Observational Study of Receptor Binding Domain Spike Antibody Responses to 3 SARS-CoV-2 Vaccinations in Noninfected Subjects: Parallel Neutralizing Antibody and Cardiac Troponin I and T Observations

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Background: Our goals were to demonstrate receptor binding domain spike 1 (RBD S1) protein antibody (Ab) kinetic responses to multiple vaccines over approximately 180 days, neutralizing Ab effectiveness, and high-sensitivity cardiac troponin I (hs-cTnI) and T (hs-cTnT) responses in postvaccinated, non-SARS-CoV-2–infected subjects.

Methods: Blood specimens were collected pre- and postvaccinations from seronegative subjects. RDB S1 Abs were measured by the novel Qorvo Biotechnologies Omnia platform. Neutralizing Abs and hs-cTnI and hs-cTnT were measured on the ET Healthcare Pylon 3D.

Results: Two-dose vaccines (Pfizer, Moderna) had peak RBD S1 Ab concentrations about 45 to 55 days after both doses and showed declines over the next 50 to 70 days. The Janssen vaccine showed lower RBD S1 Ab peak concentrations, continued to increase over time, and plateaued after 60 days. There was strong neutralizing Ab response post vaccinations, with only 3 specimens, shortly before and shortly after vaccination, not showing a response. Specimens showed no hs-cTnI (all < 3 ng/L) and hs-cTnT (all < 6 ng/L) increases or changes over time.

Conclusions: We demonstrate in seronegative SARS-CoV-2 subjects that Pfizer and Moderna vaccinations provide strong, neutralizing RBD S1 Ab effectiveness, based on 2 different assays after 2 doses, with the Janssen single-dose vaccine showing a lower RBD S1 Ab response over 4 to 6 months. No myocardial injury was associated with the Pfizer postvaccination. The Qorvo Biotechnologies RBD S1 Ab assay measured on the Omnia platform has potential as a point-of-care platform.

IMPACT STATEMENT

Our findings provide guidance regarding clinical laboratory implementation of serology testing for ruling in and out SARS-CoV-2 infection pre- and postvaccination.

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INTRODUCTION

The SARS-CoV-2 pandemic has upended healthcare globally since late 2019 and from early 2020 in the United States. New variants emerge that continue to challenge our healthcare systems and laboratory testing capabilities. Triage, management, and treatment of sick patients presenting to emergency departments and clinics with the RNA virus has mobilized research and development of diagnostics tests in the field of laboratory medicine to screen for and diagnose/detect the active SARS-COV-2 virus, primarily utilizing the molecular PCR technology. Instrumentations have been advanced to provide a large range of analytical sensitivities and turnaround times, allowing for the clinical sensitive and specific detection of the viral RNA particles, levels indirectly represented by the cycle time value. However, not all assays are created equal, and the user must be aware of the specific analytical characteristics of the assay in use and not generalize the findings from one assay with another when it comes to clinical utilization and potential of false-positive and false-negative results (1–3).

Upon SARS-COV-2 infection of an individual, the immune system initiates the synthesis of antibodies (Abs) in an attempt to neutralize the infectious virus. We have learned that Ab release into the circulation follows a timed cascade of Ab release of IgA, IgM, and IgG, demonstrating that it is optimal to follow the IgG Ab response (3). As the SARS-COV-2 virus has several antigen components, the following IgG Abs have been described from the humoral immune response of an infected individual: nucleocapsid (N protein), spike 1 (S1) protein, and the receptor binding domain (RBD) S1 protein, which has been identified with properties to neutralize the virus. The literature has shown a strong relationship between the RBD S1 Ab level (using semiquantitative assays) and neutralizing Abs (NAbs) and suggests the RBD S1 Ab as a surrogate for demonstrating effectiveness against infection from SARS-COV-2 (4–8). Further, recognizing the therapeutic (neutralizing) effectiveness of Abs directly against the RBS S1 Ab, several monoclonal Ab medications have been developed and put into clinical practice to improve outcomes of patients ill with SARS-COV-2 (9). Parallel development of SARS-COV-2 vaccines directed toward the RBD S1 protein have been developed and received Food and Drug Administration (FDA) emergency use authorization: 2 mRNA vaccines, Pfizer BioNTech BNT162b2 and Moderna mRNA-127, and 1 DNA adenovirus vector vaccine, Johnson & Johnson Janssen (10–14). Recently, the FDA cleared the Pfizer BioNTech BNT162b2 vaccine. One study demonstrated a significantly greater humoral immune response following 2 vaccine doses for the Moderna vaccine compared to the Pfizer vaccine based on the measurement of IgG RBD -S1 protein, measured by the Roche Diagnostics assays (13). However, the reader must keep in mind that no standardization or harmonization of Ab assays between different manufacturer's RBD-S1 Ab assays has been established (15, 16); however, work continues in this area (17). Further, there is no evidence to date that increased Ab concentrations translates to a difference in the duration or amount of protection from getting ill following exposure to any SARS-COV-2 variant.

