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RESPIRATORY FUNCTION AND MICROBIOME ALTERATIONS IN SEROPOSITIVE GENERALIZED MYASTHENIA GRAVIS: INTEGRATING TRADITIONAL AND EMERGING CONCEPTS

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Preface:

Myasthenia gravis (MG) is a chronic autoimmune disorder of the neuromuscular junction characterized by fluctuating muscle weakness and fatigability. The clinical expression of the disease is highly heterogeneous and extends beyond pure neuromuscular transmission failure. Respiratory involvement represents one of the most clinically relevant aspects of MG, as it significantly contributes to morbidity and mortality. Respiratory muscle weakness, impaired cough efficacy, and bulbar dysfunction can predispose patients to ventilatory insufficiency and respiratory infections, even in relatively stable phases of the disease.

Despite its clinical importance, respiratory dysfunction in MG is often under-recognized, especially in patients with mild to moderate disease managed in outpatient settings. Traditional assessment relies on neurological scales and standard pulmonary function tests, which may not fully capture the multidimensional nature of respiratory impairment. In addition to objective measures of muscle strength and ventilatory capacity, patient-reported symptoms such as dyspnea and fatigue, as well as alterations in breathing patterns, may play a significant role in determining functional limitation and quality of life. Furthermore, emerging evidence suggests that respiratory involvement in MG is influenced by multiple factors, including disease severity, serological subtype, age at onset, and comorbidities, highlighting the need for a comprehensive and integrated evaluation.

At the same time, increasing attention has been directed toward the role of host–microbiome interactions in autoimmune diseases, including MG. Most of the available evidence has focused on the gut microbiota, where dysbiosis has been associated with immune dysregulation, impaired regulatory T cell function, and systemic inflammation. However, other mucosal compartments remain largely unexplored. Among these, the oropharyngeal microbiota represents a particularly relevant interface between the external environment and the host immune system. This anatomical site is not only part of the mucosa-associated lymphoid tissue contributing to immune homeostasis but also plays a key role in respiratory health by acting as a barrier against pathogen colonization and influencing the microbial composition of the lower airways.

The relevance of the oropharyngeal niche in MG is further supported by clinical considerations. A substantial proportion of patients present with bulbar involvement and additionally, immunosuppressive therapies commonly used in MG may further impact microbial composition, potentially favoring the emergence of opportunistic pathogens. Despite these considerations, the oropharyngeal microbiome in MG has not been specifically investigated, and its relationship with respiratory dysfunction and clinical outcomes remains largely unknown.

In this context, the present doctoral project was designed to explore respiratory involvement in MG from a multidimensional perspective, integrating functional, clinical, and microbiological approaches. The first study focuses on the characterization of respiratory dysfunction in a cohort of seropositive generalized MG patients, combining objective respiratory measurements with patient-reported outcomes and functional assessments. Particular attention is given to the identification of simple and clinically applicable tools for the early detection of respiratory impairment and for risk stratification of adverse outcomes, such as hospitalization and respiratory infections, in a real-world outpatient population.

The second study investigates the composition of the oropharyngeal microbiota in MG patients compared to healthy controls, aiming to identify disease-associated microbial signatures and to explore potential links between dysbiosis and clinical features. By focusing on this underexplored anatomical site, the study seeks to provide new insights into the role of mucosal microbial communities in MG.

Together, these two complementary approaches address different but interconnected aspects of MG pathophysiology. On one hand, the functional assessment of respiratory involvement allows a better understanding of the clinical burden and its determinants. On the other hand, the analysis of the oropharyngeal microbiome provides a novel perspective on environmental and host-related factors that may influence disease expression and complications. By integrating these dimensions, this work aims to contribute to a more comprehensive and clinically relevant understanding of MG, with potential implications for patient monitoring, risk stratification, and future therapeutic strategies.

Part 1 Beyond Standard Tests: Improving Pulmonary Assessment in Myasthenia Gravis to Predict Severity and Pneumonia

Introduction:

1. Overview of Epidemiology, Classification Efforts, and Therapies in Myasthenia Gravis

MG is the most frequent autoimmune disorder involving neuromuscular junction and is characterized by poor muscle activation with repetitive movements or fatigability. Poor quality of life in MG patients can be due to fatigability and fatigue which refers to poor energy or tiredness, the latter also correlating with mood and sleep disorders. MG worldwide prevalence has increased due to improved diagnostics and ageing, ranging from 100 to 350 cases per 1 million people (Gattellari et al., 2012; Breiner et al., 2016; Park et al., 2016; Martinka et al., 2018; Zieda et al., 2018; Westerberg et al., 2020; Sciancalepore et al., 2025). Incidence peaks around 25-39 years in female patients while men are more affected after 60 years of age (Beghi et al., 1991; Bubuioc et al., 2021).

MG is not a singular disease, and several classification methods have been used to define MG subgroups, based on serological status, clinical phenotype, age at onset and thymic pathology (Gilhus and Verschuuren, 2015).

MG is considered as an antibody-mediated autoimmune disorder characterized by class II hypersensitivity reaction (Dresser et al., 2021). Serological status allows dividing MG patients into four main subgroups: a) autoantibodies against nicotinic acetylcholine receptor (AChR) in 80-85%; b) autoantibodies directed against the muscle-specific tyrosine kinase (MuSK) in 5-8%; c) autoantibodies against low-density lipoprotein receptor-related protein 4 (LRP4) in 1-3%; d) triple-negative. This classification is also relevant from a pathophysiological point of view and therapeutic perspective. AChR antibodies are IgG_1 and IgG_3 disrupting neuromuscular transmission due to three different mechanisms: direct blockade of receptors, cross-linking and internalization and complement-mediated damage of the muscle membrane by the formation of the membrane-attack complex (MAC). MuSK antibodies are IgG_4 interfering with clustering of AChR on the post-synaptic surface without activating complement; MuSK MG patients often have an early-onset disease, propensity for bulbar involvement, normal thymus pathology and poor or deleterious response to cholinesterase inhibitors. LRP4 antibodies are IgG_1 and IgG_2 responsible for mild and early-onset MG. However, beyond autoantibodies, pathogenetic studies also highlight the role of T and B cells, particularly an altered Th1/Th2 balance, increased Th17 levels, and reduced Treg and Breg populations, along with an altered cytokine profile. Together, these factors contribute to B cell activation, their differentiation into plasma cells, and the increased production of autoantibodies (Balasa and Sarvetnick, 2000; Uzawa et al., 2021).

Based on clinical phenotype, MG can be classified into ocular (oMG) and generalized (gMG) forms. oMG primarily affects the extraocular muscles, typically presenting with asymmetric ptosis and fluctuating diplopia with symptoms restricted to this group of muscles for more than two years from symptoms onset. This phenotype is typically associated with later onset, lower autoantibody titers, a longer delay between symptom onset and diagnosis, and a less intensive treatment course compared to gMG (de Meel et al., 2019; Axelsen et al., 2024). In gMG, additional patterns of muscle weakness may occur, including dysphagia, dysphonia,

chewing difficulties, neck flexor weakness, limb weakness, and respiratory involvement. Ocular, bulbar, and oculobulbar onset is more frequently observed in older male patients (de Meel et al., 2019).

Age at onset can be classified as juvenile (≤18 years), early-onset (19–50 years), late-onset (51–65 years), and very-late onset (>66 years). Juvenile MG shows a slight female predominance and more commonly presents with ocular manifestations; it is also frequently associated with thymic hyperplasia. Early-onset MG predominantly affects women, often presents with generalized disease, and is strongly associated with thymic hyperplasia. In this subgroup, thymectomy is commonly indicated and has been shown to provide clear clinical benefit. In contrast, late-onset and particularly very-late onset MG are more prevalent in men, and the role of thymectomy is less well established, as thymic pathology in these patients more frequently consists of thymic atrophy rather than hyperplasia (Gilhus and Verschuuren, 2015; Huang et al., 2025). 10-20% of MG patients have a thymoma (thymoma-associated MG or TAMG) with a moderate-severe, positive antibodies against AChR and generalized disease.

Diagnosis of MG can be obtained by at least two of three criteria: a) positive AChR, MuSK, LRP4 antibodies; b) evidence of neuromuscular transmission defect on low-frequency repetitive nerve stimulation or single fiber electromyography; c) positive effect of pyridostigmine treatment. Other supportive diagnostic tools include the ice-pack test for ocular symptoms and repetitive ocular vestibular-evoked myogenic potentials (Punga et al., 2022). Nevertheless, serological assays for autoantibodies play a central role in both diagnosis and therapeutic decision-making, while neurophysiological testing, treatment response, and ancillary investigations serve as supportive tools. The main laboratory methods used include radioimmunoprecipitation assay (RIPA), considered the gold standard for its high sensitivity and specificity, enzyme-linked immunosorbent assay (ELISA), which is simpler and avoids radioactivity, and the more complex cell-based assays (CBA), which offer higher sensitivity but are less widely available. In clinical practice, a stepwise approach is recommended: testing for anti-AChR antibodies first, followed by anti-MuSK in seronegative cases, and then other antibodies such as LRP4, always interpreting results in conjunction with clinical findings (Li et al., 2023; Vinciguerra et al., 2023; Mousavi et al., 2024).

Treatment of MG encompasses symptomatic therapy, immunosuppression, immunomodulation, and surgery. Symptomatic management with the acetylcholinesterase inhibitor pyridostigmine remains the cornerstone of therapy. Short-term immunomodulation can be achieved through plasma exchange or intravenous immunoglobulins, whereas intermediate- and long-term management typically requires immunosuppressive agents targeting different pathways: corticosteroids modulate multiple immune mechanisms; azathioprine inhibits B and T cell proliferation; tacrolimus and cyclosporine block T cell activation; mycophenolate suppresses T cell function; methotrexate promotes T cell apoptosis; and rituximab selectively depletes B cells. Long-term standard steroid and non-steroid immunosuppressive regimen has a high burden due to several contraindications and side effects (Iorio, 2024). Thymectomy is mainly addressed to patients with early-onset AChR and thymoma-associated MG, with an established benefit up to 3 years after surgery (Wolfe et al., 2016). New available therapies target two different pathways: complement activation and IgG recycling through neonatal Fc receptor (FcRn). Eculizumab, Ravulizumab and Zilocuplan target C5 convertase enzyme or block

C5b6 formation (ravulizumab); they can be used only in AChR+ MG and require vaccination against encapsulated bacteria (Howard et al., 2017; Vu et al., 2022; Howard et al., 2023). Efgartigimod (approved for AChR MG), rozanolixizumab and nipocalimab (both approved for both AChR and MuSK MG) are used to reduce circulating antibodies and work as neonatal FcRn inhibitors. Circulating antibodies bind to FcRn, are internalized but can be recycled back into circulation: these drugs can disrupt this binding, facilitating the proteolytic removal of pathogenic antibodies (Howard et al., 2021; Bril et al., 2023; Antozzi et al., 2025). However, a significant subset of MG patients (up to 30%) poorly responds to these novel strategies. Other innovative strategies target B and T lymphocytes and cytokines; additionally, chimeric antigen receptor T-cell (CAR-T) therapy is emerging as a particularly promising option, especially in refractory MG (Cavalcante et al., 2024).

Despite increasing knowledge in pathophysiology and the development of novel therapies, prognostic and progression biomarkers are lacking. Moreover, clinical presentation can be heterogenous between patients and in the same patient according to time of day or effects of symptomatic drugs. To monitor disease progression and activity, no serological biomarker is available while clinical status can be monitored by quantitative scales and patients' reported outcomes (PROs). Promising prognostic biomarkers are microRNAs (miRNA) such as miR-150-5p and miR-21-5p in early onset AChR positive MG (Punga et al., 2014) or miR-let-7a-5p and miR-let-7f-5p, miR-151a-3p and miR-423-5p in MuSK-positive MG (Punga et al., 2016). miRNAs are not being used in clinical routine and large-scale further studies using standardized methods are still needed. The identification of disease phenotype can be relevant because treatment response to novel strategies such as complement inhibitors or FcRn antagonists can be divergent. A recent study by Nelke et al. (2024) using proteomics-based consensus clustering defined a disease phenotype with high disease severity and complement activation, therefore identifying a subgroup of patients benefitting from complement-inhibiting therapies.

2. Respiratory involvement in Myasthenia Gravis: methods of assessment and current state of the art

Respiratory impairment in MG (Fig. 1) depends on several factors such as diaphragm, intercostal and accessory muscles weakness, high risk of aspiration and pneumonia due to laryngeal and pharyngeal muscles weakness, comorbidities, effects of long-term steroid and immunosuppressant therapies and sleep-disordered breathing (SDB).

Respiratory dysfunction is in turn responsible for a higher risk of respiratory infections, more frequent hospitalizations and higher mortality. About 10-20% of MG patients will develop a myasthenic crisis and in mild to moderate forms of the disease, dyspnea, either at rest or following exertion, may be present. Respiratory dysfunction is moderately-strongly related to fatigue, sleep disorders and disease severity (Dewilde et al., 2024). Risk factors for respiratory involvement in AChR MG are thymoma, older age and presence of anti-titin and ryanodine receptor antibodies (Romi et al., 2007).

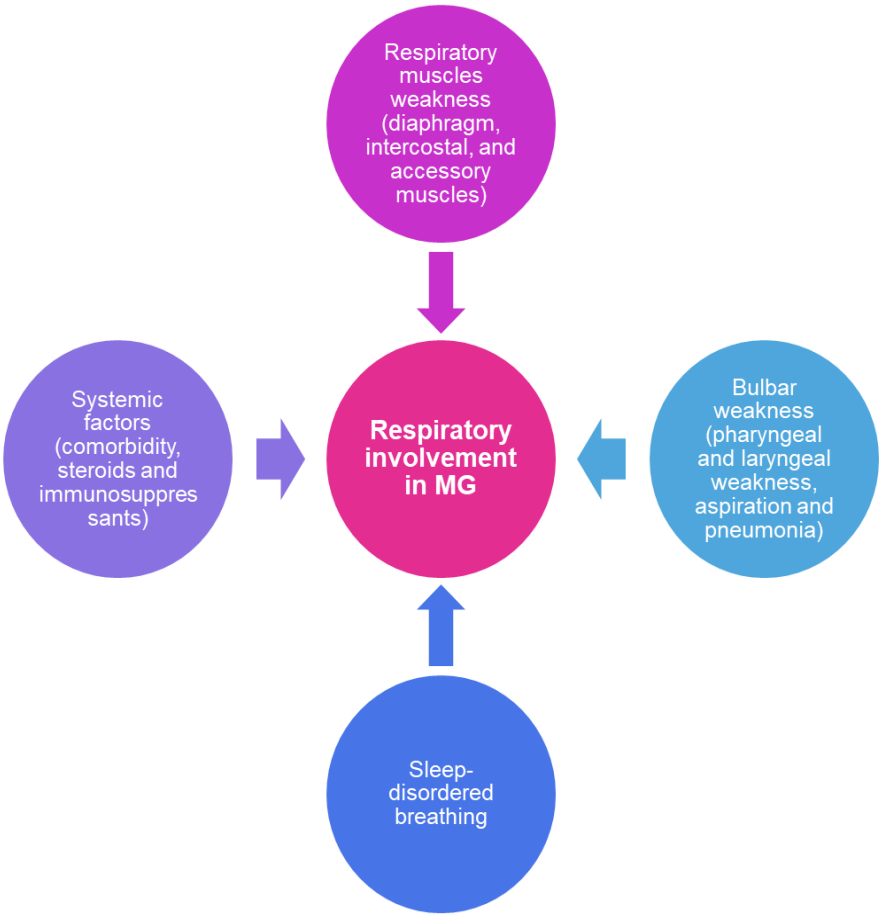


Figure 1 Determinants and clinical consequences of respiratory impairment in myasthenia gravis. Abbreviations: MG, myasthenia gravis.

Evaluating respiratory function in MG patients can be challenging, as existing MG-specific clinical scales offer limited assessment of respiratory involvement, and respiratory expertise may be lacking even in specialized neuromuscular centers (Alcantara et al., 2024).

The evaluation of respiratory dysfunction in MG relies on a wide array of clinical tools, encompassing disease-specific scales, oximetry, pulmonary function testing, and imaging modalities.

The main assessments include clinical scales, pulmonary function tests, respiratory muscle strength measurements (pressure tests), and additional techniques such as oscillometry and maximal voluntary ventilation.

a) *Clinical scales*:

The Myasthenia Gravis–Activities of Daily Living (MG-ADL) scale includes only one item related to dyspnea and is less useful for mild phenotype due to floor effect (Muppidi et al., 2011; Janssen et al., 2024). Moreover, according to a very recent Danish study, there is little agreement between spirometry measures and the respiratory item of MG-ADL (Andersen et al., 2026). On the other hand, the Quantitative Myasthenia Gravis (QMG) scale is more objective and assesses respiratory function with a single item focused on forced vital capacity (FVC) expressed as a percentage of predicted values (Bedlack et al., 2005); the Myasthenia Gravis Composite (MGC) includes a question similar to that in the MG-ADL (Burns et al., 2010). Notably, the

Myasthenia Gravis–Quality of Life 15 revised (MG-QoL15r) does not assess respiratory symptoms at all (Burns et al., 2016).

These limitations highlight that current clinical scales are insufficient to adequately capture bulbar and respiratory dysfunction but also physical fatigue in MG (Thomsen and Andersen, 2020; Regnault et al., 2023; Andersen et al., 2026).

Single breath count test (SBCT) is a simple tool to be performed, even during a phone call, to assess respiratory dysfunction (Dishnica et al., 2023; Bhandari et al., 2024). The patient is instructed to exhale while counting aloud at a rate of 2 Hz (two numbers per second) in a seated position, with the best of two attempts being recorded. A moderate positive relationship has been already observed between SBCT and FVC, negative inspiratory force, neck flexor strength in AChR-MG (Elsheikh et al, 2016) and a cut-off of 25 has a sensitivity of 80% to diagnose a MG exacerbation (Kukulka et al., 2020).

b) Pulmonary function testing

Conventional oximetry may underestimate hypoxia in MG due to the sigmoidal shape of the hemoglobin dissociation curve and the cooperative binding of oxygen, making it a less reliable indicator of respiratory dysfunction in MG population (Bhandari et al., 2024).

Spirometry is the essential part of pulmonary function testing (PFT) although it is time-consuming, requires cooperation and is influenced by decubitus and facial muscles strength. It consists of a forced expiratory maneuver after a full inspiration. The two most important dynamic lung volumes that can be measured are the forced vital capacity (FVC) and the forced expiratory volume in the first second (FEV1).

FVC is the volume of air, expressed in liters, that can be forcefully exhaled after a maximal inspiration and differs from slow vital capacity or vital capacity (SVC or VC) which is tested during a slow exhalation and inspiratory vital capacity (IVC). It depends on inspiratory and expiratory muscles strength. FVC is expressed as absolute values and/or percentage of predicted values, as it is influenced by sex, age, height, and race. It can be measured in both the seated and supine positions to help detect diaphragmatic dysfunction. A reduction in predicted FVC to < 80% is considered indicative of a restrictive ventilatory pattern in neuromuscular disorders (Boentert et al., 2017) and represents the threshold used to define respiratory involvement in the QMG score. Diaphragmatic weakness can be suspected if supine FVC drops more than 10% than seated FVC.

FEV1 is the volume of forcefully exhaled in the first second after a maximal inspiration and normally corresponds to the 80% of FVC. In obstructive disorders, FEV1 drops more significantly than FVC and FEV1/FVC ratio or “Tiffeneau” is low while in restrictive disorders, such those involving patients with neuromuscular disorders, FVC drops proportionally or more than FEV1, showing a normal FEV1/FVC ratio. Standard spirometry alone has limited diagnostic value in neuromuscular disorders, as vital capacity remains relatively preserved until there is a significant loss of respiratory muscle strength (Black and Hyatt, 1971; Griggs et al., 1981; Ward and Hill, 2001).

Peak expiratory flow (*PEF*) is the maximum speed at which a person can exhale air from the lungs after a full inhalation. PEF is often measured using a handheld peak flow meter but it can also be accurately measured using a spirometer, which provides a more comprehensive assessment of lung function: the patient is instructed

to inhale fully to total lung capacity (TLC) and then asked to forcefully and quickly exhale for at least 6 seconds. It can be measured together with the peak cough flow (PCF) and its reduction can be an earlier marker of expiratory dysfunction than FVC, predicting cough inefficacy in neuromuscular diseases (Morrow et al., 2019).

While in other neuromuscular disorders, such as Amyotrophic Lateral Sclerosis (ALS), a decline in FVC predicts reduced survival (Baumann et al., 2010), and in MG, a restrictive syndrome with FVC < 80% is frequently observed, no clear relationship between FVC decline and disease severity has been established (Aguirre et al., 2020). Moreover, no significant difference between supine and seated FVC has been clearly observed, underlying a more diffuse inspiratory than limited to diaphragm weakness (Alcantara et al., 2024). Some studies have shown that a subset of mild, well-treated MG patients may exhibit an obstructive pattern with reduced FEV1/FVC ratios (Elsais et al., 2010). This may be due to mild bronchial secretions or spasms induced by pyridostigmine, or to bronchial hyperreactivity linked to coexisting autoimmune disorders (Elsais et al., 2010).

Although FVC shows correlation with SBCT and PEF, its relationship with overall disease severity is limited. In fact, FVC does not correlate significantly with MGFA classification or MGC score (Aguirre et al., 2020).

c) Non-invasive tests of respiratory muscle function

Respiratory muscle function can be more accurately evaluated using techniques such as maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP), measured at the mouth, and sniff nasal inspiratory pressure (SNIP), measured at the nose. To assess *MIP*, the patient is instructed to exhale fully to residual volume (RV) and then inhale forcefully against a closed valve for at least 1 second. MIP is an indirect measure of diaphragm, external intercostal and accessory muscles strength. To assess *MEP*, the patient inhales to total lung capacity (TLC) and then exhales maximally against a closed valve for at least 1.5 seconds, thus allowing to evaluate expiratory muscles strength (internal intercostal and abdominal muscles). When evaluating MIP and MEP, proper mouth sealing is essential, as facial muscle weakness may affect measurement accuracy and contribute to variability in the recorded pressures. *SNIP* is measured by sniffing through one unoccluded nostril with the mouth closed, but unlike MIP and MEP, it does not require full mouth closure. It records the nasal pressure generated during a rapid, forceful inhalation from functional residual capacity, providing a reasonable approximation of esophageal, and thus intrathoracic, pressure. Although SNIP is generally strongly correlated with MIP, evidence indicates that the two are not interchangeable: MIP requires complete mouth closure, whereas SNIP is less reliable in the presence of nasal obstruction or concomitant obstructive lung disease (Fitting et al., 1996; Uldry et al., 1997). Furthermore, MIP has proven more accurate for monitoring patients with Duchenne dystrophy, while SNIP performs better in those with Myotonic dystrophy who display high variability in MIP values (Terzi et al., 2007).

Most studies in MG showed that reduced MIP and MEP are more reliable markers of respiratory dysfunction than spirometry in MGFA classes II and III, with MIP values lower than MEP ones in mild phenotypes (García Río et al., 1994; Keenan et al., 1995; Fregonezi et al., 2015; Fernandes Olivera et al., 2017; Alcantara et al., 2024).

Moreover, MIP and MEP can be altered in asymptomatic patients, having a prognostic value, without any clear association with SFEMG findings (Fernández et al., 2001; Muñoz Fernández et al., 2004). SNIP is typically the second most affected parameter following MIP (Alcantara et al., 2024).

Alcantara et al. (2024) demonstrated that MIP correlates more strongly with disease severity, as assessed by the Myasthenia Gravis Impairment Index (MGII), and with quality of life, measured by the MG-QoL15r. Moreover, both MIP and MEP are better predictors of the need for mechanical or non-invasive ventilation compared to FVC. The '20/30/40 rule'—defined as vital capacity (VC) < 20 mL/kg, negative inspiratory force (NIF) worse than –30 cm H_2O, and MEP < 40 cm H_2O—is commonly used to guide intubation decisions in cases of MG exacerbation (Thieben et al., 2005; Cabrera Serrano and Rabinstein, 2011; Claytor et al., 2023).

d) Other respiratory tests

Other pulmonary tests which have been less frequently assessed in MG are the maximal voluntary ventilation (MVV), techniques using oscillometry, the walking tests and peak cough flow.

MVV refers to the highest ventilation achieved during a voluntary, repetitive effort over a 10–15 second interval, as displayed on a volume–time curve. Patients with mild MG, classified as class IIA and IIB, exhibited a characteristic pattern of reduced MVV, with a progressive decline in volume over the course of the effort, mirroring the decremental response typically observed in repetitive nerve stimulation (Heliopoulos et al., 2003). However, MVV still requires adequate facial muscle strength to maintain a proper seal on the mouthpiece, as well as patient cooperation, and its values may be reduced in the presence of airway obstruction.

Oscillometry differs from forced respiratory maneuvers as it is performed during tidal breathing. Impulse Oscillometry (IOS) and Forced Oscillation Technique (FOT) assess respiratory mechanics by applying small pressure oscillations to the airways during normal breathing. These oscillations measure airway resistance and reactance, reflecting both central and peripheral lung properties. Low-frequency oscillations (e.g., 5 Hz) assess resistance across the entire respiratory system (parameters: R5 and X5), while high-frequency oscillations (e.g., 20 Hz) evaluate the larger airways (parameters: R20 and X20). Moreover, it measures respiratory impedance, which consists of two components: resistance (R) and reactance (X). In a single study involving patients with various neuromuscular disorders, including only four with MG, an increase in resistance and a reduction in reactance, indicative of reduced rib cage mobility, were observed by IOS (Iliaz et al., 2022). Therefore, in neuromuscular disorders, oscillometry could be useful when patients cannot perform maximal effort tests like spirometry, offering a selective advantage in children and not cooperative patients (Veldhoen et al., 2022).

Exercise capacity can also be assessed using walking tests, such as the 2-minute walking test (2MWT) and the 6-minute walking test (*6MWT*). The distance covered in the 6MWT (6MWD) shows a moderate inverse correlation with disease severity as measured by the MGC score, as well as with respiratory parameters like MEP (Calik-Kutukcu et al., 2019). In another study, both 2MWD and 6MWD were found to correlate with the QMG score, FVC, MIP, and the MG-QoL15r, highlighting the strong predictive value of walking tests in evaluating disease severity (Salci et al., 2019).

Airway clearance is a crucial defense mechanism that can be compromised in neuromuscular disorders, affecting both the upper and lower airways. In the upper airways, effective clearance depends on the ability of oronasal secretions to trap particles and on proper swallowing, while in the lower airways it relies on the mucociliary epithelium and the cough reflex. The cough reflex itself involves four coordinated phases: (1) deep inhalation; (2) glottic closure; (3) generation of high intrathoracic pressure through forced exhalation against a closed glottis; and (4) glottic opening with rapid expiratory flow to expel airway contents. Weakness at any step—due to neuromuscular impairment—can reduce tidal volumes, promote mucus retention and chronic atelectasis, impair swallowing and increase the risk of aspiration, and diminish cough effectiveness, further compromising airway clearance.

Indirect assessment of clearance includes videofluorography for swallowing, maximal inspiratory and expiratory pressures (MIP and MEP), and Peak Cough Flow (PCF) (Gipsman et al., 2023). *PCF* measures the highest airflow achieved during the compressive phase of a cough immediately following glottis opening, whereas PEF evaluates maximal expiratory flow after a full inhalation through an open glottis. Although PCF is standardized for monitoring asthma and predicting extubation success (Winck et al., 2006), it has also been studied in neuromuscular disorders such as ALS and Duchenne dystrophy (Bach, 1995; Bach et al., 1997). Self-assessed PCF, measured with a flow meter, correlates with swallowing function and standard spirometry parameters (Kuroiwa et al., 2024), and its validity has been explored using cough peak sound (CPS) in small MG patient cohorts (Recasens et al., 2024). While potentially useful for identifying aspiration risk, PCF measurement requires maximal effort and full mouth closure (Lyall et al., 2001).

Diaphragm function can be further evaluated through neurophysiology and imaging. Phrenic nerve stimulation allows recording of the compound action potential (cMAP), whose amplitude correlates with disease severity scores and improves after therapy (Suehiro et al., 2025). Repetitive nerve stimulation of the phrenic nerve may be more informative than peripheral nerve studies in patients with bulbar or generalized weakness (Pradhan et al., 2020). Additionally, diaphragmatic ultrasound after sustained breathing can assess fatigability and provide a non-invasive evaluation of respiratory muscle endurance (Ma et al., 2024).

Sleep-disordered breathing is common in neuromuscular disorders and may lead to daytime hypoventilation in advanced stages. Although high-quality studies are limited, obstructive sleep apnea has been documented even in medically stable patients (Prudlo et al., 2007; Fernandes Oliveira et al., 2017). Early detection of hypoventilation is crucial, as it represents the most prevalent form of sleep-disordered breathing in patients without obstructive apnea and often requires non-invasive ventilation (Sancho et al., 2024). Dysfunctional breathing, characterized by rapid upper-chest breaths, shoulder tension, and symptoms of hyperventilation such as dizziness and palpitations, has also been observed, though its prevalence is unclear.

Respiratory comorbidities, sleep-disordered breathing, and heart failure further contribute to respiratory dysfunction (Gilhus, 2023). Chronic pulmonary disease affects 11–21% of MG patients, and conditions such as asthma may increase susceptibility to neuromuscular disorders (Harris et al., 2022; Yingchoncharoen et al., 2021).

3. Myasthenic Crisis, risk of Respiratory Infections in MG and the role of microbioma

MG patients can show disease exacerbations or myasthenic crisis (MC), requiring mechanical ventilation or non-invasive ventilation (NIV). MC can be impending with rapid deterioration of respiratory failure or manifest, occurring in 15–20% of gMG patients within 2–3 years from disease onset (Godoy et al., 2013; Ramos-Franzi et al., 2015; Lizarraga et al., 2016). It can be the initial manifestation of MG in 18–28% of cases, while recurrence can occur in about one third of patients (Godoy et al., 2013; Neumann et al., 2020; Ntawuruhunga and Nougon, 2024; Reddy et al., 2024). From a pathophysiological perspective, microatelectasis and reduced lung volumes are accompanied by a preserved central respiratory drive. In the early phase, breathing becomes rapid and shallow, often leading to respiratory alkalosis with normal pO_2 and decreased pCO_2. As respiratory muscle weakness progresses, the ability to maintain adequate ventilation diminishes, resulting in rising pCO_2 levels and respiratory acidosis. In this context, MC is more frequent in anti-MuSK, late-onset AChR MG and thymoma-associated MG, and other risk factors include oropharyngeal weakness and a previous episode of MC. It is still the leading cause of mortality in MG (12–18%), with older age at onset, prolonged intubation and comorbidities (e.g. influenza and ischemic heart disease) as main negative prognostic factors (Liu et al., 2019; Neumann et al., 2020). Trigger factors for MC range from infections in 30–50% of cases, more often involving the upper or lower respiratory tract, to pharmacological modifications (e.g. antibiotics, immune-checkpoint inhibitors) and surgery. Weaning and extubation failure depend on older age, multiple comorbidities and the development of cardiac and pulmonary complications, concurring to poor outcome (Neumann et al., 2024). NIV can prevent intubation and reduce intensive care unit (ICU) stay (Misra et al., 2020) and early immunotherapy by plasmapheresis or intravenous immunoglobulins can prevent mechanical ventilation (Neumann et al., 2020; Huang et al., 2021), although mortality is mainly due to sepsis and consequent multiorgan failure (Neumann et al., 2020).

