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Original Research Article

What is the Impact of the Antibody Response to Glycan Alpha-Gal in Guillain-Barré Syndrome Associated with SARS-CoV-2 Infection?

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Abstract

There are several reports of the onset of Guillain-Barré syndrome (GBS) symptoms in COVID-19 patients. Previously, we reported that reduction in anti-α-Gal IgE, IgM and IgG antibody titers and alteration of anti-α-Gal antibody isotype composition correlated with COVID-19 severity. In this case study, we aimed to compare the anti-α-Gal antibody response in COVID-19 and GBS patients. The levels of anti-α-Gal IgE, IgM, IgG and IgA were measured by ELISA in the sera of GBS and COVID-19 patients. Patients diagnosed with GBS showed an increase in anti-α-Gal IgM when compared to healthy individuals and COVID-19 patients. The levels of anti-α-Gal IgA were significantly higher in COVID-19 patients. No significant differences were observed in the levels of IgE and IgG between GBS and COVID-19 patients. Profile of anti-α-Gal antibody isotypes revealed a higher representation of anti-α-Gal IgM and IgG among GBS and COVID-19 patients, respectively. Remarkably, anti-α-Gal IgM was also highly represented in SARS-CoV-2 positive patients suffering GBS. Despite the limited number of cases included in the study, we suggest that anti-α-Gal IgM and IgA responses are differentially regulated in GBS and COVID-19 patients, which could reflect the disparate etiologies of these diseases. A possible association between high anti-α-Gal IgA in GBS and COVID-19 severity is proposed.

Keywords: Alpha-Gal, Antibody, COVID-19, Guillain-Barré syndrome, SARS-CoV-2

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INTRODUCTION

The pandemic of coronavirus disease 19 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected most countries of the world with hundreds of thousands reported deaths. The typical symptoms of COVID-19 patients are associated with viral pneumonia and derived life-threatening respiratory complications with various effects on gastrointestinal, cardiovascular, neurological, hematopoietic and immune systems (Tan et al., (2020). The neurological manifestations of COVID-19 has been reviewed (Correia et al., 2020), including several cases of Guillain-Barré syndrome (GBS) with a strong association between both diseases and greater severity of symptoms in GBS associated with COVID-19 (De Sanctis et al., 2020; Trujillo Gittermann et al., 2020). Furthermore, it has been proposed that SARS-CoV-2 infection triggers GBS (Agosti et al., 2020).

Recently, Civardi et al. (2020) suggested that a broad antiganglioside antibody response is present in COVID-19-associated patients with GBS, which may be responsible for acute ascendant polyneuropathy and anosmia commonly found in COVID-19 patients. Saccharide-induced immune responses are associated with different diseases such as the GBS and the alpha-Gal syndrome (AGS) (de la Fuente et al., 2019).The AGS is a tick-induced allergy to mammalian meat triggered by the IgE antibody response against the carbohydrate Galα1-3Galβ1-(3)4GlcNAc-R (α-Gal), which is present in glycoproteins from tick saliva and tissues of noncatarrhine mammals (Cabezas-Cruz et al., 2019). However, humans evolved by losing the capacity to synthesize α-Gal to develop a strong IgM/IgG antibody response to this carbohydrate present in bacterial microbiota to confer protection to pathogens with this modification on their surface (Cabezas-Cruz et al., 2019; Hodžić et al., 2020).

A recent study showed that the reduction in anti-α-Gal IgE, IgM and IgG antibody titers and alteration of anti-α-Gal antibody isotype composition correlated with COVID-19 severity, suggesting that the inhibition of the α-Gal-induced antibody response may results into more aggressive viremia and severe disease inflammatory symptoms (Urra et al., 2020). The novel contribution of this research is the characterization of the anti-α-Gal antibody response in GBS associated with SARS-CoV-2 infection with the analysis of possible functional implications.

