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# A prospective, placebo controlled study on the humoral immune response to and safety of tetanus revaccination in myasthenia gravis

Ellen Strijbos [a,*], Maartje G. Huijbers [a,b], Inge E. van Es [b], Iris Alleman [c], Monique M. van Ostaijen-ten Dam [d], Jaap Bakker [e], Erik W. van Zwet [f], Cornelia M. Jol-van der Zijde [d], Maarten D. van Tol [d], Jan J. Verschuuren [a]

[a] Department of Neurology, Leiden University Medical Centre, The Netherlands
[b] Department of Human Genetics, Leiden University Medical Centre, The Netherlands
[c] Department of Physiotherapy, Leiden University Medical Centre, The Netherlands
[d] Department of Paediatrics, Laboratory of Immunology, Leiden University Medical Centre, The Netherlands
[e] Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Centre, The Netherlands
[f] Department of Medical Statistics and Bioinformatics, Leiden University Medical Centre, The Netherlands

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ABSTRACT

*Objective:* To investigate the humoral immune response to and safety of a tetanus revaccination in patients with myasthenia gravis or Lambert-Eaton myasthenic syndrome.
*Methods:* A tetanus revaccination was administered to 66 patients. Before and 4 weeks after revaccination a blood sample and clinical outcome scores were obtained. Anti-tetanus IgG total, IgG1 and IgG4 titres were measured with an ELISA and disease-specific antibody titres (AChR, MuSK or VGCC) with a radio-immunoprecipitation assay. A historic healthy control group was used for comparing tetanus antibody titres with that of our patients. A placebo (saline) vaccination group was used to investigate the variability of clinical outcome scores with a 4 weeks interval.
*Results:* In 60 of 65 patients, a significant increase of the anti-tetanus antibody response was measured. Thymectomy did not have an impact on this responsiveness. Patients with immunosuppressive medication had a significantly lower pre and post titre compared to healthy controls, but their response was still significant. The titres of disease-specific antibodies were unchanged 4 weeks after revaccination. The clinical outcome scores showed no exacerbation of symptoms of the disease.
*Conclusion:* A tetanus revaccination in patients with myasthenia gravis or Lambert-Eaton myasthenic syndrome is safe and induces a significant immune response, irrespectively of their immunosuppressive medication. We observed neither immunological nor clinical relevant exacerbations associated with the tetanus revaccination.
*Clinical trial registry:* The tetanus trial is listed on clinicaltrialsregister.eu under 2014-004344-35. The placebo AChR MG group was part of another clinical trial, investigating influenza vaccination in myasthenic patients. This trial is listed on clinicaltrialsregister.eu under 2016-003138-26.


## 1. Introduction

Myasthenia gravis (MG) and the Lambert-Eaton myasthenic syndrome (LEMS) are acquired autoimmune diseases of the neuromuscular junction. The clinical hallmark of MG and LEMS is fluctuating muscle weakness, often in specific muscle groups [1]. The majority of MG patients have acetylcholine receptor (AChR) antibodies. Other antibodies, are found less frequently and are directed to muscle-specific kinase (MuSK) in MuSK MG or to voltage-gated calcium channels (VGCC) in LEMS. A large part of MG and LEMS patients need long-term immunosuppressive medication, because symptomatic treatment is insufficient. Due to the immunosuppres-

*Abbreviations:* AChR, acetylcholine receptor; ELISA, enzyme-linked immunosorbent assay; GMT, geomean titre; HC, healthy controls; IM, immunosuppressive medication; LEMS, Lambert-Eaton myasthenic syndrome; MG, myasthenia gravis; MG-ADL, MG specific Activities of Daily Living; MGC, MG Composite score; MGFA, Myasthenia gravis Foundation America classification; MuSK, muscle-specific kinase; NOACs, new oral anti-coagulants; QMG, Quantitative Myasthenia Gravis score; RIA, radio immunoprecipitation assay; TT, tetanus toxoid; VGCC, voltage-gated calcium channels.

* Corresponding author at: Albinusdreef 2, 2333 ZA, Leiden, The Netherlands.
*E-mail address:* e.strijbos@lumc.nl (E. Strijbos).

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sive therapy, patients have an increased risk of infection [2], which can aggravate the symptoms, sometimes resulting in myasthenic crisis. For some of these infections vaccines are available. An example is the annual influenza vaccination which is recommended for all patients with an autoimmune disease. However, safety and efficacy of vaccination remain matter of debate [2]. Prospective studies in systemic lupus erythematosus and autoimmune vasculitis suggest that vaccination in these autoimmune diseases is effective [3,4] and safe [5]. Little is known about safety and effectiveness of vaccination in myasthenic patients. Tetanus toxoid is a frequently used vaccine with a well-known safety profile and antibody response in healthy individuals as well as in immunocompromised individuals with HIV or after stem cell transplantation [6,7]. Therefore, we choose this vaccine to prospectively investigate the clinical safety and humoral immune response in patients with MG or LEMS.