In parallel, long-term immunotherapies can contribute to increasing the risk of infections in MG patients, in turn reshaping pathogen distribution and modifying therapy responses (Chen et al., 2025). For instance, steroid treatment for more than 15 days increases the risk for respiratory tract and skin infections in the general population, especially in case of advanced age, diabetes and elevated steroid doses (Fardet et al., 2016). Consistently, an increase in hospital admissions due to infections in MG patients has been reported, leading to longer hospitalizations and increased mortality, particularly in older individuals (Sipilä et al., 2019). More detailed analyses in neuromuscular disease patients (chronic inflammatory demyelinating polyneuropathy, dermatomyositis, and MG) have shown that pneumonia is the most frequent infection, followed by sepsis and opportunistic infections; a significant association was observed between infection, plasmapheresis dose and therapy by steroid and mycophenolate (Prior et al., 2018). The most frequently identified microorganisms include Varicella-Zoster Virus (VZV), Staphylococcus aureus, Candida albicans and Pseudomonas aeruginosa (Prior et al., 2018). Similarly, although not assessing the risk of mild or moderate infections, a large cohort study involving only MG patients showed that MG carries an increased risk of severe infections, especially bacterial pneumonia, followed by sepsis and skin infections; according to this study, comorbidities (e.g. hypertension and COPD), older age and frailty contribute to this increased risk (Kassardjian et al., 2020).

Serious infection risk was also assessed in rituximab-treated MG patients, and 9 serious infections were detected in 5/30 patients, with rituximab-induced hypogammaglobulinemia concurring to infectious risk (Caballero-Avila et al., 2022).

In light of these findings, pneumonia represents the most common infection in MG patients, promoted by bulbar weakness, swallowing dysfunction leading to aspiration and reduced airway clearance (Kumai et al., 2019; Galassi and Marchioni, 2021), and ventilator-associated pneumonia can be one of the main complications occurring during the ICU stay in MG patients; the high incidence is further supported by the immunocompromised status. Clinical and microbiological characteristics (sputum or bronchoalveolar lavage fluid) have been recently analyzed in 116 MG patients with pneumonia, revealing that about 90% of infections are bacterial with 42% carbapenem-resistant bacteria; Gram-negative bacilli were the most common bacteria (Pseudomonas aeruginosa 28%, Acinetobacter baumanii 17%, Stenotrophomonas maltophila 9%), while Klebsiella pneumoniae was more prevalent in steroid-treated patients; next-generation sequencing also allowed identification of viral infections by Epstein Barr Virus (EBV) and Cytomegalovirus (CMV), respectively in 12 and 8 patients (Su et al., 2022). An unfavorable outcome was observed in case of lower lymphocyte count and high globulin levels at admission. The use of antibiotics must be considered since it contributes to complications: some antimicrobial agents (e.g. fluoroquinolones, macrolides) can exacerbate MC or can facilitate ICU-acquired weakness (e.g. aminoglycosides), highlighting an additional therapeutic challenge as certain antibiotics are able to worsen myasthenic symptoms.

Upper respiratory tract infections (URTIs) have been investigated in a limited number of studies; while a recent study found no significant difference in the incidence of URTIs between patients with MG and the general population (Alqahtani et al., 2025), and the risk of nasopharyngeal and upper respiratory tract infections was comparable in patients treated with eculizumab (Howard et al., 2017) or ravulizumab (Vu et al., 2022), a higher incidence of URTIs was reported in those receiving efgartigimod (Howard et al., 2021) and zilucoplan (Howard et al., 2023).

Objectives:

The primary objective of the first study is to comprehensively evaluate respiratory function in patients with seropositive gMG using a broad spectrum of clinical assessments and instrumental investigations at a tertiary neuromuscular clinic. By integrating standardized clinical scales, office-based respiratory measurements, and patient-reported outcomes, this study aims to identify subclinical respiratory dysfunction, assess the relative performance and sensitivity of different diagnostic tools in detecting respiratory involvement, and characterize patients at higher risk for complications, including hospitalization, pneumonia or respiratory-related mortality. This multidimensional approach is intended to inform early intervention strategies and improve risk stratification in MG.

Methodology:

Study design and setting:

This is a monocentric prospective cohort study at our neurological clinic specializing in the diagnosis and care of neuromuscular diseases in Palermo (Policlinico Paolo Giaccone). All assessments were performed in a single visit.

Participants:

Patients with seropositive gMG were included in the study and diagnosis of MG was confirmed by the combination of clinical picture (fatigable weakness of oculo-bulbar or extremity muscles or both) and serological investigations (serum AChR, MuSK, LRP4 antibodies), with abnormal electrophysiological testing or pharmacological response to cholinesterase inhibitors. Patients were excluded in the presence of ocular symptoms, negative serological antibodies, or cognitive impairment preventing completion of the questionnaire or pulmonary function testing. Pregnant or breastfeeding women were also excluded. Enrollment was conducted from September 2024 to December 2025.

The study protocol has been approved by the local ethical committee (Comitato Etico Territoriale della Regione Siciliana), and all patients were asked to sign a specific informed consent before entering the study.

Variables:

The outcome measures selected are used routinely in clinical practice. For each visit, we recorded demographics, MG diagnostic criteria, past history and medications and covariates: age at onset of MG, patient-reported sex, disease duration, classification according to MGFA, body mass index (BMI), comorbidities (COPD, asthma, OSA, cardiac and vascular, diabetes, dermatological, neurological, lipid disorders, osteoporosis, rheumatological, neurological, gastrointestinal, ophtlamological, previous extrathymic malignancies, hematological), presence of AChR, MuSK or LRP4 antibodies, history of thymectomy, current or previous smoke history, MC history, MG medications doses (pyridostigmine, steroid, azathioprine, mycophenolate, rituximab, eculizumab, ravulizumab, zilocuplan, efgartigimod, rozanolixizumab, other immunosuppressant drugs), home non-invasive ventilation (NIV) or oxygen therapy.

All assessments (listed in Figure 2) were conducted in the afternoon, generally three or four hours after pyridostigmine oral administration and included:

1. *MG outcome measures:*

   1a. **Quantitative Myasthenia Gravis** (QMG) combines clinician's and patient's outcomes, consisting of 13 items assessing ocular, bulbar, extremity function to test disease severity, with a score between 0 and 3 for every item and a total score ranging from 0 to 39 (Bedlack et al., 2005; Barnett et al., 2012).

   1b. **Myasthenia Gravis-Activities of Daily Living** (MG-ADL) is an 8-item patient-reported scale with each item assigned a score between 0 and 3, evaluating the independence and assessing severity, with a score ranging from 0 to 24 (Muppidi et al., 2011).

   1c. **Myasthenia Gravis Quality of Life-15** (MG-Qol15r) is a 15-item patient-centered outcome measuring quality of life, with each item graded from 0 to 2 and ranging from 0 to 30 (Burns et al., 2011).

2. *Dyspnea questionnaires:*

   2a. **Dyspnea-12** is a 12-item patient-reported outcome measure, already validated in Italian, that specifically assesses dyspnea, encompassing both physical and emotional domains, with each item scored from 0 to 3 (Yorke et al., 2010; Caruso et al., 2018).

   2b. **Multidimensional Profile of Dyspnea** (MDP) is a patient-reported outcome, mainly used in the experimental setting, evaluating immediate perception of respiratory problems and emotional response related to dyspnea (Banzett et al., 2015); in this study, only the total score obtained from the sensory quality (SQ) domain and the emotional response (ER) have been considered.

   2c. Dysfunctional breathing was evaluated by the **Nijmegen Questionnaire** (NQ), evaluating the possible presence of dysfunctional breathing and somatic/visceral responses associated with dysfunctional hyperventilation by 16 items (van Doorn et al., 1982); a cut-off of > 23 has been already used to identify a dysfunctional breathing pattern (Hornsveld et al., 1996) but validation studies in neuromuscular patients still lack.

3. *Sleep quality assessment* was obtained by the **Pittsburgh Sleep Quality Index** (PSQI) that is a patient-centered and 19-item scale to evaluate sleep quality over a 1-month period and can be used as a screening tool for sleep dysfunctions in several conditions with a cut-off of 5 (Buysse et al., 1989).
4. *Hospital Anxiety and Depression Scale* (HADS) is a 14-item scale for anxiety (7 items) and depression (7 items); all items have four option scores from 0 to 3, allowing to obtain a subtotal score for anxiety and depression respectively (Zigmond et al., 1982).
5. *Functional exercise capacity* was evaluated by the **6-minute walking distance** which is the total distance covered in a 6-minute frame. Patients were asked to walk as fast as possible along a flat 30-meter corridor for 6 minutes. The 6MWD was recorded in meters. Standardized instructions and encouragement were given during the test. In pre- and post-test periods, heart rate, blood pressure and oxygen saturation values were recorded and general fatigue perception in pre- e post-test was assessed by the Modified Borg Scale according to the American Thoracic Society guidelines (2002).
6. *Single breath count test* (SBCT) is a non-invasive tool to test respiratory function and its use is well established in MG; the patient was invited to count aloud while exhaling forcefully at a frequency of

two numbers per second and the best of two attempts, performed approximately 1 minute apart, was recorded (Bartfield et al., 1994).

7. *Pulmonary function and respiratory muscle testing:*

7a. Spirometry was conducted by expert clinicians after calibration with a 3-liter syringe according to the ATS/ERS guidelines (Graham et al., 2019) in the seated position (Pony Express, COSMED, Rome, Italy). Patients wore a nose clip and used a disposable mouthpiece, breathing normally to reach functional residual capacity. They then inhaled fully to total lung capacity (TLC) and performed a forced expiration. The test was repeated until three acceptable maneuvers were obtained and the highest values were recorded: we assessed **Forced Vital Capacity** (both absolute and % of predictive values) from the seated position and **FEV1**; therefore, we also calculated the **Tiffeneau** as the FEV1/FVC ratio.

7b. **Maximal Inspiratory Pressure** (MIP) is the pressure generated during maximal inspiratory effort against a closed system and is expressed in cmH_2O; it was measured at residual volume, using Pony Express (COSMED, Rome, Italy). We asked the patient to exhale to residual volume, then perform a deep forced inspiration, maintaining pressure for at least 1.5 seconds. The maneuver was repeated three times, and the best result was recorded. The values of MIP were also expressed as percentage of predicted values using the equation provided by Evans and Whitelaw (2009): $120 - 0.41 \times$ age for males and $108 - 0.61 \times$ age for females.

7c. **Maximal Expiratory Pressure** (MEP) expressed the highest pressure at the mouth during an expiratory effort at total lung capacity and is expressed in cmH_2O; the patient was instructed to breathe normally, then inhale deeply to TLC, followed by a forced expiration while maintaining pressure for at least 1.5 seconds, using the same above-mentioned device; as for MIP, the test was repeated three times and the best result was recorded. For percentage of predicted values, MEP equations from Evans and Whitelaw (2009) were applied $174 - 0.83 \times$ age (males) and $131 - 0.86 \times$ age (females).

7d. **Sniff Nasal Inspiratory Pressure** (SNIP) is the pressure, expressed in cmH_2O, measured by a nasal probe placed in a nostril and is well correlated with MIP and allows to monitor diaphragm stiffness. The subject was invited to perform a series of maximum sniffing (inhalation) by the nostrils using a portable pressure meter (MicroRPM, CareFusion, Höchberg, Germany). For percentage of predicted values, SNIP reference values were derived from Uldry and Fitting (1995): $126.8 - 0.42 \times$ age (males) and $94.9 - 0.22 \times$ age (females).

7e. **Peak Cough Flow** (PCF) is a direct measure of airway clearance and measures cough strength. It was measured by a flow-meter when the patient is invited to cough as forcefully and quickly as possible into the device (Pony Express, COSMED, Rome, Italy), after a full inspiration to TLC; the best of three recorded tests was recorded.

7f. **Maximal measurement of voluntary ventilation** (MVV) is the total volume of air exhaled during 12-15 seconds of rapid and deep breathing. Patients were instructed to breathe in and out as deeply and rapidly as possible for 12 to 15 seconds and MVV (L/min) was calculated by extrapolating the 12-

or 15-second effort to a full minute; the best of two attempts was recorded. For MVV, Araújo et al. (2024) equations were used; although the population was Brazilian and ethnically heterogeneous, this is currently the only study providing precise predictive equations. The equations applied were: 206.3 − 1.18 × age for males and 146.3 − 0.86 × age for females.

7g. Oscillometry is a non-invasive, effort-independent lung function test that assesses respiratory system mechanics during normal tidal breathing. The patient was invited to normally breath through a mouthpiece linked to a device (Resmon Pro FULL, Restech, Milan, Italy). The device applied small-amplitude pressure oscillations at multiple frequencies to measure respiratory system impedance, including resistance and reactance, according to the forced oscillation technique. The following items were measured: **resistance** (R) and **reactance** (X), respectively evaluating any obstruction or lung elasticity and peripheral airway dysfunction, resonant frequency (**FRES**) reflecting the transition point between elastic and inertive properties, **AX** which is the area under the reactance curve assessing small airway impairment, **R5-19** also reflecting small airways impairment;

8. *Arterial blood gas* analysis was obtained from nearly all participants and pH, pO2, pCO2, lactate levels, sO2, BEB data were collected.

Figure 2 Study outcome measures. Abbreviations: 6MWT, 6-minute walk test; BEB, base excess in blood; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; HADS, Hospital Anxiety and Depression Scale; MDP, Multidimensional Profile of Dyspnea; MEP, maximal expiratory pressure; MG-ADL, Myasthenia Gravis Activities of Daily Living; MG-QoL15r, Myasthenia Gravis Quality of Life 15 revised; MIP, maximal inspiratory pressure; NQ,

Nijmegen Questionnaire; PCF, peak cough flow; PSQI, Pittsburgh Sleep Quality Index; QMG, Quantitative Myasthenia Gravis; SBCT, single breath count test; SNIP, sniff nasal inspiratory pressure; sO_2, oxygen saturation; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen.

Follow-up:

The first 57 patients (enrolled up to April 2025; the remaining 38 patients will undergo follow-up telephone interviews over the subsequent 7 months) were contacted by phone and administered a standardized questionnaire by trained clinicians or research staff.

Data collection referred to events occurring within 12 months from enrollment. The following variables were recorded:

1. hospitalizations (including reasons for admission)
2. occurrence of pneumonia
3. microbiological culture results from sputum or bronchial aspirates (when available)
4. corresponding antibiotic therapy
5. urinary tract infections
6. upper urinary tract infections
7. infections at other sites
8. mortality.

Pneumonia was defined as a lower respiratory tract infection diagnosed by a physician based on clinical findings and/or confirmed by chest imaging (X-ray or computed tomography scan). Missing data were not imputed, and analyses were performed on available data.

Statistical methods:

Statistical analyses were performed mainly using Statistica software (version 8.0 for Windows). Clinical and demographic data are presented as means ± standard deviations or as medians with interquartile ranges, depending on the data distribution.

Differences in continuous variables were assessed using the Mann–Whitney U test for two-group comparisons and the Kruskal–Wallis test for multiple-group comparisons. When the Kruskal–Wallis test was significant, post-hoc pairwise comparisons were performed.

Spearman's correlation analysis was performed to determine the relationship between respiratory measurements and clinical parameters. Results were adjusted for multiplicity with the Bonferroni correction, whenever appropriate. Significance level was set in 0.05 and all comparisons were two-sided.

Two multiple regression models have been used to verify that MG severity as assessed by QMG or MG-ADL was predicted by different respiratory variables. Moreover, a third model was used to assess the multidimensional aspects of dyspnea.

For the prospective component of the study, which is currently ongoing, we preliminarily assessed the predictive ability of clinical and functional variables for key outcomes (hospitalization, pneumonia, or death) using receiver operating characteristic (ROC) curve analysis in Jamovi (version 2.6.44, Windows). The area

under the curve (AUC) with 95% confidence intervals (CI) and p-values were calculated, and optimal cut-off values were determined using the Youden index. Contingency tables were constructed based on these cut-offs to summarize outcome distribution across risk categories. Multivariable logistic regression was used to assess independent predictors of hospitalization. Results are presented as β coefficients, odds ratios, and 95% confidence intervals. All tests were two-sided, with p-values < 0.05 considered statistically significant.

Results:

1. **Descriptive statistics:**

Participants:

Study enrollment started in September 2024 and was completed in December 2025. A total of 101 patients with an initial diagnosis of seropositive gMG were screened for participation. Six patients were excluded (n = 6): serological confirmation did not support the diagnosis in three patients (n = 3), and three patients did not complete the study-specific assessments (n = 3). Ninety-five patients (n = 95) were ultimately included in the analysis. The patient selection process is summarized in Figure 3.

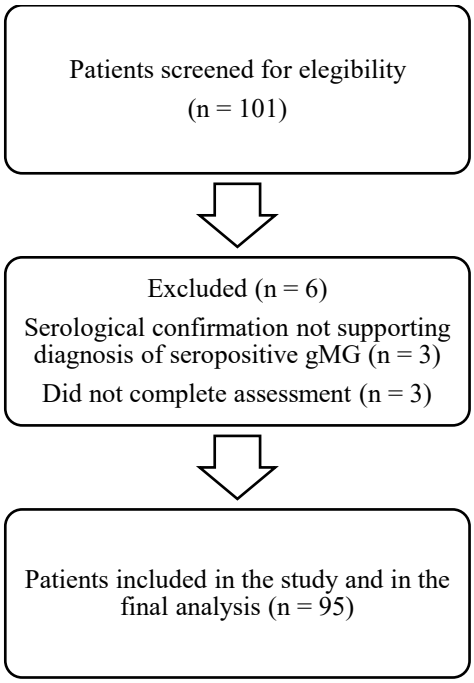


Figure 3 Patient selection diagram

Demographic and clinical characteristics are displayed in table 1.

Clinical features	**Cohort (n = 95)**
Sex (F/M)	50.5%/49.5%
Age (mean ± SD)	61.9 ± 14.9
Age at onset (mean ± SD)	53.5 ± 17.8
Age at onset (juvenile, early-onset, late-onset, very-late onset)	4% juvenile (n = 4), 29% early-onset (n = 28), 45% late-onset (n = 43), 21% very-late onset (n = 21)
Disease duration (mean ± SD)	8.6 ± 9.1
BMI (mean ± SD)	27.4 ± 5.2
History of thymectomy and thymus pathology (n (%))	32 (34%); thymoma in 13 (41%), thymic hyperplasia in 9 (28%), thymolipoma in 2 (6%)
Serological status (AChR positive n (%); MuSK positive n (%); LRP4 positive n (%))	83 (87%); 11 (14%); 1 (1%)
Pyridostigmine dose in mg (mean ± SD)	162 ± 119
Patients treated with steroid n (%) Prednisone dose in mg (mean ± SD)	71 (75%) 15.2 ± 9.8
Patients currently treated with non-steroid immunosuppressive or immunomodulator drugs: Azathioprine n (%); dose in mg (mean ± SD) Mycophenolate n (%); dose in mg (mean ± SD) Methotrexate n (%) Efgartigimod n (%) Rozanolixizumab n (%) Eculizumab n (%) Ravulizumab n (%) Zilocuplan n (%)	 27 (28%); 105 ± 21 16 (17%); 1687 ± 403 3 (3.1%) 13 (13.7%) 2 (2.1%) 2 (2.1%) 5 (5.3%) 9 (9.5%)
History of previous myasthenic crisis n (%)	15 (15.8%)
History of smoke: current smokers n (%) ex-smokers n (%)	 12 (12.6%) 29 (35.2%)
Comorbidities: COPD n (%) Asthma n (%) OSA n (%) Cardiac or vascular disease n (%) Diabetes n (%) Rheumatological disease n (%)	 8 (8.4%) 10 (10.5%) 15 (15.8%) 57 (60%) 12 (12.6%) 10 (10.5%)

Osteoporosis n (%)	17 (17.8%)
Thyroid disease n (%)	15 (15.8%)
Gastrointestinal disease n (%)	16 (16.8%)
Renal and/or urological disease n (%)	9 (9.5%)
Dermatological disease n (%)	4 (4.2%)
Psychiatric disease n (%)	6 (6.3%)
Lipid disorders n (%)	19 (20%)
Neurological disorder n (%)	10 (10.5%)
Previous extrathymic malignancy n (%)	4 (4.2%)
Current infectious disease n (%)	1 (1%)
Ophthalmic disease n (%)	7 (7.3%)
Hematological disease n (%)	5 (5.2%)
Home non-invasive ventilation n (%)	17 (17.9 %)
Home oxygen therapy n (%)	5 (5.2%)
Tracheostomy n (%)	1 (1%)
MGFA class (n (%))	IIa: 30 (31.6%); IIb: 19 (20%); IIIa 20 (21%); IIIb 25 (26.3%); IVa 0 (0%); IVb: 1 (1.1%); V: 0 (0%)

Table 1 Demographic and clinical features of patients enrolled. Abbreviations: AChR, acetylcholine receptor; BMI, body mass index; COPD, Chronic Obstructive Pulmonary Disease; LRP4, Low-Density Lipoprotein Receptor-Related Protein 4; MGFA, Myasthenia Gravis Foundation of America; MuSK, muscle specific kinase; OSA, obstructive sleep apnea; SD, standard deviation.

Our prospective gMG cohort included 95 outpatients, predominantly classified as MGFA II (n = 49) and III (n = 45), with only one patient classified as IVb. Serological testing showed that 87% of patients were AChR-positive (n = 83), 14% MuSK-positive (n = 11), and 1% LRP4-positive (n = 1).

Approximately two-thirds of patients (66%) had late- or very-late-onset disease, and 15% had a history of previous myasthenic crisis.

Regarding treatment, 75% of patients received steroid therapy, followed by immunosuppressive or immunomodulatory drugs including azathioprine (28%), mycophenolate (17%), efgartigimod (14%), and zilucoplan (10%).

Cardiac comorbidities were the most frequent (60%), followed by lipid disorders (20%), obstructive sleep apnea (16%), and gastrointestinal disorders (17%). Among respiratory comorbidities, OSA was present in 15.8%, asthma in 10.5%, and chronic obstructive pulmonary disease in 8.4%. Seventeen patients (17.9%) were on home non-invasive ventilation, and five patients (5.2%) were receiving home oxygen therapy.

Study-specific outcome measures:

Study-specific outcomes are summarized in Table 2.

Study-specific outcome measures	Results
QMG score, median (IQR, Q1 to Q3)	12 (8-17)
MG-ADL, median (IQR, Q1 to Q3)	6 (4-10)
MG-Qol 15, median (IQR, Q1 to Q3)	11 (5-17)
Dyspnea 12, median (IQR, Q1 to Q3)	6 (2-16)
MDP-SQ, median (IQR, Q1 to Q3)	11 (0-22)
MDP-ER, median (IQR, Q1 to Q3)	6 (0-19)
NQ, median (IQR, Q1 to Q3)	17 (7-28)
HADS-A, median (IQR, Q1 to Q3)	5 (2-10)
HADS-D, median (IQR, Q1 to Q3)	6 (3-10)
PSQI, median (IQR, Q1 to Q3)	8 (4-12)
6 MWD, mean ± SD	380 ± 151
SBCT, median (IQR, Q1 to Q3)	31.7 (20-43)
FVC, mean ± SD	2.9 ± 0.9
FVC %, mean ± SD	79.6 ± 29.2
FEV1, median (IQR, Q1 to Q3)	2.3 (1.8-2.9)
Tiffeneau (FEV1/FVC ratio), median (IQR, Q1 to Q3)	77% (55-84)
MIP, mean ± SD	58.9 ± 21.9
MIP %, mean ± SD	71.5 ± 24.1
SNIP, mean ± SD	58.3 ± 25.5
SNIP %, mean ± SD	56.9 ± 26.1
MEP, mean ± SD	78.3 ± 28.8
MEP %, mean ± SD	80.1 ± 29.2
PCF, mean ± SD	437 ± 121
MVV, mean ± SD	78.4 ± 29.3
MVV %, median (IQR, Q1 to Q3)	70 (48.8-84.2)
Resistance, median (IQR, Q1 to Q3)	3.0 (2.2-17.3)
Reactance, median (IQR, Q1 to Q3)	-1.1 (-1.7 - -0.7)
FRES, median (IQR, Q1 to Q3)	12.3 (9.8-16.6)
AX, median (IQR, Q1 to Q3)	3.4 (1.9-7.3)
R5-19, median (IQR, Q1 to Q3)	0.2 (-0.05-0.84)

Table 2 Study-specific outcome measures. Abbreviations: 6 MWD: 6 minute walking distance; AX: Area of Reactance; FEV1: Forced Expiratory Volume in 1 second; FRES: Resonant Frequency; FVC: forced vital capacity; HADS: Hamilton Anxiety Depression scale; IQR: interquartile range; MDP-ER: Multidimensional dyspnea profile-emotional response; MDP-SQ: Multidimensional dyspnea profile-sensory quality; MEP: Maximum Expiratory Pressure; MG-ADL: Myasthenia Gravis-Activities of Daily Living; MG-Qol15: Myasthenia Gravis-Quality of Life15; MIP: Maximum Inspiratory Pressure; MVV: Maximum Voluntary Ventilation; PCF: Peak Cough Flow; PSQI: Pittsburgh Sleep Quality Index; QMG: Quantitative Myasthenia Gravis; R5-19: Resistance measured at 5 Hz – Resistance measured at 19 Hz; SBCT: single breath count test; SNIP: Sniff Nasal Inspiratory Pressure.

The median QMG score was 12 (IQR 8–17). MG-ADL scores indicated that 16 patients had minimal symptom expression (0–2), 33 patients had mild symptoms (3–6), 28 patients had moderate impact on activities of daily living (7–10), and 18 patients had scores greater than 10. The median MG-QoL15 score was 11 (IQR 5–17), and the median Dyspnea-12 score was 6 (IQR 2–16).

Respiratory assessments showed median MDP-SQ and MDP-ER scores of 6 (IQR 0–19) and 11 (IQR 0–22), respectively. The NQ revealed a dysfunctional breathing pattern (cut-off: 24) in 33 patients (34.7%). Anxiety and depression, assessed by HADS-A and HADS-D, had median scores of 5 (IQR 2–10) and 6 (IQR 3–10), respectively. Sleep quality was impaired in 62 patients (65%), with a median PSQI of 8 (IQR 4–12).

Functional exercise capacity, as measured by the 6MWD, averaged 380 ± 151 m, while the SBCT had a median of 31.7 (IQR 20–43). Pulmonary function tests showed a mean FVC of 2.9 ± 0.9 L, FVC% of 79.6 ± 29.2%, median FEV1 of 2.3 L (IQR 1.8–2.9), and median Tiffeneau index (FEV1/FVC) of 77% (IQR 55–84). A restrictive pattern (FVC% <80%) was present in 37 patients (39%), classified as mild (65–79%) in 22 patients, moderate (50–64%) in 11 patients, and severe (<50%) in 4 patients. An obstructive pattern (FEV1/FVC <70%) was observed in 10 patients (10.5%), of whom only three had a prior diagnosis of obstructive disease (asthma, n=2; COPD, n=1).

Respiratory muscle strength, expressed as percentages of predicted values, showed a mild-to-moderate reduction in inspiratory parameters, with MIP at 71.5 ± 24.1% and SNIP at 56.9 ± 26.1%. Expiratory muscle strength was relatively preserved, with MEP at 80.1 ± 29.2%. MVV was moderately reduced, with a median value of 70% (IQR 48.8–84.2). Overall, both inspiratory and expiratory parameters were reduced to a variable extent across the cohort. PCF was 437 ± 121 L/min, indicating a relatively preserved cough efficacy across the cohort.

Airway mechanics revealed median resistance of 3.0 (IQR 2.2–17.3), reactance of −1.1 (IQR −1.7 to −0.7), FRES of 12.3 (IQR 9.8–16.6), AX of 3.4 (IQR 1.9–7.3), and R5–19 of 0.2 (IQR −0.05–0.84), indicating generally mild variations in airway impedance within the cohort.

Arterial blood gas analysis, performed in 86/95 patients, revealed respiratory alkalosis (ph>7.45, pO2 normal, pCO2 < 35 mmHg) in 11 patients, respiratory acidosis defined by pH<7.35 and pCO2 > 45 mmHg in 0 patients.

2. Comparisons between groups:

Only selected variables were included in the univariate analysis, with particular focus on serological status, MGFA class, sex, and age-at-onset classification.

Comparisons of variables according to serological status (AChR vs MuSK antibodies), performed using the Mann–Whitney U test, revealed significant differences between groups (Fig. 4). AChR-positive patients had a higher cumulative pyridostigmine dose compared to MuSK-positive patients ($U = 60.0$, $p < 0.0001$). Several pulmonary parameters were worse in MuSK-positive patients, including FEV1 ($U = 253.0$, $p = 0.015$), MIP ($U = 229.0$, $p = 0.006$), MIP % predicted ($U = 267.0$, $p = 0.02$), MEP ($U = 266.5$, $p = 0.02$), and PCF ($U = 264.5$, $p = 0.02$). These results indicate that MuSK-positive patients exhibited greater respiratory impairment than AChR-positive patients.