PATIENTS and METHODS

Patients and healthy control individuals

Seven cases diagnosed with GBS and hospitalized during COVID-19 pandemic were included in the study (Tables 1 and 2). Of them, two cases (IDs 1 and 2; Tables 1 and 2) were confirmed infected with SARS-CoV-2 by the real time reverse transcriptase-polymerase chain reaction (RT-PCR) assay from Abbott Laboratories (Abbott RealTime SARS-COV-2 assay, Abbott Park, Illinois, USA) from upper respiratory tract samples after hospital admission. Data from patients with COVID-19 (grouped from hospital discharge, n = 27; hospitalized, n = 29; intensive care unit, n = 25) cases) and healthy control individuals (sera collected from individuals without record of tick bites and allergic reactions prior to COVID-19 pandemic in April 2019, n = 37) were obtained from a previously conducted retrospective case-control study (Urra et al., 2020). The collection and use of individual's data from patients with GBS were approved by Toledo Hospital Complex (C.E.I.C Nos. 17 and 191). The use of samples and individual's data from patients with COVID-19 and healthy individuals was approved by the Ethical and Scientific Committee (University Hospital of Ciudad Real, C-352 and SESCAM C-73) (Urra et al., 2020). Written consent was obtained for all cases included in the study.

Determination of antibody titers against SARS-CoV-2

Antibodies specific for the recognition of coronavirus infection were based on IgM/IgG isotypes against SARS-CoV-2determined by ELISA using the Abbott Architect SARS-CoV-2 IgG test (Abbott. Abbott ARCHITECT SARS-CoV-2 IgG Instructions for Use. H14806R01. April 2020; https://www.fda.gov/media/137383/download) and COVID-19 Virclia IgM+Ig Amonotest (https://www.abacusdx.com/media/VIRCLIA_VCM098.pdf), respectively and following manufacturer instructions.

Determination of antibody titers against α-Gal

Antibody (IgE, IgM, IgG, IgA) titers against α-Gal were determined by ELISA in serum samples as previously described (Urra et al., 2020). Briefly, plates were coated with 50 ng of BSA coated with α-Gal (BSA-α-Gal, thereafter named α-Gal; Dextra, Shinfield, UK) per well in carbonate-bicarbonate buffer (Sigma-Aldrich, St. Louis, MO, USA) and used for ELISA. Human serum samples were diluted 1:100 in PBS with 0.05% Tween 20 (PBST; Sigma-Aldrich, St. Louis, MO, USA) with 1% human serum albumin (HAS) and 100 µl/well were added into the wells of the antigen-coated plates and incubated for 1 h at 37 °C. Plates were washed four times with PBST and 100 µl/well of goat anti-human immunoglobulins-peroxidase IgG (FC specific; Sigma-Aldrich), IgM (µ-chain specific; Sigma-Aldrich), IgE(ε-chain specific; Sigma-Aldrich), and IgA (heavy chain specific; Bio-Rad,

Table 1. Characteristics of the patients included in the study

ID, gender, age, location in Spain	Clinical diagnosis	Antibody isotype to SARS-CoV-2	Antibody titers to α-Gal (O.D.$_{450\ nm}$)
ID1 Male 44 y/o Castilla-La Mancha	AIDP	IgG-positive	IgA: 0.74 IgE: 0.49 IgM: 1.81 IgG: 0.38
ID2 Male 58 y/o Balearic Islands	AMSAN	IgM-positive	IgA: 0.19 IgE: 0.44 IgM: 1.08 IgG: 0.17
ID3 Female 65 y/o Andalucía	AIDP	Negative	IgA: 0.42 IgE: 0.45 IgM: 1.04 IgG: 0.26
ID4 Male 84 y/o Castilla-La Mancha	AMSAN	Negative	IgA: 0.43 IgE: 0.47 IgM: 1.27 IgG: 0.38
ID5 Female 17 y/o Castilla-La Mancha	AIDP	Negative	IgA: 0.20 IgE: 0.46 IgM: 1.82 IgG: 0.34
ID6 Female 54 y/o Castilla-La Mancha	AMSAN	Negative	IgA: 0.54 IgE: 0.44 IgM: 1.20 IgG: 0.31
ID7 Male 48 y/o Extremadura	AMAN	Negative	IgA: 0.63 IgE: 0.48 IgM: 1.05 IgG: 0.29

Abbreviations: AIDP, acute inflammatory demyelinating polyneuropathy; AMSAN, Acute motor and sensory axonal neuropathy; AMAN, acute motor axonal neuropathy.