## 2. Materials and methods

### 2.1. Patients

This study contained 51 patients with AChR MG, 6 patients with MuSK MG, 9 patients with LEMS, a historical control group of 20 healthy individuals (HC group) revaccinated with tetanus toxoid and 23 AChR MG patients injected with a placebo (placebo AChR MG group).

### 2.2. Prospective tetanus vaccination study protocol

This single-centre, prospective, placebo-controlled study was performed at the Leiden University Medical Centre. A group of 66 patients, of whom 51 with AChR MG, 6 with MuSK MG, and 9 with LEMS were revaccinated with tetanus toxoid and 23 AChR MG patients received a placebo, *i.e.,* saline. At day 1 serum was obtained and clinical tests were performed before revaccination. Four weeks thereafter a second serum sample was obtained and the clinical tests were repeated.

Inclusion criteria were a confirmed diagnosis of MG or LEMS, age between 18 and 65 years and stable disease during the past 3 months. Diagnosis of MG or LEMS was based on clinical signs or symptoms suggestive of MG or LEMS and a positive serological test for AChR, MuSK or VGCC antibodies. Patients continued their medication during the study. A maximum daily dose of 30 mg of prednisolone (±5 mg) was allowed as well as the use of other immunosuppressive medication (see Table 1). Time from last pyridostigmine dose to clinical testing was kept constant in one and the same patient on the two test days, but was allowed to vary between patients. Dosage of the immunosuppressive medication had to be stable in the 3 months before revaccination till at least 4 weeks after tetanus revaccination.

The exclusion criteria were: instable disease based on medication use or a Myasthenia gravis Foundation America classification (MGFA) classification of 4 or 5, presence of a thymoma, use of vitamin K antagonist or new oral anti-coagulants (NOACs), other relevant immunosuppressive/secondary immunodeficiency conditions (not applicable on screened patients), pregnancy, no previous tetanus vaccination or tetanus revaccination in the past year.

### 2.3. Placebo AChR MG group

Twenty-three AChR MG patients were intramuscularly injected with a placebo (saline). These patients fulfilled the same in- and exclusion criteria and completed the same clinical outcome scores (Quantitative Myasthenia Gravis (QMG) score, MG composite (MGC) score and the MG specific activities of daily living (MG-ADL)) at the same time points, before and 4 weeks after receiving placebo.

### 2.4. Sampling protocol and clinical scoring

The QMG, MGC and the MG-ADL are the clinical outcome measures that were used. The QMG is a 13-item scale that measures muscle strength and endurance. The MGC is a composite scale selected from existing MG-specific scales (MG-ADL, QMG and Manual Muscle Test). The MG-ADL is a scale to assess MG symptoms that patients experience in their daily activities. For all three outcome measures, higher scores indicate more severe clinical MG [8–12]. These three clinical outcome scores were performed before and 4 weeks after tetanus revaccination. The MG-ADL was repeated by the physician by telephone at 12 weeks after revaccination.

### 2.5. Tetanus vaccine

A commercially available tetanus vaccine was used, manufactured by Bilthoven Biologicals (tetanus vaccine, RVG 17639) [13].

**Table 1**
Baseline characteristics.

| | AChR MG | MuSK MG | LEMS | Total | (%) |
|---|---|---|---|---|---|
| Number of patients | 50 | 6 | 9 | 65 | |
| Gender, female (%) | 37 | 3 | 6 | 46 | (70.7) |
| Age, median years (range) | 56 | 44.5 | 49.3 | 55 | (21–65) |
| Duration of disease, mean years (SD) | 14.6 | 5.5 | 9.7 | 13.1 | (11.9) |
| MGFA classification* | | | | | |
| 0 (%) | 4 | 3 | 2 | 9 | (13,8) |
| 1 (%) | 4 | 1 | 0 | 5 | (7,7) |
| 2 (%) | 40 | 2 | 5 | 47 | (72,3) |
| 3 (%) | 2 | 0 | 2 | 4 | (6,2) |
| Use of immunosuppressive medication, % | 46 | 83.3 | 44.4 | 49.2 | |
| Prednisolone, % | 14 | 16.7 | 33.3 | 16.9 | |
| *Mean daily dose, mg (range)* | 10.3 | 7.5 | 7.5 | | (0–15) |
| Azathioprine, % | 30 | 33.3 | 22.2 | 29.2 | |
| *Mean daily dose, mg (range)* | 108 | 75 | 125 | | (25–200) |
| Mycophenolic acid, % | 4 | 33.3 | 11.1 | 7.7 | |
| *Mean daily dose, mg (range)* | 1250 | 750 | 1500 | | (500–2000) |
| Cyclosporine, % | 6 | 0 | 0 | 4.6 | |
| *Mean daily dose, mg (range)* | 140 | 0 | 0 | | (75–200) |
| Combination of immunosuppressive medication, % | 18 | 16.7 | 33.3 | 20 | |
| Thymectomy in the past (>1 year ago, N) (%) | 29 | 0 | 0 | 29 | (44.6) |
| Last tetanus vaccination, years ago (SD) | 26.4 | 13.5 | 24.1 | 24.9 | (19.5) |

