RISK FACTORS AND ANTIBODY RESPONSE ASSOCIATED WITH LONG COVID: LONGITUDINAL COHORT STUDY

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RISK FACTORS AND ANTIBODY RESPONSE ASSOCIATED WITH LONG COVID: LONGITUDINAL COHORT STUDY(Abstract): **Aims:** Although numerous studies assess risk factors for long COVID, data on humoral response are scarce and discordant. This study aimed to determine if serum antibody titers are associated with long COVID and to analyze demographic characteristics, comorbidities, treatment, and disease severity influencing its development. **Materials and methods:** We included 80 unvaccinated COVID-19 patients who contracted the disease in early 2020. Clinical data and serum samples were collected at inclusion, 33±10 days (visit 1), and 91±19 days (visit 2) post-symptom onset for symptomatic patients, and post-diagnosis for asymptomatic patients. Symptoms were tracked via questionnaires at each visit and one month after the second visit by telephone. Serum samples were analyzed to measure IgG antibodies using indirect chemiluminescent immunoassay technology. **Results:** We found 53.7% (CI95%, 42.2-65; p=0.57) of patients experienced long COVID. Female gender (OR, 1.95; CI95%, 1.12-3.41; p=0.01) and pre-existing comorbidities (OR, 1.56; CI95%, 1.02-2.38; p=0.04) were significantly associated with long COVID symptoms. Importantly, patients with long COVID had higher overall median antibody levels at both follow-up visits. While this difference was not statistically significant, it suggests a trend worth further investigation. **Conclusions:** Understanding the risk factors associated with long COVID could provide valuable insights for managing and potentially decreasing its incidence in the future. **Keywords:** LONG COVID, RISK FACTORS, GENDER, COMORBIDITIES, ANTIBODY TITER.

Long COVID, also known as chronic COVID syndrome or prolonged COVID-19 symptoms, refers to persistent health issues experienced by individuals following a COVID-19 diagnosis. These symptoms typically emerge within a few months and can significantly affect quality of life. While various definitions exist, the World Health Organization defines long COVID as the persistence or emergence of new symptoms at least three months after initial SARS-CoV-2 infection, lasting for a minimum of two months without any other clear cause (1). Estimates suggest 10–20% of

individuals may experience such symptoms (1), which encompass over 200 reported manifestations including fatigue, shortness of breath, cognitive difficulties, chest pain, and joint pain, among others (2).

Similarities to postviral syndromes observed in previous coronavirus diseases like MERS and SARS highlight the long-term impact of such infections, with symptoms such as fatigue and psychiatric impairments persisting for years (3, 4). Identifying risk factors, both pre-infection (e.g., age, sex, comorbidities) and infection-associated (e.g., disease severity, viral load), is crucial for predicting, preventing, and managing long COVID (5).

Understanding the role of antibody response dynamics is pivotal for developing effective treatments and interventions (6). However, studies on humoral responses in long COVID have yielded conflicting results, with some indicating higher antibody titers associated with persistent symptoms (7,8), while others suggesting lower titers (9).

This study aimed to investigate the association between serum antibody titers and long COVID, while analyzing demographic characteristics, comorbidities, treatment, and disease severity as potential risk factors for its development. By elucidating these factors, this research contributes to ongoing efforts in managing and potentially reducing the incidence of long COVID in the future.

