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Research Article

Immunological, Clinical, and Epidemiological Features of Guillain-Barré Syndrome Associated with SARS-CoV-2 Infection

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Objectives. There is a growing interest in understanding the association between Guillain-Barré syndrome (GBS) and SARS-CoV-2 infection. The aim of this study was to analyse various characteristics of GBS before and after the pandemic outbreak and thus identify possible distinctive features of GBS associated with SARS-CoV-2 (GBS-S). *Material and Methods*. In our centre, we retrospectively reviewed the records of patients diagnosed with GBS between January 2018 and March 2022. Epidemiological, clinical, and immunological data were analysed and compared between patients with GBS according to the time of diagnosis and antecedent events. *Results*. Thirty-nine patients with GBS were included: nine (23.1%) were diagnosed with GBS-S. GBS-S was most frequent in 2020 (6/13, 46.1%). Most of these patients developed a postinfectious classic demyelinating variant (4/9, 44.4%) with frequent bilateral facial paralysis (4/9, 44.4%). Serum antiganglioside antibodies (AGAs) were found in 1/9 patients with GBS-S. Serum anti-SSA/Ro60 antibodies were highly prevalent in GBS-S (7/9 (77.8%) vs. 3/11 (27.3%), $p = 0.019$). Three cases associated with SARS-CoV-2 vaccination (GBS-V) were detected. Of note, two had bilateral facial paralysis and anti-SSA/Ro60 antibodies. *Conclusion*. Our findings suggest that SARS-CoV-2 has become an important antecedent event associated with GBS in our setting. GBS-S shows a postinfectious demyelinating immune-mediated profile with negative serological testing for AGAs. Serum anti-SSA/Ro60 antibodies were found frequently in these patients. Bilateral facial paralysis stands out as a possible characteristic clinical feature both in GBS-S and GBS-V. Larger, prospective studies are needed for a better understanding of its immunopathogenesis.

1. Introduction

Guillain-Barré syndrome (GBS) is the most common cause of acute flaccid paralysis worldwide, with a median incidence of 1.11 cases per 100,000 person-years [1]. Although uncommon, GBS can be fatal in 5 per cent of the affected patients in high-income countries [1]. Since the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic outbreak, the ability of SARS-CoV-2 infection to induce immune dysregulation and autoimmune pathways has been thoroughly studied [2]. In this regard, several cases of GBS associated with SARS-CoV-2 infection have been reported, and numerous epidemiological studies have been published investigating its potential causality [3–5]. Patone et al. found a substantially higher risk of GBS in the first 28 days after a positive SARS-CoV-2 test [5]. Keddie et al.

found the overall case numbers reduced during the pandemic in their epidemiological and cohort study in the United Kingdom [3].

Furthermore, several studies have reported a possible association between SARS-CoV-2 vaccination and GBS, mainly linked to adenovirus-vectored vaccines [6] and a possibly higher risk of facial paralysis [7].

Therefore, there is a growing interest in understanding the relationship between GBS and SARS-CoV-2 infection. The aim of this study was to conduct a descriptive and comparative analysis of GBS over a four-year period to March 2022. Its purpose was to explore possible relevant changes in prevalence, antecedent events, and clinical and immunological features related to GBS associated with SARS-CoV-2.

2. Materials and Methods

2.1. Subjects and Data Collection. This was a single-centre retrospective study of patients aged ≥16 years and diagnosed with GBS between January 2018 and March 2022. The study was conducted in a tertiary hospital and approved by the local ethics committee (PR(AG)93/2022). Electronic patient records were reviewed for data on demographic features (age and sex) and disease variables: antecedent infections/infectious symptoms or vaccines six weeks or less before the onset of neurological symptoms [8], days elapsed between antecedent event and neurological symptoms, clinical presentation, Medical Research Council (MRC) sum score, type of treatment, and intensive care unit (ICU) admission. Clinical GBS variants were classic GBS, Miller Fisher syndrome (MFS), bifacial weakness with paraesthesia, and pharyngeal-cervical-brachial variant [9]. Disability was reviewed at admission and after 90 days using the GBS disability score (GDS), a widely accepted system for evaluating the functional ability of patients, ranging from 0 to 6 (highest level of disability) [10]. Patients able to walk independently (<3) were defined as having a good outcome, as in previous studies [11]. We also collected Brighton diagnostic criteria, ranging from levels 1 (highest level) to 4 [12]. Results of routine cerebrospinal fluid (CSF) examination were also collected. We defined an elevated CSF protein level as higher than 0.45 g/L and pleocytosis as a leukocyte count in CSF greater than five. Patients with an alternative diagnosis were excluded.

