			
Age in decades	1.80 (1.22–2.64)	0.003	Age in decades	1.76 (1.21–2.56)	0.003
Sex male, ref: female	1.43 (0.50–4.04)	0.506	Sex male, ref: female	1.43 (0.51–4.04)	0.501
Comorbidities present, ref: no comorbidities present	0.85 (0.19–3.86)	0.835	Comorbidities present, ref: no comorbidities present	0.90 (0.20–4.03)	0.889

Odds ratios (OR) and 95% confidence interval (CI) for hospitalization and death within the group of COVID-19 patients. Multivariable binary logistic regression models were calculated adjusted for age, sex and the presence of comorbidities. Patients treated with eculizumab were excluded from this analysis due to mode of action. In model 2 patients receiving rituximab were included to patients receiving first-line IST due to the low case number ($n=7$)

Abbreviations: *AZT* azathioprine, *CI* confidence interval, *CSA* cyclosporine A, *IST* immunosuppressive therapy, *MMF* mycophenolate mofetil, *MTX* methotrexate, *OR* odds ratio

Additionally, they found that long-term use of corticosteroids, especially at high dosages, together with rituximab and older age was associated with severity of COVID-19 progression [25], which is also observed in other autoimmune diseases such as RA [13] or MS [47]. In CARE-MG, no information about rituximab therapy was reported; however, of the 91 hospitalized patients, 89% had received IST [36].

The role of corticosteroids as a risk factor of SARS-CoV-2 infection is uncertain, as systemically administered corticosteroids are effective in reducing COVID-19 mortality and are particularly beneficial during acute respiratory distress syndrome (ARDS) [48]. However, corticosteroids may prolong viremia [22] in early COVID-19 stages, which could explain the higher proportion of hospitalized patients with ongoing corticosteroid treatment. It is important to emphasize that we also identified IST including first-line and escalation therapies, besides patient age, as important independent risk factor for severe progression of COVID-19 in patients with MG. It its well known that IST may have an attenuating effect in the second phase of severe COVID-19 to suppress or even prevent cytokine storm [7, 34, 51]. In consequence, dexamethasone is recommended in severe COVID-19 to be administered to modulate inflammation-mediated lung injury reducing progression to respiratory failure and death [20] and is routinely applied in severe COVID-19 in Germany. Standard IST used in MG care might be not as effective in suppressing cytokine storm syndrome and, on the other hand, increase the risk for severe outcome of SARS CoV-2 infection more than other IST. For example, the interleukin-6 receptor antagonist tocilizumab [41] has been approved for treatment of severe COVID-19. The C5 complement inhibitor eculizumab also shows beneficial effects in patients with severe COVID-19 [3, 9]. Eculizumab is effective and approved in refractory generalized acetylcholine receptor antibody-positive MG [21], and therefore, it might be considered a potentially beneficial treatment option for patients with MG with severe COVID-19, which is why we have not included this patient group in our risk analyses. Although IVIG and therapeutic plasma exchange have no significant benefit on outcome of patients with COVID-19 [29, 45], due to its proven effect in myasthenic crises or exacerbation [38], IVIG and therapeutic plasma exchange [10, 23, 39] should be considered a first-line treatment choice in patients with MG exacerbation in the course of COVID-19 [24, 27].

Data on the role of thymectomy for COVID-19 disease course in MG are scarce. In a systematic review of case series and cases, 5% of patients with a history of thymectomy died, whereas 17% of patients without a history of thymectomy died [1]. Also in our cohort, the proportion of patients who were hospitalized or died was lower with thymectomy in the history than without thymectomy (36% versus 47%). However, this may be biased by age, as patients with late MG do not undergo thymectomy.

The major limitation of previously published studies on COVID-19 in patients with MG is the lack of an uninfected control group. In addition, there seems to be a reporting bias towards severe cases in previous studies, as the CARE-MG study preliminarily found a mortality rate of 24% [36], which compares with 11% in the Czech-MG study [25], 12%

in a systematic review [1], and 12% in our study. These differences could be due to various standards of care in the countries from which the CARE-MG data are collected. Nevertheless, the COVID-19-related mortality rate of patients with MG is significantly higher compared to other autoimmune diseases such as RA [13] and MS [47], suggesting that not only IST but also MG-specific characteristics, e.g., exacerbation of myasthenic symptoms/myasthenic crisis due to COVID-19 might influence the outcome. In our study population, 10% of COVID-19-affected patients with MG suffered from exacerbation of myasthenic symptoms, which is comparable with the Czech-MG study [25], but less than in CARE-MG with a rate of 40% [36] and the systematic review with 19% [1]. One COVID-19 patient in our cohort died during the course of a myasthenic crisis, which is within the expected range of a 10% in-hospital mortality for myasthenic crises in Germany [37, 39].

Limitations

Our results obtained in German patients with MG may not be representative for other countries, because SARS-CoV-2 infection and COVID-19 may have varying effects worldwide due to different health care systems. Moreover, the data presented here are based on patients enrolled in a registry and therefore do not cover all COVID-19 affected patients with MG in Germany. Patients who were not treated at a specialized MG center may have a different risk regarding the course of an SARS-CoV-2 infection. However, baseline characteristics about age, disease duration, and MG-specific treatment are similar to another large MG study in Germany [30]. Although we included the main known influencing factors, such as age, sex, disease severity, and comorbidities, in the matching, we cannot exclude the risk of residual bias in propensity score matching. Moreover, we have not included the auto-antibody status in our risk analysis, which potentially could have an impact on COVID-19 disease course due to differences in immune response [52, 54]. Additionally, we cannot exclude a reporting bias for hospitalized and severe cases for patients with MG included in our study, although we minimized the risk by our multicentric approach. Due to the small numbers of COVID-19-affected patients with MG in the IST subgroups, we cannot draw conclusions about the risk with the single IST, which especially accounts for rituximab. We were unable to include data on IST used in the past, IST dosage, and duration of ongoing IST before infection in our analysis. We also did not collect data on social or economic factors (e.g., employed or retired) that could potentially influence virus exposure. Future studies with a larger number of patients with MG and COVID-19 are strongly needed to confirm our results and, in particular, to clarify questions regarding the impact of different immunosuppressant therapies.

Conclusions

This registry-based cohort study suggests that the current use of IST does not increase the risk of SARS-CoV-2 infection per se but, together with older age, worsens the prognosis of COVID-19-affected patients with MG. Nevertheless, corticosteroid monotherapy was not a relevant risk factor for COVID-19 severity in our study. Our data indirectly support the consequent implementation of vaccination strategies, such as early booster vaccination, especially in patients with MG treated with IST to effectively prevent COVID-19 in this high-risk group. Further studies with larger patient populations are strongly needed to better understand the risk and consequences of individual immunosuppressant subgroups for patients with MG, while the COVID-19 pandemic persists.

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Code availability Not applicable.

Declarations

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Ethical statement The study was conducted in accordance to the Declaration of Helsinki and the STROBE reporting guidelines and was registered at the WHO-licensed German clinical trial registry (DRKS00024099). The study was approved by the ethics committee of the Charité-Universitätsmedizin Berlin (EA1/025/11).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication All authors have approved the manuscript for submission; accordingly, the manuscript conforms to the journal's policies. The authors take full responsibility for the data, the analyses and interpretation, and the conduct of the research. They have full access to all data, and the right to publish any and all data separate and apart from the guidance of any sponsor.

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2.3. Komplementaktivierungsprofile bei Anti-Acetylcholinrezeptor-positiver Myasthenia gravis

Stascheit F, Chuquisana O, Keller CW, Ambrose PA, Hoffmann S, Gross CC, Lehnerer S, Wiendl H, Willcox N, Meisel A, Lünemann JD. Complement activation profiles in anti-acetylcholine receptor positive myasthenia gravis. Eur J Neurol. 2023 May;30(5):1409-1416. **https://doi.org/10.1111/ene.15730**

Das Komplementsystem spielt pathophysiologisch eine wichtige Rolle für die Störung der neuromuskulären Übertragung [45]. Die klinische Evidenz in Bezug auf die Effektivität komplementinhibierende Therapien, wie die C5-Inhibitoren, für die AChR-Ak positive gMG ist hoch [46, 47]. Dies impliziert eindeutig eine Ak-vermittelte Komplementaktivierung in der Pathogenese der MG.

In dieser explorativen Arbeit wurde in der Annahme, dass aktivierte Komplementfaktoren einen möglichen Biomarker zur Detektion der Erkrankungsaktivität und des Therapieansprechens darstellen könnten, die Konzentrationen der Faktoren des klassischen und alternativen Komplementweges bei therapienaiven Patient*innen sowie Patient*innen unter bestehender immunsuppressiver Therapie im Vergleich zu gesunden Kontrollen (HC) untersucht.

Wir konnten zeigen, dass die Plasma-Konzentrationen von C3a, C5a und sC5b9 bei therapienaiven MG-Patient*innen mit AChR-Ak im Vergleich zu gesunden Kontrollen (HC) deutlich erhöht waren, was auf eine allgemeine Aktivierung des Komplementsystems hinweist. Interessanterweise war hierbei sowohl der klassische als auch der alternative Aktivierungsweg (Faktoren Ba und Bb) involviert. Diese Anstiege waren in einer Validierungskohorte von AChR-Ak-positiven Patient*innen unter immunsuppressiver Therapie immer noch vorhanden, jedoch geringer ausgeprägt. Bei Patient*innen mit MuSK-Ak oder SNMG konnte keine signifikante Komplementaktivierung beobachtet werden. Weder die klinischen Schweregradparameter noch die AChR-Titer korrelierten signifikant mit den aktivierten Komplementspiegeln, was möglicherweise an der kleinen Fallzahl dieser explorativen Studie lag. Trotzdem liefert diese Arbeit erste Hinweise dafür, dass die Messung von aktivierten Komplementfaktoren hilfreich sein könnte, um ein objektives Therapiemonitoring von AChR-Ak positiven Patient*innen durchzuführen.

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ORIGINAL ARTICLE

Complement activation profiles in anti-acetylcholine receptor positive myasthenia gravis

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Abstract

Background and purpose: Complement component 5 (C5) targeting therapies are clinically beneficial in patients with acetylcholine receptor antibody$^+$ (AChR-Ab$^+$) generalized myasthenia gravis (MG). That clearly implicates antibody-mediated complement activation in MG pathogenesis. Here, classical and alternative complement pathways were profiled in patients from different MG subgroups.

Methods: In a case–control study, concentrations of C3a, C5a and sC5b9 were simultaneously quantified, indicating general activation of the complement system, whether via the classical and lectin pathways (C4a) or the alternative pathway (factors Ba and Bb) in MG patients with AChR or muscle-specific kinase antibodies (MuSK-Abs) or seronegative MG compared to healthy donors.

Results: Treatment-naïve patients with AChR-Ab$^+$ MG showed substantially increased plasma levels of cleaved complement components, indicating activation of the classical and alternative as well as the terminal complement pathways. These increases were still present in a validation cohort of AChR-Ab$^+$ patients under standard immunosuppressive therapies; notably, they were not evident in patients with MuSK-Abs or seronegative MG. Neither clinical severity parameters (at the time of sampling or 1 year later) nor anti-AChR titres correlated significantly with activated complement levels.

Conclusions: Markers indicative of complement activation are prominently increased in patients with AChR-Ab MG despite standard immunosuppressive therapies. Complement inhibition proximal to C5 cleavage should be explored for its potential therapeutic benefits in AChR-Ab$^+$ MG.

KEYWORDS
acetylcholine receptor, antibodies, biomarker, complement activation, myasthenia gravis

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INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease in which immunoglobulin G (IgG) antibodies (Abs) bind to acetylcholine receptors (AChRs) or their functional partners in the postsynaptic membrane at the neuromuscular junction, leading to localized or generalized muscle weakness [1]. Although the diagnosis is straightforward in most patients with typical symptoms and a positive Ab test, disease phenotype clinical courses and responses to immunotherapy are remarkably heterogeneous. In addition, the mechanisms by which neuromuscular-junction-specific Abs cause MG pathology, clinical syndromes and disease progression are incompletely understood. Pathogenic actions of AChR-specific Abs include blockade of ACh binding to the AChR and of its signalling and crosslinking, and internalization of the AChR [2]. Also, being mainly IgG1 and IgG3, the AChR-specific Abs can activate complement at the postsynaptic membrane resulting in AChR loss and membrane damage. Abs targeting the muscle-specific tyrosine kinase (MuSK), present in a separate ~3% of all MG patients, are mostly from the IgG4 subclass that does not activate the complement system. Approximately 15%–20% of MG patients are termed seronegative (SNMG), with no known serum antibodies detectable with the currently available assays. Nevertheless, complement deposits indicative of the activation of the classical IgG1-mediated pathway have recently been identified at motor-endplates in the skeletal muscle of some SNMG patients [3]. Here, complement activation pathways were systematically profiled in two independent cohorts of patients with generalized MG (gMG) with AChR or MuSK autoAbs, or with SNMG, compared to healthy donors (HDs).

PATIENTS AND METHODS

Study design and data collection

The diagnosis of MG was based on national guidelines [4]. Antibody testing was performed with the currently available assays for anti-AChR (radioimmunoassay) and anti-MuSK Abs (measured by enzyme-linked immunosorbent assay [ELISA]), as well as low-density lipoprotein receptor related protein 4 Abs (measured by indirect immunofluorescence test) [5]. The diagnosis of SNMG was established as follows: typical clinical presentation with muscle fatiguability that improves with rest, no serum Abs detected against AChR, MuSK and low-density lipoprotein receptor related protein 4, abnormal results in repetitive nerve stimulation and/or single-fibre electromyography and/or clinical response to intravenous or oral acetylcholinesterase inhibitors.

For exploratory analyses, heparinized plasmas stored at −20°C from 25 treatment-naïve patients with generalized early-onset MG (EOMG) from the National Hospital for Nervous Diseases, London, or the Department of Clinical Neurosciences, Oxford University Hospitals, UK, were tested. For the validation cohort, patients/sera were recruited from the Departments of Neurology at Charité—Universitätsmedizin Berlin, Germany, with AChR-Ab$^+$ MG (n = 51), MuSK-Ab$^+$ (n = 9) or SNMG (n = 10), and the University Hospital Münster, Germany, with MuSK-Ab$^+$ MG (n = 3) and SNMG (n = 17) (Table 1). Two cohorts of 25 and 26 age- and sex-matched HDs were recruited at the University Hospital Münster. AChR-Ab$^+$ MG patients were further subgrouped by onset age (before or after age 50, EOMG or LOMG, respectively) or with thymomas. Both German sites are certified MG centres, accredited by the German Myasthenia Society, and used identical protocols for the collection of demographic and clinical data as well as for sample processing and storing at −20°C. Sera were immediately frozen after separation. For quantification of complement protein levels, samples were thawed on ice and immediately processed. All samples underwent a maximum of two freeze/thaw cycles. Serum specimens from 10 healthy individuals that had been stored at −20°C and thawed on ice once or twice or freshly processed without freezing showed similar levels of activated complement proteins (data not shown). In preliminary experiments comparing intra-individual levels of plasma- and serum-derived activated complement proteins in 15 healthy blood donors, higher concentrations were observed in serum than in plasma samples (Figure S1). Therefore, only plasma samples from control individuals were compared to plasma samples derived from MG patients in the exploratory analysis and only serum samples from control individuals were compared to serum samples derived from MG patients in the validation analysis. MG disease severity was assessed at baseline and 1-year follow-up using the quantitative MG score and the MG-specific Activities of Daily Living scale (MG-ADL) [6]. Exclusion criteria were concomitant autoimmune diseases. Samples were thawed on ice and processed immediately.

Complement component profiling

A previously described, established multiplex ELISA based on chemiluminescence was used according to the manufacturer's recommendations (Quidel, San Diego, USA, cat. number A900) [7]; it is more fully validated than recently reported assays to detect complement deposition [8, 9]. All samples were run in duplicate, and both replicates were run in parallel; average values were used for statistical analyses and in the figures. Each plate contained samples from different clinical cohorts to control for inter-plate variations. Control samples provided by the manufacturer were included on each plate to ensure plate-to-plate consistency. Data were obtained with Imager L from Quansys, using Q-View Software 3.11 for analyses.