The purpose of our small observational study was 3-fold: (a) to demonstrate an RBD S1 Ab assay kinetic response over an approximately 180-day period, in individuals not previously infected with SARS-COV-2, to the 3 vaccinations used in the United States; (b) to show surrogate NAb assay effectiveness results for the Qorvo RBD S1 Ab testing system; and (c) to examine high-sensitivity cardiac troponin I (hs-cTnI) and T (hs-cTnT) findings in postvaccinated subjects to provide information regarding vaccine-associated myocardial injury.

METHODS AND SUBJECTS

The study received investigational review board approval by the "Pearl IRB, #21-MDC-QOR-133."

Serum and/or EDTA plasma specimens were prospectively collected pre- and postvaccination between December 2020 and April 2021 from seronegative consented subjects prior to blood draws and vaccinations.

First, we studied 11 subjects, 3 females and 8 males, ages 24 to 41 years, who were enrolled to monitor the novel Qorvo Biotechnologies RBD S1 Ab assay postvaccination kinetic response to 3 different vaccines: 5 individuals were vaccinated with 2 doses of the BNT162b2 SARS-CoV-2 mRNA vaccine (Pfizer), 3 individuals were vaccinated with 2 doses of the Moderna mRNA-1273 vaccine, and 3 individuals were vaccinated with 1 dose of the JNJ-78436735 Johnson & Johnson Janssen vaccine. Second, the NAb relationship for the Qorvo Biotechnologies RBD S1 Ab responses were assessed. Third, both hs-cTnI and hs-cTnT measurements were performed on 51 specimens remaining from 6 subjects using ET Healthcare assays.

Antibody Assay

The Qorvo Biotechnologies Ab assay (research-use only), detecting Abs to the RDB S1 antigen, was tested on the Qorvo Omnia Instrument that uses bulk acoustic wave (BAW) detection technology. This detection scheme utilizes a gravimetric sensor based on proprietary piezoelectric BAW technology in an immunoassay format previously described in the literature (18, 19). The microfluidics technology integrates the biochemistry with the BAW sensor enclosed entirely in a test cartridge. The frequency of the native sensor is approximately 3 Ghz. A positive displacement pump aspirates fluid from reagent wells on the cartridge and continually moves the fluid over the BAW sensor where the immunoassay reaction occurs, resulting in a change in the resonance frequency signal. Because the sensitivity of the sensor increases with the square of the frequency, BAW provides extremely high analytical sensitivity. Each test cartridge also includes a reference and a control channel. The dynamic range of the assay is 0 to 75 AU/mL (0–75 AU/mL absorbance units); limit of detection, 0.414 AU/mL; limit of quantitation (20% CV), 0.487 AU/mL; and positive cutoff, 1 AU/mL. Imprecision of the assay was determined by running a 3-day study with 2 runs per day and 2 replicates per run using a high and low level of pooled patient samples with results of 8.8% and 15%, respectively.

ET Healthcare Neutralizing Antibody Assay

The SARS-CoV-2 surrogate NAb assay, as previously described (20, 21), was used to measure plasma Ab levels on the TOP-Plus (Pylon 3D analyzer; ET HealthCare) as reported in 10 subjects on 45 specimens (insufficient sample volumes were not available from one subject). NAB is a surrogate NAb assay (competitive binding assay), based on the anti-SARS-CoV-2 Ab-mediated inhibition of the interaction between the angiotensin-converting enzyme 2 receptor protein and the RBD. The percentage of RBD- angiotensin-converting enzyme 2 binding is defined as %B/B0 = (sample RFU/negative control RFU) * 100%; with 85% as neutralizing cutoff. The assay had been shown to correlate well with both plaque reduction neutralization test and pseudovirus neutralization test, 2 well-established SARS-CoV-2 virus neutralization tests.

