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ORIGINAL ARTICLE

Clinical Application of a Solid-Phase Assay for SARS-CoV-2 IgG to Predict a Neutralizing Antibody Titer

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SUMMARY

Background: **To assess protective immunity among a general population against severe acute respiratory syndrome coronavirus 2, the correlation of the commercially available solid-phase assay (SPA) for SARS-CoV-2 IgG with a neutralization assay must be investigated.**
Methods: **Both the neutralization assay and SPA were performed on samples of 143 recovered coronavirus disease 2019 (COVID-19) patients. SARS-CoV-2 IgG was measured using two SPAs for the chemiluminescence immunoassay principle with different target proteins: nucleocapsid and spike protein (Architect i2000SR [Abbott] and Liaison XL [DiaSorin], respectively). The plaque reduction neutralization test (PRNT) was conducted to obtain titers for the neutralizing antibody.**
Results: **All patients had PRNT titers ranging from 10 to 2,560. Spike Ab SPA had greater sensitivity than nucleocapsid Ab SPA (81.1% [116/143] and 70.6% [101/143], respectively, $p = 0.003$). The values measured for both SPAs had a positive correlation with the PRNT titers (both $R = 0.77$, $p < 0.001$). To predict a high PRNT titer (≥ 160), cutoff values of two SPAs were adjusted based on receiver-operating characteristics curve analysis. The nucleocapsid Ab SPA (cutoff index of 4.17) attained 90.3% sensitivity and 75.9% specificity, whereas the spike Ab SPA (cutoff value of 109 unit/mL) attained 87.1% sensitivity and 89.3% specificity. Therefore, the spike Ab SPA had greater specificity than the nucleocapsid Ab SPA ($p = 0.003$).**
Conclusions: **The qualitative SPA for nucleocapsid Ab, as well as the quantitative SPA for spike Ab, had a modest positive correlation with the neutralization assay. However, spike Ab SPA was more suitable for neutralizing capacity.**
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Supplementary Data

Table S1. Clinical characteristics of the study population according to nucleocapsid Ab and spike Ab solid-phase assay results.

Characteristics	Total (n = 143)	SARS-CoV-2 IgG by solid phase assay					
		Nucleocapsid Ab			Spike Ab		
		Negative (n = 42)	Positive (n = 101)	p-value	Negative (n = 27)	Positive (n = 116)	p-value
Duration * median (IQR)	109.0 (104.0; 115.0)	109.0 (104.0; 113.0)	108.0 (104.0; 115.0)	0.915	109.0 (104.0; 113.0)	108.5 (103.5; 115.5)	0.710
Gender No. (%)				0.195			0.117
Male	68 (47.6%)	24 (57.1%)	44 (43.6%)		17 (63.0%)	51 (44.0%)	
Female	75 (52.4%)	18 (42.9%)	57 (56.4%)		10 (37.0%)	65 (56.0%)	
Height, median (IQR), cm	166.0 (158.0; 172.0)	167.0 (158.0; 174.0)	165.0 (160.0; 172.0)	0.447	171.0 (160.0; 175.0)	165.0 (158.0; 171.0)	0.069
Weight, median (IQR), kg	65.0 (56.0; 72.5)	67.0 (56.0; 75.0)	65.0 (56.0; 71.0)	0.771	68.0 (59.5; 76.5)	65.0 (56.0; 71.0)	0.191
Age median (IQR)	47.0 (30.0; 58.5)	35.5 (26.0; 46.0)	51.0 (39.0; 60.0)	< 0.001	36.0 (26.0; 45.5)	48.0 (34.0; 60.0)	0.005
Age distribution No. (%)				< 0.001			0.026
20 - 29	34 (23.8%)	16 (38.1%)	18 (17.8%)		12 (44.4%)	22 (19.0%)	
30 - 39	24 (16.8%)	12 (28.6%)	12 (11.9%)		4 (14.8%)	20 (17.2%)	
40 - 49	25 (17.5%)	8 (19.0%)	17 (16.8%)		6 (22.2%)	19 (16.4%)	
50 - 59	27 (18.9%)	3 (7.1%)	24 (23.8%)		3 (11.1%)	24 (20.7%)	
60 - 69	33 (23.1%)	3 (7.1%)	30 (29.7%)		2 (7.4%)	31 (26.7%)	
Age No. (%)				< 0.001			0.012
< 50	83 (58.0%)	36 (85.7%)	47 (46.5%)		22 (81.5%)	61 (52.6%)	
≥ 50	60 (42.0%)	6 (14.3%)	54 (53.5%)		5 (18.5%)	55 (47.4%)	
nAb titer No. (%)				< 0.001			< 0.001
10	6 (4.2%)	5 (11.9%)	1 (1.0%)		3 (11.1%)	3 (2.6%)	
20	26 (18.2%)	20 (47.6%)	6 (5.9%)		11 (40.7%)	15 (12.9%)	
40	43 (30.1%)	17 (40.5%)	26 (25.7%)		11 (40.7%)	32 (27.6%)	
80	37 (25.9%)	0 (0.0%)	37 (36.6%)		2 (7.4%)	35 (30.2%)	
160	19 (13.3%)	0 (0.0%)	19 (18.8%)		0 (0.0%)	19 (16.4%)	
320	9 (6.3%)	0 (0.0%)	9 (8.9%)		0 (0.0%)	9 (7.8%)	
640	2 (1.4%)	0 (0.0%)	2 (2.0%)		0 (0.0%)	2 (1.7%)	
2,560	1 (0.7%)	0 (0.0%)	1 (1.0%)		0 (0.0%)	1 (0.9%)	

