



HLA and immunological features of SARS-CoV-2-induced Guillain-Barré syndrome

Gian Luigi Gigli^{1,2,3} · Alberto Vogrig¹ · Annacarmen Nilo¹ · Martina Fabris⁴ · Alessia Biasotto⁴ · Francesco Curcio^{2,4} · Valeria Miotti⁵ · Carlo Tascini⁶ · Mariarosaria Valente^{1,2}

Published online: 2 October 2020

© Fondazione Società Italiana di Neurologia 2020

Abstract

We report the clinical and immunological features in a case of SARS-CoV-2-induced Guillain-Barré syndrome (Si-GBS), suggesting that (1) Si-GBS can develop even after paucisymptomatic COVID-19 infection; (2) a distinctive cytokine repertoire is associated with this autoimmune complication, with increased CSF concentration of IL-8, and moderately increased serum levels of IL-6, IL-8, and TNF- α ; (3) a particular genetic predisposition can be relevant, since the patient carried several HLA alleles known to be associated with GBS, including distinctive class I (HLA-A33) and class II alleles (DRB1*03:01 and DQB1*05:01). To the best of our knowledge, this is the first case of GBS in which SARS-CoV-2 antibodies were detected in the CSF, further strengthening the role of the virus as a trigger. In conclusion, our study suggests that SARS-CoV-2 antibodies need to be searched in the serum and CSF in patients with GBS living in endemic areas, even in the absence of a clinically severe COVID-19 infection, and that IL-8 pathway can be relevant in Si-GBS pathogenesis. Further studies are needed to conclude on the relevance of the genetic findings, but it is likely that HLA plays a role in this setting as in other autoimmune neurological syndromes, including those triggered by infections.

Keywords Covid-19 · Coronavirus · Neurological complications · Interleukin-6 · Interleukin-8 · Cytokines · Polyradiculoneuropathy · Guillain-Barré syndrome · Neurology

Gian Luigi Gigli and Alberto Vogrig contributed equally to this work.

✉ Annacarmen Nilo
annacarmen.nilo@gmail.com

¹ Clinical Neurology Unit, Santa Maria della Misericordia University Hospital, Piazzale Santa Maria della Misericordia, 15, 33010 Udine, Italy

² Department of Medicine (DAME), University of Udine Medical School, Udine, Italy

³ Department of Mathematics, Informatics and Physics (DMIF), University of Udine, Udine, Italy

⁴ Institute of Clinical Pathology, Department of Laboratory Medicine, Santa Maria della Misericordia University Hospital, Udine, Italy

⁵ Laboratory of Immunogenetics, Santa Maria della Misericordia University Hospital, Udine, Italy

⁶ Infectious Diseases Unit, Santa Maria della Misericordia University Hospital, Udine, Italy

Introduction

Neurological complications of the novel coronavirus disease 2019 (COVID-2019) are increasingly reported and include both para-infectious manifestations (i.e., developing at the same time of the acute infection by the severe acute respiratory syndrome coronavirus [SARS-CoV-2]) and post-infectious disorders. The first category includes encephalopathy and stroke [1, 2], while delayed neurological complications are often immunotherapy-responsive, including steroid-sensitive encephalitis [3] and Guillain-Barré syndrome (GBS) [4]. However, the exact pathogenesis of these neuroimmune complications remains elusive, and it is also unknown whether a clinically mild or asymptomatic COVID-19 infection is sufficient to act as a trigger.

Herein, we report the clinical and immunological features in a case of SARS-CoV-2-induced GSB (Si-GBS).

