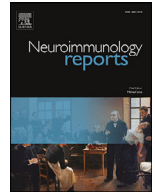




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COVID-19 Cytokine Storm in Myasthenia Gravis Treated with Mesenchymal Stem Cells: The First Philippine Experience

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) continues to plague especially the immunocompromised, and yet little is known regarding its treatment on patients who present clinically similar like those with Myasthenia Gravis (MG) in crisis.

Methods: We examined the case of a patient with MG secondary to malignant thymoma who went into COVID-19 cytokine storm and during her recovery, also suffered a postinfectious myasthenic crisis.

Results: After 10 days of intubation and completing 4 doses of mesenchymal stem cell therapy (MSc), the patient significantly improved and was discharged ambulatory with assistance oxygen-requiring on nasal cannula coexistent with a decrease in measured cytokine levels.

Discussion: Immunosuppressive treatment, defective immunoregulatory mechanisms, pro-inflammatory state and respiratory muscle weakness in MG has all shown worse outcomes in COVID-19. Both diseases share a common pathomechanism and recovery depends on a healthy T-cell regulatory and B-cell response. MSc, with its immunomodulatory and anti-inflammatory properties, is thus promising in COVID-19 treatment in the setting of autoimmunity.

Introduction

Since March 11, 2020, Coronavirus disease 2019 (COVID-19) has been declared by the World Health Organization (WHO) a pandemic (WHO, 2020). People with rare diseases such as Myasthenia Gravis, who may also have respiratory muscle weakness, are vulnerable. Interim analysis in the COVID-19-associated risks and effects in myasthenia gravis (CARE-MG) (Muppidi et al., 2020, Zubair et al., 2020) registry showed 24% mortality, with current therapies still in clinical trials at the time of this study. Current guidelines in the management of COVID-19 in this special population are also only based on expert consensus (Jacob and Muppidi, 2020). In the background of malignant thymoma, wherein autoimmunity-susceptible CD4+ cells an autoantibody-secreting B cells are produced, T-cell immunity against COVID-19 is

compromised (Weksler and Lu, 2014). We present a case of a 49-year-old female with MG secondary to malignant thymoma who suffered COVID-19 cytokine storm and the role of mesenchymal stem cell therapy (MSc) in her recovery. According to available data worldwide, this is the first report of COVID-19 in MG who received MSc.

Methods

This observational study was approved by the institutional review board of The Medical City. Diagnosis of COVID-19 was based on clinical history, inflammatory markers, and positive nasopharyngeal swab polymerase chain reaction (PCR) testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Both the inflammatory markers and cytokine levels were measured before and after MSc therapy.