* MGFA classification: Myasthenia gravis foundation America classification.

One dose of 0.5 mL contains ≥40 IU tetanus toxoid (TT), 1.5 mg aluminium phosphate and 0.05 mg thimerosal. Administration was intramuscularly, as a bolus, in the non-dominant upper arm.

### 2.6. Tetanus antibody response

IgG1, IgG4 and IgG total tetanus antibodies were quantified using a previously described ELISA [7], with the exception of using tetanus toxoid (NIBSC 02/232, National Institute for Biological Standards and Control, London, UK) for coating and the World Health Organization (WHO) 1th international standard for tetanus immunoglobulin (NIBSC TE3) for calibrating of the quantification. Titres were measured in serum samples taken at the same day of, but prior to, tetanus revaccination and 4 weeks thereafter. Criteria for a significant response against the tetanus booster were defined as either a ≥1.25-fold increase in IgG total TT antibodies and reaching a minimum titre of 5 μg/mL or a twofold increase in antibody concentration and a minimum titre of 1 μg/mL IgG total TT antibodies [7]. The pre immunization titre is considered protective above ≥0.1 IU/ml, which is equal to 0.05 μg/mL [14,15]. The TT antibody response of a historic control group of 20 TT revaccinated healthy adults served as a reference for the normal range of anti-TT titres.

### 2.7. Antibodies against AChR, MuSK and VGCC

The AChR, MuSK and VGCC antibody titres were measured with a commercially available radio immunoprecipitation assay (RIA) (RSR Ltd.) [16]. Titres were measured using multiple dilutions of each serum sample taken before and 4 weeks after tetanus revaccination.

### 2.8. Standard protocol approvals, registrations, and patient consents

The study was approved by the Local Committee on Medical Ethics of the Leiden University Medical Centre. Subjects provided written informed consent for participation in the study and received reimbursement of travel costs.

### 2.9. Statistical analysis and power

The study is powered for an expected response rate of 75% with a 95%-confidence interval of 63–87%. Statistical analysis was performed with Graph-Pad Prism software (version 7) and SPSS version 23. In all tests $p < 0.05$ was considered statistically significant. Tetanus IgG titres were log transformed. Comparison for normally distributed numerical variables was done with the ((un)paired) T-test or a one-way analysis of variance (ANOVA). Anti-TT antibody responses were compared between the AChR MG patients, the LEMS patients and the MuSK MG patients, respectively, and the healthy controls. Within the AChR MG group, responses were compared between patients with and without immunosuppression and between patients with and those without thymectomy.

## 3. Results

### 3.1. Patient characteristics

Fifty-one AChR MG patients (74% female, median age 56 years, range 21–65 years) were revaccinated with tetanus toxoid in the period from March 2015 to November 2015. One patient was excluded from analysis because of receiving other vaccinations (Diphtheria/tetanus/polio (DTP) and typhoid), before the control time point 4 weeks after tetanus revaccination. Also, 6 patients with MuSK MG and 9 with LEMS, representing more rare myasthenia subtypes, were included. There were no significant differences in baseline characteristics between patients with and without immunosuppressive medication (IM). Patients characteristics are given in Table 1.