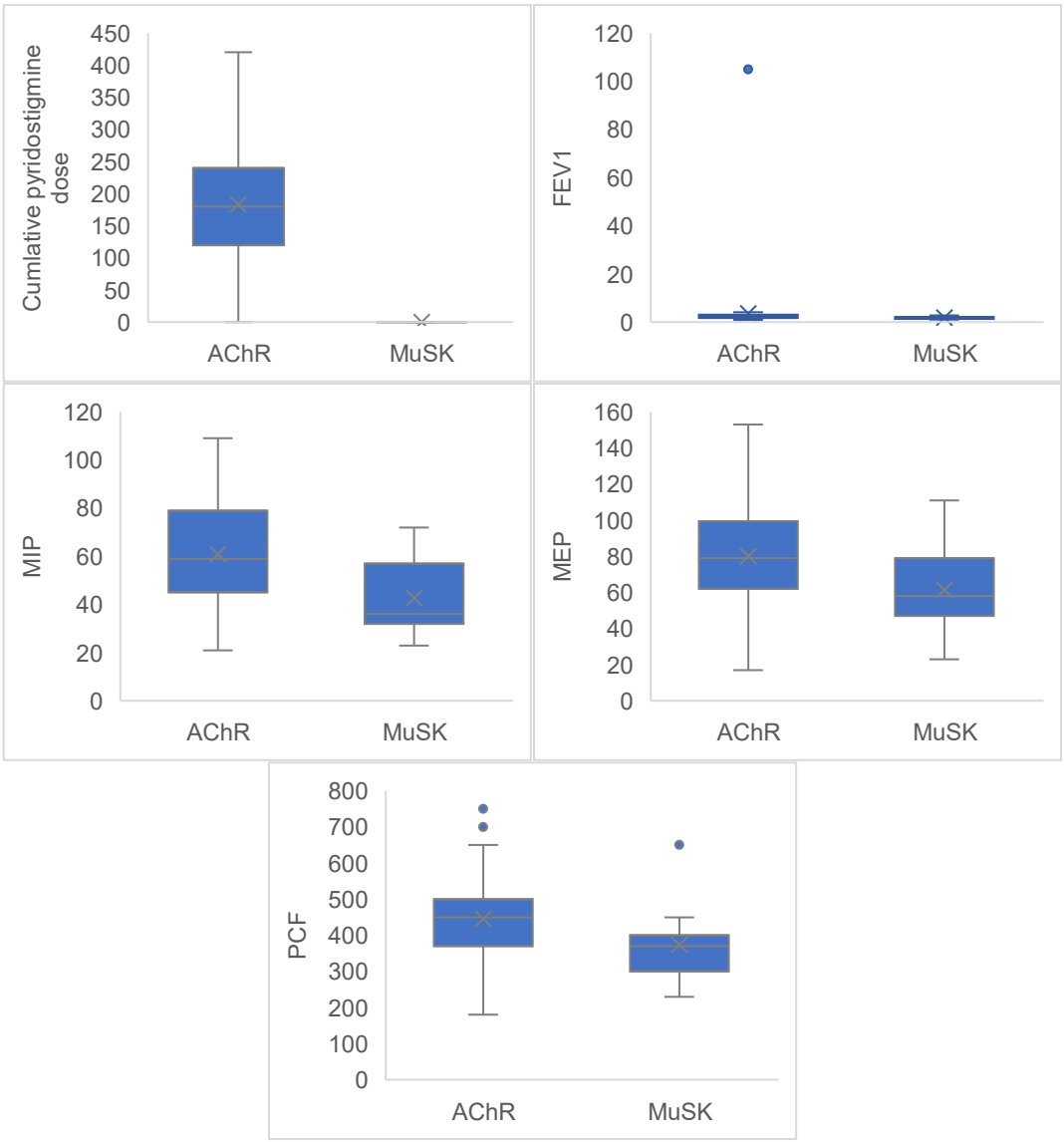
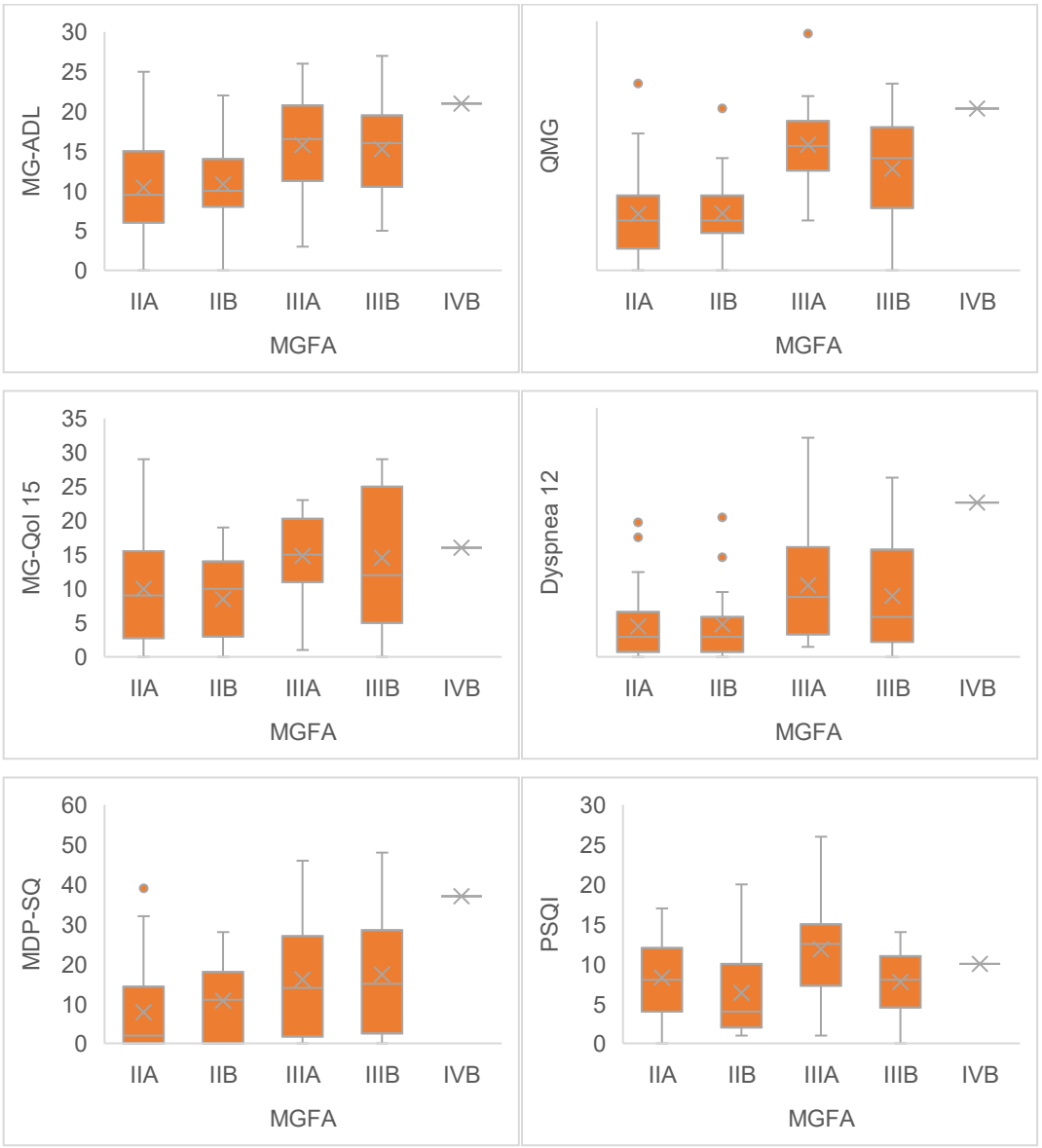


Figure 4 Box and whiskers showing statistically significant ($p < 0.05$) differences between AChR and MuSK MG patients. The boxplot displays the distribution of cumulative prednisone dose, MIP, MEP, PCF and FEV1 in the two groups: the central line represents the median, the "x" indicates the mean, the box edges correspond to the first and third quartiles, the whiskers show the range excluding outliers, and dots represent outlier values. Abbreviations: AChR, acetylcholine receptor; FEV1, forced expiratory volume in 1 second; MEP, maximal expiratory pressure; MIP, maximal inspiratory pressure; MuSK, muscle-specific kinase; PCF, peak cough flow.

Kruskal–Wallis ANOVA by ranks showed significant differences across MGFA classes (Fig.5) for QMG ($H(4, N = 95) = 17.67$, $p = 0.0014$), MG-ADL ($H(4, N = 95) = 32.31$, $p < 0.0001$), MG-QoL15 ($H(4, N = 95) = 10.78$, $p = 0.029$), Dyspnea-12 ($H(4, N = 95) = 14.34$, $p = 0.006$), MDP-SQ ($H(4, N = 95) = 11.09$, $p = 0.025$), PSQI ($H(4, N = 95) = 11.62$, $p = 0.02$), 6MWD ($H(4, N = 87) = 10.00$, $p = 0.04$), MIP ($H(4, N = 95) = 21.82$, $p = 0.0002$), MIP % ($H(4, N=95) = 16.08$, $p = 0.003$), SNIP ($H(4, N = 95) = 26.56$, $p < 0.0001$), SNIP % ($H(4, N = 95) = 27.14$, $p < 0.0001$), MEP ($H(4, N = 95) = 14.75$, $p = 0.005$), PCF ($H(4, N = 95) = 14.04$, $p = 0.007$), MVV ($H(4, N = 95) = 17.53$, $p = 0.001$) and MVV % ($H(4, N = 95) = 12.45$, $p = 0.01$). Post-hoc comparisons (multiple comparisons of mean ranks with Bonferroni correction) revealed that IIA patients had lower QMG scores than IIIA and IIIB ($p = 0.017$ and 0.03), and less disability on MG-ADL than IIIA and IIIB ($p = 0.007$ and 0.03). IIB patients showed lower MG-ADL scores than IIIA and IIIB ($p = 0.0003$ and 0.03). IIA patients

had higher Dyspnea-12 scores than IIIA ($p = 0.02$). No significant post-hoc differences were found for MG-QoL15 and MDP-SQ. Respiratory muscle strength (MIP, SNIP) was better in IIA compared to IIIA and IIIB, and in IIB compared to IIIA and IIIB (all $p < 0.05$). MEP was higher in IIA vs IIIB ($p = 0.003$), PCF was better in IIA vs IIIA ($p = 0.008$), and MVV was better in IIA vs IIIA and IIIB ($p = 0.0009$ and 0.04).

When considering percent predicted values, significant differences were observed between IIA and IIIB patients for MIP % ($p = 0.002$); between IIA and IIIA ($p = 0.03$) and IIIB patients ($p = 0.004$), as well as between IIB and IIIA ($p = 0.003$) and IIIB patients ($p = 0.0005$) for SNIP %; and between IIA and IIIA for MVV % ($p = 0.01$).



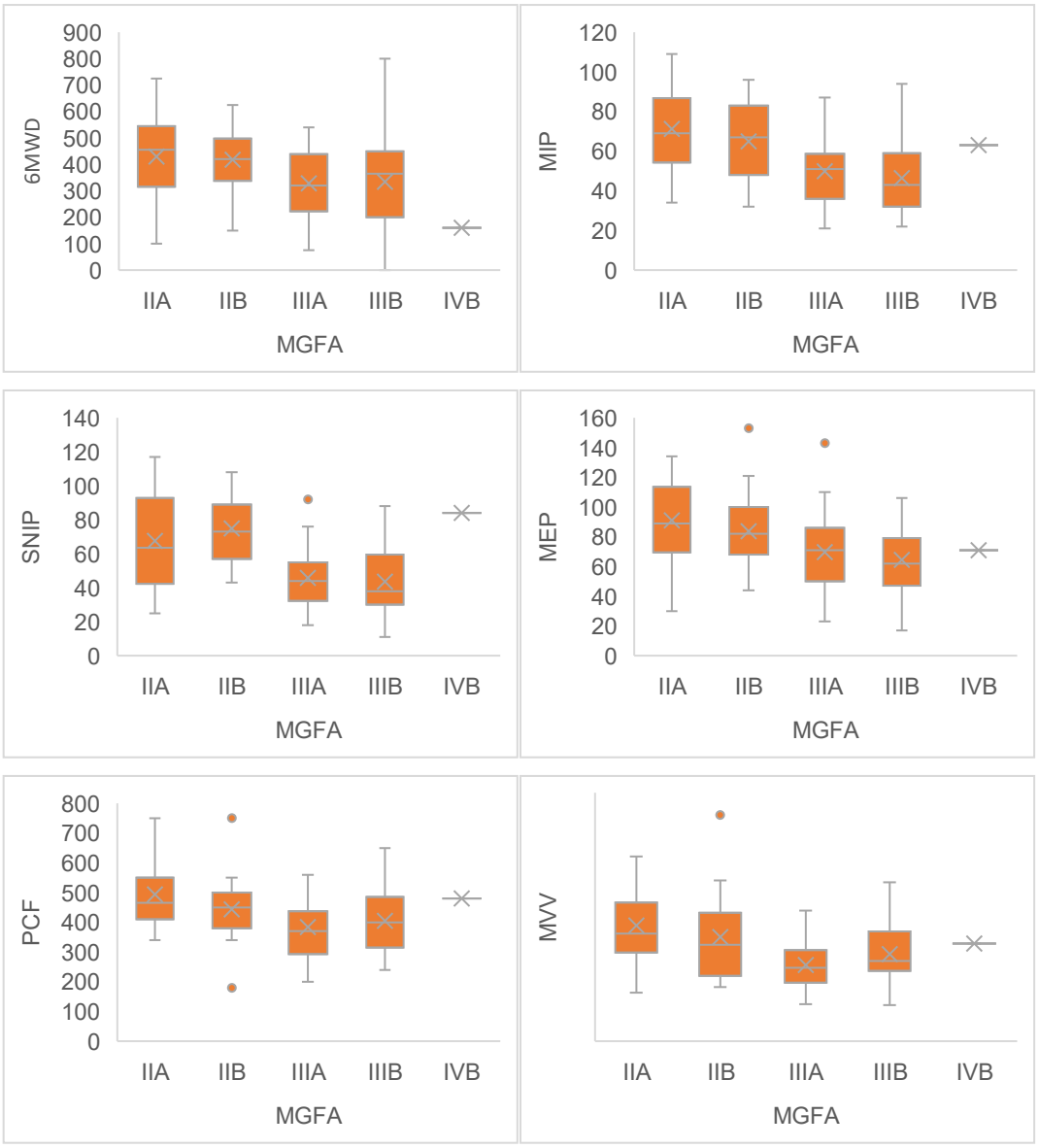


Figure 5 Box and whiskers plots showing statistically significant differences ($p < 0.05$) in several outcome scores (MG-ADL, QMG, MG-Qol15, Dyspnea 12, MDP-SQ, PSQI, 6MWD, MIP, SNIP, MEP, PCF, MVV) according to MGFA classification. The boxplot displays the distribution of outcomes in different MGFA classes: the central line represents the median, the "x" indicates the mean, the box edges correspond to the first and third quartiles, the whiskers show the range excluding outliers, and dots represent outlier values. Abbreviations: 6MWD, 6-minute walk distance; Dyspnea-12, 12-item Dyspnea Questionnaire; FEV1, forced expiratory volume in 1 second; MDP-SQ, Multidimensional Dyspnea Profile – sensory quality domain; MEP, maximal expiratory pressure; MG-ADL, Myasthenia Gravis Activities of Daily Living scale; MG-QoL15, Myasthenia Gravis Quality of Life 15-item scale; MGFA, Myasthenia Gravis Foundation of America classification; MIP, maximal inspiratory pressure; MVV, maximal voluntary ventilation; PCF, peak cough flow; PSQI, Pittsburgh Sleep Quality Index; QMG, Quantitative Myasthenia Gravis score; SNIP, sniff nasal inspiratory pressure.

Moreover, a univariate analysis was performed to investigate the role of sex across the study outcome measures. Compared with male patients, female patients were significantly older ($U = 783.5$, $p = 0.009$), had an earlier disease onset ($U = 664.5$, $p = 0.0004$), and a longer disease duration ($U = 813.5$, $p = 0.018$). Female patients also showed greater disease severity, as reflected by higher QMG ($U = 670.0$, $p = 0.0005$) and MG-ADL scores ($U = 689.5$, $p = 0.0009$). Quality of life was poorer among female patients, as indicated by higher MG-QoL15 scores ($U = 670.5$, $p = 0.0005$). They also reported more severe dyspnea, as assessed by Dyspnea-12 ($U = 855.5$, $p = 0.04$) and MDP-SQ ($U = 858.5$, $p = 0.04$). Furthermore, female patients showed more

dysfunctional breathing, as suggested by higher NQ scores (U = 1873.5, p = 0.004), and greater respiratory involvement, as indicated by worse SBCT performance (U = 627.5, p = 0.0002) and lower respiratory function parameters, including FVC (U = 574.0, p < 0.0001), FEV1 (U = 667.0, p = 0.0005), MIP (U = 721.0, p = 0.002), SNIP (U = 768.0, p = 0.007), SNIP % (U = 666.0, p = 0.0005), MEP (U = 690.5, p = 0.0009), PCF (U = 437.0, p < 0.0001), MVV (U = 610, p < 0.0001), and reactance (U = 827.0, p = 0.02).

Additional analyses examined the effect of age at disease onset on outcome measures. Significant differences across juvenile-, early-, late-, and very late-onset MG groups were observed for BMI (H(3, N = 95) = 8.29, p = 0.04), NQ (H(3, N = 95) = 9.27, p = 0.02), 6MWD (H(3, N = 95) = 10.17, p = 0.017), SBCT (H(3, N = 94) = 9.45, p = 0.02), and R5–19 (H(3, N = 95) = 13.01, p = 0.004). Post-hoc pairwise comparisons using multiple comparisons of mean ranks with Bonferroni correction revealed that late-onset patients had significantly higher BMI than early-onset patients (p = 0.048), early-onset patients had higher NQ scores than very late-onset patients (p = 0.025), very late-onset patients performed worse than early-onset patients on the 6MWT (p = 0.017). Moreover, airway resistance, as assessed by R5–19, was significantly higher in the late- and very late-onset groups compared with the early-onset group (p = 0.0046).

3. Correlation analysis

Spearman correlation analysis showed significant associations among clinical scales, symptom questionnaires, and several respiratory parameters (Table 3; Figure 6). For clarity, only moderate and strong correlations ($R \geq 0.40$) are reported.

Variables	Age	QMG	MG-ADL	MG-Qol15	Dyspnea 12	MDP-SQ	MDP-ER	NQ	HADS-A	HADS-D	PSQI	6MWD	SBCT
Age	1,00	0,01	-0,04	0,04	-0,06	-0,08	-0,19	-0,17	-0,14	0,01	0,22	-0,40	0,10
QMG	0,01	1,00	0,70	0,53	0,59	0,52	0,36	0,49	0,41	0,44	0,34	-0,54	-0,62
MG-ADL	-0,04	0,70	1,00	0,67	0,64	0,54	0,35	0,60	0,39	0,44	0,33	-0,41	-0,45
MG-Qol15	0,04	0,53	0,67	1,00	0,70	0,59	0,55	0,64	0,60	0,63	0,41	-0,54	-0,46
Dyspnea 12	-0,06	0,59	0,64	0,70	1,00	0,87	0,79	0,81	0,58	0,50	0,52	-0,42	-0,47
MDP-SQ	-0,08	0,52	0,54	0,59	0,87	1,00	0,70	0,78	0,55	0,45	0,44	-0,36	-0,46
MDP-ER	-0,19	0,36	0,35	0,55	0,79	0,70	1,00	0,67	0,54	0,45	0,45	-0,32	-0,26
NQ	-0,17	0,49	0,60	0,64	0,81	0,78	0,67	1,00	0,66	0,51	0,54	-0,29	-0,42
HADS-A	-0,14	0,41	0,39	0,60	0,58	0,55	0,54	0,66	1,00	0,64	0,48	-0,31	-0,42
HADS-D	0,01	0,44	0,44	0,63	0,50	0,45	0,45	0,51	0,64	1,00	0,45	-0,37	-0,32
PSQI	0,22	0,34	0,33	0,41	0,52	0,44	0,45	0,54	0,48	0,45	1,00	-0,35	-0,23
6MWD	-0,40	-0,54	-0,41	-0,54	-0,42	-0,36	-0,32	-0,29	-0,31	-0,37	-0,35	1,00	0,39
SBCT	0,10	-0,62	-0,45	-0,46	-0,47	-0,46	-0,26	-0,42	-0,42	-0,32	-0,23	0,39	1,00
FVC	-0,24	-0,40	-0,31	-0,37	-0,33	-0,33	-0,20	-0,27	-0,25	-0,25	-0,31	0,53	0,24
FVC%	-0,03	-0,14	-0,10	-0,14	-0,18	-0,19	-0,12	-0,14	-0,19	-0,21	-0,09	0,25	0,01
FEV1	-0,29	-0,39	-0,26	-0,30	-0,28	-0,27	-0,16	-0,18	-0,14	-0,16	-0,28	0,53	0,21
Tiffeneau	-0,17	-0,05	0,00	0,10	0,02	0,07	0,09	0,11	0,07	-0,01	-0,14	0,20	0,01
MIP	0,02	-0,45	-0,39	-0,45	-0,38	-0,40	-0,26	-0,36	-0,27	-0,30	-0,32	0,36	0,23
MIP %	0,13	-0,34	-0,29	-0,31	-0,34	-0,35	-0,29	-0,32	-0,22	-0,25	-0,24	0,21	0,11
SNIP	0,10	-0,37	-0,38	-0,44	-0,36	-0,32	-0,30	-0,35	-0,24	-0,30	-0,31	0,29	0,13
SNIP %	0,14	-0,40	-0,40	-0,46	-0,37	-0,33	-0,30	-0,37	-0,25	-0,31	-0,30	0,30	0,17
MEP	0,05	-0,45	-0,33	-0,40	-0,32	-0,35	-0,23	-0,33	-0,27	-0,24	-0,18	0,47	0,37
MEP %	0,20	-0,20	-0,05	-0,12	-0,18	-0,19	-0,23	-0,17	-0,16	-0,11	-0,06	0,22	0,14
PCF	0,03	-0,39	-0,40	-0,40	-0,33	-0,35	-0,20	-0,33	-0,20	-0,25	-0,22	0,41	0,33
MVV	-0,07	-0,50	-0,43	-0,48	-0,50	-0,48	-0,37	-0,45	-0,34	-0,37	-0,36	0,55	0,32
MVV %	0,12	-0,37	-0,28	-0,29	-0,43	-0,42	-0,40	-0,37	-0,29	-0,30	-0,23	0,38	0,17
Resistance	0,02	-0,03	-0,05	-0,02	-0,02	0,04	0,03	0,00	0,12	0,01	0,03	-0,14	0,07
Reactance	-0,07	0,07	0,07	-0,15	-0,11	-0,14	-0,12	-0,07	-0,10	0,09	-0,09	0,23	0,03
FRES	0,28	0,02	-0,04	-0,01	0,08	0,13	0,11	-0,04	-0,02	-0,03	-0,03	-0,20	0,06
AX	0,14	0,02	-0,04	0,06	0,08	0,10	0,12	0,01	0,06	-0,01	-0,03	-0,17	0,02
R5-19	0,27	-0,14	-0,17	-0,12	-0,02	0,05	0,06	-0,14	-0,08	-0,06	-0,06	-0,12	0,24

Variables	FVC	FVC%	FEV1	Tiffeneau	MIP	MIP %	SNIP	SNIP %	MEP	MEP %	PCF	MMV	MVV %
Age	-0,24	-0,03	-0,29	-0,17	0,02	0,13	0,10	0,14	0,05	0,20	0,03	-0,07	0,12
QMG	-0,40	-0,14	-0,39	-0,05	-0,45	-0,34	-0,37	-0,40	-0,45	-0,20	-0,39	-0,50	-0,37
MG-ADL	-0,31	-0,10	-0,26	0,00	-0,39	-0,29	-0,38	-0,40	-0,33	-0,05	-0,40	-0,43	-0,28
MG-Qol15	-0,37	-0,14	-0,30	0,10	-0,45	-0,31	-0,44	-0,46	-0,40	-0,12	-0,40	-0,48	-0,29
Dyspnea 12	-0,33	-0,18	-0,28	0,02	-0,38	-0,34	-0,36	-0,37	-0,32	-0,18	-0,33	-0,50	-0,43
MDP-SQ	-0,33	-0,19	-0,27	0,07	-0,40	-0,35	-0,32	-0,33	-0,35	-0,19	-0,35	-0,48	-0,42
MDP-ER	-0,20	-0,12	-0,16	0,09	-0,26	-0,29	-0,30	-0,30	-0,23	-0,23	-0,20	-0,37	-0,40
NQ	-0,27	-0,14	-0,18	0,11	-0,36	-0,32	-0,35	-0,37	-0,33	-0,17	-0,33	-0,45	-0,37
HADS-A	-0,25	-0,19	-0,14	0,07	-0,27	-0,22	-0,24	-0,25	-0,27	-0,16	-0,20	-0,34	-0,29
HADS-D	-0,25	-0,21	-0,16	-0,01	-0,30	-0,25	-0,30	-0,31	-0,24	-0,11	-0,25	-0,37	-0,30
PSQI	-0,31	-0,09	-0,28	-0,14	-0,32	-0,24	-0,31	-0,30	-0,18	-0,06	-0,22	-0,36	-0,23
6MWD	0,53	0,25	0,53	0,20	0,36	0,21	0,29	0,30	0,47	0,22	0,41	0,55	0,38
SBCT	0,24	0,01	0,21	0,01	0,23	0,11	0,13	0,17	0,37	0,14	0,33	0,32	0,17
FVC	1,00	0,58	0,89	0,14	0,53	0,30	0,42	0,44	0,46	0,10	0,69	0,71	0,46
FVC%	0,58	1,00	0,53	0,31	0,24	0,24	0,29	0,28	0,26	0,28	0,36	0,41	0,47
FEV1	0,89	0,53	1,00	0,25	0,50	0,29	0,40	0,42	0,44	0,09	0,70	0,67	0,43
Tiffeneau	0,14	0,31	0,25	1,00	-0,04	-0,07	0,03	0,02	-0,07	-0,08	0,18	0,15	0,10
MIP	0,53	0,24	0,50	-0,04	1,00	0,91	0,77	0,78	0,62	0,39	0,60	0,63	0,55
MIP %	0,30	0,24	0,29	-0,07	0,91	1,00	0,72	0,71	0,50	0,54	0,40	0,49	0,62
SNIP	0,42	0,29	0,40	0,03	0,77	0,72	1,00	0,99	0,56	0,39	0,44	0,46	0,42
SNIP %	0,44	0,28	0,42	0,02	0,78	0,71	0,99	1,00	0,57	0,36	0,49	0,48	0,41
MEP	0,46	0,26	0,44	-0,07	0,62	0,50	0,56	0,57	1,00	0,75	0,49	0,52	0,43
MEP %	0,10	0,28	0,09	-0,08	0,39	0,54	0,39	0,36	0,75	1,00	0,13	0,23	0,48
PCF	0,69	0,36	0,70	0,18	0,60	0,40	0,44	0,49	0,49	0,13	1,00	0,75	0,51
MVV	0,71	0,41	0,67	0,15	0,63	0,49	0,46	0,48	0,52	0,23	0,75	1,00	0,85
MVV %	0,46	0,47	0,43	0,10	0,55	0,62	0,42	0,41	0,43	0,48	0,51	0,85	1,00
Resistance	-0,32	-0,29	-0,28	-0,10	-0,02	0,01	0,10	0,09	0,00	0,00	-0,15	-0,17	-0,18
Reactance	0,36	0,16	0,39	0,18	0,06	-0,06	0,02	0,05	0,02	-0,10	0,33	0,24	0,10
FRES	-0,42	-0,28	-0,41	-0,13	-0,02	0,06	0,17	0,15	-0,02	0,07	-0,20	-0,20	-0,14
AX	-0,38	-0,19	-0,34	-0,05	0,01	0,08	0,15	0,13	-0,05	0,02	-0,19	-0,16	-0,10
R5-19	-0,29	-0,35	-0,35	-0,18	0,05	0,06	0,15	0,15	0,09	0,06	-0,09	-0,13	-0,14

Table 3 Spearman correlation analysis among clinical scales, symptom questionnaires and respiratory parameter. Correlation parameters (R) are shown, Abbreviations: 6MWD: 6-Minute Walk Distance; AX: area of reactance; Dyspnea-12: Dyspnea-12 questionnaire; FEV1: forced expiratory volume in 1 second; FRES: resonant frequency; FVC: forced vital capacity; FVC%: forced vital capacity expressed as percentage of predicted value; HADS-A: Hospital Anxiety and Depression Scale – Anxiety subscale; HADS-D: Hospital Anxiety and Depression Scale – Depression subscale; MDP-ER: Multidimensional Dyspnea Profile – Emotional Response; MDP-IQ: Multidimensional Dyspnea Profile – Immediate Perception; MEP: maximal expiratory pressure; MG-ADL: Myasthenia Gravis Activities of Daily Living scale; MG-QoL15: Myasthenia Gravis Quality of Life 15-item scale; MIP: maximal inspiratory pressure; MVV: maximal voluntary ventilation; NQ: Nijmegen Questionnaire; PCF: peak cough flow; PSQI: Pittsburgh Sleep Quality Index; QMG: Quantitative Myasthenia Gravis score; R5-19: difference between resistance at 5 Hz and 19 Hz; Reactance: respiratory system reactance; Resistance: respiratory system resistance; SBCT: single breath counting test; SNIP: sniff nasal inspiratory pressure; Tiffeneau: Tiffeneau index (FEV1/FVC ratio).

Variables	Age	QMG	MG-ADL	MG-Qol15	Dyspnea 12	MDP-SQ	MDP-ER	NQ	HADS-A	HADS-D	PSQI	6MWD	SBCT
Age	1,00	0,01	-0,04	0,04	-0,06	-0,08	-0,19	-0,17	-0,14	0,01	0,22	-0,40	0,10
QMG	0,01	1,00	0,70	0,53	0,59	0,52	0,36	0,49	0,41	0,44	0,34	-0,54	-0,62
MG-ADL	-0,04	0,70	1,00	0,67	0,64	0,54	0,35	0,60	0,39	0,44	0,33	-0,41	-0,45
MG-Qol15	0,04	0,53	0,67	1,00	0,70	0,59	0,55	0,64	0,60	0,63	0,41	-0,54	-0,46
Dyspnea 12	-0,06	0,59	0,64	0,70	1,00	0,87	0,79	0,81	0,58	0,50	0,52	-0,42	-0,47
MDP-SQ	-0,08	0,52	0,54	0,59	0,87	1,00	0,70	0,78	0,55	0,45	0,44	-0,36	-0,46
MDP-ER	-0,19	0,36	0,35	0,55	0,79	0,70	1,00	0,67	0,54	0,45	0,45	-0,32	-0,26
NQ	-0,17	0,49	0,60	0,64	0,81	0,78	0,67	1,00	0,66	0,51	0,54	-0,29	-0,42
HADS-A	-0,14	0,41	0,39	0,60	0,58	0,55	0,54	0,66	1,00	0,64	0,48	-0,31	-0,42
HADS-D	0,01	0,44	0,44	0,63	0,50	0,45	0,45	0,51	0,64	1,00	0,45	-0,37	-0,32
PSQI	0,22	0,34	0,33	0,41	0,52	0,44	0,45	0,54	0,48	0,45	1,00	-0,35	-0,23
6MWD	-0,40	-0,54	-0,41	-0,54	-0,42	-0,36	-0,32	-0,29	-0,31	-0,37	-0,35	1,00	0,39
SBCT	0,10	-0,62	-0,45	-0,46	-0,47	-0,46	-0,26	-0,42	-0,42	-0,32	-0,23	0,39	1,00
FVC	-0,24	-0,40	-0,31	-0,37	-0,33	-0,33	-0,20	-0,27	-0,25	-0,25	-0,31	0,53	0,24
FVC %	-0,03	-0,14	-0,10	-0,14	-0,18	-0,19	-0,12	-0,14	-0,19	-0,21	-0,09	0,25	0,01
FEV1	-0,29	-0,39	-0,26	-0,30	-0,28	-0,27	-0,16	-0,18	-0,14	-0,16	-0,28	0,53	0,21
Tiffeneau	-0,17	-0,05	0,00	0,10	0,02	0,07	0,09	0,11	0,07	-0,01	-0,14	0,20	0,01
MIP	0,02	-0,45	-0,39	-0,45	-0,38	-0,40	-0,26	-0,36	-0,27	-0,30	-0,32	0,36	0,23
MIP %	0,13	-0,34	-0,29	-0,31	-0,34	-0,35	-0,29	-0,32	-0,22	-0,25	-0,24	0,21	0,11
SNIP	0,10	-0,37	-0,38	-0,44	-0,36	-0,32	-0,30	-0,35	-0,24	-0,30	-0,31	0,29	0,13
SNIP %	0,14	-0,40	-0,40	-0,46	-0,37	-0,33	-0,30	-0,37	-0,25	-0,31	-0,30	0,30	0,17
MEP	0,05	-0,45	-0,33	-0,40	-0,32	-0,35	-0,23	-0,33	-0,27	-0,24	-0,18	0,47	0,37
MEP %	0,20	-0,20	-0,05	-0,12	-0,18	-0,19	-0,23	-0,17	-0,16	-0,11	-0,06	0,22	0,14
PCF	0,03	-0,39	-0,40	-0,40	-0,33	-0,35	-0,20	-0,33	-0,20	-0,25	-0,22	0,41	0,33
MMV	-0,07	-0,50	-0,43	-0,48	-0,50	-0,48	-0,37	-0,45	-0,34	-0,37	-0,36	0,55	0,32
MVV %	0,12	-0,37	-0,28	-0,29	-0,43	-0,42	-0,40	-0,37	-0,29	-0,30	-0,23	0,38	0,17

Figure 6 Correlation matrix between clinical severity, dyspnea perception, psychological status, functional capacity, and respiratory function parameters. Values represent correlation coefficients (R). Color scale indicates the strength and direction of correlations, with red representing positive correlations and blue representing negative correlations. Abbreviations: QMG, Quantitative Myasthenia Gravis Score; MG-ADL, Myasthenia Gravis Activities of Daily Living; MG-QoL15, Myasthenia Gravis Quality of Life-15; Dyspnea-12, Dyspnea-12 questionnaire; MDP-IQ, Multidimensional Dyspnea Profile – Immediate Perception; MDP-ER, Multidimensional Dyspnea Profile – Emotional Response; NQ, Nijmegen Questionnaire; HADS-A/HADS-D, Hospital Anxiety and Depression Scale (anxiety/depression); PSQI, Pittsburgh Sleep Quality Index; 6MWD, Six-Minute Walk Distance; SBCT, Single Breath Counting Test; FVC, Forced Vital Capacity; FEV1, Forced Expiratory Volume in 1 s; MIP, Maximal Inspiratory Pressure; SNIP, Sniff Nasal Inspiratory Pressure; MEP, Maximal Expiratory Pressure; PCF, Peak Cough Flow; MMV, Maximum Minute Ventilation; FRES, resonant frequency; AX, area of reactance; R5–19, resistance difference between 5 and 19 Hz.

QMG, MG-ADL and MG-QoL15 were moderately to strongly inter-correlated (R=0.53–0.70, p<0.0001; Fig. 7).

All MG specific scales showed moderate to strong positive correlations with dyspnea-related scales (Dyspnea-12, MDP-SQ, NQ) and psychological measures (HADS-A, HADS-D) (R≈0.40–0.70, p<0.0001). MG-QoL15 also correlated with sleep quality assessed by PSQI (R=0.41, p<0.0001).