2.2. Nerve Conduction Studies (NCS) and Electrodiagnostic Criteria. The electrophysiological evaluation was performed within the first three days of hospital admission. NCS were performed according to standardised techniques using Natus Viking EMG equipment. Distal motor latency, amplitude, and duration of the negative peak of compound muscle action potential (CMAP), motor conduction velocity, and minimal F-wave latency were measured from different stimulation sites (median, ulnar, peroneal, and tibial nerves). Sensory studies were performed orthodromically in the median and ulnar nerve and antidromically. The sensory nerve action potential amplitude was measured from the baseline to the negative peak. According to our centre's reference values, electrophysiological findings were normalised as normal upper and lower limit percentages. For the electrodiagnosis of GBS subtypes, NCS results were reviewed and classified as acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), acute motor-sensory axonal neuropathy (AMSAN), equivocal, or normal [13].

2.3. Microbiological Studies and Antecedent Events. Routine microbiological studies were performed according to standard clinical practice. *H. influenzae* and *Borrelia Burgdorferi* infection was defined as the presence of IgG serum antibodies. *C. jejuni* or *Salmonella* spp. infection was defined as positive coproculture. Infections of M. pneumoniae, hepatitis B virus, herpes simplex virus, varicella-zoster virus, EBV, CMV, and hepatitis E virus were defined as the presence of serum IgM. All patients diagnosed after January 2020 were tested at hospital admission for SARS-CoV-2 infection using rt-PCR in nasopharyngeal swab and/or CSF and IgG/IgM serology and were classified according to the SARS-CoV-2 case definitions of the European Centre for Disease Prevention and Control [14]. We used the hospital's electronic database to identify vaccine exposure. This includes vaccine type, date, and doses for all people vaccinated in Catalonia, Spain.

2.4. Autoantibody Detection in Serum. Fasting blood samples were drawn within the first three days of admission for autoantibody detection. Following our department's guidelines, we perform etiological studies and serum IgA quantification before starting intravenous immunoglobulin (IVIG). Therefore, no patient in the sample received IVIG before blood tests. Serum samples from patients were analysed for IgM and IgG anti-ganglioside antibodies (AGAs) to GM1, GM2, GM3, GD1a, GD1b, GT1b, GT1a, and GQ1b by a standard immunodot-blot assay. The detection of extractable nuclear antigen autoantibodies to topoisomerase I, Sm, RNP, Jo-1, SSA/Ro60, SSA/Ro52, and SSB-La was assayed by commercial chemiluminescent immunoassay. The rest of the immunological tests were performed using commercial techniques standardised in Spanish public healthcare (indirect immunofluorescence for ANA and ANCA and nephelometry for rheumatoid factor). Ambulatory follow-up systemic autoantibody tests were likewise performed following routine medical criteria. The hospital's electronic database was reviewed to March 2022 to detect any development of clinical or paraclinical features suggestive of systemic autoimmune diseases.

2.5. Statistical Analyses. First, we compared the numbers of institutional admissions for GBS before the pandemic outbreak with GBS patients admitted after January 2020. Additionally, we compared antecedent events and demographic and clinical data between patients in the corresponding periods.

Second, we compared demographic, clinical, and immunological data between subgroups according to the antecedent event. Subgroups were defined as GBS associated with SARS-CoV-2 infection (GBS-S) or GBS associated with SARS-CoV-2 vaccine (GBS-V) if patients with GBS were

exposed to these events in the last 6 weeks prior to the onset of neurological symptoms. The remaining patients with GBS associated with an antecedent event unrelated to SARS-CoV-2 (infection or vaccine) or with no evidence of an antecedent event were classified as GBS-O. Comparative statistical analysis was conducted between GBS-S and GBS-O. GBS-V was excluded from statistical analysis due to the low number of cases.

Categorical variables were reported as frequencies (percentages), while mean and standard deviation (SD) or median and interquartile range (IQR) were reported for continuous variables. Tests of normality for all continuous data were conducted with the Shapiro-Wilk test. Chi-square or Fisher's exact test was used to compare categorical variables between subgroups. To compare continuous variables between subgroups, an independent t-test was used for continuous variables that followed a normal distribution, and the Mann-Whitney U test was used for the remaining continuous variables. A two-sided p value below 0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics, version 22.0.