Hercules, CA, USA)secondary antibodies diluted 1:1000, v/v in blocking solution were added and incubated for 1 h at room temperature (RT). Plates were washed and revealed with 100 µl/well of 3,3,´5,5-tetramethylbenzidine TMB (Promega, Madison, WI, USA). The reaction was stopped with 50 µl/well of 2 N H_2SO_4 and the O.D. was measured in a spectrophotometer at 450 nm. The average of two technical replicates per sample was used for analysis after background (coated wells incubated with PBS and secondary antibodies) subtraction.

Statistical analysis

The ELISA O.D. at 450 nm values were compared between the 4 independent groups by one-way ANOVA test ($p \leq 0.05$; https://www.socscistatistics.com/tests/anova/default2.aspx) followed by post-hoc Tukey HSD, Scheffé, Bonferroni and Holm multiple comparisons ($p < 0.05$;inference $p < 0.05$; https://astatsa.com/OneWay_Anova_with_TukeyHSD/) .

RESULTS

The analysis of anti-SARS-CoV-2 antibodies showed that the diagnosed COVID-19 patients were positive for IgG or IgM (IDs 1 and 2; Table 1). The analysis of anti-α-Gal IgE, IgM, IgG and IgA antibody titers showed differences in serum levels and isotype composition (Figures 1A and 1B). To evaluate the effect of GBS and COVID-19 factors on anti-α-Gal antibody levels, data from COVID-19 patients and healthy individuals were included and compared with GBS cases (Figure 1A).The results of the one-way ANOVA test suggested significant differences between one or more groups for each anti-α-Gal antibody type (Figure 1A). Then, post-hoc multiple comparisons showed significant differences between different groups (Figure 1A).

As previously reported in COVID-19 cases (Urra et al., 2020), the results showed a decrease in anti-α-Gal IgE, IgM and IgG levels when compared to healthy individuals (Figure 1A). The effect of GBS was observed with an increase in IgM and a decrease in IgG antibody levels to α-Gal when compared to healthy controls (Figure 1A).