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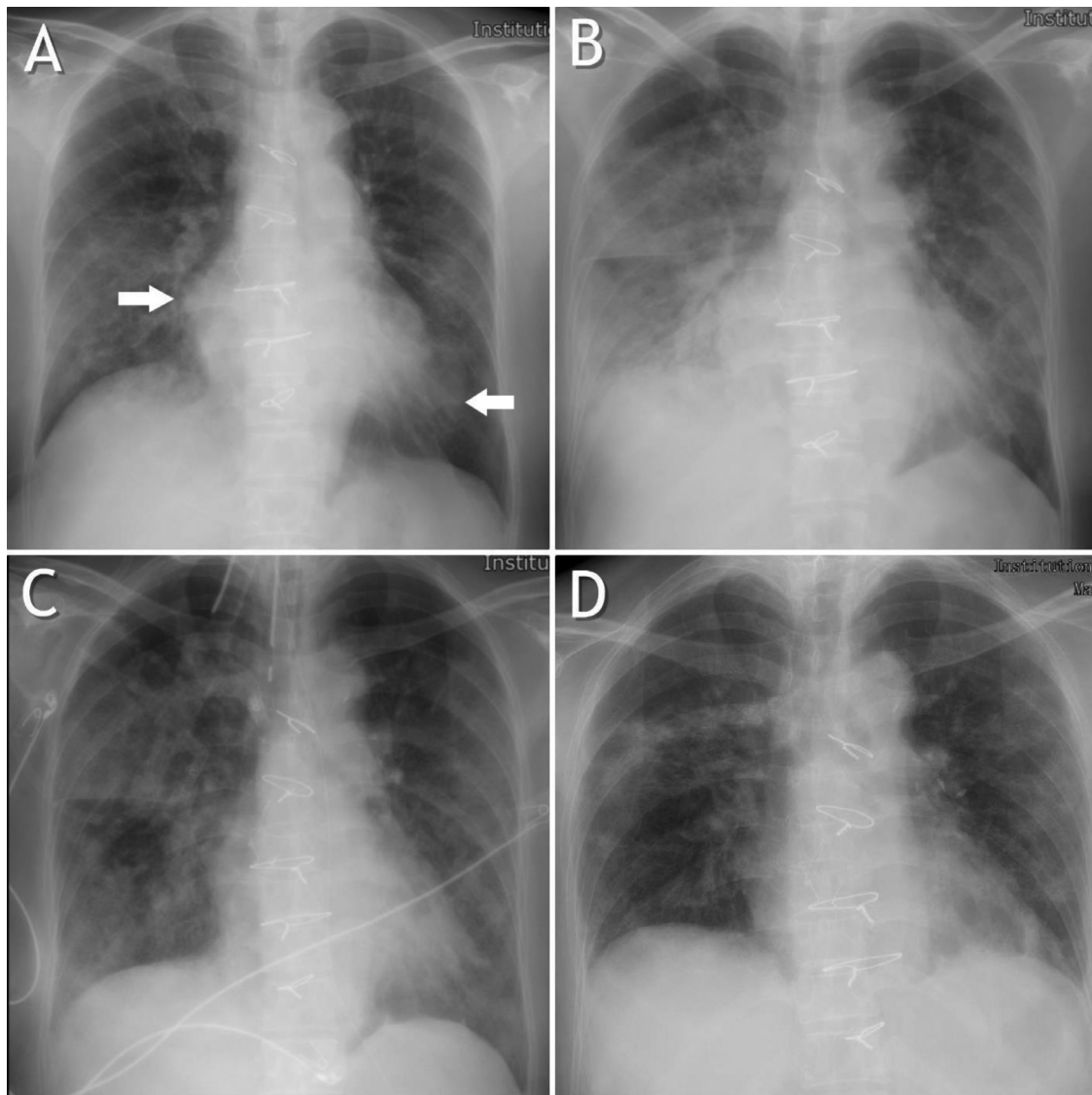


Fig. 1. Plain Chest Radiography. (A) On admission, the chest x-ray revealed bilateral pneumonia in the mid to lower lung fields; bilateral pericardial masses (arrows). (B) On the fourth hospital day, the patient was dyspneic requiring invasive ventilation and suffered COVID-19 cytokine storm. Pneumonia progressed with bilateral consolidation with co-existing pleural effusion on the right. (C) In the interim, there was further regression of pneumonia on the right, unchanged on the left, and resolution of pleural effusion. (D) On discharge planning (28th hospital day), there was further regression of pneumonia with development of minimal pulmonary fibrosis, both upper lobes.

Results

The patient is a 49-year-old female, hypertensive, known case of MG secondary to Malignant Thymoma, Mixed-type Masaoka-Koga Stage III, status-post thymectomy and radiation therapy. Her MG is controlled at MGFA Class IIb on pyridostigmine 60 mg 1 tablet and azathioprine 50 mg $\frac{1}{2}$ tablet daily. She presented with shortness of breathing and cough initially with a 5-day history of fever, anorexia, dysgeusia, anosmia, and headache, but with no myasthenic exacerbation. She was tachypneic at 22, mildly hypoxemic at 79 mmHg on blood gas; hence, was placed on 2 L of oxygen. Initial chest x-ray showed bilateral pneumonia (Fig. 1A) with *Streptococcus pneumoniae* and *Legionella* urine antigens negative. She was started on ceftriaxone and azithromycin, pyridostigmine was increased to 60 mg 1 tab twice daily, while azathioprine was put on hold. On day 2 of admission, SARS-CoV-2 RT PCR came back positive and patient was subsequently enrolled in the Solidarity Trial (WHO Public Health Emergency, 2020) randomized to receive remdesivir.

On day 4, her breathing worsened and oxygen saturation dropped to 70% wherein she required invasive ventilation (Fig. 2) and was deemed to suffer COVID-19 cytokine storm concurrent with high levels of inflammatory markers and decreased lymphocytes (Fig. 3). On neurologic examination, there was no ptosis, bulbar signs, nor proximal muscle weakness except for slight pCO₂ retention (49.5 mmHg), attributing the respiratory failure more likely to COVID-19.

