In this research, the investigators intend to figure out if spike protein IgG levels vary by peak disease severity, adjusting for gender and days since symptom onset. Participant characteristics were compared among three peak disease severity groups using ANOVA for age, the Kruskal-Wallis test for spike IgG level due to non-normality, and the Fisher's exact test for gender due to small cell size counts. There were significant differences among spike IgG levels in three peak disease severity groups ($p < .001$). There were no significant differences among peak disease severity groups for age and gender ($p > .100$).

To examine spike protein IgG levels, we conducted an ANCOVA test and did not find any interaction effects between gender, days since symptom, and peak disease severity in the model. We log-transformed spike IgG level because it is positively skewed, and spike IgG is more normally distributed after log-transformation. We compared the differences among the adjusted means of disease severity categories using Tukey's honestly significant test. There were significant differences in the adjusted mean of spike IgG levels between mild/asymptomatic disease and moderate disease ($p = .004$) and between mild/asymptomatic disease and severe disease. ($p < .001$), after adjusting for gender and days since symptom onset. There were no significant differences between moderate disease and severe disease ($p = .926$).

We conducted model diagnostics to check if the model assumptions are reasonably met and check for outliers. From the residual plots, we concluded that homoscedasticity criteria did not meet in this model, meaning that the residuals get smaller as the prediction moves from small to large. We also noticed that the variance of the mild/asymptomatic disease group is much higher than the variance of the moderate or severe disease group. From the normal Q-Q plot and Shapiro-Wilk test, we concluded that the data is not normally distributed ($p = .012$). There were several outliers detected in the model diagnostics, and we need to further examine these outliers and determine whether these outliers require to be deleted or not.

In conclusion, spike protein IgG levels are not significant between the moderate disease group and severe disease group, while they are different between the mild/asymptomatic disease group and the other two groups. Spike protein IgG levels do vary by peak disease severity, adjusting for gender and days since symptom onset. Word count: 386 words

Table 1. Summary of participant characteristics (N[a] = 175)

Variable	Total Sample (n=175), Mean ±SD [range] or No. (%)	Mild/Asymptomatic (n=162), Mean ±SD [range] or No. (%)	Moderate (n = 6), Mean ±SD [range] or No. (%)	Severe (n = 7), Mean ±SD [range] or No. (%)	P-value*
Age, years	40.9 ±13.1 [19.0-81.0]	40.8 ±13.3 [19.0-81.0]	38.5 ±10.6 [29.0-56.0]	44.9 ±12.8 [26.0-57.0]	.658
Spike IgG, AU/ml	1773.2 ±5728.8 [4.5-73076.5]	1546.6 ±5847.2 [4.5-73076.5]	3758.2 ±2513.3 [489.2-6645.7]	5315.3 ±3015.4 [1188.0-9767.2]	<.001
Gender					.751
Female	99 (56.6)	93 (57.4)	3 (50.0)	3 (42.9)	
Male	76 (43.4)	69 (42.6)	3 (50.0)	4 (57.1)	

%: percent proportion of the stratum total was displayed. [a]: 12 participants were missing data.

*We used ANOVA test for age, the Kruskal-Wallis test for Spike IgG due to non-normality, and the Fisher's exact test for gender due to small cell size counts to conduct the p-values.

Abbreviation: SD, standard deviation; No., number; Spike IgG, Spike Immunoglobin G; AU, arbitrary unit.

Table 2. ANCOVA analysis (N[a] = 175)

	Peak disease severity		
	Mild/Asymptomatic (n = 162)	**Moderate (n = 6)**	**Severe (n = 7)**
Adjusted mean [95% CI], AU/ml on log scale	6.34 [6.14, 6.53]	8.07 [7.05, 9.10]	8.34 [7.39, 9.28]
	Pairwise mean difference on log scale* [95% CI], AU/ml on log scale	**Pairwise ratio difference on original scale [95% CI], ratio**	
Mild/Asymptomatic versus Moderate	-1.74 [-2.98, -0.49]	0.18 [0.05, 0.61]	
Mild/Asymptomatic versus Severe	-2.00 [-3.15, -0.84]	0.14 [0.04, 0.43]	
Moderate versus Severe	-0.26 [-1.93, 1.40]	0.77 [0.15, 4.07]	

[a]: 12 participants were missing data.

*We used Tukey's honestly significant test for testing differences among the adjusted means. There are significant differences between mild/asymptomatic disease and moderate disease ($p = .004$), and between mild/asymptomatic disease and severe disease. ($p < .001$)

Abbreviation: CI, confidence interval; AU, arbitrary unit.