Received: 8 October 2020 | Accepted: 12 July 2021

DOI: 10.1111/jvim.16223

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Clinical features and outcome of acquired myasthenia gravis in 94 dogs

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Abstract

Background: Factors known to be associated with outcome of acquired myasthenia gravis (MG) in dogs are limited.

Hypothesis/Objectives: Of dogs with MG, advancing age and comorbid neoplasia are associated with poor long-term prognosis and low rates of remission.

Animals: Ninety-four client-owned dogs with MG diagnosed by acetylcholine receptor antibody (AChR Ab) assay between 2001 and 2019 from a university clinic and 3 private clinics in the United States.

Methods: Cases were retrospectively evaluated and data were collected to determine clinical signs, treatment, and response to therapy defined by means of a clinical scoring rubric. Immunological remission was defined as a return of the AChR Ab concentration to <0.6 nmol/L. Multivariable binary logistic regression analysis was used to identify clinical criteria predicting remission.

Results: An anticholinesterase drug was used to treat 90/94 (96%) dogs, which in 63/94 (67%) was the sole treatment; other drugs included immune modulators. Clinical remission (lack of clinical signs ≥4 weeks after treatment cessation) was observed in 29 (31% [95% confidence interval (CI): 22.4-40.8%]) dogs, clinical response (lack of clinical signs on treatment) in 14 (15% [95% CI: 9.0-23.6%]) dogs, clinical improvement (on treatment) in 24 (26% [95% CI: 17.8-35.2%]) dogs, and no clinical improvement in 27 (29% [95% CI: 20.5-38.6%]) dogs. Immunological remission was observed in 27/46 (59%) dogs, with

Abbreviations: Ab, antibody; ACh, acetylcholine; AChR, acetylcholine receptor; AD, anticholinesterase drugs; AutoAbs, autoantibodies; ID, immunomodulatory drug(s); IMPA, immune-mediated polyarthropathy; IVIG, intravenous immunoglobulin; ME, megaesophagus; MG, myasthenia gravis; MIR, main immunogenic region; PIMA, precursor-targeted immune-mediated hemolytic anemia.

clinical remission in all 27. Younger age ($P = .04$) and comorbid endocrine disease ($P = .04$) were associated with clinical remission. Initial AChR Ab concentration ($P = .02$) and regurgitation ($P = .04$) were negatively associated with clinical remission.

Conclusions and Clinical Importance: Clinical remission in MG is less likely in older dogs and dogs presenting with regurgitation or high initial AChR Ab concentration, but more likely in younger dogs and dogs with comorbid endocrine disease.

KEYWORDS

acetylcholine, autoimmune, comorbidity, junctionopathy, prognosis, remission

1 | INTRODUCTION

Acquired myasthenia gravis (MG) is 1 of the most commonly recognized neuromuscular diseases of the dog, characterized by the presence of autoantibodies (autoAbs) against nicotinic acetylcholine receptors (AChR) at the postsynaptic membrane.[1,2] The disease in human patients, rodent models, and dogs is driven by complement-fixing autoAbs with specificity for the main immunogenic region (MIR) of the α chain of the pentameric AChR.[3–6] The disease has various manifestations, including a focal form involving the esophagus and/or oropharyngeal muscles, a generalized form involving multiple striated muscles, or both.[7–9] A fulminant form of generalized MG is also observed in dogs.[8,10] The diagnostic criterion standard for MG in dogs is the demonstration of Abs against the AChR by immunoprecipitation radioimmunoassay, which is both a sensitive and specific test.[2,8] While seronegative MG occurs in approximately 2% of dogs with the generalized form, false-positive results are rare.[8]

Anticholinesterase drugs (AD) are used to treat MG, increasing the half-life of ACh in the synaptic cleft.[11,12] Immunosuppressive therapy is also administered in selected cases that do not have associated aspiration pneumonia.[13,14] A study evaluating the natural course of MG in the absence of corticosteroid or other immunosuppressive therapy, following treatment with AD and/or altered feeding procedures, reported a high rate of spontaneous clinical remission (88.7% of cases) occurring at a mean time of 6.4 months (range, 1-18 months) from inception of treatment.[15] Neoplastic comorbidities were associated with poorer outcomes in this study, but there is a general dearth of information in the literature on clinical factors predicting remission in MG. The purpose of the current work was therefore to identify clinical criteria associated with remission of MG in a typical referral population of myasthenic dogs treated with both anticholinesterase and immune-modulating drugs, including corticosteroids. We hypothesized that older dogs and dogs with comorbid neoplasia would have a poor long-term prognosis associated with low rates of remission.

2 | MATERIALS AND METHODS

2.1 | Data collection

Data from physical and electronic medical records were collected from the Matthew J. Ryan Veterinary Hospital, School of Veterinary Medicine, University of Pennsylvania; Red Bank Veterinary Hospital, Tinton Falls, New Jersey; Pieper Veterinary Hospital, Middletown, Connecticut; and Bush Veterinary Neurology Service, Leesburg, Virginia. All dogs included in the study had an AChR Ab concentration of >0.6 nmol/L, diagnostic for MG, assayed in the Comparative Neuromuscular Laboratory at the University of California, San Diego between 2001 and 2019 inclusive. Cases were only considered if they had a complete medical record that yielded information on signalment, bodyweight, treatment protocol, type of MG, and response to therapy. Thirty-seven of the original 131 cases were excluded because they were euthanized (8/37; 22%) or lost to follow-up either before (2/37; 5%) or very shortly after (5/37; 14%) treatment was initiated, precluding determination of outcome; they were also excluded if their medical record lacked sufficient detail to define treatment and/or outcome information, or was absent altogether (22/37; 60%). Cases were followed based on retrospective data collection from the time they were diagnosed with MG based on AChR Ab concentration to the date in which they were either lost to follow-up, euthanized, or died.