Statistical analyses

Descriptive statistics are reported and means and standard deviations (SDs) or medians and interquartile ranges (IQRs) for continuous variables, and absolute and relative frequencies for nominal data. All statistical analyses were performed using GraphPad Prism V9.1.2. Mann–Whitney tests were performed to compare levels

TABLE 1 Demographic and clinical characteristics of patients with AChR-Ab$^+$ gMG, MuSK-Ab$^+$, seronegative and healthy donors

	Exploratory analysis		Validation analysis			
	AChR	HDs	AChR	HDs	MuSK	SN
n	25	25	51	26	12	27
Sex, female, *n* (%)	23 (92)	21 (84)	34 (67)	16 (62)	9 (75)	18 (67)
Age in years, mean (SD)	21 (8)	31 (7)	48 (16)	46 (13)	45 (19)	56 (16)
EOMG, *n* (%)	25 (100)	–	22 (43)	–	–	–
LOMG, *n* (%)	–	–	16 (31)	–	–	–
Disease duration in months, median (IQR)	33 (13–88.5)	–	22 (15–37)	–	64 (32–134.5)	28 (14–84)
Thymectomy, *n* (%)	25 (100)	–	35 (69)	–	0 (0)	4 (15)
Thymoma, *n* (%)	0 (0)	–	13 (26)	–	n.a.	0 (0)
MGFA classification at time point of sampling						
0	0	–	6 (12%)	–	2 (17%)	0 (0%)
I	0	–	2 (4%)	–	1 (8%)	13 (48%)
II A	n.a.[a]	–	18 (35%)	–	2 (17%)	6 (22%)
II B	n.a.	–	14 (27%)	–	5 (42%)	4 (15%)
III A	n.a.	–	0 (0%)	–	1 (8%)	1 (4%)
III B	n.a.	–	11 (22%)	–	1 (8%)	2 (7%)
QMG, mean (SD)	n.a.	–	9.5 (6.5)	–	6 (6.7)	6 (5.9)
MG-ADL, mean (SD)	n.a.	–	5.8 (4.4)	–	5 (3.4)	4 (4)
MG specific therapy at baseline						
Corticosteroids, *n* (%)	0	–	34 (67)	–	7 (58)	9 (33)
Azathioprine, *n* (%)	0	–	25 (49)	–	6 (50)	6 (22)
Mycophenolate mofetil, *n* (%)	0	–	7 (14)	–	1 (8)	1 (4)
Methotrexate, *n* (%)	0	–	3 (6)	–	1 (8)	2 (7)
Rituximab, *n* (%)	0	–	5 (10)	–	1 (8)	1 (4)
Eculizumab, *n* (%)	0	–	0 (0)	–	0 (0)	0 (0)

Note: Data are mean (SD) and *n* (%) for the baseline variables and median (IQR) for disease duration. Disease duration is the time from diagnosis until baseline. The percentage of the thymus histology results is related to the number of thymectomized patients. Score 0 (MGFA), remission.

Abbreviations: Ab, antibody; AChR, acetylcholine receptor; EOMG, early-onset myasthenia gravis, gMG, generalized myasthenia gravis; HDs, healthy donors; IQR, interquartile range; LOMG, late-onset myasthenia gravis; MG, myasthenia gravis; MG-ADL, MG Activities of Daily Living score; MGFA, Myasthenia Gravis Foundation of America classification; MuSK, muscle-specific kinase; *n*, number of included patients; n.a., not available; QMG, Quantitative Myasthenia Gravis score; SD, standard deviation; SN, seronegative.

[a]All patients were classified MGFA II A or higher.

of complement components between patients and controls. Their correlations with clinical disease parameters were analysed using Spearman's rank correlation coefficient, and their capacity to predict disease severity by logistic regression analysis. A two-tailed *p* value <0.05 was considered statistically significant. The conservative Bonferroni correction method was applied to adjust for multiple comparisons.

Standard protocol approval, registrations and patient consents

The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committees of the Universities of Münster (registration no. 2010-262-f-S, 2011-665-f-S, 2013-350-f-S, 2014-068-f-S and 2016-053-f-S), London and Oxford; written informed consents were given by all participants.

RESULTS

Complement activation was first compared in an exploratory study on 25 immunotherapy-naïve patients with AChR-Ab$^+$ generalized EOMG and 25 HDs matched by age and sex (Table 1). Levels of components indicating activation of both the classical and alternative complement pathways were substantially increased in most of the EOMG patients (Figure 1) and were highly significant overall. These increases were observed for the soluble terminal membrane attack

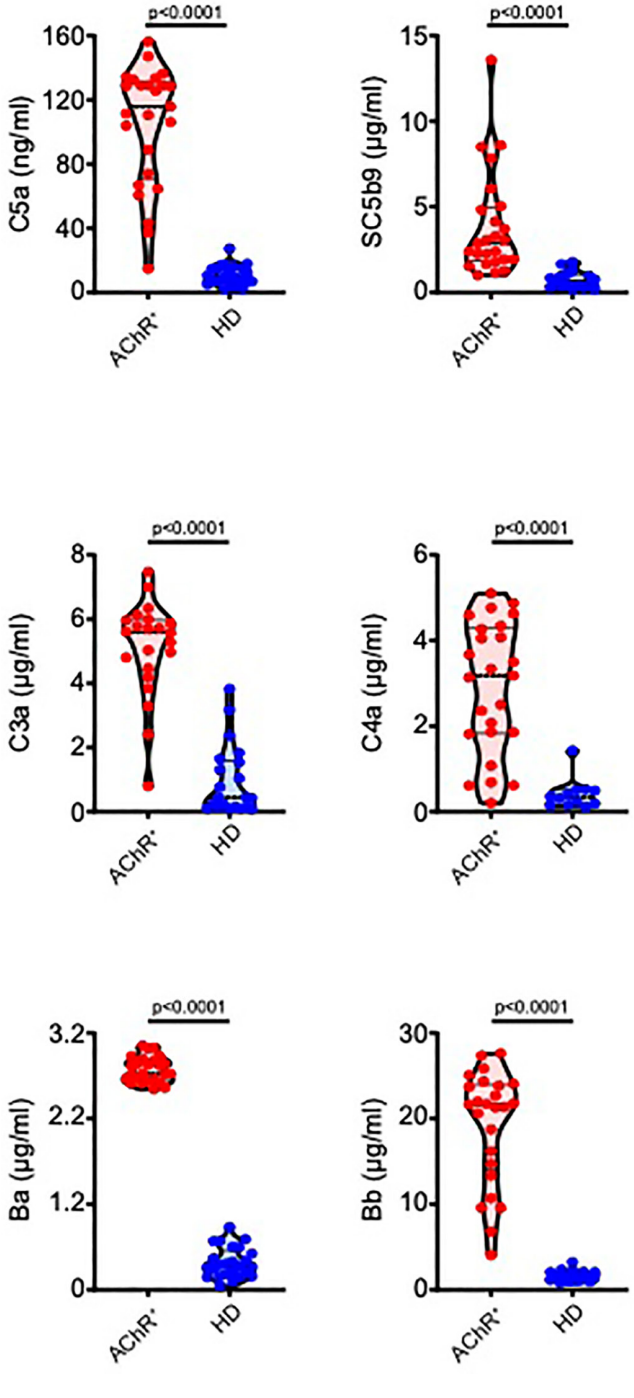

FIGURE 1 Increased levels of activated complement components in patients with AChR-Ab$^+$ MG. Exploratory analysis in immunotherapy-naïve patients with AChR-Ab$^+$ EOMG (n = 25) and HDs (n = 25). The Mann–Whitney test was used for statistical analyses.

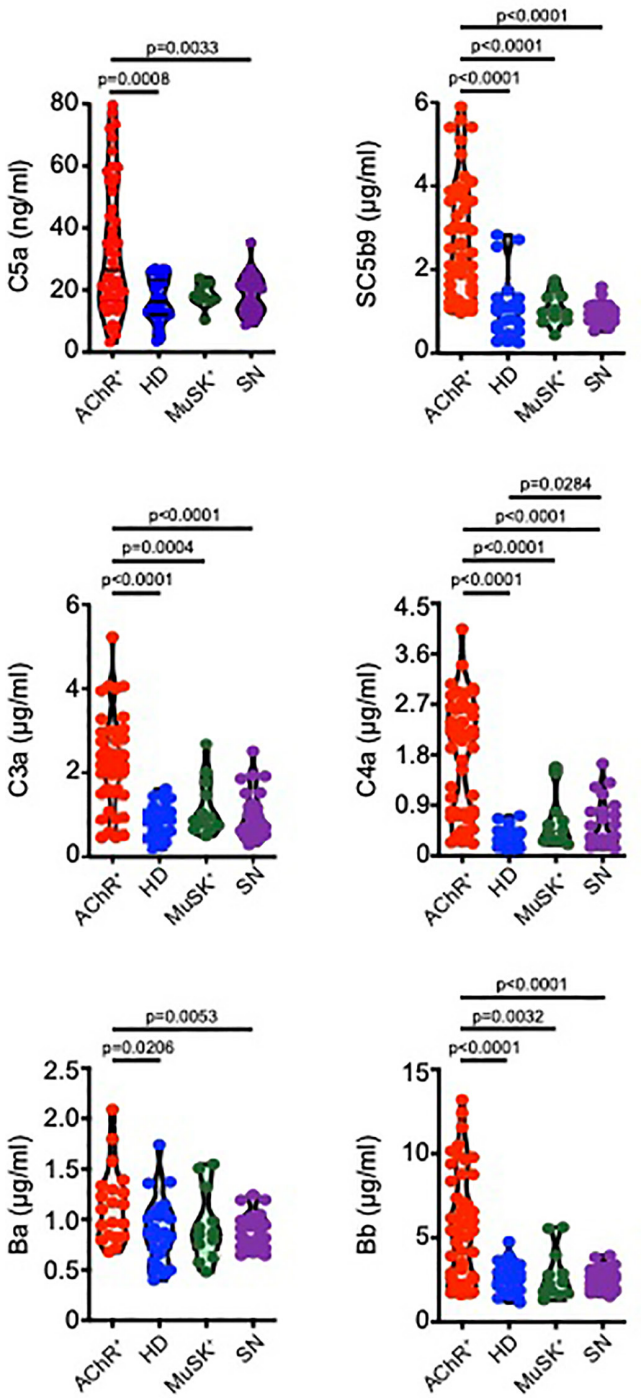

FIGURE 2 Increased levels of activated complement components in patients with AChR-Ab$^+$ MG receiving standard immunosuppressive therapies. Patients with AChR-Ab$^+$ MG (n = 51) receiving standard immunosuppressive therapies compared to MuSK-Ab$^+$ MG patients (n = 12), seronegative MG (SN, n = 27) and HDs (n = 26). For Ba, serum concentrations in 31 of the 51 patients with AChR-Ab$^+$ EOMG exceeded the upper limit of quantification and were excluded from the plot and analysis. The Mann–Whitney test was used for statistical analyses.

complex sC5b9, components close to its initiation (C5a) and for more proximal components such as C3a, C4a, Ba and Bb.

A replication analysis was next performed in an independent cohort of 51 patients with AChR-Ab$^+$ gMG compared to 12 patients with MuSK-Ab$^+$ MG and 27 with SNMG, all of them receiving standard immunosuppressive therapies, and 26 HDs (Table 1).

Levels of activated complement components were again strikingly and highly significantly higher in most of the AChR-Ab$^+$ MG cohort than in the demographically matched HDs (Figure 2).

Elevation of C3a appeared to be marginally higher in patients with EOMG and LOMG than with thymomas but not significantly so after correction for multiple comparisons (data not shown). In contrast to patients with AChR-Ab$^+$ MG, those with MuSK-Ab$^+$ MG or SNMG did not differ from HDs in complement activation profiles (Figure 2).

These results from the validation cohort (Figure 2) clearly support the provisional findings from the treatment-naïve patients shown in Figure 1. Whilst levels of activated complement components appear lower in sera from patients receiving immunotherapy in the validation cohort (Figure 2) than in the plasmas from the immunotherapy-naïve patients in our exploratory cohort (Figure 1), this may have been partly due to differences in the serum versus plasma sampling [10]. Patients with AChR-Ab$^+$ stratified by their different immunosuppressive therapies (azathioprine, mycophenolate mofetil, methotrexate or rituximab) did not show significant differences in their complement activation profiles (not shown). Nor did baseline levels of individual activated complement components correlate significantly with disease severity parameters such as the Quantitative Myasthenia Gravis score and MG-ADL, whether at the time of sampling or 1 year later, after correction for multiple comparisons (Table 2). In addition, correlations of complement activation levels with AChR-Ab titre have not been seen (Table 2).

DISCUSSION

Substantially increased levels of markers were detected indicative of IgG-mediated complement activation in AChR-Ab$^+$ gMG, not only in therapy-naïve but also in treated patients. The elevations in these six components indicate activation of proximal and terminal components of the classical and alternative pathways. Our findings are supported by the clinical efficacy of eculizumab, the first and so far only monoclonal C5-targeting Ab approved for MG therapy, in patients with AChR-Ab$^+$ gMG [11].

The complement signature in patients with AChR-Ab$^+$ gMG identified here is consistent with IgG-mediated activation of the classical pathway. In addition, higher levels of activated components of the alternative pathway, that is, Ba and Bb, were observed. It is continuously activated and can act as an amplification loop for all three pathways as it is initiated by the binding of C3b [12, 13]. Initial Ab-mediated activation of the classical complement cascade can therefore amplify alternative pathway activation, which might explain the raised levels of Ba and Bb proteins in AChR-Ab$^+$ MG. The latter, however, could be further increased through recognition of apoptotic and necrotic cell surfaces at the neuromuscular junction which are rich in carbohydrates [14].

Despite the clinical efficacy of its inhibition, roles of the complement system in MG are still poorly understood, but in vivo data on its activation in MG patients are increasing. Iacomino et al. reported increased levels of C3, C3b and C5a in the plasmas of patients with AChR-Ab$^+$ MG, but not in MuSK-Ab$^+$ MG patients,

TABLE 2 Spearman correlations of demographic and clinical characteristics, including AChR-Ab titres, with levels of activated serum complement proteins (validation analysis)

	C4a	C3a	C5a	sC5b9	Ba	Bb
Age	$r = -0.01$; $p = 0.97$	$r = -0.05$; $p = 0.74$	$r = -0.08$; $p = 0.60$	$r = -0.04$; $p = 0.78$	$r = 0.10$; $p = 0.48$	$r = -0.07$; $p = 0.64$
Disease duration (months)	$r = -0.01$; $p = 0.92$	$r = 0.03$; $p = 0.81$	$r = -0.18$; $p = 0.21$	$r = -0.16$; $p = 0.26$	$r = -0.15$; $p = 0.30$	$r = -0.26$; $p = 0.07$
Anti-AChR-Ab titre	$r = 0.12$; $p = 0.45$	$r = -0.13$; $p = 0.44$	$r = 0.20$; $p = 0.22$	$r = 0.12$; $p = 0.47$	$r = -0.07$; $p = 0.68$	$r = 0.14$; $p = 0.40$
QMG baseline	$r = -0.22$; $p = 0.12$	$r = -0.32$; $p = 0.02$	$r = 0.03$; $p = 0.83$	$r = -0.11$; $p = 0.46$	$r = -0.20$; $p = 0.17$	$r = -0.11$; $p = 0.43$
QMG year 1	$r = 0.21$; $p = 0.16$	$r = -0.02$; $p = 0.89$	$r = 0.07$; $p = 0.63$	$r = 0.03$; $p = 0.82$	$r = -0.09$; $p = 0.55$	$r = -0.06$; $p = 0.69$
MG-ADL baseline	$r = -0.16$; $p = 0.25$	$r = -0.23$; $p = 0.11$	$r = -0.01$; $p = 0.94$	$r = -0.15$; $p = 0.31$	$r = -0.26$; $p = 0.07$	$r = -0.18$; $p = 0.22$
MG-ADL year 1	$r = 0.28$; $p = 0.05$	$r = -0.01$; $p = 0.92$	$r = 0.11$; $p = 0.46$	$r = 0.03$; $p = 0.83$	$r = -0.14$; $p = 0.33$	$r = -0.03$; $p = 0.84$

Note: Spearman correlation coefficients are shown. The conservative Bonferroni correction method was applied to adjust for multiple testing.

Abbreviations: anti-AChR-Ab titre, anti-acetylcholine receptor antibody titre; MG-ADL, MG Activities of Daily Living score; QMG, Quantitative Myasthenia Gravis score.

which is in line with our findings [15]. Additionally, they also found no correlation of plasma levels of the altered complement components (C2, C3, C3b, C5 and C5a) with disease severity or AChR-Ab titres. Nor did they observe any differences in plasma concentrations of C2, C3 and C5 between corticosteroid-naïve and -treated AChR-MG patients or any correlations with the durations of immunosuppressive treatment. Additionally, their study population was rather small with 18 AChR-Ab positive and five MuSK-Ab positive patients.

Moreover, Ozawa et al. reported higher serum levels of sC5b9 in AChR-Ab positive patients with gMG than in healthy volunteers [16]. Conversely, Romi et al. [17] and Liu et al. [18] reported lower serum levels of the uncleaved C3 or C4 components in AChR-Ab$^+$ MG patients than in healthy volunteers, again suggesting increased consumption due to activation. Assaying C3, C4 and C5a in patients with AChR-Ab$^+$ MG, most of them receiving standard immunosuppressive therapies, Aguirre et al. observed a positive correlation of plasma C5a levels with higher scores in the MG-ADL scale [19]. By contrast, Fichtner et al. [20] found no difference between AChR-Ab$^+$ MG patients and HD sera in the lysis of IgM-sensitized red blood cells, an assay for overall complement activity; ~12% even showed reduced activity, although with no obvious clinical or serological correlates. Furthermore, Ozawa et al. [16] reported higher serum levels of sC5b9 in patients with AChR-Ab$^+$ MG than in HDs. Some discrepancies in these data might reflect the different biomaterials used, such as plasma and serum. Ethylenediaminetetraacetic acid plasma is optimal for quantification of individual components and activation products [21, 22], and the use of serum in our validation cohort is certainly a limitation of our study. However, our results strongly support the concept that complement activation networks are deregulated in patients with AChR-Ab$^+$ MG.

