PLASMA ANTIBODY AND N ANTIGEN STATUS PREDICT OUTCOMES IN OUTPATIENTS WITH COVID-19

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Nikolaus Jilg[1-3], Mark J. Giganti[4], Kara W. Chew[5], Katy Shaw-Saliba[6], Justin Ritz[4], Carlee Moser[4], Teresa Evering[7], Eric S. Daar[8], Joseph J. Eron[9], Judith S. Currier[5], Michael D. Hughes[4], H. Cliff Lane[6], Robin Dewar[10]*, Davey M. Smith[11]*, Jonathan Z. Li[2,3]*

[1]Massachusetts General Hospital, [2]Brigham and Women's Hospital, [3]Harvard Medical School, [4]Harvard T.H. Chan School of Public Health, [5]University of California Los Angeles, [6]National Institute of Allergy and Infectious Diseases, [7]Weill Cornell Medicine, [8]Lundquist Institute at Harbor-UCLA Medical Center, Torrance, [9]University of North Carolina, [10]Frederick National Laboratory, [11]University of California San Diego; **equal contribution*

BACKGROUND

- Reliable biomarkers of COVID-19 severity and outcomes are critically needed.
- We evaluated associations of anti-Spike and SARS-CoV-2 nucleocapsid antigen (N Ag) in plasma with clinical outcomes in non-hospitalized patients with mild-to-moderate COVID-19.

METHODS

- We studied 229 symptomatic, non-hospitalized adults with COVID-19 at high risk for progression enrolled in the US from Jan-Jul 2021 who received placebo within the ACTIV-2/A5401 platform trial.
- Baseline plasma samples were analyzed by the quantitative Simoa SARS-CoV-2 IgG antibody assay (lower limit of quantification [LLoQ] 0.77 µg/mL) and the quantitative Simoa SARS-CoV-2 N Protein Advantage (Quanterix) (LLoQ 3 pg/mL).
- Associations of SARS-CoV-2 anti-Spike, N Ag levels and outcomes (hospitalization/death, time to symptom improvement for 2 consecutive days) were evaluated by binomial, robust Poisson and Cox regression models.
- Adjusted hazard ratio (aHR) estimates are from models adjusted for age (<60/≥60), race (White/Non-white), ethnicity, sex, and time from symptom onset to enrollment (>5/≤5 days).

Table 1. Baseline Characteristics

	N=229
Age, years median (Q1, Q3) <60, n	 50 (41, 59) 172 (75%)
Female sex, n	116 (51%)
Cisgender, n	229 (100%)
Race, n Asian Black or African American White Multiracial/other	 6 (3%) 18 (8%) 196 (86%) 7 (3%)
Hispanic/Latinx, n	119 (52%)
BMI [kg/m^2], median (Q1, Q3)	30.5 (26.5, 36.3)
High risk for progression, n	221 (97%)
COVID-19 Vaccination, n	8 (3%)
Symptom onset ≤5 days, n	107 (47%)
Anti-Spike [µg/mL] Median (Q1, Q3) Min, max ≥0.77*, n	 <LLoQ (<LLoQ, 3.61)* <LLoQ, 413.33* 91 (40%)
N Antigen [pg/mL] Median (Q1, Q3) Min, max ≥3*, n	 17.0 (<LLoQ, 139) <LLoQ, 16,135.0 147 (64%)

*Lower limit of quantification (LLoQ) for anti-Spike: 0.77 µg/mL, for N antigen: 3 pg/mL; Q1, Q3, 1st/3rd quartile; BMI, body mass index.

Absence of Spike antibodies and higher levels of plasma SARS-CoV-2 N antigen predict hospitalization/death in outpatients with COVID-19

RESULTS

- At day 0, 40% had anti-Spike levels ≥LLoQ, and 64% had plasma N Ag levels ≥LLoQ (**Table 1**).
- Higher risk of hospitalizations/death was seen in those with unquantifiable plasma anti-Spike ab levels (15.9% vs 2.2%, RR [95% CI] 7.1 [1.8,33.3], p=0.006) (**Fig. 1A**), and quantifiable plasma N Ag (15.6% vs 1.2%, RR [95% CI] 12.8 [1.8, 93.3], p=0.012) (**Fig. 1B**). Combined markers showed the highest risk in those with unquantifiable anti-Spike and quantifiable N Ag, while those with quantifiable anti-Spike and unquantifiable N Ag were relatively protected (18.4% vs 0% risk of hospitalization/death) (**Fig. 1C**).
- Increasing risk of hospitalizations/death was seen with increasing levels of plasma N Ag (RR [95% CI] 3.94 [2.47,6.26] per +1 $\log_{10}$ pg/mL, p<0.001), and even higher with quantifiable anti-Spike (**Fig. 2**).
- We found longer times to symptom improvement with unquantifiable anti-Spike (median [Q1, Q3] 14 [8, >27] days vs 9 [4, 16] days (aHR [95% CI] 0.65 [0.47, 0.88], p=0.007) (**Fig. 3A**), and with increasing plasma N Ag (aHR [95% CI] 0.78 [0.63, 0.97] per +1 $\log_{10}$ pg/mL N Ag level, p=0.024) (**Fig. 3B).**