The following information was collected for each individual case: signalment, bodyweight, type of MG (generalized, focal), initial and follow-up AChR Ab concentrations, presence of and time to immunological remission if applicable, presence of megaesophagus (ME) and time to resolution of ME if applicable, presenting clinical signs, treatment type, presence of relapse as defined in Table 1, cause of death or euthanasia, and comorbidities, including the presence of a thymoma. Based on the presenting clinical signs and results of diagnostic imaging, including thoracic radiographs, dogs were characterized as having focal MG if they had ME; generalized MG if they had experienced weakness but no ME; and generalized MG with ME if they had a combination of the 2. Radiographic interpretation of the initial diagnosis of ME and the resolution of ME was made based on written radiographic reports and assessments from either board-certified radiologists, neurologists, and/or general veterinary practitioners. Treatment types were defined as treatment with AD alone, immunomodulatory drugs (ID) alone, a combination of AD and ID, or other, including thymectomy or other medical treatment. All dogs with comorbidities were also identified, including those with evidence of a thymoma or mediastinal mass, other neoplasms, other immune-mediated, neurologic, systemic, or orthopedic disease, or an endocrinopathy.

TABLE 1 Clinical scoring rubric used to categorize myasthenic dogs' response to treatment (clinical group)

	Clinical signs (by scores)	Period	Treatment
No clinical improvement	No resolution or improvement	≥1 week, unless euthanized or died	On any type of treatment
Clinical improvement	Migration to a lower severity category (down to and including mild)	≥1 week	Still on any type of treatment
Clinical response	Migration to category score = 0	≥4 weeks	Still on acetylcholinesterase inhibitor and/or immunosuppressive treatment
Clinical remission	Migration to category score = 0, or migration of AChR Ab to <0.6 nmol/L	≥4 weeks	After cession of all treatment if migration to category score 0
Relapse	Migration up from 0, after ≥4 weeks, to any other category, or if AChR Ab concentration increased from <0.6 nmol/L to >0.6 nmol/L	≥1 week	On or off treatment

Notes: Using the findings from Table 2 (Muscle Weakness Scoring System), the duration of response to treatment was used to place individuals into a clinical group. For the purposes of analysis, 1 week was defined as a duration of 7 days.

TABLE 2 Muscle Weakness Scoring System to assess clinical status of dogs with myasthenia gravis

	0	1	2	3
Ambulation	Normal	Weakens after >3 steps	Weakens after <3 steps	Unable to maintain sternal recumbency or hold head up
Breathing	Normal	Shortness of breath with exertion	Shortness of breath at rest	Ventilation required
Chewing	Normal	Fatigues with solid food	Fatigues with soft food	Feeding tube or parenteral nutrition required
Swallowing	Normal	Rare episode of choking, gagging, or trouble swallowing	Frequent trouble swallowing or gagging, necessitating changes in diet	Feeding tube or parenteral nutrition required
Score	Normal (0)	Mild (1-4)	Moderate (5-8)	Severe (9-12)

Notes: The Muscle Weakness Scoring System was used to calculate individual patient scores before and after treatment for MG to determine their place in the clinical scoring rubric and thus their clinical group (Table 1).

2.2 | Clinical scoring rubric

A muscle weakness scoring rubric was created to categorize each dog's response to treatment, assessing individual clinical signs over the sum of the documented treatment period (Table 2). The treatment period was defined from the date of diagnosis to the point at which the dog was last evaluated or the last owner communication occurred. Categories of response based on the scoring system were defined as clinical remission if they reached a category score of 0 (normal/no clinical signs) from another score after cession of all treatment for ≥4 weeks; clinical response if they reached a category score of 0 while still receiving acetylcholinesterase inhibitor and/or immunosuppressive treatment for ≥4 weeks; clinical improvement if they migrated to a lower category score (down to and including mild) while still receiving any treatment for ≥1 week; or no clinical improvement if they failed to show migration to a lower category score for a period of ≥1 week, or were euthanized or died at any time after failing to migrate to a lower category score, regardless of treatment (Table 1). Immunological remission was defined by a decrease in AChR Ab concentration to <0.6 nmol/L. In addition, dogs were placed in the category relapse if they experienced a return of clinical signs after a previous clinical score of 0 for ≥4 weeks, or if the AChR Ab concentration increased from <0.6 to >0.6 nmol/L for ≥1 week on or off treatment.

2.3 | Statistical analysis

All data were analyzed using R software (version 3.6.1). Summary statistics are presented as median (minimum, maximum) for numerical variables, and frequency (percentage) for categorical variables. Dogs' signalment, clinical signs, initial AChR Ab concentration, and comorbidities were used to predict outcome. Univariable and multivariable binary logistic regression analysis was used to evaluate predictors of lack of clinical remission. In addition, outcome was coded as clinical remission = 1, clinical response = 2, clinical improvement = 3 and no clinical improvement = 4. Univariable and multivariable ordinal logistic regression analysis was used to evaluate predictors of worse response to treatment. Variables with $P < .2$ were evaluated in the multivariable analysis using the backward elimination method. Odds ratios (OR) and their 95% confidence intervals (CIs) are reported. Significance was set at 5% in all analyses.

3 | RESULTS

Ninety-four of 131 dogs satisfied our inclusion criteria, including 21 cases from the University of Pennsylvania, 15 cases from Red Bank, 24 cases from Pieper Veterinary, and 34 cases from Bush Neurology (Supplementary Table 1). Of the 94 dogs, 45 (48%) were spayed females, 45 (48%) were neutered males, 2 (2%) were intact females, and 2 (2%) were intact males. Thirty-two different breeds were represented, of which the 5 most common were mixed breed dogs (MBD; 25), Labrador Retrievers (12), Golden Retrievers (7), Boxers (5), and Pitbull Terriers (5). The median and mean age at presentation was 6 years (8 months to 14 years). Figure 1 shows the age distribution for individual response to treatment, revealing that younger animals were more likely to be represented in the clinical remission group compared to the no clinical improvement group. Presenting clinical signs were defined as weakness, regurgitation, coughing, vomiting, ptyalism, dysphagia, collapse, or facial nerve weakness/paralysis (Table 3).