Characteristics	Total (n = 143)	SARS-CoV-2 IgG by solid phase assay					
		Nucleocapsid Ab			Spike Ab		
		Negative (n = 42)	Positive (n = 101)	p-value	Negative (n = 27)	Positive (n = 116)	p-value
nAb ≥ 40 No. (%)				< 0.001			< 0.001
No	32 (22.4%)	25 (59.5%)	7 (6.9%)		14 (51.9%)	18 (15.5%)	
Yes	111 (77.6%)	17 (40.5%)	94 (93.1%)		13 (48.1%)	98 (84.5%)	
nAb ≥ 80 No. (%)				< 0.001			< 0.001
No	75 (52.4%)	42 (100.0%)	33 (32.7%)		25 (92.6%)	50 (43.1%)	
Yes	68 (47.6%)	0 (0.0%)	68 (67.3%)		2 (7.4%)	66 (56.9%)	
nAb ≥ 160 No. (%)				< 0.001			0.006
No	112 (78.3%)	42 (100.0%)	70 (69.3%)		27 (100.0%)	85 (73.3%)	
Yes	31 (21.7%)	0 (0.0%)	31 (30.7%)		0 (0.0%)	31 (26.7%)	
nAb titer value	40.0 (40.0;80.0)	20.0 (20.0; 40.0)	80.0 (40.0;160.0)	< 0.001	20.0 (20.0; 40.0)	80.0 (40.0; 160.0)	< 0.001
Abbott index value	3.3 (1.2; 4.8)	0.7 (0.3; 1.0)	4.2 (3.0; 5.8)	< 0.001	-	-	
DiaSorin index value	59.0 (20.0; 114.0)	-	-		9.7 (5.1; 12.2)	81.2 (42.7; 126.5)	< 0.001
Severity category No. (%)				< 0.001			0.015
Asymptomatic	8 (5.6%)	6 (14.3%)	2 (2.0%)		2 (7.4%)	6 (5.2%)	
Mild	90 (62.9%)	35 (83.3%)	55 (54.5%)		24 (88.9%)	66 (56.9%)	
Moderate	29 (20.3%)	1 (2.4%)	28 (27.7%)		0 (0.0%)	29 (25.0%)	
Severe	12 (8.4%)	0 (0.0%)	12 (11.9%)		1 (3.7%)	11 (9.5%)	
Critical	4 (2.8%)	0 (0.0%)	4 (4.0%)		0 (0.0%)	4 (3.4%)	
ICU admission history				0.333			0.605
No	138 (96.5%)	42 (100.0%)	96 (95.0%)		27 (100.0%)	111 (95.7%)	
Yes	5 (3.5%)	0 (0.0%)	5 (5.0%)		0 (0.0%)	5 (4.3%)	
Oxygen treatment history							0.303
No	127 (88.8%)	42 (100.0%)	85 (84.2%)	0.014	26 (96.3%)	101 (87.1%)	
Yes	16 (11.2%)	0 (0.0%)	16 (15.8%)		1 (3.7%)	15 (12.9%)	
MV treatment history							0.741
No	139 (97.2%)	42 (100.0%)	97 (96.0%)	0.452	27 (100.0%)	112 (96.6%)	
Yes	4 (2.8%)	0 (0.0%)	4 (4.0%)		0 (0.0%)	4 (3.4%)	
DM				0.397			1.000