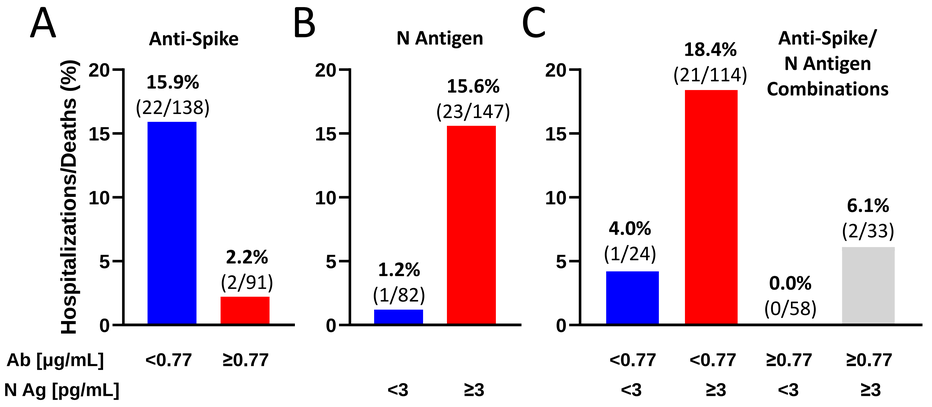


Figure 1. Hospitalizations/deaths at 28 days for values below vs equal to/above lower limit of quantification (LLoQ) evaluating (**A**) anti-Spike (Ab) (LLoQ=0.77 µg/mL), (**B**) N antigen (LLoQ=3 pg/mL), and (**C**) combinations of anti-Spike and N Ag.

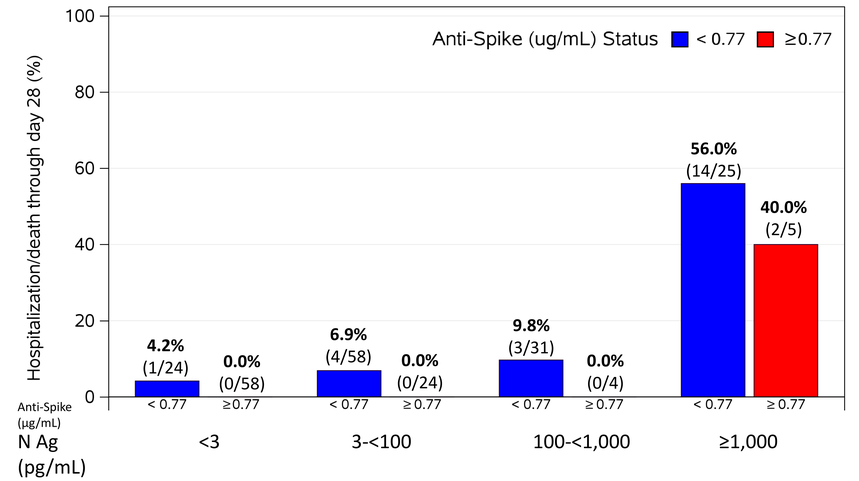


Figure 2. Correlation of N antigen categories, serostatus and hospitalization/death. Lower limit of quantification (LLoQ) for N antigen (N Ag) is 3 pg/mL, LLoQ for anti-Spike is 0.77 µg/mL.

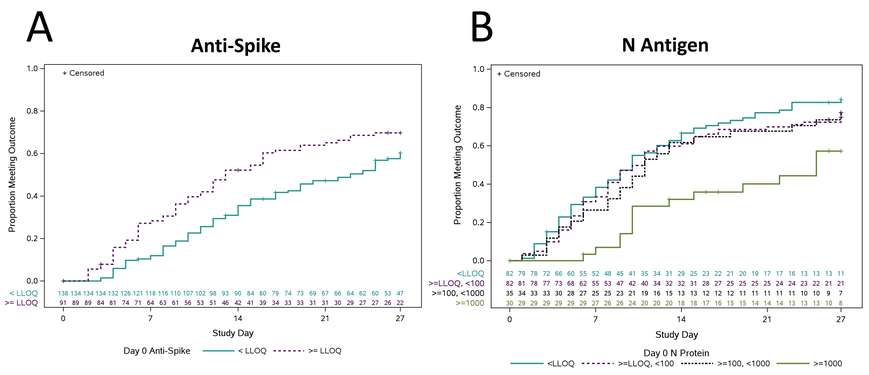


Figure 3. Time to improvement of all targeted symptoms for 2 consecutive days **(A)** for anti-Spike (below lower limit of quantification [LLoQ] vs ≥ LLoQ), and **(B)** for N antigen levels by 4 categories, <LLoQ, ≥LLoQ-<100, ≥100-<1,000, ≥1,000 [pg/mL]. LLoQ for anti-Spike is 0.77 µg/mL and for N antigen 3 pg/mL. Q1, Q3, 1st and 3rd quartile.

CONCLUSIONS

- Absence of anti-Spike and higher levels of plasma SARS-CoV-2 N Ag predicted hospitalization/death and delayed symptom improvement in COVID-19 outpatients.
- Anti-SARS-CoV-2-Spike and plasma SARS-CoV-2 N antigen may serve as informative biomarkers for risk stratification in the evaluation of outpatients with COVID-19.

Contact: *Nikolaus Jilg, Massachusetts General Hospital, njilg@mgh.harvard.edu*

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