Immune Response and Safety of SARS-CoV-2 mRNA-1273 Vaccine in Patients With Myasthenia Gravis

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Abstract

Background and Objectives
Evidence regarding the safety and efficacy of messenger RNA (mRNA) vaccines in patients with myasthenia gravis (MG) after immunosuppressive therapies is scarce. Our aim is to determine whether the mRNA-1273 vaccine is safe and able to induce humoral and cellular responses in patients with MG.

Methods
We performed an observational, longitudinal, prospective study including 100 patients with MG of a referral center for MG in our country, conducted from April 2021 to November 2021 during the vaccination campaign. The mRNA-1273 vaccine was scheduled for all participants. Blood samples were collected before vaccination and 3 months after a second dose. Clinical changes in MG were measured using the MG activities of daily life score at baseline and 1 week after the first and second doses. A surveillance of all symptoms of coronavirus disease 2019 (COVID-19) was conducted throughout the study. Humoral and cellular immune responses after vaccination were assessed using a spike-antibody ELISA and interferon gamma release assay in plasma. The primary outcomes were clinically significant changes in MG symptoms after vaccination, adverse events (AEs), and seroconversion and T-cell immune response rates.

Results
Ninety-nine patients completed the full vaccination schedule, and 98 had 2 blood samples taken. A statistically significant worsening of symptoms was identified after the first and second doses of the mRNA-1273 vaccine, but this was not clinically relevant. Mild AEs occurred in 14 patients after the first dose and in 21 patients after the second dose. Eighty-seven patients developed a humoral response and 72 patients showed a T-cell response after vaccination. A combined therapy with prednisone and other immunosuppressive drugs correlated with a lower seroconversion ratio (OR = 5.97, 95% CI 1.46–24.09, p = 0.015) and a lower T-cell response ratio (OR = 2.83, 95% CI 1.13–7.13, p = 0.024).

Discussion
Our findings indicate that the mRNA vaccination against COVID-19 is safe in patients with MG and show no negative impact on the disease course. Patients achieved high humoral and cellular immune response levels.

*These authors contributed equally to this work.


Go to Neurology.org/NN for full disclosures. Funding information is provided at the end of the article.

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Myasthenia gravis (MG) is a rare neuromuscular disease caused by autoantibodies against postsynaptic receptors in the neuromuscular junction, which leads to muscular weakness. The disease is characterized by the fluctuations of symptoms over time, but triggering factors such as infections, surgery, and drugs may induce exacerbations. Several immunosuppressive and immunomodulator drugs are commonly used for the treatment of the disease.

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has prompted the development of effective and safe vaccines based on new messenger RNA (mRNA) technology. These vaccines not only produce high seroconversion rates but are also capable of inducing a T-cell immunologic response against SARS-CoV-2.

The need for massive vaccination has reignited the debate about the vaccine's safety in patients with MG and its efficacy in patients with autoimmune disease taking immunosuppressive therapies. Moreover, in view of the hypothetical role of the SARS-CoV-2 infection as a triggering factor of a myasthenic exacerbation that could induce respiratory insufficiency in addition to the severe respiratory distress that COVID-19 may cause, it is of interest to prevent the coronavirus infection in this group of patients.

In this study, we aimed to longitudinally investigate humoral and cellular responses to mRNA-1273 vaccine in patients with MG and assess the vaccine safety regarding MG worsening and side effects.

Methods

Study Design and Patients

In this prospective longitudinal study, we prospectively recruited 100 unvaccinated patients with MG scheduled for SARS-CoV-2 vaccination between April and May 2021. In accordance with the health authorities’ recommendations at that moment, patients with significant comorbidities, including MG, were offered vaccination, except for patients with a confirmed COVID-19 infection in the previous 6 months who were not considered candidates to immunization then.

We excluded patients who had presented significant clinical or therapeutic changes in the previous 6 months.

Demographics (sex and age), date of MG onset, Myasthenia Gravis Foundation of America (MGFA) score at onset, highest MGFA score to date, MGFA score at baseline visit, and presence of thymoma were obtained from clinical records. Active and former immunosuppressive treatments since MG onset were also collected from the clinical records. Patients taking prednisone alone were classified as receiving monotherapy, and patients on prednisone combined with another immunosuppressive drug (e.g., azathioprine, mycofenolate mofetil, cyclosporine, tacrolimus, or rituximab) were considered to be on combination therapy. Patients were considered drug-refractory as defined by Sanders et al. COVID-19 symptoms were monitored using a telematic system.