Characteristics	Total (n = 143)	SARS-CoV-2 IgG by solid phase assay					
		Nucleocapsid Ab			Spike Ab		
		Negative (n = 42)	Positive (n = 101)	p-value	Negative (n = 27)	Positive (n = 116)	p-value
No	126 (88.1%)	39 (92.9%)	87 (86.1%)		24 (88.9%)	102 (87.9%)	
Yes	17 (11.9%)	3 (7.1%)	14 (13.9%)		3 (11.1%)	14 (12.1%)	
HTN				0.038			0.156
No	116 (81.1%)	39 (92.9%)	77 (76.2%)		25 (92.6%)	91 (78.4%)	
Yes	27 (18.9%)	3 (7.1%)	24 (23.8%)		2 (7.4%)	25 (21.6%)	
CKD				1.000			0.605
No	138 (96.5%)	41 (97.6%)	97 (96.0%)		27 (100.0%)	111 (95.7%)	
Yes	5 (3.5%)	1 (2.4%)	4 (4.0%)		0 (0.0%)	5 (4.3%)	
Liver disease				0.301			0.245
No	133 (93.0%)	41 (97.6%)	92 (91.1%)		27 (100.0%)	106 (91.4%)	
Yes	10 (7.0%)	1 (2.4%)	9 (8.9%)		0 (0.0%)	10 (8.6%)	
Hematologic disease				1.000			1.000
No	143 (100.0%)	42 (100.0%)	101 (100.0%)		27 (100.0%)	116 (100.0%)	
Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
Solid tumor				1.000			1.000
No	143 (100.0%)	42 (100.0%)	101 (100.0%)		27 (100.0%)	116 (100.0%)	
Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
CVA				0.625			0.921
No	140 (97.9%)	42 (100.0%)	98 (97.0%)		27 (100.0%)	113 (97.4%)	
Yes	3 (2.1%)	0 (0.0%)	3 (3.0%)		0 (0.0%)	3 (2.6%)	
COPD				1.000			0.425
No	142 (99.3%)	42 (100.0%)	100 (99.0%)		26 (96.3%)	116 (100.0%)	
Yes	1 (0.7%)	0 (0.0%)	1 (1.0%)		1 (3.7%)	0 (0.0%)	
Heart disease				1.000			0.921
No	140 (97.9%)	41 (97.6%)	99 (98.0%)		27 (100.0%)	113 (97.4%)	
Yes	3 (2.1%)	1 (2.4%)	2 (2.0%)		0 (0.0%)	3 (2.6%)	

Ab - antibody, nAb - neutralizing antibody, ICU - intensive care unit, MV - mechanical ventilator, DM - Diabetes mellitus, HTN – Hypertension, CKD - Chronic kidney disease, CVA - Cerebrovascular accident, COPD - Chronic obstructive pulmonary disease, SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2.

*** Duration: Duration from symptom onset or diagnosis to blood sample collection.**

Table S2. Clinical characteristics of asymptomatic patients (n = 8).

Gender	Age	Duration * (days)	PRNT titer	SARS-CoV-2 IgG by solid phase assay			
				Nucleocapsid Ab		Spike Ab	
				index value	results	measured value AU/mL	results
F	27	99	20	0.37	negative	4.9	negative
F	22	100	40	1.19	negative	28.8	positive
M	36	104	40	0.75	negative	8.3	negative
M	40	104	40	1.85	positive	51.1	positive
F	26	108	80	2.51	positive	39.9	positive
M	47	113	40	1.29	negative	43.8	positive
F	37	107	10	0.42	negative	20.4	positive
M	30	117	10	1.22	negative	16.4	positive

Abbreviations: PRNT - plaque reduction neutralization test, Ab - antibody.
*** Duration: COVID-19 diagnosis to sample collection.**

Table S3. Sensitivity, Specificity, PPV, and NPV of two solid-phase assays according to PRNT results.