3. Results

3.1. Sample Description and Patient Classification. A total of 39 patients with GBS were recruited with neurological symptom onset between 1 January 2018 and 31 March 2022. Table 1 summarises detailed clinical and paraclinical characteristics of the total sample and the comparative analysis between subgroups as explained in Materials and Methods. An antecedent event was found in 31/39 (79.5%) cases of GBS. A total of 9/39 (23.1%) patients were affected by GBS-S. 3/39 (7.7%) patients were affected by GBS-V. The remaining 27/39 (69.2%) patients were affected by GBS-O.

3.2. GBS and Antecedent Events according to Time of Diagnosis. The number of GBS cases fluctuated from six in 2018 to 13 in 2020 (Figure 1(a)). GBS-S cases predominate in the first year of the pandemic; however, new cases occurred throughout these three years. 2020, the year with the highest total number of GBS cases, coincided with the highest number of patients with GBS-S. Before the start of the SARS-CoV-2 pandemic, the most frequent infectious antecedent event was upper respiratory tract infection (URTI) in 7/16 (44%) of cases, followed by gastroenteritis in 4/16 (25%). After the onset of the pandemic, SARS-CoV-2 infection has become the main antecedent event associated with GBS in our hospital in 9/23 (39.1%) cases (Figure 1(b)). Moreover, cases related to gastroenteritis (3/23, 13%) or URTI (3/23, 13%) have decreased. The proportion of cases of GBS without a known antecedent event has remained stable over time, accounting for approximately a quarter of the patients. Additionally, we report three cases of GBS-V: specifically, two after the Moderna booster and one after the first dose of the Oxford-AstraZeneca vaccine. Regarding electrodiagnosis GBS subtypes, there is no significantly increased prevalence of the AIDP variant (50% vs. 56.5%) after the start of the SARS-CoV-2 pandemic (Figure 1(c)), and the overall proportion of patients with axonal variants (31.3% vs. 26.1%) also remains stable before and after the pandemic ($p = 0.91$).

3.3. Features Associated with GBS-S and Comparison with the Patients with GBS-O (Table 1). SARS-CoV-2 infection diagnosis was confirmed in eight patients and considered probable in one (1/9, 11.1%). Coronavirus disease 2019 (COVID-19) was symptomatic in all patients with GBS-S. However, 5/9 (55.5%) did not develop respiratory distress and did not require oxygen therapy. No patient with GBS-S had microbiological evidence of a recent concurrent infection. SARS-CoV-2 was not found in CSF in patients with GBS-S when tested (0/4, 0%). The mean time interval between COVID-19 symptoms and neurological symptoms was 20.8 days (SD: 10.5 days) (Supplementary Table 2). Most patients with GBS-S had a classic GBS variant (6/9, 66.7%), although other variants were also collected. No patient with MFS was reported in GBS-S. Facial paralysis was found in all patients with GBS-S and cranial neuropathies (4/9, 44%), with bilateral involvement in all of them (4/4, 100%). Although not statistically significant, bilateral facial paralysis (Figure 2(a)) was more frequent in patients with GBS-S compared to GBS-O patients (5/27, 18.5%; $p = 0.23$).

An electrophysiological examination was performed on all 39 patients. AIDP (4/9, 44.4%) was the most common electrodiagnosis in GBS-S. Axonal subtypes were found in 3/9 (33.3%) patients. Lumbar puncture was performed in all patients with GBS-S. No CSF analysis was compatible with pleocytosis, and albumin-cytological dissociation (ACD) was found in 8/9 (88.8) patients. All patients with GBS-S received treatment with IVIG. 2/39 (5.1%) patients with GBS-S underwent plasma exchange after IVIG infusion ($p = 0.05$). At day 90 postadmission, most of them had a good outcome (7/9 (78.8%)). Although not statistically significant, ICU admission was more frequent in GBS-S compared to other patients (3/9 (33.3%) vs. 4/27 (11.1%), $p = 0.15$)). Three fatalities were recorded, none of them being patients with GBS-S.