Currently, there are no established biological markers that allow reliable prediction of disease progression in patients with MG. Levels of complement activation products did not correlate obviously with parameters of clinical severity at the time of sampling or 1 year later, possibly because of small numbers in our validation analysis. The potentially reduced complement activation after treatment initiation warrants longitudinal measurements in larger cohorts of patients the better to correlate the predictive value of levels of individual components with MG subgroup, severity, clinical outcomes and/or therapeutic efficacies and thus in guiding treatment decisions and even prognostication.

Studies in such larger series could additionally focus on the various AChR epitopes recognized by the patients' Abs, which can vary between MG subgroups [23] and might affect complement activation. Additionally, our SNMG patients were not tested in the cell-based assays that are currently confined to specialized research centres [24–26]; as they reportedly have higher sensitivities [27, 28], especially for Abs specific for clustered AChRs [22], they should be used in future studies on SNMG patients.

Our finding that complement activation is increased in AChR-Ab$^+$ MG despite standard immunosuppression supports the use of terminal complement inhibition as a therapeutic strategy, potentially early during the disease course or in refractory cases. More proximal members of both classical and alternative pathways exert powerful immunoregulatory functions, for example through recognition by complement receptors C5aR or C3aR expressed on myeloid and activated T and B cells. Hence, our data indicate that therapeutic complement inhibition might yield additional benefits, especially for patients with AChR-Ab$^+$ MG.

In conclusion, markers indicating complement activation are prominently increased—despite standard immunosuppressive therapies—in patients with AChR-Ab$^+$, although not in those with MuSK-Ab$^+$ or SNMG. Evidently, the therapeutic potential of complement inhibition proximal to C5 cleavage warrants further assessment in patients with AChR-Ab$^+$ MG.

AUTHOR CONTRIBUTIONS

Frauke Stascheit: Investigation, statistical analysis, writing—original draft preparation, writing—review and editing. Omar Chuquisana: Investigation, statistical analysis, writing—original draft preparation, writing—review and editing. Christian W. Keller: Investigation, writing—review and editing. Philip Alexander Ambrose: Writing—review and editing. Sarah Hoffmann: Investigation, writing—review and editing. Catharina C. Gross: Investigation, writing—review and editing. Sophie Lehnerer: Investigation, writing—review and editing. Heinz Wiendl: Writing—review and editing. Nick Willcox: Investigation, resources, writing—review and editing. Andreas Meisel: Resources, investigation, writing—review and editing. Jan D. Lünemann: Conceptualization, methodology, writing—original draft preparation, writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The authors declare that anonymized data will be shared from the corresponding author upon reasonable request from any qualified investigator.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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2.4. Calprotectin als potenzieller Biomarker für die Myasthenia gravis

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Neben genetischen Faktoren [14] sowie der fundamentalen, pathophysiologischen Rolle des Thymus für die MG [18], bleibt der komplexe Einfluss weiterer umweltbedingter Faktoren für die Entstehung der MG unklar. Es mehren sich jedoch die Hinweise, dass das Darmmikrobiom eine wichtige pathophysiologische Rolle bei der MG spielen könnte, da dieses kritisch ist für die Aufrechterhaltung der Immunität und Selbsttoleranz [48]. So konnten mehrere Studien zeigen, dass bei therapierten als auch unbehandelten MG-Patient*innen eine geringere mikrobielle Diversität vorliegt [48, 49]. Diese führt zu einer erhöhten Permeabilität der intestinalen Mukosa und Dysbalance proinflammatorischer Th17- sowie regulatorischer T-Zellen [49]. Da Serum Calprotectin (CLP) mit dem Grad der Dysbiose korreliert [50], hatten wir die Hypothese, dass CLP als potenzieller Biomarker zur Detektion der Erkrankungsaktivität bei der MG fungieren könnte.

Seren von 251 MG-Patient*innen und 90 HC wurden in einem explorativen Querschnittsdesign analysiert und der Zusammenhang zwischen CLP-Spiegeln und soziodemografischen Merkmalen, Krankheitsaktivität (aktuelle MGFA-Klassifikation, QMG- und MG-ADL-Score), Ak-Status und Therapie untersucht. Serum CLP war bei MG-Patient*innen unabhängig vom Antikörperstatus im Vergleich zu den HC signifikant höher und korrelierte schwach mit der Erkrankungsschwere, gemessen anhand der MGFA-Klassifizierung und des QMG-Scores zum Zeitpunkt der Probenentnahme. Die Ergebnisse dieser Studie geben Hinweise für eine wichtige pathophysiologische Rolle des Darmmikrobioms und der intestinalen Immunität bei MG. CLP kann ein Biomarker für (hoch-) aktive MG-Verläufe darstellen. Der klinische Stellenwert von Serum CLP muss jedoch weiter geprüft und validiert werden, wozu bereits eine prospektive Studie in Vorbereitung ist.

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Calprotectin as potential novel biomarker in myasthenia gravis

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ABSTRACT

Myasthenia gravis (MG) is the most common autoimmune disease affecting the neuromuscular junction by specific autoantibodies. The etiology of MG and its heterogeneity in clinical courses are poorly understood, although it was recently shown that gut microbial dysbiosis plays a critical role. Since levels of Calprotectin (CLP) seem to correlate with level of dysbiosis, we hypothesize that CLP may serve as potential disease activity biomarker in MG. Sera from 251 patients with MG and 90 controls were analyzed in an explorative, cross-sectional design. Prospectively, we tested CLP levels in MG patients up to 3 years. Association of CLP levels with socio-demographics, disease activity (quantitative myasthenia gravis (QMG) score, myasthenia gravis-specific Activities of Daily Living scale (MG-ADL)), antibody (Abs) status, history of myasthenic crisis, treatment regime, and history of thymectomy were investigated using univariate analysis. Mean baseline serum levels of CLP were significantly higher in MG patients compared to controls (4.3 μg/ml vs. 2.1 μg/ml; $p < 0.0001$). Higher levels of CLP were associated with a higher clinical disease severity measured by MGFA classification and QMG score. Nevertheless, the only weak correlation of CLP with clinical outcome parameters needs confirmation in future studies. Currently, there are no validated blood biomarkers for MG. The significantly elevated CLP and mild correlation with parameters of disease activity suggests that CLP holds promise as a biomarker for measurement of individual disease severity.

1. Introduction

Myasthenia gravis (MG) is an autoimmune disease affecting the neuromuscular junction by specific autoantibodies [1,2]. While the final pathways of the disease and its effectors disturbing the functions of the neuromuscular junction are relatively well known, the etiology of MG and its heterogeneity in clinical course are poorly understood. Importantly, there is an urgent need for a sensitive biomarker in MG that reliably predicts the individual disease course and exacerbation, as well as guiding immune suppressive treatment, especially in the light of emerging and more specific therapy options for MG patients [3].

Both, genetic and environmental factors have been considered crucially involved in the etiology of MG [4]. While the exact factors responsible for predisposition to MG remain elusive, a crucial role for gut microbiota in the pathogenesis of MG has been hypothesized, since MG patients show a high level of microbial dysbiosis [5–7].

Similarly to inflammatory bowels diseases (IBD) [8], the incidence of MG is increasing in newly industrialized countries [9,10], supporting further an association between "westernization of lifestyles", gut microbiome and MG.

Calprotectin (CLP), a calcium-binding protein of the S100 family, performs various biological functions via interaction with Toll-like receptor 4 [11] on the surface of leukocytes and is manly released by activated neutrophils, monocytes and early differentiation states of macrophages [12]. CLP has been shown to perform various biological functions, especially in triggering signaling pathways involved in inflammatory processes and inhibition of microbial growth [12]. In IBD, levels of CLP not only correlated with the level of microbial dysbiosis

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[13], but were also significantly increased in patients with active disease [14] and strongly predicted disease relapse and treatment response [15]. CLP was also investigated in other autoimmune diseases, mainly rheumatoid arthritis (RA) [16], where prediction of disease activity and treatment outcome was proven high. Of highlight, CLP leads to the release of pro-inflammatory cytokines [17,18] and induction of auto-reactive $CD8^+$ T cells [18,19], which play a key role in the pathogenesis of MG [20].

Therefore, we want to test the hypothesis that CLP may serve as a potential disease activity biomarker using a cohort of MG patients compared to controls.

2. Methods

2.1. Standard protocol approvals, registrations, and patient consent

The study was approved by the ethics committee of the Charité-Universitätsmedizin Berlin (EA1/281/10). All patients gave written informed consent in accordance with the Declaration of Helsinki in its currently applicable form.

2.2. Study design

This is an explorative cross-sectional and prospective study comparing serum CLP levels of MG patients and controls to assess the potential of CLP to measure disease activity as assessed by MGFA classification system, quantitative myasthenia gravis (QMG) score, and myasthenia gravis-specific Activities of Daily Living scale (MG-ADL).

2.3. Patients and controls

This study was performed at the certified integrated Center for Myasthenia gravis (iMZ) of the Charité-Universitätsmedizin Berlin, Germany. Patients over the age of 18 years with confirmed diagnosis of myasthenia gravis based on the current guidelines of the German Neurological Society [21] were included independent of disease duration and severity. Overall, 251 patients were consecutively screened at the iMZ clinic between March 2016 and May 2020 and were further categorized in subgroups according to age at onset (early-onset MG [EOMG] was defined as onset at $\leq$50 years of age, late-onset MG was defined as onset >50 years of age [22]) and thymus pathology (thymoma-associated MG [TAMG]). Prospectively, we tested CLP levels in an explorative design in a limited cohort of 58 MG patients over 3 years.

Socio-demographics (age, sex, disease duration), history of myasthenic crisis, antibody status (acetylcholine receptor antibody [AChR-Abs], muscle specific receptor tyrosine kinase antibody [MuSK-Abs], lipoprotein-related protein 4 [LRP4], seronegative), current MG specific medication (cholinesterase inhibitors, glucocorticoids, and long-term immunosuppressant's), history of thymectomy, and comorbidities were collected in a database. Exclusion criteria were age <18, previous history of cancer except thymoma [23], and diagnosis of RA or IBD due to potential influence on CLP levels. 77 age and gender matched voluntary HC were enrolled as a healthy control group, as well as 13 patients with non-inflammatory neurological diseases (NC) recruited from the outpatient clinic for polyneuropathies as a diseased control group. Exclusion criteria for controls were history of autoimmune disorders, neurological diseases other than polyneuropathies, obesity, cardiovascular diseases as well as history of cancer.

2.4. Clinical assessment

Clinical outcome was assessed using the MGFA classification for disease classification [24] and the QMG score for disease severity. Using the MGFA classification, patients were grouped into remission (MGFA 0), ocular (MGFA I) or generalized MG patients (MGFA II-IV) at time of study inclusion and blood sampling. We have not used the MGFA classification by employing the most severely affected muscles of disease history but for current disease severity to define the patient's MGFA class [24].

The QMG score was developed as a tool for assessing disease severity as well as the pattern of deficits based on quantitative testing of sentinel muscle groups [24,25]. Its reliability and validity have been demonstrated in several studies [25,26]. QMG scores were assessed at baseline and follow up to evaluate disease severity. Moreover, patient reported outcome regarding impact on daily living was assessed at both corresponding time points using the MG-ADL [27,28].

2.5. Laboratory testing

Blood samples were collected from patients with MG and controls immediately centrifuged and stored at −80 °C until being analyzed in May 2020. Serum levels of CLP were measured using the fCAL turbo method® on a COBAS 8000 semi-automated analyses (Bühlmann Laboratories AG, Schönenbuch, Switzerland) according to manufacturer's protocol [29,30]. The fCAL turbo method® has been validated for accurate measurement of CLP levels in serum [29].

2.6. Statistical analysis

All statistical analyses were performed using GraphPad Prism (version 8.2.1, GraphPad Software, San Diego, CA, USA) and SPSS (version 25; SPSS Inc., Chicago, USA). Continuous data are presented as means and standard deviation (SD) and categorical variables as absolute frequencies and percentages. Baseline serum levels of CLP between MG patients and HC were compared using the one-way analysis of variance (ANOVA) with corrections for multiple comparisons. Correlation between CLP levels, clinical, and laboratory assessments were examined using nonparametric Spearman correlation analysis. A univariate analysis was performed using Mann-Whitney nonparametric test to analyze the differences between groups, and the Kruskal-Wallis test was used to analyze the differences between three or more groups. To illustrate the predictive performance of CLP in regard to disease severity as measured by QMG and MG-ADL, we calculated a delta for changes in QMG, MG-ADL and CLP levels, performed a correlation using Spearman correlation coefficient as well as a Mann-Whitney test. A p-value<0.05 was considered statistically significant.

2.7. Data availability

Anonymized data will be shared upon reasonable request from a qualified investigator.

3. Results

3.1. Demographics and baseline characteristics of MG patients

Overall, we included 251 patients with MG, 77 HC and 13 NC (Table 1) and for prospective analysis 58 MG patients (Table 2). Mean age was 54.4 years (SD 17.4), 147 (59 %) were female. Median disease duration was 4.0 years (2.0–10.0). Mean age at disease onset was 46.1 years (SD 19.0). 208 (83 %) of MG patients were positive for AChR-Abs, 10 (3 %) for MusK-Abs, 0 for LRP4-Abs (0 %), and 34 (15 %) remained seronegative (SN). Disease severity at time of sampling ranged from MGFA class 0–IIIB (median II), mean QMG was 8.1 (SD 6.5) and mean MG-ADL was 4.9 (SD 3.9). 50 MG patients (20 %) had a history of myasthenic crisis as defined by rapid worsening of muscle weakness and potential airway compromise from ventilatory or bulbar dysfunction [31].

At time of sampling, 129 (52 %) of MG patients had already undergone a thymectomy, 56 MG patients (16 %) used symptomatic monotherapy with cholinesterase inhibitors, while the majority of patients additionally used oral corticosteroids (n = 75; 42 %) and/or steroid

Table 1
Baseline characteristics and medical history of MG patients and controls.

	Total MG	EOMG	LOMG	TAMG	Controls	HC	NC
Demographics	251	97 (39%)	154 (61 %)	30 (12 %)	90	77 (86 %)	13 (14 %)
Sex	147 (59 %)	77 (69 %)	69 (45 %)	19 (68 %)	52 (58 %)	46 (60 %)	6 (38 %)
Female							
Age at diagnosis (YEARS)	54.4 ± 1.4	36.1 ± 8.4	66.2 ± 9.9	58.1 ± 12.5	51.9 ± 11.8	47.2 ± 7.7	65.6 ± 10.9
Disease duration (years)	4.0 (2-0-10.0)	4.0 (2.0–7.0)	4.0 (2.0–11.3)	3.5 (1.3–8.3)	–	–	–
History of myasthenic crisis	50 (20 %)	21 (22 %)	27 (18 %)	11 (39%)	–	–	–
MGFA classification at time point of sampling	38 (15 %)	20 (21 %)	18 (12 %)	0 (0 %)	–	–	–
0	42 (17 %)	13 (13 %)	24 (16 %)	5 (18 %)			
I	144 (57 %)	43 (44 %)	80 (52 %)	21 (75 %)			
II	27 (11 %)	13 (13 %)	13 (8 %)	1 (4 %)			
III	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)			
IV	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)			
V							
QMG- Score	8.1 ± 6.5	8.1 ± 6.3	7.9 ± 6.5	9.4 ± 6.7	–	–	–
MG-ADL- Score	4.9 ± 3.9	5.0 ± 3.9	4.5 ± 3.7	4.6 ± 4.2	–	–	–
History of thymectomy	129 (52 %)	66 (68 %)	62 (40 %)	28 (100 %)	–	–	–
MG-specific treatment at baseline	56 (16 %)	30 (31 %)	32 (21 %)	2 (7 %)	–	–	–
Cholinesterase inhibitors monotherapy	75 (43 %)	28 (29 %)	83 (54 %)	10 (36 %)			
Corticosteroids	86 (29 %)	22 (23 %)	48 (31 %)	12 (43 %)			
Azathioprine	28 (11 %)	9 (9 %)	17 (13 %)	2 (7 %)			
MycophenolatE mofetil	12 (5 %)	6 (6 %)	6 (4 %)	0 (0 %)			
Methotrexate	10 (4.0 %)	6 (6 %)	3 (3 %)	4 (14 %)			
Rituximab	7 (3 %)	2 (2 %)	5 (3 %)	0 (0 %)			
Eculizumab							

Data are mean (SD) and n (%) for the baseline variables and median (IQR) for disease duration. Disease duration is the time from diagnosis until baseline. *Abbreviations*: EOMG = early onset myasthenia gravis, HC = healthy controls, IQR = interquartile range; LOMG = late onset myasthenia gravis; MG = myasthenia gravis; MGFA = Myasthenia gravis foundation of America classification; MG-ADL = MG-activity of daily life score; NC = non-inflammatory neurological controls, SD−standard deviation, TAMG = thymoma - associated myasthenia gravis, QMG = quantitative myasthenia gravis score.