ET Healthcare hs-cTnI and hs-cTnT Assays

cTnI and cTnT were measured in plasma using high-sensitivity assays on the Pylon 3D analyzer (ET HealthCare), as previously described (22). For hs-cTnI, assay characteristics were: limit of detection, 1.4 ng/L; 99th percentile upper reference limits, male 27 ng/L and female 21 ng/L; 10% CV, 10 ng/L; and 20% CV, 2 ng/L. For hs-cTnT, assay characteristics were: limit of detection, 0.8 ng/L; 99th percentile upper reference limits, male 14 ng/L and female 13 ng/L; 10% CV, 4 ng/L; and 20% CV at 1 ng/L.

Data Analysis

RBD S1 Ab assay kinetic response over the study period was fit with LOESS (locally estimated

scatterplot smoothing) polynomial regression in R (version 4.1.3).

RESULTS

Goal 1

Figure 1 shows the Qorvo RBD S1 Ab responses prevaccination and approximately 150 days post the initial vaccination for 11 seronegative subjects prior to vaccination with 2 doses of the Pfizer vaccine, 2 doses of the Moderna vaccine, and 1 dose of the Johnson & Johnson Janssen vaccine. Figure 1, A shows individual RBD S1 Ab concentration points, while Fig. 1, B shows estimated scatterplot, smoothing fitted curves to individual RBD S1 Ab concentrations, for all 3 vaccinations. The vertical line in Fig. 1, B shows that the median time to second dose was 21 days. It further illustrates that for the 2-dose vaccines, the peak RBD S1 Ab concentration was about 20 days after second dose. The RBD S1 Ab responses for Pfizer and Moderna were similar in values. The RBD S1 Ab responses post the Johnson & Johnson Janssen vaccine showed lower RBD S1 Ab responses than the 2-dose vaccines and plateaued at approximately 41 days after vaccination.

Goal 2

Figure 2 shows the strong NAb relationship for the Qorvo RBD S1 Ab responses utilizing the ET Healthcare's indirect NAb assay in 45 specimens from 10 subjects obtained over time postvaccination. Only 2 of the subjects did not show a neutralizing response, which represented one subject's prevaccine sample and another subject's prevaccine sample and day 2 postvaccination sample. All postvaccinated subjects maintained a neutralizing response, even after 100 to 150 days postvaccination, based on the previously reported neutralizing response as <85%, with the lower percentage representing a greater neutralizing effect.

Goal 3

hs-cTnI and hs-cTnT measurements were obtained on 57 specimens from 8 different seronegative subjects vaccinated with 2 doses of the Pfizer vaccine. Insufficient sample volumes were available from 3 subjects vaccinated with the Johnson & Johnson Janssen vaccine. For all pre- and postvaccination specimens, all results for all subjects were found to be <3 ng/L for hs-cTnI and <6 ng/L for hs-cTnT (absolute concentration data not shown), indicating that no myocardial injury was associated with the Pfizer vaccination over 150 days postvaccination.

DISCUSSION

Our findings are unique in several aspects. First, we have demonstrated in seronegative SARS-COV-2 subjects that both the Pfizer and Moderna vaccinations provide initial strong, neutralizing RBD S1 Ab effectiveness (Fig. 2), based on a novel, research-use only RBD S1 Ab assay, for at least 4 to 6 months after 2 doses of vaccination. This is the first report of the Qorvo Biotechnologies RBD S1 Ab assay measured on the Omnia instrument. The Omnia instrument has been previously cleared by the FDA for measurement of SARS-COV-2 antigen. RBD S1 Ab was not detected in prevaccinated specimens from all subjects.

Second, both the Pfizer and Moderna RBD S1 Ab concentrations decrease over time, supporting previous studies that have demonstrated declining Ab concentrations over time following 2-dose vaccinations (23). However, both the Pfizer and Moderna RBD S1 Ab concentrations generally remained greater than the 1-dose Johnson & Johnson Janssen vaccinated subjects after 120-plus days.