### 3.2. Response to tetanus revaccination

The AChR MG group had a significantly lower geomean titre (GMT) of IgG total anti-TT before ($p = 0.003$) and after ($p = 0.03$) tetanus revaccination compared to healthy controls (HC) (Fig. 1A). The AChR MG group also had a significantly ($p = 0.02$) lower mean IgG1 titre before revaccination than the HC group, but not 4 weeks after revaccination. No significant difference in IgG4 titres before and after revaccination was found between the AChR MG and HC groups (Fig. 1A). Nevertheless, even before revaccination all patients had a protective IgG total anti-TT titre (>0.05 μg/mL according to the World Health Organization (WHO) [15]). To investigate the effect of immunosuppressive medication (IM), we divided the AChR MG group in a subgroup with ($n = 23$, Table 1) and one without ($n = 27$) IM (IM+ and IM-, respectively). Both subgroups had a significant lower GMT before revaccination compared to HC (IM-, $p = 0.02$; IM+, $p < 0.01$), but only the IM+ group had a significantly ($p < 0.01$) lower GMT 4 weeks after revaccination. There was no significant difference between the IgG total anti-TT GMT of the IM- and the IM+ subgroups (Fig. 1B). The increase factor of the IgG total anti-TT titre after revaccination was not significantly different between the HC group (mean 23-fold increase, range 1.25–313), the IM- (mean 31-fold, range 1.51–445) and the IM+ subgroups (mean 14-fold, range 0.68–70), although patients with a lower increase factor were mostly IM+ patients. The increase factor was lower in individuals with higher

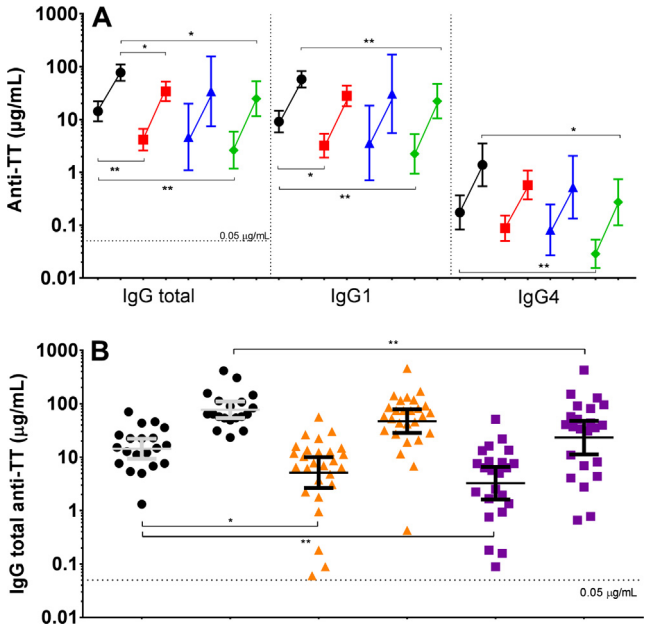


**Fig. 1.** (**A**) Response to tetanus revaccination. Geomean titres (GMT) of tetanus toxoid (TT) specific IgG total, IgG1 and IgG4, pre and 4 weeks post-vaccination with a 95%CI. Groups consist of: 20 healthy controls (●), 50 patients with AChR MG (■), 6 patients with MuSK MG (▲) and 9 with LEMS (◆). The dotted line is the minimal IgG total anti-TT titre that is considered as protective (0.05 μg/mL). Anti-TT titres were log transformed. $^{*}p < 0.05$, $^{**}p < 0.01$. (**B**) Effect of immunosuppressive medication. Geomean titres of IgG total anti-TT in HC (●) and AChR MG with (■) and without (▲) immunosuppressive medication (IM). The dotted line is the minimal IgG total anti-TT titre that is considered as protective (0.05 μg/mL). Anti-TT titres were log transformed. $^{*}p < 0.05$, $^{**}p < 0.01$.

pre-vaccination titres (Fig. 2A). Four weeks after tetanus revaccination, 46 AChR MG patients did significantly respond to tetanus revaccination (Fig. 2B). Thus, the response rate in the AChR MG group is 92% (95%CI 81–98%).

The healthy controls had a significantly ($p < 0.0001$) lower median age (median age 33 years, range 20–55 years) than AChR MG patients (median age 56 years, range 21–65 years). From the HC group 55% is female vs. 74% in the AChR MG group. In the HC group the TT response showed a tendency ($p = 0.07$) to be age-dependent. The controls in the age category >50 years had a lower post IgG total TT titre (mean GMT 43.4 μg/mL, 95%CI 20.7–90.4) than the controls <30 years of age (mean GMT 109.9 μg/mL, 95%CI 61.5–196.3). In the AChR MG group, containing only a few young patients, such a difference based on age groups was not observed. The years passed since the last tetanus revaccination did not affect the increase factor of the TT titre.

Since the response to tetanus toxoid is T-cell dependent and almost half of our AChR MG group (58%) underwent a thymectomy in the past (Table 1), we tested whether a thymectomy impacted the antibody response. We found no significant difference in pre ($p = 0.8$) and post IgG total TT titre ($p = 0.2$) between the groups with (pre mean GMT 4.0 μg/mL, 95%CI 2.1–7.5; post mean GMT 27 μg/mL, 95%CI 14.9–49) and without (pre mean GMT 4.5 μg/mL, 95%CI 2.1–9.5; post mean GMT 46.8 μg/mL, 95%CI 25–87.5) a thymectomy.