On day 7, due to progressive dyspnea and pneumonia (Fig. 1C) with increasing inflammatory markers, she was given MSC from umbilical cord blood (5×10^5 cells/kg body weight) intravenously every other day for 4 doses (Day 1, 3, 5, 7) based on the hospital's standard protocol. After Day 3, she was weaned off from the ventilator coexistent with a decrease in the measured cytokine levels and inflammatory markers (Fig. 3). It was only at this time that the patient developed ptosis, proximal muscle weakness, and fluctuating intolerance to CPAP, necessitating pyridostigmine to be titrated up to 240 mg/day. Thereafter, she was extubated following a total of 10 days on the ventilator.

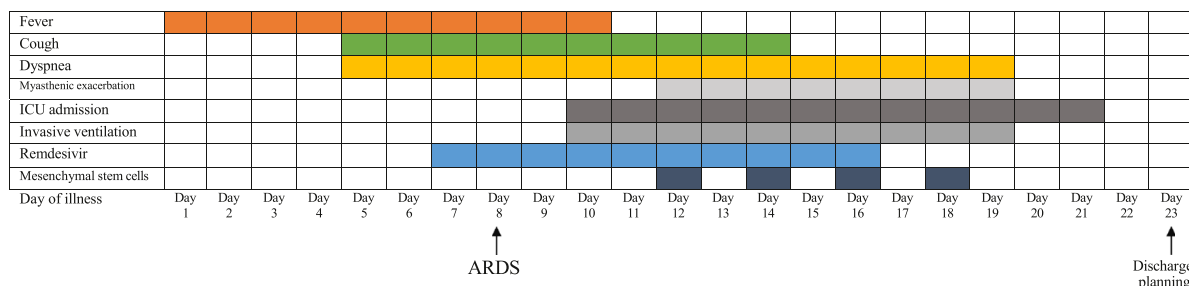
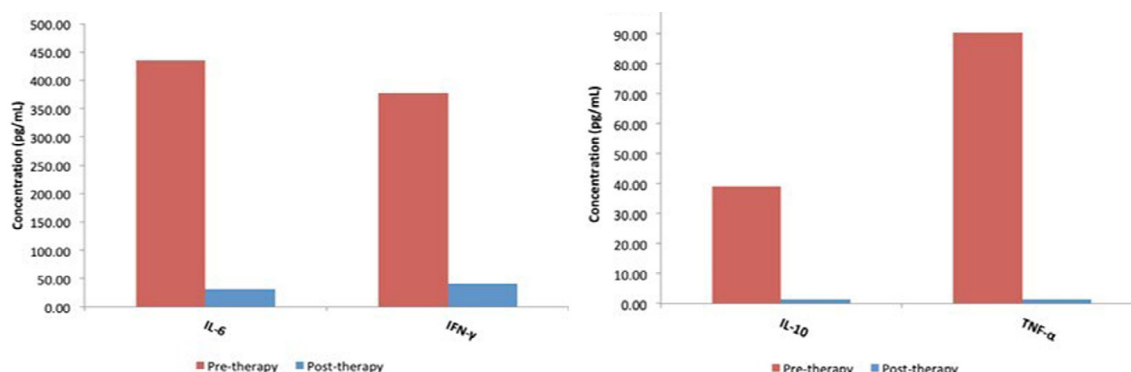


Fig. 2. Clinical Course of the Patient's Hospital Stay. Note that the postinfectious Myasthenic Crisis experienced by the patient on day 32 of illness is not reflected here.

A.

TEST	PRE	POST	% DECREASE	NORMAL VALUES
Neutrophil	0.89	0.80		0.56 – 0.66
Lymphocyte	0.05	0.13		0.22 – 0.40
Neutrophil/ Lymphocyte ratio	17.80	6.20	65.43	
ESR	31			0 – 15 (mm/hr)
Ferritin	304	45	85.20	17.90 – 464.00 (ng/mL)
C-reactive protein	251.60	7.14	97.16	1.00 - 3.00 (mg/L)



B.

CYTOKINE	PRE*	POST*	% DECREASE
IL-6	434.95	31.47	92.8
IL-10	39.35	1.39	96.5
IFN- γ	377.88	40.67	89.2
TNF- α	90.52	1.36	98.5

Fig. 3. Inflammatory markers and cytokine levels pre- and post-mesenchymal therapy with corresponding percent change. (A) Inflammatory markers. (B) Cytokine levels in pg/ml* Samples used were in triplicates, with 3-7% relative standard deviation.