Based on the sum of the treatment period available in individual medical records, each dog's individual response to treatment was defined using the Muscle Weakness Scoring System. Dogs were evaluated for a total of 3 to 3861 days, over a median (mean) duration of 169 (451) days (Figure 2). Clinical remission was observed in 29/94 (31% [95% CI: 22.4-40.8%]) dogs, clinical response in 14/94 (15% [95% CI: 9.0-23.6%]) dogs, clinical improvement in 24/94 (26% [95% CI: 17.8-35.2%]) dogs, and no clinical improvement in 27/94 (29% [95% CI: 20.5-38.6%]) dogs. Relapse was observed in 3/94 (3%) dogs.

Fifty-seven (61%) of the 94 dogs had both generalized MG and ME, 26/94 (28%) dogs had solely generalized MG, and 11/94 (12%) dogs had focal MG with ME only. Comorbid neoplasms included thymoma in 10/94 (11%) dogs (1 of which also had 2 pulmonary masses), and other or unknown neoplasia in 5/94 (5%) dogs, including 2 with a current or historical mast cell tumor, and 1 case each of cranial mediastinal mass (unspecified), adrenal mass, and pulmonary mass (unspecified). Comorbid neurologic disease or neurologic manifestations were observed in 11/94 (12%) dogs, and included seizures (5), idiopathic epilepsy (1), spinal cord disease (2), and laryngeal paralysis (3). Comorbid endocrine disease was observed in 8/94 (9%) dogs, and included hypothyroidism (6), diabetes mellitus (1), and hyperadrenocorticism (1). Method of diagnosis of endocrine diseases was not specified in the data collected for each individual case. Systemic disease was observed in 8/94 (9%) dogs, including suspected allergic skin disease (2), of which 1 dog also had a history of pyloric stenosis; urinary tract infection (UTI) and a nonspecific arrhythmia (1); and 1 each with a history of collapsing trachea, chronic diarrhea, inflammatory bowel disease, neosporosis, and campylobacteriosis. Comorbid immune-mediated diseases were observed in 4/94 (4%) dogs, including 2 with current or historical masticatory myositis, 1 with pemphigus foliaceus, and 1 with a history of both immune-mediated polyarthritis, and precursor-targeted immune-mediated anemia (the same dog also had a history of neosporosis). Finally, 4/94 (4%) dogs had comorbid orthopedic diseases, including hip dysplasia (2) and cruciate ligament disease (2) (Table 3 and Figure 3).

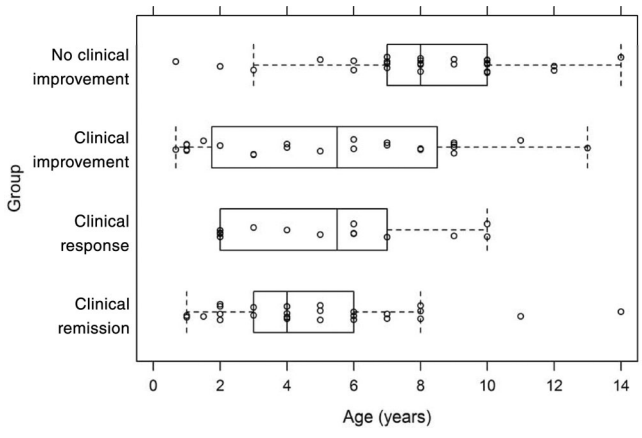

FIGURE 1 Age in relation to clinical group of myasthenia gravis. The distribution of ages in each clinical group is shown, the ends of the box representing the upper and lower quartiles; median age is marked by the vertical line inside the box. The length of whiskers represents either 1.5 times the interquartile range, or the range from minimum to maximum, whichever is shorter. Younger animals were more likely to be represented in the clinical remission group compared to the no clinical improvement group

Most dogs were treated with AD (90/94, 96%); of those, 60/94 (64%) dogs were treated with AD alone. Fifteen (15/94; 16%) dogs in total were treated with prednisone, most often in combination with AD (11/94, 12%). The corticosteroid doses administered to dogs in this study were predominantly anti-inflammatory (0.5-1.0 mg/kg/day; 12/15, 80%). One dog (1/15, 7%) was administered an intermediate dose between anti-inflammatory and immunosuppressive (1.5 mg/kg/day), 1 (7%) received an immunosuppressive dose (≥2 mg/kg/day), and 1 (7%) received an unknown dose. Other treatments included a combination of AD with other ID (cyclosporine, azathioprine, or mycophenolate; 12/94, 13%); AD, prednisone, and ID (cyclosporine, azathioprine, or mycophenolate; 1/94, 1%); AD and prednisone with thymectomy in the case of thymoma (3/94, 3%); AD with thymectomy in the case of thymoma (2/94, 2%); ID alone (2/94, 2%); and 1 each of thymectomy alone, mycophenolate with 2 human intravenous immunoglobulin infusions, and chemotherapy drugs alone (carboplatin and toceranib in a dog with pulmonary and cranial mediastinal neoplasia).

Baseline AChR Ab concentrations for each of the clinical groups are depicted in Figure 4. Forty-six of 94 (49%) dogs had follow-up AChR Ab assays, which yielded subsequent normal values (representing immunological remission) in 27/46 (59%) dogs. AChR assays were repeated in 36 dogs showing lower, but not yet normal, AChR Ab concentrations, of which 29/36 (81%) subsequently decreased to normal. Individual changes from baseline to follow-up AChR Ab concentration for each of the clinical score categories are presented in Figure 5. The median (mean) time to clinical remission from diagnosis was 220 (355) days (range, 61-1065 days). Thirty-five of 68 (51%) cases received follow-up thoracic radiographs after diagnosis of ME at varying frequencies and timepoints, ranging from 1 week to 39 months after initial diagnostic radiographs. Radiographic resolution of ME was documented in 10/68 (15%) dogs with ME (10/35 [29%]