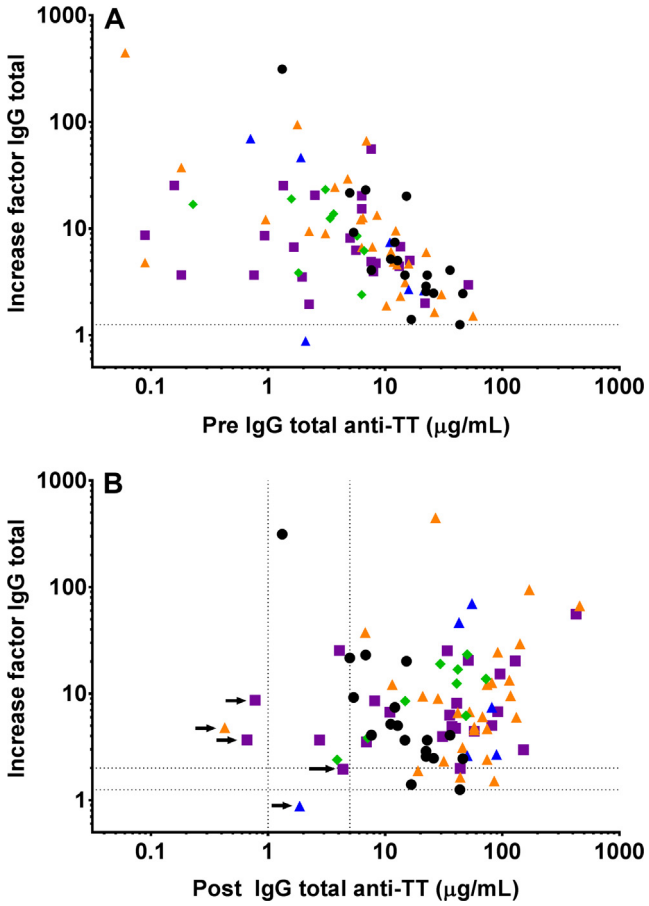


**Fig. 2.** (**A**) The factor increase of the IgG total anti-tetanus toxoid (TT) titre in the healthy controls (●), in patients with AChR MG with (■) and without immunosuppressive medication IM (▲) and in the patients with MuSK MG (▲) and LEMS (◆) is dependent on the pre revaccination IgG total anti-TT titre. (**B**) To fulfil the criteria of a significant response, a factor increase of 1.25 or 2 times the pre revaccination IgG total anti-TT titre (horizontal dotted lines) and a post IgG total anti-TT titre > 1 μg/mL or 5 μg/mL (vertical dotted lines), respectively. The arrows indicate patients who don't meet one of these criteria.

In the LEMS group, the mean GMT of pre and post-revaccination IgG total (pre, $p < 0.01$; post, $p < 0.01$), IgG1 (pre, $p < 0.01$; post, $p < 0.01$) and IgG4 (pre, $p < 0.01$; post, $p = 0.03$) TT titre was significantly lower, compared to that of healthy controls (Fig. 1A). There was no significant difference in pre and post-revaccination IgG total, IgG1 and IgG4 anti-TT titre in the MuSK MG group, compared to healthy controls (Fig. 1A).

### 3.3. Antibodies against AChR, MuSK and VGCC

To investigate if tetanus revaccination affects auto-antibody levels, AChR, MuSK and VGCC antibody titres were measured. No changes in all these antibody titres were observed 4 weeks after revaccination compared to the day of revaccination (Fig. 3).

### 3.4. Clinical scores

The MGC score, QMG score and MG-ADL were obtained at the visit of the revaccination and 4 weeks after revaccination to measure the impact of revaccination on disease severity. Individual scores and the delta of the scores of the AChR MG group ($n = 50$) and the placebo AChR MG group ($n = 23$) are shown in Fig. 4. Total scores for these 3 outcome measures pre-revaccination were comparable between the tetanus revaccination group and the placebo group. There was no significant change of the mean score of the MGC and MG-ADL after revaccination/placebo administration in these respective groups. The QMG score showed a significant increase ($p < 0.01$) at 4 weeks in the AChR MG revaccinated group (Fig. 4D). The delta of the QMG in the AChR MG revaccinated group also showed a statistically significant increase ($p = 0.01$) compared with the delta of the placebo group. Mean increase of the QMG in the AChR MG revaccinated group was 1.08 points, 95%CI 0.5–1.7 (Fig. 4F). The MG-ADL was also evaluated after 12 weeks in the tetanus revaccination group. The mean MG-ADL score showed a significant decrease of 0.86 point (95%CI 1.6–0.2) after 12 weeks compared with the MG-ADL score before revaccination (data not shown). At individual level there was a large variation between the three clinical outcome scores. Only one patient showed a clinical relevant increase in all tests. But all patients who had a worse score on the MG-ADL after 4 weeks, normalised to the pre-vaccination MG-ADL score after 12 weeks. We also obtained clinical outcome scores for the MuSK MG and LEMS patients before and 4 weeks after revaccination. The clinical scores were not statistically different in these two groups (data not shown).