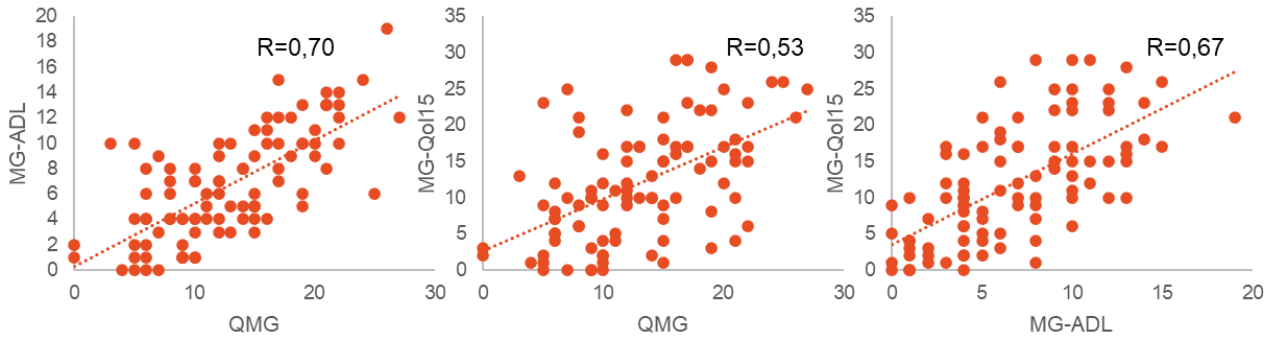


Figure 7 Associations between clinical severity and patient-reported outcomes in myasthenia gravis. Scatter plots showing the relationships between QMG and MG-ADL (left), QMG and MG-QoL15 (center), and MG-ADL and MG-QoL15 (right). Each dot represents an individual patient; dashed lines indicate linear regression trends, showing positive associations between clinical severity, functional impairment, and quality-of-life burden Abbreviations: QMG, Quantitative Myasthenia Gravis Score; MG-ADL, Myasthenia Gravis Activities of Daily Living scale; MG-QoL15, Myasthenia Gravis Quality of Life 15-item scale.

Functional exercise capacity showed inverse correlations with disease severity and symptom burden. In particular, the 6MWD correlated negatively with QMG (R=-0.54), MG-QoL15 (R=-0.54) and Dyspnea-12 (R=-0.42) (all p<0.0001) (Fig. 8A).

Similarly, the SBCT showed moderate negative correlations with QMG (R=-0.62) and MG-ADL (R=-0.45) (Fig. 8B).

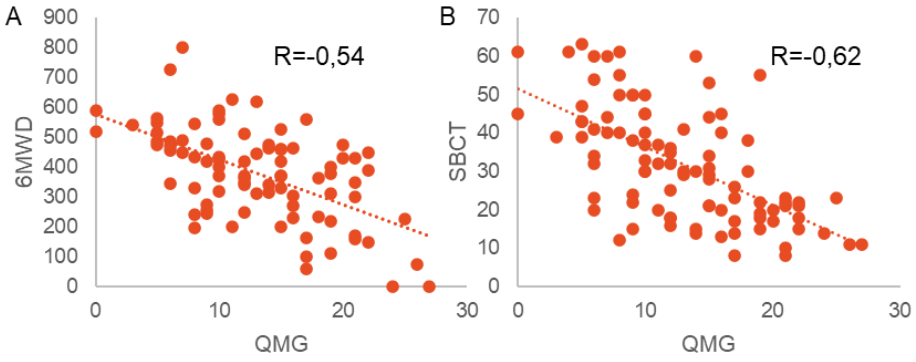


Figure 8 Associations between clinical severity and functional capacity as evaluated by the 6MWD (A) and clinical severity and single breath respiratory count (B) Abbreviations: 6MWD, 6-minute walking distance; QMG, Quantitative Myasthenia Gravis Score; SBCT, single breath respiratory count.

Several respiratory muscle and ventilatory parameters were inversely associated with clinical severity. Moderate negative correlations were observed between QMG and MIP (R = -0.45), SNIP % (R = -0.4), MEP (R=-0.45) and MVV (R=-0.50) (all p<0.0001) (Fig. 9), with similar patterns for MG-ADL and MG-QoL15.

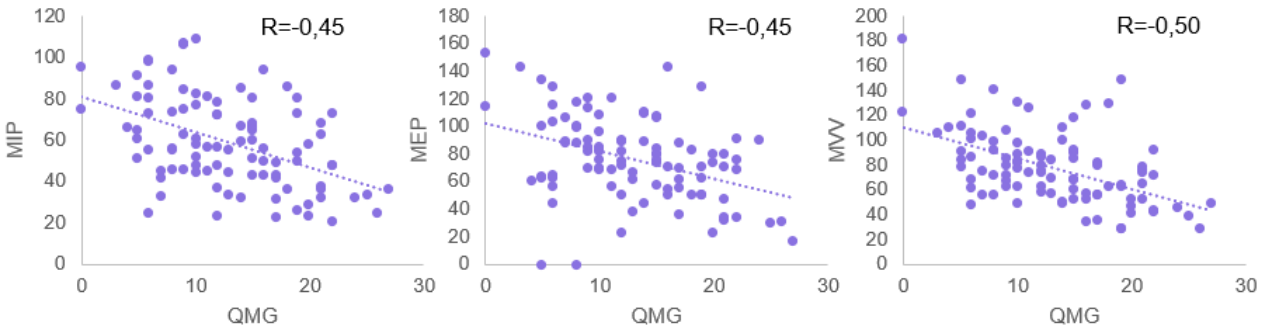


Figure 9 Associations between clinical severity and inspiratory pressure (left), expiratory pressure (center) and maximal voluntary ventilation (right). Abbreviations: MEP, Maximal Expiratory Pressure; MIP, Maximal Inspiratory Pressure; MVV, Maximal Voluntary Ventilation; QMG, Quantitative Myasthenia Gravis.

By contrast, oscillometry-derived parameters (Resistance, Reactance, FRES, AX and R5-19) showed no meaningful correlations with clinical severity scales.

Finally, age was moderately associated with reduced exercise capacity, showing a negative correlation with 6MWD (R=-0.40, p<0.0001).

4. Regression analysis

Model 1 – QMG

Two multiple linear regression models were performed to assess predictors of disease severity, as measured by the QMG score.

In the first model, which included sex, age at inclusion, serological status, SBCT, FVC, and *MVV* as independent variables, the overall model was statistically significant ($F(6,87) = 15.53$, $p < 0.0001$), explaining 51.7% of the variance in QMG scores ($R^2 = 0.517$; adjusted $R^2 = 0.483$). The standard error of the estimate was 4.31. Within this model, SBCT was the strongest predictor of QMG ($\beta = -0.198$, $p < 0.001$), followed by MVV ($\beta = -0.070$, $p = 0.0028$) and serological status ($\beta = 0.155$, $p = 0.04$). Sex, age, and FVC were not significantly associated with QMG scores.

In the second model, sex, age at inclusion, serological status, SBCT, FVC, and *MIP* were included as predictors. This model was also statistically significant ($F(5,88) = 18.18$, $p < 0.001$), accounting for 50.8% of the variance in QMG ($R^2 = 0.508$; adjusted $R^2 = 0.480$), with a standard error of 4.33. SBCT again emerged as the strongest predictor ($\beta = -0.510$, $p < 0.001$), followed by MIP ($\beta = -0.269$, $p = 0.003$). Other variables, including sex, age, serological status, and FVC, did not reach statistical significance.

Model 2 – MG-ADL

A multiple linear regression model was performed to investigate the association between MG-ADL score (dependent variable) and sex, age at inclusion, serological status, Dyspnea-12 score, HADS-A score, and SBCT.

The model was statistically significant ($F(6,84) = 11.96$, $p < 0.001$), explaining 46.1% of the variance in MG-ADL scores ($R^2 = 0.461$; adjusted $R^2 = 0.422$). The standard error of the estimate was 3.21.

In this model, Dyspnea-12 showed the strongest positive association with MG-ADL ($\beta = 0.511$, $p < 0.001$). Significant associations were also observed for sex ($\beta = 0.225$, $p = 0.012$) and SBCT ($\beta = -0.207$, $p = 0.030$). No significant associations were found for age at inclusion ($\beta = 0.100$, $p = 0.232$), serological status ($\beta = 0.107$, $p = 0.198$), or HADS-A ($\beta = -0.070$, $p = 0.507$).

Model 3 – Dyspnea-12

A multiple linear regression model was conducted to assess factors associated with Dyspnea-12 score (dependent variable), including sex, age at inclusion, serological status, QMG score, NQ score, HADS-A score, and SBCT.

The model was statistically significant ($F(7,83) = 25.62$, $p < 0.001$), explaining 68.4% of the variance in Dyspnea-12 scores ($R^2 = 0.684$; adjusted $R^2 = 0.657$). The standard error of the estimate was 5.92.

Within the model, NQ emerged as the strongest predictor of Dyspnea-12 ($\beta = 0.639$, $p < 0.001$). QMG was also significantly associated with Dyspnea-12 ($\beta = 0.176$, $p = 0.048$). HADS-A showed a borderline association ($\beta = 0.163$, $p = 0.057$), while sex showed a trend toward significance ($\beta = -0.117$, $p = 0.096$). No significant associations were observed for age at inclusion ($\beta = 0.021$, $p = 0.748$), serological status ($\beta = -0.044$, $p = 0.507$), or SBCT ($\beta = -0.007$, $p = 0.934$).

5. Follow-up analysis:

Since enrollment, only 57 patients (30 female, 27 male, age 61.9 ± 14.3) completed the 12-month observation time frame. Of these, 47 patients completed the interview, while 9 patients were unreachable and 1 declined to participate, corresponding to a response rate of 82.5%. A total of 47 patients (82%) were positive for AChR antibodies, 9 patients (16%) had MuSK-positive MG, and 1 patient (2%) had LRP4-positive MG. Regarding treatment, 19 patients (33.3%) were receiving steroids only, 17 patients (29.8%) were on combined steroid and/or classical immunosuppressive therapy, 17 patients (29.8%) were receiving innovative treatments, either alone or in combination with other therapies, and 4 patients (6.1%) were taking only symptomatic treatment.

When evaluating events, percentages are calculated on the 47 patients who completed follow-up.

The reasons for admission, along with the overall incidence of infections, antibiotic use, and other clinical events, are summarized in Table 4.

Event/Infection type	**N patients**	**Percentage (of 47)**	**Notes/antibiotic therapy**
Hospitalization (any cause)	12	25.5%	
Reasons for hospitalization			
Pneumonia	5	10.6%	Antibiotics: ceftriaxone (n = 2), piperacillin/tazobactam

			(n = 2), clarithromycin (n = 1)
Exacerbation of MG	4	8.5%	2 with concomitant pneumonia; 0 requiring ventilatory support
Cerebral ischemia	1	2.1%	
Pacemaker implantation	1	2.1%	
Abdominal pain	1	2.1%	
Pneumonia (overall)	7	14.9%	Antibiotics listed above; no culture available
Upper respiratory tract infections	28	59.6%	8 received antibiotics
Urinary tract infections	11	23.4%	
Other infections	4	8.5%	1 salpingo-oophoritis, 1 otitis, 1 cholecystitis and 1 dental infection
Death	0	0%	

Table 4 Summary of hospitalizations, infections, and antibiotic use among 47 patients who completed follow-up. Data are presented as number of patients (percentage). Antibiotic therapy is specified only for pneumonia.

Among the 47 patients who completed the interview, 12 (25.5%) required hospitalization within 12 months from enrollment. The number of hospitalizations was 1 for all the 12 patients within the 12-month period. The reasons for admission were as follows: pneumonia in 5 patients (10.6%); exacerbation of myasthenia gravis in 4 patients (8.5%; including 2 cases [4.3%] with concomitant pneumonia), none of which required ventilatory support; suspected cerebral ischemia in 1 patient (2.1%); pacemaker implantation in 1 patient (2.1%); and abdominal pain in 1 patient (2.1%).

Overall, 7 cases of pneumonia were reported (14.9%). Antibiotic therapy for pneumonia included ceftriaxone in 2 patients (4.3%), piperacillin/tazobactam in 2 patients (4.3%), clarithromycin in 1 patient (2.1%), and cefixime in 1 patient (2.1%); 1 patient (2.1%) was unable to recall the specific antibiotic used in the home-based treatment. No microbiological culture results were available for analysis.

Upper respiratory tract infections were reported in 28 patients (59.6%), of whom 8 (17.0%) received antibiotic therapy. Urinary tract infections occurred in 11 patients (23.4%). Infections at other sites were observed in 4 patients (8.5%), including salpingo-oophoritis, otitis, cholecystitis, and dental infection (1 case each; 2.1% each). No deaths were recorded during the follow-up period.

Two receiver operating characteristic (ROC) curves were constructed to evaluate the predictive ability of selected baseline outcome measures (age, QMG, MG-ADL, Dyspnea-12, MIP, MVV, SBCT) in this study. The first ROC analysis focused on hospitalizations (Fig. 10).

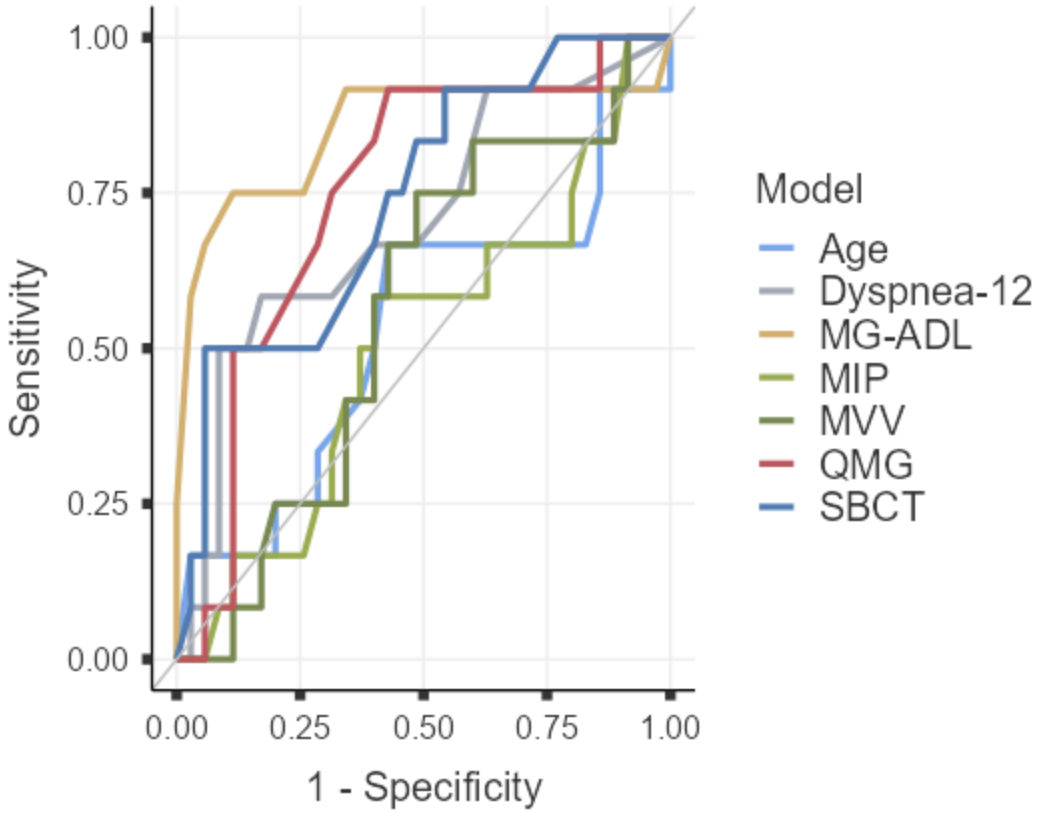


Figure 10 Receiving Operating Characteristics (ROC) curve evaluating hospitalization evaluating different baseline assessments (age, Dyspnea-12, Myasthenia Gravis-Activities of Daily Living (MG-ADL), Maximal Inspiratory Pressure (MIP), Maximal Voluntary Ventilation (MVV), Quantitative Myasthenia Gravis (QMG) and Single Breath Respiratory Count (SBCT) values).

Among the predictors, MG-ADL showed the highest discriminative ability, with an area under the curve (AUC) of 0.852 (95% confidence interval [CI] 0.686–1.000; $p < 0.001$), indicating good predictive performance. QMG score and SBCT also demonstrated significant predictive value, with AUCs of 0.749 (95% CI 0.589–0.908; $p = 0.002$) and 0.739 (95% CI 0.575–0.903; $p = 0.004$), respectively. Dyspnea-12 showed moderate predictive ability (AUC 0.702, 95% CI 0.518–0.887; $p = 0.031$). In contrast, age, MIP, and MVV did not significantly discriminate for hospitalization (AUC 0.532, 0.507, and 0.561, respectively; $p > 0.05$).

Optimal cut-off values were determined using the Youden index.

For MG-ADL, a threshold of 11 identified patients at higher risk of hospitalization: among patients with MG-ADL ≥11 (n = 13), 9 were hospitalized and 4 were not, while among patients with MG-ADL <11 (n = 34), 3 were hospitalized.

For QMG, a cut-off of 14 distinguished risk groups: among patients with QMG ≥14, 11 were hospitalized and 15 were not, whereas among patients with QMG <14, 1 was hospitalized and 20 were not.

For SBCT, a threshold of 16 identified higher-risk patients: among patients with SBCT ≥16, 6 were hospitalized and 2 were not, while among patients with SBCT <16, 6 were hospitalized and 33 were not.

Regarding Dyspnea-12, a cut-off of 20 identified patients at higher risk: among patients scoring ≥20, 6 were hospitalized and 3 were not, whereas among patients with Dyspnea-12 <20, 6 were hospitalized and 32 were not. These results suggest that functional and disease-specific measures, particularly MG-ADL and QMG

scores, provide greater predictive information than demographic or basic respiratory variables in identifying patients at risk of hospitalization in this cohort.

The predictive ability of baseline clinical and functional variables for pneumonia within 12 months was evaluated using ROC curve analysis (Fig. 11). Among the variables tested, SBCT showed the highest discriminative ability, with an area under the curve (AUC) of 0.893 (95% CI 0.785–1.000; $p < 0.001$), indicating excellent predictive performance. MG-ADL and QMG scores also demonstrated good predictive value, with AUCs of 0.859 (95% CI 0.726–0.992; $p < 0.001$) and 0.846 (95% CI 0.713–0.980; $p < 0.001$), respectively. MVV showed moderate predictive ability (AUC 0.754, 95% CI 0.602–0.906; $p = 0.001$). Dyspnea-12 had a borderline predictive value (AUC 0.691, 95% CI 0.492–0.890; $p = 0.060$), while age and MIP did not significantly discriminate for pneumonia (AUC 0.530 and 0.459, respectively; $p > 0.05$).

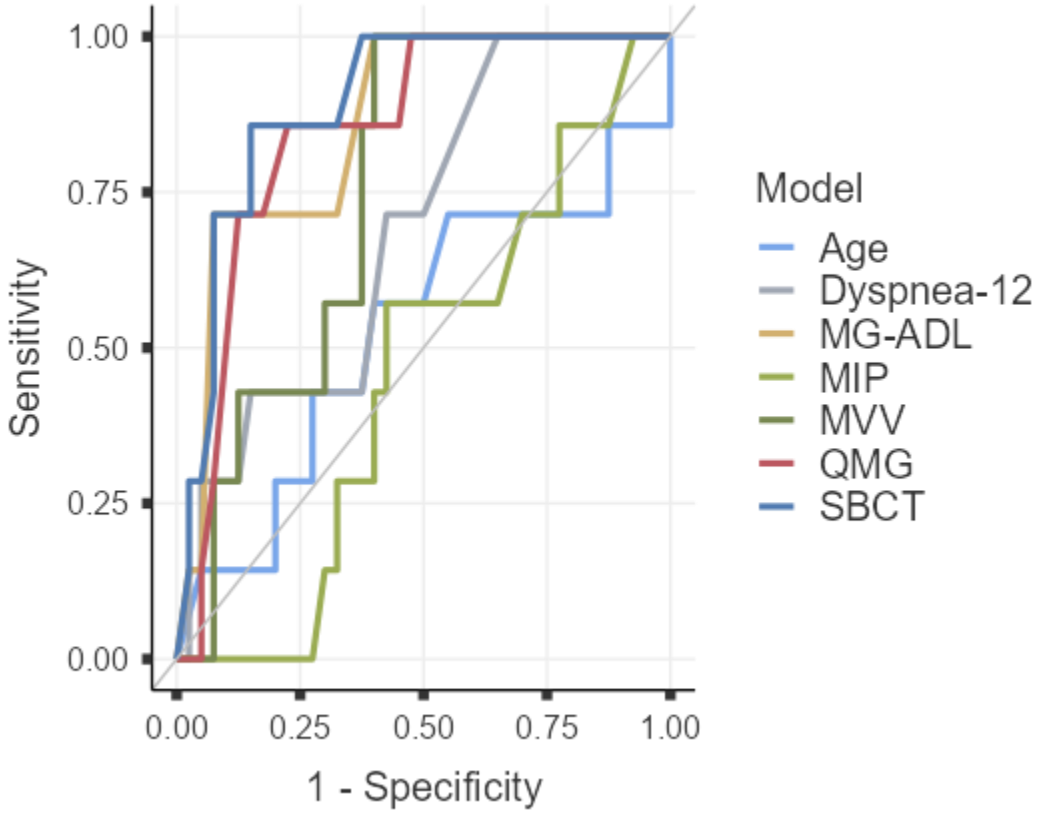


Figure 11 Receiving operating characteristics (ROC) curves evaluating pneumonia in our subgroup. Variables included in the analysis included age, Dyspnea-12, Myasthenia Gravis-Activities of Daily Living (MG-ADL), Maximal Inspiratory Pressure (MIP), Maximal Voluntary Ventilation (MVV), Quantitative Myasthenia Gravis (QMG) and Single Breath Respiratory Count (SBCT) values.

Optimal cut-off values for baseline functional and disease-specific measures were determined using the Youden index to stratify patients according to pneumonia risk.

For SBCT, a threshold of 19 distinguished higher-risk patients: among those with SBCT ≤ 18 (n = 12), 6 developed pneumonia and 6 did not, whereas among patients with SBCT >18 (n = 35), only 1 developed pneumonia. For MG-ADL, a cut-off of 13 identified higher-risk patients: among patients with MG-ADL ≥ 13

(n = 8), 5 developed pneumonia and 3 did not, while among patients with MG-ADL < 13 (n = 39), 2 developed pneumonia. For QMG, a threshold of 18 was used: 6 of 15 patients with QMG ≥ 18 developed pneumonia, compared with 1 of 32 patients with QMG <18. Finally, for MVV, a cut-off of 79.3 was identified: all 7 pneumonia cases occurred in patients with MVV ≤79.3, while no cases occurred among patients with MVV >79.3.

These contingency tables illustrate that lower SBCT and MVV scores, as well as higher MG-ADL and QMG scores, are associated with an increased risk of pneumonia in this cohort. Functional performance measures, particularly SBCT and MVV, together with disease-specific scores (MG-ADL and QMG), appear more informative than demographic variables in predicting pneumonia.

Although preliminary, a multivariable logistic regression analysis was performed to evaluate the combined predictive effect of baseline clinical and functional measures on hospitalization within 12 months (Table 5). Analysis concerning pneumonia was not performed due to low event rate.

Predictor	**Estimate (β)**	**SE**	**OR**	**95% CI**	**p-value**
Intercept	-3.587	5.054	0.028	1.38e-6 – 554.92	0.478
Age	0.023	0.038	1.024	0.951 – 1.10	0.533
QMG score	-0.254	0.255	0.776	0.471 – 1.28	0.320
MG-ADL score	0.821	0.372	2.27	1.10 – 4.71	0.027
Dyspnea-12	-0.040	0.075	0.961	0.831 – 1.11	0.595
MIP	0.038	0.047	1.039	0.947 – 1.14	0.421
MVV	-0.013	0.041	0.988	0.911 – 1.07	0.760
SBCT	-0.190	0.115	0.827	0.661 – 1.04	0.098
Male sex (vs female)	4.427	1.849	83.65	2.23 – 3137.95	0.017

Table 5 Multivariable logistic regression analysis for predictors of hospitalization within 12 months. The model included baseline clinical and functional variables. Results are reported as regression coefficients (β), standard errors (SE), odds ratios (OR), and 95% confidence intervals (CI). Statistically significant associations were observed for MG-ADL score and male sex, while all other variables were not significantly associated with hospitalization.

In the multivariable logistic regression analysis, higher MG-ADL scores and male sex were independently associated with an increased risk of hospitalization within 12 months. All other baseline clinical and functional variables, including age, QMG score, Dyspnea-12, MIP, MVV, and SBCT, were not significantly associated with the outcome, although SBCT showed a trend toward a protective effect.

Discussion:

This is a monocentric multidimensional study evaluating respiratory dysfunction in a cohort of seropositive gMG patients, aimed at assessing the diagnostic accuracy of several patient-centered outcomes together with more objective neurological and respiratory parameters. A 12-month follow-up is currently ongoing and will

be completed in December 2026. However, an exploratory analysis using ROC curves and logistic regression was performed to preliminarily evaluate the ability of respiratory parameters and questionnaires to predict severe outcomes such as pneumonia, death or hospitalization.

Our cohort mainly consisted of patients with mild to moderate MG and showed a good representation of different autoantibody profiles. According to age at onset, nearly two-thirds of the patients had late-onset (50–65 years) or very-late-onset (>65 years) gMG. The proportion of very-late-onset MG was lower than that reported in previous studies describing a higher prevalence of this subgroup (Cortés-Vicente et al., 2020). This difference may be explained by the exclusion of ocular MG from the selection process, as patients with very-late-onset MG often present with isolated ocular symptoms both at disease onset and during clinical worsening. Evaluation of comorbidity is line with the literature data, showing that 13% of patients had received a diagnosis of thymoma and that autoimmune disorders, especially thyroid disease, are very common in MG (Gilhus et al., 2015; Gilhus, 2016). Cardiovascular comorbidities, including blood hypertension, and lipid disorders were as common as in the adult general population (Couch et al., 2025).

Even among respiratory comorbidities, our cohort showed a high prevalence of COPD, asthma, and OSA, consistent with previous reports (Di Stefano et al., 2024). The prevalence of COPD and OSA was slightly lower than reported by Harris et al. (2022) and Nicolle et al. (2006) respectively. In our cohort of patients with gMG, asthma was present in 10.5% of cases, slightly higher than in most previously described MG cohorts (~5%), but within the range observed in the general adult population (5–10%) (To et al., 2012; Di Stefano et al., 2024). Notably, asthma has been associated with an increased risk of developing MG (Yingchoncharoen et al., 2021).

Furthermore, the variety of therapeutic regimens, including steroids, conventional immunosuppressants, and innovative agents, highlights the individualized approach to management in this population. Notably, a substantial proportion of patients were receiving innovative therapies, reflecting the evolving treatment landscape in MG and raising the possibility that different immunomodulatory strategies could impact clinical outcomes and infection risk.

The median QMG score of 12 (IQR 8–17) and the distribution of MG-ADL scores indicate that most patients had mild to moderate functional impairment, consistent with previous reports in similar real world outpatient cohorts (Howard et al., 2024; Zhu et al., 2025). Similarly, the median MG-QoL15 score of 11 (IQR 5–17) suggests a moderate impact of the disease on health-related quality of life.

In this study, we propose, for the first time, the use of respiratory symptom questionnaires in gMG to bridge patients' subjective experience with functional impairment and pulmonary physiology. We employed three instruments assessing respiratory dysfunction, physical symptoms, and affective components of dyspnea. The Dyspnea-12 and MDP have been previously validated in ALS and other neuromuscular conditions, showing good performance in dyspnea assessment (Young et al., 2024; Dangers et al., 2017; Morélot-Panzini et al., 2018). The Dyspnea-12 is a multidimensional measure of breathlessness, whereas the MDP offers a more granular evaluation of its sensory and affective components (Banzett et al., 2015; Williams et al., 2022; Yorke et al., 2010). The Nijmegen Questionnaire, originally developed for dysfunctional breathing, has not previously

been applied in neuromuscular diseases (Van Doorn et al., 1983; van Dixhoorn et al., 2015). It may help identify abnormal breathing patterns potentially contributing to symptom burden in gMG. Dysfunctional breathing is characterized by dyspnea disproportionate to organic disease and may present with nonspecific symptoms such as air hunger or sighing (Boulding et al., 2016). In MG, such patterns may coexist with neuromuscular impairment and could be relevant for targeted respiratory rehabilitation strategies (Farrugia et al., 2020; Freitag et al., 2018). Overall, patient-reported outcomes showed mild-to-moderate respiratory symptom burden, with median MDP-SQ and MDP-ER scores of 6 and 11, respectively, and variable Dyspnea-12 scores across patients. Dysfunctional breathing, as assessed by an NQ score ≥ 24 (cut-off defined in non-neuromuscular populations), was present in approximately one-third of patients, suggesting that altered respiratory patterns may be a relevant contributor to symptom perception in gMG.

The prevalence of impaired sleep quality (65% with median PSQI of 8) and the presence of mild anxiety and depression (median HADS-A 5, HADS-D 6) is similar to that observed by other groups (Dede et al., 2025; Yan et al., 2026; Lehnerer et al., 2022; Li et al., 2025), further underscoring the multidimensional impact of the disease beyond pure neuromuscular weakness.

Functional exercise capacity, measured by the 6MWD, was reduced compared to population norms, averaging 380 ± 151 m (Calik-Kutukcu et al., 2019; Salci et al., 2019), and the SBCT (median 31.7, IQR 20 – 43) demonstrated a wide variability, reflecting heterogeneity in respiratory muscle performance (Dishnica et al., 2023).

Pulmonary function tests revealed a mean FVC% of 79.6 ± 29.2%, with a restrictive pattern present in 39% of patients, mostly mild to moderate in severity, and an obstructive pattern in 10.5%, of whom only a minority had a prior diagnosis of asthma or COPD. These findings confirm that respiratory involvement in gMG is predominantly characterized by reduced lung volumes consistent with neuromuscular weakness rather than intrinsic pulmonary disease and highlight the importance of systematic respiratory assessment even in patients without known pulmonary comorbidities. Notably, even in our sample, an obstructive pattern was quite prevalent thus suggesting the presence of a pseudo-obstructive pattern, previously observed in MG patients with mild disease and partly associated with pyridostigmine administration (Elsais et al., 2010); however, it may also simply reflect the combined effects of intrinsic respiratory muscle weakness, variability in performing forced respiratory maneuvers, and comorbidities.

In our cohort, a comprehensive assessment of respiratory muscle strength using MIP, MEP, SNIP, and PCF proved useful for identifying subclinical respiratory impairment. While some patients showed reduced respiratory muscle strength, most maintained moderate function, consistent with the mild-to-moderate respiratory involvement commonly reported in gMG. Inspiratory muscle strength, as assessed by MIP and SNIP, was slightly more impaired than MEP, suggesting that mild inspiratory weakness was accompanied by relatively preserved expiratory strength in our outpatient cohort.

Notably, MIP and SNIP values below ~60 cmH_2O are generally associated with an increased risk of respiratory insufficiency, particularly in the presence of bulbar weakness or myasthenic crisis; in our outpatient cohort,

most patients remained above this threshold. The observed MVV of 78.4 ± 29.3 L/min suggests preserved global ventilatory capacity, in line with the relatively stable clinical status of the participants.

As regards oscillometry, its advantage lies in being noninvasive and effort-independent as it is performed in tidal breathing conditions; to date only one previous study has shown subtle changes in 4 MG patients, with good reproducibility and correlations with other respiratory measures (Iliaz et al., 2022). Oscillometric parameters—including a median resistance of 3.0 kPa·s/L, reactance of −1.1 kPa·s/L, FRES 12.3, AX 3.4, and R5–19 0.2—revealed mild variations in airway impedance, without evidence of significant airway obstruction. These findings indicate that, in this population, respiratory limitations are primarily driven by muscle weakness rather than structural or obstructive airway disease, highlighting the importance of detailed functional assessment even in clinically stable outpatients.