Case report

A 53-year-old man presented with a 6-day history of lower limb paresthesia, followed by distal weakness, ataxia, and areflexia. The patient did not manifest fever, cough, diarrhea, nor fatigue in the 4 weeks prior to hospitalization. However, he reported a close contact with COVID-19-infected colleagues 17 days before the onset of the neurological symptoms. He also recalled a flu-like syndrome starting 55 days before GBS onset. The latter was characterized by low-grade fever and diarrhea for 7 days. Despite the fact that the patient never experienced respiratory symptoms, chest computed tomography (CT) scan revealed the presence of bilateral ground-glass opacities. Nasopharyngeal swab was negative for SARS-CoV-2 in two independent tests performed on real-time reverse transcriptase–polymerase chain reaction (RT-PCR) assay. The presence of SARS-CoV-2 was subsequently excluded also in the gastric aspirate and in the sputum. Additionally, PCR for influenza A and B viruses and serology for *Borrelia* and tick-borne encephalitis (TBE) resulted negative. CSF analysis revealed albumin-cytologic dissociation (increased protein content [193 mg/dL] and normal white cell count), while nerve conduction studies were compatible with a demyelinating process (Table 1). A brain magnetic resonance

imaging (MRI) excluded an involvement of the central nervous system. The patient was treated with intravenous immunoglobulin with gradual clinical improvement. At last follow-up, 25 days after onset, only mild difficulty in tandem gait and diffuse hyporeflexia persisted.

Immunological investigations

The presence of antibodies for SARS-CoV-2 was investigated using 3 different techniques in both serum and cerebrospinal fluid (CSF): (1) rapid serological test (Cellex, USA); (2) enzyme-linked immunosorbent assay—ELISA (Eurospital Diagnostic, Italy); (3) paramagnetic particle chemiluminescent immunoassay—CLIA (YHLO, China).

Serum resulted IgG and IgM highly positive showing specific reactivity against SARS-CoV-2 nucleocapsid and spike 1 and 2 glycoproteins. CSF resulted strongly positive for IgG and IgM by rapid test and IgG positive with specific reactivity against nucleocapsid and spike 2 glycoprotein by ELISA.

A large panel of autoantibodies was also tested in serum, revealing negative anti-ganglioside IgG and IgM antibodies (GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b, GQ1b), low-positive ANA (1:80, fine-speckled

Table 1 Clinical course and paraclinical studies

Patient sex, age (years)	Onset of neurologic syndrome	Neurologic signs and symptoms	Other clinical and paraclinical features	CSF findings	SARS-CoV-2 analysis	Treatment
M (53)	(a) 55 days after fever/diarrhea (living in an Italian region with high incidence for COVID-19). (b) 17 days after contact with COVID-19-infected colleagues	Lower limb paresthesia (day 1) and weakness (day 3) with ataxia (day 4), areflexia (day 6)	NCS: prolongation of distal latencies and F waves (day 6) Routine laboratory tests: normal, increase CRP (day 13) Chest CT: mild interstitial pneumonia (day 14) Brain MRI: normal (day 17) Erythema nodosum (day 13) and lower limb skin petechiae (day 17)	Day 6: increased protein level, 193 mg/dL; white cell count, 2 per mm ³	Day 7: first RT-PCR assay on nasopharyngeal swab negative Day 14: second RT-PCR assay on nasopharyngeal swab negative Day 16: serologic test on blood and CSF positive Day 17: RT-PCR assay on sputum and gastric aspirate negative	1 cycle of IVIG with clinical improvement of ataxia and lower limb paresthesia/-weakness

COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CSF, cerebrospinal fluid; CT, computed tomography; IVIG, intravenous immunoglobulin; M, male; NCS, nerve conduction study; RT-PCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Table 2 Serum and CSF levels of cytokines

Cytokine	Serum concentration, pg/mL (range)*	Interpretation	CSF concentration, pg/mL (range)*	Interpretation	CSF/serum ratio
IL-1b	0.39 (<0.21)	↑	0.10 (0.1–0.5)	Normal	0.26
IL-6	49 (0.76–6.38)	↑	2 (2.1–9.6)	↓	0.04
IL-8	26 (6.7–16.2)	↑	121 (32.6–88)	↑	4.65
TNF- α	16 (7.78–12.2)	↑	2 (0.2–3.7)	Normal	0.12

CSF, cerebrospinal fluid; IL, interleukin; TNF, tumor necrosis factor

*Ranges obtained from healthy subjects provided by the manufacturer (ELLA™, Bio-Techne, USA)

pattern), low-positive anti-SSA Ro52 and Ro60 antibodies, positive p-ANCA (1:160) with negative anti-myeloperoxidase and proteinase 3 antibodies, and negative anti-dsDNA antibodies. Cryoglobulins and HCV antibodies resulted also negative, while C3 and C4 complement components were within normal range.