TABLE 3 Synopsis of myasthenia gravis dogs by clinical group

	Clinical remission (n = 29)	Clinical response (n = 14)	Clinical improvement (n = 24)	No clinical improvement (n = 27)
Signalment				
Median age (years)	4.0 (1.0, 14.0)	5.5 (2.0, 10.0)	5.5 (0.7, 13.0)	8 (0.7, 14.0)
Female : male	16 : 13	4 : 10	11 : 13	16 : 11
Altered : intact	28 : 1	13 : 1	22 : 2	27 : 0
Median weight (kg)	27.9 (3.8, 72.7)	11.5 (3.2, 37.0)	23.7 (4.3, 43.7)	28.0 (1.7, 56.6)
Breeds	MBD: 7 Labrador: 5 Collie: 2 Golden Retriever: 2 Other breeds x1: 13	MBD: 6 Other breeds x1: 8	MBD: 6 GSD: 3 Labrador: 2 Golden Retriever: 2 Dachshund: 2 Boxer: 2 Pitbull: 2 Other breeds x1: 5	MBD: 6 Labrador: 4 Golden Retriever: 3 Boxer: 3 Cocker Spaniel: 2 Other breeds x1: 9
Clinical signs				
Weakness	24 (83%)	11 (79%)	22 (92%)	18 (67%)
Regurgitation	7 (24%)	7 (50%)	9 (38%)	11 (41%)
Coughing	5 (17%)	1 (7%)	3 (13%)	2 (7%)
Vomiting	1 (3%)	1 (7%)	2 (8%)	3 (11%)
Ptyalism/hypersalivation	0	0	0	3 (11%)
Dysphagia	1 (3%)	0	1 (4%)	1 (4%)
Collapse	3 (10%)	1 (7%)	0	1 (4%)
Facial nerve weakness/ paralysis	1 (3%)	1 (7%)	3 (13%)	1 (4%)
Initial AChR Ab concentration (nmol/L)	1.67 (0.51, 8.28)	2.26 (0.74, 7.84)	2.47 (0.75, 6.89)	3.54 (0.78, 10.17)
Type of MG				
Generalized only	12 (39%)	1 (8%)	6 (25%)	7 (26%)
Focal (megaesophagus only)	3 (14%)	3 (23%)	1 (6%)	4 (19%)
Generalized + megaesophagus	14 (70%)	10 (83%)	17 (94%)	16 (76%)
Comorbidities				
Endocrine dz	5 (17%)	0	1 (4%)	2 (7%)
Other immune-mediated dz	2 (7%)	1 (7%)	0	1 (4%)
Neurologic dz	3 (10%)	1 (7%)	3 (13%)	1 (4%)
Neoplasia	4 (14%)	2 (14%)	2 (8%)	3 (11%)
Cranial mediastinal mass	1 (3%)	0	0	0
Thymoma	2 (7%)	2 (14%)	1 (4%)	5 (19%)
Other systemic dz	2 (7%)	1 (7%)	1 (4%)	3 (11%)
Orthopedic dz	2 (7%)	0	0	2 (7%)
Treatments				
AD alone	19 (65.5%)	8 (57.1%)	12 (50.0%)	21 (77.8%)
ID alone	0	0	1 (4.2%)	1 (3.7%)
AD + ID	9 (31.0%)	4 (28.6%)	9 (37.5%)	2 (7.4%)
Other	1 (3.4%)	2 (14.3%)	2 (8.3%)	3 (11.1%)

Abbreviations: Ab, antibody; AChR, acetylcholine receptor; AD, anticholinesterase drug(s); ID, immunomodulatory drug; dz, disease; GSD, German Shepherd Dog; ID, immunomodulatory drug(s); MBD, mixed breed dog; MG, myasthenia gravis.

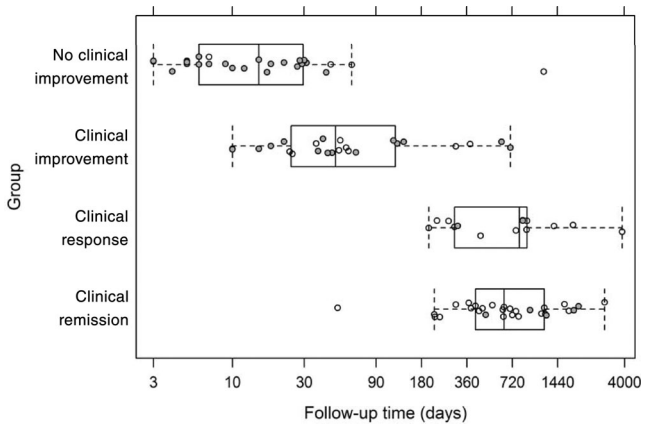

FIGURE 2 Duration of follow-up in relation to clinical group of myasthenia gravis. The distribution of follow-up durations in each clinical group is shown, the ends of the box representing the upper and lower quartiles; median age is marked by the vertical line inside the box. The length of whiskers represents either 1.5 times the interquartile range, or the range from minimum to maximum, whichever is shorter. Follow-up time is represented by a logarithmic *x* axis for clarity. Dogs that were euthanized or died are represented by ; those lost to follow-up are represented by white dots

of those with follow-up thoracic radiographs), at a median (mean) of 86 (169) days from diagnosis (range, 24-666 days). Twenty-six of 94 (28%) dogs were euthanized and 7/94 (7%) dogs died from MG-related causes, with aspiration pneumonia (16/33; 48.5%) being the most common cause of death or euthanasia.

Univariable binary logistic regression analysis identified age (OR [95% CI]: 1.15 [1-1.33]; $P = .04$) and initial AChR Ab concentration 1.37 [1.09-1.84]; $P = .006$) as being negatively associated with clinical remission (Supplementary Table 2). In contrast, the presence of endocrine disease was positively associated with clinical remission (0.23 [0.04-1.02]; $P = .05$). Additional factors with a *P* value of <.2 were regurgitation (2.23 [0.87-6.32]; $P = .10$), collapse (0.28 [0.03-1.75]; $P = .17$) and bodyweight (0.97 [0.94-1.01]; $P = .12$). All other factors were associated with *P* values of >.2 (Supplementary Table 2). Univariable ordinal logistic regression analysis identified only age (1.21 [1.08-1.37]; $P = .0009$) and initial AChR Ab concentration (1.36 [1.14-1.64]; $P = .0004$) as significant factors (Supplementary Table 2). Endocrine disease (0.35 [0.07-1.53]; $P = .16$) and collapse (0.28 [0.04-1.63]; $P = .16$) were also associated with *P* values of <.2; all other factors were associated with *P* values of >.2 (Supplementary Table 2). There was not a significant association between the 4 treatments (AD, AD + ID, ID or Other) and either clinical remission ($P = .33$) or worse outcome ($P = .24$).