On day 18, the patient was considered recovered with development of COVID IgG antibodies on electrochemiluminescence immunoassay (ECLIA) despite a positive repeat SARS-CoV-2 RT PCR testing. Nine days on discharge planning, she developed tachypnea with CO2 retention (73-120 mmHg) again requiring intubation and was managed as postinfectious Myasthenic crisis. Plasmapheresis was not an option due to the recent MSc transplantation; hence Intravenous Immune globulin (IVIG) at 0.4 mg/kg/day for 5 cycles was administered, and azathioprine was resumed and increased to 50 mg once daily. Two weeks later, she was discharged ambulatory with assistance, oxygen-requiring on nasal cannula.

Discussion

Is mesenchymal stem cell therapy a valid treatment option?

MG and COVID-19 present clinically similar, with dyspnea occurring in 14-18% versus 45.6% of cases, respectively (Rodriguez-Morales et al., 2020). Both illnesses show elevated proinflammatory cytokines such as IL-6, IL-17, and IFN γ along with B- and T-cell depletion (Fig. 4), expectedly worsening outcomes (Ye et al., 2020). Mesenchymal stem cells (MSc), with its immunomodulatory and anti-inflammatory properties, has thus shown promise especially in this subset of patients who failed to improve after standard life support measures (Liu et al., 2020).