### 3.5. Non-responders

There were 5 non-responders to tetanus upon revaccination, 4 with AChR MG and 1 with MuSK MG (arrows in Fig. 2B). One AChR MG patient did not reach the required factor of increase (1.95-fold instead of 2-fold increase) of the IgG total TT titre and reached a post TT titre of 4.36 μg/mL. This patient used cyclosporine A (a daily dose of 200 mg) and mycophenolic acid (daily dose of 2000 mg) and was the only one with this combination of IM. Three other non-responsive AChR MG patients showed an adequate ratio between post and pre IgG total TT titre (>2-fold), but the post titre was below the lower threshold of 1 μg/mL. These patients received their last tetanus boost at the age of 9, which was >50 years ago. Two of them used prednisolone at a dose of 10 and 15 mg every other day, without other immunosuppressive agents. These conditions may have interfered with their response to tetanus. The fifth non-responder was a patient with MuSK MG who received rituximab 22 months before vaccination.

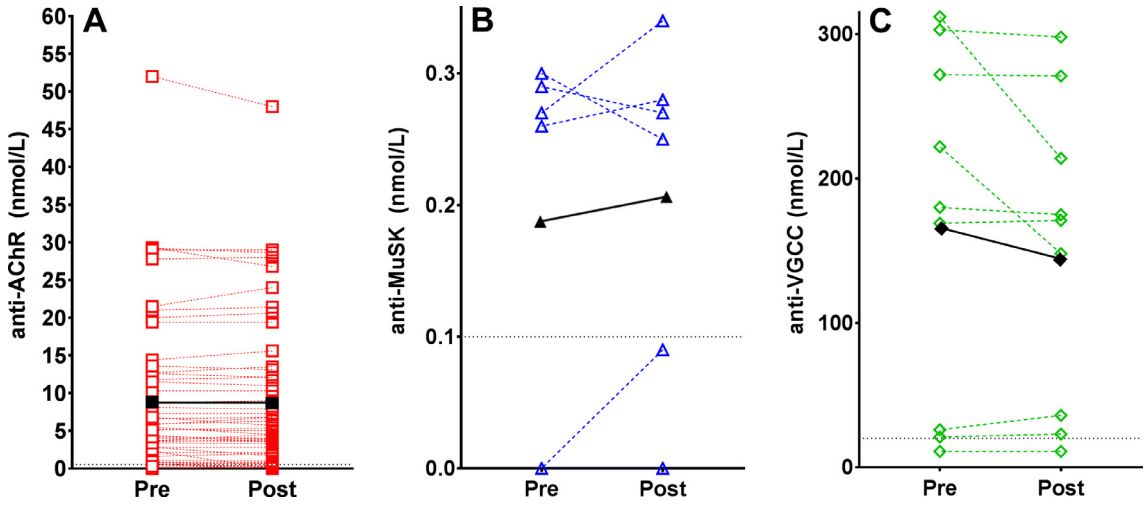


**Fig. 3.** Anti-AChR (A), anti-MuSK (B) and anti-VGCC (C) antibody concentrations before and 4 weeks after revaccination with tetanus. The dotted lines indicate the minimal titre that is considered as positive (anti-AChR: 0.5 nmol/L; anti-MuSK: 0.1 nmol/L and anti-VGCC: 20 nmol/L). Black: mean titres of the group, in colour individual titres are depicted.

## 4. Discussion

In this prospective study we showed that tetanus revaccination is safe and effective in patients with MG. The vaccinated population consisted of patients who had a stable disease, based on the MGFA classification and a stable medication regime in the past 3 months. Tetanus revaccination evoked a significant antibody response in 92.3% (60/65) of the study cohort. We found that immunosuppressive medication slightly lowered pre and post tetanus antibody titres. However, in the subgroups with and without immunosuppressive medication there was a median 6-fold increase factor of the tetanus antibody titre**.** No immunological exacerbation was found as the AChR, MuSK or VGCC antibody titres did not change after revaccination. Overall, tetanus revaccination proved to induce a significant humoral response and to be safe in this study cohort with stable disease. Patients with more severe or instable disease or receiving a higher dose of immunosuppressive medication might respond differently.