Multivariable binary logistic regression analysis confirmed that age, regurgitation, and initial AChR Ab concentration were all independent negative predictors of clinical remission, in contrast to the presence of endocrine disease, which was an independent positive predictor of clinical remission (Table 4). Multivariable ordinal regression analysis identified age and initial AChR Ab concentration as independent negative predictors of better outcome; regurgitation and endocrine disease did not achieve significance in this analysis (Table 4). Of note, there was no association—positive or negative—between neoplasia in general, or thymoma in particular, and clinical remission. Variables influencing relapse could not be analyzed because only 3 dogs relapsed in the study sample.

When the sample of myasthenic dogs treated only with AD was considered as a separate group, concordant observations were made. Univariable binary logistic regression analysis identified age (1.25 [1.04-1.53]; $P = .02$) as a significant factor, and initial AChR Ab concentration (1.29 [0.99-1.80]; $P = .09$), collapse (0.13 [0.01-1.13]; $P = .09$), and coughing (0.30 [0.07-1.30]; $P = .11$) as factors with *P* values of <.2 (Supplementary Table 3). Univariable ordinal regression analysis identified age (1.29 [1.11-1.53]; $P = .0007$), initial AChR Ab concentration (1.47 [1.16-1.94]; $P = .0009$), and collapse (0.11 [0.01-.79]; $P = .03$) as significant factors, and coughing (0.35 [0.08-1.34]; $P = .12$) as a factor with a *P* value of <.2 (Supplementary Table 3). Multivariable logistic regression analysis identified age as an independent negative predictor of clinical remission (binary analysis) and better outcome in general (ordinal analysis; Table 5). Initial AChR Ab concentration was an independent negative predictor, and collapse an independent positive predictor, of better outcome (Table 5).

4 | DISCUSSION

This study analyses the factors associated with outcome of MG in dogs. Clinical remission was experienced in 31% of the study sample, reflecting our anecdotal impressions in a referral context. These dogs had all last received treatments of any nature at least 4 weeks before their normal clinical assessments or had reached immunological remission with an AChR Ab concentration decreasing to <0.6 nmol/L. Nevertheless, the impact of type of treatment on their clinical trajectories remains an important unanswered question. In particular, the risks and benefits of corticosteroid and other ID needs to be carefully weighed up in individual cases, not only because of the possibility of concurrent aspiration pneumonia but also because routine immunosuppressive doses of prednisolone or prednisone tend to exacerbate the clinical manifestations of neuromuscular disease by contributing to weakness and muscle atrophy.[16–18] The majority of dogs in the current study receiving corticosteroids were treated by specialist neurologists administering cautious, predominantly anti-inflammatory doses.

Of the dogs for which multiple AChR Ab assays were submitted, 59% experienced immunological remission. Not all dogs had follow-up AChR Ab assays, precluding definitive statements on the rate of immunological remission, but 27 of the 29 dogs experiencing clinical remission that were retested also experienced immunological remission. An immunological remission rate in dogs with MG of almost 90% has been previously recorded and confirmed on the basis of AChR Ab titers within the reference interval.[15] Clinical and immunological remission was attained with a mean of 4.1 and 6.4 months, respectively, compared to the median of 7.2 (mean 11.7) months to reach immunological remission in the current cohort of dogs. However, the 2 studies are fundamentally different, precluding direct comparison of