Table 2. Medication and functional status of the patients included in the study

ID	Medication Date of physical strength examination at HNP Functional status
ID1	Duphalac, 1/8 h; Metamizol, 1/8 h; Trazodona, 100 mg/24 h;IVIg before hospitalization at HNP April 22, 2020 Tetraparesis, Superior limbs: right upper limb 4/5-; left upper limb 3-/5. Lower limbs: right lower limb 4-/5, left lower limb 3-/5. EGM shows an increase in distal latencies and F wave latencies. Decrease in the amplitudes of the CMAP in most of the nerves scanned. The absence of signs of acute or chronic denervation suggests that amplitude decreasing in CMAP recorded are due to conduction blocks by demyelination without associated axonal involvement
ID2	Sertraline; Paracetamol; Lorazepam; Neurontin; Enoxaparin; Zolpidem; Gutron; Sumial; Dulcolax O; Aerored; Famotidine; Lioresal; IVIg before hospitalization at HNP May 20, 2020 Superior limbs: 1-2/5 proximal, better left. Lower limbs: There is no mobilization of the lower limbs. Sensory neurography: Right median and ulnar nerves showed a severe decrease in potential amplitude, with normal conduction velocity; right sural nerve without CMAP. Motor neurography: right median, ulnar, peroneal and tibial nerves without CMAP. EMG showed abundant spontaneous denervation activity, scarcer in the proximal muscle in the right upper limb, and signs of reinnervation only in this muscle
ID3	Lorazepam, at night if necessary; Neurontin 600 (Gabapentin); Ginecanesten; Nolotil, if required; Ipatropium bromide, inhaled; Bisoprolol; Levothyroxine; Losartan; Duloxetine; Movicol; Atorvastatin; Paracetamol; IVIg before hospitalization at HNP May 11, 2020 Superior limbs: right proximal 2-3/5; left proximal 3/5; bilateral distal 2-3/5.Lower limbs: Bilateral distal 1-2/5; the rest 0/5.Sensory neurography: Right ulnar nerve showed a decrease in amplitude and conduction speed; right median nerve not obtaining potential, right sural nerve within normal limits. Motor neurography study of the right median, ulnar, peroneal and tibial nerves showing a severe decrease in the amplitude of the CMAP, with severely increased distal motor latency, and a severe decrease in conduction velocity and absence of F wave. EMG showed few signs of denervation in myotome explored and and slight signs of renewal in myotome of the right upper limb that correspond to demyelinating polyradiculoneuropathy
ID4	Metformin; Movicol; Mastical D; Hydrochlorothiazide; Amlodipine; Acfol; Adiro; Irbesartan; IVIg before hospitalization at HNP May 5, 2020 Superior limbs: proximal 2/5; distal 3-4/5; lower limbs: proximal 2-3/5, distal 3-4/5. Sensory neurography study of the right median, ulnar and sural nerves showed a moderate decrease in the potential amplitude, with conduction velocity within normal limits. Motor neurography study: median, ulnar and right peroneal nerves showed a moderate decrease in the amplitude of the CMAP, with distal motor latency and conduction velocity within normal limits, including F wave; right tibial nerve within normal limits. The EMG study is compatible with a moderate sensory-motor axonal polyneuropathy, with signs of denervation and reinnervation in the myotomes explored
ID5	Lorazepam; Tryptizol; Cymbalta; Lyrica (Pregabalin); Pantoprazole; Atenolol; Movicol; Enoxaparin; IVIg before hospitalization at HNP June 1, 2020 Superior limbs: 4/5, except fingers with 3-4/5. Lower limbs: 4/5, except distal with 2/5 for ankle extension and 3-4/5 for ankle flexion. Sensory neurography: Right median nerve within normal limits; right ulnar nerve showed a slight decrease in potential amplitude, with normal conduction velocity; Right sural nerve showed a severe decrease in potential amplitude, with normal conduction velocity. Motor neurography: Right median nerve showed a normal distal CMAP amplitude, while the proximal had severely decreased amplitude and conduction velocity, with normal distal motor latency. The right ulnar nerve showed a slightly decreased distal CMAP amplitude, while the proximal nerve showed a severely decreased amplitude, with severely decreased conduction speed and normal distal motor latency. Right tibial median nerve showed severely decreased distal CMAP amplitude, proximal without potential, and normal distal motor latency, here no F wave was obtained. In right peroneal nerve, CMAP was not obtained. In both phrenic nerves, a normal CMAP amplitude was observed, with an increase in latency, being light on the right side and moderate on the left.EMG showed signs of denervation in the myotomes explored, with early signs of reinnervation in the myotome of the right upper limb, and reduction in conduction velocity in 2 motor nerves, conduction blocks in 3 nerves, and absence of F responses in 2 nerves, thus meeting criteria for demyelinating polyradiculopathy