AGAs were tested in all patients with GBS-S. One (11.1%) patient tested positive (Supplementary Table 1). Table 2 shows detailed information on systemic autoantibodies in the sample and the correspondent comparative analysis. None of the patients had a history of systemic autoimmune disease. Positivity for any kind of systemic autoantibody was higher in GBS-S than in GBS-O. These differences did not reach statistical significance (8/9 (88.9%) vs. 6/13 (46.1%), $p = 0.07$)). All ANA-positive GBS-S cases (4/4, 100%) showed speckled staining in contrast to the more heterogeneous immunostaining patterns found in the GBS-O patients. This type of staining is more characteristic of the presence of autoantibodies to extractable nuclear antigens, including those against Ro antigen [15]. Anti-SSA/Ro60 autoantibodies were significantly more prevalent in GBS-S than in GBS-O (7/9 (77.8%) vs. 1/9 (11.1%), $p = 0.015$)) (Figure 2(b)). Follow-up systemic autoantibody tests were performed in 9/16 (56.2%) GBS patients, with a mean of 2.3 months elapsed between blood tests (SD: 1.1 months). Persistent seropositivity was seen only in one patient (1/9, 11.1). According to the hospital's database, until 31 March 2022, none of the originally seropositive patients had developed clinical or paraclinical features suggestive of systemic autoimmune diseases.

Table 1: Demographic, clinical, and laboratory findings of patients with GBS.

		Total ($n = 39$)	GBS-S ($n = 9$)	GBS-V ($n = 3$)	GBS-O ($n = 27$)	p^a
Age (years), mean (SD)		60.2 (15)	60.3 (9.6)	60 (6.5)	60.2 (17.2)	0.98
Sex	Female, *n* (%)	16 (41)	4 (44.4)	1 (33.3)	11 (40.7)	1
Days from antecedent event to symptom onset, mean (SD)		23.1 (10.3)	20.8 (10.5)	24 (14.9)	15.7 (9.1)/$n = 18$	0.15
Limb paresis	Tetraparesis	21 (53.8)	4 (44.4)	3 (100)	14 (51.9)	0.66
	Paraparesis	6 (15.4)	2 (22.2)	0 (0)	4 (14.8)	
	Upper limbs	2 (5.1)	1 (11.1)	0 (0)	1 (3.7)	
	Normal	10 (25.6)	2 (22.2)	0 (0)	8 (29.6)	
MRC sum score (median/IQR)		52 (59)	56 (33)	32 (-)	54 (14)	0.47
Sensory impairment	Yes, *n* (%)	33 (84.6)	8 (88.9)	3 (100)	22 (81.5)	1
Cranial neuropathies	Oculomotor, *n* (%)	7 (17.9)	1 (11.1)	1 (33.7)	5 (18.5)	1
	Facial, *n* (%)	13 (33.3)	4 (44.4)	2 (66.7)	7 (25.9)	0.4
	Bilateral, *n* (%)	11 (28.2)	4 (44.4)	2 (66.7)	5 (18.5)	0.23
	Bulbar, *n* (%)	4 (10.2)	1 (11.1)	1 (33.3)	2 (7.4)	1
	Normal, *n* (%)	24 (61.5)	5 (55.6)	1 (33.3)	18 (66.7)	0.69
Clinical diagnosis	Classical GBS, *n* (%)	29 (74.3)	6 (66.7)	3 (100)	20 (74.1)	0.68
	MFS, *n* (%)	5 (12.8)	0 (0)	0 (0)	5 (18.5)	0.30
	Bifacial weakness, *n* (%)	4 (10.2)	2 (22.2)	0 (0)	2 (7.4)	0.25
	PCB, *n* (%)	1 (2.5)	1 (11.1)	0 (0)	0 (0)	0.23
Electrodiagnosis	AIDP, *n* (%)	21 (53.8)	4 (44.4)	2 (66.7)	15 (55.6)	0.70
	AMAN, *n* (%)	6 (15.4)	1 (11.1)	0 (0)	5 (18.5)	0.48[b]
	AMSAN, *n* (%)	5 (12.8)	2 (22.2)	1 (33.3)	2 (7.4)	
	Equivocal, *n* (%)	7 (17.9)	2 (22.2)	0 (0)	5 (18.5)	0.57
CSF ACD, *n* (%)	Yes, *n* (%)	29/37 (78.4)	8 (88.9)	1/2 (50)	20/26 (76.9)	0.65
Serum AGA, *n* (%)	Yes, *n* (%)	9/37 (24.3)	1 (11.1)	1 (33.3)	7/25 (28)	0.40
Serum systemic autoantibodies, *n* (%)	Yes, *n* (%)	16/25 (64)	8 (88.9)	2 (66.6)	6/13 (43.2)	0.07
Brighton criteria	Level 1	31 (79.5)	8 (88.9)	2 (66.7)	21 (77.8)	0.65
	Level 2	8 (20.5)	1 (11.1)	1 (33.3)	6 (22.2)	
GDS: ≥3	Admission	29 (74.4)	5 (55.6)	3 (100)	21 (77.8)	0.22
	90 days	9 (23.1)	2 (22.2)	1 (33)	7 (25.9)	1
Treatment	PLEX[c]	2 (5.1)	2 (22.2)	0 (0)	0 (0)	0.05
	IVIG	37 (94.9)	9 (100)	3 (100)	25 (92.6)	1
	Corticosteroids	1 (2.6)	0 (0)	0 (0)	1 (3.7)	1
	No treatment	1 (2.6)	0 (0)	0 (0)	1 (3.7)	1
ICU admission	Yes, *n* (%)	7 (17.9)	3 (33.3)	1 (33.3)	3 (11.1)	0.15
IMV	Yes, *n* (%)	4 (10.2)	2 (22.2)	0 (0)	2 (7.4)	0.25
Mortality	Yes, *n* (%)	3 (7.7)	0 (0)	0 (0)	3 (11.1)	1