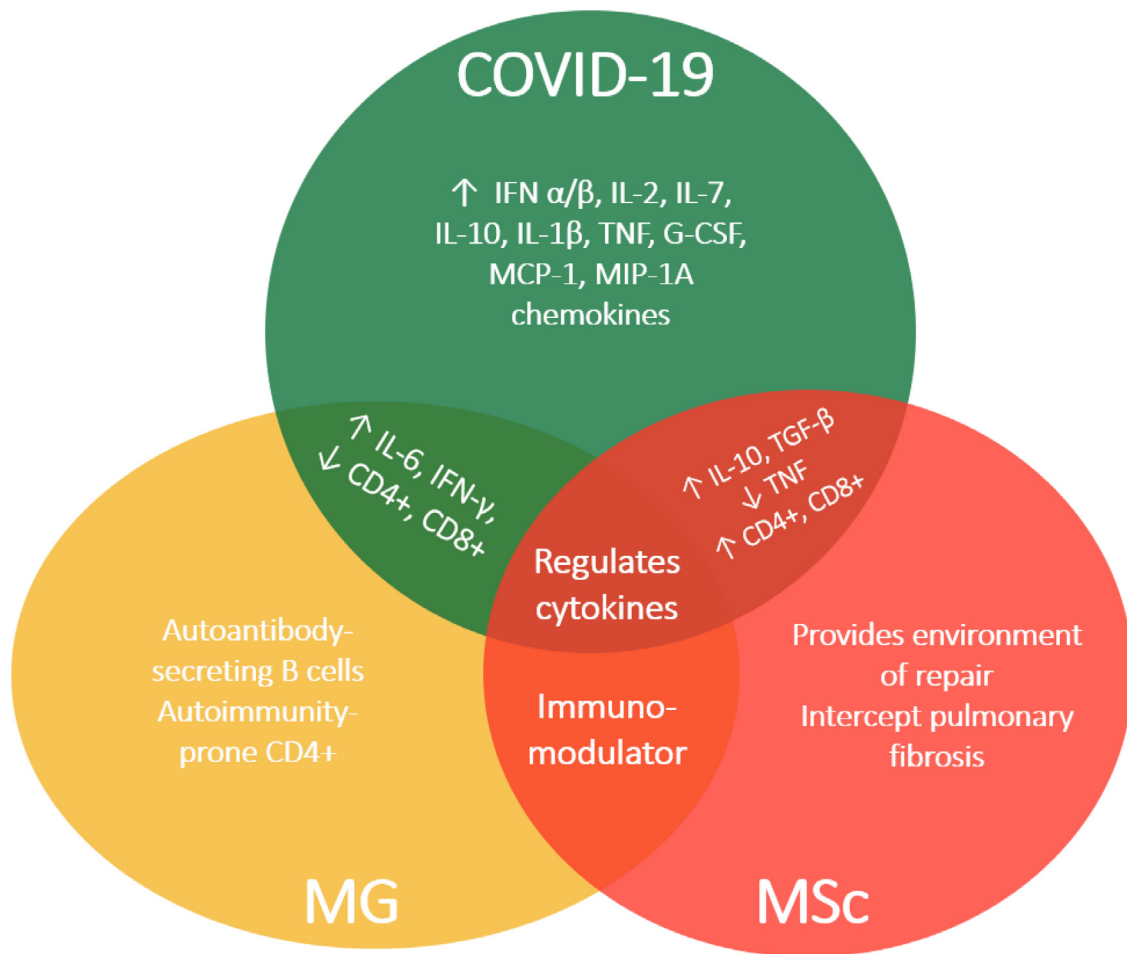


Fig. 4. Overlapping Pathophysiology of COVID-19 Cytokine Storm and Myasthenia Gravis vis-à-vis Mechanism of Action of Mesenchymal Stem Cell Therapy. COVID-19, novel coronavirus disease; MG, myasthenia gravis; MSc, mesenchymal stem cell.

The first randomized, double-blind, placebo-controlled trial on umbilical cord (UC) MSc (4×10^7 cells/kg) has shown it to be safe with marked improvement in 65 severe COVID-19 patients with overall whole lung lesion volume and integrated reserve capacity (Shi et al., 2020). Studies have shown that with a marked improvement of pulmonary lesions, there is subsequent recovery of lung function (Shi et al., 2020). A smaller cohort study has shown zero deaths in severe COVID-19 patients given UC-MSc ($n = 12$) compared to 10.34% 28-day mortality rate in the control group ($n = 29$), where 4 were intubated and 3 died. Improvement of clinical symptoms began on day 3 of infusion, similar to our case, and reached a significant difference on day 7 concurrent with a significant decrease of CRP and IL-6 levels (Shu et al., 2020). In a pilot study of MSc transplantation in China, there was also significant improvement of 7 patients in 2 days (4 were severe, 1 was critical, and 2 were common type) (Leng et al., 2020).

The underlying mechanisms of MSc are inhibition of abnormal T lymphocyte and macrophage activation and regulation of cytokine secretion such as TNF- α , IL-6, IL-1, IL-12, and IFN- γ (Fig. 4). It also promotes endogenous repair of damaged lung tissues by improving its microenvironment (Rajarshi et al., 2020). Interestingly, since it's negative for angiotensin-converting enzyme 2 (ACE2) and serine protease TMPRSS2, which are both necessary for virus entry into the cells, this makes them an ideal treatment (Rajarshi et al., 2020).

Taken separately, MSc has shown promise to both COVID-19 and MG. With the overlapping pathophysiology of both diseases, its use is thus a valid treatment option. In severe and refractory MG, symptom- and treatment-free remission has been documented in 7 patients who received autologous hematopoietic stem cell (Bryant et al., 2016). There

was a reduced level of anti-acetylcholine receptor (AChR) and a restoration of the AChR expression at the muscle endplate (Sudres et al., 2017) seen in humanized immunodeficient mice. In our patient, after day 3 of infusion, she was weaned off from the ventilator and extubated four days later coexistent with a decrease in the measured cytokine levels and inflammatory markers (Fig. 3), which showed a notable nearly 100% decrease from baseline.

Conclusion

The pro-inflammatory state and dysregulated immune process in both COVID-19 and MG leads to more severe disease. To effect virus clearance, a robust immune system is necessary. MSc is therefore a promising option for MG patients since it both counters a dysregulated immunity and provides an environment of repair.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

ABG: Arterial blood gas
 ACE2: Angiotensin-converting enzyme 2
 AChR: Anti-acetylcholine receptor
 ARDS: Acute respiratory distress syndrome
 CARE-MG: COVID-19-associated risks and effects in myasthenia gravis
 COVID-19: Coronavirus disease 2019
 CPAP: Continuous positive airway pressure
 CRP: C-reactive protein
 ECLIA: Electrochemiluminescence immunoassay
 IFN- γ : Interferon gamma
 IgG: Immunoglobulin
 IL-6: Interleukin-6
 IL-12: Interleukin-12
 IL-17: Interleukin-17
 IVIG: Intravenous Immune globulin
 MSc: Mesenchymal stem cell therapy
 MG: Myasthenia Gravis
 MGFA: Myasthenia Gravis Foundation of America Clinical Classification
 pCO₂: Partial pressure of carbon dioxide
 RT-PCR: Reverse Transcription Polymerase Chain Reaction
 SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
 TNF- α : Tumor necrosis factor - alpha
 TMPRSS2: Transmembrane protease, serine 2
 WHO: World Health Organization

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