PRNT	Solid-phase assays	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Chi^2	95% confidence intervals	p-value
≥ 40	Nucleocapsid antibody	84.7	78.1	93.1	59.5	3.38	-0.37 - 12.55	0.064
	Spike antibody	88.3	43.8	84.5	51.9	0.5	-4.7 - 11.0	0.473
≥ 80	Nucleocapsid antibody	100	56.0	67.3	100	31.0	18.2 - 23.1	< 0.001
	Spike antibody	97.1	33.3	56.9	92.6	42.5	26.8 - 36.0	< 0.001
≥ 160	Nucleocapsid antibody	100.0	37.5	30.7	100	68.0	43.9 - 49.0	< 0.001
	Spike antibody	100.0	24.1	26.7	100	83.0	54.4 - 59.4	< 0.001

Abbreviations: PRNT - plaque reduction neutralization test, PPV - positive predictive value, NPV - negative predictive value.

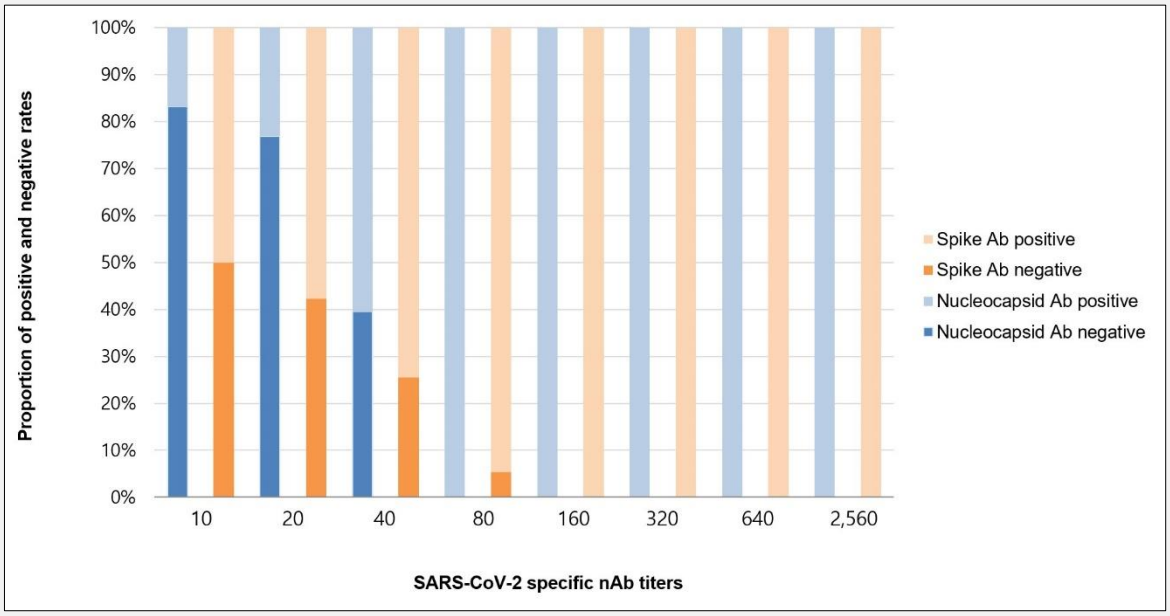

Figure S1. Proportion of nucleocapsid antibody (Ab) and spike Ab solid-phase assay positive rates according to severe acute respiratory syndrome coronavirus 2-specific neutralizing Ab titers in recovered patients with coronavirus disease 2019.