Third, the quantitative Qorvo Biotechnologies RBD S1 Ab concentrations results are reportable, compared to "qualitative" positive/negative tests that do not allow providers and patients a real sense of an approximate, indirect titer of the

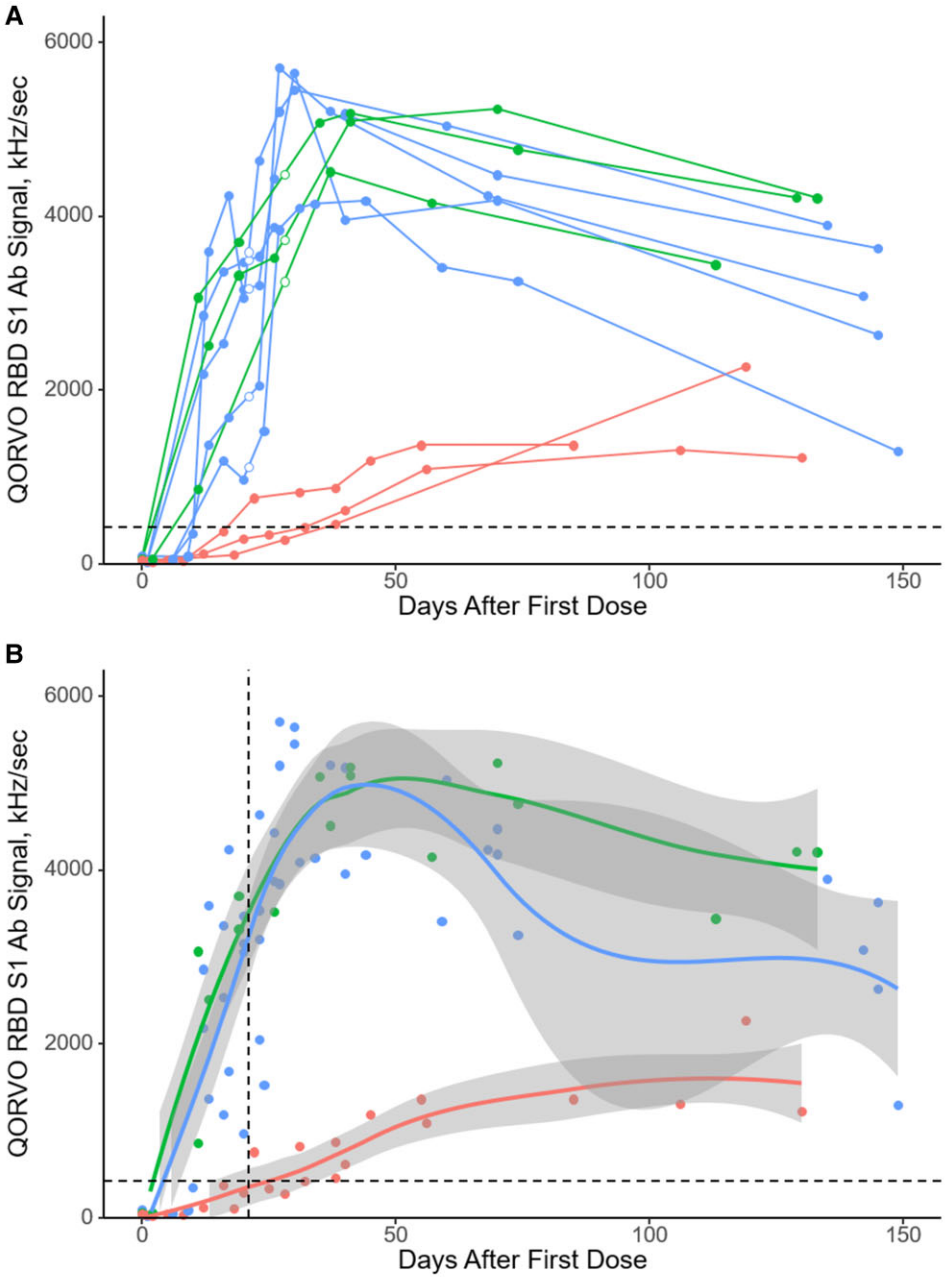

Fig. 1. Qorvo RBD S1 Ab responses prevaccination and approximately 120 to 150 days post initial vaccination for 11 seronegative subjects (Pfizer [blue], Moderna [green], Johnson & Johnson Janssen [red]) based on (A) individual RBD S1 Ab concentration points and (B) estimated scatterplot, smoothing fitted curves to individual RBD S1 Ab concentrations. The horizonal dashed line indicates the normal cutoff. Open boxes in (A) indicate day of second dose. The vertical line in (B) represents the median time to second dose.