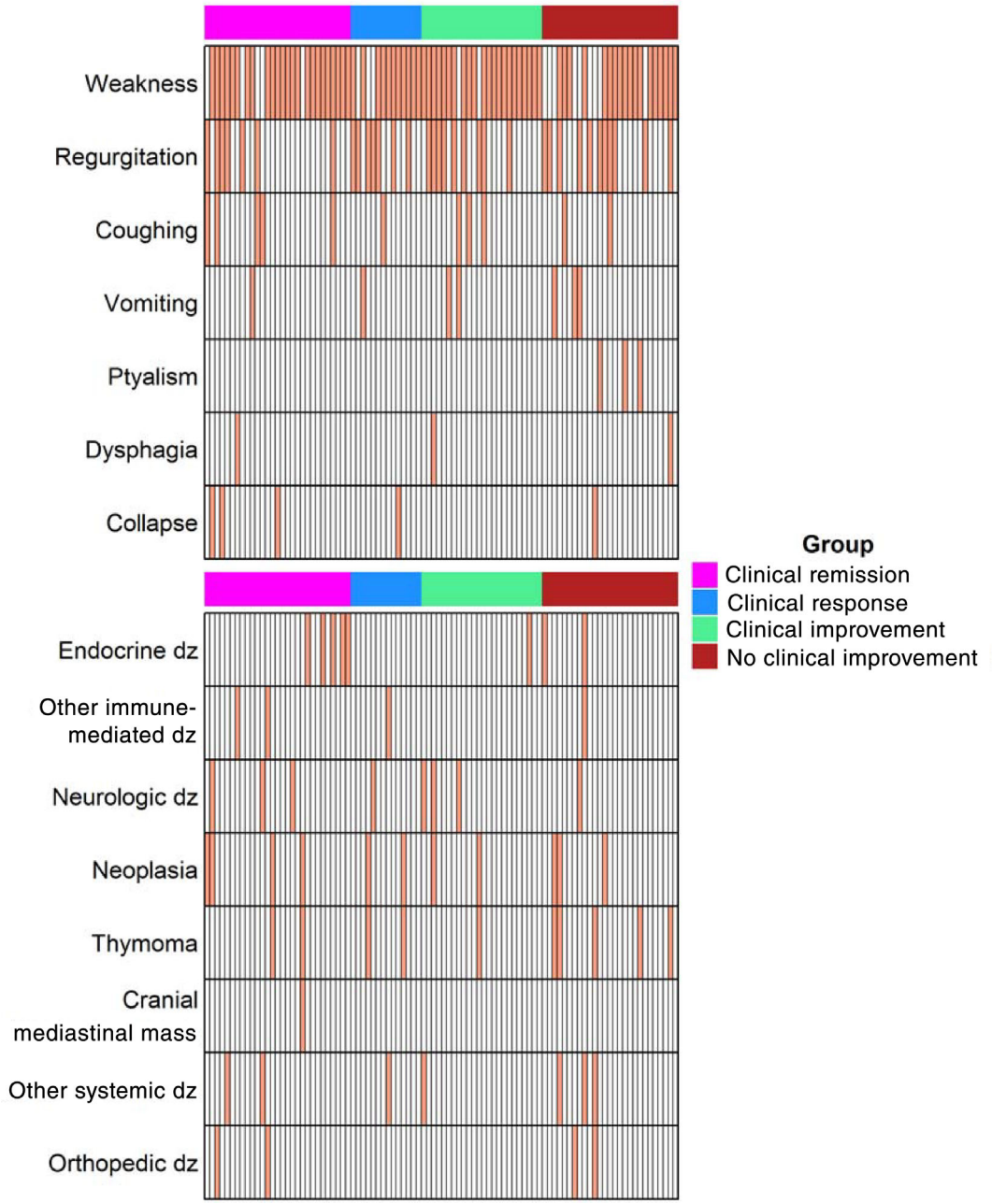

FIGURE 3 Distribution of presenting clinical signs and comorbidities with clinical group of myasthenia gravis. The clinical scoring groups are organized by colored bars, shaded areas below the bars representing the cases within that group with the corresponding presenting clinical signs (upper rows) or comorbidities (lower rows). dz, disease

remission rates. The specific aim of the 2001 study[15] was to evaluate the natural course of disease in the absence of immunosuppression; dogs were excluded if they received corticosteroids or other immunosuppressive drugs. In contrast, our study included a more heterogeneous sample of dogs, some of which received ID. Chronic prednisone therapy could have induced steroid myopathy in a percentage of our dogs, influencing outcome or clinical scoring. An earlier, smaller retrospective study[10] yielded outcomes similar to ours. In that study, 12/25 (48%) dogs were euthanized or died from poor response to treatment, compared to the current study in which 33/94 (35%) dogs were euthanized or died. Within the previous case sample, ME was found in 21/25 (84%) dogs, compared with our 68/94 (72%).

The age range of our current sample of myasthenic dogs was similar to that in previous studies.[7,10] However, in previous retrospective studies female dogs outnumbered male dogs,[10,15,19] in contrast to the even distribution of both sexes present in this cohort. Many autoimmune diseases, including MG, more commonly affect females,[19,20] but the spayed status of most of our dogs calls into question the relevance of hormonal influences. Labradors and Golden Retrievers appeared to be overrepresented, consistent with previous studies that

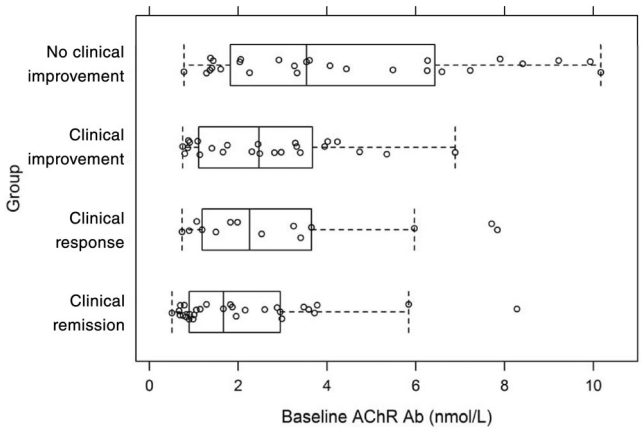

FIGURE 4 Baseline acetylcholine receptor antibody concentration in relation to clinical group of myasthenia gravis. The distribution of AChR Ab concentrations in each clinical group is shown, the ends of the box representing the upper and lower quartiles; the median AChR Ab concentration is marked by the vertical line inside the box. The length of whiskers represents either 1.5 times the interquartile range, or the range from minimum to maximum, whichever is shorter. The no clinical improvement group had higher baseline AChR Ab concentrations than the clinical remission group. Ab, antibody; AChR, acetylcholine receptor

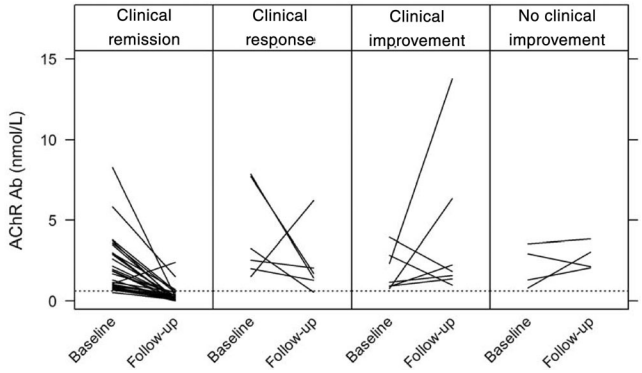

FIGURE 5 Baseline and follow-up acetylcholine receptor antibody concentrations in individual dogs across the clinical groups of myasthenia gravis. Each line represents the baseline and follow-up AChR Ab concentration for dogs within the respective groups. The dotted line represents the upper reference limit of AChR Ab concentration (0.6 nmol/L). Ab, antibody; AChR, acetylcholine receptor

have found these breeds to be at higher prevalence of developing MG,[7,10,21] but normalization to a control sample of hospital dogs was not undertaken to account for the popularity of these breeds. The novel information conveyed in the current study was the evaluation of canine clinical characteristics in the context of remission, of importance because factors predisposing to autoimmune disease and those promoting its progression are not necessarily the same.[14,19]