[a]Comparisons were made between GBS-S and GBS-O. GBS-V is excluded from statistical comparison. [b]Comparison was made between axonal and nonaxonal electrodiagnosis. [c]Additional to intravenous immunoglobulins. Abbreviations: ACD: albumin-cytological dissociation; AGA: anti-ganglioside antibodies; AIDP: acute inflammatory demyelinating polyradiculoneuropathy; AMAN: acute motor axonal neuropathy; AMSAN: acute motor and sensory axonal neuropathy; CSF: cerebrospinal fluid; GDS: GBS disability score; GBS: Guillain-Barré syndrome; ICU: intensive care unit; IQR: interquartile range; IMV: invasive mechanical ventilation; IVIG: intravenous immunoglobulin; MFS: Miller Fisher syndrome; MRC: Medical Research Council; NCS: nerve conduction study; O: other antecedent infections; PCB: pharyngo-cervico-brachial GBS variant; PLEX: plasma exchange; V: SARS-CoV-2 vaccination; S: SARS-CoV-2 infection; SD: standard deviation.

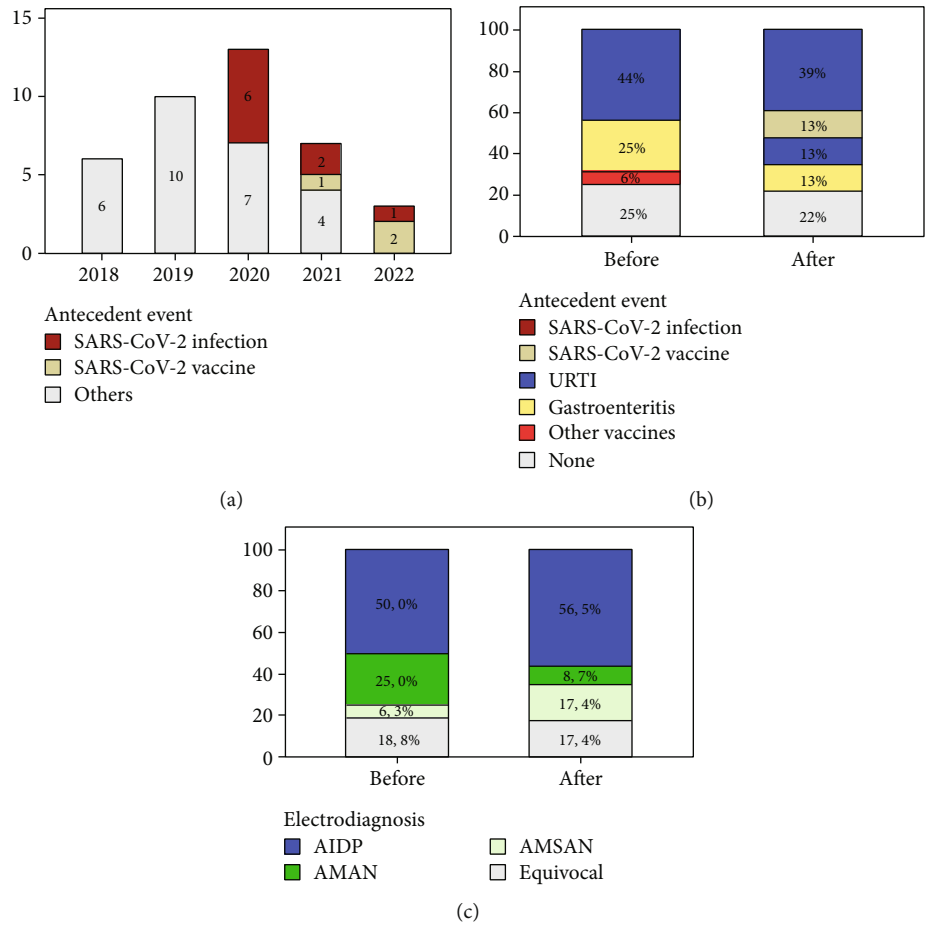

Figure 1: The epidemiological findings regarding GBS diagnosis and associated antecedent event. (a) Number of patients with GBS and SARS-CoV2-related antecedent event according to the year of diagnosis. (b, c) Proportion of patients with GBS according to the associated antecedent event (b) and electrodiagnosis (c) before and after the SARS-CoV-2 pandemic outbreak. AIDP: acute inflammatory demyelinating polyradiculoneuropathy; AMAN: acute motor axonal neuropathy; AMSAN: acute motor and sensory axonal neuropathy; GBS: Guillain-Barré syndrome; URTI: upper respiratory tract infection.

