Correlates of immunity in SARS-CoV-2 post-vaccine infections in the Prospective Assessment of SARS-CoV-2 Seroconversion (PASS) Study

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Background

We sought to determine pre-infection correlates of immunity against SARS-CoV-2 post-vaccine infections in a prospective cohort of healthcare workers.

Inclusion criteria:
- ≥ 18 years old
- Generally healthy
- Works at Walter Reed National Military Medical Center (WRNMMC)

Exclusion criteria:
- Severely immunocompromised
- History of COVID-19
- SARS-CoV-2 seropositive at time of study entry

Research Design
- Baseline peripheral blood mononuclear cells (PBMCs), serum, plasma, saliva
- Monthly serum antibody testing for SARS-CoV-2 (changed to quarterly in Fall 2021)
- PBMCs drawn at scheduled intervals
- PCR testing at the WRNMMC COVID-19 center every time a participant has symptoms of viral respiratory infection
- Baseline and then serial symptom & risk exposure/PPE use/social distancing questionnaires

Methods

Single-center, observational cohort of 183 healthcare workers.

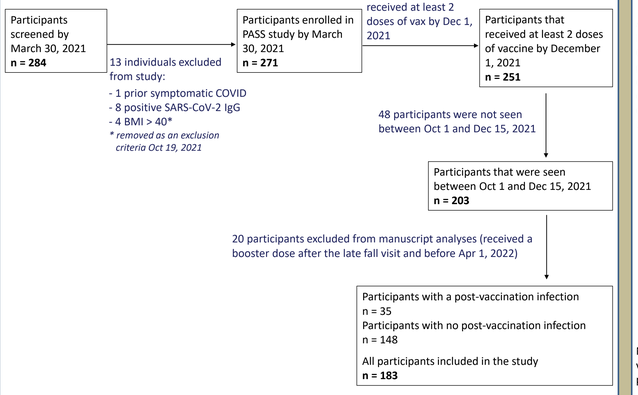
Testing for ancestral (WT) SARS-CoV-2 Spike (S)-specific IgG antibodies was performed on serum collected during the Fall 2021 visit and conducted using a microsphere-based multiplex immunoassay (research assay) interpolated against an internal standard curve for binding antibody units and then calibrated to the US National Standard (readout: WHO Binding Antibody Units/ml - BAU/ml).

Testing for WT SARS-CoV-2 S-specific IgG, IgA and secretory IgA was performed on saliva collected during the same visit and using the same microsphere-based multiplex immunoassay interpolated against an internal standard curve for binding antibody units/ml.

Serum samples were also tested using a commercial assay in collaboration with Quest Diagnostics (Elecsys® anti-SARS-CoV-2 S for use on the Roche Cobas analyzer). This electrochemiluminescence immunoassay is intended for qualitative detection of total antibodies to SARS-CoV-2 S protein receptor binding domain (RBD).

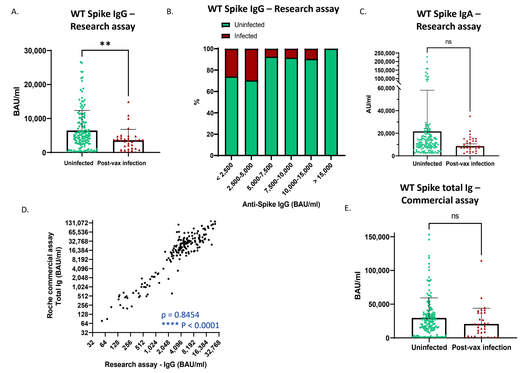
Neutralizing antibody titers against the wild-type virus (D614G) were determined by microneutralization assays, and against the Delta variant (B.1.617.2), and 2 Omicron subvariants (BA.1, BA.1.1) by lentiviral pseudovirus neutralization assays.

STROBE flowchart of the study cohort.



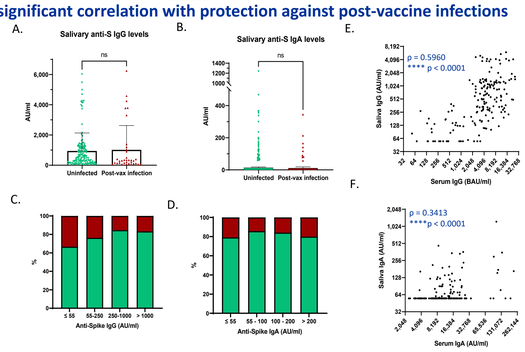
Results

Pre-Omicron epidemic serum anti-S IgG antibody levels correlate with protection against post-vaccine infections