Younger age, absence of regurgitation, the presence of comorbid endocrine disease, and lower initial concentration of AChR Abs were independent positive prognostic factors in our cohort of myasthenic dogs, associated with a better clinical outcome and clinical remission (Table 4 and Figure 1). Largely concordant conclusions were made if the dogs treated with AD alone were considered as a separate group, suggesting that these outcomes were not predicated on the administration of ID (Table 5). Acquired MG has a bimodal age distribution in both humans and dogs,[11,19] with increasing incidence in older human patients,[22] but it can occur at any age. The age of dogs in the current study was broad, ranging from 8 months to 14 years (Table 3). The association of younger age with a higher rate of remission was an expected finding based on the premise that younger dogs would have fewer underlying comorbidities, especially those of neoplastic etiology. Supportive treatment, in some cases coupled with ID, presumably allows a window of opportunity for regulation and mitigation, or extinction, of autoaggressive immune responses in the absence of death from more serious comorbidities. In human patients with MG, studies have revealed conflicting data on optimal outcome and age of MG onset, but most recent studies have revealed a better outcome with older age.[23,24] However, most data on outcome in relation to age in human MG pertain to MG associated with thymoma or thymectomy.[22,25]

Regurgitation as a presenting clinical sign negatively predicted remission in this study, thought to reflect the likelihood of underlying ME and aspiration pneumonia, a potentially fatal complication of this disease in dogs.[10,11] Megaesophagus is uncommon in human myasthenic patients.[26] The prevalence of dogs with MG presenting with ME is reported to be ~90%,[7] a greater proportion than observed in our study (72%). The cause of this manifestation in the focal subtype of MG is unknown[21] and the prognosis of ME in dogs is generally poor[27]; however, a recent study found a better outcome in dogs with MG and ME, with a mortality rate of 33% to 40%, concordant with previous studies.[10,27,28] The morbidity and death in dogs with ME would reduce the probability of ultimate remission, underlining the need for aggressive treatment to prevent regurgitation and aspiration pneumonia. Further studies are required to determine an optimal treatment protocol for dogs with ME before they develop aspiration pneumonia, addressing whether ID are indicated in these cases.

A positive association between the presence of an endocrinopathy and likelihood of remission was an unexpected finding (Figure 3), though the weight of this conclusion is unclear given the unknown circumstances of diagnosis of endocrine disease in this group. In this study sample, 8/94 (9%) dogs were diagnosed with an endocrinopathy before diagnosis of MG. Six of these 8 (75%) dogs had a diagnosis of hypothyroidism. MG has been associated with hypothyroidism in dogs[12,29] and in human patients.[30] Thyroid disorders causing both hypothyroidism and hyperthyroidism are more frequently associated with MG compared to other autoimmune diseases,[14,30,31] present in about 5% to 10% of human patients with MG.[32] The association of thyroid autoimmunity and MG is likely to be multifactorial, predicated on shared trigger factors, common defects in regulation, and other unknown variables.[30,33] In human patients, the early-onset and ocular subgroups of MG are most frequently associated with thyroid disease or thyroiditis.[22,32] The relevance of this observation to canines, in which these clinical phenotypes are not

TABLE 4 Multivariable logistic regression analysis evaluating predictors of clinical outcome and lack of remission of myasthenia gravis

	Binary		Ordinal[a]	
Risk factor	**OR [95% CI]**	***P***	**OR [95% CI]**	***P***
Age (years)	1.18 [1.01-1.16]	.04	1.17 [1.03-1.32]	.009
Regurgitation	2.93 [1.04-9.13]	.04	Not significant	
Initial AChR Ab concentration (nmol/L)	1.31 [1.02-1.80]	.02	1.30 [1.08-1.58]	.004
Endocrine dz	0.17 [0.03-0.91]	.04	Not significant	

[a]Outcome is coded as clinical remission = 1, clinical response = 2, clinical improvement = 3, and no clinical improvement = 4. Older age and increased initial AChR Ab concentration were associated with lack of clinical remission.
Abbreviations: Ab, antibody; AChR, acetylcholine receptor; CI, confidence interval; dz, disease; OR, odds ratio.

TABLE 5 Multivariable logistic regression analysis evaluating predictors of clinical outcome and lack of remission for dogs with myasthenia gravis treated with anticholinesterase drug(s) only

	Binary		Ordinal[a]	
Risk factor	**OR [95% CI]**	***P***	**OR (95% CI)**	***P***
Age (years)	1.25 [1.04-1.53]	.02	1.27 [1.08-1.52]	.003
Initial AChR Ab concentration (nmol/L)	Not significant		1.50 [1.15-2.06]	.002
Collapse	Not significant		0.03 [0.00-0.46]	.01

[a]Outcome is coded as clinical remission = 1, clinical response = 2, clinical improvement = 3, and no clinical improvement = 4. Older age was associated with lack of clinical remission.
Abbreviations: Ab, antibody; AChR, acetylcholine receptor; CI, confidence interval; OR, odds ratio.

recognized as separate entities, remains unclear. While human MG is associated with increased risk for thyroid autoimmunity, its role in prognosis and outcome is not well elucidated,[34] but MG in association with other autoimmune disorders might actually have a less favorable prognosis.[35] In human MG, a higher incidence of autoimmune disease than the normal population has been observed,[22,32] and occurrence of associated autoimmune disease predicts MG relapse.[36] Of note, reliability of the diagnosis of hypothyroidism in our study represents an important confounding variable: sick euthyroidism is sometimes overlooked as a differential diagnosis. Further studies to confirm the potential link between endocrine disease and MG are warranted.