Table 2
Demographical and clinical characteristics of MG patients of prospective analysis.

	V1*	V2**
Demographics	58	58
Sex	23 (40 %)	23 (40 %)
Female		
Age at diagnosis (YEARS)	57.6 ± 16.4	60.5 ± 16.1
Disease duration (years)	5.0 (1.8–14.0)	8.0 (2.0–7.0)
History of myasthenic crisis	8 (14 %)	8 (14 %)
MGFA classification at time point of sampling	6 (10 %)	13 (22 %)
0	16 (28 %)	11 (19 %)
I	33 (57 %)	31 (54 %)
II	2 (4 %)	3 (5 %)
III	0 (0 %)	0 (0 %)
IV	0 (0 %)	0 (0 %)
V		
QMG- Score	6.6 ± 4.9	7.2 ± 5.7
MG-ADL- Score	4.2 ± 3.4	4.0 ± 3.7
History of thymectomy	26 (45 %)	33 (57 %)
TYMOMYA	6 (22 %)	8 (24 %)
THYMITIS	10 (39 %)	10 (30 %)
WITHOUT PATHOLOGY	10 (39 %)	15 (46 %)
MG-specific treatment at Sample time	7 (12 %)	10 (17 %)
Cholinesterase inhibitors monotherapy	8 (14 %)	9 (16 %)
Corticosteroids	12 (21 %)	21 (36 %)
Azathioprine	1 (2 %)	8 (14 %)
MycophenolatE mofetil	5 (9 %)	9 (16 %)
Methotrexate	1 (2 %)	2 (3 %)
Rituximab	0 (0 %)	0 (2 %)
Eculizumab		

Data are mean (SD) and n (%) and median (IQR) for disease duration. Disease duration is the time from diagnosis until baseline. *Abbreviations*: IQR = interquartile range; MG = myasthenia gravis; MGFA = Myasthenia gravis foundation of America classification; MG-ADL = MG-activity of daily life score; SD−standard deviation, QMG = quantitative myasthenia gravis score, V1 = baseline visit, V2 = follow-up visit after 3 years.

sparing immunosuppressive therapy (n = 86; 29 %). In 17 MG patients (7 %), escalation therapies (rituximab; eculizumab) were required.

3.2. CLP levels are higher in MG

Baseline serum CLP levels were significantly higher in MG patients with a mean of 4.3 μg/ml (SD 3.0, 95 % CI 3.8–4.6) compared to HC (mean 2.1 μg/ml (SD 1.1, 95 % CI 1.2–2.2) and NC (mean 2.0 μg/ml (SD 1.2, 95 % CI 1.6–2.3); $p < 0.0001$; Fig. 1), with an area under the receiver operating curve (AUC) of 0.77 (95 % confidence interval (CI) 0.70–0.83; $p < 0.0001$). With a cut-off-value of 1.55 μg/ml CLP discriminated MG patients from controls with a sensitivity of 90.4 % and a specificity of 45.1 %.

There was a trend of highest levels in TAMG patients (4.9 μg/ml (SD 3.7, 95 % CI 3.4–6.2)), but not reaching statistical significance ($p = 0.218$, Kruskal-Wallis test).

Serum CLP levels in MG patients and controls were neither correlated to age ($r = -0.04$, $p = 0.459$) nor associated to gender ($p = 0.9246$; Mann-Whitney test).

Mean CLP levels in AChR-Abs positive patients (n = 208) were 4.2 μg/ml (SD 3.0), in MuSK-Abs positive patients (n = 10) 4.6 μg/ml (SD 4.1), and in seronegative MG patients (n = 34) 4.9 μg/ml (SD 2.9) showing no significant in between group differences ($p = 0.21$, Kruskal-Wallis test). Moreover, in AChR-Abs positive and MuSK-Abs positive MG patients, CLP levels did not correlate with Abs level ($r = 0.03$, $p = 0.677$ for AChR-Abs; $r = 0.04$, $p = 0.55$ for MuSK-Abs) (Spearman correlation coefficient). There was no significant difference in CLP levels in patients with (n = 51) and without (n = 200) a history of myasthenic crisis (n = 51) ($p = 0.213$, Mann-Whitney test).

3.3. Baseline CLP levels correlates with disease severity

Clinical severity measured by MGFA classification ranging from remission, I– III at time point of sampling were compared regarding CLP levels and revealed significant higher CLP levels in patients with a generalized compared with pure ocular MG or patients in remission ($p = 0.0435$, Kruskal-Wallis test, Fig. 2A). QMG score at baseline correlated

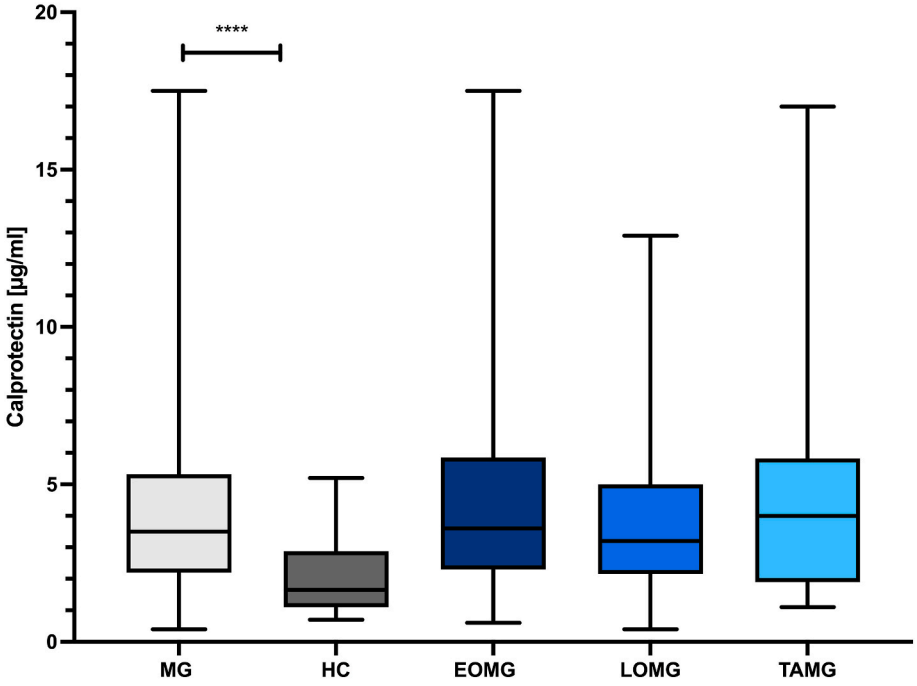

Fig. 1. Calprotectin levels are elevated in MG patients compared to controls. The box plot bar represents the mean baseline serum calprotectin (CLP) level with SD in all myasthenia gravis (MG) patients (n = 251) and subgroups compared to healthy controls (HC) (n = 77), n = number of patients with evaluable data. Mean CLP levels were significantly increased in all MG patients compared to HC regardless of MG-subtype ($p < 0.001$). Abbreviations: EOMG = early onset myasthenia gravis, LOMG = late onset myasthenia gravis; MG = myasthenia gravis; TAMG = thymoma - associated myasthenia gravis.

weakly, but significantly with serum CLP levels (r = 0.134, $p = 0.043$, Spearman correlation coefficient, Fig. 2B). However, there was no significant correlation with the patient outcome parameter MG-ADL (r = 0.09, $p = 0.1664$, Spearman correlation coefficient, Fig. 2C).

3.4. Relationship between CLP levels and treatment regime

Patients receiving only symptomatic monotherapy showed the highest levels of CLP at baseline (n = 75; 42 %; 4.1 µg/ml (SD 2.8)), whereas patients treated with eculizumab (n = 7 (3 %); 2.1 µg/ml (SD 0.4)) had the lowest, although not reaching statistical significance ($p = 0.072$; Kruskal-Wallis test). MG patients with a history of thymectomy (at least >2 years) had similar mean baseline CLP levels compared to patients without history of thymectomy (4.3 µg/ml (SD 3.3) vs. 4.2 µg/ml (SD 2.7); Mann-Whitney test).

3.5. CLP not predictive with individual disease severity activity

To investigate the predictive performance of CLP regarding to individual changes in clinical (QMG) as well as patient reported outcome parameters (MG-ADL), we calculated a delta for each time point as well as a delta for individual change in CLP level and performed a Mann-Whitney test. We did not observe significant delta changes as defined by ≥ 2 points for QMG and ≥3 for MG-ADL in correlation with delta changes of CLP using Spearman correlation coefficient (r = 0.241 for QMG, r = 0.495 for MG-ADL). There was only a tendency for correlation in regard to changes over time for QMG score in total MG population at baseline (n = 251) and year 3 (n = 58) ($p = 0.410$) (Fig. 3).

4. Discussion

This explorative cross-sectional and prospective study revealed that serum CLP levels of MG patients were significantly higher in comparison to controls. Moreover, baseline CLP levels correlated weakly, but positively with clinical disease activity as measured by QMG score and MGFA classification. Nevertheless, the only weak correlation of CLP with clinical outcome parameters needs confirmation in future studies.

It should be emphasized, that baseline CLP levels were elevated in all MG patients regardless of Abs status (AChR-Abs, anti-MuSK-Abs, SN), which might be helpful in suspected MG cases, since about 15 % of the MG patients remain SN [1] and we have not found elevated CLP levels in the control group. Nevertheless, CLP is a sensitive but unspecific marker of inflammation. Its potential value therefore does not lie in the ability to discriminate different autoimmune diseases, but rather to reflect different degrees of disease activity, as proposed in other conditions like IBD [14] and RA [16,32], where CLP has become a routinely measured biomarker for disease severity. In addition, CLP has the potential to be a marker of microbial dysbiosis [13], which has been proposed to play a critical pathophysiological role in MG [7,33,34].

CLP leads to induction of auto-reactive $CD8^+$ T cells, IL-17 [35] and other pro-inflammatory cytokines like IL-1β. The imbalance of inflammatory cytokines are involved in the pathogenesis of MG and play a central role in the development of inflammation at the neuromuscular junction [20,35]. IL-1β was reported to be a key cytokine which promotes Th17 cell generation, which is crucially involved in pathogenesis of MG [36]. In an experimental autoimmune MG mouse model, IL-17-knock out mice were developing fewer myasthenic symptoms and less pathogenic AChR-specific Abs [35]. Furthermore, increased IL-17 levels have been observed in MG patients [36], and an increased frequency of IL-17- producing $CD4^+$ T cells has been demonstrated in particular for TAMG [37], which might explain the trend towards higher mean CLP level in TAMG patients in our cohort.

CLP was elevated in MG patients with high disease activity as scored with MGFA and QMG, which can effectively reflect the severity of the disease. This important finding relates to studies of CLP in IBD and RA, where CLP is routinely used as a disease activity and treatment response marker, especially for biologicals [15,38]. Although not statistically significant, this fact is further supported by the finding of lowest CLP levels in patients receiving eculizumab, providing additional evidence of the strong therapeutic efficiency of complement inhibition [39].

Socio-demographic parameters, history of myasthenic crisis, as well as Abs-status and levels had no effect on CLP levels, although several longitudinal studies in RA patients observed higher CLP levels in patients being positive for rheumatoid factor [16]. In addition, patients with a history of thymectomy >2 years showed no relevant difference regarding CLP level, which might be due to the lower classification regarding MGFA- and QMG-score at time of sampling in comparison to time of diagnosis.

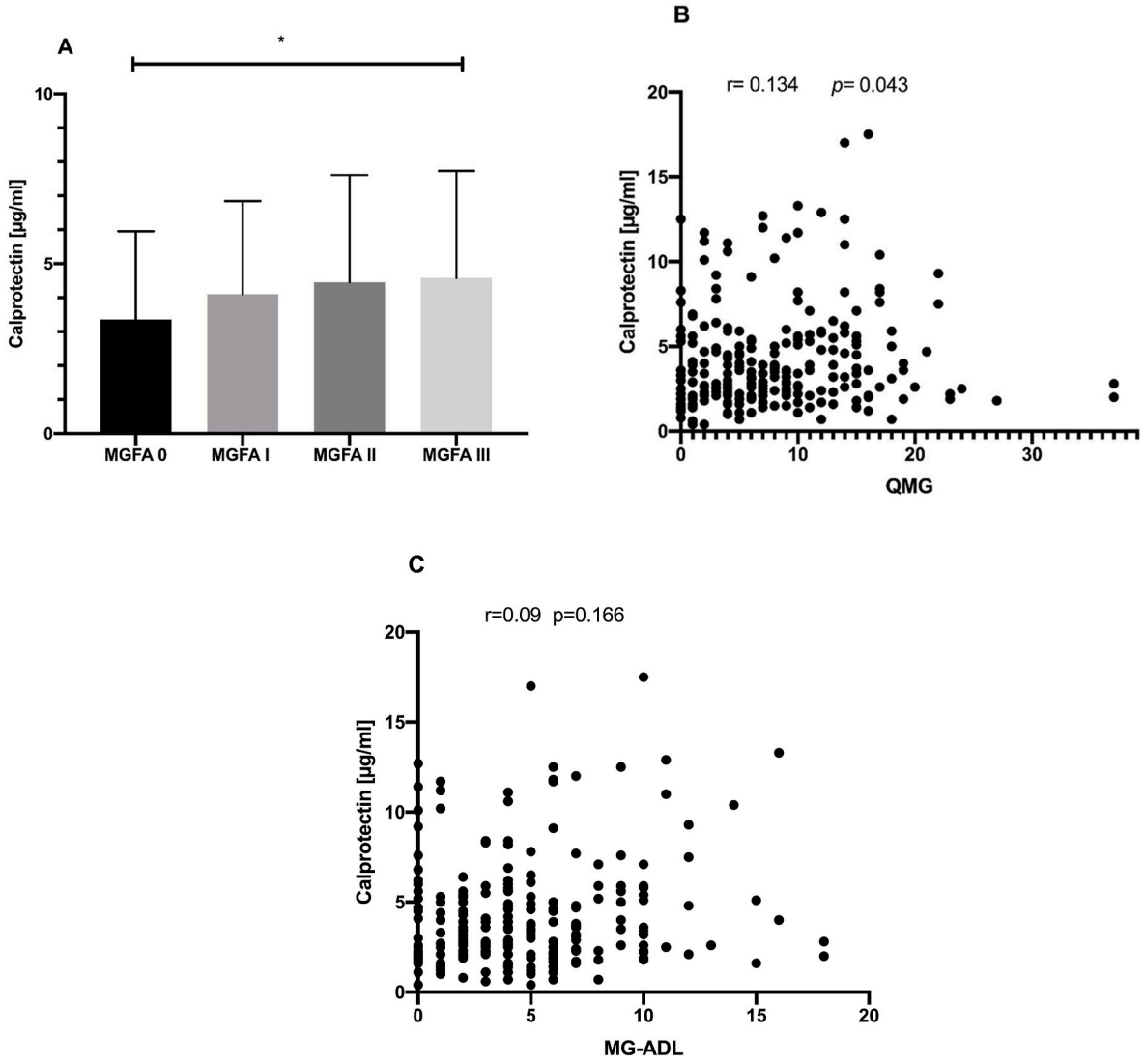

Fig. 2. Baseline CLP and clinical disease severity. **A**: Column bar graph of association of CLP level with clinical severity measured by Myasthenia gravis foundation of America classification (MGFA) using Kruskal-Wallis test. **B**: Correlation analysis of quantitative myasthenia gravis (QMG) score with serum level of CLP using Spearman correlation coefficient. C: Correlation analysis of myasthenia gravis activity of daily life (MG-ADL) score with serum level of CLP using Spearman correlation coefficient.

In the longitudinal analysis, the individual disease activity did not show a significant correlation in regard to delta changes. However, mean changes in primary outcome parameters QMG and MG-ADL were not significantly differing over the observed follow up time in our cohort, which might be mainly due to the low number of therapy naïve patients. In addition, the precise cut-off value for significant delta changes of QMG and MG-ADL score remains unclear and need to be explored in larger, longitudinal studies.

There are several limitations to our study. Although we included a rather high number of MG-patients in the cross-sectional design, our findings are limited to the rather small sample size in subgroups as well as the low number of included patients for the follow up assessment, which was due to the explorative design of our study. Future conformational and larger prospective studies are strongly needed to further examine the potential utility of CLP as a disease activity biomarker in MG.,. In addition, the main proportion of included patients was not therapy naïve as being heterogeneous in regards to disease duration and clinical severity. Nevertheless, this diversity reflects the typical demography of a specialized MG clinic. The control population was rather small, although in line with studies examining CLP in other autoimmune diseases.