Vaccination Protocol and Immune Response Evaluation

The Moderna COVID-19 (mRNA-1273) vaccine (Moderna TX, Inc., Cambridge, MA) was administered in accordance with the protocol at our center, and the recommended schedules were followed. All participants received 2 doses separated by 28 days.

Blood samples were collected in serum and heparin tubes at baseline visit before the first vaccine dose and at 90 days after the second vaccine dose administration (Figure 1). Immunoglobulin G (IgG) positivity against nucleocapsid protein (NP) was detected in baseline blood samples using the SARS-CoV-2 NP-IgG ELISA kit in accordance with the manufacturer’s instructions (MyBioSource, Inc., San Diego, CA, MBS398004), and a sample was considered positive when OD readings were greater than 0.2. A high-sensitive SARS-CoV-2 S1-IgG ELISA kit (MyBioSource, Inc., MBS398013) was used to detect spike-IgG in the 90 days after vaccination, following the manufacturer’s instructions. A sample is considered positive if values were $\geq$ 5 IU/mL.

The cellular immune response was evaluated using an interferon gamma release assay (IGRA) method. A commercial kit (Quan-T-Cell SARS-CoV-2 EUROIMMUN, order no. ET 2606-3003. and Quan-T-Cell ELISA EUROIMMUN, order...
no. EQ 6841-9601) was used. According to the manufacturer’s specifications, the EUROIMMUN recommends basing results on a borderline range. Positive results are considered those with >200 mIU/mL and negative results as those with <100 mIU/mL. Borderline results are those with levels between 100 and 200 mIU/mL, and indeterminate results are those with a nonvalid stimulation control. Finally, we grouped the participants as having either a T-cell IGRA positive or T-cell IGRA nonpositive result. Negative, borderline, and indeterminate results were included in the former group.

**Clinical Changes in Myasthenia Gravis and Adverse Events**

Clinical changes during the study were assessed using the validated myasthenia gravis activities of daily life (MG-ADL) score administered through telephone call. This is a quantitative, reliable, and simple-to-administer scale that reflects the severity of symptoms in MG and their effect on daily living activities.²⁰

The MG-ADL score was obtained at 3 time points referred to MG symptoms occurring in the 7 days before the baseline visit, in the 7 days after the first dose administration, and in the 7 days after the second dose (Figure 1) in all patients. Changes in symptoms were considered clinically relevant when an increase in MG-ADL score of 3 points or more was observed.

All patients were instructed to contact the team if any symptoms of MG appeared or worsened within the first 2 months of the study. Adverse events (AEs) related to the vaccination were self-reported and collected from each patient in the 2 weeks after the first and second doses of the vaccine by telephone call or email. Severe AEs were those that resulted in hospitalization, disability, or a life-threatening event. To differentiate between generalized fatigue or other AEs and MG symptoms, patients who reported fatigue or flu-like symptoms for more than 72 hours were clinically assessed by neurologic examination. Patients were also instructed to contact the study team if COVID-19 symptoms and/or positive SARS-CoV-2 testing appeared during the study period and were telematically assessed.

**Statistical Analysis**

A descriptive statistics of the demographic variables, AEs, humoral and cellular immune responses, and statistical analyses were performed using IBM-SPSS Statistics v.21 software (IBM, Armonk, NY).

The time course of MG-ADL scores was explored using 1-way repeated measures analysis of variance. Greenhouse-Geisser correction was applied. We performed post hoc multiple comparisons by means of Bonferroni correction. Generalized linear models were used to study the effect of several variables on Spike-IgG and IGRA response. To discern between models, we considered plausibility of estimation and Akaike information criterion. We finally chose a Poisson log-linear model that included the following variables: sex, age, thymectomy, time of disease evolution, and the various types of immunosuppressive and immunomodulator treatment (prednisone, azathioprine, mycophenolate mofetil, cyclosporine, tacrolimus, periodic endovenous immunoglobulins, and periodic plasma exchange).

The Fisher exact test was used to compare the number of events between categorical groups of patients. The relative
risk was also calculated to evaluate the risk of not developing a serologic or cellular immune response.

**Standard Protocol Approvals, Registrations, and Patient Consents**

The study was approved by the “Hospital de la Santa Creu i Sant Pau” ethics committee (IIBSP-COV-2021-26). All participants gave written informed consent to participate in the study.