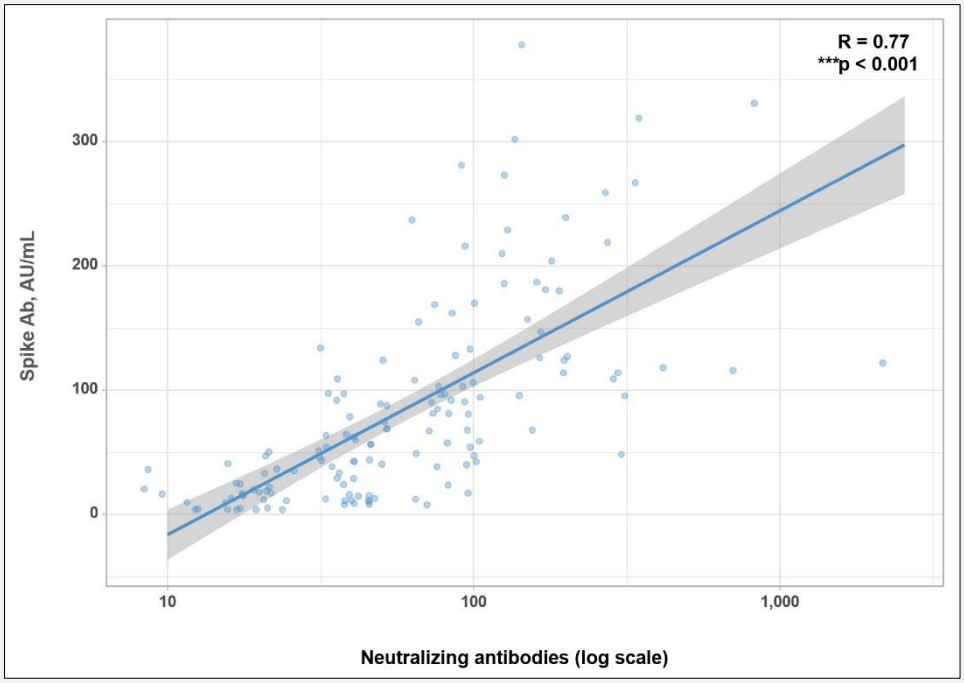

Figure S2. Distribution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike antibody (Ab) results according to SARS-CoV-2-specific neutralizing Ab titers in recovered patients with coronavirus disease 2019.

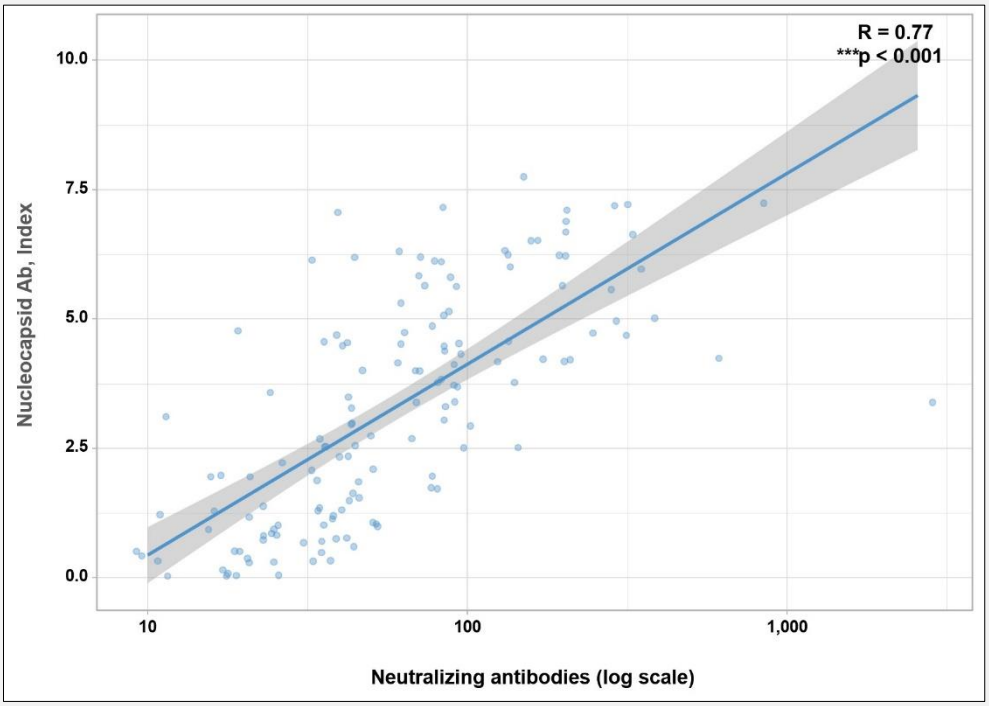

Figure S3. Distribution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid antibody (Ab) index values according to SARS-CoV-2-specific neutralizing Ab titers in recovered patients with coronavirus disease 2019.