Table 2. Continue

ID6	Lorazepam; Tryptizol; Zolpidem; Famotidone; Movicol; Ipratropium Bromide, inhaled; Salbutamol, inhaled; Dulcolax; IVIg before hospitalization at HNP March 13, 2020 Superior limbs: Trapezoids 2/5. Fingers 1-2/5. Bilateral biceps, 1/5; the rest, 0/5. Lower limbs: 4/5 for plantar flexion and 2-3/5 for bilateral ankle and quadriceps extension 1-2/5. Sensory neurography: normal right sural and superficial peroneal nerves, right ulnar nerve with slight decrease in potential amplitude and conduction velocity, right median nerve with severe decrease in potential amplitude, and slight decrease in conduction velocity. Motor neurography study of the right peroneal and tibial nerves showed a severe decrease in the amplitude of the CMAP, with a slight decrease in the conduction velocity, and normal distal motor latency. Median and ulnar nerves without CMAP. EMG showed signs of reinnervation in myotome of the right lower limb and abundant signs of denervation in all myotomes explored. The findings are compatible with an axonal GBS, with sensory and motor involvement
ID7	Pantoprazole; Neurontin; Paracetamol; Lorazepam; Enoxaparin; Ibuprofen; Plasmapheresisbefore hospitalization at HNP April 30, 2020 Right upper limb: initiates carpal extension and finger extension flexion. Lower right limb: minimal quadriceps contraction, ankle flexion-extension 1/5. Left upper limb: finger flexion 1/5; lower left limb: slight ankle movements. Sensory neurography study: median, ulnar, superficial peroneal and right sural nerves within normal limits. Motor neurography study: right median and ulnar nerves showed a severe decrease in the amplitude of the CMAP, with distal motor latency and conduction velocity within normal limits. The right tibial nerve showed a very severe decrease in the amplitude of the CMAP, with an increase in distal motor latency and a decrease in conduction velocity. Right peroneal nerve without CMAP. EMG showed abundant signs of denervation in all myotomes explored, with signs of reinnervation more abundant in the scanned muscles of the right upper limb. It is currently compatible with a predominantly axonal motor polyradiculoneuropathy with signs of reinnervation

Abbreviations: EMG, electromyography; CMAP, compound muscle action potentials; IVIg, intravenous immunoglobulins; HNP, Hospital Nacional de Parapléjicos (National Paraplegic Hospital). International standards for neurological classification of spinal cord injury (ISNCSCI) was used (https://asia-spinalinjury.org/international-standards-neurological-classification-sci-isncsci-worksheet/).