5. Conclusion

In conclusion, this explorative cross-sectional and prospective study demonstrates that CLP levels were significantly higher in MG compared to controls. Additionally, we provide evidence, that CLP might reflect disease severity. There is an unmet need of a validated, non-invasive biomarker to assess disease activity and potentially guiding treatment. Further multicentric, longitudinal investigations are strongly needed to determine the potential utility of CLP as a biomarker for better care of patients with MG.

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Author contributions

Frauke Stascheit, MD: Design and conceptualized study, analyzed and interpreted the data, drafted the manuscript for intellectual content; the author takes full responsibility to the integrity of the data analyzed,

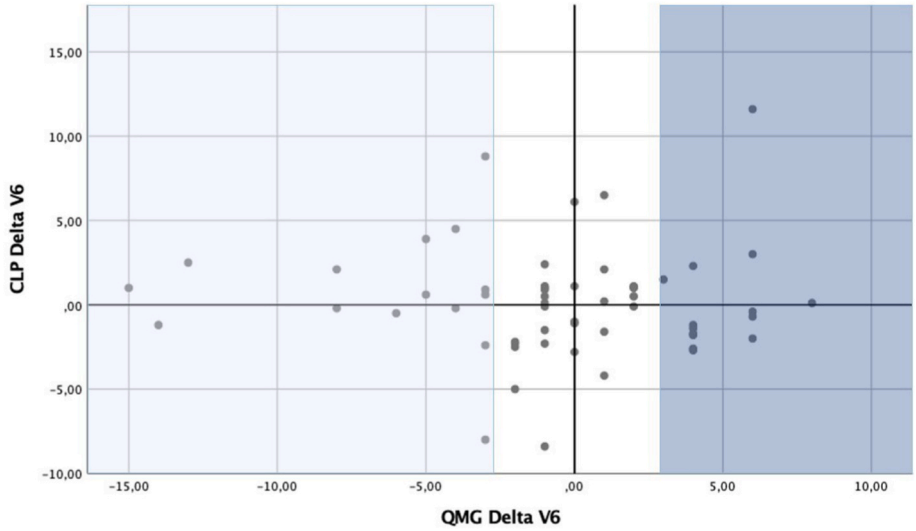

Fig. 3. Correlation of QMG score with CLP levels over time. Using grouped scattered plot we examined correlation in individual change (delta score) between clinical outcome parameter QMG score and CLP over two time points (baseline (n = 251 patients; year 3 (n = 58 patients). A significant change in delta QMG was defined as improvement ≥2 points presented as a positive delta (dark blue); significant change in CLP level as a negative delta (light blue).

Benjamin Hotter, MD: Analyzed and Interpreted the data, revised the manuscript for intellectual content; Sarah Hoffman, MD: Major role in acquisition of data; interpreted the data; revised the manuscript for intellectual content; Siegfried Kohler, MD: Major role in acquisition of data; interpreted the data; revised the manuscript for intellectual content; Sophie Lehnerer, MD: interpreted the data, revised the manuscript for intellectual content; Andreas Sputtek, MD: Laboratory analysis, revised the manuscript for intellectual content; Andreas Meisel, MD: Design and conceptualized study, interpretetd the data, revised the manuscript for intellectual content, funding acquisition.

Declaration of competing interest

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2.5. Serum Neurofilament light chain in Myasthenia gravis-Untergruppen: Eine explorative Kohorten- und Fall-Kontroll-Studie

Stascheit F, Aigner A, Mergenthaler P, Hotter B, Hoffmann S, Lehnerer S, Meisel C, Meisel A. Serum neurofilament light chain in myasthenia gravis subgroups: An exploratory cohort and case-Control study. Front Neurol. 2023 Jan 11;13:1056322.
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Serum Neurofilament light chain (sNfl) ist ein Marker axonalen Schadens und stellt sowohl bei neurodegenerativen [51] als auch inflammatorischen Erkrankungen wie der Multiplen Sklerose (MS) [52] oder der chronisch-inflammatorisch demyelinisierenden Polyneuropathie [53, 54] einen prognostischen Biomarker dar. Da die Auto-Ak der MG direkt pathogen an der NME wirken [22] wollten wir in dieser Studie untersuchen, ob sNfl ein Marker für den Schweregrad der Destruktion der NME darstellen könnte.
Hierzu wurden Seren von 134 Patient*innen mit unterschiedlichem Schweregrad und Ak-Status in einem Querschnittsdesign im Vergleich zu alters- und geschlechtsgematchten HC analysiert. Zudem wurde prospektiv über einen Zeitraum von bis zu 3 Jahren sNfl gemessen. Mittels Korrelationskoeffizienten und gemischter linearer Regression wurde der Zusammenhang zwischen sNfl-Werten, Soziodemografie, Krankheitsaktivität (QMG-Score, MG-ADL), Ak-Titer und Therapie untersucht.
Wir konnten in dieser Arbeit zeigen, dass die sNfl-Werte bei Patient*innen mit MG höher als bei HC waren, wobei AChR-Ak positive Patient*innen die höchsten Spiegel aufwiesen. Wir fanden keinen relevanten Zusammenhang zwischen individuellen Veränderungen in der Höhe des sNfl-Wertes und der klinischen Erkrankungsaktivität in der prospektiven Analyse.
Die Ergebnisse dieser Studie legen nahe, dass sNfl als prognostischer Marker, insbesondere bei der AChR-Ak positiven MG, eingesetzt werden könnte, um frühzeitig eine irreversible Destruktion der NME zu detektieren und zu vermeiden.

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Serum neurofilament light chain in myasthenia gravis subgroups: An exploratory cohort and case–Control study

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Background: This study aimed to evaluate the association of neurofilament light chain (Nfl) with neuromuscular destruction and disease severity in the serum of patients with myasthenia gravis (MG).

Materials and methods: Sera from 134 patients with MG with varying degrees of disease severity and autoantibody (Abs) status were analyzed and compared to controls in a cross-sectional design. Prospectively, we additionally measured serum NfL (sNfl) levels in patients with MG longitudinally for up to 3 years. Based on linear regression, differences between patients and controls were assessed. With correlation coefficients and mixed linear regression, the association among sNfl levels, socio-demographics, disease activity (Quantitative Myasthenia Gravis (QMG) score and Myasthenia Gravis Activities of Daily Living (MG-ADL) scale), Abs-status (acetylcholine receptor antibody (AChR-Abs), muscle-specific receptor tyrosine kinase antibody (MuSK-Abs), lipoprotein-related protein 4 (LRP4), and seronegative), Abs titer, treatment regime (pyridostigmine, steroids, and immunosuppressive therapies), and thymectomy were investigated.

Results: sNfl levels were higher in patients with MG compared to controls (median: 11.2 vs. 7.88), where sNfl levels were highest in anti-AChR-Abs positive patients (median 12.6), followed by anti-MuSK-Abs positive, anti-LRP4-Abs positive, and seronegative patients. Adjusting for age and sex, sNfl levels of patients with MG were on average 35% higher compared to controls (35.1, 95% CI: 8.4;68.3) and highest for patients with seronegative MG (44.35; 95% CI 16.47; 78.90). We found no relevant relationship between individual changes in sNfl and changes in QMG and MG-ADL scores.

Conclusion: sNfl levels are higher in patients with MG than in controls but were not consistently associated with clinical severity. Thus, sNfl is not a suitable biomarker to monitor individual disease progression in patients with MG.

KEYWORDS

serum neurofilament light chain, myasthenia gravis, antibody status, biomarker, disease severity

1. Introduction

Myasthenia gravis (MG) is an autoimmune disease affecting the neuromuscular junction (NMJ) by specific autoantibodies (1, 2). While the effector mechanisms of the disease disturbing the functions of the NMJ are relatively well known, the etiology of MG and its heterogeneity in the clinical course are poorly understood. Importantly, there is an urgent need for sensitive biomarkers in MG predicting treatment responses and outcomes, especially in light of emerging and more specific treatment options (3, 4).

Neurofilaments (Nfl) are important structural elements of neurons and are released into the extra-cellular environment upon neuronal injury (5). Nfl has been studied in several neurodegenerative and central neuroinflammatory conditions (6–12).

Less is known about the role of Nfl in peripheral nervous system disorders, but there is increasing evidence that sNfl potentially has diagnostic and prognostic value in acquired polyneuropathies (13, 14), inherited peripheral neuropathy (15), and Guillain-Barré syndrome (16).

Although MG is not a typical disorder characterized by neuronal injury, histopathological studies in patients with MG demonstrate neurogenic changes regardless of MG subtype (17, 18). Additionally, it is known that antibodies (Abs) against the acetylcholine receptor (AChR), the muscle-specific kinase (MuSK), and the lipoprotein-related protein 4 (LRP4) are directly pathogenic, inducing accelerated degradation of these receptors (19–21) and leading to local membrane damage at the NMJ (22). These mechanisms destabilize the signaling pathways at the NMJ, leading to ACh deprivation, which is crucial for proper muscle innervation (23).

As the neuromuscular terminal seems to be enriched with proteins critical for NMJ structure and function, including cytoskeleton-associated proteins like Nfl (24–27), the different extent of neuromuscular destruction on the NMJ could have a measurable effect on Nfl release (28, 29). Here, we investigate whether sNfl is increased in patients with MG compared to controls and whether sNfl could serve as a biomarker of disease severity.

2. Materials and methods

2.1. Standard protocol approvals, registration, and patient consent

The study was approved by the ethics committee of the Charité—Universitätsmedizin Berlin (EA1/281/10). All patients gave written informed consent in accordance with the Declaration of Helsinki in its currently applicable form. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines (Supplementary material).

2.2. Study design

This is an explorative cohort study examining sNfl levels in patients with MG with different Abs statuses. Longitudinally we followed the patients and assessed sNfl as a potential biomarker for MG disease severity as measured by the Quantitative Myasthenia Gravis (QMG) score and Myasthenia Gravis Activities of Daily Living (MG-ADL) scale. Based on the cohort design, patients were matched with controls at baseline.

2.3. Patients and controls

This study was carried out at the certified integrated Center for Myasthenia gravis (iMZ) of the Charité—Universitätsmedizin Berlin, Germany. Patients over the age of 18 years with a confirmed diagnosis of MG, based on the current guidelines of the German Neurological Society (30), were included independent of disease duration and severity. Patients with MG were consecutively screened at the iMZ clinic from March 2011 to October 2020. Due to the potential influence on sNfl levels, patients with a previous history of cancer were excluded, excepting thymoma (31). Other exclusion criteria were diagnosis of polyneuropathy, neurodegenerative diseases (atypical and typical parkinsonian disorders, frontotemporal dementia, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS)), central nervous

inflammatory diseases (multiple sclerosis and autoimmune encephalitis), traumatic brain injury, and previous stay on intensive care unit (<3 months) (32). Overall, 134 patients could be included for baseline analysis and were followed up to 3 years. For exploratory longitudinal analysis, we tested sNfl in a total of 59 patients. Age- and sex-matched controls without comorbidities recruited from the clinical staff of the Charité-Universitätsmedizin Berlin were enrolled as a control group ($n = 31$).

2.4. Clinical assessment

Patients were categorized into subgroups according to their Abs status (anti-AChR-Abs, anti-MuSK-Abs, anti-LRP4-Abs, and seronegative). None of the patients were double positive for an MG-Abs. Age, sex, disease duration, history of myasthenic crisis, current MG-specific medication (cholinesterase inhibitors, glucocorticoids, and long-term immunosuppressants), history of thymectomy, and comorbidities were collected. Disease severity was assessed using the Myasthenia gravis foundation of America (MGFA) classification, the QMG, and the MG-ADL score (33, 34). According to the MGFA classification, patients were grouped into ocular (MGFA I) or generalized patients with MG (MGFA II-IV) at the time of study inclusion and blood sampling. The MGFA classification system served as an assessment of patients' disease severity at the time of data sampling as a simple scoring system (33). The QMG score was developed as a tool for assessing disease severity as well as the pattern of deficits based on quantitative testing of sentinel muscle groups (33, 35). It is a 13-item score with a total score range of 0–39 points and shows good interrater variability (36). Its reliability and validity have been demonstrated in several studies (35, 37). The MG-ADL is an eight-question survey of symptom severity, with each response graded from 0 (normal) to 3 (most severe) (34). Questions include ocular, oropharyngeal, respiratory, and extremity functions. The total MG-ADL score ranges from 0 to 24. All scores were assessed at the time of sample collection.

2.5. sNfl measurement

Serum samples were collected from patients with MG and controls, clotted for 30 min at room temperature and then centrifuged, aliquoted at room temperature, and stored at −80°C. The temperature of the freezers was continuously monitored, and samples were thawed only before analysis. sNfl concentrations were measured using the SIMOA Nf-light kit® in SR-S immunoassay analyzer, SIMOA™ (Quanterix Corp, Boston, MA, USA), according to the manufacturer's protocol (38). SIMOA Nf-light kit® is an ultrasensitive paramagnetic bead-based enzyme-linked immunosorbent assay and is at least 125 times more sensitive than conventional immunoassays and maintains a high analytical performance (39). All samples were analyzed in one measurement in March 2021. Calibration curves for assay calibration were set up according to the manufacturer's instructions. As recommended by the manufacturer, the use of stored calibration curves for a maximum of 3 weeks has been validated in a curve storage study over 4 weeks during assay validation. Calibration curve validity was assessed during each analytical run by assaying controls of known concentrations at two different levels (low and high controls provided by the manufacturer with sNF-L concentrations ranging between 2 and 5 pg/ml and between 100 and 200 pg/ml, respectively). Assay variation over time was low as indicated by the coefficient of variation (CV) values of <7.9 and 12% for high and low controls, respectively, during their shelf life of up to 6 months over different control lots.

2.6. Statistical analysis

Continuous data are presented as the median and interquartile range (IQR), and categorical variables as absolute frequencies and percentages. Age-adjusted z-scores were calculated for cases and controls based on a reference database of 4,532 healthy controls from the serum neurofilament light chain reference app (40–42). Comparisons of sNfl levels between MG subgroups and recruited controls were based on log-linear regression analysis, adjusted for age and sex to control for confounding by these variables. These models apply the natural logarithm to the dependent variable, for example, if the dependent variable is skewed. Based on them, we derive adjusted estimates of percentage change in sNfl levels comparing cases and controls additionally stratified by Abs-status. Association between sNfl levels of MG patients with clinical and laboratory assessments at baseline and during follow-up were analyzed by computing Spearman correlation and repeated measure Spearman correlation coefficients, respectively. Additionally, log-linear mixed models were used to assess the association between patient characteristics and sNfl, where again the results are derived as the percentage change in sNfl levels. All derived effect sizes are reported along with 95% confidence intervals (CI). All statistical analyses were performed using R (43) and R packages (44–48).

2.7. Primary research question

Are sNfl levels elevated in patients with MG compared to controls and can sNfl be used as a biomarker of neuromuscular destruction indirectly reflecting disease severity in patients with MG?

TABLE 1 Baseline characteristics and medical history of patients with myasthenia gravis and controls.