Although pre and post titres are lower in a part of our patients, all patients were protected for a tetanus infection according to WHO guidelines [15]. This is similar to our historic control group of healthy controls. It also corresponds with a previous study that measured the IgG level of diphtheria and tetanus antibodies in patients with MG, without revaccination [14]. In the latter study no significant difference in the protection rate between healthy controls, patients with systemic lupus erythematosus (SLE) or MG was found [14]. Other prospective vaccination studies in patients with autoimmune diseases were performed in SLE and ANCA+ vasculitis, which also suggest that vaccination is safe and effective [3–5]. However, lower response rates than in healthy controls were observed. This differs from our study, but might be due to the type of vaccine (Pneumococcal polysaccharides, a T-cell independent vaccine, and Influenza, respectively) and the kind of treatment that patients received.

Part of our patients with immunosuppressive medication had lower levels of tetanus antibody titres, but immunosuppressive medication did not affect the ability to respond to revaccination. Due to small size of treatment subgroups it was not possible to investigate the effect of specific treatment modalities on tetanus antibody titres. Studies in other autoimmune diseases described the effect of immunosuppressive medication, like rituximab, azathioprine or TNF-α blockers [17–19]. These studies showed only a modestly impaired immune response in patients with TNF-α blockers, but a long-term effect of rituximab [17,18]. Indeed, one of our non-responding patients was treated with rituximab 22 months before revaccination. A study in inflammatory bowel disease patients reported that azathioprine limits the immune response to hepatitis B vaccine. In the group without azathioprine, 88% (103/117 patients) reached protective titres (anti-HBs titres >10 IU/L) compared to only 55% (47/86 patients) in the group with azathioprine [19]. Prednisone has a dose-dependent effect on the immune system; a daily dose of less than 10 mg is considered non-immunosuppressive [20]. Overall, the results of our study add to these observations that immunosuppressive medication influences the height of the humoral immune response, but affects tetanus toxoid responsiveness as such in only a very limited number of cases.

The primary clinical outcome measure in our study was the MGC, which showed no change 4 weeks after revaccination compared to the day of revaccination. The QMG was the only secondary clinical outcome measure that suggested some worsening of the MG. This showed a statistically significant increase of 1 point at 4 weeks, which is less than the minimal clinically relevant difference of 2.3 points described in literature, and fits within normal fluctuation of MG [8,9]. In contrast, the MG-ADL showed a marginal improvement at 4 weeks, and even a statistically significant improvement at 12 weeks after revaccination, compared to the MG-ADL score before revaccination. Our placebo group did not show a statistically significant difference for any outcome measure at 4 weeks. Therefore, after revaccination an individual patient might experience a temporarily, clinically insignificant worsening of symptoms, which in all cases recovered 12 weeks after revaccination. These conclusions are supported by the observation that tetanus revaccination has no impact on titres of disease-specific antibodies. At an individual level, variation between pre- and post- revaccination clinical outcome scores was quite large in both the revaccination and the placebo group. This likely reflects characteristic disease fluctuation in MG, but also demonstrates limitations of the use of these clinical outcome scores as primary outcome measures. Of note, in our study anti-TT antibody responses were determined at 4 weeks and clinical outcome measures at 4 and 12 weeks after revaccination. In most clinical trials of new vaccines data are collected up to 6 weeks after vaccination [21]. Currently, a similar study is performed on the antibody response upon the yearly influenza virus (re)vaccination and its safety in MG patients.