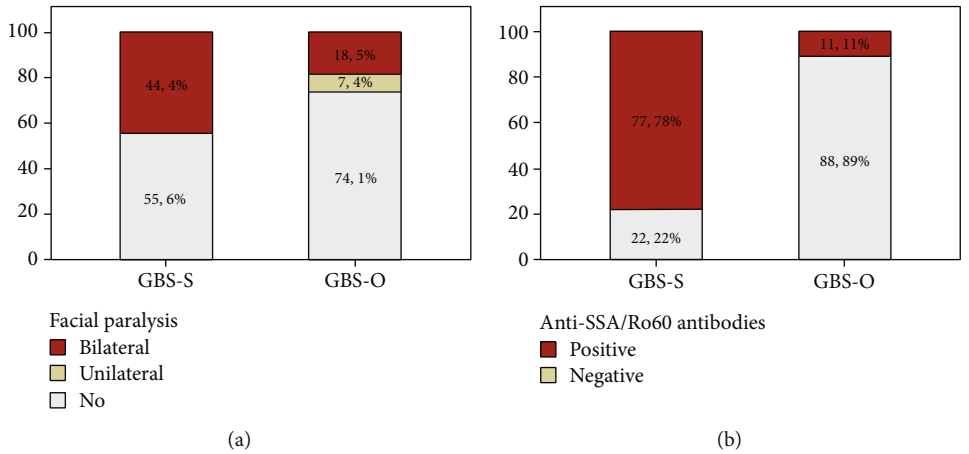

Figure 2: The proportion of patients with GBS with facial paralysis (a) and anti-SSA/Ro60 antibodies (b) according to SARS-CoV-2-related event. FP: facial paralysis; GBS: Guillain-Barré syndrome; O: other antecedent infection; S: SARS-CoV-2 infection; V: SARS-CoV-2 vaccination.

Table 2: Serum systemic autoantibodies in GBS patients according to antecedent event.

		Total ($n = 39$)	GBS-S ($n = 9$)	GBS-V ($n = 3$)	GBS-O ($n = 27$)	p
Serum systemic autoantibodies	Positive test, n (%)	16/25 (64)	8 (88.9)	2 (66.6)	6/13 (43.2)	0.07
	ANAs, n (%)	9/25 (36)	4 (44.4)	1 (33.3)	4/13 (30)	0.66
	Nucleolar, n (%)	1/9 (11.1)	0/4 (0)	0/1 (0)	1/4 (25)	
	Speckled, n (%)	6/9 (66.7)	4/4 (100)	1/1 (100)	1/4 (25)	0.14
	Homogenous, n (%)	2/9 (22.2)	0/4 (0)	0/1 (0)	2/4 (50)	
	ANA titer, median (range)	1/80 (1/80-1/640)	1/80 (1/80-1/160)	1/160 (-)	1/160 (1/80-1/640)	1
	Anti-SSA/Ro60, n (%)	10/21 (47.6)	7 (77.8)	2 (66.6)	1/9 (11.1)	*0.015*
	>20.00 CU, median (IQR)	69.6 (61.6)	59.9 (58.8)	43.3 (-)	34 (-)	1
	Anti-SSA/Ro52, n (%)	2/21 (9.5)	2 (22.2)	0 (0)	0/9 (0)	—
	ANCA, n (%)	1/15 (6.6)	0/5 (0)	0/2 (0)	1/8 (12.5)	1
	RF, n (%)	2/10 (20)	1/5 (20)	0/2 (0)	1/3 (33.3)	1
	aPL, n (%)	0/7 (0)	0/5 (0)	0/1 (0)	0/1 (0)	—
Persistent seropositivity, n (%)		1/9 (11.1)	1/5 (20) (ANAs)	0/1 (0)	0/3 (0)	—