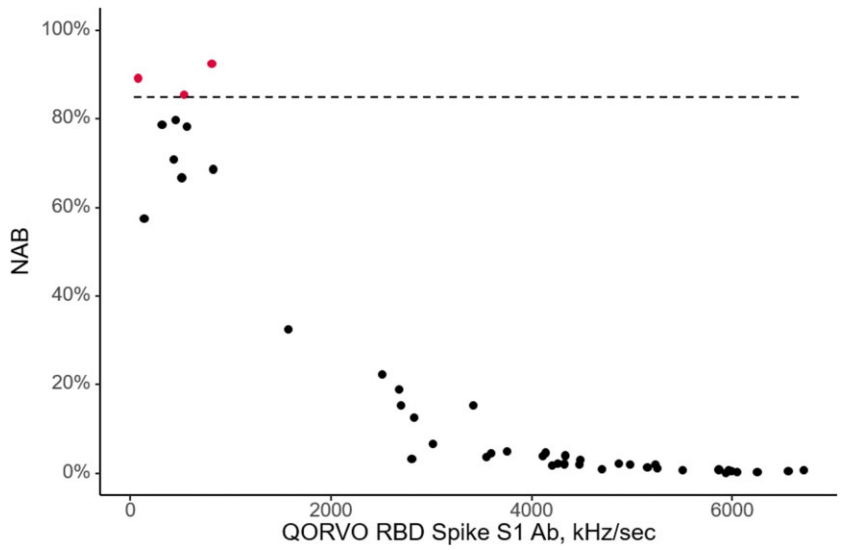

Fig. 2. NAb relationship for the Qorvo Biotechnologies RBD S1 Ab responses utilizing the ET Healthcare's indirect NAb assay in 45 specimens from 10 subjects obtained over time post Pfizer vaccination. Dashed line indicates 85% cutoff. Red circles indicate nonneutralizing.

amount of RBD S1 Abs present. This provides the ability to follow increasing or decreasing Ab concentrations that may be important for determining when booster vaccinations may be necessary. We recognize, as a limitation, that studies with a larger number of subjects, with further statistical analyses, are needed.

Fourth, we demonstrate that both cTnI and cTnT concentrations, measured by high sensitivity assays (24), do not reflect any myocardial injury in the seronegative subjects studied postvaccination, as has been described in SARS-COV-2–infected patients (25, 26). We do recognize the limitation that our subject numbers are small, but our observations are unique and promisingly nullify the perception and misinformation often reported that vaccinations are frequently associated with myocardial injury, particularly myocarditis.

Fifth, we provide parallel RBD S1 Ab measurements by a novel assay (Qorvo Biotechnologies) and both hs-cTnI and hs-cTnT assays (ET Healthcare) postvaccination. At present, the Qorvo Biotechnologies RBD S1 Ab assay is not cleared by the FDA or other regulatory agency for clinical use. However, the assay has excellent potential for assisting providers and the general population with a better understanding of Ab neutralization (a) postvaccination and (b) following SARS-COV-2 infection. The RBD S1 Ab assay has promise as a point-of-care, near-patient capability in urban and rural settings.

Regarding the ET Healthcare hs-cTnI and hs-cTnT assays utilized in the current study, both of which are China FDA cleared for clinical use, including point of care, we also suggest that in the current pandemic setting of the reemergence of SARS-COV-2 infections with new variants, that emergency use authorization of hs-cTn assays in a point-of-care format should be considered, as have SARS-COV-2 PCR and antigen tests in the United States. This would be a valuable cardiac biomarker tool for assisting emergency department providers in the triage of acutely presenting patients with complications from SARS-COV-2.

Nonstandard Abbreviations: Abs, antibodies; N, nucleocapsid; S1, spike 1; RBD, receptor binding domain; NAbs, neutralizing antibodies; FDA, Food and Drug Administration; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T.

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