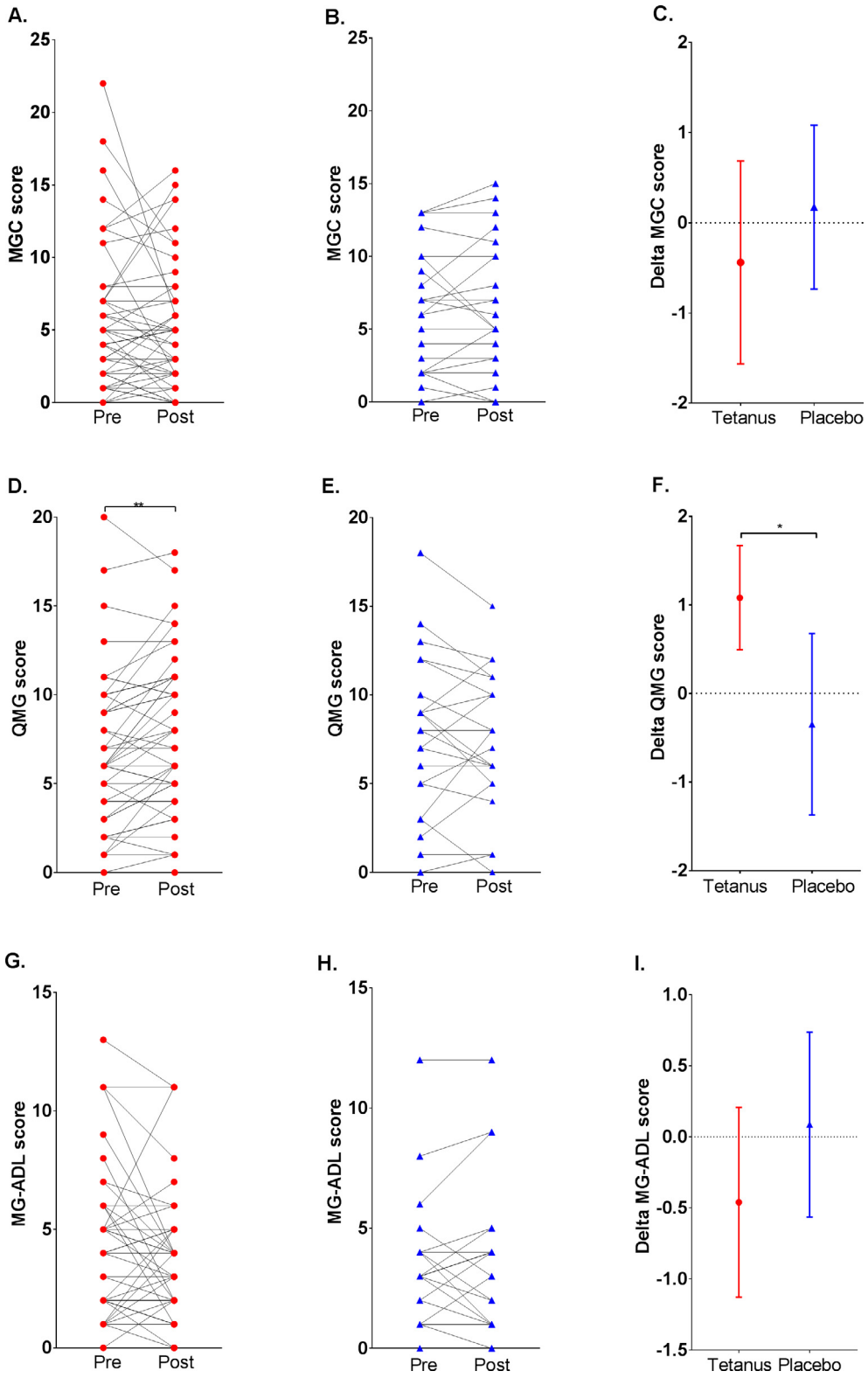


**Fig. 4.** Individual clinical scores of MG Composite score (MGC), (A: AChR MG with tetanus revaccination, B: AChR MG with placebo), Quantitative Myasthenia Gravis score (QMG), (D: AChR MG with tetanus revaccination, E: AChR MG with placebo) and Myasthenia Gravis Activities of Daily Living (MG-ADL) (G: AChR MG with tetanus revaccination, H: AChR MG with placebo) at the day of tetanus revaccination or placebo administration and 4 weeks thereafter. Delta of the scores for the group of AChR MG with a tetanus revaccination and the AChR MG group who received placebo is shown (C, F, I). $^{*}p < 0.05$, $^{**}p < 0.01$.

In conclusion, patients with AChR MG are able to mount an antibody response to a tetanus revaccination, irrespective of immunosuppressive medication. Tetanus revaccination does not induce an immunological exacerbation of AChR MG. At group level, clinical relevant worsening is absent, and does not impair daily activities of patients.

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## Author contributions

(1) Conception and design of the study: ES, MH, MOD, EZ, MOD, EJZ, MDT, JJGMV.
(2) Acquisition and analysis of data: ES, MH, IE, IA, JB, EJZ, MDT, JJGMV.
(3) Drafting a significant portion of the manuscript or figures: ES, MH, MOD, EJZ, MDT, JJGMV.
(4) Approval of final version: All authors have approved the finale version of the article.

## Conflict of interest

J.J.G.M.V. was involved in the National Institutes of Health–sponsored thymectomy trial. J.J.G.M.V. and M.G.H. received grants from Princes Beatrix fund, outside the submitted work. J.J.G.M.V. and M.G.H. has participated in research collaboration with argenx. The Leiden University Medical Centre (LUMC) received royalties from IBL for antibody tests, which were not used in this study. All reimbursements were received by the LUMC.

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