[a]Italic-typed p values represent statistically significant differences between GBS-S and GBS-O. GBS-V has been excluded from statistical analysis. Abbreviations: ANAs: antinuclear antibodies; ANCA: antineutrophil cytoplasmic antibodies; aPL: antiphospholipid antibodies; CU: chemiluminescent unit; GBS: Guillain-Barré syndrome; IgG: immunoglobulin G; IQR: interquartile range; O: other antecedents or none; RF: rheumatoid factor; V: vaccine against SARS-CoV-2; S: SARS-CoV-2 infection; SD: standard deviation.

3.4. Features of GBS Associated with SARS-CoV-2 Vaccine (Table 1). All patients with GBS-V (3/39, 7.7%) showed a classic variant, while 2/3 (66.7%) showed cranial nerve involvement and demyelinating features on NCS (2/3, 66.7). Of note, facial paralysis was found in 2/3 (66.6%) patients with GBS-V, with bilateral involvement in both of them. AGAs were tested in all patients with GBS-V and were positive in one (33.3%). 2/3 (66.7%) GBS-V showed seropositivity for anti-SSA/Ro60 autoantibodies.

4. Discussion

In the present study of patients with GBS presenting to a tertiary hospital over the last four years, SARS-CoV-2 became a highly prevalent antecedent event related to predominantly classic demyelinating postinfectious GBS responsive to immunomodulatory treatment, with a good outcome at day 90 post-admission and negative serological testing for AGAs.

In 2020, there was a significant increase in the total number of GBS cases secondary to the appearance of GBS-S, which accounted for almost half of the total cases that year. While some studies show no increase in GBS cases during the first months of the pandemic [3], others show, as in our study, a clear increase in GBS cases at the expense of a significant number of GBS-S [16]. However, during 2021, there was no increase in the total number of GBS, although SARS-CoV-2 infection has remained one of the main associated antecedent events. This could be explained by a decrease in the number of cases of GBS associated with other infections, most likely due to strategies implemented to combat SARS-CoV-2, such as wearing masks, hand hygiene, and social distancing. These measures to prevent SARS-CoV-2 infection have been effective in reducing infection by other pathogens, demonstrated by a dramatic decrease in cases of influenza, enterovirus, and other causes of pneumonia during the COVID-19 pandemic [17, 18].

In the present study, most patients with GBS-S developed a classic demyelinating variant with frequent cranial neuropathy, especially bilateral facial paralysis. This clinical and neurophysiological profile is consistent with previous reports of GBS-S [4]. The predominant demyelinating phenotype linked to GBS-S is also consistent with that found in GBS associated with other antecedent viral infections such as Zika virus [19], thus suggesting that prominent demyelination may be the neurophysiological signature of an antecedent viral infection in GBS, in contrast to axonal GBS associated with bacterial infections [20]. Regarding cranial nerve involvement, Uncini et al. already reported a highly frequent bilateral involvement of facial paralysis in GBS-S (13/16, 81.2%) [21], in line with the findings in the present study. However, demyelinating features in NCS and facial paralysis are also suggestive features of most cases of GBS [1]. Thus, these electroclinical features seem to be representative of GBS-S but do not suffice to distinguish them from others [22]. The onset of neurological symptoms in patients with GBS-S occurred within an approximated mean time of three weeks after COVID-19 symptoms began, in line with most scientific literature [4, 22, 23]. On the other hand, CSF pleocytosis and viral replication were absent in all tested subjects. Taken as a whole, the above does not support the parainfectious hypothesis suggested by the first reported case in Wuhan [24]. Finally, GBS-S exhibits a satisfactory response to immunomodulatory treatment with a good midterm outcome, as reported in previous GBS-S reviews and meta-analyses [4, 16]. All the above favours a postinfectious autoimmune aetiology in GBS-S, in accordance with current scientific literature [23].