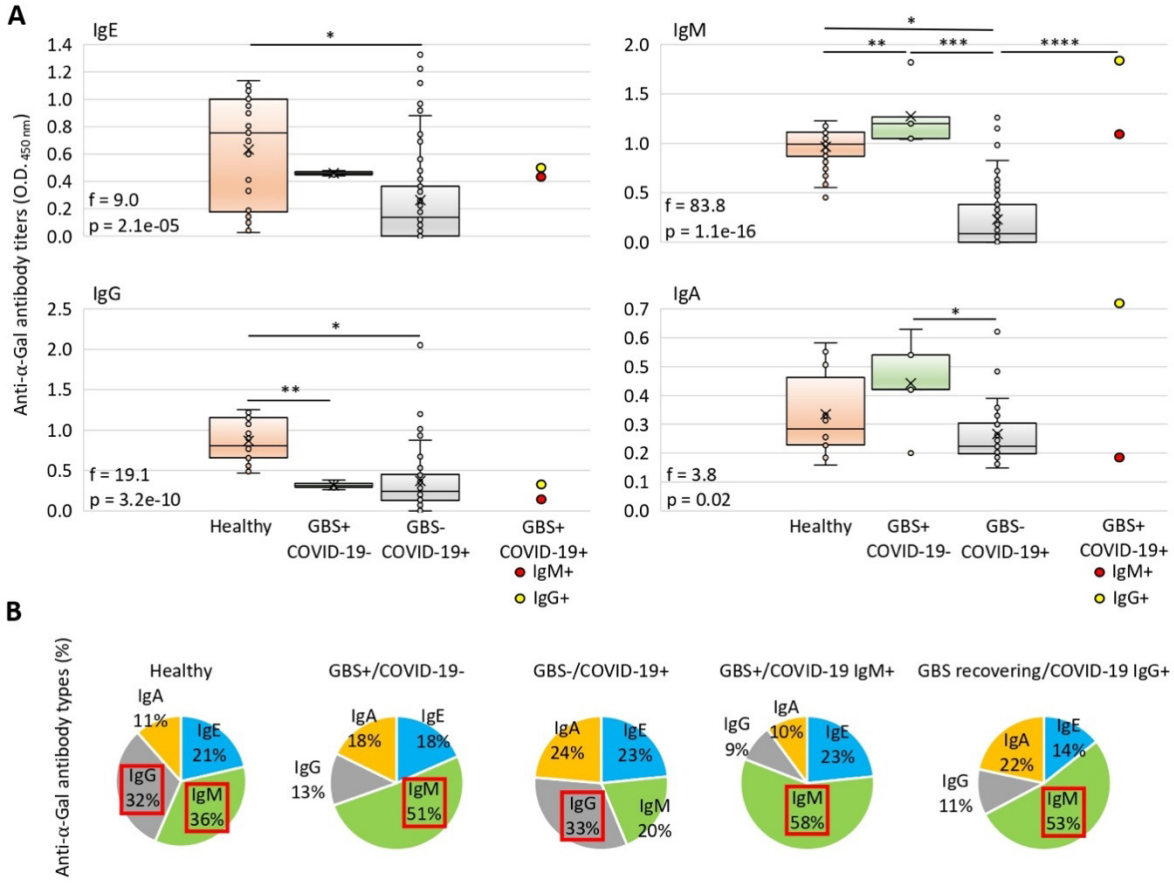

Figure 1. Anti-α-Gal antibody response in GBS associated with COVID-19. (A) Anti-α-Gal IgE, IgM, IgG and IgA serum antibody levels were determined by ELISA in healthy individuals and patients with GBS, COVID-19 and GBS/COVID-19. The ELISA O.D. at 450 nm values were compared between the 4 independent groups by one-way ANOVA test ($p < 0.05$; f-ratio and p-values are shown) followed by post-hoc Tukey HSD (T), Scheffé (S), Bonferroni (B) and Holm (H) multiple comparisons ($p < 0.05$; inference $p < 0.05$). The p-values are shown for significant differences (*IgE: Tp = 0.001; Sp = 2.3e-05; Bp = 6.0e-06; Hp = 6.0e-06. *IgM: Tp = 0.001; Sp = 1.1e-16; Bp = 0.0e+00; Hp = 0.0e+00. **IgM: Hp = 0.04. ***IgM: Tp = 0.001; Sp = 7.1e-12; Bp = 9.3e-13; Hp = 7.8e-13. ****IgM: Tp = 0.001; Sp = 2.3e-07; Bp = 4.4e-08; Hp = 3.0e-08. *IgG: Tp = 0.001; Sp = 8.1e-10; Bp = 1.2e-10; Hp = 1.2e-10. **IgG: Tp = 0.005; Sp = 0.01; Bp = 0.005; Hp = 0.005. *IgA: Tp = 0.04). (B) Profile of anti-α-Gal antibody isotypes representation. Highest representation values for each group/case are shown in red squares.

The comparative analysis between GBS and COVID-19 patients revealed higher anti-α-Gal IgM and IgA antibody levels in GBS patients (Figure 1A). The levels of anti-α-Gal IgM antibodies in GBS+/COVID-19+ cases were higher than in GBS-/COVID-19+ cases, supporting an increase in anti-α-Gal IgM levels associated with the GBS (Figure 1A). Finally, the profile of anti-α-Gal antibody isotypes was qualitatively compared between groups and showed that the most represented immunoglobulins corresponded to IgM/IgG in healthy individuals, IgG in GBS-/COVID-19+ cases, and IgM in all GBS patients with or without COVID-19 (Figure 1B).