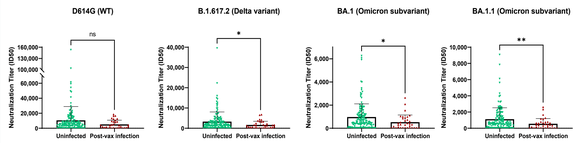
A) Comparison of ancestral (WT) anti-Spike (S)-serum IgG (BAU/ml) between the uninfected group (n=144) and the post-vaccination infection group (n=34) using the research assay. P value determined using the Mann-Whitney U test (** p = 0.0045). B) Percentages of uninfected vs post-vaccination infected participants depending on the serum levels of anti-S IgG (BAU/ml). C) Comparison of anti-S-serum IgA (AU/ml) between the uninfected group (n=144) and the post-vaccination infection group (n=34) using the research assay. P value determined using the Mann-Whitney U test (p = 0.0903). D) Strong correlation (Spearman ρ = 0.8454) between research assay anti-S IgG levels with the Roche commercial assay for serum levels of total anti-RBD antibodies. E) Comparison of anti-RBD-serum total antibodies (BAU/ml) between the uninfected group (n=144) and the post-vaccination infection group (n=34) using the Roche commercial assay. P value determined using the Mann-Whitney U test (p = 0.0996).

Pre-Omicron epidemic saliva anti-S antibody levels do not show statistically significant correlation with protection against post-vaccine infections



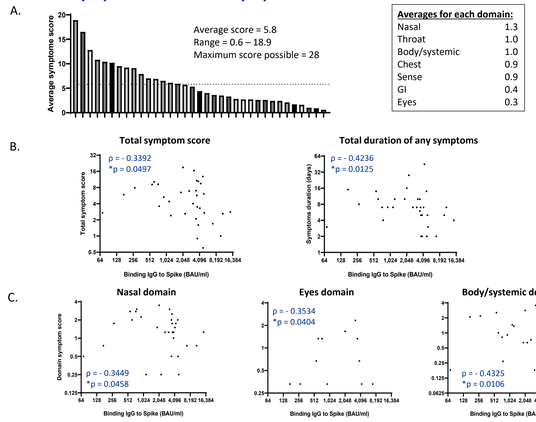
A) Comparison of anti-Spike (S)-saliva IgG (AU/ml) between the uninfected group (n=147) and the post-vaccination infection group (n=35) using the research assay. P value determined using the Mann-Whitney U test (ns p = 0.1195). B) Comparison of anti-Spike (S)-saliva IgA (AU/ml) between the uninfected group (n=147) and the post-vaccination infection group (n=35) using the research assay. P value determined using the Mann-Whitney U test (ns p = 0.4731). C) Percentages of uninfected vs post-vaccination infected participants depending on the saliva levels of anti-S IgG (AU/ml). D) Percentages of uninfected vs post-vaccination infected participants depending on the saliva levels of anti-S IgA (AU/ml). E) Spearman correlation (ρ) to determine whether the anti-S serum IgG correlated with the anti-S saliva IgG. F) Spearman correlation (ρ) to determine whether the anti-S serum IgA correlated with the anti-S saliva IgA.

Pre-infection neutralization titers against Omicron subvariants provide robust correlation with protection against post-vaccine infections



Neutralization assays used lentiviral pseudoviruses bearing SARS-CoV-2 S proteins from WT D614G (ns p=0.0627), Delta variant (* p=0.0379) or Omicron subvariants BA.1 (* p=0.0114) and BA.1.1 (** p=0.0074)(n = 144 uninfected group; n= 34 post-vaccination infection group). P values determined using the Mann-Whitney U test.

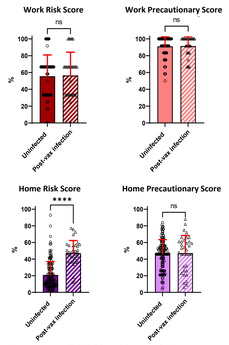
Pre-Omicron epidemic serum anti-S IgG antibody levels correlate with reduced duration of symptoms and fewer symptoms



A) Total symptom scores and average scores for each symptom domains for the 35 post-vaccination infections. B) Spearman correlations (ρ) to determine whether the anti-S serum IgG (research assay) correlated with the total symptom scores and the total duration of any symptoms. C) Spearman correlations (ρ) to determine whether the anti-S serum IgG (research assay) correlated with each domain symptom scores. No correlation was found for the gastrointestinal, chest, smell/taste, and throat domains.