A conceptual comparison of the respiratory tests used in our study highlights their complementary roles in the assessment of respiratory function in MG, with spirometry primarily reflecting airflow limitation, MIP and MEP capturing inspiratory and expiratory muscle strength, PCF providing an index of cough effectiveness, and oscillometry offering an effort-independent evaluation of airway mechanics but with limited diagnostic performance in MG (Fig. 12).

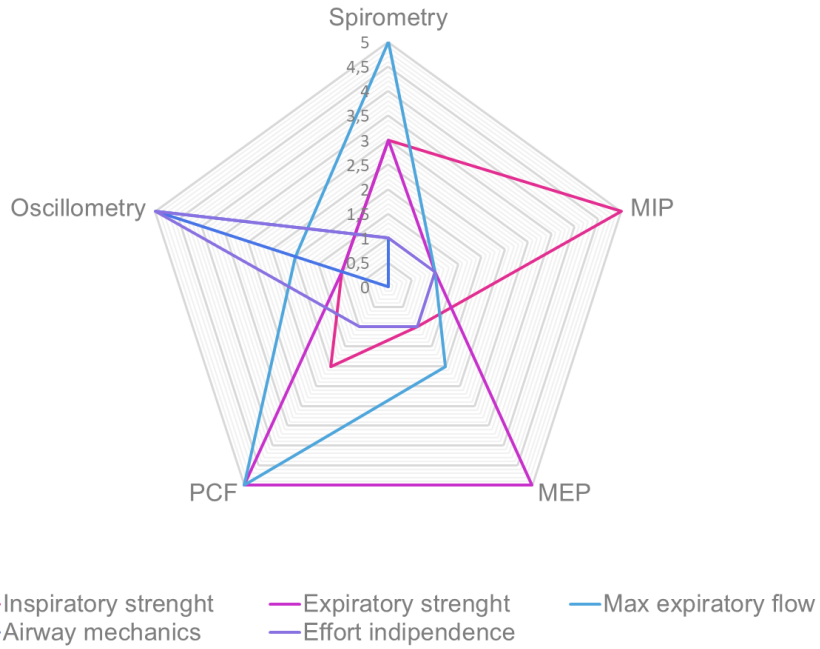


Figure 12 Conceptual comparison of respiratory tests used in the study. Radar chart illustrating the main functional domains assessed by each respiratory test. Spirometry primarily reflects expiratory flow and ventilatory function; MIP and MEP measure inspiratory and expiratory muscle strength; PCF reflects cough effectiveness and expiratory flow generation; and oscillometry provides an effort-independent assessment of airway mechanics. Abbreviations: MEP, maximal expiratory pressure; MIP, maximal inspiratory pressure; PCF, peak cough flow.

The univariate analysis provided additional insight into clinical and respiratory heterogeneity within the cohort. Differences according to serological status showed that MuSK-positive patients exhibited a more pronounced impairment in respiratory function compared with AChR-positive patients. Expiratory and inspiratory muscle strength, peak cough performance, and expiratory airflow were significantly lower in the former group. These findings are consistent with previous observations indicating that this serological subtype is often associated

with more prominent bulbar and respiratory involvement (Deymeer et al., 2007). Interestingly, despite this greater functional impairment, AChR-positive patients had received a higher cumulative dose of symptomatic therapy, possibly reflecting low response/no response or adverse reactions to acetylcholinesterase inhibitors (Rodolico et al., 2020).

Disease severity also emerged as a major determinant of functional outcomes. Increasing MGFA class was associated with worse motor scores, greater disability in daily activities, and poorer perceived quality of life. In parallel, respiratory involvement progressively increased with disease severity, as reflected by reduced inspiratory and expiratory muscle strength, lower cough effectiveness, and decreased ventilatory capacity. Exercise performance also declined across severity classes. Together, these findings reinforce the concept that respiratory muscle involvement parallels overall disease burden and becomes more evident in patients with more advanced clinical presentations.

As regards sex-related differences, our study confirmed that female patients had an earlier disease onset, longer disease duration, and greater clinical severity (Suh et al., 2013; Beeching et al., 2025; Alonso-Jiménez et al., 2026). Moreover, women reported a worse quality of life as assessed by patient-reported outcomes (Dong et al., 2020; Thomsen et al., 2021), although no differences were observed in anxiety or depressive symptoms. Interestingly, several respiratory parameters—including questionnaire-based assessments, SBCT, and more objective measures—were more impaired in female than in male patients, reflecting a higher degree of respiratory involvement in women that was not explained by sex differences in psychological status and replicating what was observed by Alcantara et al. (2024). The female predominance observed in MG, particularly in early-onset disease, may be related to several biological mechanisms, including sex hormone differences and skewed X-chromosome inactivation (Nicoli et al., 2022).

Age at disease onset also appeared to influence several functional parameters. Patients with later onset tended to have higher body mass index and increased airway resistance, whereas those with very late onset showed poorer walking performance. Although these differences may partly reflect physiological changes associated with aging, they also suggest that the clinical phenotype and respiratory profile of the disease may vary across onset groups.

Overall, the univariate analysis highlights the multifactorial determinants of respiratory involvement in gMG. Serological subtype, disease severity, sex, and age at onset all contribute to shaping the respiratory phenotype, underscoring the need for a comprehensive and multidimensional assessment of respiratory function in this population.

The correlation analysis highlighted a coherent relationship between clinical severity, patient-reported outcomes, functional capacity, and respiratory function. Greater disease severity was consistently associated with worse quality of life, higher dyspnea perception, and greater psychological burden, supporting the multidimensional impact of the disease on patients' daily functioning (Dewilde et al., 2023; Dewilde et al., 2024; Pesa et al., 2024).

Dyspnea-related questionnaires showed strong interrelationships in our cohort and were closely associated with dysfunctional breathing scores. This finding suggests that altered breathing perception and abnormalities

in breathing patterns may represent an important component of respiratory symptomatology in this population. Dysfunctional breathing and hyperventilation symptoms, only sparsely described in patients with moderate MG (García-Río et al., 1994; Farrugia et al., 2020), can have a substantial impact on daily functioning. In our cohort, respiratory alkalosis was observed in 11 patients, while approximately one-third of patients showed a NQ score >24. These findings highlight the importance of early detection of this breathing pattern, which may benefit from targeted respiratory training programs (Farrugia et al., 2020). Further research is needed to better define the role of dysfunctional breathing in MG and neuromuscular disorders, and a dedicated validation study is currently underway at our center.

Functional performance measures also reflected overall disease burden. Both walking capacity and breath-counting performance were associated with clinical severity and disability, supporting their value as simple functional indicators of respiratory involvement and global disease impact (Aguirre et al., 2020; Bandhari et al., 2024; Dishnica et al., 2023; Elsheikh et al., 2016; Gilhus et al., 2023; Kukulka et al., 2020; Octaviana et al., 2023; Calik-Kutukcu et al., 2019; Salci et al., 2019).

In our cohort mainly constituted by MGFA class II or III patients, SBCT range was very wide and resembled what already observed by other authors (Elsheikh et al., 2016). However, the modest correlations observed with more objective respiratory assessments (R 0.32 for MVV–0.37 for MEP) suggest that it only partially reflects respiratory function, likely due to its dependence on phonatory control, speech rate, and articulatory efficiency, which may be impaired even with subtle bulbar involvement. Compared with prior reports showing moderate correlations with FVC and MIP (Elsheikh et al., 2016), our findings support a multifactorial interpretation. A language-related bias may also contribute. SBCT was developed in English, whereas Italian number words are longer and more phonologically demanding, potentially reducing counts within a single breath. This, combined with bulbar fatigability, may lead to lower scores despite preserved respiratory function. Thus, SBCT likely reflects a composite of respiratory and speech performance and should be interpreted cautiously in Italian-speaking MG patients. Further studies are needed to address language effects and define standardized, language-specific references (Bhandari et al., 2024).

Respiratory muscle strength and ventilatory capacity were moderately associated with clinical severity and symptom burden, reinforcing the role of neuromuscular respiratory weakness as a key determinant of functional impairment. Specifically, reduced FVC, MIP, SNIP, MEP, MVV and PCF correlated with higher disease severity scores and worse patient-reported outcomes, consistent with previous studies highlighting the impact of impaired ventilatory and cough function on activities of daily living and risk of respiratory complications (Aguirre et al., 2020; Alcantara et al., 2024; Fernandes Oliveira et al., 2017; Heliopoulos et al., 2003; Keenan et al., 1995; Kuroiwa et al., 2024; Recasens et al., 2024). In line with previous results, MIP and MVV showed better correlation with disease severity, disability and quality of life, followed by FVC, MEP and PCF (Alcantara et al., 2024). In contrast, oscillometry-derived parameters did not show meaningful associations with clinical severity or symptom burden, suggesting that airway mechanical alterations play a limited role in the respiratory phenotype. This distinction emphasizes that respiratory compromise in MG primarily reflects neuromuscular weakness rather than intrinsic airway obstruction, and that objective

measures of respiratory muscle strength (MIP, MEP, SNIP), ventilatory capacity (FVC), and cough efficacy (PCF) remain crucial tools for clinical assessment, early detection of respiratory involvement, and monitoring response to interventions. A robust body of evidence demonstrates that reductions in MIP and MEP are predictive of an increased risk of acute respiratory failure. Similarly, decreased PCF is associated with impaired airway clearance and a heightened susceptibility to respiratory infections, highlighting the clinical relevance of these measures beyond traditional spirometric parameters (Claytor et al., 2023). Incorporating these assessments into routine MG management also in mild-moderately affected patients may facilitate timely respiratory interventions, including inspiratory muscle training, noninvasive ventilation, and cough augmentation strategies, potentially mitigating the multidimensional impact of respiratory muscle weakness on patients' daily functioning.

This study includes the largest cohort of patients with MG evaluated using MVV. MVV represents the pulmonary function counterpart of repetitive nerve stimulation, demonstrating a progressive decline in inspiratory and expiratory volumes during exercise and thereby capturing respiratory fatigability more effectively than FVC (Heliopoulos et al., 2003; Santy et al., 2022). Historically, MVV has been considered contraindicated in MG due to concerns about triggering myasthenic crisis (American Thoracic Society, 1991); however, no adverse effects were observed in our cohort. These findings suggest that patients with mild to moderate MG can safely undergo MVV under controlled conditions, enabling a more comprehensive assessment of respiratory dysfunction in this population.

The regression models provided further insight into these relationships. Measures of respiratory performance, particularly SBCT, MVV, and MIP, independently predicted clinical severity as assessed by the QMG score, with SBCT surprisingly demonstrating the strongest association. MVV and MIP, which reflect distinct aspects of respiratory muscle function, were analyzed separately to avoid collinearity and both emerged as significant predictors; however, they differed in effect size. Notably, MIP showed a stronger association ($\beta = -0.269$) compared to MVV ($\beta = -0.070$), suggesting that inspiratory muscle strength may represent a more sensitive indicator of disease severity in this cohort. In contrast, sex did not contribute to the variance in QMG, although it was a significant predictor of functional disability as assessed by the MG-ADL, consistent with previous reports showing that clinical scales tend to be worse in women than in men (Thomsen et al., 2021).

Dyspnea perception emerged as a major determinant of disability in daily activities and dysfunctional breathing as assessed by NQ appeared to be the strongest contributor to dyspnea perception. Age and serological status did not significantly influence clinical severity, disability, or dyspnea perception. Collectively, these findings support a multidimensional model in which respiratory symptoms arise from the interplay between neuromuscular weakness, abnormal breathing patterns, and subjective symptom perception.

The 12-month follow-up was completed by the majority of the cohort, with an overall response rate of 82.5% (47/57), suggesting that telephone-based follow-up is a feasible approach for longitudinal monitoring of patients in real-world settings. Nonetheless, the small number of non-responders and refusals points to the need for strategies to minimize attrition, particularly when studying infrequent but clinically significant events such as hospitalization or severe infections.

In our small prospective cohort, no deaths were observed, but a substantial proportion of patients (12/47) required hospitalization. In comparison, previous studies report hospitalization rates (length of stay ≥ 3 days) of around 10% in MG patients (Wartmann et al., 2023) although a recently published work reported a hospitalization rate up to 42% (Sobieszczuk et al., 2022). Hospitalization rate in our cohort may be explained by several factors. First, our patients were recruited from a tertiary neuromuscular center and, aside from four individuals not receiving steroids or other immunosuppressive therapies, the cohort largely included patients at higher risk of complications. Second, only patients with generalized symptoms were included, further enriching the cohort for individuals more likely to experience severe clinical events. It should also be noted that hospitalizations were ascertained through telephone interviews, and we did not collect information on whether the length of stay was three days or longer. Finally, the follow-up period was limited to one year, which may have influenced comparisons with studies reporting longer-term outcomes. MG-related hospitalizations (8.5%) were slightly lower than those reported in other cohorts stratified by MGFA classification (Pesa et al., 2024), possibly reflecting the evolving landscape of immunological therapies in MG. The prospective but still incomplete part of this study provides preliminary insights into the clinical and functional predictors of hospitalization and pneumonia in gMG patients over a 12-month follow-up period. The findings highlight that measures of disease severity and functional impairment, such as MG-ADL and QMG scores, as well as simple bedside tests like SBCT and respiratory assessments such as MVV, can provide meaningful information to identify patients at higher risk of pneumonia. Importantly, these measures appear more informative than basic demographic variables or isolated respiratory parameters, underscoring the value of comprehensive functional assessment in routine clinical practice.

The observed associations between higher MG-ADL scores and hospitalization, and between lower SBCT or MVV scores and pneumonia, underscore the multifactorial nature of clinical vulnerability in MG. MG-ADL reflects the overall disease burden by capturing the impact of muscle weakness on daily activities, explaining why patients with higher scores are more likely to be hospitalized when facing acute stressors such as infections or exacerbations. Despite its weak association with pulmonary function testing and pressures in our MG cohort, SBCT emerged as a strong predictor of pneumonia, able to capture clinically meaningful vulnerability rather than pure respiratory function, which may explain its predictive value for adverse respiratory outcomes. MVV, although reflecting ventilatory reserve, requires patient cooperation and upright posture, making it less feasible in frailer individuals. Its predictive value suggests that, when measurable, reduced ventilatory capacity may further identify patients at risk, but practical limitations mean that SBCT might be a more universally applicable tool in clinical practice. These findings emphasize the need to integrate both disease-specific functional assessments and feasible respiratory measures to stratify risk, allowing clinicians to identify patients who might benefit from closer monitoring or early interventions to prevent hospitalization and infectious complications.

Binomial logistic regression reached statistical significance for MG-ADL, reinforcing the notion that disease severity contributes meaningfully to outcome risk. Unlike isolated clinical or respiratory measurements, MG-ADL integrates multiple aspects of muscle weakness and functional limitation, providing a comprehensive

snapshot of a patient's baseline vulnerability. Although male sex was significantly associated with hospitalization, the magnitude of the effect should be interpreted cautiously given the imprecision of the estimate (wide confidence interval). This finding contrasts with previous observations reporting higher hospitalization rates in female patients (Sobieszczuk et al., 2022) but aligns with other studies showing that from the sixth decade of life, males have a higher risk of hospitalization (Alshekhlee et al., 2009).

According to our preliminary prospective analysis, we suggest using SBCT, MVV, MG-ADL and QMG as practical tools for early identification of patients at higher risk for complications, supporting proactive monitoring and intervention in routine clinical practice.

Conclusions:

This monocentric, multidimensional study provides a comprehensive characterization of respiratory involvement in seropositive gMG, integrating patient-reported outcomes with objective neurological and pulmonary measures. Our findings show that even patients with mild-to-moderate disease exhibit measurable respiratory impairment, reflected by reduced inspiratory and expiratory muscle strength, decreased cough efficacy, and altered ventilatory capacity. Importantly, respiratory symptom perception, as assessed by Dyspnea-12, MDP, and NQ scores, was associated with functional impairment, highlighting the multidimensional nature of respiratory involvement in gMG.

Our results further suggest that neuromuscular weakness, abnormal breathing patterns, and perceived dyspnea interact to determine respiratory burden, with serological subtype, disease severity, sex, and age at onset contributing to phenotypic heterogeneity. Functional performance measures, including SBCT, 6MWD, and respiratory muscle testing, were closely related to disease severity and patient-reported disability, supporting their role as practical, office-based markers of respiratory involvement and potential indicators of adverse outcomes.

Despite spirometry being the most widely used test for respiratory assessment in MG and an integral component of the QMG scale, our findings suggest that more detailed measures, such as MIP and MVV, may provide a more sensitive evaluation of respiratory involvement in gMG and may also contribute to risk stratification, particularly MVV. Although SBCT showed weaker correlations with formal respiratory parameters, it demonstrated relevant prognostic value, particularly for pneumonia, suggesting that it may capture a broader construct of functional vulnerability encompassing respiratory, bulbar, and speech-related performance. This may explain its utility in risk stratification beyond conventional ventilatory measures. These observations also highlight the need for dedicated validation studies, including in Italian-speaking populations, to address potential language-related effects and establish robust, language-specific reference values.

Finally, our results highlight the potential value of early identification of dyspnea, enabling targeted interventions such as breathing retraining, inspiratory muscle training, or cough augmentation, which may help mitigate the impact of respiratory symptoms on daily functioning and quality of life. Overall, these findings support the integration of comprehensive, patient-centered respiratory assessment into routine clinical

management and provide a basis for future longitudinal studies aimed at validating predictive models of respiratory risk in gMG.

This study has several limitations. First, patients with purely ocular myasthenia were excluded, despite the possibility of subclinical respiratory involvement. This may have led to an underestimation of the overall prevalence and spectrum of respiratory impairment in the broader myasthenia population. Inclusion of these patients in future studies may help identify earlier or subtler patterns of respiratory dysfunction relevant for preventive strategies. Although NQ scores were elevated in our cohort, validation studies in MG are needed to define disease-specific cut-offs. Longitudinal follow-up is still ongoing, and 12-month outcomes have not yet been fully analyzed. Therefore, the predictive value of baseline respiratory assessments for clinical events remains preliminary, and caution is warranted in extrapolating these findings to long-term prognosis. Completion of follow-up and longer-term analyses will provide more robust insights into the temporal evolution of respiratory involvement and the prognostic utility of both patient-reported outcomes and simple office-based measures.

Funding:

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Part 2 The Hidden Player: Oropharyngeal Microbiome and Its Role in Myasthenia Gravis

Introduction:

1. Microbiota human sites:

The human body is colonized by a complex and dynamic community, including bacteria, viruses, fungi and protozoa, the so-called microbiota. Alteration of this microbial diversity or dysbiosis is presumed to be involved in the pathogenesis of several autoimmune diseases such as multiple sclerosis or rheumatoid arthritis, triggering the immunological response (Preiningerova et al., 2022; Bergot et al., 2019). The human microbiome is not a single entity but includes multiple microbial communities located in different body sites, each with specific composition and functions. The main types of microbiome include: (a) Gut microbiome which is the largest and most studied, mainly involved in metabolism, immune regulation, thus participating to the modulation of the gut–brain axis; (b) Oral microbiome, highly diverse, involved in oral and systemic health, and a potential contributor to systemic inflammation; (c) Skin microbiome which varies by body site and plays a role in barrier function and immune defense; (d) Respiratory microbiome including upper (nasal, pharyngeal) and lower airways, involved in modulation of immune response, in respiratory health and disease in the so-called gut-lung axis; (e) Urogenital microbiome which comprises vaginal and urinary microbiota, important for local immunity and infection prevention (Reynoso-García et al., 2022; Oh et al., 2023). Other less frequently studied sites include the placental, breast milk, and blood microbiome, although their existence and role are still under investigation (Reynoso-Garcìa et al., 2022).

Several factors can influence microbiota composition, including diet, aging, physical activity, and antibiotic use. The Mediterranean diet, for instance, promotes microbial diversity and increases beneficial SCFA-producing bacteria, whereas a Western diet is associated with reduced diversity and a pro-inflammatory microbial profile (Schirò et al., 2023). Excessive antibiotic use can also induce dysbiosis by reducing beneficial bacteria and impairing immune regulation.

2. Detection techniques of microbiome:

Several techniques are currently available to study the human microbiome, each providing different types of information on microbial composition and function.

The most widely used method for studying bacterial communities is 16S ribosomal RNA (rRNA) gene sequencing, which allows both identification and relative quantification of bacteria. The 16S rRNA is a component of the 30S small subunit of the prokaryotic ribosome and contains nine hypervariable regions (V1–V9), each approximately 30–100 base pairs long, which contribute to the secondary structure of the ribosome. Highly conserved regions flanking the hypervariable regions enable the design of universal primers for PCR amplification, while sequence variation within hypervariable regions allows discrimination across taxonomic ranks:

- Phylum and Family: conserved regions provide reliable classification;

- Genus and Species: hypervariable regions allow finer resolution, though species-level identification may have lower accuracy.

Sequence variation in the amplicon is widely used to characterize microbial diversity, and with high-throughput sequencing platforms such as Illumina, amplicon sequencing has become a key tool for analyzing microbiome composition and structure (Caporaso et al., 2011; Youssef et al., 2009; Hess et al., 2011). This approach supports the reconstruction of bacterial phylogenies and classifications from phylum to species. Microbial diversity can be assessed through two main approaches: alpha diversity, which measures the richness and distribution of microbial species within a single sample, and beta diversity, which evaluates differences in microbial composition between samples or groups. Together, these measures provide a comprehensive view of the microbiome, including the relative abundance of taxa at multiple levels, overall diversity, and potential ecological or functional shifts.

A more advanced technique is shotgun metagenomic sequencing, which analyzes all genetic material present in a sample. Unlike 16S rRNA sequencing, it provides higher taxonomic resolution (often at species or strain level) and allows the evaluation of microbial functional potential by identifying genes involved in metabolic pathways (Human Microbiome Project Consortium, 2012; Thomas et al., 2012; Quince et al., 2017).

Other "omics" approaches include Metatranscriptomics, which evaluates gene expression by analyzing microbial RNA, thus providing information on active metabolic processes (Zhang et al., 2021), Metaproteomics, which studies the proteins produced by the microbiota, reflecting functional activity (Lai et al., 2019) and Metabolomics, which analyzes microbial metabolites (e.g., short-chain fatty acids, SCFAs), offering insight into the biochemical interactions between microbiota and host (Bauermeister et al., 2022). In addition, culture-based methods are still used, although limited, as many microorganisms are not easily cultivable. Newer approaches such as culturomics aim to improve microbial isolation using multiple culture conditions (Lagier et al., 2018).

In summary, multiple complementary techniques are used to study the microbiome, ranging from taxonomic profiling to functional analysis, and different anatomical sites host distinct microbial communities with specific biological roles.

3. Evidence of altered microbiome in MG

Dysbiosis can contribute not only to disease onset but also to progression in MG (Schirò et al., 2023). Main studies have focused on gut microbiota and only one study evaluated the oral microbiome.

The gut microbiota is mainly composed of bacteria belonging to the Firmicutes, Bacteroidetes, and Actinobacteria phyla and plays a crucial role in metabolic and immunomodulatory processes. Kapoor et al. (2023) analyzed gut microbiota in MG patients and observed that altered microbial composition (increased abundance of Fusobacteria, Bacteroidetes and Proteobacteria and reduced composition of Actinobacteria and Firmicutes) can contribute to alter gut permeability and facilitate the immunological response. Certain bacterial groups such as Clostridia produce SCFAs, which are essential for immune regulation. SCFAs promote the differentiation of regulatory T cells (Tregs) and contribute to maintaining intestinal barrier integrity as well as

the blood–brain barrier (Kim, 2021; Hu et al., 2022). The interaction between microbiota, the immune system, and the central nervous system occurs through the gut–brain axis, involving neural, endocrine, and inflammatory pathways (Fock and Parnova, 2023). In patients with MG, a reduction in SCFA-producing bacteria (particularly Clostridia) and an increase in pro-inflammatory taxa such as Bacteroides and Streptococcus have been observed (Qiu et al., 2018). These changes are associated with decreased SCFA levels (Qiu et al., 2018) and an imbalance between Tregs and T helper 17 (Th17) cells, promoting a pro-inflammatory immune response and autoantibody production against neuromuscular junction components (Chen and Tang, 2021).

In addition to gut microbiota, oral microbiota may also play a role in MG, although evidence is still limited. Preliminary findings suggest an increased abundance of genera such as Streptococcus and Rothia in MG patients, which may contribute to systemic inflammation and immune activation (Huang et al., 2022). Moreover, the oral microbiota may influence gut microbiota composition, further contributing to dysbiosis.

From a clinical perspective, dysbiosis may not only be involved in MG pathogenesis but also in the development of comorbidities such as cognitive impairment, anxiety, and depression, possibly through alterations in the gut–brain axis (Schirò et al., 2023). Experimental studies have shown that transplantation of microbiota from MG patients into animal models can induce behavioral changes, supporting a direct role of microbiota in modulating brain function (Zhang et al., 2022).

To date, no studies have specifically evaluated the microbiome of the human upper respiratory tract in MG. However, clinical features commonly observed in MG patients, such as impaired swallowing and increased risk of aspiration, together with frequent use of antibiotics and immunosuppressive therapies, may promote dysbiosis in the oral cavity and upper airways, potentially contributing to disease heterogeneity and different clinical outcomes.

Materials and methods:

- **Study population:**

In this single center study, 25 patients with a diagnosis of seropositive gMG and 25 neurologically healthy controls (HC) were enrolled at the tertiary neuromuscular outpatient clinic of the University of Palermo between September 2024 and January 2026. The study was approved by the Local Ethical Committee (Comitato Etico Regione Sicilia 1), and written informed consent was obtained from all participants prior to inclusion.

Participants were excluded if they were using probiotics, had received antibiotic therapy within the previous three months, had recently undergone oral surgery, had active oral infections, or had used antibacterial mouthwash in the previous month and in case of pregnancy.

The diagnosis of MG was established based on neurological evaluation and serological testing for AChR or MuSK antibodies. All enrolled patients had previously participated in the first subproject of the study and were not treatment-naïve. For this sub-project, we only accounted for some of the variables, including QMG, MG-ADL, MG-QoL15r, MGFA classification, and FVC%.

In parallel, 25 neurologically healthy controls were recruited. Exclusion criteria for controls included pregnancy, a history of neuromuscular or autoimmune disorders, recent antibiotic use (<3 months), systemic or inhaled steroid therapy within the previous month, current immunosuppressant therapy, active or recent malignancy, chronic infectious diseases (including Human Immunodeficiency Virus [HIV], hepatitis B or C), oral infections. Healthy participants were matched based on sex, age, and smoking status.

- **Sample collection and DNA extraction:**

Oropharyngeal swabs were collected from the tonsils and posterior walls of pharynx during the visit, then immediately placed on ice and stored at -80°C to preserve DNA integrity until downstream processing. Genomic DNA was extracted using standardized protocols, quantified and quality-checked using Qubit and Bioanalyzer. RNA contamination was removed by RNase digestion (3 μL stock DNA + 6 μL ddH_2O + 1 μL RNase A [10 mg/mL], 37°C for 15 min), followed by electrophoretic verification.

- **PCR amplification and sequencing:**

Bacterial community composition was assessed by amplifying multiple regions of the 16S rRNA gene. Specifically, the V4, V3–V4, V4–V5, and V5–V7 regions were targeted using the following primer pairs: 16Sv4 (GTGCCAGCMGCCGCGGTAA/ GGACTACHVGGGTWTCTAAT), 16Sv34 (CCTAYGGGRBGCASCAG/ GGACTACNNGGGTATCTAAT), 16Sv45 (GTGCCAGCMGCCGCGGTAA/ CCGTCAATTCCTTTGAGTTT), and 16Sv57 (AACMGGATTAGATACCCKG / ACGTCATCCCCACCTTCC).

Polymerase chain reaction (PCR) amplification of targeted regions was performed by using specific primers connecting with barcodes. The PCR products of proper size were selected through 2% agarose gel electrophoresis. The same amount of PCR products from each sample was pooled, end-repaired, A-tailed, and further ligated with Illumina adapters. Libraries were sequenced on a paired-end Illumina platform (Novogene, Beijing, China), and raw data were processed for quality filtering, chimera removal, and downstream microbial community analysis.

Each step from DNA extraction to final sequencing data, including sample preparation, PCR amplification, purification, library construction, and sequencing, could affect data quality. Quality control (QC) at every step was essential to ensure accurate and reliable downstream analysis.

- **Bioinformatic analysis and statistical analysis:**

After sequencing, raw data contain some errors, so they were first filtered and cleaned to obtain reliable data. These clean data were then processed using tools such as Divisive Amplicon Denoising Algorithm 2 (DADA2) or Deblur, which reduce noise and identify Amplicon Sequence Variants (ASVs).

For sequence denoising, the DADA2 method (Callahan et al., 2016) performs dereplication instead of traditional clustering approaches based on similarity-based clustering. This method allows 100% sequence similarity grouping. Each unique sequence obtained after denoising is defined as an ASV, also referred to as a feature sequence (analogous to an OTU, Operational Taxonomic Unit, representative sequence). The abundance of these sequences across samples is summarized in a feature table. Compared to OTU-based methods, DADA2 provides higher sensitivity and specificity, allowing detection of true biological variation

while reducing spurious sequences (Callahan et al., 2019). Consequently, ASVs improve the accuracy, resolution, and reproducibility of marker gene analyses (Amir et al., 2017).

Taxonomic classification of ASVs was performed using the classify-sklearn algorithm implemented in QIIME2 (Quantitative Insights Into Microbial Ecology 2) (Bokulich et al., 2018; Bolyen et al., 2019), which applies a pre-trained Naive Bayes classifier. The reference database used for taxonomic assignment was SILVA version 138.2.

Based on ASV annotations and feature tables, taxonomic abundance tables were generated at multiple levels, including domain, phylum, class, order, family, genus, and species (Fig. 12). These annotated abundance tables represent the core output of amplicon sequencing analysis.

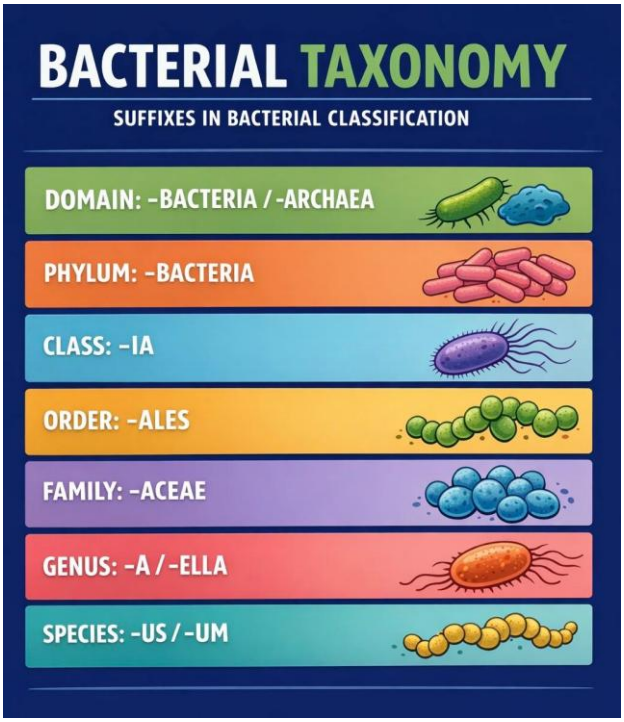

Figure 12 Bacterial taxonomy hierarchy with standard suffixes used in nomenclature. Typical suffixes include: Phylum (–bacteria), Class (–ia), Order (–ales), Family (–aceae), Genus (–a/–ella), Species (–us/–um). Note that genus and species names follow binomial nomenclature (Gen. sp.), with species written in lowercase and both italicized.

Depending on the experimental objectives, key taxa of interest, typically at the phylum and genus levels, could be selected for further investigation. These analyses included species composition profiling, differential abundance analysis between sample groups, and cluster analysis.

The ASVs were analyzed in two main ways: the composition analysis and the evolutionary/structural analysis. Alpha diversity indices (observed features, Chao1, Pielou, Simpson and Shannon) were calculated to assess microbial richness and diversity within samples. Differences between groups were evaluated using the non-parametric Wilcoxon rank-sum test, considering p-values < 0.05 as statistically significant.