**Data Availability**

Anonymized data not published within this article will be made available by request from any qualified investigator.

**Results**

**Demographics and Baseline Characteristics of Patients With MG**

From our registry of 378 patients with MG, 70 patients older than 80 years were already vaccinated when we started the study protocol. One patient was pregnant and did not meet vaccination criteria then, and 8 patients refused vaccination. One hundred patients were therefore finally included in the study.
Table 1 summarizes the baseline characteristics of these 100 patients. Blood samples were collected from all of them at the first time point and at the first and second MG-ADL. Ninety-nine patients completed the full vaccination protocol (see further). The remaining patient, who was on prednisone and azathioprine, was lost to follow-up after the first scheduled blood sample.

Four patients reported confirmed COVID-19 or PCR positive asymptomatic infection before the baseline visit. The mean time (SD) from infection to the first blood sample was 8.25 months (4.19). One patient reported close contact with a SARS-CoV-2–positive individual but did not develop symptoms.

Table 1 summarizes ongoing treatments in our cohort. Sixty-seven patients were on prednisone, 37 together with some type of concomitant immunosuppressive therapy: prednisone and mycophenolate in 20 patients; prednisone and azathioprine in 5; prednisone and cyclosporine in 6; and prednisone and tacrolimus in 2. Four further patients had been on prednisone and rituximab at some point in the last 10 years (September 2017, April 2018, May 2019, and November 2019). In addition, 1 patient on cyclosporine and prednisone at baseline received rituximab.

The mean prednisone dose (SD) was 12.6 mg daily (8.27). Nine patients were on high prednisone doses (daily doses greater than 20 mg). The mean doses of other immunosuppressant drugs were as follows: azathioprine, 110.71 mg/24 h (38.87); mycophenolate, 1,976 mg/24 h (109.11); cyclosporine, 166.67 mg/24 h (87.56); and tacrolimus, 4.14 mg/24 h (1.46).

One anti-MusK–positive female patient was undergoing rituximab treatment when the study started, with the most recent dose being administered in the month before immunization. This patient developed severe COVID-19 1 week after the first vaccine; she required ICU admission and mechanical ventilation but made a full recovery in 1 month. She did not receive the second dose of the vaccine in view of the recommendations of her infectious disease specialist. The remaining 8 patients on rituximab, treated between 2015 and 2019, received their second vaccine dose as scheduled, and none developed COVID-19.

Clinical Evaluation
The mean MG-ADL score (SD) at the beginning (n = 100) of the study was 2.34 (3.22). A week after the first dose (n = 100), it was 2.65 (3.52), and after the second dose, it was 2.72 (3.57) (n = 99). The effect of occasion was statistically significant (F = 5.074; p = 0.011). Further post hoc tests showed a statistically significant difference between the MG-ADL score at baseline and that after the first vaccine (p = 0.018) and between the MG-ADL score at baseline and that after the second dose (p = 0.046). However, no difference was observed between the first and second vaccine doses (p > 0.05). Nevertheless, the differences between baseline MG-ADL score and MG-ADL score after the first and second doses were less than 2 points.

We observed an increase of 3 or more points in MG-ADL scores in 8 patients after the first vaccine dose and in 10 patients after the second vaccine dose. Five patients worsened after both doses, 3 with only the first dose and 5 with only the second one. In these cases, the mean worsening was 3.25 points after the first dose (2–7 range) and 3.9 points (2–7 range) after the second dose. Nonetheless, when we focused on baseline clinical characteristics, patients with an MG-ADL score greater than 3 points at baseline were prone to MG-ADL worsening after the first and second doses (Figure 2). In all but 1 patient, this worsening was self-limited—lasting less than 7 days—and mild, affecting ocular and limb items on the MG-ADL score, and they did not need...
any therapeutic interventions. So we did not considere those patients to have an MG exacerbation for the current analysis. The remaining case was a generalized MG patient with anti-AChR–positive antibodies MG-ADL 4 at baseline (diplopia 2 and ptosis 2) treated with 20 mg of prednisone every other day and pyridostigmine on demand. This patient experienced an MG exacerbation after the second dose of the vaccine with mild limb weakness together with moderate to severe worsening in ptosis and diplopia, reaching an MG-ADL score of 7 and requiring a modification of treatment comprising an increase in prednisone to 25 mg every other day and 6 pyridostigmine tablets. This worsening lasted for 4 months, and the number of pyridostigmine tablets was lowered to 1 tablet, achieving an MG-ADL 2 of ptosis and diplopia.