	Total MG	Anti-ACHR-aBS+	Anti-mUsk-ABS+	Anti-lrp4-ABS+	Seronegative	Controls
n (%)	134	79 (59%)	18 (13%)	11 (8%)	26 (19%)	31
Sex						
Female, *n* (%)	85 (63%)	46 (55%)	15 (79%)	6 (68%)	25 (81%)	21 (68%)
Age at time point of sampling, median (IQR)	52.5 (39–68)	54 (39–70)	56 (33–63)	49 (42–58)	51 (39–60)	46.0 (39–58)
Disease duration (years), median (IQR)	4.0 (2.0–10.5)	3.0 (2.0–10.5)	6.0 (2.0–18.0)	1 (1.0–5.0)	3.5 (2.0–8.0)	–
History of myasthenic exacerbation/CRISIS, *n* (%) (missing)	32 (25%) (11)	19 (14%) (0)	9 (7%) (0)	0 (0%) (11)	4 (3%) (0)	–
MGFA classification at time point of sampling, Median (IQR) (missing)	2 (1.0–2.0) (1)	2 (1.0–2.0) (0)	2 (2.0–2.0) (0)	3 (2.0–3.0) (0)	2 (1.0–2.0) (1)	–
QMG, median (IQR) (missing)	7 (2.75–13.0) (10)	6 (3.0–12.0) (1)	7 (1.5–12.0) (0)	10 (2.5–13.75) (5)	10 (4.5–14.0) (4)	
MG-ADL-score, median (IQR) (missing)	5 (2.0–8.0) (3)	4 (1.0–7.0) (0)	5 (3.0–7.75) (0)	13 (12.25–24.5) (1)	6 (3.0–9.25) (2)	–
History of thymectomy	52 (39%)	41 (52%)	0 (0%)	3 (27%)	8 (31%)	–
Thymoma, *n* (%) (missing)	1 (2%) (76)	1 (2%) (32)	0 (0%) (18)	0 (0%) (9)	0 (0%) (17)	
MG-specific treatment at baseline, *n* (%)						
Cholinesterase inhibitors	110 (82%)	13 (16%)	1 (6%)	3 (27%)	8 (26%)	
Corticosteroids Mono	35 (28%)	19 (14%)	7 (5%)	2 (2%)	6 (5%)	–
Azathioprine	45 (34%)	30 (38%)	3 (16.7%)	2 (18.2%)	10 (38.5%)	
Mycophenolate mofetil	15 (11%)	9 (11.4%)	3 (16.7%)	1 (9.1%)	2 (7.7%)	–
Methotrexate	6 (4%)	4 (5.1%)	1 (5.6%)	1 (9.1%)	0 (0%)	
Rituximab	4 (3%)	1 (1%)	3 (3%)	0 (0%)	0 (0%)	–
Eculizumab	1 (1%)	1 (0.7%)	0 (0%)	0 (0%)	0 (0%)	
SNfl (PG/ML), median (IQR)	11.2 (6.8–22.3)	12.6 (7.0–24.2)	9.1 (6.6–22.4)	8.7 (4.4–20.9)	12.3 (7.0–16.1)	7.8 (6.5–9.5)
Age-adjusted z-score (missing)	0.81 (−0.12, 1.62) (1)	1.18 (0.05, 1.75) (1)	0.70 (−0.49, 1.79) (0)	0.52 (−1.26, 1.36) (0)	1.04 (0.25, 1.62) (0)	0.08 (−0.66, 0.36) (0)

Results are presented as median (IQR) or *n* (%). Disease duration is the time from diagnosis until baseline.
AChR-+, acetylcholine receptor antibody positive MG-patients; IQR, interquartile range; LRP4+, lipoprotein related peptide 4 positive MG-patients; MG, myasthenia gravis; MG-ADL, MG activity of daily life score; MGFA, Myasthenia gravis foundation of America classification; MuSK+, muscle specific positive MG patients; sNfl, serum neurofilament light chain; QMG, quantitative myasthenia gravis score; –, not applicable.

3. Results

3.1. Demographics and characteristics of patients with MG

We included 134 patients with MG and 31 controls (Table 1). The median age of patients with MG was 52.5 years (IQR 39.0–68.8), 63% ($n = 85$) were female, whereas the median age of controls was 46.0 years (IQR 39.0–58.0), and 68% were female. The median disease duration was 4.0 years (IQR 2.0–10.5). The main proportion of the study population was anti-AChR-Abs positive ($n = 79$, 59%), followed by anti-MuSK-Abs ($n = 18$, 13%) and anti-LRP4-Abs ($n = 11$, 8%), while 26 patients (19%) remained seronegative. For 37 anti-AChR-Abs positive, 14 seronegative, and 8 anti-MuSk-Abs positive patients, we had follow-up data. Median disease severity at the time of blood sampling according to the MGFA classification system was II (IQR I-II), median QMG 7 (IQR 2.75–13.0), and median MG-ADL 5 (IQR 2.0–8.0). About 25% of patients with MG ($n = 30$) had a history of myasthenic exacerbation/crisis as

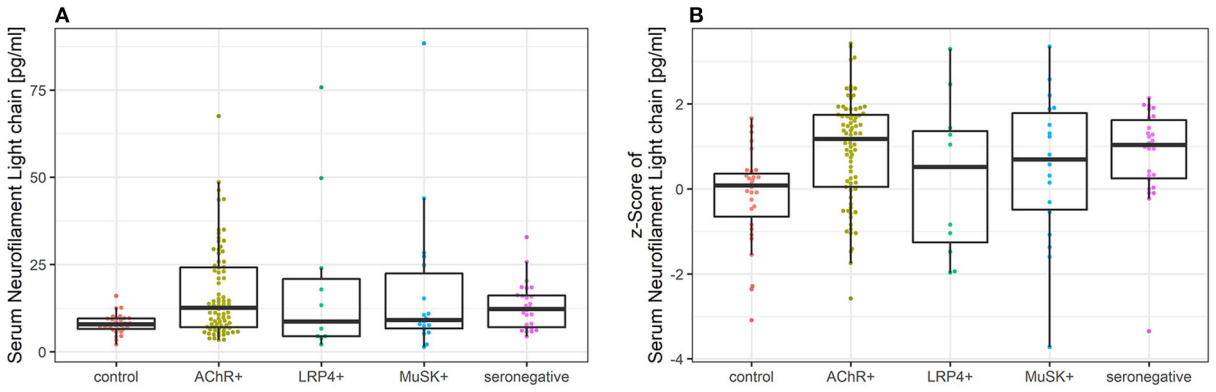

FIGURE 1
(A) Comparison of sNfl levels in patients with myasthenia gravis by antibody status and controls. Boxplot of sNfl levels for patients with MG by antibody status in comparison to controls. Anti-AChR-positive patients ($n = 79$) had the highest sNfl level with a median of 12.6 pg/ml (IQR 7.0–24.2), followed by seronegative (12.3 pg/ml, IQR 7.0–16.1; $n = 26$), anti-MuSK-Abs-positive patients (9.1 pg/ml, IQR 6.6–22.4; $n = 18$), and anti-LRP4-positive patients (8.7 pg/ml, IQR 4.4–20.9; $n = 11$). **(B)** Comparison of age-adjusted z-scores of sNfl levels in patients with myasthenia gravis by antibody status and controls using Boxplot of age-adjusted z-scores of sNfl levels in MG patients by antibody status in comparison to controls. The z-scores quantify the deviation of sNfl in comparison to controls of the same age based on a reference database of sNfl measured in 4,532 persons. AChR-+, acetylcholine receptor antibody positive MG-patients, Abs, antibody, IQR, interquartile range, LRP4+, lipoprotein related peptide 4 positive MG-patients, MuSK+, muscle specific positive MG patients, sNfl, serum neurofilament light chain.

defined by rapid worsening of muscle weakness and potential airway compromise from ventilatory or bulbar dysfunction (49). Thymectomy as an immunomodulatory therapy has been undergone by 52 patients (39%). The majority of patients received immunosuppressive therapy at baseline either with corticosteroids monotherapy ($n = 38$, 28%) or with standard immunosuppressive therapy with azathioprine ($n = 45$; 34%), mycophenolate mofetil ($n = 15$, 11%), or methotrexate ($n = 6$, 4%). Escalation therapy with rituximab was administered to 3% ($n = 4$) of patients, of which, three of them were positive for anti-MuSK-Abs. One of the patients (anti-AChR-Abs positive) received eculizumab.

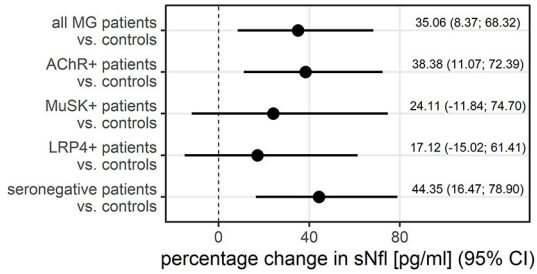

FIGURE 2
The adjusted difference between patients with MG and controls additionally stratified by antibody status, displayed as the percentage change in sNfl, derived from log-linear regression adjusted for age and sex in a logistic regression. AChR−+, acetylcholine receptor antibody positive MG-patients, CI, 95% confidence interval, LRP4+, lipoprotein related peptide 4 positive MG-patients, MuSK+, muscle specific positive MG patients, sNfl, serum neurofilament light chain.

3.2. sNfl levels in patients with myasthenia gravis compared to controls

The median sNfl levels were elevated by 3.3 pg/ml and about 1.4 times higher in patients with MG compared to controls with a median of 11.2 pg/ml (IQR 6.8–22.3) vs. 7.9 pg/ml in controls (IQR 6.5–9.5). Median sNfl levels were highest for anti-AChR-positive (median 12.6) and seronegative patients (12.3), compared to anti-MuSK-Abs (9.1) and anti-LRP4-positive patients (8.7) (Figure 1A, Table 1). Based on age-adjusted z-scores of sNfl levels, these observations were confirmed—with a median of 1.18 (IQR = 0.05, 1.75) for anti-AChR-positive, 1.04 (IQR = 0.2–1.62) for seronegative patients, 0.70 (IQR = −0.49–1.79) for anti-MuSK-Abs, and 0.52 (IQR = −1.26–1.36) for anti-LRP4-positive patients. A median of 0.08 for the recruited controls (IQR = −0.66–0.36) indicates a good similarity between the controls and the reference dataset used for the z-scores (Figure 1B, Table 1). Adjusting for age and sex, sNfl levels of patients with MG are on average 35% higher compared to controls (35.06%, 95% CI: 8.4;68.3) (Figure 2), where this difference compared to controls was even higher for seronegative patients (44.35; 95% CI 16.47; 78.90). The adjustment only for age yielded very similar estimated effects.

Additionally, we analyzed clinical characteristics of patients with MG with extensively elevated sNfl levels above 95% quantile with a median sNfl level of 49.8 pg/ml (IQR 47.5–71.6; Supplementary Table 1). Outliers were observed in all

TABLE 2 Spearman correlation coefficients for the association of patient characteristics and sNfl levels.

	Number of observations	Correlation with sNfl levels spearman correlation coefficient (95% CI)
Patient characteristics		
Age at baseline	134	0.68 (0.57; 0.79)
Age at visit*	236	0.42 (0.24; 0.57)
Disease duration at baseline	134	0.01 (−0.16; 0.19)
Disease duration*	236	0.36 (0.17; 0.52)
Mgfa at baseline	133	0.03 (−0.16; 0.22)
Anti-AChR-ABS level	70	0.20 (−0.04; 0.43)
Anti-MuSK-ABS level	16	0.28 (−0.36; 0.92)
Disease severity		
MG-ADL at baseline	131	0.03 (−0.14; 0.21)
MG-ADL*	210	−0.11 (−0.30; 0.09)
Diff MG-ADL*#	101	−0.20 (−0.48; 0.12)
QMG at baseline	124	0.18 (0.00; 0.36)
QMG*	210	−0.04 (−0.23; 0.16)
Diff QMG*#	77	−0.04 (−0.41; 0.35)

∗Repeated measure correlation coefficient.
#Correlation between differences of measurements between two subsequent visits of a patient.
AChR-+, acetylcholine receptor antibody positive MG-patients, CI, confidence interval, MG-ADL, MG activity of daily life score, MuSK+, muscle specific positive MG patients, *n*, number of included patients, QMG, quantitative myasthenia gravis score.

MG subgroups with higher median sNfl levels in anti-MuSK-Abs (median sNfl 66.2 pg/ml; IQR 55.2–77.3) and anti-LRP4-Abs positive patients (median sNfl of 62.75 pg/ml; IQR 56.3–69.3) compared to anti-AChR-Abs positive patients (48.6 pg/ml; IQR 47.5–58.1). In addition, disease duration was shorter, MGFA classification higher, and patients had a lower rate of thymectomy and immunosuppressive drugs. However, because sNfl is not normally distributed and the number of outliers was very low, no clear clinically relevant conclusions can be drawn from this observation.

3.3. sNfl levels and patient characteristics

Older age was strongly correlated with higher sNfl levels [at baseline only: $r = 0.68$, 95% CI: 0.57; 0.79, over the entire study period: 0.43 (0.26; 0.58)], as was disease duration over the entire study period: 0.36 (0.17; 0.52), but not disease duration at baseline and MGFA score. The Spearman correlation between sNfl levels and levels of anti-AChR-Abs titer was weakly positive (0.20, −0.04; 0.43), as was for anti-MuSK-Abs levels (0.28, −0.36; 0.92) (Table 2). Based on a mixed log-linear regression model, we found that independent of other factors, male patients had 14% higher sNfl levels compared to female patients (13.9%, 95% CI: −9.8; 43.7). A 10-year increase in age is associated with ∼31% (31.4, 23.2; 40.1) higher sNFl values (Figure 3).

Anti-LRP4-Abs positive patients had the lowest sNfl levels compared to the other MG subgroups. Adjusting for all other variables in this model, thymectomy and disease duration had no relevant effect on sNfl levels (Figure 3).

There was a trend for lowest sNfl levels in patients receiving symptomatic monotherapy with cholinesterase inhibitors (median of 7.00 pg/ml, IQR 5.8–21.0), followed by patients on corticosteroid monotherapy (median of 10.6 pg/ml, IQR 5.5–20.5), standard immunosuppressive therapy (azathioprine, mycophenolate mofetil, methotrexate; median of 13.4 pg/ml, IQR 7.99, 24.65), and patients receiving escalation therapy with rituximab (median of 21.9 pg/ml, IQR 6.85–37.2). Adjusting for all other variables in the log-linear model, there was no relevant effect of immunosuppression at baseline. The use of escalation therapies at baseline is associated with lower sNFl levels (coefficient (coefficient= −17.4; −52.1; 42.3), but due to uncertainty in this estimate, this finding cannot be generalized (Figure 3).

3.4. sNfl levels and disease severity

MG-ADL scores and sNfl levels did not correlate [values at baseline only; $r = 0.03$ (−0.14; 0.21)] (Figure 4A) or correlated weakly negatively [all available measurements; −0.11 (−0.30; 0.09)]. In contrast, there was a moderate positive correlation

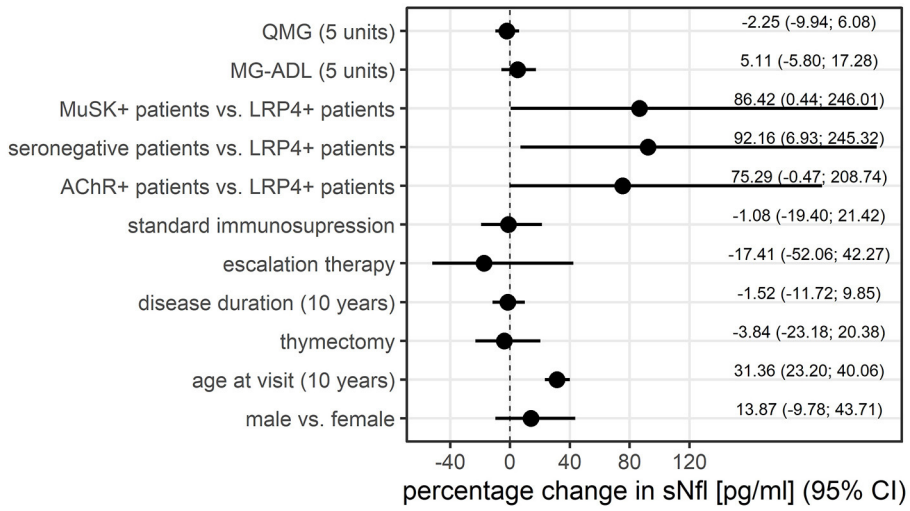

FIGURE 3
Association between patient characteristics and sNfl levels, displayed as the percentage change in sNfl, derived from mixed log-linear regression. AChR-+, acetylcholine receptor antibody positive MG-patients, LRP4+, lipoprotein related peptide 4 positive MG-patients, MG-ADL, myasthenia gravis activity of daily life score, MuSK+, muscle specific positive MG patients, sNfl, serum neurofilament light chain, QMG, quantitative myasthenia gravis score.

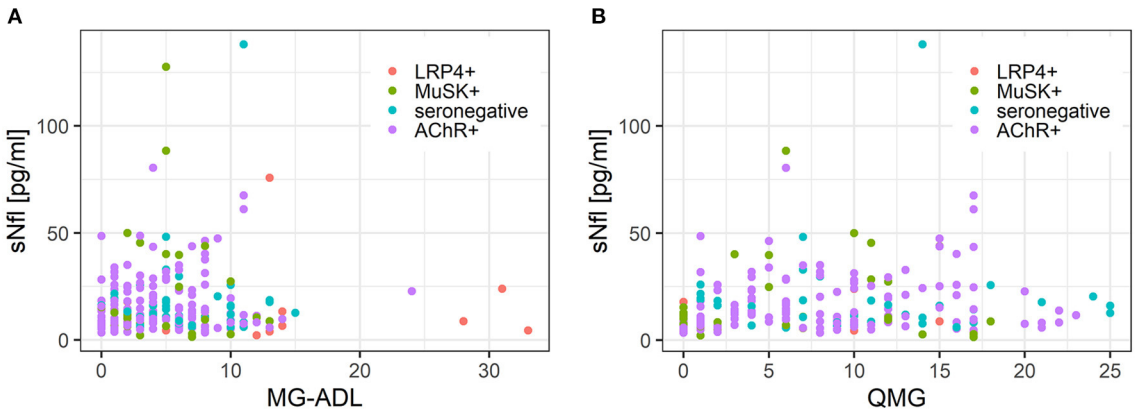

FIGURE 4
(A) Scatterplot of the association between sNfl levels and disease severity measured with QMG score in MG subgroups. **(B)** Scatterplot of the association between sNfl levels and disease severity measured with MG-ADL score in MG subgroups. AChR-+, acetylcholine receptor antibody positive MG-patients, LRP4+, lipoprotein related peptide 4 positive MG-patients, MuSK+, muscle specific positive MG patients, sNfl, serum neurofilament light chain, QMG, quantitative myasthenia gravis score, MG-ADL = myasthenia gravis activity of daily life score.

between QMG scores and sNfl levels at baseline [0.18 (0.00; 0.36)] (Figure 4B), whereas this was not found when all follow-up visits were taken into account (Table 2). When investigating the association between individual changes of sNfl with changes in MG-ADL and QMG scores, we found a negative correlation with both scores, but higher for MG-ADL [−0.20 (−0.48; 0.12), and QMG (−0.04 (−0.23; 0.16)] (Table 2). In a mixed log-linear regression model, sNFl levels were not relevantly associated with either MG-ADL or QMG levels (Figure 3).