Beta diversity analyses were conducted using phylogenetic distance metrics, including weighted and unweighted UniFrac distances, to evaluate differences in microbial community composition among samples. Visualization techniques included heatmaps, hierarchical clustering (UPGMA), Principal Coordinates Analysis

(PCoA), and Non-metric Multidimensional Scaling (NMDS). Group differences in community structure were statistically tested using Analysis of Similarities (ANOSIM), Multi-response Permutation Procedure (MRPP), and Permutational Multivariate Analysis of Variance (PERMANOVA, Adonis), with significance set at $p < 0.05$. Similarity Percentage (SIMPER) analysis was applied to decompose overall community dissimilarity into contributions by individual bacterial taxa, allowing identification of taxa driving differences between groups. All statistical analyses were performed using R software (version 4.0.3) with relevant packages for ecological and microbiome data analysis.

Results:

Characteristics of study participants:

A total of 50 participants were included in the study, comprising 25 gMG patients and 25 HC. The two groups were well matched for sex, age, and smoking status, with no statistically significant differences observed (Table 6). In both groups, females accounted for 52% of participants, and the mean age was comparable (MG: 63.0 ± 13.4 years; HC: 62.7 ± 13.7 years). Smoking habits were similarly distributed, with most subjects being nonsmokers (MG: 60%; HC: 68%), followed by former smokers (MG: 32%; HC: 24%) and current smokers (8% in both groups).

Among MG patients, AChR antibodies were detected in 84% of cases (n = 21), while 16% were MuSK-positive (n = 4). Thymic pathology varied within the MG group, including normal thymus (4%), thymic hyperplasia (8%), and thymoma (20%). Regarding current treatment, 8% of patients were not receiving disease-modifying therapy, 40% were on steroids, 28% were treated with steroids and/or other immunosuppressants, and 20% were receiving innovative therapies.

Disease severity was heterogeneous, with patients distributed across MGFA classes II–III, and a predominance of more advanced classes (IIIB: 36%). Consistently, clinical scores indicated a moderate disease burden, as reflected by mean QMG (15.8 ± 4.23), MG-ADL (8.1 ± 3.9), and MG-QoL15 (14.2 ± 8.9) values. Respiratory and functional measures (SBCT and FVC%) further supported the presence of clinically relevant impairment. Overall, MG patients and HC were comparable in baseline demographic and lifestyle characteristics, while disease-specific variables highlighted a cohort with predominantly moderate and clinically established myasthenia gravis. Differences in categorical data (e.g. sex and smoke habit) were calculated by χ^2 while differences in continuous variables such as age were obtained by 2-tailed Student's t-test.

Characteristics	**MG**	**HC**	**p-value**
Sample size	25	25	-
Female, n (%)	13 (52%)	13 (52%)	1.000
Age, mean ± SD	63.0 ± 13.4	62.7 ± 13.7	0.950
Smoke habit: - Nonsmokers, n (%) - Current smokers, n (%)	 15 (60%) 2 (8%)	 17 (68%) 2 (8%)	0.814

- Former smokers, n (%)	8 (32%)	6 (24%)	
Autoantibodies: - Anti-AChR, n (%) - Anti-MuSK, n (%)	 21 (84%) 4 (16%)	-	-
Thymic pathology - Normal, n (%) - Thymic hyperplasia, n (%) - Thymoma, n (%)	 1 (4%) 2 (8%) 5 (20%)	-	-
Current therapy: - No therapy, n (%) - Steroids, n (%) - Steroids and/or other immunosuppressants, n (%) - Innovative therapies, n (%)	 2 (8%) 10 (40%) 7 (28%) 5 (20%)	-	-
MGFA class: - IIA, n (%) - IIB, n (%) - IIIA, n (%) - IIIB, n (%)	 4 (16%) 5 (20%) 7 (28%) 9 (36%)	-	-
QMG, mean ± SD	15.8 ± 4.23	-	-
MG-ADL, mean ± SD	8.1 ± 3.9	-	-
MG-Qol15, mean ± SD	14.2 ± 8.9	-	-
FVC%, mean ± SD	70.9 ± 20.3	-	-

Table 6 Demographic and clinical characteristics of Myasthenia Gravis (MG) patients and Healthy controls (HC) undergoing oropharyngeal swabs. Continuous variables are expressed as mean ± standard deviation (SD), categorical variables are expressed as number (percentage). Differences between groups were obtained by 2-tailed Student's test and χ^2 test based on type of variable. Abbreviations: MG, myasthenia gravis; HC, healthy controls; Anti-AChR, anti–acetylcholine receptor antibodies; Anti-MuSK, anti–muscle-specific kinase antibodies; MGFA, Myasthenia Gravis Foundation of America classification; QMG, Quantitative Myasthenia Gravis score; MG-ADL, Myasthenia Gravis Activities of Daily Living; MG-QoL15, Myasthenia Gravis Quality of Life 15-item scale; SBCT, Single Breath Counting Test; FVC%, forced vital capacity (percentage of predicted).

Microbial diversity in the MG and HC Groups:

Bacterial richness, estimated as the number of observed ASVs, ranged from 99 to 402 in HC samples and from 20 to 279 in MG samples, indicating substantial variability in community complexity across samples.

This method identified significant differences between the two groups ($p = 0.014$) indicating a more elevated richness in healthy controls, then confirmed by the chao1 index ($p = 0.017$) (Fig. 13).

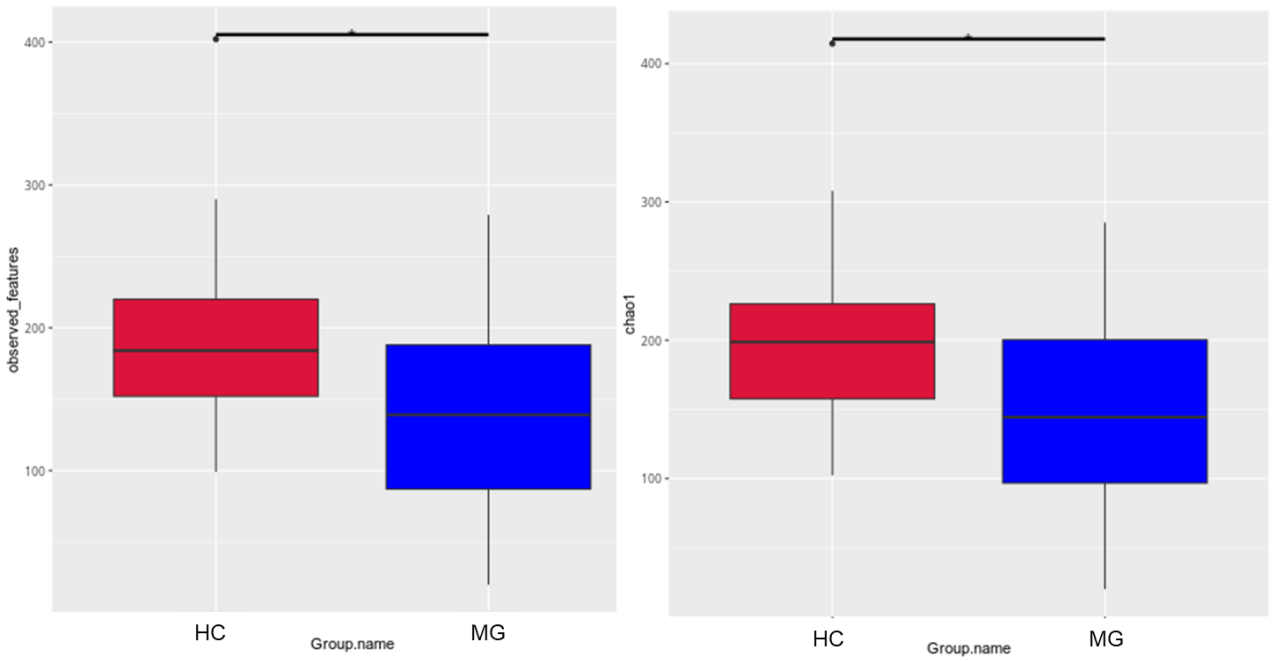

Figure 13 Box plots drawing alpha diversity measures of richness. Both observed features and chao1 index were significantly higher in the healthy control (HC, red) group than in the myasthenia gravis (MG, blue) group, indicating higher microbial variability in the HC group.

Evenness measures evaluate the uniformity of species abundances in the groups. Pielou's evenness was significantly higher in the HC group ($p = 0.013$) as well as Simpson's index ($p = 0.021$) (Fig. 14).

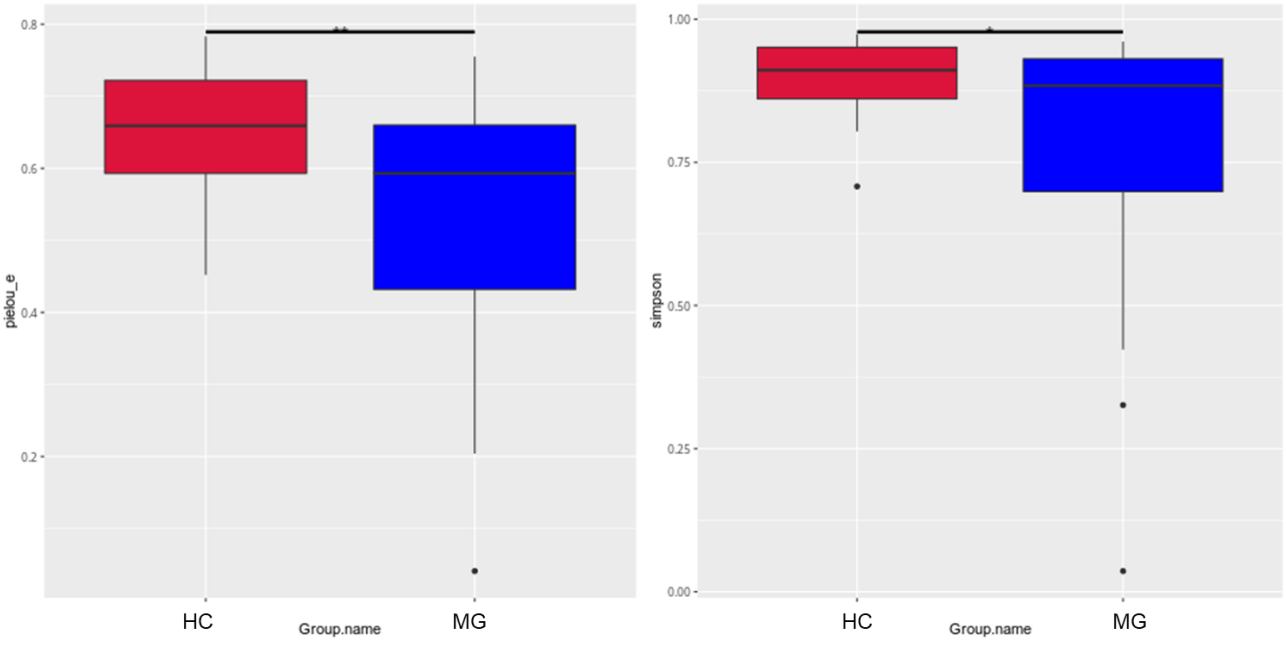

Figure 14 Box plots evaluating alpha diversity measures of evenness (uniformity). Both Pielou's and Simpson's indexes were significantly higher in the healthy control (HC, red) than in the myasthenia gravis (MG, blue group).

Shannon's index, which evaluates both richness and evenness within the microbial community, also showed significantly higher values in the HC group than in MG group ($p = 0.009$) (Fig. 15).

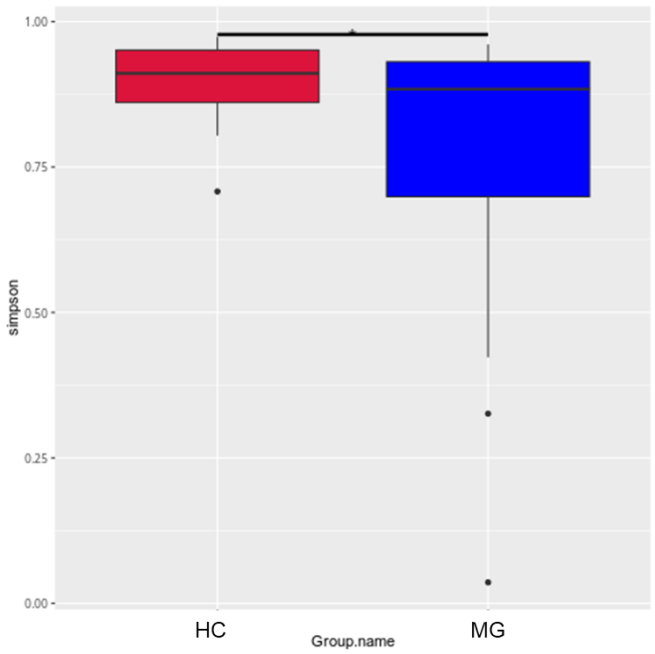

Figure 15 Shannon index differences in healthy controls (HC, red) and myasthenia gravis (MG) patients (blue).

Beta diversity analysis, based on phylogenetic distance metrics, revealed differences in microbial community composition between groups. Both weighted and unweighted UniFrac distances were used to assess inter-sample dissimilarity. Visualization through hierarchical clustering (UPGMA) (Fig. 16) showed a tendency toward partial segregation of samples according to group, although with some overlap.

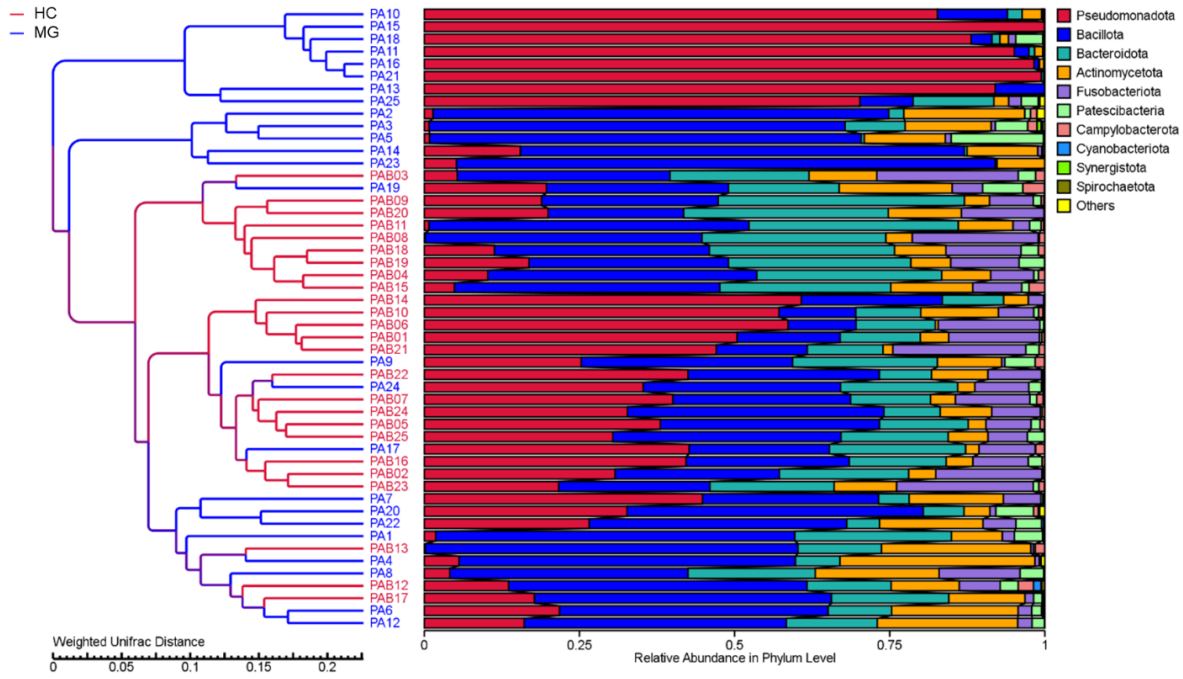

Figure 16 UPGMA clustering of bacterial communities based on weighted UniFrac distances. The hierarchical clustering was performed using the Unweighted Pair-group Method with Arithmetic Mean (UPGMA), which groups samples according to similarity in microbial community composition. The tree on the left shows the clustering relationships among samples, while the bar plot on the right represents the relative abundance of bacterial phyla in each sample. Myasthenia gravis (MG) patients are indicated in blue, and healthy controls (HC) are indicated in red.

Consistent patterns were observed with ordination methods, including PCoA (Fig. 17), with PCA and NMDS analyses (not shown) yielding similar results. Principal Coordinates Analysis (PCoA) based on UniFrac distances showed a tendency toward group-related clustering. In PCoA, samples from the two groups exhibited partial separation along the principal coordinates, with HC more tightly clustered and MG patients displaying greater dispersion. However, a degree of overlap between groups was observed, indicating that differences in microbial community structure, although present, were not absolute. Overall, these findings indicate group-related differences in microbial community structure.

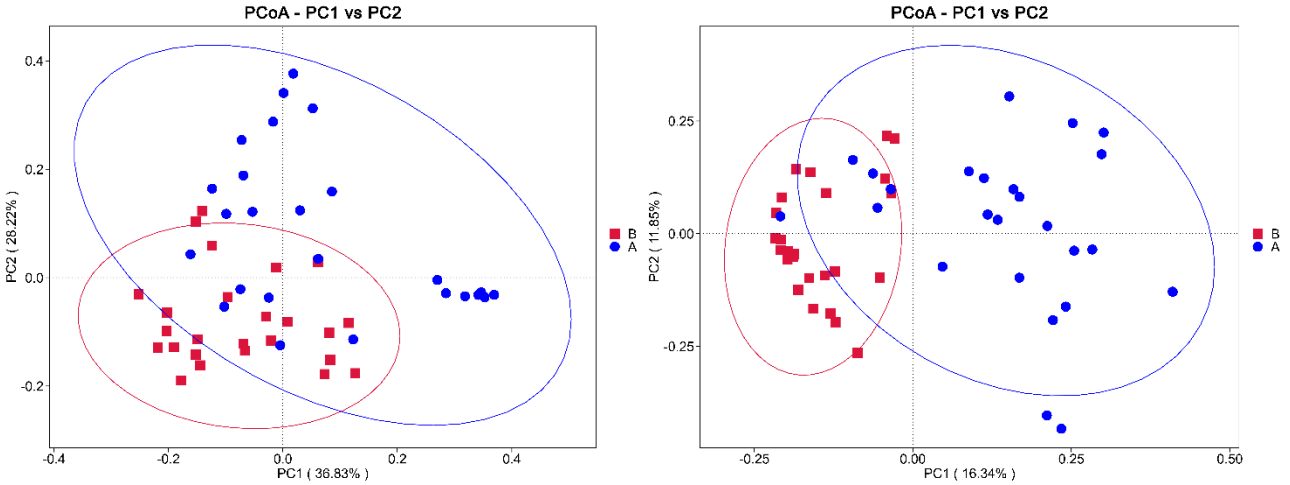

Figure 17 Principal Coordinates Analysis (PCoA) of bacterial communities based on UniFrac distances. PCoA was performed using weighted UniFrac (left) and unweighted UniFrac (right) distances to visualize differences in microbial community composition among samples. Each point represents a sample, plotted along the first and second principal coordinates, with the percentage on each axis indicating the contribution of that coordinate to overall variation. Myasthenia gravis patients (Group A) are shown in blue, and healthy controls (Group B) are shown in red.

Statistical testing confirmed these observations, with significant differences between groups detected by ANOSIM ($R = 0.232$, $p = 0.001$), MRPP ($A = 0.0339$, $p = 0.001$), and PERMANOVA ($R^2 = 0.0798$, $p = 0.001$), indicating modest but significant differences in microbial community structure.

Moreover, the relative contributions of bacterial orders to differences between MG patients and HC were assessed using the SIMPER (Similarity Percentage) analysis. SIMPER decomposes the overall dissimilarity between groups into contributions from each taxon, identifying those taxa most responsible for driving group differences. In our dataset, orders such as Pseudomonadales, Lactobacillales, Burkholderiales, and Enterobacterales were highlighted as key contributors to the microbial community variation (Fig. 18). Specifically, Pseudomonadales were more abundant in MG patients, while Lactobacillales were more prevalent in healthy controls.

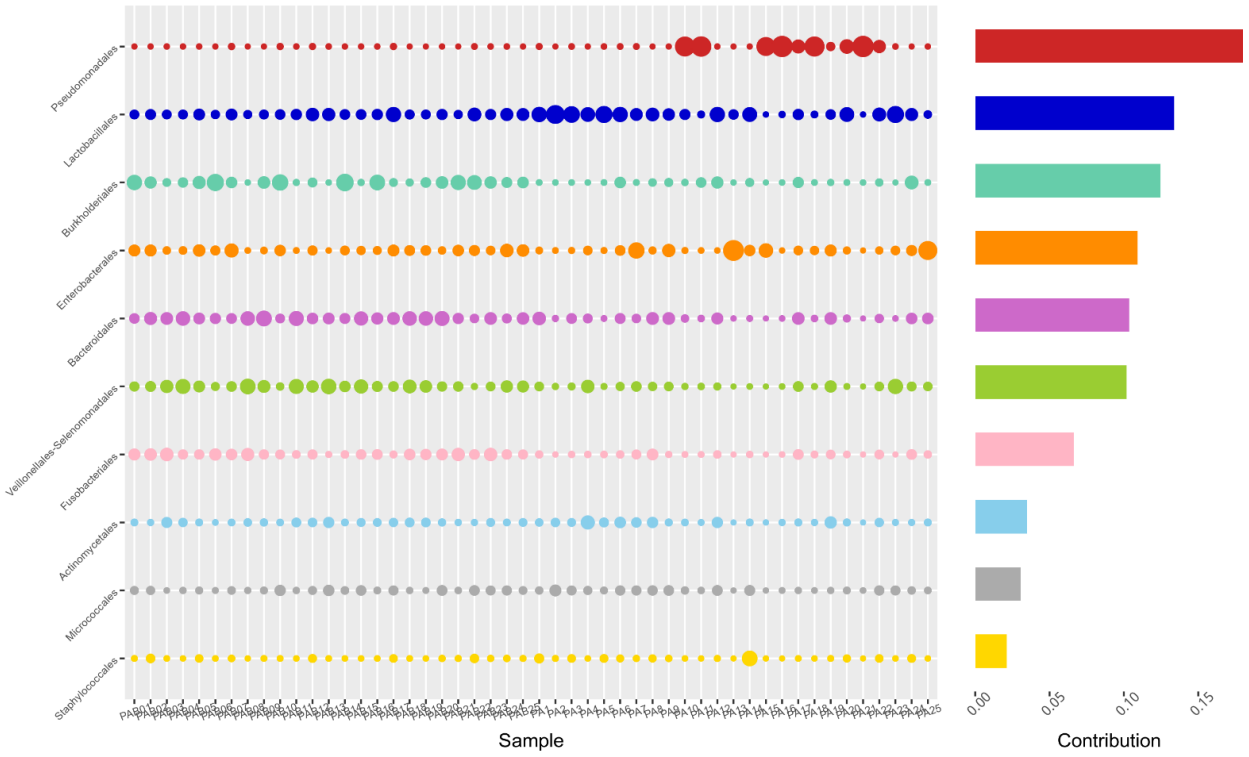

Figure 18 SIMPER (Similarity Percentage) analysis showing the relative contribution of bacterial orders to differences between samples (25 samples on the left: healthy controls; 25 samples on the right: myasthenia gravis patients). The bubble plot displays the relative abundance of dominant bacterial orders across individual samples with bubble size proportional to abundance. The bar chart on the right summarizes the overall contribution of each bacterial order to the dissimilarity between groups. Orders such as Pseudomonadales, Lactobacillales, Burkholderiales, and Enterobacterales contribute most to the observed microbial community differences. Samples are arranged along the x-axis and bacterial orders along the y-axis.

The genus-level contribution analysis of beta diversity (Fig. 19) revealed clear differences between healthy individuals and patients. *Pseudomonas* emerged as the main driver of between-group variability, showing low and relatively stable abundance in healthy samples, but marked increases in a subset of patient samples. This uneven distribution resulted in the highest contribution to overall compositional dissimilarity.

Core genera of the oral microbiome, including *Neisseria*, *Streptococcus*, *Veillonella*, and *Prevotella*, were consistently detected across both groups but exhibited notable shifts in relative abundance. These variations contributed substantially to beta diversity, indicating that differences between healthy and diseased states are largely driven by changes in the relative balance of common taxa rather than their presence or absence.

Genera such as *Haemophilus*, *Fusobacterium*, and *Rothia* displayed comparatively stable abundance patterns across all samples, resulting in a lower contribution to between-group differences.

In contrast, *Serratia* showed a sporadic distribution, with low abundance in most healthy samples and occasional enrichment in patients, further contributing to compositional heterogeneity. Overall, these results indicate that microbial differences between HC and MG are driven by both reduced diversity and compositional shifts involving core and opportunistic taxa.

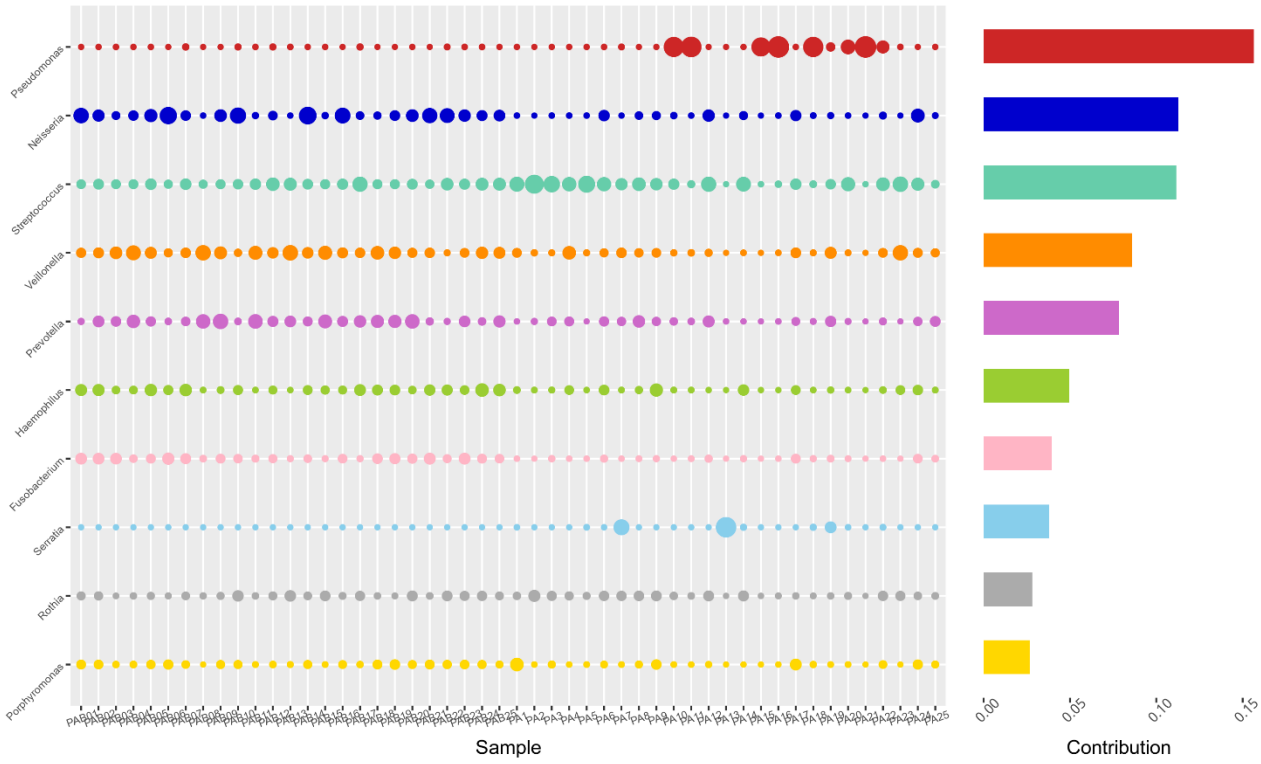

Figure 19 SIMPER (Similarity Percentage) analysis showing the relative contribution of bacterial genus to differences between samples (25 samples on the left: healthy controls; 25 samples on the right: myasthenia gravis patients). The bubble plot displays the relative abundance of dominant bacterial genus across individual samples, with bubble size proportional to abundance. The bar chart on the right summarizes the overall contribution of each bacterial order to the dissimilarity between groups. Genus such as Pseudomonas, Neisseria, Streptococcus, Vielonella and Prevotella contribute most to the observed microbial community differences. Samples are arranged along the x-axis and bacterial orders along the y-axis.

Differences across MG patients:

An exploratory analysis stratifying patients by MGFA classification (IIA vs IIB+III classes) and respiratory dysfunction (based on % predicted FVC classes on QMG) showed no significant differences within the MG group in either alpha or beta diversity, suggesting a limited contribution of disease severity and respiratory impairment to the observed oropharyngeal dysbiosis.

Given the small number of MuSK-positive patients (n = 4), a separate exploratory analysis by serological status was performed. No differences were observed in alpha diversity indices (all $p > 0.05$). Consistent with SIMPER results, comparisons of the top 10 taxa (by relative abundance) at the order and genus levels revealed no significant differences at the order level. At the genus level, *Streptococcus* showed a nominal difference between groups ($p = 0.019$), which did not remain significant after post-hoc analysis.

Discussion:

In this study, we investigated the composition of the oropharyngeal bacterial communities in MG patients compared to HC. This anatomical site offers several advantages over other microbiota niches, considering both the nature of the disease and methodological reproducibility.

First, sampling of the oropharyngeal microbiota can be easily performed by non-invasive and highly reproducible procedures, such as oropharyngeal swabs. This makes it particularly suitable for longitudinal

studies and for monitoring microbiome changes associated with immunosuppressive therapies or disease exacerbations.

Furthermore, the oropharyngeal mucosa represents a key component of the mucosa-associated lymphoid tissue (MALT), which plays a crucial role in shaping both local and systemic immune responses (Zhou et al., 2025). The MALT system is characterized by the activation of immune cells and the production of immunoglobulins, particularly IgA, thus contributing to immune homeostasis and host–microbiome interactions.

Despite its relevance, the interaction between the oropharyngeal microbiome and the immune system remains less extensively studied compared to the gut microbiota in MG. Another important rationale for focusing on the upper respiratory microbiome is that a substantial proportion of patients exhibit bulbar involvement, and respiratory complications represent a major cause of morbidity and mortality. The oropharyngeal microbiota may therefore better reflect clinically relevant features, including susceptibility to respiratory infections, alterations related to dysphagia, and disease-related changes in the upper airway microbial ecosystem. In addition, the oropharyngeal microbiota plays a critical role in respiratory health, acting as a gatekeeper against pathogen colonization and influencing infection risk. Alterations in this microbial community have been associated with increased susceptibility to respiratory infections and disease severity (Paulo et al., 2023). Moreover, oral and oropharyngeal microorganisms can directly influence the composition of the lower respiratory tract microbiota, further highlighting their relevance in respiratory diseases (Pulvirenti et al., 2024). Finally, it has been hypothesized that autoimmune processes may be initiated following antigen exposure at mucosal surfaces, including the oral and respiratory mucosa (Jiang et al., 2025). Therefore, the study of the oropharyngeal microbiota may help identify early triggers of autoimmunity or potential biomarkers of disease onset and progression.

In the context of MG, most of the available evidence on host–microbiome interactions come from studies investigating the gut microbiota. These studies have consistently demonstrated the presence of dysbiosis, typically characterized by reduced abundance of SCFA-producing bacteria, including members of the class Clostridia, and a relative enrichment of pro-inflammatory taxa such as Bacteroides and Proteobacteria (Qiu et al., 2018; Chen and Tang, 2021). Such alterations have been linked to impaired regulatory T cell (Treg) function, increased intestinal permeability, and enhanced systemic immune activation, ultimately contributing to the breakdown of immune tolerance.

Despite these findings, the current literature remains largely restricted to the gut ecosystem, with very limited exploration of other mucosal compartments. Only a small number of studies have investigated the oral microbiome in MG, and these are primarily exploratory, reporting shifts toward potentially pro-inflammatory genera such as Streptococcus and Rothia (Huang et al., 2022).