MATERIALS AND METHODS

Patients and collection of serum samples and clinical data

Adult patients aged 18 years or older diagnosed with SARS-CoV-2 infection via positive reverse transcription polymerase chain reaction (PCR) tests from nasopharyngeal swabs were consecutively recruited for this study. The study population was drawn from a larger investigation conducted at the onset of the COVID-19 pandemic, focusing on the clinical performance of six commercially available assays, including the chemiluminescent immunoassay (CLIA) IgG antiS1/S2 (10). Enrollment commenced in May 2020, with baseline blood samples collected during hospitalization: 6+/-10 days post-symptom onset (PSO) for symptomatic patients, and post-day of diagnosis (PDD) defined as the first positive PCR for asymptomatic patients. Subsequent serum samples were obtained at 33+/-10 days (visit 1) and 91+/-19 days (visit 2) PSO for symptomatic patients, and PDD for asymptomatic patients. Demographic and clinical data were collected concurrently. Only patients who completed visit 2 were included in the analysis. Specimens were processed within 5 days of collection: stored at 4 °C, centrifuged, aliquoted, and frozen at −20 °C until analysis. Data on demographic characteristics (age, gender), comorbidities, and COVID-19 clinical classifications (mild, moderate, severe) were collected using a questionnaire based on WHO criteria. Persistent or new symptoms were assessed at each visit, with a follow-up telephone questionnaire (Annex 1- Post-COVID Questionnaire) conducted one month after visit 2 for symptomatic patients.

Long COVID was defined as the persistence or emergence of signs, symptoms, or conditions beyond three months post-acute COVID-19 infection, lasting over one month without an apparent cause. Patients reporting at least one persistent symptom fitting these criteria were classified as having long COVID.

Ethical approval was obtained from the hospital's local ethics committee, and all participants provided written informed consent for serial blood sampling.

Assays. Nasopharyngeal swabs were analyzed for SARS-CoV-2 RNA using the Allplex™ SARS-CoV-2 Assay and the CFX96 Real-Time PCR Detection System. Serum samples were tested for IgG antibodies against SARS-CoV-2 S1/S2 using the LIAISON® SARS-CoV-2 S1/S2 IgG assay on the Liaison XL platform, with results reported in arbitrary units (AU/mL). Samples with a value of ≥15 AU/ml were interpreted as positive, 12.0 ≤ AU/mL < 15.0 equivocal, and < 12.0 AU/mL as negative.

Statistical analysis employed IBM® SPSS® Statistics version 26.0. Descriptive statistics included median values with interquartile range (IQR). Results are presented as total values and percentages with 95% confidence intervals. Mann-Whitney U test compared categorical variables, and Fisher's exact test analyzed categorical variables between patients with and without long COVID symptoms. Odds ratios (ORs) with 95% confidence intervals were calculated. A p value <0.05 indicated significance.

RESULTS

A total of 93 patients were initially recruited in the study, but by the end of the study two patients died and 11 were lost to follow-up. Among them, 44 were males (55%, p=0.43), median age 49.5 (IQR 38.2-57). Five patients (6.25%, p=0) had been asymptomatic, 32 (40%, p=0.09) had a mild form of the disease, 25 (31.25%, p=0.001) had moderate disease and 18 of them (22.5%, p=0) had severe disease. Comorbidities were recorded in 45 patients (56.25%, p=0.31) as illustrated in Table 1, with arterial hypertension being the most common one.

Forty-three patients (53.75%, p=0.57) had long COVID symptoms; refer to Table 2 for specific symptoms.

Other Long Covid symptoms: diaphoresis, arterial hypertension, alopecia, headache, visual acuity disturbances (hypermetropia, myopia), ulnar neuropathy, sneeze, and rash

TABLE I.

Associated comorbidities

Comorbidities	N (%)
Arterial hypertension	26 (32.5)
Diabetes mellitus	12 (15)
Dyslipidemia	9 (11.2)
Obesity	8 (10)
Chronic Liver Disease	7 (8.7)
Thyroid Dysfunction	6 (7.5)
Asthma	3 (3.7)
Neoplasia	3 (3.7)

TABLE II.