No patients presented worsening of bulbar items of the MG-ADL score (dysarthria, swallowing, and dyspnea). Another patient who had an ocular phenotype and was asymptomatic at baseline (MG-ADL = 0) with 17.5 mg of prednisone every other day and 150 mg of azathioprine a day, presented with diplopia and ptosis, scoring 2 points in the MG-ADL 1 week after the last MG-ADL score was obtained. A change in therapy was required, with an increasing dose in prednisone until 30 mg per day. This worsening was excluded from the current analysis because it did not occur within the time limits of our study protocol.

**Immune Response to the Vaccine**

We assessed the immune response to vaccination in 98 patients. Four showed positive NP antibodies in baseline blood samples and spike-IgG seroconversion and IGRA positivity in their postvaccination blood sample.

Eighty-seven of the 98 patients developed spike-IgG antibodies in the blood sample collected after vaccination (88.77%). The frequency of spike-IgG positivity in the different treatment groups is shown in Figure 3A. A statistically significant difference (the Fisher exact p = 0.015) was found between the percentage of seroconverted patients when they were grouped as taking either immunosuppressive monotherapy or combined therapy (prednisone and another immunosuppressant), with an OR = 5.97 (95% CI 1.46–24.09) of not seroconverting in this second group. None of the other variables analyzed were statistically significant in the generalized linear model.

Of the nonimmunosuppressed patients, including patients on cholinesterase inhibitor monotherapy, 15/15 developed antispike antibodies after vaccination. No statistically significant differences were found (the Fisher exact p = 0.206) compared with those in patients on immunosuppressants (72/83 patients seroconverted).

Eight of the 98 patients had a negative IGRA result according to the manufacturer’s recommendations. Eight other patients had a borderline result and 10 had an indeterminate result. Therefore, 72 of the 98 patients (73.47%) were IGRA positive. Figure 3B shows the frequency of IGRA results in each treatment group. The difference in the frequency of IGRA positive and IGRA nonpositive results between patients taking immunosuppressive monotherapy and those taking combined therapy was statistically significant. The Fisher exact test yielded a p value of 0.024 with an OR value of 2.83 (95% CI 1.13–7.13). None of the other variables included in the generalized linear model showed a statistically significant correlation (p > 0.05).
Fourteen of the 15 patients in the subgroup not on immunosuppressive therapy had a positive IGRA result after vaccination compared with 60/83 in the group of patients taking immunosuppressive therapy. This difference was not statistically significant (the Fisher exact $p = 0.065$).

**Vaccination-Related AEs**

Fourteen patients reported AEs (14%) after the first dose, and 21 patients (21%) reported such events after the second dose. No statistically significant differences were found in the rates of reported AEs between patients on immunosuppressant therapy and patients not receiving immunosuppression after the first dose (the Fisher exact $p = 0.687$) and the second dose (the Fisher exact $p = 0.301$). Table 2 summarizes the main AEs and their frequency. No severe AEs were reported.

**Vaccine Efficacy Against Symptomatic COVID-19 After Vaccination**

None of the 99 patients who received the full vaccination scheme developed symptomatic COVID-19 in the following 6 months.

**Discussion**

In this study, we prospectively studied a representative population of patients with MG after administration of the mRNA-based vaccine against COVID-19. Our results showed the participants did not develop clinically significant worsening of MG symptoms or severe side effects after vaccination. Moreover, patients achieved high rates of humoral and cellular immune responses, and none of those who completed the vaccination scheme had confirmed SARS-CoV-2 infection or suggestive symptoms during a high-incidence period in our country.

The use of vaccines in the autoimmune disease population has been a matter of debate for many years. Specifically in MG, vaccines have been considered a potential trigger of exacerbation, although scientific evidence is conflicting. Clinical case reports suggest that mRNA vaccines can lead to MG debut and moderate exacerbations and that COVID-19 may also lead to MG worsening. In our daily clinical practice, we observed that patients were concerned that the new mRNA vaccines could lead to clinical worsening of their illness, and this fear had an impact on their willingness to receive the vaccination against COVID-19. The findings from our study showed that the SARS-CoV-2 mRNA-1273 vaccine is safe in patients with MG. In our series, only 8% of patients had MG-ADL worsening ≥3 points after the first dose and only 10% after the second dose. No patients required hospitalization, and only 2 patients (2%) from among the whole cohort needed therapeutic intervention to control symptoms. Most of these patients had higher MG-ADL scores at baseline, probably reflecting poorer control of the disease and a greater predisposition to clinical fluctuations. Another finding of interest is that the patients experienced AEs less frequently than the healthy population studied in the mRNA-1273 clinical trial. We hypothesize that the effect of systemic corticosteroid therapy may have limited the development of systemic AEs.