In this context, our study provides novel insights into the composition of the oropharyngeal microbiota in MG patients. Notably, the study population was characterized by a relatively high disease burden, with more than half of patients classified as moderate disease (MGFA class III), and a substantial proportion (14 out of 25, including IIB and IIIB patients) presenting with bulbar involvement. This is particularly relevant, as bulbar dysfunction may directly influence the oropharyngeal microenvironment through impaired swallowing, altered

salivary clearance, and increased risk of microaspiration, all of which can contribute to shaping microbial communities.

From a microbiological perspective, alpha diversity analyses revealed statistically significant differences between MG patients and healthy controls. Specifically, both richness and evenness were significantly reduced in the MG group, indicating a less diverse and more uneven microbial community. This finding suggests a shift toward a simplified and potentially dysbiotic ecosystem in MG patients, a pattern commonly observed in chronic inflammatory and autoimmune conditions (Wang et al., 2024).

In contrast, beta diversity analyses demonstrated differences in microbial community structure between groups, indicating the presence of a distinct oropharyngeal microbial signature associated with MG. Although the proportion of variance explained was modest, these differences were consistent across multiple analytical approaches (ANOSIM, MRPP, PERMANOVA), supporting the robustness of the observed shift while also suggesting that additional host- or environment-related factors contribute to overall microbiome variability.

Taxonomic analyses further highlighted specific bacterial orders contributing to this separation. In particular, the enrichment of Pseudomonadales and Enterobacterales in MG patients suggests a shift toward a more opportunistic and pro-inflammatory microbial profile. These taxa, largely belonging to the Proteobacteria phylum, are commonly associated with respiratory infections and have been linked to mucosal inflammation and impaired host defenses (Li et al., 2019; Qin et al., 2025).

Conversely, the relative enrichment of Lactobacillales in healthy controls supports a role for these taxa in maintaining mucosal homeostasis. Members of this order, particularly Lactobacillus spp., are known to exert anti-inflammatory effects, enhance epithelial barrier integrity, and modulate both innate and adaptive immune responses, including the promotion of regulatory T cell activity (Belkaid and Hand, 2014; Bron et al., 2017; O'Callaghan and van Sinderen, 2016). Their relative depletion in MG patients may therefore reflect a loss of protective microbial functions, potentially favoring a more permissive environment for inflammation and pathogen colonization.

The present analysis highlights distinct compositional differences between healthy individuals and patients, driven by both opportunistic taxa and fluctuations within the core microbiome. The prominent contribution of Pseudomonas suggests a potential association with the diseased state, as its increased abundance in patient samples may reflect dysbiosis, environmental exposure, or opportunistic colonization under altered ecological conditions.

In addition to this genus-specific effect, the substantial contribution of core genera such as Neisseria, Streptococcus, Veillonella, and Prevotella indicates that disease-associated changes are not limited to the appearance of new taxa but involve significant shifts in the relative proportions of established community members. This supports the concept that microbiome-associated diseases are often characterized by imbalances within a conserved microbial framework, rather than complete community restructuring.

Importantly, these findings suggest that the observed dysbiosis operates at two complementary levels. On one hand, a "core dysbiosis" is evident, driven by coordinated shifts in the relative abundance of dominant commensal genera. On the other hand, a subset of patients exhibits a marked expansion of opportunistic taxa,

particularly Pseudomonas and, to a lesser extent, Serratia, which are unevenly distributed across samples and disproportionately contribute to beta diversity. This pattern indicates that, beyond a shared baseline alteration of the microbiome, there is an additional layer of inter-individual variability characterized by opportunistic enrichment. Such a configuration may reflect differences in immune status, local microenvironmental conditions, or susceptibility to respiratory colonization, potentially identifying a subgroup of patients at higher risk of microbial imbalance and related complications.

The relatively minor contribution of genera such as Haemophilus and Rothia suggests that some members of the microbiome remain stable regardless of health status, potentially representing a resilient core component.

The sporadic enrichment of Serratia in patient samples further emphasizes the role of low-prevalence, high-variability taxa in shaping beta diversity, consistent with the long-tail distribution typical of microbial ecosystems.

The clinical characteristics of the cohort further reinforce the biological plausibility of these findings. The high prevalence of bulbar symptoms suggests that local functional impairment, including dysphagia and reduced airway protection, may contribute to microbial imbalance. In addition, exposure to immunosuppressive therapies, although unavoidable, may represent an additional factor influencing microbial composition.

Taken together, these findings indicate that MG is associated with a reorganization of the oropharyngeal microbiota, characterized by both reduced microbial diversity and consistent compositional shifts. Such alterations suggest a transition toward a less complex and more unbalanced microbial ecosystem, which may have both local and systemic implications, potentially contributing to increased susceptibility to respiratory complications as well as to immune dysregulation through mucosal pathways. While causality cannot be established, these results support the hypothesis that the oropharyngeal microbiome may represent both a marker and a modulator of disease processes.

Moreover, our findings suggest that neither disease severity nor respiratory impairment, as defined in our cohort, are major drivers of oropharyngeal microbiota composition in gMG, while differences according to serological status remain inconclusive due to the limited number of MuSK-positive patients.

Further longitudinal and functional studies will be essential to clarify these relationships and to explore the potential of microbiome-based approaches in risk stratification and therapeutic modulation.

Conclusions:

This study identifies a distinct oropharyngeal microbiome signature in MG, characterized by reduced diversity and compositional shifts involving both core and opportunistic taxa. A major strength is the focus on an underexplored but clinically relevant site, along with the inclusion of predominantly ambulatory patients, providing insight into microbiome alterations in real-world clinical settings.

Limitations include the small sample size, cross-sectional design, and potential confounding factors such as therapy and lifestyle variables.

Overall, these findings suggest that oropharyngeal dysbiosis is present even in clinically MG outpatients and may contribute to disease-related processes, warranting further longitudinal and functional investigation.

Clinical severity, serological status and respiratory dysfunction appear to have a limited impact on oropharyngeal microbiota composition in gMG, warranting further investigation in larger cohorts.

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References:

Aguirre F, Fernández RN, Arrejoría RM, Manin A, Cores VE, Sivori M, Villa AM. Peak expiratory flow and the single-breath counting test as markers of respiratory function in patients with myasthenia gravis. Neurologia (Engl Ed). 2020:S0213-4853(20)30432-1. English, Spanish. doi: 10.1016/j.nrl.2020.09.007.

Alcantara M, Barnett-Tapia C, Bril V, Mannan S, Shabanpour J, Riaz S, Ng E, Ryan C, Katzberg H. Office-based respiratory assessment in patients with generalized myasthenia gravis. Neuromuscul Disord. 2024;40:1-6. doi: 10.1016/j.nmd.2024.05.005.

Alonso-Jiménez A, De Ridder W, Van Schil P, Baets J, Mercelis R. Gender differences in Myasthenia Gravis in a neuromuscular reference center in Belgium. Acta Neurol Belg. 2026. doi: 10.1007/s13760-025-02977-8.

Alqahtani HM, Alanazy TM, Namshah AS, Alshuaibi FT, Albulaihe H, Alhammad RM, Alanazy MH. Frequency of upper respiratory tract infections in patients with myasthenia gravis compared to the general population. Clin Neurol Neurosurg. 2025;248:108649. doi: 10.1016/j.clineuro.2024.108649.

Alshekhlee A, Miles JD, Katirji B, Preston DC, Kaminski HJ. Incidence and mortality rates of myasthenia gravis and myasthenic crisis in US hospitals. Neurology. 2009;72(18):1548-54. doi: 10.1212/WNL.0b013e3181a41211.

American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. Am Rev Respir Dis. 1991;144(5):1202-18. doi: 10.1164/ajrccm/144.5.1202.

Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Zech Xu Z, Kightley EP, Thompson LR, Hyde ER, Gonzalez A, Knight R. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. mSystems. 2017;2(2):e00191-16. doi: 10.1128/mSystems.00191-16.

Andersen LK, Birnbaum S, Missel M, Petersen KG, Mohringer C, Deurell E, Witting N, Vissing J. Assessing respiratory status in myasthenia gravis: limited value of the MG-ADL as a standalone tool compared with spirometry in a Danish cohort. J Neurol. 2026;273(4):231. doi: 10.1007/s00415-026-13757-6.

Antozzi C, Vu T, Ramchandren S, Nowak RJ, Farmakidis C, Bril V, De Bleecker J, Yang H, Minks E, Park JS, Grudniak M, Smilowski M, Sevilla T, Hoffmann S, Sivakumar K, Suzuki Y, Youssef E, Sanga P, Karcher K, Zhu Y, Sheehan JJ, Sun H; Vivacity-MG3 Study Group. Safety and efficacy of nipocalimab in adults with generalised myasthenia gravis (Vivacity-MG3): a phase 3, randomised, double-blind, placebo-controlled study. Lancet Neurol. 2025;24(2):105-116. doi: 10.1016/S1474-4422(24)00498-8.

Araújo PRS, Fonseca JDMD, Marcelino AA, Moreno MA, Dornelas de Andrade AF, Yañez MO, Torres-Castro R, Resqueti VR, Fregonezi GAF. Reference values for respiratory muscle strength and maximal voluntary ventilation in the Brazilian adult population: A multicentric study. PLoS One. 2024;19(11):e0313209. doi: 10.1371/journal.pone.0313209.

ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166(1):111-7. doi: 10.1164/ajrccm.166.1.at1102. Erratum in: Am J Respir Crit Care Med. 2016;193(10):1185. doi: 10.1164/rccm.19310erratum.

Axelsen KH, Kjær Andersen R, Andersen LK, Vissing J, Witting N. Ocular versus generalized myasthenia gravis: a continuum associated with acetylcholine receptor antibody titers. Neuromuscul Disord. 2024;43:39-43. doi: 10.1016/j.nmd.2024.07.002.

Bach JR, Ishikawa Y, Kim H. Prevention of pulmonary morbidity for patients with Duchenne muscular dystrophy. Chest. 1997;112(4):1024-8. doi: 10.1378/chest.112.4.1024.

Bach JR. Amyotrophic lateral sclerosis: predictors for prolongation of life by noninvasive respiratory aids. Arch Phys Med Rehabil. 1995;76(9):828-32. doi: 10.1016/s0003-9993(95)80547-8.

Balasa B, Sarvetnick N. Is pathogenic humoral autoimmunity a Th1 response? Lessons from (for) myasthenia gravis. Immunol Today. 2000 Jan;21(1):19-23. doi: 10.1016/s0167-5699(99)01553-4.

Banzett RB, O'Donnell CR, Guilfoyle TE, Parshall MB, Schwartzstein RM, Meek PM, Gracely RH, Lansing RW. Multidimensional Dyspnea Profile: an instrument for clinical and laboratory research. Eur Respir J. 2015;45(6):1681-91. doi: 10.1183/09031936.00038914.

Barnett C, Katzberg H, Nabavi M, Bril V. The quantitative myasthenia gravis score: comparison with clinical, electrophysiological, and laboratory markers. J Clin Neuromuscul Dis. 2012;13(4):201-5. doi: 10.1097/CND.0b013e31824619d5.

Bartfield JM, Ushkow BS, Rosen JM, Dylong K. Single breath counting in the assessment of pulmonary function. Ann Emerg Med. 1994;24(2):256-9. doi: 10.1016/s0196-0644(94)70138-5.

Bauermeister A, Mannochio-Russo H, Costa-Lotufo LV, Jarmusch AK, Dorrestein PC. Mass spectrometry-based metabolomics in microbiome investigations. Nat Rev Microbiol. 2022;20(3):143-160. doi: 10.1038/s41579-021-00621-9.

Bedlack RS, Simel DL, Bosworth H, Samsa G, Tucker-Lipscomb B, Sanders DB. Quantitative myasthenia gravis score: assessment of responsiveness and longitudinal validity. Neurology. 2005;64(11):1968-70. doi: 10.1212/01.WNL.0000163988.28892.79.

Beghi E, Antozzi C, Batocchi AP, Cornelio F, Cosi V, Evoli A, Lombardi M, Mantegazza R, Monticelli ML, Piccolo G, et al. Prognosis of myasthenia gravis: a multicenter follow-up study of 844 patients. J Neurol Sci. 1991;106(2):213-20. doi: 10.1016/0022-510x(91)90260-e.

Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157(1):121-41. doi: 10.1016/j.cell.2014.03.011.

Bergot AS, Giri R, Thomas R. The microbiome and rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2019;33(6):101497. doi: 10.1016/j.berh.2020.101497.

Bhandari SK, Bist A, Ghimire A. Single breath count test and its applications in clinical practice: a systematic review. Ann Med Surg (Lond). 2024;86(4):2130-2136. doi: 10.1097/MS9.0000000000001853.

Black LF, Hyatt RE. Maximal static respiratory pressures in generalized neuromuscular disease. Am Rev Respir Dis. 1971;103(5):641-50. doi: 10.1164/arrd.1971.103.5.641.

Boentert M, Wenninger S, Sansone VA. Respiratory involvement in neuromuscular disorders. Curr Opin Neurol. 2017;30(5):529-537. doi: 10.1097/WCO.0000000000000470.

Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory Caporaso J. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. 2018;6(1):90. doi: 10.1186/s40168-018-0470-z.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld

JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852-857. doi: 10.1038/s41587-019-0209-9. Erratum in: Nat Biotechnol. 2019;37(9):1091. doi: 10.1038/s41587-019-0252-6.

Breiner A, Widdifield J, Katzberg HD, Barnett C, Bril V, Tu K. Epidemiology of myasthenia gravis in Ontario, Canada. Neuromuscul Disord 2016; 26: 41–46.

Bril V, Drużdż A, Grosskreutz J, Habib AA, Mantegazza R, Sacconi S, Utsugisawa K, Vissing J, Vu T, Boehnlein M, Bozorg A, Gayfieva M, Greve B, Woltering F, Kaminski HJ; MG0003 study team. Safety and efficacy of rozanolixizumab in patients with generalised myasthenia gravis (MycarinG): a randomised, double-blind, placebo-controlled, adaptive phase 3 study. Lancet Neurol. 2023;22(5):383-394. doi: 10.1016/S1474-4422(23)00077-7. Erratum in: Lancet Neurol. 2023;22(10):e11. doi: 10.1016/S1474-4422(23)00336-8.

Bron PA, Kleerebezem M, Brummer RJ, Cani PD, Mercenier A, MacDonald TT, Garcia-Ródenas CL, Wells JM. Can probiotics modulate human disease by impacting intestinal barrier function? Br J Nutr. 2017;117(1):93-107. doi: 10.1017/S0007114516004037.

Bubuioc AM, Kudebayeva A, Turuspekova S, Lisnic V, Leone MA. The epidemiology of myasthenia gravis. J Med Life. 2021;14(1):7-16. doi: 10.25122/jml-2020-0145.

Burns TM, Conaway M, Sanders DB; MG Composite and MG-QOL15 Study Group. The MG Composite: A valid and reliable outcome measure for myasthenia gravis. Neurology. 2010;74(18):1434-40. doi: 10.1212/WNL.0b013e3181dc1b1e.

Burns TM, Grouse CK, Wolfe GI, Conaway MR, Sanders DB; MG Composite and MG-OL15 Study Group. The MG-QOL15 for following the health-related quality of life of patients with myasthenia gravis. Muscle Nerve. 2011;43(1):14-8. doi: 10.1002/mus.21883.

Burns TM, Sadjadi R, Utsugisawa K, Gwathmey KG, Joshi A, Jones S, Bril V, Barnett C, Guptill JT, Sanders DB, Hobson-Webb L, Juel VC, Massey J, Gable KL, Silvestri NJ, Wolfe G, Cutter G, Nagane Y, Murai H, Masuda M, Farrugia ME, Carmichael C, Birnbaum S, Hogrel JY, Nafissi S, Fatehi F, Ou C, Liu W, Conaway M. International clinimetric evaluation of the MG-QOL15, resulting in slight revision and subsequent validation of the MG-QOL15r. Muscle Nerve. 2016;54(6):1015-1022. doi: 10.1002/mus.25198.

Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213. doi: 10.1016/0165-1781(89)90047-4.

Caballero-Ávila M, Álvarez-Velasco R, Moga E, Rojas-Garcia R, Turon-Sans J, Querol L, Olivé M, Reyes-Leiva D, Illa I, Gallardo E, Cortés-Vicente E. Rituximab in myasthenia gravis: efficacy, associated infections and risk of induced hypogammaglobulinemia. Neuromuscul Disord. 2022;32(8):664-671. doi: 10.1016/j.nmd.2022.06.006.

Cabrera Serrano M, Rabinstein AA. Usefulness of pulmonary function tests and blood gases in acute neuromuscular respiratory failure. Eur J Neurol. 2012;19(3):452-6. doi: 10.1111/j.1468-1331.2011.03539.x.

Calik-Kutukcu E, Salci Y, Karanfil E, Fil-Balkan A, Bekircan-Kurt CE, Armutlu K. Expiratory muscle strength as a predictor of functional exercise capacity in generalized myasthenia gravis. Neurosciences (Riyadh). 2019;24(2):95-100. doi: 10.17712/nsj.2019.2.20180024.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. NatMethods. 2016;13(7):581-583. doi:10.1038/nmeth.3869.

Callahan BJ, Wong J, Heiner C, Oh S, Theriot CM, Gulati AS, McGill SK, Dougherty MK. High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution. Nucleic Acids Res. 2019;47(18):e103. doi: 10.1093/nar/gkz569.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A. 2011 Mar 15;108 Suppl 1(Suppl 1):4516-22. doi: 10.1073/pnas.1000080107.

Caruso R, Arrigoni C, Groppelli K, Magon A, Dellafiore F, Pittella F, Grugnetti AM, Chessa M, Yorke J. Italian version of Dyspnoea-12: cultural-linguistic validation, quantitative and qualitative content validity study. Acta Biomed. 2018;88(4):426-434. doi: 10.23750/abm.v88i4.6341.

Cavalcante P, Mantegazza R, Antozzi C. Targeting autoimmune mechanisms by precision medicine in Myasthenia Gravis. Front Immunol. 2024;15:1404191. doi: 10.3389/fimmu.2024.1404191.

Chen P, Tang X. Gut Microbiota as Regulators of Th17/Treg Balance in Patients With Myasthenia Gravis. Front Immunol. 2021;12:803101. doi: 10.3389/fimmu.2021.803101.

Chen X, Ding J, Zhang J, Dai H, Yu L. Myasthenia gravis and community-acquired pneumonia: therapeutic challenges. Front Pharmacol. 2025;16:1695526. doi: 10.3389/fphar.2025.1695526.

Claytor B, Cho SM, Li Y. Myasthenic crisis. Muscle Nerve. 2023;68(1):8-19. doi: 10.1002/mus.27832.

Cortés-Vicente E, Álvarez-Velasco R, Segovia S, Paradas C, Casasnovas C, Guerrero-Sola A, Pardo J, Ramos-Fransi A, Sevilla T, López de Munain A, Gómez MT, Jericó I, Gutiérrez-Gutiérrez G, Pelayo-Negro AL, Martín MA, Mendoza MD, Morís G, Rojas-Garcia R, Díaz-Manera J, Querol L, Gallardo E, Vélez B, Albertí MA, Galán L, García-Sobrino T, Martínez-Piñeiro A, Lozano-Veintimilla A, Fernández-Torrón R, Cano-Abascal Á, Illa I. Clinical and therapeutic features of myasthenia gravis in adults based on age at onset. Neurology. 2020;94(11):e1171-e1180. doi: 10.1212/WNL.0000000000008903.

Couch CA, Mascarenhas R, Stierman B, Fryar CD. Prevalence of Cardiovascular Disease Risk Factors in Adults: United States, August 2021-August 2023. NCHS Data Brief. 2025;(540):10.15620/cdc/174623. doi: 10.15620/cdc/174623.

Dangers L, Laviolette L, Georges M, Gonzalez-Bermejo J, Rivals I, Similowski T, Morelot-Panzini C. Relieving dyspnoea by non-invasive ventilation decreases pain thresholds in amyotrophic lateral sclerosis. Thorax. 2017;72(3):230-235. doi: 10.1136/thoraxjnl-2016-208544.

de Meel RHP, Tannemaat MR, Verschuuren JJGM. Heterogeneity and shifts in distribution of muscle weakness in myasthenia gravis. Neuromuscul Disord. 2019;29(9):664-670. doi: 10.1016/j.nmd.2019.07.006.

Dede Y, Koskderelioglu A, Gedizlioglu M. Sleep-related quality of life in patients with myasthenia gravis. Neurological Sciences and Neurophysiology. 2025;42(1):7–13. doi:10.4103/nsn.nsn_126_24.

Dewilde S, Philips G, Paci S, Beauchamp J, Chiroli S, Quinn C, Day L, Larkin M, Palace J, Berrih-Aknin S, Claeys KG, Muppidi S, Mantegazza R, Saccà F, Meisel A, Bassez G, Murai H, Janssen MF. Patient-reported burden of myasthenia gravis: baseline results of the international prospective, observational, longitudinal real-world digital study MyRealWorld-MG. BMJ Open. 2023;13(1):e066445. doi: 10.1136/bmjopen-2022-066445.

Dewilde S, Phillips G, Paci S, De Ruyck F, Tollenaar NH, Janssen MF. The Burden Patients with Myasthenia Gravis Experience in Terms of Breathing, Fatigue, Sleep, Mental Health, Discomfort and Usual Activities in Comparison to the General Population. Adv Ther. 2024;41(1):271-291. doi: 10.1007/s12325-023-02704-w.

Deymeer F, Gungor-Tuncer O, Yilmaz V, Parman Y, Serdaroglu P, Ozdemir C, Vincent A, Saruhan-Direskeneli G. Clinical comparison of anti-MuSK- vs anti-AChR-positive and seronegative myasthenia gravis. Neurology. 2007;68(8):609-11. doi: 10.1212/01.wnl.0000254620.45529.97.

Di Stefano V, Iacono S, Militello M, Leone O, Rispoli MG, Ferri L, Ajdinaj P, Lanza P, Lupica A, Crescimanno G, Monastero R, Di Muzio A, Brighina F. Comorbidity in myasthenia gravis: multicentric, hospital-based, and controlled study of 178 Italian patients. Neurol Sci. 2024;45(7):3481-3494. doi: 10.1007/s10072-024-07368-0.

Dishnica N, Vuong A, Xiong L, Tan S, Kovoor J, Gupta A, Stretton B, Goh R, Harroud A, Schultz D, Malycha J, Bacchi S. Single count breath test for the evaluation of respiratory function in Myasthenia Gravis: A systematic review. J Clin Neurosci. 2023;112:58-63. doi: 10.1016/j.jocn.2023.04.011.

Dong D, Chong MK, Wu Y, Kaminski H, Cutter G, Xu X, Li H, Zhao C, Yin J, Yu S, Zhu J. Gender differences in quality of life among patients with myasthenia gravis in China. Health Qual Life Outcomes. 2020;18(1):296. doi: 10.1186/s12955-020-01549-z.

Dresser L, Wlodarski R, Rezania K, Soliven B. Myasthenia Gravis: Epidemiology, Pathophysiology and Clinical Manifestations. J Clin Med. 2021;10(11):2235. doi: 10.3390/jcm10112235.

Elsais A, Johansen B, Kerty E. Airway limitation and exercise intolerance in well-regulated myasthenia gravis patients. Acta Neurol Scand Suppl. 2010;(190):12-7. doi: 10.1111/j.1600-0404.2010.01369.x.

Elsheikh B, Arnold WD, Gharibshahi S, Reynolds J, Freimer M, Kissel JT. Correlation of single-breath count test and neck flexor muscle strength with spirometry in myasthenia gravis. Muscle Nerve. 2016;53(1):134-6. doi: 10.1002/mus.24929.

Evans JA and Whitelaw WA. The assessment of maximal respiratory mouth pressures in adults. Respir Care. 2009;54(10):1348-59.

Fardet L, Petersen I, Nazareth I. Common Infections in Patients Prescribed Systemic Glucocorticoids in Primary Care: A Population-Based Cohort Study. PLoS Med. 2016;13(5):e1002024. doi: 10.1371/journal.pmed.1002024. ù

Farrugia ME, Goodfellow JA. A Practical Approach to Managing Patients With Myasthenia Gravis-Opinions and a Review of the Literature. Front Neurol. 2020;11:604. doi: 10.3389/fneur.2020.00604.

Fernandes Oliveira E, Nacif SR, Alves Pereira N, Fonseca NT, Urbano JJ, Perez EA, Cavalcante V, Santos Oliveira C, Insalaco G, Oliveira AS, Oliveira LV. Sleep disorders in patients with myasthenia gravis: a systematic review. J Phys Ther Sci. 2015;27(6):2013-8. doi: 10.1589/jpts.27.2013.

Fernández CM, Tejedor ED, Frank A, Pino JM, Conde CP, Barreiro P. Maximal respiratory pressures in myasthenia gravis. Relation to single fiber electromyography. Acta Neurol Scand. 2001;103(6):392-5. doi: 10.1034/j.1600-0404.2001.103006392.x.

Fitting JW, Héritier F, Uldry C. Evaluation de la force musculaire inspiratoire par la pression nasale lors du sniff [Evaluation of the inspiratory muscle strength using the nasal pressure of the sniff]. Rev Mal Respir. 1996;13(5):479-84.

Fock E, Parnova R. Mechanisms of Blood-Brain Barrier Protection by Microbiota-Derived Short-Chain Fatty Acids. Cells. 2023;12(4):657. doi: 10.3390/cells12040657.

Fregonezi G, Azevedo IG, Resqueti VR, De Andrade AD, Gualdi LP, Aliverti A, Dourado-Junior ME, Parreira VF. Muscle impairment in neuromuscular disease using an expiratory/inspiratory pressure ratio. Respir Care. 2015;60(4):533-9. doi: 10.4187/respcare.03367.

Freitag S, Hallebach S, Baumann I, Kalischewski P, Rassler B. Effects of long-term respiratory muscle endurance training on respiratory and functional outcomes in patients with Myasthenia gravis. Respir Med. 2018;144:7-15. doi: 10.1016/j.rmed.2018.09.001.

Galassi G, Marchioni A. Myasthenia gravis at the crossroad of COVID-19: focus on immunological and respiratory interplay. Acta Neurol Belg. 2021;121(3):633-642. doi: 10.1007/s13760-021-01612-6.

García Río F, Prados C, Díez Tejedor E, Díaz Lobato S, Alvarez-Sala R, Villamor J, Pino JM. Breathing pattern and central ventilatory drive in mild and moderate generalised myasthenia gravis. Thorax. 1994;49(7):703-6. doi: 10.1136/thx.49.7.703.

Gattellari M, Goumas C, Worthington JM. A national epidemiological study of myasthenia gravis in Australia. Eur J Neurol 2012; 19: 1413–20.

Gilhus NE and Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015;14(10):1023-36. doi: 10.1016/S1474-4422(15)00145-3.

Gilhus NE, Nacu A, Andersen JB, Owe JF. Myasthenia gravis and risks for comorbidity. Eur J Neurol. 2015;22(1):17-23. doi: 10.1111/ene.12599.

Gilhus NE. Myasthenia gravis, respiratory function, and respiratory tract disease. J Neurol. 2023;270(7):3329-3340. doi: 10.1007/s00415-023-11733-y.

Gilhus NE. Myasthenia Gravis. N Engl J Med. 2016;375(26):2570-2581. doi: 10.1056/NEJMra1602678.

Gipsman AI, Lapinel NC, Mayer OH. Airway clearance in patients with neuromuscular disease. Paediatr Respir Rev. 2023;47:33-40. doi: 10.1016/j.prrv.2023.02.002.

Godoy DA, Mello LJ, Masotti L, Di Napoli M. The myasthenic patient in crisis: an update of the management in Neurointensive Care Unit. Arq Neuropsiquiatr. 2013;71(9A):627-39. doi: 10.1590/0004-282X20130108.

Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, Hallstrand TS, Kaminsky DA, McCarthy K, McCormack MC, Oropez CE, Rosenfeld M, Stanojevic S, Swanney MP, Thompson BR. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med. 2019;200(8):e70-e88. doi: 10.1164/rccm.201908-1590ST.

Griggs RC, Donohoe KM, Utell MJ, Goldblatt D, Moxley RT 3rd. Evaluation of pulmonary function in neuromuscular disease. Arch Neurol. 1981;38(1):9-12. doi: 10.1001/archneur.1981.00510010035004.

Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. Muscle Nerve. 2008;37(2):141-9. doi: 10.1002/mus.20950.

Harris L, Graham S, MacLachlan S, Exuzides A, Jacob S. A retrospective longitudinal cohort study of the clinical burden in myasthenia gravis. BMC Neurol. 2022;22(1):172. doi: 10.1186/s12883-022-02692-4.

Heliopoulos I, Patlakas G, Vadikolias K, Artemis N, Kleopa KA, Maltezos E, Piperidou H. Maximal voluntary ventilation in myasthenia gravis. Muscle Nerve. 2003;27(6):715-9. doi: 10.1002/mus.10378.

Hess M, Sczyrba A, Egan R, Kim TW, Chokhawala H, Schroth G, Luo S, Clark DS, Chen F, Zhang T, Mackie RI, Pennacchio LA, Tringe SG, Visel A, Woyke T, Wang Z, Rubin EM. Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science. 2011;331(6016):463-7. doi: 10.1126/science.1200387.

Hornsveld HK, Garssen B, Dop MJ, van Spiegel PI, de Haes JC. Double-blind placebo-controlled study of the hyperventilation provocation test and the validity of the hyperventilation syndrome. Lancet. 1996;348(9021):154-8. doi: 10.1016/s0140-6736(96)02024-7.

Howard JF Jr, Bresch S, Genge A, Hewamadduma C, Hinton J, Hussain Y, Juntas-Morales R, Kaminski HJ, Maniaol A, Mantegazza R, Masuda M, Sivakumar K, Śmiłowski M, Utsugisawa K, Vu T, Weiss MD, Zajda M, Boroojerdi B, Brock M, de la Borderie G, Duda PW, Lowcock R, Vanderkelen M, Leite MI; RAISE Study Team. Safety and efficacy of zilucoplan in patients with generalised myasthenia gravis (RAISE): a randomised, double-blind, placebo-controlled, phase 3 study. Lancet Neurol. 2023;22(5):395-406. doi: 10.1016/S1474-4422(23)00080-7.

Howard JF Jr, Bril V, Vu T, Karam C, Peric S, De Bleecker JL, Murai H, Meisel A, Beydoun SR, Pasnoor M, Guglietta A, Van Hoorick B, Steeland S, T'joen C, Utsugisawa K, Verschuuren J, Mantegazza R; ADAPT+ Study Group. Long-term safety, tolerability, and efficacy of efgartigimod (ADAPT+): interim results from a

phase 3 open-label extension study in participants with generalized myasthenia gravis. Front Neurol. 2024; 17;14:1284444. doi: 10.3389/fneur.2023.1284444.

Howard JF Jr, Bril V, Vu T, Karam C, Peric S, Margania T, Murai H, Bilinska M, Shakarishvili R, Smilowski M, Guglietta A, Ulrichts P, Vangeneugden T, Utsugisawa K, Verschuuren J, Mantegazza R; ADAPT Investigator Study Group. Safety, efficacy, and tolerability of efgartigimod in patients with generalised myasthenia gravis (ADAPT): a multicentre, randomised, placebo-controlled, phase 3 trial. Lancet Neurol. 2021;20(7):526-536. doi: 10.1016/S1474-4422(21)00159-9. Erratum in: Lancet Neurol. 2021;20(8):e5. doi: 10.1016/S1474-4422(21)00216-7.

Howard JF Jr, Utsugisawa K, Benatar M, Murai H, Barohn RJ, Illa I, Jacob S, Vissing J, Burns TM, Kissel JT, Muppidi S, Nowak RJ, O'Brien F, Wang JJ, Mantegazza R; REGAIN Study Group. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. Lancet Neurol. 2017;16(12):976-986. doi: 10.1016/S1474-4422(17)30369-1. Epub 2017 Oct 20. Erratum in: Lancet Neurol. 2017;16(12):954. doi: 10.1016/S1474-4422(17)30377-0.