Long COVID symptoms

Symptoms	N (%)
Fatigability	29 (67.4)
Arthralgia	11 (25.5)
Lack of concentration memory attention	11 (25.5)
Dyspnea	11 (25.5)
Smell disorders	8 (18.6)
Anxiety	7(16.2)
Cough	6(13.9)
Sleep disorders	5 (11.6)
Palpitations	4 (9.3)
Mialgii	4 (9.3)
Taste disorders	3 (6.9)
Emotional lability	3 (6.9)
Lack of interest/Social isolation	1 (2.3)
Others	11 (25.5)

Female gender, OR 1.95 (95% CI 1.12-3.41, p 0.01), severe disease, OR 2.23 (95% CI 0.88-5.68, p 0.07), and comorbidities, OR 1.56 (95% CI 1.02-2.38, p 0.04), especially dyslipidemia, OR 6.88 (95% CI 0.90-52.51, p 0.03), were associated with long COVID. Table III illustrates factors linked to long COVID symptoms.

Steroid treatment and Tocilizumab did not correlate with long COVID onset (OR 1.28 (95% CI 0.74-2.21), p 0.53 and OR

1.15 (95% CI 0.51-2.57), p 1, respectively). Patients with severe disease showed elevated IgG anti-S1/S2 titers, but no significant difference was found between groups or disease severity at any evaluation point (tab. IV).

TABLE III.

Factors associated with developing long COVID

Characteristics	Long COVID N=43 / N (%)	Non-long COVID N=37 / N (%)	OR (95% CI)	p
Sex (F)	25 (58.1)	11 (29.7)	1.95 (1.12-3.41)	0.01
Age (≤50 years)	26(60)	14(37)	1.64 (0.99-2.70)	0.07
Disease form				
Asymptomatic	1 (2.3)	4 (10.8)	0.21 (0.02-1.84)	0.12
Mild	17 (39.5)	15 (40.5)	0.97 (0.57-1.66)	0.92
Moderate	12 (27.9)	13 (35.1)	0.79 (0.41-1.52)	0.49
Severe	13 (30.2)	5 (13.5)	2.23 (0.88-5.68)	0.07
Presence of comorbidities	29 (67.4)	16 (43.2)	1.56 (1.02-2.38)	0.04
Arterial Hypertension	13 (44.8)	13 (81.2)	0.86 (0.45-1.61)	0.81
Dyslipidemia	8 (27.5)	1 (2.7)	6.88 (0.90- 52.51)	0.03
Diabetes mellitus	8 (27.5)	4 (10.8)	1.72 (0.56-5.25)	0.36
Obesity	5 (17.2)	3 (8.1)	1.43 (0.36-5.60)	0.71
Chronic liver disease	5 (17.2)	2 (5.4)	2.15 (0.44-11.44)	0.44
Thyroid dysfunction	4 (13.8)	2 (5.4)	1.72 (0.33-8.86)	0.68
Asthma	2 (6.9)	1 (2.7)	1.72 (0.16-18.22)	1.00
Neoplasia	1 (3.4)	2 (5.4)	0.43 (0.41-4.55)	0.60

TABLE IV.

Median CLIA detected IgG antiS1/S2 concentration according to disease severity and time PSO for both Long COVID and non-Long COVID groups

Disease form	Median CLIA IgG antiS1/S2 (AU/mL), IQR								
	Baseline (6+/-10 days) N=80			Visit 1 (33 +/-10 days) N=80			Visit 2 N=74		
	Long COVID N=43	Non-Long COVID N=37	p	Long COVID N=43	Non-Long COVID N=37	p	Long COVID N=40	Non-Long COVID N=34	p
Asymptomatic / Mild	3.8 3.8-4.9	3.8 3.8-4.7	0.82	48.7 33.2-76.3	63.7 19.1-104	0.61	59.4 26.2-91.4	37.8 11.4-79.3	0.61
Moderate	3.8 3.8-9.2	3.8 3.8-8.9	0.92	76.6 42.7-160	80.6 20.6-115	0.90	91.4 41.6-177.7	69.2 17-92.6	0.25
Severe	3.8 3.8-5.3	3.8 35-21	0.41	76.4 42.3-108.7	80 22.9-107.7	0.61	71.6 51.9-127	72.6 22.7-101.1	0.89
Overall	3.8 3.8-9.2	3.8 3.8-17.8	0.56	95.2 44.5-159	82.8 27.5-121.5	0.26	91.4 56.3-171.7	77.1 25.8-137.7	0.47

DISCUSSION

Long COVID poses challenges for healthcare systems, demanding a multidisciplinary approach (5). Ongoing research has identified risk factors like older age, female gender, and pre-existing conditions (11), but much remains unknown about why symptoms persist variably among individuals. Literature on chronic COVID reflects diverse symptom persistence due to

sample characteristics, study methodologies, and the fact that long COVID exhibits significant heterogeneity.