**Table 2 Frequency of Adverse Events Related to Vaccine Administration**

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<th>Event</th>
<th>First dose</th>
<th>Second dose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache, n (%)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>3</td>
</tr>
<tr>
<td>Moderate to severe pain at injection site, n (%)</td>
<td>3 (3)</td>
<td>1 (1)</td>
<td>4</td>
</tr>
<tr>
<td>Fatigue without weakness (less than 72 h), n (%)</td>
<td>2 (2)</td>
<td>6 (6)</td>
<td>8</td>
</tr>
<tr>
<td>Flu-like symptoms (chills, myalgia, and fatigue), n (%)</td>
<td>4 (4)</td>
<td>7 (7)</td>
<td>11</td>
</tr>
<tr>
<td>Abdominal pain or vomits/diarrhea, n (%)</td>
<td>3 (3)</td>
<td>1 (1)</td>
<td>4</td>
</tr>
<tr>
<td>Fever, n (%)</td>
<td>3 (3)</td>
<td>9 (9)</td>
<td>12</td>
</tr>
<tr>
<td>Skin rash/urticaria</td>
<td>1</td>
<td>2*</td>
<td>2*</td>
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* One patient presented a generalized popular erythematous rash after both vaccine doses, but this was resolved with antihistamines and local steroids. Another patient, with a history of allergies, developed generalized urticaria after the second dose.
immune responses has been recently published. Our results showed a 100% seroconversion rate in untreated patients and a slightly lower rate (86.7%) in immunosuppressed patients. Although this lower rate, none of these patients developed COVID-19 symptoms during a 6-month follow-up in a period of high COVID-19 incidence in our country. In contrast with previous studies, our findings may be due to the use of fewer or lower doses of immunosuppressive drugs. Nevertheless, in agreement with other studies in highly immunosuppressed populations, a therapy combined with prednisone and another immunosuppressive drug resulted in an almost 6 times higher risk of not developing antibodies against spike protein.

Our study includes data about cellular T-cell response to mRNA-1273, another important immune trait that may constitute a long-lasting feature in the prevention of COVID-19. As shown in preliminary studies of the mRNA-1273 vaccine, many patients developed a T-cell response measured by quantifying cytokines and IF-gamma in serum after vaccination. In our study, 73.5% of our patients developed a T-cell response measured by IGRA. Patients on combined immunosuppressive therapy had nearly 3 times more risk of not achieving a T-cell immune response after vaccination than patients on monotherapy. Because of the difficulties in studying the vaccine efficacy in preventing COVID-19 infection in rare diseases such as MG, serologic and cell assays emerge as reliable surrogate biomarkers. These biomarkers may be useful to assess vaccine efficacy in rare autoimmune diseases.

The main limitation of our study is the lack of a proportional control group. Nevertheless, our cohort includes a group of patients with MG who were not taking immunosuppressive treatment and had a 100% rate of seroconversion and 93% of T-cell response measured by IGRA. A second limitation is the absence of a standardized method to assess the humoral and cellular immune responses, and none of those who completed the vaccination scheme developed COVID-19 during a period of high incidence. Taken together, these findings support the safety and efficacy of the mRNA-1273 vaccine in MG.

Our results indicate that the mRNA-1273 vaccine does not cause significant AEs or relevant worsening in the clinical status of patients with MG. Despite receiving immunosuppressive therapy, the patients in this study achieved significant humoral and cellular immune responses, and none of those who developed a T-cell response measured by IGRA. A second limitation is the absence of a standardized method to assess the humoral and cellular immune responses, and none of those who completed the vaccination scheme developed COVID-19 during a period of high incidence. Taken together, these findings support the safety and efficacy of the mRNA-1273 vaccine in MG.

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Disclosure
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<td>Drafting/revision of the article for content, including medical writing for content; and study concept or design</td>
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References

Immune Response and Safety of SARS-CoV-2 mRNA-1273 Vaccine in Patients With Myasthenia Gravis

David Reyes-Leiva, Joaquín López-Contreras, Esther Moga, et al.

*Neurol Neuroimmunol Neuroinflamm* 2022:9;
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