Human leukocyte antigen (HLA) was analyzed, showing the following genotype: A*02:01,*33:01; B*14:02,*51:01; C*07:01,*08:02; DRB1*01:02,*03:01; DQA1*01:XX,*05:01; DQB1*02:01,*05:01.

Cytokines were measured in serum and CSF by multiplex ELISA assay (Bio-Techne, USA), demonstrating moderate increased levels of serum IL-6 (49 pg/mL), IL-8 (26 pg/mL), and TNF- α (16 pg/mL). In CSF, only IL-8 showed a significant increase (121 pg/mL) (Table 2).

Discussion

We report herein the clinical and immunological findings in a case of Si-GBS, suggesting that (1) GBS can develop even after paucisymptomatic COVID-19 infection; (2) a distinctive cytokine repertoire is associated with this complication, with increased CSF concentration of IL-8; and (3) a particular genetic predisposition can be relevant in this setting; therefore, we provided the associated HLA of the patient, paving the way for future studies exploring his role in COVID-19-associated-GBS.

On a clinical standpoint, it is possible that the patient was either infected 2 months before GBS with a mild clinical infection (a much longer delay than expected) [5] or, more likely, that he developed an asymptomatic infection approximately 2 weeks before GBS. The possibility of asymptomatic COVID-19 infection [6], along with infection spreading and positive chest CT for asymptomatic individuals [7], was previously demonstrated and raised the important clinical question on whether we should test for SARS-CoV-2-antibody patients with GBS without symptomatic preceding infection.

To the best of our knowledge, this is the first case of Si-GBS in which SARS-CoV-2 antibodies were detected in the

CSF, further strengthening the role of the virus as a trigger. Moreover, despite the great focus on IL-6 pathway in COVID-19 [8], we observed a much greater increase of the CSF concentration of IL-8. This cytokine was found to be highly elevated in a case of steroid-responsive encephalitis induced by SARS-CoV-2 [3].

Even if it is not possible to establish firm conclusions on the role of HLA in Si-GBS based on the data of a single patient, it is interesting to note that the presented patient harbored several HLA alleles known to be associated with GBS. In particular, regarding class I alleles, HLA-A33 was previously associated with acute inflammatory demyelinating polyradiculoneuropathy (AIDP) in China [9]. The class II allele DRB1*03:01 was also associated with GBS in Iraq [10], while DQB1*05:01 was linked with severe GBS in a study performed in Germany [11]. Further studies are needed to conclude on the relevance of these findings, but it is likely that HLA plays a role in this setting as in other autoimmune neurological syndromes [12], including those triggered by infections.

In conclusion, our study suggest that SARS-CoV-2 Abs need to be searched in the serum and CSF in patients with GBS living in endemic areas, even in the absence of a clinically severe COVID-19 infection, and that IL-8 pathway can be relevant in Si-GBS pathogenesis.

Acknowledgments This work is dedicated in memory of Prof. Arrigo Moglia, neurologist, who died during the fight to COVID-19.

Author contribution Prof. Gigli: designed and conceptualized the study; acquisition of data; interpreted the data; drafted and revised the manuscript for intellectual content; study supervision

Dr. Vogrig: designed and conceptualized the study; interpreted the data; drafted and revised the manuscript for intellectual content

Dr. Nilo: acquisition of data; interpreted the data; revised the manuscript for intellectual content

Dr. Fabris: acquisition of data; interpreted the data; revised the manuscript for intellectual content

Dr. Biasotto: acquisition of data; revised the manuscript for intellectual content

Prof. Curcio: interpreted the data; revised the manuscript for intellectual content

Dr. Miotti: acquisition of data; interpreted the data; revised the manuscript for intellectual content

Prof. Tascini: acquisition of data; revised the manuscript for intellectual content

Prof. Valente: interpreted the data; revised the manuscript for intellectual content; study supervision

Data availability Annacarmen Nilo had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All procedures were performed in accordance with the Declaration of Helsinki. The patient's consent was obtained for publication of this report.