Long COVID's mechanisms may involve sustained tissue damage and inflammation (5). Survivors often experience multi-organ complications, such as pulmonary (12), neurological (13), cardiac (14), and others (15). Pathological inflammation, including prolonged virus shedding (16), immune dysregulation involving T and B cells (17, 18), and disrupted gut microbiome (19), likely contributes to persistent symptoms.

Ideal for long COVID research is a diverse cohort of individuals recovering from SARS-CoV-2 infection, ranging from mild to severe cases, with persistent symptoms post-acute phase. Longitudinal tracking is crucial to monitor symptom evolution across organ systems. Including hospitalized and non-hospitalized patients, diverse demographics, and comorbidities would capture comprehensive long COVID manifestations and risk factors. Our cohort includes 80 patients recovering from various SARS-CoV-2 clinical forms, from asymptomatic to severe, with ongoing symptoms three to four months post-acute phase. It features balanced gender representation and diverse comorbidity profiles.

Regarding long COVID symptoms in survivors, fatigue was prominently reported in our patient cohort, consistent with findings from other studies (20, 21). Approximately half of our patients experienced fatigue three months post-SARS-CoV-2 infection. Dyspnea, cognitive impairment, and arthralgia affected nearly a quarter of our cohort. Studies with higher dyspnea prevalence, up to 61%, likely included more severe cases and ICU admissions (20, 22), unlike our cohort where severe cases were 22.5% and no ICU admissions occurred. Other symptoms like anosmia, anxiety, and cough were less frequent (23, 24).

Numerous studies are focused on demonstrating that specific epidemiological and clinical risk factors are associated with an increased likelihood of developing long COVID symptoms.

Our study identified a significant association between female gender and increased risk of long COVID symptoms compared to males (OR, 1.95; CI95%, 1.12 to 3.41; p =0.014). This finding is consistent across several individual studies (8, 25-27,) and meta-analyses (11, 28). Studies such as Bai *et al.* (25) reported females having 2.7 times higher odds of long COVID symptoms (CI95%, 1.68 to 4.62), while Asadi-Pooya *et al.* (26), Chudzik *et al.* (27), and Peghin *et al.* (8) reported odds ratios of 1.26 (CI 95%, 1.12 to 1.43), 1.44 (CI95%, 1.20 to 1.70), and 1.55 (CI95%, 1.05 to 2.27), respectively. Meta-analyses by Kin Israel Notarte *et al.* (11) and Maglietta *et al.* (28) also showed significant associations with approximately 50% higher risk for females. The reasons for this gender disparity in long COVID risk are not fully understood, but potential factors include the influence of female hormones on sustaining post-recovery inflammation (29) and higher acute phase mortality rates among males during COVID-19 (30).

The influence of age on long COVID is still debatable, despite the well-established understanding that older age is a significant risk factor for adverse outcomes during COVID-19 hospitalization (31). In our study as well as in the study conducted by Peghin *et al.* (8), who compared young adults under 40 with those older than 40, no association between age and long-COVID was observed. Furthermore, no such association was found in the meta-analysis conducted by Kin Israel Notarte *et*

al. (11) (OR, 0.86; 95%CI 0.73 to 1.03, p = 0.17) who compared individuals under the age of 60 with those aged 60 and above. One possible explanation for this discrepancy includes the inadequate control of confounding factors observed in older individuals, such as a higher prevalence of medical comorbidities or longer hospitalization stays, which may also contribute to developing long COVID (11).