Home risk score provides robust correlation with protection against post-vaccine infections

Work		Home	
1. Risk Exposure	**2. Precautionary Measures**	**3. Risk Exposure**	**4. Precautionary Measures**
1. Days working in the hospital (0-4)	1. Consistent PPE use during patient interactions (0-4)	1. Member of household tested COVID+ (0-4)	1. Mask wearing outside of the home (grocery stores) (0-4)
2. Direct contact with COVID+ patients (0-4)	2. PPE use when not directly interacting with patients (0-4)	2. Member of household had symptoms consistent with COVID and hasn't been tested (0-2)	2. Mask wearing while outside doing solitary activities (0-4)
3. Conducted high-risk activities on COVID+ patients (0-4)	3. Consistency of applying hand sanitizer/washing hands before and after interacting with patients (0-4)	3. Number of times in an out of house activity (grocery, restaurant, gym, bus…) (0-4 averaged for all 6 responses to this question)	3. Consistently practicing social-distancing outside home (0-4)
		4. Size and number of social gatherings (0-4 averaged for all 5 responses to this question)	4. Disinfecting mail/packages delivered to home (0-4)
			5. Type of mask typically used (1-4 averaged for all 4 responses to this question)
Work Risk Score= (Total score/12)x100%	**Work Precautionary Score= (Total score/12)x100%**	**Home Risk Score= (Total score/14)x100%**	**Home Precautionary Score= (Total score/20)x100%**



Risk exposure and precautionary scores were collected during the visit following the Late Fall 2021 visit. Work risk score, ns p=0.8333 (n=144 uninfected group; n=35 post-vaccination infection group); Work precautionary score, ns p=0.7664 (n=138 uninfected group; n=33 post-vaccination infection group); Home risk score, *** p<0.0001 (n=144 uninfected group; n=35 post-vaccination infection group); Home precautionary score, ns p=0.5705 (n=144 uninfected group; n=35 post-vaccination infection group). P values determined using the Mann-Whitney U test.

Pre-Omicron epidemic serum anti-S IgG antibody levels, and neutralization titers against the Delta variant B.1.617.2 and Omicron subvariant BA.1.1 are strong covariates of protection against post-vaccine infections

Immunologic Marker	OR	Lower CI	Upper CI	AUC	P-value
Serum Binding IgG to Spike (BAU/ml)	0.655	0.467	0.918	0.919	0.014
Serum Binding IgA to Spike (AU/ml)	0.346	0.079	1.513	0.908	0.159
Roche Serum anti-S total Igs (BAU/ml)	0.720	0.497	1.041	0.903	0.081
ID50 neutralizing titer BA.1.1	0.476	0.239	0.945	0.914	0.034
ID50 neutralizing titer D614G	0.308	0.095	1.000	0.907	0.050
ID50 neutralizing titer B.1.617.2	0.365	0.134	0.995	0.907	0.049
ID50 neutralizing titer BA.1	0.663	0.418	1.051	0.908	0.081
Saliva Binding IgG to Spike (AU/ml)	0.972	0.770	1.227	0.887	0.811
Saliva Binding IgA to Spike (AU/ml)	0.790	0.329	1.898	0.891	0.597

COVID-19 risk by immunologic marker. The pre-Omicron wave covariates were adjusted for age, sex and home risk score. Odds ratios (OR) represent the relative change in the odds of infection corresponding to an increase in the independent variable of 1/10 of its range. CI, confidence interval; AUC, area under the curve; ID50, 50% inhibitory dilution. ROC analyses (not shown) showed that a 90% protection was associated with anti-S IgG serum levels ≥ 7200 BAU/ml, B.1.617.2 ID50 > 1:1200 and BA.1.1 ID50 > 1:1400. An 80% protection was associated with anti-S IgG serum levels ≥ 4900 BAU/ml, B.1.617.2 ID50 > 1:1100 and BA.1.1 ID50 > 1:900.

Conclusions

Anti-spike IgG binding antibody (bAb) serum levels correlate with protection against symptomatic infection during the 1st Omicron wave, but complete protection was only observed with very high levels (> 15,000 BAU/ml).

There is a high correlation between the research assay and the commercial Roche assay, raising the prospect of translational clinical implementation.

Spike IgG bAb serum levels correlate with reduced duration of symptoms and fewer symptoms in the nasal, eyes, and body/systemic domains.

Pre-Omicron epidemic saliva levels of anti-spike IgG and IgA antibodies do not show statistically significant correlation with protection against post-vaccine infection.

Neutralization titers against the Omicron subvariants provide robust correlation of protection against post-vaccine infection, but are not markedly better correlates than binding antibody levels.

The Home risk score provides a robust correlate of protection against post-vaccine infection.

Regression analysis for immunologic markers after adjustment for non-immunologic markers (sex, age, and home risk score) shows that pre-Omicron wave Spike IgG bAb serum levels (research assay) and neutralization titers against the Delta variant B.1.617.2 and Omicron subvariant BA.1.1 are strongly correlated with protection against post-vaccine infection (p=0.014, p=0.049, and p=0.034, respectively).

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