References

- Morassi M, Bagatto D, Cobelli M, D'Agostini S, Gigli GL, Bnà C, Vogrig A (2020) Stroke in patients with SARS-CoV-2 infection: case series. *J Neurol* 267(8):2185–2192. <https://doi.org/10.1007/s00415-020-09885-2>
- Vogrig A, Bagatto D, Gigli GL, Cobelli M, D'Agostini S, Bnà C, Morassi M (2020) Causality in COVID-19-associated stroke: a uniform case definition for use in clinical research. *J Neurol* 1:1–4. <https://doi.org/10.1007/s00415-020-10103-2>
- Pilotto A, Odolini S, Stefano Masciocchi S, et al. (2020) Steroid-responsive encephalitis in coronavirus disease 2019. *Ann Neurol*; published online May 17. <https://doi.org/10.1002/ana.25783>
- Toscano G, Palmerini F, Ravaglia S, Ruiz L, Invernizzi P, Cuzzoni MG, Franciotta D, Baldanti F, Daturi R, Postorino P, Cavallini A, Micieli G (2020) Guillain-Barré syndrome associated with SARS-CoV-2. *N Engl J Med* 382(26):2574–2576. <https://doi.org/10.1056/NEJMc2009191>
- Willison HJ, Jacobs BC, van Doorn PA (2016) Guillain-Barré syndrome. *Lancet* 388(10045):717–727. [https://doi.org/10.1016/S0140-6736\(16\)00339-1](https://doi.org/10.1016/S0140-6736(16)00339-1)
- Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W, Seilmaier M, Drosten C, Vollmar P, Zwirgmaier K, Zange S, Wölfel R, Hoelscher M (2020) Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *N Engl J Med* 382(10):970–971. <https://doi.org/10.1056/NEJMc2001468>
- Meng H, Xiong R, He R, Lin W, Hao B, Zhang L, Lu Z, Shen X, Fan T, Jiang W, Yang W, Li T, Chen J, Geng Q (2020) CT imaging and clinical course of asymptomatic cases with COVID-19 pneumonia at admission in Wuhan, China. *J Inf Secur* 81(1):e33–e39. <https://doi.org/10.1016/j.jinf.2020.04.004>
- McGonagle D, Sharif K, O'Regan A, Bridgewood C (2020) The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. *Autoimmun Rev* 19(6):102537. <https://doi.org/10.1016/j.autrev.2020.102537>
- Guo L, Wang W, Li C, Liu R, Wang G (2002) The association between HLA typing and different subtypes of Guillain Barré syndrome. *Zhonghua Nei Ke Za Zhi* 41(6):381–383
- Hasan ZN, Zalzal HH, Mohammedsalih HR, Mahdi BM, Abid LA, Shakir ZN, Fadhel MJ (2014) Association between human leukocyte antigen-DR and demyelinating Guillain-Barré syndrome. *Neurosciences (Riyadh)* 19(4):301–305
- Schirmer L, Worthington V, Solloch U, Loleit V, Grummel V, Lakdawala N, Grant D, Wassmuth R, Schmidt AH, Gebhardt F, Andlauer TFM, Sauter J, Berthele A, Lunn MP, Hemmer B (2016) Higher frequencies of HLA DQB1*05:01 and anti-glycosphingolipid antibodies in a cluster of severe Guillain-Barré syndrome. *J Neurol* 263(10):2105–2113. <https://doi.org/10.1007/s00415-016-8237-6>
- Muñiz-Castrillo S, Vogrig A, Honnorat J (2020) Associations between HLA and autoimmune neurological diseases with autoantibodies. *Autoimmun Highlights* 11(1):2. <https://doi.org/10.1186/s13317-019-0124-6>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.