

## Project 2

### **Do spike protein IgG levels vary by peak COVID-19 disease severity?**

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The objective of this study is to examine the effect of peak COVID-19 disease severity on spike protein IgG (SpikelgG) levels, controlling for gender and days since symptom onset (daysPSO). This research question was addressed by conducting a two-way ANCOVA with log-transformed SpikelgG (Log SpikelgG) as the dependent variable since the original scale is positively skewed.

In this study, analyses were restricted to the first measurement from each participant and only those participants with non-missing values for all variables of interest ( $n = 175$ ).

The sample characteristics, as shown in Table 1, were compared among the peak disease severity categories using Fisher's exact test for gender, ANOVA F-test for age and Log SpikelgG, and Kruskal-Wallis test for SpikelgG (due to the non-normality). These comparisons found that the three groups differed in SpikelgG and Log SpikelgG (both  $p < .001$ ). Additionally, distributions of continuous variables (Log SpikelgG and daysPSO) did not indicate strong outliers that should be removed according to sensitivity analysis.

The effect of peak disease severity on SpikelgG levels was estimated using a two-way ANCOVA, adjusting for gender as another factor and daysPSO as a covariate. Two interactions of interest, between peak disease severity and gender, and peak disease severity and daysPSO, were dropped in the model as they were both insignificant. We also conducted model diagnostics by examining a scatter plot of residuals on the fitted values and normal Q-Q plot. Although there appeared to be a few outliers, these plots indicated that the assumptions of homogeneity and normality were reasonably met. Table 2 provides (adjusted) means for Log SpikelgG and pairwise differences on Log SpikelgG and SpikelgG among groups using the Tukey-Kramer method. Participants with mild/asymptomatic disease had a median SpikelgG that was 0.18 (95% confidence interval = 0.05–0.61) and 0.14 (0.04–0.43) times lower than those with moderate and severe disease ( $p < .01$  and  $< .001$  in the log-scale), respectively. On the other hand, there was an insignificant difference between moderate and severe groups.

In conclusion, there is sufficient evidence to show that spike protein IgG levels vary by peak COVID-19 disease severity between mild/asymptomatic disease and moderate or severe categories, controlling for gender and daysPSO.

(360 words)

**Table 1. Summary of sample characteristics**

Variable	Peak disease severity				P*
	Mild/Asymptomatic (N=162)	Moderate (N=6)	Severe (N=7)	Total (N=175)	
<b>Gender, n (%)</b>					.75
Female	93 (57.4%)	3 (50.0%)	3 (42.9%)	99 (56.6%)	
Male	69 (42.6%)	3 (50.0%)	4 (57.1%)	76 (43.4%)	
<b>Age, years</b>					.66
Mean (SD)	40.8 (13.3)	38.5 (10.6)	44.9 (12.8)	40.9 (13.1)	
Range	19.0, 81.0	29.0, 56.0	26.0, 57.0	19.0, 81.0	
<b>SpikelgG, AU/ml</b>					<.001
Mean (SD)	1546.6 (5847.2)	3758.2 (2513.3)	5315.3 (3015.4)	1773.2 (5728.8)	
Median	509.2	4259.7	6099.1	563.9	
Range	4.5, 73076.5	489.2, 6645.7	1188.0, 9767.2	4.5, 73076.5	
<b>log (SpikelgG), AU/ml</b>					<.001
Mean (SD)	6.3 (1.4)	7.9 (1.1)	8.4 (0.7)	6.4 (1.4)	
Range	1.5, 11.2	6.2, 8.8	7.1, 9.2	1.5, 11.2	

\* P values were obtained using Fisher's exact test for gender, Kruskal-Wallis test for SpikelgG, and ANOVA F-test for age and log SpikelgG.

Abbreviations: SD = Standard Deviation; SpikelgG = Spike protein Immunoglobulin G.

**Table 2. Two-way ANCOVA results without interactions for SpikelgG (AU/ml)**

Peak disease severity category	Adjusted mean log SpikelgG (95% CI)	Estimate by pairwise comparison			
		Difference of means log SpikelgG (95% CI)		Ratio of medians SpikelgG (95% CI)	
		Moderate	Severe	Moderate	Severe
Mild/Asymptomatic (N=162)	6.34 (6.14, 6.53)	-1.74* (-2.98, -0.49)	-2.00** (-3.15, -0.84)	0.18 (0.05, 0.61)	0.14 (0.04, 0.43)
Moderate (N=6)	8.07 (7.05, 9.10)	-	-0.26 (-1.93, 1.40)	-	0.77 (0.15, 4.07)
Severe (N=7)	8.34 (7.39, 9.28)	-	-	-	-

\* p < .01; \*\* p < .001 adjusted for a multiple comparison: Tukey-Kramer method.

Abbreviations: CI = Confidence Interval; SpikelgG = Spike protein Immunoglobulin G.