The severity of acute COVID-19 illness does not appear to predict the development of long COVID. Research indicates that long COVID can impact individuals with mild-to-moderate cases, including younger adults treated as outpatients (5). Consistent with other studies (32, 33), we found no association between initial disease severity and long COVID. In contrast, Tsampasian *et al.*'s meta-analysis highlighted severe illness as a significant risk factor (34). ICU survivors may experience post-intensive care syndrome, overlapping with long COVID symptoms (35). Our study, with few severe cases and none in the ICU, underscores the need for further exploration into these complexities.

Comorbidities are recognized as potential risk factors for long COVID. Studies have linked conditions like diabetes (36), obesity (34, 37), cardiovascular disease (36, 37), and respiratory disorders (38) to a higher likelihood of persistent symptoms post-COVID-19 recovery. Consistent with this research, our study found that having comorbidities significantly increased the risk of long COVID (OR, 1.56; CI95% 1.02 to 2.38; p 0.04). Dyslipidemia showed a particularly strong association (OR, 6.88; CI 95% 0.90 to 52.51; p 0.03), though our cohort size limited statistical significance for other conditions. Pretorius *et al.* also noted elevated cholesterol and conditions like hypertension and type 2 diabetes as significant in long COVID (36). Lipid metabolism disorders and obesity represented age-independent risk factors for long COVID syndrome indicating that metabolic changes play a significant role in determining the severity of the disease throughout all stages of COVID-19 in another study conducted by Loosen *et al.* (37).

Similar to other research findings, we found no association between the treatment administered during the acute phase of SARS-CoV-2 infection and long COVID (39).

The long COVID group revealed overall higher median titers of IgG anti-S1/S2 antibody at visit 2 and visit 3, compared to baseline where no disparities were noticed, with no statistically significant differences observed at any time point. Also, when comparing median antibody antiS1/S2 concentration according to the disease severity and time PSO between the two groups, no statistically significant differences were observed. Similar to our results, no association was observed between increased specific immunity evaluated by qualitative IgG antibodies directed against S1 protein using in vitro SARS-CoV-2 ELISA assay and long-term complications in a study conducted by Rank *et al.* (7). Interestingly, Klein *et al.*, found that the long COVID state positively predicted the anti-spike humoral response with significant differences among the various epitopes (40). Potential explanations suggest that long-term complications following COVID-19 may be due to virus-specific pathophysiological changes, immunological dysregulation, and inflammatory damage initiated by the acute infection. However, the involvement of the SARS-CoV-2 specific humoral immune response in post-COVID syndrome remains unclear, given the incomplete and sometimes conflicting data, potentially arising from differences in

methodological approaches.

Our study's strength lies in its focus on unvaccinated patients recruited early in the pandemic, ensuring antibody responses were not influenced by prior COVID-19 infection or vaccination. Further studies are needed to determine the usefulness of measurement of antibody titers for prognosis of long COVID symptoms.This research surpasses the examination of recognized risk factors and extends its focus to explore the complex interaction between antibody titers and the emergence of long COVID over different timeframes. A limitation of this study is its small sample size, although it included participants with varying disease severities from asymptomatic to severe cases. Due to the limited number of patients, not all potential long COVID symptoms may have been captured. While we followed up with patients for at least three months, longer-term observation is needed to understand symptom persistence fully. Another limitation is the use of a single serological method, LIAISON® SARS-CoV-2 S1/S2 IgG (DiaSorin), chosen based on its superior sensitivity and specificity compared to other serological assays, as supported by recent literature (10, 41).

CONCLUSIONS

The study identified female gender and pre-existing medical conditions as potential risk factors for long COVID symptoms. Factors such as age, disease severity during acute COVID-19, immunomodulatory treatment, and antibody levels were not associated with long COVID symptoms. Nonetheless, the current literature does not conclusively identify the risk factors associated with long COVID symptoms. Through the inclusion of analyses conducted at various intervals, the study provides insight into the possible role of antibody dynamics in the development of this condition.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no external funding regarding this scientific research.

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