DISCUSSION

Despite previous reports about the association of SARS-CoV-2 infection with clinical manifestations of GBS (recently reviewed with 72 patients reported in 52 publications) (Abu-Rumeileh et al., 2020), a causal relationship between viral infection and GBS has not been established. However, the time elapsed between the onset of COVID-19 related symptoms and neurological complaints (min. 2 days –max. 33 days) (Urra et al., 2020) suggests that COVID-19-associated GBS is triggered via a secondary immune mediated mechanism rather than via direct viral neuropathic damage (Abu-Rumeileh et al., 2020). It is worth mentioning that, although with very low frequency, parainfectious profile in which the onset of GBS overlapped with the period of SARS-CoV-2 infection and onset of GBS before COVID-19 symptoms appeared, were also reported (Abu-Rumeileh et al., 2020). Considering that GBS is caused by the production of

autoreactive antibodies (Van den Berg et al., 2014) and that human cells including neurons do not produce the antigen α-Gal, we rule out the possibility that anti-α-Gal antibodies that may be partly associated with SARS-CoV-2 infection could directly cause GBS. This idea is further supported by the fact that COVID-19 inflammatory response is associated with reduction in anti-α-Gal IgE, IgM and IgG antibody titers (Urra et al., 2020). Remarkably, our results showed that GBS is associated with a significant increase in the levels of anti-α-Gal IgA when compared with COVID-19 patients. A higher representation of anti-α-Gal IgA antibodies was observed in COVID-19 patients that needed intensive care unit (ICU) attention (Urra et al., 2020). Furthermore, most GBS cases reported to this day have been symptomatic for COVID-19 with various disease severity and a significant proportion (63.8%, 46/72) developed pneumonia (Abu-Rumeileh et al., 2020). Invasive pulmonary aspergillosis was also reported among COVID-19 patients admitted to the ICU in Southern Netherlands (van Arkel et al., 2020). Abrogation of anti-α-Gal IgA production in lungs was previously associated with an anti-inflammatory response in endogenous α-Gal-negative turkeys experimentally infected with *Aspergillus fumigatus* (Mateos-Hernández et al., 2020).

These findings lead us to the hypothesis that a potential increase of anti-α-Gal IgA levels in COVID-19-associated GBS may aggravate the inflammatory response in the lungs of SARS-CoV-2-infected patients and therefore aggravates disease morbidity. However, the increase in anti-α-Gal IgM antibody levels associated here with GBS may be explained by co-occurrent host-related factors such as gut microbiota composition, ABO blood group or treatment with intravenous immunoglobulins (IVIg) (Gortázar et al., 2020; Korem et al., 2020; de la Fuente et al., 2020; Indikova et al., 2015; Cabezas-Cruz et al., 2017; Hodžić et al., 2020). Nevertheless, higher anti-α-Gal IgM antibody levels may constitute a protective response to COVID-19 and co-infections with pathogens containing α-Gal (Hodžić et al., 2020).

The main limitations of this study are the low number of cases included in the study and analysis of other co-occurrent factors affecting anti-α-Gal IgM antibody levels. Therefore, although these results cannot be generalized until a higher number of GBS+/COVID-19+ patients are evaluated, these findings suggest that it may be relevant to monitor anti-α-Gal IgA and IgM antibody levels to predict COVID-19 severity particularly in patients with GBS.

CONCLUSION

Although results need to be confirmed in a larger number of cases, the findings of this study suggest that anti-α-Gal IgM and IgA responses are differentially regulated in GBS and COVID-19 patients, which could reflect the disparate etiologies of these diseases. A possible association between high anti-α-Gal IgA in GBS and COVID-19 severity is proposed, which supports the suggestion of monitoring anti-α-Gal IgA and IgM antibody levels in patients with GBS and COVID-19.

Ethics approval and consent to participate

The collection and use of individual's data from patientswith GBS were approved by Toledo Hospital Complex (C.E.I.C Nos. 17 and 191). The use of samples and individual's data from patients with COVID-19 and healthy individuals was approved by the Ethical and Scientific Committee (University Hospital of Ciudad Real, C-352 and SESCAM C-73) (Urra et al., 2020). Written consent was obtained from all cases included in the study.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

EDP, CG, ACC and JF contributed to the conception and design of the study. EDP, CGH, EVB, JBG, JRG, AVG and JF participated in acquisition of data. MC and CGH performed the ELISA tests. All authors participated in the analysis and interpretation of results and critical revision of the article for intellectual content. All authors approved the final version of the manuscript for publication.

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