Hu M, Alashkar Alhamwe B, Santner-Nanan B, Miethe S, Harb H, Renz H, Potaczek DP, Nanan RK. Short-Chain Fatty Acids Augment Differentiation and Function of Human Induced Regulatory T Cells. Int J Mol Sci. 2022;23(10):5740. doi: 10.3390/ijms23105740.

Huang C, Gao F, Zhou H, Zhang L, Shang D, Ji Y, Duan Z. Oral Microbiota Profile in a Group of Anti-AChR Antibody-Positive Myasthenia Gravis Patients. Front Neurol. 2022;13:938360. doi: 10.3389/fneur.2022.938360.

Huang X, Yu ZH, Cui Y, Zhang H, Fu M, Zhao Y, Liu P, Fan M, Wu T, Zhang C, Qi G, Shi FD. Outcomes in Relation to the Age at Onset in Patients With Myasthenia Gravis. Neurology. 2025;105(12):e214428. doi: 10.1212/WNL.0000000000214428.

Huang Y, Tan Y, Shi J, Li K, Yan J, Guan Y. Patients With Myasthenia Gravis With Acute Onset of Dyspnea: Predictors of Progression to Myasthenic Crisis and Prognosis. Front Neurol. 2021;12:767961. doi: 10.3389/fneur.2021.767961.

Human Microbiome Project Consortium. A framework for human microbiome research. Nature. 2012;486(7402):215-21. doi: 10.1038/nature11209.

Iliaz S, Yunisova G, Cakmak OO, Celebi O, Bulus E, Duman A, Bayraktaroglu M, Oflazer P. The clinical use of impulse oscillometry in neuromuscular diseases. Respir Med. 2022;200:106931. doi: 10.1016/j.rmed.2022.106931.

Iorio R. Myasthenia gravis: the changing treatment landscape in the era of molecular therapies. Nat Rev Neurol. 2024;20(2):84-98. doi: 10.1038/s41582-023-00916-w.

Janssen MF, Dewilde S, Wolfe GI, Muppidi S, Phillips G. Psychometric properties of MG-ADL items and MG-ADL score: An assessment of distributional characteristics, validity and factor structure in two large datasets. J Neurol Sci. 2024;463:123135. doi: 10.1016/j.jns.2024.123135.

Jiang X, Zhang S. Respiratory microbiota, host immunity, respiratory viral infections and malignant tumors. Front Microbiol. 2025;16:1626077. doi: 10.3389/fmicb.2025.1626077.

Kapoor B, Gulati M, Gupta R, Singla RK. Microbiota dysbiosis and myasthenia gravis: Do all roads lead to Rome? Autoimmun Rev. 2023;22(5):103313. doi: 10.1016/j.autrev.2023.103313.

Kassardjian CD, Widdifield J, Paterson JM, Kopp A, Nagamuthu C, Barnett C, Tu K, Breiner A. Serious infections in patients with myasthenia gravis: population-based cohort study. Eur J Neurol. 2020;27(4):702-708. doi: 10.1111/ene.14153.

Keenan SP, Alexander D, Road JD, Ryan CF, Oger J, Wilcox PG. Ventilatory muscle strength and endurance in myasthenia gravis. Eur Respir J. 1995;8(7):1130-5. doi: 10.1183/09031936.95.08071130.

Kim CH. Control of lymphocyte functions by gut microbiota-derived short-chain fatty acids. Cell Mol Immunol. 2021;18(5):1161-1171. doi: 10.1038/s41423-020-00625-0.

Kukulka K, Gummi RR, Govindarajan R. A telephonic single breath count test for screening of exacerbations of myasthenia gravis: A pilot study. Muscle Nerve. 2020;62(2):258-261. doi: 10.1002/mus.26987.

Kumai Y, Miyamoto T, Matsubara K, Samejima Y, Yamashita S, Ando Y, Orita Y. Assessment of oropharyngeal swallowing dysfunction in myasthenia gravis patients presenting with difficulty in swallowing. Auris Nasus Larynx. 2019;46(3):390-396. doi: 10.1016/j.anl.2018.10.004.

Kuroiwa R, Shibuya K, Inagaki T, Nara T, Nemoto M, Doi Y, Yasuda M, Uzawa A, Shiko Y, Murata A, Yamanaka Y, Kuwabara S. Reliability and validity of cough peak flow measurements in myasthenia gravis. Neuromuscul Disord. 2024;41:29-34. doi: 10.1016/j.nmd.2024.06.003.

Lagier JC, Dubourg G, Million M, Cadoret F, Bilen M, Fenollar F, Levasseur A, Rolain JM, Fournier PE, Raoult D. Culturing the human microbiota and culturomics. Nat Rev Microbiol. 2018;16:540-550. doi: 10.1038/s41579-018-0041-0.

Lai LA, Tong Z, Chen R, Pan S. Metaproteomics Study of the Gut Microbiome. Methods Mol Biol. 2019;1871:123-132. doi: 10.1007/978-1-4939-8814-3_8.

Lai YC, Chen JY, Wu HD, Yang CC, Lin CH, Lee PL. Sleep Disordered Breathing Mimicking Myasthenia Crisis in a Patient with Myasthenia Gravis. J Clin Sleep Med. 2016;12(5):767-9. doi: 10.5664/jcsm.5816. PMID: 26943715; PMCID: PMC4865565.

Lehnerer S, Jacobi J, Schilling R, Grittner U, Marbin D, Gerischer L, Stascheit F, Krause M, Hoffmann S, Meisel A. Burden of disease in myasthenia gravis: taking the patient's perspective. J Neurol. 2022;269(6):3050-3063. doi: 10.1007/s00415-021-10891-1.

Li KJ, Chen ZL, Huang Y, Zhang R, Luan XQ, Lei TT, Chen L. Dysbiosis of lower respiratory tract microbiome are associated with inflammation and microbial function variety. Respir Res. 2019;20(1):272. doi: 10.1186/s12931-019-1246-0.

Li M, Shao D, Zhou J, et al. Signatures within esophageal microbiota with progression of esophageal squamous cell carcinoma. Chinese Journal of Cancer Research. 2020;32(6):755-767. doi:10.21147/j.issn.1000-9604.2020.06.09.

Li X, Li Z, Zhang C, Li L, Liang Y, Zhang X, Liu F, Lin X, Jiang Q. Quality of life and social support as key determinants of anxiety and depression in Myasthenia Gravis: evidence from a Chinese cohort. Front Neurol. 2025;16:1670436. doi: 10.3389/fneur.2025.1670436.

Li Y, Peng Y, Yang H. Serological diagnosis of myasthenia gravis and its clinical significance. Ann Transl Med. 2023 Apr 15;11(7):290. doi: 10.21037/atm-19-363.

Liu F, Wang Q, Chen X. Myasthenic crisis treated in a Chinese neurological intensive care unit: clinical features, mortality, outcomes, and predictors of survival. BMC Neurol. 2019;19(1):172. doi: 10.1186/s12883-019-1384-5.

Lizarraga AA, Lizarraga KJ, Benatar M. Getting Rid of Weakness in the ICU: An Updated Approach to the Acute Management of Myasthenia Gravis and Guillain-Barré Syndrome. Semin Neurol. 2016;36(6):615-624. doi: 10.1055/s-0036-1592106.

Lyall RA, Donaldson N, Polkey MI, Leigh PN, Moxham J. Respiratory muscle strength and ventilatory failure in amyotrophic lateral sclerosis. Brain. 2001;124(Pt 10):2000-13. doi: 10.1093/brain/124.10.2000.

Ma J, Zhang H, Pang X, Zhang J, Zhao R, Wang J, Chang X, Guo J, Zhang W. Diaphragmatic ultrasonography as a predictor of respiratory muscle fatigue in myasthenia gravis. Muscle Nerve. 2024;69(2):199-205. doi: 10.1002/mus.28020.

Martinka I, Fulova M, Spalekova M, Spalek P. Epidemiology of myasthenia gravis in Slovakia in the years 1977–2015. Neuroepidemiology 2018; 50: 153–59.

Misra UK, Kumar S, Singh VK, Dubey D, Kalita J. Noninvasive Ventilation in Myasthenia Gravis. Neurol India. 2020;68(3):648-651. doi: 10.4103/0028-3886.289001.

Morélot-Panzini C, Perez T, Sedkaoui K, de Bock E, Aguilaniu B, Devillier P, Pignier C, Arnould B, Bruneteau G, Similowski T. The multidimensional nature of dyspnoea in amyotrophic lateral sclerosis patients with chronic respiratory failure: Air hunger, anxiety and fear. Respir Med. 2018;145:1-7. doi: 10.1016/j.rmed.2018.10.010.

Morrow BM, Angelil L, Forsyth J, Huisamen A, Juries E, Corten L. The utility of using peak expiratory flow and forced vital capacity to predict poor expiratory cough flow in children with neuromuscular disorders. S Afr J Physiother. 2019;75(1):1296. doi: 10.4102/sajp.v75i1.1296.

Mousavi A, Kumar P, Frykman H. The changing landscape of autoantibody testing in myasthenia gravis in the setting of novel drug treatments. Clin Biochem. 2024;133-134:110826. doi: 10.1016/j.clinbiochem.2024.110826.

Muñoz Fernández C, Díez Tejedor E, Frank Garcia A, Pino JM, Pérez Conde C, Barreiro Tella P. Evaluation of maximal respiratory pressures in myasthenia gravis. Prognostic value. Eur Neurol. 2004;52(3):136-40. doi: 10.1159/000081464.

Muppidi S, Wolfe GI, Conaway M, Burns TM; MG COMPOSITE AND MG-QOL15 STUDY GROUP. MG-ADL: still a relevant outcome measure. Muscle Nerve. 2011;44(5):727-31. doi: 10.1002/mus.22140.

Nelke C, Schroeter CB, Barman S, Stascheit F, Masanneck L, Theissen L, Huntemann N, Walli S, Cengiz D, Dobelmann V, Vogelsang A, Pawlitzki M, Räuber S, Konen FF, Skripuletz T, Hartung HP, König S, Roos A, Meisel A, Meuth SG, Ruck T. Identification of disease phenotypes in acetylcholine receptor-antibody myasthenia gravis using proteomics-based consensus clustering. EBioMedicine. 2024;105:105231. doi: 10.1016/j.ebiom.2024.105231.

Neumann B, Angstwurm K, Dohmen C, Mergenthaler P, Kohler S, Schönenberger S, Lee DH, Gerner ST, Huttner HB, Thieme A, Steinbrecher A, Dunkel J, Roth C, Schneider H, Reichmann H, Fuhrer H, Kleiter I, Schneider-Gold C, Alberty A, Zinke J, Schroeter M, Linker R, Meisel A, Bösel J, Stetefeld HR; Initiative of German NeuroIntensive Trial Engagement (IGNITE). Weaning and extubation failure in myasthenic crisis: a multicenter analysis. J Neurol. 2024;271(1):564-574. doi: 10.1007/s00415-023-12016-2.

Neumann B, Angstwurm K, Mergenthaler P, Kohler S, Schönenberger S, Bösel J, Neumann U, Vidal A, Huttner HB, Gerner ST, Thieme A, Steinbrecher A, Dunkel J, Roth C, Schneider H, Schimmel E, Fuhrer H, Fahrendorf C, Alberty A, Zinke J, Meisel A, Dohmen C, Stetefeld HR; German Myasthenic Crisis Study Group. Myasthenic crisis demanding mechanical ventilation: A multicenter analysis of 250 cases. Neurology. 2020;94(3):e299-e313. doi: 10.1212/WNL.0000000000008688. Erratum in: Neurology. 2020;94(16):724. doi: 10.1212/WNL.0000000000009262.

Nicolì V, Tabano SM, Colapietro P, Maestri M, Ricciardi R, Stoccoro A, Fontana L, Guida M, Miozzo M, Coppedè F, Migliore L. Preferential X Chromosome Inactivation as a Mechanism to Explain Female Preponderance in Myasthenia Gravis. Genes (Basel). 2022;13(4):696. doi: 10.3390/genes13040696.

Nicolle MW, Rask S, Koopman WJ, George CF, Adams J, Wiebe S. Sleep apnea in patients with myasthenia gravis. Neurology. 2006;67(1):140-2. doi: 10.1212/01.wnl.0000223515.15691.26.

Ntawuruhunga IN, Nougon G. Acute Respiratory Failure Due to Inaugural Myasthenia Crisis. Cureus. 2024;16(8):e68090. doi: 10.7759/cureus.68090.

O'Callaghan A, van Sinderen D. Bifidobacteria and Their Role as Members of the Human Gut Microbiota. Front Microbiol. 2016;7:925. doi: 10.3389/fmicb.2016.00925.

Octaviana F, Safri AY, Wiratman W, Indrawati LA, Fadli N, Hakim M. Pulmonary Function Assessment in Myasthenia Gravis Patients in a National Referral Hospital in Indonesia. Int J Gen Med. 2023;16:4477-4483. doi: 10.2147/IJGM.S426321.

Oh S, Lee HJ, Park KU. Metagenomic characterization of the microbiomes in five different body habitats of otherwise healthy individuals with periodontal disease. Front Cell Infect Microbiol. 2023;13:1257816. doi: 10.3389/fcimb.2023.1257816.

Oliveira EF, Nacif SR, Urbano JJ, Silva AS, Oliveira CS, Perez EA, Polaro MN, Valerio BCO, Stirbulov R, Insalaco G, Oliveira ASB, Oliveira LVF. Sleep, lung function, and quality of life in patients with myasthenia gravis: A cross-sectional study. Neuromuscul Disord. 2017;27(2):120-127. doi: 10.1016/j.nmd.2016.11.015.

Park SY, Lee JY, Lim NG, Hong YH. Incidence and prevalence of myasthenia gravis in Korea: a population-based study using the National Health Insurance claims database. J Clin Neurol 2016; 12: 340–44.

Paulo AC, Lança J, Almeida ST, Hilty M, Sá-Leão R. The upper respiratory tract microbiota of healthy adults is affected by Streptococcus pneumoniae carriage, smoking habits, and contact with children. Microbiome. 2023;11(1):199. doi: 10.1186/s40168-023-01640-9.

Pesa J, Choudhry Z, de Courcy J, Barlow S, Gibson G, Chatterton E, Birija SL, Hahn B, Govindarajan R. Clinical characteristics and impairment of activities of daily living among patients with myasthenia gravis with differing degrees of muscle weakness: a real-world study of patients in the US and five European countries. BMC Neurol. 2024;24(1):385. doi: 10.1186/s12883-024-03869-9.

Pradhan S, Anand S. Respiratory Assessment of Myasthenia Gravis Patients Using Repetitive Nerve Stimulation of Phrenic and Intercostal Nerves. Neurol India. 2020;68(6):1394-1399. doi: 10.4103/0028-3886.304128.

Preiningerova JL, Jiraskova Zakostelska Z, Srinivasan A, Ticha V, Kovarova I, Kleinova P, Tlaskalova-Hogenova H, Kubala Havrdova E. Multiple Sclerosis and Microbiome. Biomolecules. 2022;12(3):433. doi: 10.3390/biom12030433.

Prior DE, Nurre E, Roller SL, Kline D, Panara R, Stino AM, Davis JA, Freimer ML, Arnold WD. Infections and the relationship to treatment in neuromuscular autoimmunity. Muscle Nerve. 2018;57(6):927-931. doi: 10.1002/mus.26032.

Pulvirenti F, Giufrè M, Pentimalli TM, Camilli R, Milito C, Villa A, Sculco E, Cerquetti M, Pantosti A, Quinti I. Oropharyngeal microbial ecosystem perturbations influence the risk for acute respiratory infections in common variable immunodeficiency. Front Immunol. 2024;15:1371118. doi: 10.3389/fimmu.2024.1371118.

Punga AR, Maddison P, Heckmann JM, Guptill JT, Evoli A. Epidemiology, diagnostics, and biomarkers of autoimmune neuromuscular junction disorders. Lancet Neurol. 2022;21(2):176-188. doi: 10.1016/S1474-4422(21)00297-0.

Punga T, Bartoccioni E, Lewandowska M, Damato V, Evoli A, Punga AR. Disease specific enrichment of circulating let-7 family microRNA in MuSK+ myasthenia gravis. J Neuroimmunol. 2016;292:21-6. doi: 10.1016/j.jneuroim.2016.01.003.

Qin M, Ding W, Qin L, Liang R, Guo Y, Zhao Y, Xu H, Wen Y, Pang Y, Li L. Dysbiosis associated with enhanced microbial mobility across the respiratory tract in pulmonary tuberculosis patients. BMC Microbiol. 2025;25(1):499. doi: 10.1186/s12866-025-04206-x.

Qiu D, Xia Z, Jiao X, Deng J, Zhang L, Li J. Altered Gut Microbiota in Myasthenia Gravis. Front Microbiol. 2018;9:2627. doi: 10.3389/fmicb.2018.02627.

Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. Nat Biotechnol. 2017 Sep 12;35(9):833-844. doi: 10.1038/nbt.3935. Erratum in: Nat Biotechnol. 2017;35(12):1211. doi: 10.1038/nbt1217-1211b.

Ramos-Fransi A, Rojas-García R, Segovia S, Márquez-Infante C, Pardo J, Coll-Cantí J, Jericó I, Illa I; Myasthenia NMD-ES Study Group. Myasthenia gravis: descriptive analysis of life-threatening events in a recent nationwide registry. Eur J Neurol. 2015;22(7):1056-61. doi: 10.1111/ene.12703.

Recasens BB, Balañá Corberó A, Llorens JMM, Guillen-Sola A, Moreno MV, Escobar GG, Umayahara Y, Soh Z, Tsuji T, Rubio MÁ. Sound-based cough peak flow estimation in patients with neuromuscular disorders. Muscle Nerve. 2024;69(2):213-217. doi: 10.1002/mus.27987.

Reddy BSK, Jadhav U, Wagh P, Bhanushali J, Sarkar S. Atypical Presentations of Myasthenia Gravis as Acute Respiratory Failure: A Rare Case. Cureus. 2024;16(8):e67635. doi: 10.7759/cureus.67635.

Regnault A, Morel T, de la Loge C, Mazerolle F, Kaminski HJ, Habib AA. Measuring Overall Severity of Myasthenia Gravis (MG): Evidence for the Added Value of the MG Symptoms PRO. Neurol Ther. 2023;12(5):1573-1590. doi: 10.1007/s40120-023-00464-x. Erratum in: Neurol Ther. 2023;12(5):1591-1592. doi: 10.1007/s40120-023-00508-2.

Reynoso-García J, Miranda-Santiago AE, Meléndez-Vázquez NM, Acosta-Pagán K, Sánchez-Rosado M, Díaz-Rivera J, Rosado-Quiñones AM, Acevedo-Márquez L, Cruz-Roldán L, Tosado-Rodríguez EL, Figueroa-Gispert MDM, Godoy-Vitorino F. A complete guide to human microbiomes: Body niches, transmission, development, dysbiosis, and restoration. Front Syst Biol. 2022;2:951403. doi: 10.3389/fsysb.2022.951403.

Rodolico C, Bonanno C, Toscano A, Vita G. MuSK-Associated Myasthenia Gravis: Clinical Features and Management. Front Neurol. 2020;11:660. doi: 10.3389/fneur.2020.00660.

Romi F, Aarli JA, Gilhus NE. Myasthenia gravis patients with ryanodine receptor antibodies have distinctive clinical features. Eur J Neurol. 2007;14(6):617-20. doi: 10.1111/j.1468-1331.2007.01785.x.

Salci Y, Karanfil E, Balkan AF, Kütükçü EÇ, Ceren AN, Ayvat F, Bekircan-Kurt CE, Armutlu K. Functional exercise capacity evaluated by timed walk tests in myasthenia gravis. Muscle Nerve. 2019;59(2):208-212. doi: 10.1002/mus.26345.

Sancho J, Ferrer S, Quezada A, Gimenez E, Signes-Costa J. Home Noninvasive Ventilation in Myasthenia Gravis. Respir Care. 2024;69(3):333-338. doi: 10.4187/respcare.11308.

Santy L, Janssens W, Buyse B. A late diagnosis of myasthenia gravis at the respiratory practice: back to basics. Breathe (Sheff). 2022;18(2):210167. doi: 10.1183/20734735.0167-2021.

Sciancalepore F, Sciancalepore F, Lombardi N, Valdiserra G, Bonaso M, Cappello E, Hyeraci G, Crescioli G, Celani MG, Cantisani TA, Brunori P, Vecchi S, Bacigalupo I, Locuratolo N, Lacorte E, Vanacore N, Kirchmayer U. Prevalence, Incidence, and Mortality of Myasthenia Gravis and Myasthenic Syndromes: A Systematic Review. Neuroepidemiology. 2025;59(5):579-592. doi: 10.1159/000539577.

Sipilä JOT, Soilu-Hänninen M, Rautava P, Kytö V. Hospital admission and prevalence trends of adult myasthenia gravis in Finland in 2004-2014: A retrospective national registry study. J Neurol Sci. 2019;407:116520. doi: 10.1016/j.jns.2019.116520.

Sobieszczuk E, Napiórkowski Ł, Szczudlik P, Kostera-Pruszczyk A. Myasthenia gravis-treatment and severity in nationwide cohort. Acta Neurol Scand. 2022;145(4):471-478. doi: 10.1111/ane.13576.

Su M, Jin S, Jiao K, Yan C, Song J, Xi J, Zhao C, Zhou Z, Zheng J, Luo S. Pneumonia in myasthenia gravis: Microbial etiology and clinical management. Front Cell Infect Microbiol. 2022;12:1016728. doi: 10.3389/fcimb.2022.1016728.

Suehiro H, Sekiguchi K, Noda Y, Matoba S, Watanabe S, Hayashi M, Yoshikawa M, Sugisawa R, Matsumoto R. Diaphragmatic Compound Muscle Action Potentials Correlate With the Clinical Severity of Myasthenia Gravis. Muscle Nerve. 2025;71(6):1043-1051. doi: 10.1002/mus.28397.

Suh J, Goldstein JM, Nowak RJ. Clinical characteristics of refractory myasthenia gravis patients. Yale J Biol Med. 2013;86(2):255-60.

Terzi N, Orlikowski D, Fermanian C, Lejaille M, Falaize L, Louis A, Raphael JC, Fauroux B, Lofaso F. Measuring inspiratory muscle strength in neuromuscular disease: one test or two? Eur Respir J. 2008;31(1):93-8. doi: 10.1183/09031936.00094707.

Thieben MJ, Blacker DJ, Liu PY, Harper CM Jr, Wijdicks EF. Pulmonary function tests and blood gases in worsening myasthenia gravis. Muscle Nerve. 2005;32(5):664-7. doi: 10.1002/mus.20403.

Thomas T, Gilbert J, Meyer F. Metagenomics - a guide from sampling to data analysis. Microb Inform Exp. 2012;2(1):3. doi: 10.1186/2042-5783-2-3.

Thomsen JLS, Andersen H. Outcome Measures in Clinical Trials of Patients With Myasthenia Gravis. Front Neurol. 2020;11:596382. doi: 10.3389/fneur.2020.596382.

Thomsen JLS, Vinge L, Harbo T, Andersen H. Gender differences in clinical outcomes in myasthenia gravis: A prospective cohort study. Muscle Nerve. 2021;64(5):538-544. doi: 10.1002/mus.27331.

To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, Boulet LP. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC Public Health. 2012 Mar 19;12:204. doi: 10.1186/1471-2458-12-204. Erratum in: BMC Public Health. 2021;21(1):1809. doi: 10.1186/s12889-021-11859-6.

Uldry C and Fitting JW. Maximal values of sniff nasal inspiratory pressure in healthy subjects. Thorax. 1995;50(4):371-5. doi: 10.1136/thx.50.4.371.

Uldry C, Janssens JP, de Muralt B, Fitting JW. Sniff nasal inspiratory pressure in patients with chronic obstructive pulmonary disease. Eur Respir J. 1997;10(6):1292-6. doi: 10.1183/09031936.97.10061292.

Uzawa A, Kuwabara S, Suzuki S, Imai T, Murai H, Ozawa Y, Yasuda M, Nagane Y, Utsugisawa K. Roles of cytokines and T cells in the pathogenesis of myasthenia gravis. Clin Exp Immunol. 2021;203(3):366-374. doi: 10.1111/cei.13546.

van Dixhoorn J, Folgering H. The Nijmegen Questionnaire and dysfunctional breathing. ERJ Open Res. 2015;1(1):00001-2015. doi: 10.1183/23120541.00001-2015.

Van Doorn P, Colla P, Folgering H. Een vragenlijst voor hyperventilatieklachten. De Psycholoog 1983. 18(10), 513-517.

Veldhoen ES, Roos JH, Bekkema R, van der Pol LW, Tinnevelt MHB, Verweij-van den Oudenrijn LP, Wösten-van Asperen RM, Hulzebos EHJ, Wijngaarde CA, van der Ent CK. Oscillometry: A substitute of spirometry in children with neuromuscular diseases? Pediatr Pulmonol. 2022;57(7):1618-1624. doi: 10.1002/ppul.25923.

Vinciguerra C, Bevilacqua L, Lupica A, Ginanneschi F, Piscosquito G, Rini N, Rossi A, Barone P, Brighina F, Di Stefano V. Diagnosis and Management of Seronegative Myasthenia Gravis: Lights and Shadows. Brain Sci. 2023;13(9):1286. doi: 10.3390/brainsci13091286.

Vu T, Meisel A, Mantegazza R, Annane D, Katsuno M, Aguzzi R, Enayetallah A, Beasley KN, Rampal N, Howard JF. Terminal Complement Inhibitor Ravulizumab in Generalized Myasthenia Gravis. NEJM Evid. 2022;1(5):EVIDoa2100066. doi: 10.1056/EVIDoa2100066.

Wang T, Sternes PR, Guo XK, Zhao H, Xu C, Xu H. Autoimmune diseases exhibit shared alterations in the gut microbiota. Rheumatology (Oxford). 2024;63(3):856-865. doi: 10.1093/rheumatology/kead364.

Ward NS, Hill NS. Pulmonary function testing in neuromuscular disease. Clin Chest Med. 2001;22(4):769-81. doi: 10.1016/s0272-5231(05)70065-4.

Wartmann H, Hoffmann S, Ruck T, Nelke C, Deiters B, Volmer T. Incidence, Prevalence, Hospitalization Rates, and Treatment Patterns in Myasthenia Gravis: A 10-Year Real-World Data Analysis of German Claims Data. Neuroepidemiology. 2023;57(2):121-128. doi: 10.1159/000529583.

Westerberg E, Punga AR. Epidemiology of myasthenia gravis in Sweden 2006–2016. Brain Behav 2020; 10: e01819.

Williams MT, Lewthwaite H, Paquet C, Johnston K, Olsson M, Belo LF, Pitta F, Morelot-Panzini C, Ekström M. Dyspnoea-12 and Multidimensional Dyspnea Profile: Systematic Review of Use and Properties. J Pain Symptom Manage. 2022;63(1):e75-e87. doi: 10.1016/j.jpainsymman.2021.06.023.

Winck JC, LeBlanc C, Soto JL, Plano F. The value of cough peak flow measurements in the assessment of extubation or decannulation readiness. Rev Port Pneumol (2006). 2015;21(2):94-8. doi: 10.1016/j.rppnen.2014.12.002.

Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, Ströbel P, Mazia C, Oger J, Cea JG, Heckmann JM, Evoli A, Nix W, Ciafaloni E, Antonini G, Witoonpanich R, King JO, Beydoun SR, Chalk CH, Barboi AC, Amato AA, Shaibani AI, Katirji B, Lecky BR, Buckley C, Vincent A, Dias-Tosta E, Yoshikawa H, Waddington-Cruz M, Pulley MT, Rivner MH, Kostera-Pruszczyk A, Pascuzzi RM, Jackson CE, Garcia Ramos GS, Verschuuren JJ, Massey JM, Kissel JT, Werneck LC, Benatar M, Barohn RJ, Tandan R, Mozaffar T, Conwit R, Odenkirchen J, Sonett JR, Jaretzki A 3rd, Newsom-Davis J, Cutter GR; MGTX Study Group. Randomized Trial of Thymectomy in Myasthenia Gravis. N Engl J Med. 2016;375(6):511-22. doi: 10.1056/NEJMoa1602489. Erratum in: N Engl J Med. 2017;376(21):2097. doi: 10.1056/NEJMx170003.

Yan J, Choi K, Fu P, Lin J, Gui M, Li Y, Luo L, Hu Z, Bu B, Li Z. Sleep disturbances in patients with myasthenia gravis: A cross-sectional study. Neurologia (Engl Ed). 2026;41(1):501925. doi: 10.1016/j.nrleng.2025.501925.

Yingchoncharoen P, Charoenngam N, Ponvilawan B, Thongpiya J, Chaikijurajai T, Ungprasert P. The Association Between Asthma and Risk of Myasthenia Gravis: A Systematic Review and Meta-analysis. Lung. 2021;199(3):273-280. doi: 10.1007/s00408-021-00444-8.

Yorke J, Moosavi SH, Shuldham C, Jones PW. Quantification of dyspnoea using descriptors: development and initial testing of the Dyspnoea-12. Thorax. 2010;65(1):21-6. doi: 10.1136/thx.2009.118521.

Young CA, Chaouch A, Mcdermott CJ, Al-Chalabi A, Chhetri SK, Talbot K, Harrower T, Orrell RW, Annadale J, Hanemann CO, Scalfari A, Tennant A, Mills R; TONIC-ALS STUDY GROUP. Dyspnea (breathlessness) in amyotrophic lateral sclerosis/motor neuron disease: prevalence, progression, severity, and correlates. Amyotroph Lateral Scler Frontotemporal Degener. 2024;25(5-6):475-485. doi: 10.1080/21678421.2024.2322545.

Youssef N, Sheik CS, Krumholz LR, Najar FZ, Roe BA, Elshahed MS. Comparison of species richness estimates obtained using nearly complete fragments and simulated pyrosequencing-generated fragments in 16S rRNA gene-based environmental surveys. Appl Environ Microbiol. 2009;75(16):5227-36. doi: 10.1128/AEM.00592-09.

Zhang H, Li Y, Zheng P, Wu J, Huang Y, Tan X, Hu X, Wen L, Xie P, Zhou X, Yu G, Zhao L, Zhou C, Fang L, Xie P. Altered Metabolism of the Microbiota-Gut-Brain Axis Is Linked With Comorbid Anxiety in Fecal Recipient Mice of Myasthenia Gravis. Front Microbiol. 2022;13:804537. doi: 10.3389/fmicb.2022.804537.

Zhang Y, Thompson KN, Branck T, Yan Yan, Nguyen LH, Franzosa EA, Huttenhower C. Metatranscriptomics for the Human Microbiome and Microbial Community Functional Profiling. Annu Rev Biomed Data Sci. 2021;4:279-311. doi: 10.1146/annurev-biodatasci-031121-103035.

Zhao L, Luo JL, Ali MK, Spiekerkoetter E, Nicolls MR. The Human Respiratory Microbiome: Current Understandings and Future Directions. Am J Respir Cell Mol Biol. 2023;68(3):245-255. doi: 10.1165/rcmb.2022-0208TR.

Zhou X, Wu Y, Zhu Z, Lu C, Zhang C, Zeng L, Xie F, Zhang L, Zhou F. Mucosal immune response in biology, disease prevention and treatment. Signal Transduct Target Ther. 2025;10(1):7. doi: 10.1038/s41392-024-02043-4.

Zhu W, Chen Y, Tian C, Wang J, Wen X, Lou H, Jiang Y, Di L, Gu S, Wang Y, Wang Y, Lu Y, Wang M, Xu M, Chen H, Zhao Y, Qi G, Da Y. Factors associated with early response to efgartigimod in patients with generalized myasthenia gravis: a multicenter retrospective observational study. J Neurol. 2025;272(9):622. doi: 10.1007/s00415-025-13367-8.

Zieda A, Ravina K, Glazere I, et al. A nationwide epidemiological study of myasthenia gravis in Latvia. Eur J Neurol 2018;25: 519–26.

Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67(6):361-70. doi: 10.1111/j.1600-0447.1983.tb09716.x.