4. Discussion

In this explorative cohort and case–control study, we demonstrated that sNfl levels of patients with MG were relevantly higher in comparison to controls. After adjusting for age and sex, median sNfl levels were highest for seronegative patients compared to controls. We did not find robust associations between sNfl levels and clinical disease severity measured as QMG or MG-ADL score. Therefore, our data

suggest that sNfl is not a suitable biomarker for monitoring individual disease activity in MG.

There is limited evidence for neurogenic involvement in MG, but early histopathological studies suggested neurogenic changes in muscle biopsies (18), which was confirmed in a recent review regardless of MG subtype (17). One potential cause is complete or partial atrophy of muscle fibers based on permanent local acetylcholine (ACh) deficiency because ACh could exert a trophic influence on muscle fibers in addition to transmitting the impulse at the NMJ (17, 18). The pathophysiological mechanisms leading to ACh deficiency are well known: direct blockade of the receptors through MG auto-Abs, complement activation leading to destruction and loss of AChR content at the postsynaptic membrane, and depletion of AChR receptors by Abs-mediated crosslinking (29, 50, 51). These mechanisms depend on the specific IgG auto-Abs subclasses, Abs titer, and specific epitopes (29, 51). Another explanation for a possible neurogenic change might be the increasing evidence for presynaptic involvement in the pathogenesis of MG (29, 52). Presynaptic proteins such as synaptic vesicle glycoprotein 2A (SVP2) are enriched in patients with MG (29). Notably, in the presence of high anti-AChR-Abs titers, these presynaptic proteins may be activated as a bystander effect of complement activation (29). An example that complement activation can lead to bystander-induced neuronal injury was recently described in aquaporin-4-positive neuromyelitis optica suggesting that a complement-bystander injury may be a general mechanism for early neuronal injury (28). Nfl, a cytoskeletal protein of the presynaptic membrane, could thus be released into extracellular fluids and potentially serve as a biomarker for NMJ destruction.

We have found that levels of sNfl in the group of patients with MG were found to be on average 35% higher than those of controls. In the subgroup of patients with seronegative MG, they were even 44% higher than in controls. However, precise cutoff values to use sNFl as an additional marker for diagnosis need to be defined in analogy to other neuroinflammatory and neurodegenerative disorders. Since sNfl is a sensitive but unspecific marker of axonal loss, its potential diagnostic value lies mainly in distinguishing between a healthy and pathological state, as well as between diseases with different neurodegenerative damage potential. The best evidence for reliable cutoff values exists for ALS, where an sNfl value of 62 pg/ml was shown to have a sensitivity of 85.5% (95% CI 78–91.2%) and specificity of 81.8% (95% CI 74.9–87.4%) to discriminate ALS mimics such as chronic inflammatory demyelinating polyneuropathies (CIDP) or multifocal motor neuropathy (MMN) (53). In CIDP and MMN, a median sNfl value of about 28 pg/ml was described, although the majority of the patients were receiving immunosuppressive therapy at the time of sampling (13, 14, 54).

sNfl levels in blood depend on age, increasing by an average of 2.2% per year between the ages of 18 and 70 years in controls, mainly due to physiological age-dependent neuronal loss, which needs to be considered when defining cutoffs (55, 56). Therefore, the establishment of age-dependent thresholds for sNfl concentration is also necessary for its diagnostic use. As the difference in median age between patients with MG and controls was 6.5 years, we adjusted for age in our analyses to minimize this potential confounder. In addition, although the association between disease duration and sNfl is weak, the association with age is already taken into account, we adjusted for this variable as it is yet a potential confounder.

We found a tendency toward higher sNfl levels in anti-AChR-Abs positive patients as compared to the other subgroups. This finding might be related to the different pathogenetic mechanisms at NMJ, depending on the causative autoantibody. Anti-AChR-Abs predominantly belong to the IgG1 and IgG3 subtypes (22, 57). The binding of these antibodies results in the activation of the classical complement pathway with the assembly of the membrane attack complex (MAC) leading to local membrane damage and loss of AChRs at the NMJ (22). Especially in the presence of high anti-AChR-Abs titers, presynaptic proteins may be activated as a bystander effect of complement activation (29), leading to Nfl release in extracellular fluids, explaining the tendency of higher levels of sNfl in patients with positive anti-AChR-Abs status. The neuromuscular terminal seems to be enriched with proteins critical for NMJ structure and function, including cytoskeleton-associated proteins, like Nfl (24–27). In contrast, anti-MuSK-Abs belong mainly to the IgG4 subtype (58), which is not able to activate the complement system and act directly pathogenic by blocking the natural activation of MuSK, leading to progressive loss of AChRs from the motor endplate (22). This might explain the tendency of lower levels of sNfl in this subgroup. Additionally, muscle biopsies of patients with anti-MuSK-Abs MG show myopathic signs, whereas neurogenic features and atrophy are more frequently found in patients with anti-AChR-Abs-positive MG (17, 18, 59, 60). Additionally, we found a moderate positive correlation between sNfl levels and levels of anti-AChR-Abs and anti-MuSK-Abs titers, which is an interesting clinical finding as data on the correlation of clinical severity with Abs-titer remains controversial (61–63).

It should be emphasized that after adjustment for age and sex, we found the highest median sNfl levels in seronegative MG patients. This observation might support the recent finding that in up to 60% of previously negative-tested patients with MG clustered auto-Abs against the AChR at the NMJ can be detected with cell-based essays (64–66), and that complement deposition is found at the NMJ (67). There is *in vitro* evidence that clustered auto-Abs can strongly activate complement-causing severe NMJ destruction and muscle weakness in a passive transfer MG rat model (29), which might explain why seronegative MG patients presented with the highest sNfl levels in our study. It might also provide novel opportunities for biomarkers of critical exacerbation in this understudied patient population (68). Nevertheless, the accessibility of cell-based essays is still

limited and confined to specialized research centers. Thus, sNfl might be of diagnostic value, since a seronegative Abs-status carries is the risk of diagnostic uncertainty, but it is also of therapeutic relevance given an increasing antibody-specific treatment (3, 4).

It is established that MG is most active in the first 2–3 years after diagnosis (51). These findings relate to previous research on sNfl in ALS and multiple sclerosis, where sNfl levels are higher in active disease stages serving as an individual marker of disease progression (40, 69). However, although we found a positive association of sNfl with QMG scores at baseline, in our longitudinal analyses, we found contradictory results regarding the associations of sNfl levels with the MG-ADL and QMG scores in our study population. Therefore, no clear relationship of sNfl with parameters of disease activity can be drawn based on our results. Nevertheless, it should be mentioned that the majority of our study population received immunosuppressive therapies at sampling time and that the main proportion of patients experienced no significant change in QMG and MG-ADL scores during follow-up, which might have had a confounding effect.

We did not find a definite association between sNfl levels and MG-specific treatment. No association was shown for thymectomy history. Patients without immunosuppressive therapy tended to have lower nNfl levels while patients with escalation therapy had the highest sNfl levels. However, we cannot reliably assess the effects of MG-specific treatments on sNfl levels because of the small number of patients in the treatment subgroups. This should be investigated in larger, prospective studies, preferably in a treatment-naïve cohort.

There are several limitations to our study. Our cohort study was rather small with respect to the antibody subgroups, although we included a rather high number of patients with MG in our study. In this exploratory study, we nevertheless analyzed samples from patients with different Abs status, thymus pathology, and age at onset. In addition, the majority of included patients were not treatment naïve and therefore heterogeneous with respect to treatment regime and disease duration. Although patients with MG with pre-existing neuro-inflammatory and neurodegenerative diseases, as well as recent ICU stay, were excluded from the study, we cannot exclude the possibility that some patients, as well as controls, may have suffered from a subclinical neurodegenerative or neuroinflammatory disease that may have influenced sNfl levels. The control population was small and the median age was lower than in the MG population, for which we, however, adjusted in all analyses. In addition, age-adjusted z-scores were derived for cases and controls based on a reference database (40–42) to correct for age.

In conclusion, this exploratory cohort and case–control study demonstrates that sNfl levels were relevantly higher in patients with MG compared to controls. sNfl levels were descriptively higher in anti-AChR-Abs positive patients than in other MG subgroups. Adjusting for age and sex, seronegative patients had the highest sNfl levels in comparison to controls. Although sNfl levels are significantly increased in patients with MG compared to controls, the difference is small. Furthermore, sNfl levels do not correlate robustly with the clinical severity of MG. Therefore, sNfl is not a suitable biomarker for monitoring individual disease activity in patients with MG. However, as we found interesting differences in sNfl levels associated with auto-abs status, our study may prompt further studies in larger, treatment-naïve cohorts to evaluate the potential of sNfl as a biomarker of disease progression in specific MG subgroups.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Charité—Universitätsmedizin Berlin (EA1/281/10). The patients/participants provided their written informed consent to participate in this study.

Author contributions

FS: designed and conceptualized the study, had a major role in the acquisition of data, analyzed and interpreted the data, drafted the manuscript for intellectual content, and takes full responsibility for the integrity of the data analyzed. AA and BH: analyzed and interpreted the data and revised the manuscript for intellectual content. PM: acquisition of data, interpreted the data, and revised the manuscript for intellectual content. SH and SL: acquisition of data and revised the manuscript for intellectual content. CM: laboratory analysis and revised the manuscript for intellectual content. AM: designed and conceptualized the study, acquisition of data, interpreted the data, and revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

CM was employed by Charité Vivantes GmbH.

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The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1056322/full#supplementary-material

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3. Diskussion

Nach Jahrzehnten der Stagnation ist es mit dem Einzug des C5-Komplement-Inhibitors Eculizumab zu großen Fortschritten in der Entwicklung innovativer Therapeutika zur Behandlung der MG gekommen. Nichtsdestotrotz existieren in der klinischen Praxis weiterhin große Herausforderungen bei der Versorgung von Patient*innen mit MG, auch wenn die MG als Prototyp einer Ak-vermittelten Autoimmunerkrankungen gilt, bei der die Effektormechanismen der „klassischen“ AChR-Ak vermittelten MG als gut charakterisiert gelten.

Die Erkrankungslast ist bei den betroffenen Patient*innen, auch im langfristigen Verlauf, nach wie vor sehr hoch und nicht nur bedingt durch ein unzureichendes klinisches Ansprechen [8, 55]. In der Versorgung der Patient*innen besteht zum einen das Problem, dass zum Zeitpunkt der Diagnose kein prädiktiver klinischer oder blutbasierter Biomarker zur Verfügung steht, welcher den weiteren klinischen Verlauf vorhersagt. Zum anderen existieren bislang keine Biomarker, die das Therapieansprechen monitoren können.

Nach der neuen Leitlinie ist erklärtes Therapieziel, die „bestmögliche Krankheitskontrolle unter Wiederherstellung der Lebensqualität“ zu erreichen. Nichtsdestotrotz fehlt es bislang an objektiven Biomarkern zur Messung der Erkrankungsaktivität, sodass therapeutische Entscheidungen weiterhin, neben dem zeitlichen Verlauf und dem Erreichen eines MMS unter immunsupprimierender Therapie und/oder Thymektomie, anhand des regelmäßigen klinischen Monitorings (aktuelle MGFA-Klassifikation, QMG- und MG-ADL-Score) stattfinden. Diese haben jedoch Ihre Limitationen, da sie nur bedingt die Erkrankungsschwere aufgrund der tageszeitlichen Fluktuation der myasthenen Symptome widerspiegeln können. Unklar bleibt darüber hinaus, ob das Konzept einer frühzeitigen, intensivierten Immuntherapie in die klinische Praxis Einzug halten wird, um therapierefraktäre Verläufe und insbesondere eine myasthenen Krise zu verhindern. Die Identifikation von prädiktiven Risikofaktoren kann hier einen besonderen Stellenwert ausüben. Vor dem Hintergrund der dynamischen und auch hochpreisigen Entwicklungen in der Therapie der MG gewinnt daher die Biomarkerforschung eine immer weiterreichende Bedeutung.

Die in dieser Habilitationsschrift zusammengefassten Arbeiten sollen zum einen zur Identifikation von klinischen Risikofaktoren für einen schlechten MG-Verlauf als auch zur Erforschung möglicher Biomarker zur Detektion der Erkrankungsaktivität, des

Therapieansprechens sowie der Prädiktion des Erkrankungsverlaufes beitragen. Vor dem Hintergrund der aktuellen Leitlinie zur Therapie der MG und der derzeitigen Dynamik in der pharmakologischen Entwicklung von Therapeutika, haben diese Arbeiten eine hohe klinische Relevanz, um die Behandlung der Patient*innen zielgerichteter zu machen. Die in dieser Habilitationsschrift zusammengefassten Arbeiten stellen zudem die Grundlage für weitere klinische und experimentelle Forschungsprojekte dar.

3.1. Risikofaktoren für eine myasthene Exazerbation und Krise

Obwohl in den letzten Jahren deutliche Fortschritte in der Behandlung der gMG erzielt wurden, kommt es nach wie vor bei ca. 20% der MG-Patient*innen zu einer myasthenen Krise [3, 4, 56], welche mit einer hohen Erkrankungslast einhergeht. Daher ist die Identifizierung von Patient*innen mit hohem Risiko für eine Exazerbation und myasthenen Krise, sowie von Faktoren, die eine rasche Remission fördern, für die klinische Praxis von großer Bedeutung. Darüber hinaus lagen bislang nur Beobachtungsstudien für bestimmte Untergruppen der MG vor, vor allem für Patient*innen, die eine Thymektomie erhielten [5, 57]. Neben bereits bekannten Risikofaktoren wie das Vorhandensein eines Thymoms sowie einer MuSK-Ak positiven MG [58] konnten wir in dieser bislang größten retrospektiven Beobachtungsstudie zeigen, dass der Schweregrad der Erkrankung zum Zeitpunkt der Diagnose ein leicht zugänglicher und zuverlässiger Prädiktor für eine myasthenen Krise ist. Die Behandlungsstrategien sollten auf die Schwere der anfänglichen Symptome zugeschnitten sein, um möglicherweise die Wahrscheinlichkeit einer myasthenen Krise oder Exazerbation zu verringern. Dies unterstützt das Konzept der frühen intensivierten Immuntherapie zum raschen Erreichen eines MMS. Darüber hinaus unterstreichen unsere Daten, dass die Prävention und rasche Behandlung von Infektionen ein entscheidender Faktor für das Outcome der myasthenen Krise, neben dem Patientenalter und Anzahl der Komorbiditäten, darstellt.

3.2. Patient*innen mit bestehender immunsuppressiver Therapie und COVID-19-Infektion haben ein höheres Risiko für einen schlechten Verlauf

Zu Beginn der COVID-19 Pandemie lagen nur begrenzte Daten zum mit COVID-19 verbundenen Risiko von Patient*innen mit MG vor. Diese waren potenziell anfällig für einen schweren COVID-19-Verlauf (Hospitalisation oder Tod) aufgrund einer vorbestehende

bulbären und/oder respiratorischen muskulären Schwäche, dem Risiko der Entwicklung einer infektgetriggerten myasthenen Krise sowie einem möglicherweise erhöhten Risiko für eine Infektion aufgrund einer vorbestehenden, immunsuppressiven Therapie [43, 44].

Die Handlungsempfehlungen beruhten zu Beginn der Pandemie vor allem auf Expertenmeinung [59], da nur begrenzte *real-world* Daten zum mit COVID-19 verbundenen Risiko für Patient*innen mit MG vorlagen. Insbesondere gab es Hinweise darauf, dass Patient*innen, die Rituximab zur Therapie ihrer vorbestehenden rheumatologischen oder neurologischen Autoimmunerkrankung erhielten, ein höheres Risiko für einen schlechten COVID-19 Verlauf aufwiesen [60, 61].

Auf der anderen Seite war zum Zeitpunkt der Durchführung der Studie bereits bekannt, dass durch eine COVID-19 Infektion eine exzessive Produktion pro-inflammatorischer Zytokine stattfindet, die durch die Verabreichung von immunsuppressiver Therapien, wie beispielsweise Steroide, abgemildert werden kann [62].