Despite the above, the most characteristic immunological marker of GBS, AGAs, was found only in one patient

in the present study and has been rarely reported in patients with GBS-S, about 13.8% according to a recent review [25]. This fact contrasts with most GBS cases in general, particularly after *Campylobacter jejuni* infection, in which AGAs are found in more than half of GBS cases [11, 20]. Thus, molecular mimicry between SARS-CoV-2 antigens and peripheral nerve glycolipids might not be the driving force underlying GBS-S pathogenesis, as already pointed out by Palaiodimou et al. and Ariño et al. [4, 23], despite the scarce evidence pointing to this possibility [26]. Systemic autoantibodies of any kind were present in this study in all but one case of GBS-S, primarily due to the transient presence of anti-SSA/Ro60 antibodies. The prevalence of systemic autoantibodies in hospitalised COVID-19 patients, especially antinuclear antibodies, has been reported to be up to 50% or more and has been linked in some case series to the worst prognosis [27, 28]. However, anti-SSA/Ro60 seropositivity in COVID-19 patients is less well characterized [29–32]. Anti-SSA/Ro60 antibodies in systemic autoimmune diseases, especially Sjögren syndrome, are intimately linked to the upregulation of the type-I interferon (IFN) pathway and its regulated genes, i.e., the IFN signature [33]. Type-I IFN is crucial in antiviral response by activating and modulating innate immunity and adaptative antibody pathways [34]. We hypothesise that patients with GBS-S could have a distinct immunopathogenesis, related to an aberrant delayed upregulation of the IFN signature after the viral phase [2, 35], which could result in autoimmune inflammatory nerve damage [33, 36]. Gigli et al. reported the presence of anti-SSA/Ro60 antibodies and other immunological features of a patient with GBS-S [37]. We should note that anti-SSA/Ro60 antibodies were found in GBS-V in this study and other previously published works as well [38, 39].

According to previous literature, GBS-V had a predominant phenotype that shared similar clinical and paraclinical features with GBS-S [40, 41]. It has been shown in epidemiological and clinical studies that adenovirus-vectored vaccines harboured an increased risk of association with GBS and bilateral facial paralysis compared to mRNA-based vaccines [5, 7, 41, 42]. Therefore, the antigens in the adenovirus vector could be responsible for GBS development and not the spike protein itself, despite the reports of GBS associated with mRNA-based vaccination [43]. However, it must be borne in mind that the inevitable temporal association between SARS-CoV-2 vaccination and many cases of GBS does not mean causation. The link between vaccinations of any type and GBS is weak, especially if compared to correspondent infections, including SARS-CoV-2 [5, 42].

The main limitations of our study are its small sample size and retrospective design, which inherently lead to bias and reduced statistical power for comparison. Additionally, being a tertiary referral hospital might have altered the referral pattern during the pandemic and thus partially explain the increasing number of GBS patients in 2020. Furthermore, the exceptionally high prevalence of SARS-CoV-2 in Spain during the pandemic might imply a coincidental time association with GBS in some cases. Moreover, further high-powered studies would be needed to explain the relationship between the presence of anti-SSA/Ro60 autoantibodies in patients with SARS-CoV-2 infection and the potential development of GBS. Given the above, the present study does not either pretend nor can it prove a causal relationship between GBS and SARS-CoV-2 infection or vaccination. Instead, our study shows immunological and clinical findings that could lead to future research to better understand GBS epidemiology and aetiopathogenesis in the pandemic era.

5. Conclusions

Our findings suggest that SARS-CoV-2 has become an important antecedent event associated with GBS in our setting. GBS-S shows a postinfectious, mostly demyelinating immune-mediated profile with negative serological testing for AGAs. Bilateral facial paralysis stands out as a possible clinical characteristic feature both in GBS-S and GBS-V. Serum anti-SSA/Ro60 autoantibodies were frequently found in these patients. Larger and prospective studies are needed to understand its immunopathogenesis further.

Data Availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Approval

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

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors' Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Juan Luis Restrepo-Vera and Arnau Llauradó, who also wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

Additional supporting information may be found online in the Supporting Information section at the end of the article. Supplementary Table 1: serum anti-ganglioside antibodies in GBS patients according to antecedent event. Supplementary Table 2: individual values for time elapsed between COVID-19 symptoms and neurological symptom onset. *(Supplementary Materials)*

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