With increasing initial AChR Ab concentration, there was a diminishing likelihood of optimal clinical outcome in this study (Table 4 and Figure 4). This was an unexpected finding given that AChR Ab concentration is generally accepted to have poor correlation to severity of MG in both humans and dogs.[8] Moreover, in human AChR-positive MG cases low concentrations are not associated with a higher probability of an optimal outcome.[24,37] However, individual studies have shown some variation. An increased AChR Ab concentration at the time of diagnosis was associated with a decrease in likelihood of pharmacological remission in another canine retrospective case series.[13] A significantly higher AChR Ab concentration was found in dogs with the acute fulminating form of MG in a second retrospective study.[10] Most of the autoAbs in MG in dogs are directed against the MIR, which is readily accessible in vivo to Abs on the extracellular surface of the AChR.[5,38] Thus demonstration of serum AChR Abs against native AChR by immunoprecipitation radioimmunoassay (RIA) is diagnostic for MG, but Ab concentrations between patients are highly variable, despite correlating with disease severity within individual human patients in some studies.[38,39] Because the criterion standard diagnostic test provides no information on the magnitude or nature of the specific autoimmune response, this is often not resubmitted during the follow-up period after inception of treatment. Nevertheless, the withdrawal of treatment while AChR Ab concentrations are >0.6 nmol/L would be injudicious, and resolution of clinical signs, including ME, is usually accompanied by a return of AChR Ab concentrations to <0.6 nmol/L.[8] Repeated AChR Ab concentrations therefore allow more confident prediction of treatment duration, and in human MG they might be useful as a marker for nonresponse or inadequate immunotherapy[37,40]; an increase in AChR Ab concentration in an MG dog might predict clinical deterioration[40] or a pending thymoma. When comparing all of the dogs with follow-up AChR Ab concentrations, our data also showed that the AChR Ab concentrations generally trended downward for all of the dogs that went into immunological remission (Figure 4), but this trend was not consistently observed in dogs undergoing clinical remission, clinical response, or clinical improvement, underlining the need for better biomarkers of disease severity. Novel, isotype-specific AChR Ab assays, which measure the concentration of complement-fixing pathogenic autoAbs against the MIR of the AChR, might offer the potential to guide treatment in a more objective fashion in both human patients and canines. However, the value of such assays currently remains speculative.

Approximately 10% to 30% percent of human myasthenic patients have a thymoma, with increasing prevalence with age.[22,34,41] While there seemed to be no correlation with age, the prevalence of thymoma (11%) in our study mirrors these data. The association

between the presence of thymoma and MG in dogs is well characterized,[11,42] with paraneoplastic syndromes of MG and ME reported in over 40% dogs with thymoma.[43,44] In human thymoma patients, deficits in intracellular signaling, expression of MHC II molecules, and polymorphisms in immunoregulatory genes within the thymus might lead to failure of central tolerance to AChR antigens, and thus to MG.[40,45,46] The pathophysiology of MG in dogs with thymoma remains unknown; however, some authors suggest that, as in humans, these dogs might mount a humoral immune response against myoid cells of the thymus, which are antigenically similar to the receptor-bearing myocytes at the neuromuscular junction.[40,43] Human thymoma with MG has been associated with a more severe clinical disease and a poorer prognosis than thymomas without MG.[34,40] In a previous study, the presence of thymoma and ME in dogs with MG appeared to predict a poor prognosis,[42] but our data did not reveal any impact of this comorbidity on remission. This might be due, at least in part, to the small number of dogs with a thymoma in this study, increasing the tendency for a type II error. The relationship between thymectomy and remission of MG in dogs with thymoma appears controversial, with different outcomes in different dogs with MG studies.[8,43,44,46] In a recent study performed to elucidate prognostic factors for ocular MG in human patients, younger age of onset postthymectomy was associated with remission.[47] Studies of thymectomy in human MG, with and without thymoma, have also been associated with variable outcomes in patients of different age and forms of MG; some patients have developed MG postthymectomy.[22,25,41,48] In human MG, the general consensus supports thymectomy, especially in patients with thymoma, in generalized and ocular MG; older patients and those with B-type thymomas tend to have worse outcomes.[41,47,49–51] In the current study, not all dogs that underwent thymectomy went into remission or had a positive outcome. Of interest, dogs with MG with a thymoma—as in equivalent human patients—might have autoAbs to titin and ryanodine receptors, which can worsen clinical status.[52]

In the current study, the addition of ID to standard anticholinesterase treatment did not influence outcome (Table 4), and many dogs remained on their respective treatments for months to years. The introduction of ID and impact on clinical course of disease in dogs with MG is still unclear and lacks consensus. Previous clinical studies investigating the addition of different ID to anticholinesterase treatment in MG in dogs have shown mixed results.[11,13,28,53,54] In animal models, evidence suggests that autoAb production against the AChR at the neuromuscular junction is T cell-dependent.[53,55] Dogs with MG might therefore benefit from IDs that target T cell activation, with the caveat that routine immunosuppressive doses of corticosteroids exacerbate clinical manifestations. This is especially true of the weakness accompanying steroid myopathy.[16,17]

Limitations of the current study included the lack of consistent follow-up AChR Ab assays to document immunological remission in an unbiased manner. In addition, not all dogs were followed until death, and several were eventually lost to follow-up. The variable duration of follow-up would also have reduced the power of this study to identify prognostic factors, especially in those dogs euthanized soon after presentation, some of which might have turned a corner if given sufficient time. The study data contained cases from 4 different facilities. Variability in individual clinician assessments of the dogs would have yielded differences in documentation of historical and physical examination findings, as well as other clinical variables required to apply our Muscle Weakness Scoring System and clinical scoring rubric. Both scoring protocols are new instruments that rely on clinician perception to gauge response to treatment, requiring formal validation before they can be more widely used in studies of MG in dogs.

5 | CONCLUSION

The goal of this study was to advance our knowledge of the factors that predict clinical outcome and remission in a typical referral population of myasthenic dogs. Our data suggest that younger dogs presenting without regurgitation and with lower AChR Ab concentrations at the time of diagnosis experience better clinical outcomes in MG, including remission. Comorbid endocrine disease also appears to predict better outcome, with the important caveat that method of diagnosis was often unclear in the study sample, calling into question the reliability of this observation: further, prospective studies are required to corroborate this association and to elucidate the underlying pathomechanisms. We speculate that additional biomarkers of MG in dogs, including isotype-specific assays of complement-fixing pathogenic autoAbs against the MIR, could be useful to guide treatment of this disease by enabling more objective documentation of the resolution of autoimmunity. Such assays might be particularly helpful in those dogs suffering from regurgitation and at risk of aspiration pneumonia.

ACKNOWLEDGMENT

No funding was received for this study.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

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

How to cite this article: Forgash JT, Chang Y-M, Mittelman NS, et al. Clinical features and outcome of acquired myasthenia gravis in 94 dogs. *J Vet Intern Med*. 2021;35(5): 2315-2326. https://doi.org/10.1111/jvim.16223