Mittels der Daten des MyaRegs konnten wir unter Berücksichtigung des Alters und Anzahl der Komorbiditäten zeigen, dass eine immunsuppressive Therapie *per se* keinen Risikofaktor für eine COVID-19 Infektion darstellt. COVID-19-infizierte Patient*innen mit bestehender immunsuppressiver Therapie wiesen jedoch ein im Vergleich zu anderen Autoimmunerkrankungen, wie der rheumatoiden Arthritis (RA) oder MS, hohes Mortalitätsrisiko von knapp 12% auf. Letzteres suggeriert, dass krankheitsspezifische Faktoren, wie die Entwicklung einer myasthenen Krise, die Letalität stark beeinflussen. Zudem konnten wir insbesondere bei den mit Rituximab vorbehandelten Patient*innen eine hohe Letalitätsrate finden.

Die Ergebnisse dieser Studie stellen bis dato die größte *real-world* Kohorte dar, die den Einfluss einer COVID-19 Infektion auf den Verlauf der MG untersucht hat, was unterstreicht, wie wichtig Registerdaten für eine verbesserte Patientenversorgung sind. Die Ergebnisse führten unmittelbar zu einer verbesserten Beratung und Versorgung der Patient*innen und unterstreichen einmal mehr, dass Infektionen einen wichtigen Risikofaktor für die Entwicklung einer myasthenen Krise darstellen. Präventionsstrategien, wie z.B. Impfungen, sollten daher in dieser Hochrisikogruppe implementiert werden [6].

3.3. Komplementaktivierung als möglicher Biomarker zur therapeutischen Stratifikation von Patient*innen mit Acetylcholinrezeptor-Antikörper positiver Myasthenia gravis

Die MG gilt als pathophysiologisch die am besten charakterisierteste Autoimmunerkrankung. Die „klassischen“ Antikörper gegen AChR können bei 75% der MG-Patient*innen nachgewiesen werden. Aufgrund der fundamentalen diagnostischen und pathophysiologischen Rolle der AChR-Ak lag frühzeitig nahe, dass die Höhe der AChR-Ak mit der Symptomschwere korrelieren und somit als potenzieller Biomarker zur Erfassung der Erkrankungsaktivität dienen könnten. Die Studienlage diesbezüglich bleibt derzeit jedoch unklar, da auf Gruppenebene berichtet wurde, dass die AChR-Ak Titer mit der klinischen Symptomschwere korrelieren, aber aktuellere Arbeiten darauf hindeuten, dass solch ein Zusammenhang nicht besteht [63]. Das europäische Expertengremium empfiehlt daher nicht den routinemäßigen klinischen Einsatz der AChR-Ak-Titer-Testung [64].

Seit der Zulassung und der hohen klinischen Effektivität des C5-Komplementinhibitors Eculizumab [46] ist jedoch klar, dass die Komplementaktivierung wahrscheinlich den wichtigsten pathophysiologischen Mechanismus darstellt. Trotz der klinischen Wirksamkeit fehlten bislang Daten zum Ausmaß und Höhe der Komplementaktivierung bei MG-Patient*innen.

Ziel dieser explorativen Arbeit war es daher, bei Patient*innen mit gMG mit unterschiedlichen Ak-Status (AChR-Ak, MuSK-Ak, SNMG) eine systematische Untersuchung von Komplementaktivierungsprofilen des klassischen und alternativen Weges durchzuführen.

Wir konnten zeigen, dass sowohl proximale als auch distale Marker des klassischen und alternativen Komplementwegs bei Patient*innen mit AChR-Ak positiven MG-Patient*innen deutlich erhöht sind. Therapienaive MG-Patient*innen wiesen dabei höhere Plasmaspiegel als Patient*innen unter immunsuppressiver Therapie auf. Im Gegensatz hierzu konnten wir bei Patient*innen mit MuSK-Ak, die vorrangig vom IgG4-Typ und daher das Komplementsystem nicht aktivieren können [65] sowie SNMG-Patient*innen, bei welcher der involvierte IgG-Subtyp unklar bleibt, keine systemische Komplementaktivierung finden. Insbesondere der fehlende Nachweis einer systemischen Komplementaktivierung im peripheren Blut bei SNMG muss weitergehend untersucht werden, da in der Interkostalmuskelbiopsie von SNMG-Patient*innen regelhaft Komplementablagerungen nachgewiesen werden können [29]. Möglicherweise sind bei SNMG-Patient*innen

vorrangig Auto-Ak vom IgG1-Subtyp involviert, da diese das Komplementsystem nicht so stark aktivieren können wie Ak vom IgG3-Subtyp, zu denen die AChR-Ak vorwiegend gehören [66]. Wir fanden zudem keine Korrelation der Komplementfaktoren mit der Höhe des AChR-Ak Titers oder Parametern der klinischen Erkrankungsaktivität.

Unsere Daten sprechen für eine systemische Komplementaktivierung bei MG-Patient*innen. Weder der zugrundeliegende Mechanismus noch die Bedeutung ist bisher ausreichend verstanden. Die verringerte Komplementaktivierung nach Behandlungsbeginn mit immunsuppressiven Therapien sollte in prospektiven Studien weitergehend untersucht werden, um den prädiktiven Wert der Spiegel mit dem Schweregrad und dem therapeutischen Ansprechen zu korrelieren. Möglicherweise könnten somit Behandlungsentscheidungen und sogar Prognosen besser gesteuert werden.

Die Ergebnisse der Studie unterstützen zudem den Einsatz der terminalen Komplementhemmung als therapeutische Strategie, möglicherweise auch schon zu Beginn des Krankheitsverlaufs. Sowohl Komponenten des klassischen, als auch des alternativen Weges können starke immunregulatorische Funktionen ausüben, beispielsweise durch Erkennung der Komplementrezeptoren C5aR oder C3aR, welche auf myeloischen und aktivierten T- und B-Zellen exprimiert werden [67]. Eine therapeutische Komplementhemmung könnte daher zusätzliche Vorteile bringen. Zudem deuten die Ergebnisse dieser Arbeit darauf hin, dass eine Komplementhemmung proximal der Spaltung von C5 einen potenziellen therapeutischen Nutzen bei der AChR-Ak positiven MG haben könnte.

3.4. Calprotektin als potenzieller Biomarker für die Myasthenia gravis

Das menschliche Darmmikrobiom ist für die Erhaltung der Homöostase des Immunsystems von entscheidender Bedeutung. Störungen in der Zusammensetzung und Funktion des Mikrobioms wurden mit mehreren Autoimmunerkrankungen in Verbindung gebracht [48]. Darüber hinaus mehreren sich die Hinweise, dass eine gestörte Zusammensetzung des Darmmikrobioms zur Pathogenese der MG beiträgt [49]. Im Vergleich zu HC war bei MG-Patient*innen die relative Häufigkeit der Bakterientaxa im Darmmikrobiom verändert [48], insbesondere konnte kürzlich festgestellt werden, dass die mikrobielle Diversität bereits bei erstdiagnostizierten und unbehandelten MG-Patient*innen deutlich geringer war [68]. Aufgrund der mikrobiellen Dysbalance kommt es zur Störung der Permeabilität der intestinalen Mukosa und infolgedessen zu einer Dysbalance von proinflammatorischen

Th17- sowie regulatorischen T-Zellen, die zur Pathogenese der MG beitragen [49]. Zudem führt CLP zur Induktion autoreaktiver CD8+ T-Zellen und anderer proinflammatorischer Zytokine wie IL-17, die eine zentrale Rolle in der Pathophysiologie der MG spielen [69, 70]. In dieser explorativen Studie fanden wir erhöhte Serum CLP-Spiegel bei MG-Patient*innen im Vergleich zu HC, unabhängig vom Ak-Status. Darüber hinaus korrelierte CLP mit der Erkrankungsschwere gemessen an der aktuellen MGFA-Klassifikation und dem QMG-Score. Die individuelle Veränderung des Serum CLP waren jedoch nicht prädiktiv für die Erkrankungsaktivität über einen Beobachtungszeitraum von 3 Jahren, wobei der Großteil der Patient*innen keine signifikanten Änderungen im QMG- und MG-ADL- Score in dem Beobachtungszeitraum aufwiesen. Zukünfitge Biomarker-Studien sollten daher vorrangig therapienaive MG-Patient*innen einschließen, um prospektiv die Validität von Serum CLP zu analysieren, da CLP auch bei anderen Autoimmunerkrankungen, wie der RA oder chronisch-entzündlichen Darmerkrankungen, gut mit der Erkrankungsschwere und dem Therapieansprechen korreliert und daher bereits routinemäßig zum Monitoring eingesetzt [71, 72].

Die Ergebnisse dieser Studie legen eine wichtige pathophysiologische Rolle des Darmmikrobioms und der intestinalen Immunität bei der MG nahe. Therapeutisch bietet sich an, den Einfluss einer Modulation des Darmmikrobioms (z.B. Einnahme von Probiotika, Ernährungsumstellung) auf die Progression der MG im Kontext von CLP zu untersuchen [49]. Möglicherweise ergeben sich hierdurch komplementäre Therapieansätze zur Verbesserung der Symptomlast und Lebensqualität der MG-Patient*innen.

3.5. Serum Neurofilament light chain als Marker der neuromuskulären Destruktion

Neurofilamente (Nfl) sind wichtige Strukturelemente von Neuronen und werden bei neuronaler Schädigung in die extrazelluläre Umgebung freigesetzt [51]. Nfl wurde bei mehreren neurodegenerativen [73] sowie bei zentral und peripher entzündlichen Erkrankungen untersucht [52, 53]. Insbesondere für die MS ist die Evidenz hoch, dass Nfl sowohl mit der Erkrankungsaktivität korreliert als auch als prognostischer Biomarker fungieren kann [52] und daher Einzug in die klinische Routine haben wird. Kürzlich wurden zudem altersadjustierte Referenzwerte publiziert, sodass eine individuelle Nutzung von sNfl möglich scheint [52]. Obwohl seit langem bekannt ist, dass Auto-Ak gegen AChR v.a. über die Komplementaktivierung direkt pathogen an der NME wirken, wurde die Rolle von Nfl bei der MG bislang nicht untersucht. Histopathologische Studien legen jedoch nahe, dass

neurogene Veränderungen bei MG-Patient*innen, unabhängig vom MG-Subtyp, zu beobachten sind [74, 75]. Wir nahmen daher an, dass sNfl ein Marker für die Destruktion der NME darstellen könnte, da v.a. der prä- und postsynaptische Spalt reich an Strukturproteinen wie Nfl ist [76].

In dieser explorativen Studie konnten wir zeigen, dass die sNfl-Werte bei Patient*innen mit MG im Vergleich zu alters- und geschlechtsgematchten HC relevant erhöht sind, insbesondere bei AChR-Ak positiven MG-Patient*innen. Dies könnte darin begründet sein, dass durch die AChR-Ak abhängige Komplementaktivierung als „bystander Effekt“ Nfl extrazellulär freigesetzt wird und suggeriert die Durchführung einer frühen und effektiven immunmodulierenden Therapie zur Vermeidung einer langfristigen Destruktion der NME.

Unsere Daten konnten nicht zeigen, dass sNfl ein geeigneter Biomarker für die Überwachung der individuellen Krankheitsaktivität bei der MG darstellt. Der mögliche klinische Wert liegt jedoch in der frühzeitigen Detektion einer komplementvermittelten Destruktion der NME. Daher könnte sNfl als möglicher prognostischer Biomarker bei Patient*innen mit hochaktiver AChR-Ak positiver MG unter intensivierter Therapie fungieren, um eine (irreversible) Schädigung der neuromuskulären Endplatten zu vermeiden.

4. Zusammenfassung

Mit dem Einzug moderner Therapieoptionen eröffnen sich viele neue therapeutische Optionen für die MG. Eine verbesserte Behandlung der MG-Patient*innen ist dringend notwendig, da deren Lebensqualität und Teilhabe am Leben gerade auch im langfristigen Verlauf erheblich eingeschränkt ist. Dies ist zum einen bedingt an der nicht ausreichenden Wirkung sowie Nebenwirkungen vor allem der Standardtherapien. Dies liegt zum anderen aber auch an dem Fehlen von prädiktiven Biomarkern, die nicht nur den Erkrankungsverlauf, sondern auch das individuelle Ansprechen auf die verschiedenen Therapien vorhersagen können. Das Ziel muss sein, sowohl die Standard- als auch die intensivierten Therapien mit Hilfe von Biomarkern zu stratifizieren, um das optimale Behandlungsergebnis unter gesundheitlichen und ökonomischen Gesichtspunkten erreichen zu können.

Im Rahmen dieser Habilitationsschrift sind die Ergebnisse meiner bisherigen Arbeiten zur Analyse von klinischen Risikofaktoren für einen schlechten MG-Verlauf sowie Biomarker-Studien zur Erfassung der Erkrankungsaktivität und möglichen Therapieansprechen zusammenfassend dargelegt, welche das Ziel haben einer individualisierten Patientenversorgung näher zu kommen.

Das Risiko eine myasthene Krise im Verlauf der MG zu entwickeln kann nach wie vor nicht prädiziert werden. Neben bekannten Risikofaktoren, wie eine TAMG und einer MuSK-Ak MG, konnte die Erkrankungsschwere zum Diagnosezeitpunkt als wichtigster prädiktiver Risikofaktor für die Entwicklung einer myasthenen Krise festgestellt werden. Dies suggeriert, dass in dieser Patientengruppe das Konzept der frühzeitigen intensivierten Immuntherapie zum Erreichen eines MMS umgesetzt werden sollte, um das Auftreten einer myasthenen Krise zu vermeiden.

Mit Hilfe der Daten des Deutschen Myasthenie-Registers konnten wir zu Beginn der COVID-19 Pandemie zeigen, dass MG-Patient*innen mit einer COVID-19-Infektion und bestehender immunsuppressiver Therapie ein schlechteres Outcome gemessen an der Rate der Hospitalisation und insbesondere eine höhere, krankheitsspezifische Letalität im Vergleich zu anderen Autoimmunerkrankungen aufweisen. Die Arbeit konnte damit zu einer verbesserten Aufklärung beitragen, die im Wesentlichen die Implementierung wirksamer Präventionsstrategien für diese Hochrisiko-Gruppe empfiehlt.

Sowohl Marker der klassischen als auch der alternativen Komplementaktivierung sind bei der AChR-Ak positiven MG erhöht und sinken unter immunsuppressiver Therapie. Die MuSK-Ak positive und seronegative MG weisen keine erhöhten Komplementspiegel auf. Die Messung der Komplementaktivierung könnte daher ein möglicher prädiktiver Biomarker zur Detektion des therapeutischen Ansprechens der AChR-Ak positiven MG darstellen.

Serum Calprotectin (CLP) ist ein etablierter Marker der mikrobiellen Dysbiose und korreliert mit der klinisch aktiven MG. Dieser Marker könnte daher helfen, neben den klinischen Skalen, die Krankheitsaktivität zu bestimmen. Darüber hinaus unterstreicht diese Studie die pathophysiologische Bedeutung der Darm-Dysbiose für die MG und bietet die Grundlage zur weiteren Erforschung möglicher neuer modulierender Therapien des Darmmikrobioms [49].

Serum Neurofilament light chain (sNfl) ist insbesondere bei der AChR-Ak positiven MG deutlich erhöht, bedingt durch die komplementvermittelte Destruktion der NME. Ebenso wie Serum CLP stellt sNfl einen für die medizinische Routine breit verfügbaren Biomarker dar, welcher frühzeitig das Ausmaß der komplementvermittelten Destruktion der NME bei der AChR-Ak positiven MG widerspiegelt. sNfl könnte somit als prognostischer Marker, insbesondere bei aktiven MG-Patient*innen unter intensivierter Therapie eingesetzt werden, um eine irreversible Destruktion der NME zu vermeiden.

Wir brauchen in der Zukunft dringend prospektive, multizentrische Biomarker-Validierungsstudien, welche vorrangig therapienaive MG-Patient*innen einschließen sollten, da hier noch keine Immuneffekte durch die immunsupprimierenden Therapien zu erwarten sind. Zudem könnte mit Hilfe technologischer Verfahren wie Proteomics und Transcriptomics, potenziell Kandidaten neuer pathophysiologisch relevanter Biomarker identifiziert werden.
Perspektivisch werden für eine individualisierte Patientenversorgung eher Biomarker-Signaturen und als einzelne Biomarker und Scores zur Messung der Krankheitsaktivität eingesetzt werden, um prognostische und therapeutische Entscheidungen zu treffen.
Darüber hinaus werden die Daten des Deutschen Myasthenie-Registers dazu beitragen können, die Versorgungsqualität und Sicherheit der neuen Therapien zu bewerten, sodass die Lebensqualität der MG-Patient*innen nachhaltig verbessert werden kann.

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06. Februar 2024

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